EFFECT OF EXOGENOUS APPLICATION OF TRIACONTANOL AND HYDROGEN SULPHIDE ON *BRASSICA JUNCEA* L. EXPOSED TO SALINITY STRESS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Botany

By

Tunisha Verma

Registration Number: 11919631

Supervised By

Dr. Bilques Farooq (29717)

Department of Botany (Assistant Professor)

Lovely Professional University, Punjab

Co-supervised by

Dr. Dhriti Kapoor

Department of Biological and Environmental Sciences, (Assistant Professor)

Shoolini University, Solan

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2024

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Effect of exogenous application of Triacontanol and Hydrogen sulphide on *Brassica juncea* L. exposed to salinity stress**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr Bilques Farooq, working as Assistant Professor, in the Department of Botany/School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)
Name of the scholar: Tunisha Verma
Registration No.: 11919631
Department/school: Department of Botany,
School of Bioengineering and Biosciences
Lovely Professional University, Punjab

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Effect of exogenous application of Triacontanol and Hydrogen sulphide on *Brassica juncea* L. exposed to salinity stress**" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Botany, School of Bioengineering and Biosciences, is a research work carried out by Tunisha verma 11919631, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)
Name of supervisor: Dr. Bilques Farooq
Designation: Assistant Professor
Department/school: Department of Botany/School of Bioengineering and Biosciences
University: Lovely Professional University, Punjab

(Signature of Co-Supervisor)
Name of Co-Supervisor: Dr Dhriti Kapoor
Designation: Assistant Professor
Department/school: School of Biological and Environmental sciences
University: Shoolini University, Solan, Himachal Pradesh

ABSTRACT

Soil salinity is growing global hazard to plant productivity worldwide. Growth of plant was found to be exposed to salinity could be either due to ionic effect on metabolism related due to relations of water. Therefore, strategies are being used to study the effect plant growth under soil salinity. The production and cultivation of agricultural crops has been set up by many environmental pressure cause a substantial decline in crop production and quality. In order to increase food security and reduce economic losses it is necessary to understand crop responses to significant soil and plant stress. Exogenous administration of hormones provides a different strategy for combating stressful situations because of reduced plant growth under salt stressed conditions as a result of disrupted hormonal balance. In order to understand physiological and biochemical parameters affected by combined treatment of phytohormone (Triacontanol) and evolving signalling molecule (H_2S). Thus, present study was created with consideration of the function of triacontanol and hydrogen sulphide in various aspects of growth attributes, the utility of *Brassica juncea*.

There is need for some cost effective, eco-friendly and adaptable technique to confer salt stress. Keeping in mind the ill-effects of salt stress on plants, the current research work was planned to reduce salinity. Seeds of *Brassic*a were supplied with triacontanol solution for 8 h, and left over seeds were submerged in distilled water for same amount of time. Salt stress was applied in the form of three different concentrations 50, 100, and 150 mM concentrations in the soil. Seedlings and plants of *Brassica* were supplied with H_2S foliar spray applied exogenously at 25 µM concentration. Seedlings and plants of *Brassica juncea* harvested after 7, 30, and 60 days was used for evaluating different morphological, biochemical and molecular parameters.

Different morphological parameters like photosynthetic pigments and gas exchange attributes were measured. Likewise, content of metabolites and oxidative stress markers was also assessed. In addition to this nuclear and cellular damage was examined by using confocal microscope. Content of osmolytes, total sugar and protein was altered under salt stress. Activity of different antioxidative and non-

antioxidants enzymes was evaluated and gene expression of different stress related genes was analyzed. However, TRIA and H_2S used alone or in combination improved the growth attributes, photosynthetic system, osmolytes, total sugar, and protein contents. ROS production was diminished by stimulation of different antioxidants under stressed conditions. Expression of various stress related genes was upregulated by treatment of TRIA and H_2S.

Therefore, data concluded that treatment with TRIA and H_2S is a valuable approach for ameliorating ill- effects of salinity stress in *Brassica juncea* by improving their morphological, physiological, biochemical, and molecular attributes.

ACKNOWLEDGEMENTS

First and foremost, I would acknowledge all my gratitude to Almighty for his blessings with whose grace I was able to complete my study

I feel privileged to express my sense of gratitude to my major supervisor, Dr Bilques Farroq, Department of Botany, Lovely Professional University, Phagwara, for her guidance, valuable suggestions, generous support and persistent motivation.

I am also grateful to my co-supervisor Dr Dhriti Kapoor for her valuable guidance, consistent support, keen supervision, expertise and suggestions in conducting my research work. Her association, untiring efforts and appreciate patience helped me in completing the degree programme and this study in its wholeness.

I would like to acknowledge Dr Neeta Raj Sharma, Dean and Head of School of Bioengineering and Biosciences, for her kind support. I would like to acknowledge the generous help, co-operation and support extended by Dr Joydeep Datta, HOD and all the other faculty members of the department.

I would like to show my deepest concern of regards to Prof Renu Bhardwaj, Director of Research, Guru Nanak Dev University, Amritsar, for providing me facilities to carry out some part of my research work there.

Loving thanks to my dear friend **Ms. Savita Kumari** for her help, cooperation, moral support and propitious company during my entire research work. I am also thankful to my friends (Sahima and monika) seniors and lab mates for their support, co-operation and affection. I am extremely thankful to non-teaching staff of the university for their assistance.

Words are too little to express my appreciation to my adorable parents (Mr**. Manoj Kamal Verma** and Mrs**. Meena verma**), my sister (**Disha verma**) and my fiancé **(Hitesh**) for their continuous support, inspiration, encouragement and assistance through entire course of my work.

Apart from above mentioned, there are many hands and good heart that have helped me to walk on the right track of life. I will remain indebted to them.

(Tunisha)

TABLE OF CONTENTS

Sr. No	Contents	Page No.
Chapter 1	Introduction	1
Chapter 2	Review of Literature	7
2.1	Status of Saline areas across the world	7
2.2.1	Growth	10
2.2.2	Photosynthesis	10
2.2.3	Oxidative Stress	11
2.2.4	Ionic toxicity	12
2.2.5	Osmotic stress	13
2.2.6	Nutrient Imbalance	14
2.2.7	Antioxidant defense system	14
2.3	Role of triacontanol in mediating salinity stress in plants	16
2.3.1	Mechanism of action of triacontanol	16
2.3.2	Plant growth	18
2.3.3	Photosynthetic system	20
2.3.4	Oxidative stress	23
2.3.5	Carbohydrates and protein content	25
2.3.6	Osmolytes	26
2.3.7	Antioxidative defense system	27
2.3.8	Gene expression	30
2.4	Role of hydrogen-sulphide in mediating salinity stress in plants	32
2.4.1	Plant growth	32
2.4.2	Photosynthetic system	33
2.4.3	Oxidative stress	36
2.6.4	Carbohydrates and protein content	37
2.6.5	Osmolytes	39
2.6.6	Antioxidant defense system	41
2.6.7	Gene expression	44
Chapter 3	**Hypothesis**	46
Chapter 4	**Objectives**	47
4.1	Background	47
4.2	Objectives	47
Chapter 5	**Materials and Methods**	48
5.1	Location and climate	48
5.2	Plan of work	48
5.3	Combination of treatments	48
5.4	Raising of plant material for experimental studies	49
5.4.1	Surface sterilization	49
5.4.2	In vitro Raising of seedlings	49
5.4.3	In-vivo Raising of plants	49
5.5	Growth attributes	49

5.5.1	Germination (%)	49
5.5.2	Plant Growth:	50
5.5.3	Vigor index	50
5.4.4	Relative water content	50
5.5	Photosynthetic pigments and gas exchange parameters	51
5.5.1	Pigments	51
5.5.2	Gaseous exchange parameters	52
5.6	Metabolites	53
5.6.1	Oxidative damage	53
5.6.2	Oxidative stress markers	53
5.6.3	Histochemical studies by confocal microscope	54
5.7	Oxidative damage	54
5.7.1	Malondialdehyde (MDA) content	54
5.7.2	Hydrogen peroxide content (H2O2)	54
5.7.3	Histochemical studies by confocal microscope	54
5.8	Estimation of Osmolytes	55
5.8.1	Proline	55
5.8.2	Glycine betaine content	55
5.9	Total carbohydrates content	55
5.10	Protein content and Antioxidant defense system	56
5.10.1	Protein content	56
5.10.2	Enzymatic antioxidants	56
5.10.3	Superoxide dismutase	56
5.10.4	Catalase	57
5.10.5	Ascorbate peroxidase	57
5.10.6	Guaiacol peroxidase	57
5.10.7	Glutathione reductase	58
5.10.8	Glutathione peroxidase	58
5.10.9	Dehydroascorbate reductase	59
5.10.10	Monodehydroascorbate reductase	59
5.10.11	Glutathione-S-transferase	59
5.10.12	Polyphenol oxidase	60
5.11	Non-enzymatic antioxidants	60
5.11.1	Ascorbic acid	60
5.11.2	Glutathione content	60
5.11.3	Tocopherol	61
5.12	Gene expression analysis by qRT-PCR	61
5.13	Statistical analysis	61
Chapter 6	**Results and Discussion**	62
6.1.1	In vitro grown seedlings	62
6.1.1.1	Plant growth	62
6.1.1.2	Photosynthetic pigments	68
6.1.1.3	Metabolites	72
6.1.1.4	Oxidative stress markers	75
6.1.1.5	Histochemical studies	77

6.1.1.6	Osmolytes	80
6.1.1.7	Total carbohydrates content	82
6.1.1.8	Protein content and Antioxidative Enzymes	84
6.1.1.9	Non-enzymatic antioxidants	93
6.1.2	30-days old plants	96
6.1.2.1	Plant growth	96
6.1.2.2	Photosynthetic activity	101
6.1.2.2.1	Photosynthetic pigments	101
6.1.2.2.2	Gas exchange parameters	106
6.1.2.3	Metabolites	109
6.1.2.4	Oxidative stress markers	112
6.1.2.5	Osmolytes	113
6.1.2.6	Total carbohydrates	115
6.1.2.7	Protein content and antioxidant defense system	117
6.1.2.7.1	Protein content and antioxidative enzymes	117
6.1.2.7.2	Non Enzymatic Antioxidants	126
6.1.2.8	Gene expression	129
6.1.3	60-days old plants	132
6.1.3.1	Plant growth	132
6.1.3.2	Photosynthetic activity	136
6.1.3.2	Photosynthetic pigments	136
6.1.3.3	Gas exchange parameters	140
6.1.3.4	Metabolites	143
6.1.3.5	Oxidative stress markers	146
6.1.3.6	Osmolytes	148
6.1.3.7	Total carbohydrates	150
6.1.3.8	Protein content and antioxidant defense system	151
6.1.3.8.1	Protein content and antioxidative enzymes	151
6.1.3.8.2	Non-enzymatic antioxidants	160
Chapter 7	**Summary and Conclusions**	173-176
Chapter 8	**Bibliography**	177-221

LIST OF TABLES

Sr. No	Tables	Page No.
2.1	Effect of NaCl on antioxidant defense system in various plant species	15
2.2	Effect of TRIA on plant morphological parameters in different plant species	18
2.3	Effect of TRIA on photosynthetic system of various plant species	21
2.4	Effect of TRIA application on oxidative stress in different plant species	23
2.5	Effect of TRIA on total carbohydrates and protein content in different plant species	25
2.6	Effect of TRIA on osmolytes in different plant species	26
2.7	Effect of TRIA application on plant's antioxidant defence system in different plant species	28
2.8	Effect of TRIA application on gene expression in different plant species	30
2.9	Effect of H_2S application on growth parameters in different plant species	32
2.10	Effect of H_2S on the photosynthetic system of various plant species	34
2.11	Effect of H_2S application on oxidative damage in plant species	36
2.12	Effect of H_2S application on total carbohydrates and content of protein in different plant species	37
2.13	Effect of H_2S application on osmolytes in different plant species	39
2.14	Effect of H_2S application on antioxidant defense system in different plant species	41
2.15	Effect of H_2S application on gene expression in different plant species	44
5.3.1	Different treatments selected for the experiment	48
6.1	Effect of TRIA and H_2S on morphological parameters of *B. juncea* under salinity stress	63
6.2	Effect of TRIA and H_2S on germination percentage, vigor index, and relative water content of *B. juncea* under salt stress	66
6.3	Effect of TRIA and H_2S on photosynthetic pigments of *B. juncea* seedlings under salt stress	69
6.4	Effect of TRIA and H_2S on photosynthetic pigments of *B. juncea* seedlings under salt stress	71
6.5	Effect of TRIA and H_2S on metabolites of *B. juncea* seedlings under salt stress	73
6.6	Effect of TRIA and H_2S on oxidative stress markers of *B. juncea* seedlings under salt stress	76
6.7	Effect of TRIA and H_2S on osmolytes of *B. juncea* seedlings under salt stress	81

6.8	Effect of TRIA and H_2S on total carbohydrates of *B. juncea* seedlings under salt stress	83
6.9	Effect of TRIA and H_2S on protein content and antioxidative enzymes of *B. juncea* seedlings under salt stress	85
6.10	Effect of TRIA and H_2S on antioxidative enzymes of *B. juncea* seedlings under salt stress	87
6.11	Effect of TRIA and H_2S on antioxidative enzymes of *B. juncea* seedlings under salt stress	91
6.12	Effect of TRIA and H_2S on non-enzymatic antioxidants of *B. juncea* seedlings under salt stress	94
6.13	Effect of TRIA and H_2S on morphological parameters of 30-days old plants of *B. juncea* under salt stress	97
6.14	Effect of TRIA and H_2S on germination percentage and relative water content of 30-days old plants of *B. juncea* under salt stress	100
6.15	Effect of TRIA and H_2S on photosynthetic pigments of 30-days old plants of *B. juncea* under salt stress	102
6.16	Effect of TRIA and H_2S on photosynthetic pigments of 30-days old plants of *B. juncea* under salt stress	104
6.17	Effect of TRIA and H_2S on gas exchange parameters of 30-days old plants of *B. juncea* under salt stress	106
6.18	Effect of TRIA and H_2S on metabolites of 30-days old plants of *B. juncea* under salt stress	110
6.19	Effect of TRIA and H_2S on oxidative stress markers of 30-days old plants of *B. juncea* under salt stress	112
6.20	Effect of TRIA and H_2S on osmolytes of 30-days old plants of *B. juncea* under salt stress	114
6.21	Effect of TRIA and H_2S on total carbohydrates of 30-days old plants of *B. juncea* under salt stress	116
6.22	Effect of TRIA and H_2S on protein content and antioxidative enzymes of 30-days old plants of *B. juncea* under salt stress	118
6.23	Effect of TRIA and H_2S on antioxidative enzymes of 30 days old plants of *B. juncea* under salt stress	121
6.24	Effect of TRIA and H_2S on antioxidative enzymes of 30-days old plants of *B. juncea* under salt stress	125
6.25	Effect of TRIA and H_2S on non-enzymatic antioxidants of 30-days old plants of *B. juncea* under salt stress	127
6.26	Effect of TRIA and H_2S on relative gene expression of SOD and CAT in *B. juncea* plants under salt stress	129
6.27	Effect of TRIA and H_2S on morphological parameters and 60 days old plants of *B. juncea* under salt stress	133
6.28	Effect of TRIA and H_2S on morphological parameters and of 60-days old plants of *B. juncea* under salt stress	137
6.29	Effect of TRIA and H_2S on photosynthetic pigments of 60-days old plants of *B. juncea* under salt stress	139
6.30	Effect of TRIA and H_2S on gas exchange parameters of 60-days	141

	old plants of *B. juncea* under salt stress	
6.31	Effect of TRIA and H_2S on metabolites of 60-days old plants of *B. juncea* under salinity stress	144
6.32	Effect of TRIA and H_2S on oxidative stress markers of 60-days old plants of *B. juncea* under salt stress	146
6.33	Effect of TRIA and H_2S on osmolytes of 60-days old plants of *B. juncea* under salinity stress	148
6.34	Effect of TRIA and H_2S on total carbohydrates of 60-days old plants of *B. juncea* under salt stress	150
6.35	Effect of TRIA and H_2S on protein content and antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress	152
6.36	Effect of TRIA and H_2S on antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress	155
6.37	Effect of TRIA and H_2S on antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress	157
6.38	Effect of TRIA and H_2S on non-enzymatic antioxidants of 60-days old plants of *B. juncea* under salt stress	161

LIST OF FIGURES

Sr. No	Figures	Page No.
2.1	Diagrammatic representation of different ill-effects of salinity stress	9
2.2	Diagrammatic representation of plant response to salt stress	13
2.3	Diagrammatic representation of mechanism of action of Triacontanol	17
6.1	Effect of TRIA and H_2S on root and shoot length and fresh weight in 7-days old seedlings of *B. juncea* under salt stress	64
6.2	Effect of TRIA and H_2S on dry weight in 7-days old seedlings of *B. juncea* under salt stress	65
6.3	Effect of TRIA and H_2S on germination percentage, vigor index and relative water content in 7-days old seedlings of *B. juncea* under salt stress	67
6.4	Effect of TRIA and H_2S on total chlorophyll, chl a and chl b in 7-days ld seedlings of *B. juncea* under salt stress	70
6.5	Effect of TRIA and H_2S on carotenoid and xanthophyll content in 7-days old seedlings of *B. juncea* seedlings under salt stress	72
6.6	Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 7-days old seedlings of *B. juncea* under salt stress	75
6.7	Effect of TRIA and H_2S on MDA and H_2O content in 7-days old seedlings of *B. juncea* under salt stress	77
6.8	Effect of TRIA and H_2S on membrane damage in *B. juncea* seedlings under salt stress	78
6.9	Effect of TRIA and H_2S on nuclear damage in *B. juncea* seedlings under salt stress	79
6.10	Effect of TRIA and H_2S on proline and glycine betaine content in 7-days old seedlings of *B. juncea* under salt stress	82
6.11	Effect of TRIA and H_2S on total carbohydrates in 7-days old seedlings of *B. juncea* under salt stress	83
6.12	Effect of TRIA and H_2S on protein, SOD and CAT enzyme activity in 7-days old seedlings of *B. juncea* under salt stress	86
6.13	Effect of TRIA and H_2S on APX, POD and GR enzyme activity in 7-days old seedlings of *B. juncea* under salt stress	89
6.14	Effect of TRIA and H_2S on GPOX, DHAR and MDHAR enzyme activities in 7-days old plants of *B. juncea* under salt stress	92
6.15	Effect of TRIA and H_2S on GST and PPO enzyme activities in 7-days old plants of *B. juncea* under salt stress	93
6.16	Effect of TRIA and H_2S on ascorbic acid, glutathione and tocopherol content in 7-days old seedlings of *B. juncea* under salt stress	95

6.17	Effect of TRIA and H_2S on root length, fresh and dry weight in 30-days old plants of *B. juncea* under salt stress	99
6.18	Effect of TRIA and H_2S on germination percentage and relative water content of 30-days old plants of *B. juncea* under salt stress	101
6.19	Effect of TRIA and H_2S on total chlorophyll, chl a and chla b content in 30-days old plants of *B. juncea* under salt stress	103
6.20	Effect of TRIA and H_2S on carotenoid and xanthophyll content in 30-days old plants of *B. juncea* under salt stress	105
6.21	Effect of TRIA and H_2S on photosynthetic rate, stomatal conductance and intercellular CO_2 concentration in 30-days old plants of *B. juncea* under salt stress	108
6.22	Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 30-days old plants of *B. juncea* under salt stress	111
6.23	Effect of TRIA and H_2S on MDA and H_2O_2 content in 30-days old plants of *B. juncea* under salt stress	113
6.24	Effect of TRIA and H_2S on proline and glycine betaine content in 30-days old plants of *B. juncea* under salt stress	115
6.25	Effect of TRIA and H_2S on carbohydrates content in 30-days old plants of *B. juncea* under salt stress	117
6.26	Effect of TRIA and H_2S on protein content, SOD and CAT enzyme activity in 30-days old plants of *B. juncea* under salt stress	119
6.27	Effect of TRIA and H_2S on APX, POD and GR activity in 30-days old plants of *B. juncea* under salt stress	122
6.28	Effect of TRIA and H_2S on GPOX, DHAR, and MDHAR enzyme activity in 30-days old plants of *B. juncea* under salt stress	124
6.29	Effect of TRIA and H_2S on GST and PPO enzyme activity in 30-days old plants of *B. juncea* under salt stress	126
6.30	Effect of TRIA and H_2S on ascorbic acid, glutathione and tocopherol content in 30-days old plants of *B. juncea* under salt stress	128
6.31	Effect of TRIA and H_2S on relative gene expression of SOD and CAT genes in *B. juncea* plants under salt stress	130
6.32	Indiviual and combined effect of TRIA, H_2S and NaCl in 7 seedlings and 30 and 60 days old plants of *B. juncea*	131
6.33	Effect of TRIA and H_2S on root length, fresh and dry weight in 60-days old plants of *B. juncea* under salt stress	135
6.34	Effect of TRIA and H_2S on relative water content in 60-days old plants of *B. juncea* under salt stress	139
6.35	Effect of TRIA and H_2S on total chlorophyll, chl a and chl b in 60-days old plants of *B. juncea* under salt stress	140
6.36	Effect of TRIA and H_2S on carotenoid and xanthophyll content in 60-days old plants of *B. juncea* under salt stress	143
6.37	Effect of TRIA and H_2S on photosynthetic rate, stomatal	145

	conductance and intercellular CO_2 concentration in 60-days old plants of *B. juncea* under salt stress	
6.38	Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 60-days old plants of *B. juncea* under salt stress	147
6.39	Effect of TRIA and H_2S on MDA and H_2O content in 60-days old plants of *B. juncea* under salt stress	149
6.40	Effect of TRIA and H_2S on proline, and glycine betaine in 60 days old plants of *B. juncea* under salt stress	151
6.41	Effect of TRIA and H_2S on carbohydrates content in 60-days old plants of *B. juncea* under salt stress	153
6.42	Effect of TRIA and H_2S on protein content, SOD and CAT enzyme activities in 60-days old plants of *B. juncea* under salt stress	156
6.43	Effect of TRIA and H_2S on APX, POD and GR enzyme activities in 60-days old plants of *B. juncea* under salt stress	158
6.44	Effect of TRIA and H_2S on GPOX, DHAR and MDHAR enzyme activities in 60-days old plants of *B. juncea* under salt stress	160
6.45	Effect of TRIA and H_2S on GST and PPO enzyme activites in 60-days old plants of *B. juncea* under salt stress	162

ABBREVIATIONS

$AlCl_3$	Aluminum chloride
APX	Ascorbate peroxidase
AAS	Atomic absorption spectrophotometer
CDNB	1-chloro-2,4-dinitrobenzene
CAT	Catalase
Cm	Centimeter
DHAR	Dehydroascorbate reductase
DMA	Dimethylarsinic acid
DDW	Double distilled water
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylenediamine tetraacetic acid
GPOX	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione S transferase
POD	Guaiacol peroxidase
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
IRGA	Infrared gas analyzer
µg	Microgram
µM	Micromolar
Mg	Milligram
mM	Millimole
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
HNO_3	Nitric acid
NBT	Nitroblue tetrazolium
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
PS II	Photosystem II

PPO	Polyphenol oxidase
PBG	Porphobilinogen
PI	Propidium iodide
P5CS1	Pyrroline-5-carboxylate synthetase
ROS	Reactive oxygen species
RWC	Relative Water Content
H_4SiO_4	Silicic acid
NaOH	Sodium hydroxide
$NaNO_2$	Sodium nitrite
NBT	Nitroblue
SOD	Superoxide dismutase
TRIA	Triacontanol
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
TPTZ	2,4,6-tripyridyl-S-triazine
WUE	Water use efficiency

Chapter-1

Introduction

Global Climatic Change and anthropogenic activities have resulted in widespread contamination of soil and water resources. Due to these environmental disturbances, plants are continuously subjected to number of biotic (bacteria, fungi, oomycetes, nematode) and abiotic stresses (flooding, radiation, drought, temperature stress and metal stress) during their lifecycle (Hasanuzzaman et al., 2013; Vardhini and Anjum 2015; Sytar et al., 2019). It was found that external environmental conditions that adversely affect agricultural productivity, limit economic production and quality of crop (Verma et al., 2013). Abiotic stress conditions have significant impact on crop production and biomass since they are considered to be predominant reason of major yield diminishments which influence differential distribution of plant cultivars across the ecosystem (Kerchev et al., 2020, Ashraf et al., 2008). Economic potential of the crop is loss due to various stresses was found to be 40% due to hot temperature, 15% due to cold temperature, 20% due to salinity and 8% due to other stresses (Ashraf and Harris 2005). The effect of these abiotic pressures is becoming prominent over time as more and more land becomes contaminated with salt as a result of poor irrigation methods, industrialization, mining and smelting (Ajibade et al., 2021). Salinization has emerged as the foremost and exacerbating topic for restricting crop production as it is associated with harsh climatic variations occurring consecutively and concurrently (Javaid et al., 2019). The current demand for food and energy requirement has been greatly strained by notable rise in global population, combined with speedy industrialization and urbanization in emergent countries (Ghose et al., 2014). As a result, the population will confront tremendous problems on many fronts, among which attaining food security is a high priority issue (Kim et al., 2021). This situation poses great risk to global food security and is expected to get worse with expected acceleration of climatic change in the future years, which further causes challenges for food production (Zulfiqar and Ashraf, 2021).

Salinity is becoming a major concern on a daily basis as a result of inadequate management of natural resources in dry regions where soils are saline and have agricultural productivity. This problem is further exacerbated by salinity due to worldwide due to higher evapo-transpiration rate of salinity (Glick et al., 2007). According Salinity Research Institute of India nearly major land area is affected by salinity which includes states of Punjab, Haryana, Rajasthan, Uttar Pradesh, Bihar

(Kumar and Sharma, 2020). The accumulation of salt in these areas has a negative impact on soils, rendering them unproductive in nature. Salinity-affected soils are major factor limiting agriculture industry, as they can degrade newly irrigated land over time (Bacilio et al., 2004).

Salinity caused by higher concentration of soluble salts such as sodium chloride (NaCl), from both natural and anthropogenic activities. Reduced agricultural productivity are some of the detrimental effects of salinity (Hu and Schmidhalter, 2002). These detrimental effects of salinity are associated with excessive ion formation. Ions like Na^+ and Cl^- produce required condition for plant survival by undermining numerous plant systems. Despite of the fact that both Na^+ and Cl^- are the major ions that causes variety of physiological responses. Cl^- ion is considered to be more harmful than Na^+ ion as it causes both hyerionic and hyperosmotic stress which can kill plants (Tavakkoli et al., 2010).

Agricultural crops exhibit spectrum of response under salinity. It is known to be serious limiting factors which affects germination of seeds, vegetative growth and reproductive development, agricultural productivity is lowered due to physiological characterstics and water absorption (Akbarimoghaddam et al., 2011, Vimal et al., 2019, Prittesh et al., 2020). Thus, growth inhibition due to salinity is the result of osmotic and ionic stress. Several studies pertaining to salinity have reported reduction in growth rate as it alters metabolism of proteins, change the enzymatic and non–enzymatic activities, cause hormonal imbalance and reduce utilization of seed reserves (Poss et al., 2010, James et al., 2011). Salt stress generated due to accumulation of salts not only effect crop productivity but also effect different physiological activities of plant such as water deficit, toxicity of specific ion (Na and Cl ion) (Shahbaz et al., 2011; Babu et al., 2017), nutrient deficiency, disrupted relationship between leaf and water (Carpici et al., 2010), oxidative stress (Klein et al., 2018), difference in the activities of antioxidant enzymes, which ultimately culminates into cellular damage, membrane disorganization, stunted growth, wilting, sterility and plant death (Mane et al., 2010). High levels of salinity enhance the formation of ROS which affected lipds, proteins and nucleic acid (Gill and Tuteja, 2010).

Brassica crops are the most affected by salinity among the major food crops because they are grown in dry regions. It is ranked third among the oilseed crops of the world. It is herb grown for its diverse soil types and adaptability to varying climatic conditions. *B. juncea* is widely consumed all over globe and is recognized as imperative portion of human nutrition (Rai et al., 2022).

It has gained lot of importance because it contains numerous valuable metabolites, which are directly allied to diverse recognized biological activities like anti-bacterial, anti-malarial, anti-aging, anti-inflammatory and anti-oxidant activities as it has active constituents like glucosinolates, flavonoids, phenolic compounds, phytosterols, essential oils and fixed oils. Plant leftover is frequently used as biofuel and feed for cattle (Jat et al., 2019). Production of the crop is severly owing to complex nature of salinity stress and lack of appropriate technique for introgression (Mahdavi and Sanavy,2007).

In view of on growing demand of the nutritive and therapeutic value of *B. juncea* and soil salinity is putting heavy strain on the market. In the upcoming years, the supply might not be enough to satisfy demand. If at all possible, the improved output with value addition is highly sought to escape out of this dire situation. One of the greatest solution to handle this bad issue is to cultivate these plants on large scale according to scientific principles. This would not only ensure continuous supply but also augment growth and yield. As a result, effective strategy is needed to alleviate salt stress in *B. juncea* and to satisfy the species growing requirement for sustainable agricultural production (Ashraf & McNeilly, 2004). Use of growth regulators and signaling molecule is beneficial approach towards stress which is found to play effective role in plant protection.

Plant growth regulators are naturally found substances which can be synthesized synthetically regulate a wide range of enviornmentl situations either directly or indirectly. These chemical messengers vary in their chemical structures and properties. Based on their beneficial role, these growth regulators are broadly categorized as planthormone and secondary plant growth regulator (Sabagh et al., 2021). PGRs are the substances, which when applied in specific formulations to plants or seeds, inhibit or promote the growth, development and stress responses. Suitable

PGRs which play essential roles in imparting salt stress tolerance include auxins, cytokinins, gibberelic acid, abscisic acid, ethylene, nitric oxide, jasmonic acid. Plant growth regulator use in agriculture increase plant growth and production (Ashraf et al., 2011). Apart from traditional PGRs, which are mostly plant hormones like methyl jasmonate, brassinosteriods and strigolactones act as PGRs. Due to their role in growth and development of plant under stressed conditions by transmitting signal from cell to cell (Sharma et al. 2011, Peleg and Blumwald, 2011). All these growth regulators mitigate salinity by bringing up changes in its mechanism of action. As endogenous concentration and ratio of different PGRs are greatly influenced by numerous internal and external stimuli (Ashraf et al., 2010). Furthermore, the use of phytohormones is considered as one of the best approaches for coping salt stress and has also shown in various studies with huge success in mitigating toxicity of saline condition (Iqbal et al., 2014; Fatma et al., 2016; Ahanger et al., 2020; Sehar et al., 2023; Jahan et al., 2020). In order to meet the rising need for biochemical developmental processes, novel PGR is needed because of its significant function in plant as growth regulator. Thus, TRIA control variety of physio-biochemical processes and lessens the stress (Verma et al., 2022).

Triacontanol a 30 carbon chain of primary alcohol prepared naturally and is known to improve final productivity by applying exogenously in plants (Naeem et al., 2011). It chemically prepared by Kolbe coupling of stearic acid which brings about certain physiological changes and give rise to desirable yield and quality of crops (Taştan et al., 2016). It is found in wax of plant alfalfa (Chibnall et al., 1933) and is also found in many crops as 3% of total free alcohols e.g., wheat (Digruber et al., 2018). It is applied to seeds of different crops by process of soaking or foliar spray in order to develop stress tolerance (Asadi Karam et al. 2017b; Perveen et al. 2012b; Waqas et al. 2016; Khanam and Mohammad, 2018). It is found in the market by different trade names such as Paras, Golden, Jeevan, Nutron, etc. TRIA is known to be eco-friendly and economic meet the growing demand. Out of variety of PGRs, TRIA is recognized as potent plant growth regulator that results in favourable growth and exerts beneficial effects on different agro-horticultural crops. It not only improves the plant's growth, physio-biochemical characteristic, metabolic activities but also improves consistency (Naeem et al., 2010). Apart from its role in mitigation of stress using defense genes.

TRIA play important role in improving productivity of crop by affecting plant morphology, seed germination, water and nutrient absorption, photosynthesis, antioxidant enzymatic activity, nutrient balance, stability of membrane and defence gene. It promote growth of different species of plant as an effective growth regulator by promoting development of shoot and root and production of secondary metabolites (Reddy et al., 2002; Giridhar et al., 2005; Malabadi et al., 2005; Malabadi and Nataraja., 2007; Parimalan et al. 2009). It is non-poisonous and not harmful to human and animals. For large storage it can be kept for 3 years under cool, shade and dry conditions. Foliar treatment of TRIA is variable in different plant species It is known for improving different factors like enzymatic rate, chlorophyll content, photosynthesis rate, dry substrate accumulation, water absorption, protein and sugar content, seed germination, budding rate, root and leaf formation, cell division, tiller quality, fruit ripening and resistance to different kind of environmental stresses. The stimulatory effect of TRIA is only regulated when it is applied in diluted form (Kumaravelu et al., 2000; Khan et al., 2007).

These gaseous signaling molecule are the most significant moieties which include hormones, regulators, proteins and nucelotides (Mittler et al., 2004). The response to stimuli by these molecules is produced in plant cell or tissue either exogenously or endogenously. Signaling molecules modulate specific gene expression by activating signaling cascades and metabolites like CO (Hancock and Neill, 2019), CH_4 (Kou et al., 2018) are widely used signaling moieties. Among different types of gasotransmitters, H_2S have emerged as an important moiety in plants which regulates various signalling pathways (Delledonne et al., 2005). It is colourless, odourless, soluble and highly flammable gas with rotten egg odour, Because of its pungent odor it is classified as poisonous gas and toxic pollutant (Vandiver and Snyder, 2012) but recently it has received great recoganization as a novel gas signalling molecule that is directly or indirectly involved in improving yield under various stress like conditions by generating responses like stomatal movement and chloroplast formation in plants (Christou et al., 2014). Various studies conducted on biosynthesis of H_2S revealed that cysteine desulphydrases are the major substrates involved in production of H_2S. Enzymes like cystathionine is involved in the H_2S generation has positive impact on l-cysteine (Hughes et al., 2009; Mancardi et al., 2009). Furthermore, endogenous

generation of H_2S has expanded it studies in mitigating stress by improving enzymatic action (Corpas et al., 2021, Aroca et al., 2018). It is able to improve antioxidant defense mechanism and balance antioxidant pools by mediating sulphur to cells due to its gaseous nature.

In the past few years' beneficial role of H_2S as a signaling molecule has been widely explored in the plants. A slew of plant related studies revealed that H_2S has protective effect against variety of abiotic stresses (oxidative stress, metal stress, heat stress) has found to have major impact in improving plant's ability in response to stressful environmental conditions by modifying different types of cellular and molecular mechanism which in turn boost plant (Sharma et al., 2019). Apart from mitigating toxic effect of different kind of stresses it is directly or indirectly used in improving major growth factors like rate of photosynthesis, germination rate of seeds, photo-morphogenesis, fruits and flowers (Chen et al., 2011; Garcia-Mata and Lamattina 2010).

H_2S has come to the light because of its adaptive relationship with plant system. Tiny dimensions and easily diffusible properties of H_2S against abiotic stresses at different physiological concentrations make it a beneficial signaling moiety (Jin et al., 2015). Increased generation of ROS under stressed conditions cause oxidative damage. Numerous studies have demonstrated that exogenous application of H_2S under minute concentrations like i.e., nM and µM, significantly improve tolerance against abiotic stresses. The positive impact of H_2S is directly linked with defense mechanism (Arif et al., 2020). It has been found that H_2S alleviates antioxidant activities and expressions of various enzymes like SOD, CAT, GR, GPX, DHAR and MDHAR under stressed conditions (Aghdam et al., 2018; Christou et al., 2014; Guo et al., 2020; Khan et al. 2018; Luo et al. 2015;). It has been found that H_2S improve the efficiency of these enzymatic antioxidants in plants by undergoing H_2S-mediated post-translational modification (PTM) which might be attributed to the widespread distribution of H_2S under different subcellular sections (Dawood et al., 2012 Amooaghaie et al., 2017).

Chapter-2

Review of Literature

Salinity is one of the most brutal enviornmental factors which limit agriculture productivity by affecting economically significant crops, fruits and vegetables required for feeding the growing population (Mane et al., 2010). However, this agriculture sector experiences serious challenges because of salinty such as scarcity of seeds, cost-effectiveness of small scale production and issue of growing crops under saline soils. Land degradation with respect to accumulation of ions like Na^+ and Cl^- is considered to be one of the biggest ecological issues. Rise in the level of salt concentration accelerates degradation of land which in turn reduce crop yield (Jamil et al., 2011; Ivushkin et al., 2019). Soil salinity mainly focus on salts such as Na^+ and Cl^- and salinization due to anthropogenic activities increase the process (Ayers and Westcot, 1985; Ghassemi et al., 1995).

Soil salinity is mainly divided into 3 main categories defined as low, moderate and high (Rogers et al., 2005). It is dynamic process and is spreading globally, land area of million hectares all around the globe is too saline to produce economic yield and more land is becoming unproductive every year (Metternicht and Zinck 2003). Salinity is mainly found in 75 countries (Ghassemi et al., 1995). Soil salinity is caused by two kinds of processes one is primary salinity and other is secondary salinity. Primary salinity is caused due to weathering of rocks. Break down of rocks from parent material release soluble salts of various types such as sodium, calcium and magnesium (Garg and Manchanda, 2008). These soluble salts are carried away by wind and mainly deposited by rainfall to form basins of oceans. Secondary salinity is considered to be more serious as compared to primary salinity as it is the result of human activities like deforestation (Said-Al and Omer, 2011), excessive use of fertilizer, poor irrigation practices, waterlogging, inadequate drainage. However, in comparison to biophysical elements (Zekri et al., 2010). The availability of little knowledge on quantifying the impacts of land deterioration affectand by income and farming activities (Zekri et al., 2010; Metternicht and Zinck., 2003; Ivits et al., 2013).

2.1 Status of Saline areas across the world

Extent of salinity around the world is broadly categorized into two main types, one is termed as oceanic salinity which is caused due to changing sea temperature from place to place on oceans and the other type is termed as terrestrial salinity which is

caused due to salinity on surface of land and in ground water (Yihdego et al., 2016), Around 77 Mha of the productive area is under effect of salinity (Selvakumar et al., 2014; Arora, 2017; Miransari, 2017) and this percentage is increasing due to extensive utilization of land in dry regions (Rengasamy 2006, 2010). Recent investigation by FAO have found that around 1-2% of agriculture area is becoming unproductive every year and this issue is aggravated due over utilization of ground water for irrigation purposes in saline areas (Koohafkan, 2012). According to recent estimates it has been found land is affected by salt and this percentage is going to increase in the coming thirty years (Wang et al., 2020). Due to salinization, agricultural loss is increasing upto US$ 27 billion worldwide (Wang et al., 2021). It has found that around 1billion hectare of land is under salinization and this percentage is increasing a rapid rate (Hopmans et al., 2021; Tian et al., 2020). This problem of salinization has extended to 100 other countries (Hammam and Mohamed, 2020). Which include countries like India, China, Pakistan and Turkey (Seifi et al., 2020). According to the report by Rengasamy (2006), it has been found that salinity is an extensive problem which is spreading over an area of 9 Mha globally. It is expected that by 2025 around 11.7 Mha of area in India will be affected by salinity (Sharma and Chaudhari., 2012). Among all the states of India, Uttar pradesh, Kerala, Orrisa, West Bengal and Andaman Nicobar Islands are the states having major issue as salinity. Around 25% groundwater is saline specially in countries like Haryana and Rajasthan (Sharma et al., 2014).

2.2 Effect of salinity in plants

Salinity is an extensive problem which is caused due to problems like poor management of agricultural lands, excessive rate of evaporation and lack of drainage facilities. Due to excessive concentration of salts in the soils the effect plant growth, development, yield and seed quality is increasing making them more prone to salinization (Culha et al., 2011). Plant under effect of salinity suffer different kind of damage from seedling stage to germination stage. NaCl salts cause salinity in the agricultural lands. High level of salinity stress cause ionic stress and osmotic stress which cause negative impact on plant developmental processes which includes growth attributes, morphology, physiological aspects, biomas, flowering, fruting and

biochemical processes damaged by ROS production (Rahneshan et al., 2018). Salinity tends to activate the ionic ratio of ions, the rise in the ionic ratio of these ions ultimately damage regular activities in plants. Raised level of Na^+ in soil limit water uptake and nutrient absorption in the plant. Salt stress is also known to effect photosynthesis by impacting LHC (Light Harvesting Complex). Together all these primary stresses activate oxidative stress which in turn cause series of secondary stresses which impedes plant growth by decreasing photosynthetic activity, causing closure of stomata, ROS generation and PCD (Zhu et al., 2002).

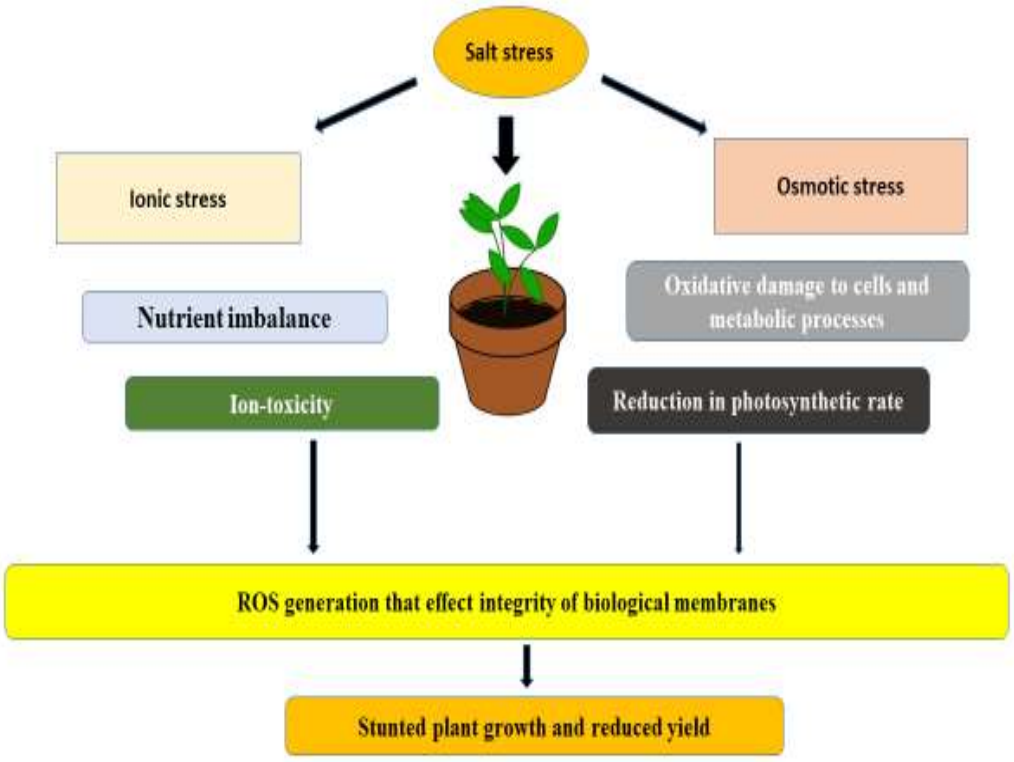


Fig. 2.1 Diagrammatic representation of different ill-effects of salinity stress

Ionic stress causes secondary metabolic changes in plant which affects development in plant. Plants affected by salinity are known to show symptoms of necrosis and chlorosis as it disrupts the normal functioning of PS I and PS II system due to higher concentration of sodium ions (Chen et al., 2002). Salinity effect the activity of key enzymes like RUBisCO which is required for carrying out glycation. Apart from this salinity is also known to change the level of sugar by the modulating the process of glycolysis. Salinity manage the level of different types of sugars such as fructose,

sucrose which in turn influence the sugar signaling required for the process of glycolysis (Shumilina et al., 2019). Salinity tend to exert negative impact on plant productivity by affecting all major processes which are discussed under separate headings.

2.2.1 Growth

Salt stress significantly affect yield of crop and decline food availability globally (Gharsallah et al.. 2016). Plant growth in saline soils cause changes in morphological parameters and effect plant biomass as reported by numerous studies. One such study done on *Brassica campestris* L. by Memon et al. (2010). which stated that low concentration of NaCl led to increase in plant heights. Whereas higher concentration of NaCl resulted in decrease plant height. A decrease in plant biomass was found under salinity in selected cultivars of water dropwort. The results showed that under salt treated condition (V11E0135) showed higher reduction than control (V11E0022) (Kumar et al., 2021). Higher Concentration of salt resulted in shortage of root- shoot length followed by leaf area reduction in different types of genotypes of tomato like *Ailsa Craig* (Wild type) and *notabilis* (ABA-deficit mutant) (Shahid et al., 2016). Similar suppression under salt stress was observed in root and shoot growth was observed in case of *Solanum tuberosum* as studies by (Gao et al., 2016) It has found that changes in growth pattern is the main indicator of salinity change. Root growth is found to be more affected to saline conditions in contrast to shoot growth (Caines and Shennan, 1999). Salinity is known to affect leaf area of plant directly. It has been found that number of leaves were found to decrease under different concentration of NaCl like 50 and 100 mM. Excessive salt concentration was observed in the cytoplasm and vacuole of cell sap of older leaves resulted in cell death (Munns, 2002).

2.2.2 Photosynthesis

Photosynthesis is known to be one of the major biological pathway which is known to convert sunlight into chemical energy. The photosynthetic rate in plants is significantly inhibited by salt stress. Excessive concentration of salt affect chlorophyll content and photosynthetic efficiency which in turn effect plant health (Zhang et al., 2005). The chlorophyll content in plants decline under salinity due to lack of

regulation of PSI and II. Salinity cause reduction in content of Chl Decrease in the content of chl has been reported in rice (Cha-um et al., 2010), tomato (Doganler et al., 2010) Likwise, a study done on cucumber found that chlorophyll content in leaves was found to decline when the concentration of salt stress increased drastically in response to control (Khan et al., 2013). The chlorophyll degradation under salt stress is found to be caused because of the enzyme chlorophyllase (Yan et al., 2013). The decrease in the content of pigment is one of the main reason responsible for causing deterioration of membrane (Mane et al. 2010) Photosynthetic parameter underwent different alterations in *Scenedesmus obliques* which declined accumulation of biomass (Demetriou et al., 2007). Salinity decreased rate of photosynthesis, stomatal conductance and effected efficiency of process of photosynthesis (Lopez-Climent et al., 2008). In wheat, PSII activity is affected by salinity (Mehta et al. 2010). Higher rate of salinity level that caused alterations in enzymatic activity of photosynthesis and decrease in the level of chlorophyll and carotenoids (Lycoskoufis et al. 2005). The reduced photosynthetic rate affected the productivity level and final yield of the crop in grape (Hatami et al., 2010), commonbean (Gama et al., 2007), *Arthrocnemum Macrostachyum* (Redondo-Gómez., 2010).

2.2.3 Oxidative Stress

Salinity leads to generation of ROS which cause oxidative damage by disturbing redox homeostasis in in the cells. Salt stress is known to produce different kind of ROS like H_2O_2, $O2^{\bullet-}$and $OH^{\bullet}$ (Parida and Das., 2005). These kinds of water loss conditions that leads ROS generation, highly toxic and reactive nature results in membrane deteioration due to oxidation of fats and lipds (Apel and Hirt 2004). Generation of ROS due to salt stress cause oxidative stress which in turn cause deterioration of the membrane because of malondialdehyde (MDA) formation and lipid peroxidation (Abbasi et al., 2016). It has been found MDA cause damage to membrane due to oxidation as both are linked to each other Higher concentration of salt increase the content of MDA in leaves which in turn cause oxidative damage in the cells (Hasanuzzaman et al., 2018). Several cases of membrane disruption have been reported due higher salt concentration which is known to trigger oxidative stress. The study done on tomatoes found that salinity stress caused activation of ROS which

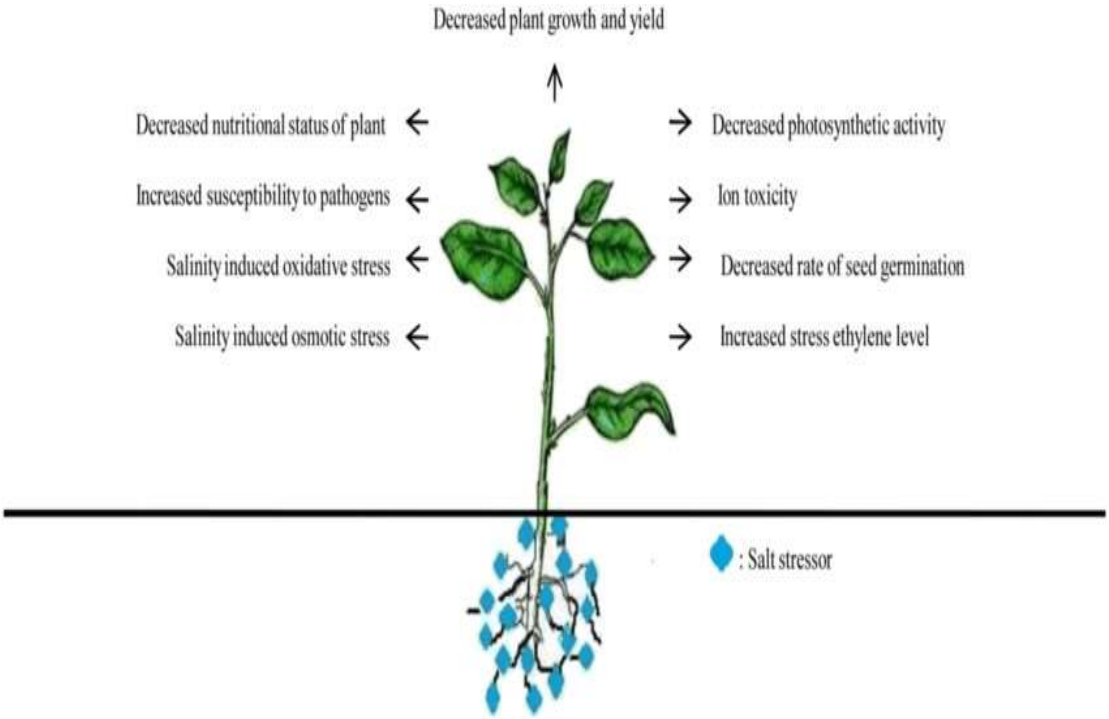
is known to increase the level H_2O_2 in plant which disrupt the normal functioning of cell membrane in plants (Manai et al., 2014). Burst of ROS signal result in oxidative stress and cause membrane permeability. Increase in H_2O_2 is another important factor which determines the level of salinity in different genotypes of plant. Similarly, level of MDA was found to increase under saline conditions (Nagesh and Devraj, 2008). Increase in the leakage of ions has been reported to increase salinity (Sairam et al., 2002). Peroxidation of lipd is the major reason of damage to membrane due to oxidative stress (Khan and Panda., 2008). Numerous studies have shown that ROS cause oxidative stress due to negative impact of salt stress on legumes (Jungklang *et al*., 2004) and vascular plants (Mittova *et al*., 2004).

2.2.4 Ionic toxicity

Highly soluble salts in the soil disrupt the normal mineral nutrient absorption and plant metabolism which is important for their growth (Wang et al., 2003). Ionic balance is the prime reason which is responsible for causing cell stability by maintaining balance of major processes in plants. Balance of mineral nutrient is essential nutrient is important for maintaining. Salt stress is caused due to ionic toxicity which is transferred to cell and inside cell organelle and tend to have ill-effect on ion accumulation in the cell (Tanveer et al., 2019). Salinity tends raise Na^+ ion and decline K^+ ion (Yassin et al., 2019). Different investigation stated that higher rise of Na^+ in different plants like wheat (Yassin et al., 2019). In recent research conducted under salt stress stated that root and shoot of carrot showed higher uptake of Na^+ ion and decrease in K^+ ion (Menezes et al. 2017). It was noted that root maintains the level of NaCl and manage its export level to shoot and soil. The movement of Na^+ towards shoot is controlled by transpiration (Tester and Davenport, 2003). Increase in concentration of ions like Na^+ and Cl^- in root shoot and leaves in plant *Atriplex griffithii* suggested a positive relationship between different ion concentration. It was stated that content of Ca^{2+} ion was found to get reduced in leaves and shoot under high concentration of salt stress whereas content of Mg^{2+} ion showed no variation in stem and roots however it was found to decrease in leaves (Khan et al., 2000). Likewise, it was found that ion toxicity reduced growth of barley under salinity stress by exclusion of Na^+ and Cl^- which affect the permeability of the membrane due to

electronic leakage of ions (Tavakkoli et al., 2011).

Fig. 2.2 Diagrammatic representation of plant response to salt stress



2.2.5 Osmotic stress

Water balance in plants is maintained by an important physiological factor called water potential which is known to estimate status of water in plants (Parida and Das, 2005). Salinity in the rooting medium is known to cause decline in the water potential which in turn effect different processes by causing oxidative stress in plant induced due to salinity stress. This conditions becomes worse with increase in concentration of salt. In recent study conducted *Cucumins sativa* found that water potential reduced due to rise in salinity (Khan et al., 2013). Ionic rich soil generates the ROS which is prime reason for oxidative stress caused due to leakage of electrolytes in plant, resulting in membrane deterioration (Ganie et al., 2019). Salinity is found to be negatively co-related with osmotic potential which means that when salinity increase osmotic potential decreases resulting in osmotic stress (Shahzad et al., 2019). Growth reduction is caused due to reduced water uptake and osmotic effect under salt stress. These kinds of conditions which help in preserving water content is the major reason for determining osmotic stress (Negrao et al., 2017). Osmotic stress related to salinity

stress caused closure of stomata affecting the turgor pressure and result in crop yield loss (Zheng et al., 2002).

2.2.6 Nutrient Imbalance

Salinity induces nutritional imbalance in crops. The crop is affected due to salinity as it reduces the nutritional status of crops. Nutritional disturbance is caused due to different types of factors such as availability, uptake and transport of nutrients to the. Various studies have been reported reduce accumulation and uptake by nutrient (Rogers et al., 2008; Hu and Schmidhalter 2005). Recent work perdormed by Hanin et al. 2016 found that salinity leads to injury of leaves and show symptoms of necrosis. Higher level of NaCl in the roots affect the assimilation of major like K, Ca and Mg nutrients which in turn cause nutrient imbalance (Keutgen and Pawelzik, 2009). Studies have found that salinity decline the uptake of Ca and Mg ion concentration to different organs of plant (Hussain et al., 2013). Likewise, In case of maize where rise in salinity caused interference in the transfer of essential mineral nutrients (Shahzad et al., 2012). Deficiency of micronutrients due to high pH under salinity is very common (Zhu et al., 2004). Salinity causes decrease in N and rise of essential nutrient like Na^+, NH_4^+, Cl^- and NO_3^- which ultimately result in yield loss (Rozeff, 1995).

2.2.7 Antioxidant defense system

Salt stress increase antioxidant enzymatic activities like SOD, APX, and GR activities (de Azevedo Neto, 2006). Enzymes like CAT, SOD and APX decreased under salinity in case of *Beta vulgaris* (Zhang et al., 2021). Similary, *B. parviflora* treatment with salt enhanced the activity of APX, GPX and SOD (Parida et al., 2004a). Decrease in the antioxidative enzymatic activities by production of ROS is the main reason of yield loss (Polash et al., 2019).

Table 2.1 Effect of NaCl on antioxidant defense system in various plant

Plant	**Family**	**Concentration of NaCl**	**Effect**		**References**
			Antioxidative enzyme	Effect	
Spinacia oleracea	Amaranthaceae	20 mM and 50 mM	SOD	Increase	Venkat et al. (2023)
			CAT	Increase	
			APX	Increase	
			GR	Increase	
Solanum lycopersicum	Solanaceae	150 mM	SOD	Decrease	Faisal et al. (2023)
			APX	Decrease	
			CAT	Decrease	
Tritcum aestivum	Poaceae	0.0, 30, or 60 (mM)	SOD	Increased	Sadak et al. (2023)
			CAT	Increased	
			POD	Increased	
Sorghum	Poaceae	Hoagland's nutrient solution + 9 $g \cdot L^{-1}$ NaCl	SOD	Elevated	Wang and Wei (2022)
			CAT	Elevated	
			APX	Elevated	
Zea Mays	Poaceae	Control, 6 dS m^{-1} and 12 dS m^{-1}	APX	Increase	Chattha et al. (2023)
			CAT	Increase	
			POD	Increase	
Zea Mays	Poaceae	200mM	CAT	Decrease	Gul at al. (2023)
			APX	Decrease	
Brassica juncea	Brassicaceae	0, 100, and 200 mM	GR	Reduced	Ahmad et al. (2015)
			GSH	Reduced	

			DHAR	Decrease	
			MDHAR	Decrease	
Glycine Max	Fabaceae	200 and 300 mM	SOD	Decreased	Rahman et al. (2015)
			CAT	Decreased	
			APX	Reduced	
			POX	Reduced	
			DHAR	Reduced	
			MDHAR	Reduced	
			GST	Decreased	
			GPX	Decreased	
Triticum durum	Poaceae	200 mM	SOD	Decreased	Esfandiari et al. (2011)
			CAT	Decreased	
Hordeum vulgare	Poaceae	6.0 and 12.0 dS m^{-1}	SOD	Increased	Talaat et al. (2023)
			CAT	Increased	
			POD	Increased	
			MDHAR	Decreased	
			DHAR	Decreased	

2.3 Role of triacontanol in mediating salinity stress in plants

2.3.1 Mechanism of action of triacontanol

Phytohormones enhance plant productivity. Different types of growth regulators are used to improve quality of plant (Kefeli and Dashek, 2008). Triacontanol is new type of plant growth regulator whose exogenous application tend to improve all major growth parameters like height, biomass, photosynthetic pigments, transpiration, activity of antioxidant enzymes, water use efficiency, stomatal conductance active (Aftab et al., 2010). All these beneficial roles of TRIA has led to recoganization of

this phytohormone as secondary messenger TRIM (Ries et al., 1990). The formation of TRIM from TRIA is known to be the intiation step in the mechanism of TRIA. In recent study conducted on *Oryza sativa* commonly known as rice found that TRIA elicits the TRIM, it was depicted from the study conducted that plants responds to TRIA was similar to that of TRIA at nanomolar dosage (Ries and Wert, 1992). TRIA is known to increase the ratio of isomer. It has been found that L (+)-adenosine is derived from the sources like adenosine monophosphate/ diphosphate or triphosphate (AMP). Studies conducted on adenosine deaminase revealed that L (+)-adenosine exist in racemic form (Ries et al., 1991).

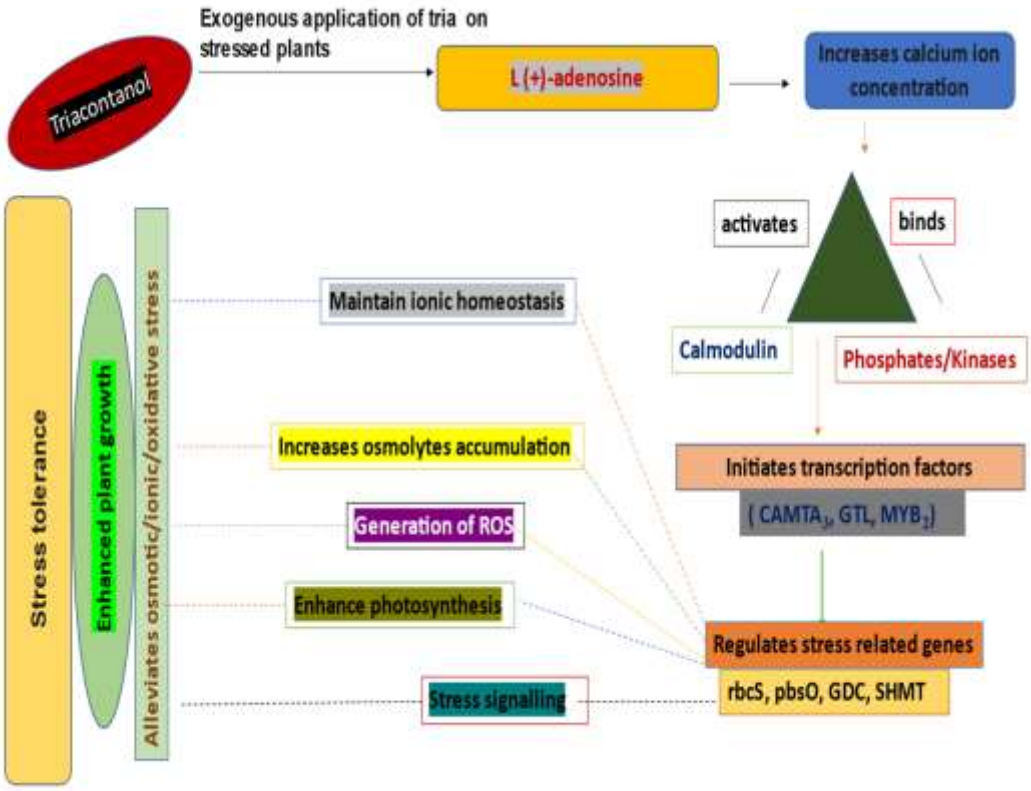


Fig. 2.3 Diagrammatic representation of mechanism of actin of Triacontanol

Treatment of TRIA has non-racemic affect, which affect plant growth. In line to this study, it was further found that TRIA affected plant processes, however the racemic mixtures like (D-adenosine were not able to stimulate major plant processes. Increased adenosine activates the content of major ions like Ca^{2+} and K^{+}. Elevated levels of Ca^{+} help in binding Calmodulin accompanied by enhancement in the activity

of kinases and phosphatases. Apart from these transcription factors of different genes like rbsc and pbsco which are known to play important role in improving water absorption ratio and formation of organic compound which enhance different antioxidant enzymatic mechanism under different conditions (Islam et al. 2020). These beneficial roles of TRIA help in alleviating salinity stress by exogenous application of TRIA on plants (Chen et al. 2002, 2003)

2.3.2 Plant growth

TRIA is known to regulate important physiological processes which in turn improve plant height, leaf area and number of leaves (Kumaravelu et al., 2000). Recent study conducted on TRIA found that application of TRIA resulted in rise in node number, shoots of *Capsicum frutescens* and *Decalepis hamiltonii* leaves (Malabadi et al., 2005). TRIA application of 0.5 mg dm^{-3} under stressed and unstressed conditions promoted the flowering. Even lower concentrations of TRIA were found to biologically active in promoting plant growth. In case of flowers of *Chrysanthemum morifolium*, foliar application of TRIA improved the quality, inflorescence and growth in the flowers (Skogen et al., 1982). Likewise, it was found that application of TRIA negatively affected plant height are under salt stressed conditions (Shahbaz et al., 2013).

Table 2.2 Effect of TRIA on plant morphological parameters in different plant species

Sr No	**Plant species**	**Family name**	**TRIA concentration**	**Effect**	**Reference**
1.	*Brassica oleracea*	Brassicaceae	0 mL L_1 Control, 1 mL L_1 , 1.5 mL L_1 and 2 mL L_1	TRIA treatment raised height, number, leaves, leaf area at 40 day stage in plants	Bhandari et al. (2021)
2.	*Zea mays*	Poaceae	15 µM and 25 µM TRIA	Exogenous application of TRIA enhanced the growth	Iqbal et al. (2023)

				attributes i.e., Plant length, Biomass and photosynthetic pigments in 45 old plants of maize	
3.	*Phaseolus vulgaris*	Fabaceae	Control, 10 µmol L^{-1}, 20 µmol L^{-1}, 30 µmol L^{-1}	Seed priming with TRIA alleviated Pb stress by increasing rate of germination, growth, yield and biomass	Ahmad et al. (2020)
4.	*Spinacia oleracea*	Amaranthaceae	25 nM and 1 µM	Supplementation of TRIA raised germination percentage, plant length, biomass and yield.\	Tompa et al. (2021)
5.	*Zea mays*	Poaceae	25 µM and 50 µM	Treatment of TRIA to Zea mays enhanced different types of morphological parameters when exposed to Nickel toxicity	Younis et al. (2022)
6.	*Helianthus Annuus*	Asteracece	0, 20, 40 µM	Increase in biomass and growth was observed in case of cultivars sunflower by foliar spray of	Khan et al. (2020)

				TRIA under salt stress	
7.	*Cucumis sativus*	Cucurbitaceae	0.20, 0.40, 0.60, 0.80, 1.00 and 1.20 mg L-1	Foliar feeding of triacontanol significantly affected growth attributes reduced in cucumber and was found in mitigating salinity in plants	Sarwar et al. (2019)
8.	*Solanum lycopersicum*	Solanaceae	100, 200, and 600 μmol m−2 s^{-1}	Exogenous application of TRIA increased plant biomass and height, branching growth and yield.	Zambrano et al. (2020)

2.3.3 Photosynthetic system

The important physiological activity like photosynthesis in green plants is regulated by important parameters like photosynthetic pigments, stomatal conductance, gas exchange characterstics (Bhardawaj et al., 2015). Application of TRIA on seeds of *Brassica napus* L. resulted in increase in photosynthetic activity under salt stressed condition. It was found that content of chl in the green plants was found to be enhanced by photochemical quenching and electron transport rate under salinity in plants of canola (Shahbaz et al., 2013). TRIA application alleviated salinity stress in plants of *Oryza sativa* L. (rice) and has positive impact on yield and harvest by enhancing content of chlorophyll and photosynthetic rate (Chen et al., 2002). In case of *Vigna radiata*, it was found that pigments like cholorophyll content, carotene, xanthophyll was found to be increased by ameliorating salinity stress in plants which resulted in decrease in content of pigments (Saha et al., 2010). Similarly, it was found that treatment of TRIA (1000ppm) raised the content of chlorophyll in the

Coriandrum sativum L. (Meena et al., 2014, 2015). TRIA application balances the stomatal conductance in plants under salt stressed conditions by regulating functioning of stress-related proteins and genes (Chen et al., 2002).

Table 2.3 Effect of TRIA on photosynthetic system of various plant species

Sr No	**Plant species**	**Family**	**TRIA concentration**	**Effect**	**Reference**
1.	*Brassica oleracea*	Brassicaceae	10, 20 and 30 μmol L^{-1}	Exogenous application of TRIA had positive impact on activity of photosynthesis regulating metabolic functioning, growth and productivity, and stomatal conductance in plants which improves stress tolerance under Pb stress.	Ahmad et al. (2022)
2.	*Coriandrum sativum*	Apiaceae	5, 10, and 20 μmol L^{-1}	Cd-induced stress resulted in stunted growth and decreased photosynthetic activity and synthesis of chlorophyll pigment which was improved by applying different concentrations of TRIA	Sardar et al. (2022)
3.	*Mentha arvensis*	Lamiaceae	10^{-6} M	Deleterious effect of As toxicity on Photosynthetic apparatus, growth and	Nabi et al. (2022)

				productivity was mitigated by foliar spray of TRIA by directly influencing ROS generation in plants	
4.	*Cucumis sativus*	*Cucurbitaceae*	0.25 and 50 µM	Photosynthetic rate, CO_2 assimilation, stomatal conductance, content of chlorophyll and carotenoid, Increase in number of chloroplast, water use efficiency of Rubisco was found to be enhanced by TRIA in leaves under salinity	Sarwar et al. (2017)
5.	*Mentha piperita L.*	Lamiaceae	1 µM	TRIA application enhanced chlorophyll content, stomatal conductance (gs), transpiration rate and photosynthetic rate, CO_2 which improved photosynthesis under environmental circumstances	Khanam and Mohammad (2018)
6.	*Triticum aestivum*	Poaceae	1 µM	TRIA application improved adverse effects caused due to arsenic stress in plants. Photosynthetic activity	Perveen at al. (2013)

				was found to be improved by different types of photosynthetic pigments like chl a,b, flavonoids, anthocyanin etc followed by transpiration rate, stomatal conductance	
7.	*Glycine max*	Leguminosae	10 mM	Foliar spray of TRIA mitigated by activity of photosynthetic pigments like chlorophyll and carotenoid which improved rate of photosynthesis in plants	Krishnan and Kumari (2008)

2.3.4 Oxidative stress

TRIA application was lowering oxidative stress. The effect of oxidative stress has been presented in table 2.4

Table 2.4 Effect of TRIA application on oxidative stress in different plant species

Sr No	**Plant species**	**Family**	**TRIA concentration**	**Effect**	**Reference**
1.	*Zea mays*	Poaceae	25 and 50 µM	Level of MDA and H_2O_2 was found to be reduced by Nickel stress but the application of TRIA mitigated adverse effect of	Younis and ismail (2019)

				nickel toxicity.	
2.	*Triticum aestivum*	Poaceae	0, 10, and 20 µM	Content of MDA and H_2O_2 was found to be reduced due to salt stress. TRIA in reducing salinity when applied at different stages in plants	Perveen et al. (2014)
3.	*Coriandrum sativum*	Apiaceae	6 and 12 mM	Application of TRIA under As-induced oxidative stress reduced the ROS by increasing the level H_2O_2 and MDA under As stress	Keramat et al. (2017)
4.	*Linum Usitatissimum*	Linaceae.	0, 1.0 and 0.1 µM	TRIA diminished the level of MDA and H_2O_2 under Drought induced stress.	Perveen et al. (2020)
5.	*Dracocephalum forrestii*	Lamiaceae	2.5, 5 and 10 µM	MDA and H_2O_2 content declined in TRIA supplemented shoots under stressed conditions.	Weremczuk-Jeżyna (2022)

6.	*Glycine max*	Leguminosae	0, 5 and 10 μM TRIA	A decline in MDA and H_2O_2 levels was observed by the addition of TRIA	Mozafri et al. 2017

2.3.5. Carbohydrates and protein content

Table 2.5 represents TRIA supplementation on content of carbohydrates and protein in different species of plant.

Table 2.5 Effect of TRIA on total carbohydrates and protein content in different plant species

Sr No	**Plant species**	**TRIA concentration**	**Effect**	**Reference**
1.	*Ocimum basilicum*	10^{-8}, 10^{-6} and 10^{-4}	Content of carbohydrates was found be significantly increased under application of TRIA	Hashmi et al. (2010)
2.	*Sesamum indicum*	2, 4, 6, 8 and 10 ppm	Level of protein and carbohydrates was enhanced by application of TRIA	Singh and Raghava (2022)
3.	*Helianthus annuus*	0 (Control) 50 and 100 μM	Improved production of protein was detected in TRIA-applied flowers under stressed environments.	Aziz et al. (2013)
4.	*Arabidopsis thaliana*	0.3 μM	TRIA application efficiently augmented the protein and carbohydrate content under stressed	He et al. (2020)

			conditions.	
5.	*Lablab purpureus*	10^{-0} (Control), 10^{-8},10^{-7},10^{-6} and 10^{-5} M	Content of carbohydrates and protein was found to be enhanced by TRIA application	Naeem et al. (2009)
6.	*Phaseolus vulgaris*	CN, 10, 20, 30 µmol L^{-1}	Foliar spray of Triacontanol increased protein content under Pb stress stating that TRIA plays important role in providing plant tolerance	Ahmed et al. (2023)

2.3.6 Osmolytes

Plants tend to cope up with different kind of environmental stresses by regulating level of osmolytes in plants. Various studies have reported that presence of different kind of osmolytes like proline and glycinebetaine protect plant from different kind of abiotic stresses by keeping osmotic balance. The osmolytes tend to maintain the integrity of the membrane by stabilizing these membranous structure like proteins, enzymes and controlling production of ROS (Ashraf et al., 2011; De la TorreGonzalez et al., 2018).

Table 2.6 Effect of TRIA on osmolytes in different plant species

Sr No	**Plant species**	**Family**	**TRIA concentration**	**Effect**	**Reference**
1.	*Brassica napus*	Brassicaceae	0, 0.5, and 1 mg L^{-1}	Content of proline and glycinebetaine was higher in TRIA under nonsaline and saline conditions	Shahbaz et al. (2012)
2.	*Zea mays*	Poaceae	5 µM	The addition of	Perveen et al.

				TRIA enhanced the proline level under stressed conditions.	(2018)
3.	*Cucumis sativus*	Cucurbitaceae	50 µM	Treatment of TRIA increased the proline content under saline conditions by increasing tolerance towards salinity	Sarwar et al. (2017)
4.	*Mentha piperita*	Lamiaceae	1 µM	Addition of TRIA enhanced proline content in salt regimes	Khanam and Mohammad (2018)
5.	Zea mays	Poaceae	0, 2 and 5 µM	TRIA improved the content of compatible solutes in glycinebetaine amd proline content	Perveen et al. (2016)

2.3.7 Antioxidative defense system

Triacontanol application improved the functioning of major antioxidant enzymes in case of stressed conditions by increasing activity major enzymes like SOD, POX, APX and CAT (Perveen et al., 2011). Furthermore, it was found that TRIA induced improvement in growth of tomato and maize under salinity stress (Mittova 2002). Various reports showed effect of different concentration of TRIA application by modifying antioxidative enzymes like CAT, APX, SOD and POD on plants like *Coriandrum sativum* L. (Karam and Keramat, 2017). CAT activity was found to increase under application of TRIA in Tulsi under cold stress. (Borowski and Blamowski., 2009). Likewise, different genotypes of wheat showed different response in case of different stressful environment (Abdel Latef., 2010). Lipid peroxidation

enhanced activity of different types of antioxidative system in case of stressed condition for controlling generation of ROS during different environmental stress conditions (Sanchezviveros, 2010).

Table 2.7 Effect of TRIA application on plant's antioxidant defence system in different plant species

Sr No	**Type of Plant**	**Family**	**TRIA concentration**	**Effect**	**Reference**
1.	*Triticum aestivum*	Poaceae	10 and 20 µM	TRIA application increased the enzymatic activities of major enzymes like CAT, POD and SOD.	Perveen et al. (2011)
2.	*Linum Usitatissimum*	Linaceae	1.0 and 0.1 µM	Activities and functioning of major antioxidant enzymes like CAT, SOD and POD was found to be enhanced under drought stress due to application of TRIA	Perveen et al. (2022)
3.	*Brassica napus*	Brassicaceae	10 and 20 µM	TRIA addition enhanced activity of like SOD, CAT, GPX and MDHAR, DHAR and enzymes like GSH and ASA	Karam et al. (2017)

				under cd toxicity	
4.	*Zea mays*	Poaceae	2 and 5 µM	CAT, ASA, SOD and GSH activity improved performance of different cultivars under drought stress by application of TRIA	Perveen et al. (2016)
5.	*Brassica juncea*	Brassicaceae	20 µM	Enzymes significantly raised SOD and CAT by using TRIA whivh mitigated drought induced oxidative stress.	Ahmad et al. (2021)
6.	*Solanum melongea*	Solanaceae	10µM	SOD, POD and CAT response was found to be decreased under high hight conditions	Faiz et al. (2022)
7.	*Hellianthus annus*	Asteraceae	0, 20 and 40 µM	Levels of GPOX and POD enzymes were amplified while SOD activity was reduced by the application of TRIA against	Yazdani et al. (2021)

				stressed environments.	
8.	*Zea Mays*	Poaceae	0, 2 and 5 µM	TRIA application improved activity of SOD, POD and CAT non-antioxidative enzymes like glycinebetaine was found to be increased under lead stressed conditions.	Iqbal at al. (2023)
9.	*Dracocephalum forrestii*	Lamiaceae	2.5, 5, and 10 µM	Antioxidative enzymes CAT, POD and CAT was improved at the affect of salinity under application of TRIA.	Weremczuk-Jeżyna et al. 2022

2.3.8 Gene expression

TRIA induced alteration in gene expression is responsible for causing mitigation of salt stress in plants.

Table 2.8 Effect of TRIA application on gene expression in different plant species

Sr No	**Stress**	**Plant species**	**Effect**	**Reference**
1.	Drought stress	*Oryza sativa*	Stress related *PIP* genes were stimulated in TRIA treated plants under stressed environments.	Alhrabi et al. (2021)

			PIP1,2 gene showed greater expression in TRIA-primed stressed roots.	
2.	Salt stress	*Triticum aestivum*	Genes like *MYB* regulate ionic homeostasis in plant which mitigate salt stress by production of ROS	Song et al. (2020)
3.	Oxidative stress	*Brassica juncea*	*MYB46* and *PAL* gene expressions were stimulated and inhibited respectively, in TRIA-applied plants under drought-induced oxidative stress conditions.	Ahmad et al. (2021)
4.	Drought stress	*Helianthus annus*	Treatment of TRIA alleviated drought stress in seedlings of sunflower by regulating expression of gene *RBCS*	Ismail and Younis (2021)
5.	Salt stress	*Zea mays*	Supplementation of TRIA increased expression of gene *ZmPAL1* in order to deal against salt stressed conditions	Ertani et al. (2013)
6.	Salt stress	*Triticum aestivum*	TRIA treatment increased the *P5CS* and *W36* expression of gene under NaCl concentration	Goharrizi et al. (2020)
7.	Metal stress	*Zea mays*	Stress related genes such as *ZmRBCS* and *ZmASR1* were reported decrease of Ni toxicity in plants	Younis and Ismail. (2022)

2.4 Role of hydrogen-sulphide in mediating salinity stress in plants

2.4.1 Plant growth

Supplementation of H_2S increased at different concentration like 0.01and 1.0Mm increased leaf length and size in Kandelia *obovate* (Li et al, 2021). Greamination of seeds of wheat and length of coleoptiles increased under H_2S treatment (Zhang et al. 2010a, b). Morphology of the root was found to be in plant of *Brassica napus* by application of H_2S (Li et al., 2012a). Aluminum induced toxicity was found to be mitigated by application of H_2S in barley (Dawood et al., 2012). Length of radicle and hypocotyl was found to be enhanced by Foliar spray of NaHS in *Cucumis sativus* (Sun and Luo, 2014).

Table 2.9 Effect of H_2S application on growth parameters in different plant species

Sr No	**Plant name**	**Stress**	**H_2S concentration**	**Effect**	**Reference**
1.	*Brassica rapa*	Cd-stress	5 μM	NaHS application reported increase in root length	Zhang et al. (2015)
2.	Gossiypium *hirusutum*	Pb- stress	0, 50, and 100 µM	Treatment of H_2S resulted in improved height, length, leaf area and number of leaves.	Bharwana et al. (2013)
3.	*Arabidopsis thaliana*	Cd-stress	50 µM	H_2S application increased the root length and alleviated toxic effects of environmental stressed condition	Jia et al. (2016)

4.	*Brassica oleracea*	Cr-stress	200 µM	Supplementation of TRIA resulted in improvement in length of roots, leaf no and leaf area	Ahmad et al. (2019)
5.	*Artemisia annua*	Copper stress	200 µM	Plant length, and biomass was found to be improved in H_2S applied plants.	Nomani et al. (2021)
6.	*Capsicum annum*	Salt stress	100 µM	Combined treatment of H_2S with NO improved the plant length and biomass against salt stressed conditions.	Kaya et al. (2020)

2.4.2 Photosynthetic system

H_2S application play important role in regulating photosynthesis by regulating opening and closing of stomata. Similar case was reported in *Arabidopsis thaliana*, where H_2S produced enzymes inhibited closing of stomata by inducing NO-mediated pathway (Honda et al., 2015). H_2S is known to have direct impact on PSII which conferred sharp and abrupt decline in FV/FM studies done on lichens (Bertuzzi and Tretiach., 2013). Opening and closing of stomata is regulated by PSII receptor which in turn affects stomatal conductance (Busch., 2014). H_2S improves photosynthetic machinery in plants by regulating chloroplast and fixation of CO_2 (Mostofa et al., 2015). H_2S treatment in the seedlings of *Spinacia oleracea* improved photosynthetic activity in the plants by activation of enzyme Rubisco (Chen et al., 2011). Various reports have indicated that H_2S treatment at lower concentration regulate movement of stomata as it has direct influence on ABA and NO which control movement of stomata (Duan et al., 2015).

Table 2.10 Effect of H_2S on the photosynthetic system of various plant species

Sr No	**Stress**	**Plant species**	**H_2S concentration**	**Effect**	**Reference**
1.	Co- stress	*Triticum aestivum*	600 µM	Photosynthetic parameters like RWC, osmotic potential, stomatal conductance and intercellular concentration was found to be improved by application of NAHS	Konakci et al. (2020)
2.	Temprature stress	*Vaccinium sect*	100 µM	H_2S application increased photochemical activities of PSI and PSII under stressed conditions.	Tang et al. (2005)
3.	Temprature stress	*Oryza sativa*	200 µM	Treatment of H_2S augmented the net photosynthetic rate by increasing gene expression of photosynthetic genes	Gautam et al. (2022)

4.	Nickel stress	*Cucurbita pepo*	0, 50, 100, 200, 400 µM	Treatment of NaHS intensified the content of photosynthetic pigments	Valivand et al. (2014)
5.	Salinity stress	*Cucumis sativus*	0, 25, 50, 75 and 100 µM	Photosynthetic apparatus was found to be improved by application of H_2S under salinity	Liu et al. (2020)
6.	Salt stress	*Oryza sativa*	0.2 mM	Combined application of H_2S and JIL 321 improved chlorophyll content and photosynthetic rate in H_2S applied plants.	Wang et al. (2022)
7.	Pb- stress	*Gossypium herbaceum*	0 and 200 µM	Chlorophyll content of and photosynthetic was found to be enhanced under Pb-stress by application of H_2S.	Bharwana et al. (2013)
8.	Cd-stress	*Nicotiana tabacum*	0.3, 0.6, 0.9, and 1.2 µM	H_2S improved PSII, Electron	Chen et al. (2021)

				transport chain, Photochemical Conductance, Transpiration and mechanism of photosynthesis	

2.4.3 Oxidative stress

H_2S alleviate drought stress in the seedlings of *Triticum aestivum* by regulating ascorbic acid and glutathione content (Shan et al., 2018). Level of H_2O_2 and MDA was found improved in seedlings of strawberry. However, H_2S treatment alleviated thermotolerance by regulating aquaporins and HSP (Christou et al., 2014).

Table 2.11 Effect of H_2S application on oxidative damage in plant species

Sr No	**Type of Stress**	**Plant species**	**H_2S concentration**	**Effect**	**Reference**
1.	Cr-stress	*Triticum aestivum*	0, 0.4, 0.8, 1.2, 1.6, 2.0 mM	Level of MDA and H_2O_2 was found to be reduced by endogenous production of H_2S application under Cr stress.	Zhang et al. (2010)
2.	Salinity stress	*Brassica oleracea*	0.2 m mol. L^{-1}	Application of H_2S decreased the content of H_2O_2 and MDA alleviating salinity stress.	Shalaby et al. (2023)
3.	Cu-stress	*Triticum aestivum*	0.0, 0.2, 0.8, 1.4 mM	Supplementation with H_2S in wheat	Zhang et al. (2008)

				plants reduced the level of MDA and H_2O_2.	
4.	Zn-regime	*Capsicum annum*	0.2 mM	MDA content and level of H_2O_2 were found to be decreased in H_2S plants under high toxic state	Kaya et al. (2018)
5.	Al-stress	*Brassica napus*	0 and 0.3 mM	Pretreatment with H_2S reported that H_2O_2 and MDA level decreased in seedling stage of root and leaves	Qian et al. (2014)
6.	Temprature stress	*Cucumis sativus*	10, 20, 40 or 80 µM	Content of MDA and H_2O_2 under chilling stress decrased drastically.	Nasibi et al. (2019)

2.6.5 Carbohydrates and protein content

Table 2.12 represents hydrogen sulphide impact on total carbohydrates and protein content in various species

Table 2.12 Effect of H_2S application on total carbohydrates and content of protein in different plant species

Sr No	**Metal name**	**Plant species**	**Effect**	**Reference**
1.	Metal stress	*Glycine max*	Application of H_2S raised the level of carbohydrates in *Glycine max* under	Zhang et al. (2020)

			deficiency of N	
2.	Drought stress	*Carthamus tinctorius*	Total carbohydrate content raised in H_2S cultivars.	Jabbari et al. (2020)
3.	Cd-stress	*Vigna radiata*	Carbohydrate and protein metabolism were significantly regulated by NO application to enhance barley tolerance to Cd.	Alp et al. (2022)
4.	Heat stress	*Triticum aestivum*	Content of carbohydrates was reduced in H_2S-treated seedlings under heat stress.	Yang et al. (2016)
5.	$NaHCO_3$ stress	*Cucumis sativus*	H_2S foliar spray increased the content of carbohydrates by alleviating toxic damage caused due to stress	Sun et al. (2014)
6.	Salt stress	*Cucumis sativus*	In case of salt stress, H_2S treatment promoted improvement in carbohydrate content and protein level	Liu et al. (2022)
7.	Drought stress	*Triticum aestivum*	Pre-treatment of NaHS alleviated in seedlings of triticum in case of dry conditions by increasing content of carbohydrate and protein under stress like conditions	Ding et al. (2017)

2.6.5 Osmolytes

Plants response to stressed situations by generating solutes like glycine betaine and proline which act as defense mechanism against stressful environmental conditions to enhance tolerance (Shahbaz et al., 2012). H_2S application raised the content of proline and glycinebetaine by regulating the level of ions and H_2S metabolism in plants. (Jiang et al., 2019). Likewise, roots of *Malus hupehenis* under salt induced oxidative stress (Su et al., 2016).

Table 2.13 Effect of H_2S application on osmolytes in different plant species

Sr No	**Stress**	**Plant name**	**H_2S concentration**	**Effect**	**Reference**
1.	Drought stress	*Spinacia oleracea*	100 μM	NaHS application raised the level of glycinebetaine and proline under Drought induced conditions.	Chen et al. (2016)
2.	Temprature stress	*Zea mays*	0, 5, 10, 15, 20, and 25 mM	Level of proline and glycinebetaine was enhanced by application of H_2S under high temperature conditions	Zhou et al. (2018)
3.	Cd-stress	*Populus euphratica*	200 μM	Treatment of H_2S increased the content of proline and glycinebetaine against Cd toxicity .	Sun et al. (2017)
4.	As-stress	*Pisum*	0.4 and 0.6	Content of proline	Alsahli et

		sativum	mM	and glycinebetaine was found to be enhanced by H_2S application.	al. (2018)
5.	Drought stress	*Eruca sativa*	2 mM	Level of proline and glycine betaine was enhanced by H_2S treatment against toxic effects of dehydration	Khan et al. (2018)
6.	Salt stress	*Phaseolus vulgaris*	50 and 100 µM	Osmolyte content was found be enhanced by addition of H_2S	Dawood et al. (2019)
7.	Salt stress	*Cucumis sativus*	25, 50, 100 and 150 µM	H_2S treatment improved the proline and glycinebetaine content against stressful conditions	Liu et al. (2022)
8.	Drought stress	*Medicago sativa*	100 µM	H_2S released synthetic compounds which increased the level of proline and glycinebetaine against stressful conditions	Antoniou et al. (2020)

2.6.6 Antioxidant defense system

Antioxidant enzymatic activities of different enzymes was improved and mitigatede stress by increasing activity of ROS scavengers such as CAT, POD, SOD and APX by enhancing antioxidative defense mechanism (Apel and Hirt., 2004). Expression and functioning of different antioxidative enzymes was found to be improved in strawberry and cut flowers (Hu et al., 2012). Under salinity, In case of cucumber Addition of NaHS donor up-regulated the activities of SOD, GR, and POX. Antioxidative enzymes were stimulated activities of enzymes like APOX and GR in *Zea mays* subjected under temperature stress. NaHS treatment improved CAT, POD and SOD activity under Cd toxicity (Mostofa et al., 2015). Further, APX, SOD and POD response improved by treatment of NaHS under CuO NP stress (Li et al., 2020).

ASA and GSH content was found to be declined in oak leaves by application of H_2S under saline-alkali stress due to enzyme dehydrogenase (Liu et al., 2021). The functioning of CAT, GPX, GR, GSH and ASA content was found to be improved under heat stress by application of H_2S donor (NaHS) (Li et al., 2014).

Table 2.14 Effect of H_2S application on antioxidant defense system in different plant species

Sr No	**Type of stress**	**Plant name**	**H_2S concentration**	**Effect**	**Reference**
1.	Heat stress	*Triticum aestivum*	0–1.5 m mol L^{-1}	H_2S application enhanced SOD, POD, CAT, and APX enzymes response under heat stress	Yang et al. (2016)
2.	Salinity stress	*Phaseolus vulgaris*	50 and 100 μM	APX, CAT, POD, SOD and GR, NR enzymes and ascorbic acid and glutathione content was improved under salinity stress in case of H_2S	Dawood et al. (2008)

				treated plants.	
3.	Osmotic stress	*Capsicum annuum*	100 µM	Addition of H_2S increased the SOD, CAT, POD, and APX activities under Cu pollution.	Kaya et al. (2018)
4.	Oxidative stress	*Arabidopsis thaliana*	500 µM	SOD, CAT, APX enzymatic and non-antioxidant enzymes like ascorbic acid and glutathione were noticed to be boosted in H_2S applied plants.	Ozfidan-Konakci et al. (2023)
5.	Heat stress	*Zea mays*	0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.5, 2.0 and 3.0 mM	Treatment of NaHS improved the functioning of different enzymesin under heat stress i.e., CAT, SOD, GR APX including ASA and GSH	Li et al. (2014)
6.	As-stress	*Solanum lycopersicum*	0.2 mM	H_2S application alone or in combination enhanced enzymatic activities of CAT, SOD, DHAR , MDHAR and GST by H_2S addition under As toxicity.	Kaya et al. (2020)
7.	Salt stress	*Malus hupehensis*	0.05 mM	Antioxidant enzymatic activities like SOD, POD, CAT, improved	Wei et al. (2018)

				under NaCl by application of H_2S.	
8.	$AlCl_3$ stress	*Triticum aestivum*	0.3mM	Enzymatic activity of antioxidants like SOD, CAT, APX and GPX was found to be improved under metal stress	Zhang et al. (2010)
9.	Temprature stress	*Nicotiana tabacum*	0.05 mM	H_2S treatment increased activity of enzymes like SOD, CAT, GPX, and GR under high temperature conditions	Li et al. (2015)
10.	Salt stress	*Zea mays*	0.6 mM	Salt stress was alleviated by application of H_2S by using non-enzymatic antioxidant defense mechanism which include enzyme like GSH and ASC acid	Shan et al. (2014)

2.6.7 Gene expression

Table 2.15 represents hydrogen sulphide impact on gene on various types of plants

Table 2.15 Effect of H_2S application on gene expression in different plant species

Sr No	**Type of stress**	**Plant species**	**Effect**	**Reference**
1.	Al -stress	*Oryza sativa*	Expression of genes like *OsSATR, OsALS1* and *OsSTAR2* was found to be up-regulated and gene like *OsNRAT1* was downregulated	Zhu et al. (2018)
2.	Oxidative stress	*Arabidopsis thaliana*	H_2S upregulated the expression of genes like *PM H^+ATPase, SOS1* and *SKOR* under excessive salt stress conditions.	Jiang et al. (2019)
3.	Osmotic stress	*Solanum lycopersicum*	H_2S inhibit the expression of *LeACO* genes and particularly gene like *LeACO1* and *LeACO2* under stressed conditions by regulation of ethylene biosynthesis	Jia et al. (2018)
4.	Metal stress	*Oryza sativa*	*Lsi1* and *Lsi2* gene expressions were up-regulated by H_2S treatment in rice under copper oxide nanoparticle phytotoxicity	Rai et al. (2021)
5.	Nickel stress	*Cucurbita pepo*	Expression of gene Ca^{2+}-dependent protein kinase (CDPK) and phytochelatin (PCs) was improved under nickel stress.	Valivand et al. (2019)

6.	Salt stress	*Malus hupehensis*	Expression of gene like *MhSOS1* and *MhSKOR* in case of salinity	Li et al. (2020)
7.	Chiling stress	*Cucumis sativus*	H_2S regulated genes like *MAAI, Tau, GR, GS, MDHAR* under chilling stress it also included enzymes like MDHAR and GR	Liu et al. (2021)

Chapter-3

Hypothesis

Stress is main factor which affect yield. One such major agro-economic problem that affects plant is salinization. It is the main cause to worry since it builds up in soil and seriously disrupts the plant growth and productivity everywhere. Due to rising food demand, it is important ameliorate negative impact of salinity stress as it accumulates in soil and water and is extremely harmful to plant growth and productivity globally. Most common source of salinity is irrigation. Irrigation of crop fields with excessive concentration of sodium chloride has gained a great importance in the present time because of it has severe impact on productivity.

Food security is one of the major concerns that can never be disregarded by the human civilization. Rise in environmental damage, poor agricultural practices puts intense pressure on human population and have unlucky consequence on global food production making it inadequate to feed the growing population. Furthermore, no research has yet been done on *Brassica juncea* about the combined effects of TRIA and H_2S. Exogenous administration of TRIA and H_2S may counteract the detrimental effects of salinity and synergistic approach powerfully demonstrate the substantial character in the mitigation of salinity. Therefore, present research may work provides a novel method for assessing the salinity allevation based on hypothesis.

- Reduction in soil salinity is problem to be solved for improving cultivar's growth, productivity and accomplishing the food demand.
- Ameliorating the toxic effect of salinity and improving the productivity of *Brassica juncea*, which is significantly important crop by synergistic association of both Triacontanol (Phytohormone) and H_2S (Signaling molecule).
- Determining the influence of TRIA and H_2S on morphological, biochemical, physiological and molecular aspects of *Brassica juncea* under salinity is the main goal of current research.

Chapter-4

Objectives

4.1 Background

Salt stress is one of the most serious factor limiting agricultural production by disrupting water uptake, translocation and accumulation of salt from soil has lately emerged as global concern for enhancing their development and productivity as well as growing demand of food production (Bali et al., 2021). In order to increase salt tolerance in plant, different methods are utilized by plants to reduce the salinity stress but these methods have not yet proven to be successful. That is why mitigation of salinity stress is much needed with cost effective and environment friendly application. Hence use of TRIA and H_2S could be an advantageous strategy for this.

4.2 Research objectives

The present study was designed to meet the below-mentioned objectives

1. Analysis of Triacontanol and H_2S in mitigating malicious effect of salinity stress on *Brassica juncea*
2. Assessment of Triacontanol and H_2S induced growth attributes and physiochemical aspect of *Brassica juncea* in vitro and in vivo under salinity stress.
3. Comparative study of gene expression of salt stress related genes in *Brassica juncea* in response to Triacontanol and H_2S.

Chapter-5

Materials and Methods

5.1 Location and Climate

Phagwara is situated between 31°-55' Latitude, 75°-54' Longitude and 247 meters above the sea level. It has semi-arid and subtropical type of climate with wide range of variation in climatic conditions. Beginning of the month is marked by post-monsoon showers. The soothing and pleasant climate is experienced in the month of October and November. Maximum day temperature of the city reaches upto 32-35°C and at night temperature falls down to 16-18°C.

5.2 Plan of Work

(i) Experiment Name: Effect of exogenous application on triacontanol and hydrogen sulphide on *Brassica juncea* L. exposed to salinity stress.

(ii) Location and place of work: Certified seeds of *Brassica juncea* var PBR-91 were procured from Punjab Agricultural University, Ludhiana, Punjab. Experiments were conducted at Research farms, Lovely Professional University, Phagwara.

(iii) Methodology

5.3 Combinations of treatments

Different treatments selected are mentioned in table 5.3.1.

Table 5.3.1 Different treatments selected for the experiment.

S. No.	Treatment	NaCl (mM)	TRIA (µM)	H_2S (µM)
1.	CN	0	0	0
2.	NaCl I	50	0	0
3.	NaCl II	100	0	0
4.	NaCl III	150	0	0
5.	TRIA	0	150	0
6.	TRIA + NaCl I	50	150	0
7.	TRIA + NaCl II	100	150	0
8.	TRIA + NaCl III	150	150	0
9.	H_2S	0	0	25
10.	H_2S + NaCl I	50	0	25
11.	H_2S + NaCl II	100	0	25
12.	H_2S + NaCl III	150	0	25
13.	TRIA + H_2S	0	150	25
14.	TRIA + H_2S + NaCl I	50	150	25
15.	TRIA + H_2S + NaCl II	100	150	25
16.	TRIA + H_2S + NaCl III	150	150	25

5.4 Raising of plant material for experimental studies

5.4.1 Surface sterilization

0.01% sodium hypochlorite for 5 minutes was used for surface sterilization and then washed with distilled water 3 times.

5.4.2 *In vitro* Raising of seedlings

B. juncea seeds were sterlizied by pre-soaking in TRIA (150 µM) for 8 hours and the other seeds were dipped in distilled water for same time duration. NaCl solution of different concentrations i.e. 50, 100 and 150 mM was used to supplied in Petri-plates which were lined with *Whatmann* no.1 filter paper. Pre-treated seeds of TRIA were cleaned and then placed in petri-plates containing NaCl solutions of varied concentrations. H_2S (in the form of sodium hydrosulphide) was applied as foliar spray. Control seeds were supplied with distilled water. Under carefully controlled conditions, petri- dishes were placed inside seed germinator at 25 ±0.5 ^oC, 16 h photoperiod, 175 µmol m^{-2} s^{-1} of light intensity, and 68–70%. humidity level. 7 days old seedlings were harvested for research analysis.

5.4.3 *In-vivo* Raising of plants

Similar treatment process was followed for *in-vivo* study. Grow bags (24 cm diameter and 40 cm height) were filled with soil + organic manure in the ratio of 3:1, were used for sowing seeds. Plants were then harvested after 30 and 60 days, for further evaluations.

No of replications/treatment :3

Observations to be recorded

The observations were made at 7 days (Seedling emergence stage), 30 days and 60 days (Plant emergence stage).

5.5 Growth attributes

5.5.1 Germination (%)

It was determined by using the formula

$$= \frac{total\ seeds\ germinated}{number\ of\ initial\ seeds\ used} \times 100$$

5.5.2 Plant Growth:

Plant length (cm)

B. juncea seedlings were used to measure growth parameters. Root and shoot length were measured in cm using scale.

Fresh and Dry weight (g^{-1})

Fresh weight was measured in gm using a weighing balance. Drying was done at 80 ^{0}C for 24 h described as dry weight. Similar measurements were made on plants that were 30 and 60 days old

5.5.3 Vigor index

Vigor index was calculated using

$$\text{Vigor index} = (Root\ length + shoot\ length) \times germination\ percentage$$

5.5.4 Relative water content

It was calculated both in seedling and plants by formula

$$\text{RWC} = \frac{FW-TW}{FW-DW} \times 100$$

5.5 Photosynthetic pigments and gas exchange parameters

5.5.1 Pigments

5.5.1.1 Chlorophyll content

Arnon (1949) method was followed for the evaluation of chlorophyll. 0.5g tissue was crushed in 80% acetone (4ml) in chilled pestle and mortar. Centrifugation at 13, 000 rpm (4 ^{0}C) for 20 minutes and Total chlorophyll, chl a and b contents was calculated by reading absorbance at 645 and 663 nm with spectrophotometer.

Chlorophyll contents were measured in mg g^{-1} FW by using below-mentioned equations

Chl a $= \{(Abs_{663} \times 12.7) - (Abs_{645} \times 2.69)\} \times {}^{v}/_{1000 \times w}$

Chl b $= \{(Abs_{645} \times 22.9) - (Abs_{663} \times 4.68)\} \times {}^{v}/_{1000 \times w}$

Total chlorophyll $= \{(Abs_{645} \times 20.2) + (Abs_{663} \times 8.02)\} \times {}^{v}/_{1000 \times w}$

Where, v = volume of the extract (ml)

w = weight of fresh leaf tissue (g)

5.5.1.2 Total carotenoid content

Maclachlan and Zalik (1963) method was used to measure total carotenoid content. The estimation was done from 0.5 g fresh plant tissue. The tissue was then finely chopped and homogenized in acetone (4ml) and centrifuged at 13,000 rpm (4 ^{0}C) for 20 minutes. Supernatant was collected for the evaluation of total carotenoid content and reading at 480 and 510 nm. Total carotenoid content was measured by using the below-mentioned formula

Carotenoid content$= \{(Abs_{480} \times 7.6) - (Abs_{510} \times 1.49)\} \times {}^{v}/_{1000 \times w}$

v = volume of plant extract

w = weight of plant sample

5.5.1.3 Total xanthophyll content

AOAC method given by Lawrence (1990) was used for the determination of xanthophyll content. Plant sample was dried and grounded into powdered paste. After

that, leaf powder of 50 mg was transferred to flask (100 ml). To the plant sample, (30ml) extract containing hexane (10 ml), acetone (7ml), absolute alcohol (6 ml) and toluene was pipette into the flask followed by continuous shaking for 10-15 min. 2ml of methanolic KOH (40%) in the flask added to plant extract. After that flask was refuxed in hot water bath for 20 min at 56 ^{o}C. 30 ml of hexane was added before this it was placed for 1 h in dark. 10% sodium sulphate solution was added to make volume to 100 ml by continuous shaking of the flask. The flask was placed in dark for an hour.

Top layer was added 50 ml to male volume to 50 ml in flask by adding hexane. All contents were mixed and reading was done at 474 nm wavelength using spectrophotometer.

Calculations

Total xanthophyll content $= \frac{Abs_{474} \times D}{w \times 236}$

Where,

Abs_{474}= Absorbance at 474

D= final dilution

W= weight of sample taken

236= specific-absorptivity (trans-lutein in gl^{-1})

5.5.2 Gaseous exchange parameters

Gaseous exchange characteristics were determined by using LI-COR LI-6400XT. Following measurements were made;

- Photosynthetic rate (Pn)
- Stomatal conductance (Gs)
- Inter-cellular CO_2 (C_i)
- Transpiration rate (Et)

Procedure

Conditions during data measurements in an open system IRGA were at constant CO_2

level, air coming from the same source is allowed to enter into analysis and reference lines. IRGA compares the concentration of CO_2 and H_2O in the air entering into the reference chamber to the air coming out of the sample chamber. The measurement of all gaseous exchange parameters were taken in the sunlight 11:00 am to 1:00 pm. Instrument was set at ambient conditions to measure photosynthetic activities

- Air temperature = 25 ^oC,
- Photon flux density = 1000 μmol m^{-2} s^{-1}
- Air relative humidity = 80-90%
- CO_2 concentration = 400 μmol mol^{-1}

5.6 Metabolites

5.6.1 Anthocyanin content

Anthocyanin content was estimated by method given by Mancinelli (1984). Fresh plant tissue of 1g was crushed. Homogenization was done by adding methanol: H_2O: HCl in the ratio 79:20:1 subjected to 20 min at centrifugation of 13,000 rpm. Absorbance was read at 530 nm and 657 nm wavelengths.

Calculations

$$A = Abs_{530} - (0.25 \times Abs_{657})$$

Anthocyanin content (mg g^{-1} FW) = A×MW×1000 / ε

Where; Abs_{530} = Absorbance at 530

Abs_{657}= Absorbance at 657

MW = molecular weight of cyanidin-3-glucoside (449.2)

ε = molar absorptivity (cyanidin-3-glucoside, 26900)

5.6.2 Flavonoid content

Flavonoid content was evaluated by following the method of Kim et al. (1999). Briefly, homogenization of 500 mg of plant tissue was carried out in 3 ml of absolute methanol. After centrifugation, supernatant was collected to which DDW (4 ml) + $NaNO_2$ (0.3ml) + $AlCl_3$ (3 ml) was mixed. Then 2 ml of NaOH and 2.4 ml of DDW

were added after which pink color appeared. Absorbance was then recorded at 510 nm wavelength and rutin was utilized as standard.

5.6.3 Phenolic content

Malick and Singh (1980) method was used to analyze phenolic content. Briefly, 0.5g of fresh leaves were crushed in (80%) ethanol, followed by centrifugation for 20 minutes. Supernatant, was prepared by adding Folin ciocalteau reagent (5ml) + 20% Na_2CO_3 (2ml). After incubating for 5 minutes, the optical density was taken at absorbance of 650 nm. Standard was formed using Gallic acid

5.7 Oxidative damage

5.7.1 Malondialdehyde (MDA) content

MDA content was measured using Heath and Packer (1968) protocol. Briefly, 0.1 g finely sample was crushed in 0.1% TCA by centrifugation at 5,000 rpm. 20% TCA containing 0.5% TBA was followed by incubation at 95^0C followed by reading density at 532 and 600 nm

MDA content was evaluated by using 155 mM^{-1} cm^{-1} as an extinction coefficient.

Calculations

$$\mathbf{MDA} = \frac{Absorbance \times total\ volume \times 1000}{Ext\ coeff \times sample\ volume \times wt\ of\ plant\ tissue}$$

5.7.2 Hydrogen peroxide content (H_2O_2)

Velikova et al. (2000) was used for H_2O_2 content. 100 mg of tissue was homogenized in 0.1% of TCA. The homogenate was then centrifuged. To 0.5 ml of supernatant, 0.4 PPB and 0.8 ml of and potassium iodide was added. Refrence H_2O_2 was used as standard at 390 nm density.

5.7.3 Histochemical studies by confocal microscope

Histochemical studies were done on roots of *B. juncea* seedlings to study the membrane and nuclear damage by the method of Callard et al. (1996) and Gutierrez-Alcala et al. (2000) using confocal microscope (Nikon AIR). Roots of 1 cm were cut from each sample and washed with water. To evaluate membrane and nuclear damage, roots of *B. juncea* seedlings. 0.1 mg DAPI was used to evaluate in 100 ml

PBS and propidium iodide (50 µM) was used for staining. Incubation was done for 30 minutes in the dark after that PBS washing was done. Stained slides of roots mounted with water were prepared and observed the effect of salinity stress under confocal microscope. Magnification was set to 10X to observe stained slides under microscope.

5.8 Estimation of Osmolytes

5.8.1 Proline

Proline content was found by using Bates et al. (1973) method. Plant tissue of 0.25g was crushed in 3% sulfosalicylic acid followed by centrifugation. To the 2 ml of filtrate, ninhydrin and glacial acetic acid (2 ml) was added followed by 60 min incubation. Extracted mixture contains toluene and proline and absorbance of 520 nm and graph was plotted using standard curve. Standard solution was prepared using L-proline.

5.8.2 Glycine betaine content

Glycinebetaine content was determined by following method of Grieve and Grattan (1983). Briefly, 1 g of dried plant sample was homogenized. After filtration, 1 ml of 2M HCl and 0.2 ml of PI_3 solution was mixed to supernatant (1ml). Shaking and cooling was done for 90 min. 2.0 ml and 20 ml of iced DW and 1-2 dichloromethane, respectively were added to it. Wavelength was set at 365 nm and the top layer was discarded. Standard curve was calculated for glycine betaine.

5.9 Total carbohydrates content

Total carbohydrates were analyzed by following method of Scott and Melvin (1953). Fresh tissue of 25 mg was added to flask of 100 ml containing 1.25ml of HCl (2.5 N) followed by cooling at room temperature. After this Na_2CO_3 was added to 25 ml of volume. 4 ml of anthrone reagent to was added to 1ml of supernatant. For 8 minutes the mixture was heated. After cooling, 630 nm was used as optical density when the dark green color appeared. Standard was calculated using glucose and by plotting standard graph.

5.10 Protein content and Antioxidant defense system

5.10.1 Protein content

Lowry et al. (1951) protocol was used for evaluating protein content. Briefly, in 3 ml of phosphate buffer, 500 mg plant tissue was crushed. Centrifugation was done at 10,000 rpm for 10 minutes. Then, volume was made up to 1 ml by adding distilled water (0.9 ml) to 0.1ml supernatant. Reagent C (5 ml) was prepared by mixing of reagent A and B. Blue color appeared after addition of reagent D in 0.5 ml quantity i.e. FC reagent. 660 nm was used an absorbance to calculate optical density.

Sodium carbonate in sodium hydroxide= Reagent A

Copper sulphate in potassium sodium tartarate= Reagent B.

5.10.2 Enzymatic antioxidants

Antioxidant enzymes extraction

For SOD activity, enzyme was extracted by finely grinding 1 g of fresh leaves to 3 ml Na_2CO_3 followed centrifugation at the speed of 5,000 rpm for 20 minutes. In case of other antioxidant enzymes i.e., CAT , POD, APX, GR, GPOX, DHAR, MDHAR, GST, and PPO. The homogenate was used as 1g of fresh plant tissue in PB of 3 ml followed by centrifugation. Supernatants were used for analysis

5.10.3 Superoxide dismutase (SOD)

SOD activity was determined by using Kono method (1978) to inhibit photochemical reduction. Briefly, 300 µl NBT (96 µM) and 300 µl Triton X-100 (0.6 %) and 1700 µl of Na_2CO_3 buffer (50 mM, Ph 10), mixed to the test cuvettes. After that, 300 µl of $HONH_2$ and 300 µl of EDTA (0.1 mM), were added to start the reaction. The addition of a 100 µl plant sample was done after 2 minutes. Absorbance was read when reaction mixture was subjected to 540 nm.

The percentage inhibition of NBT reduction was calculated by using following formula

$$x = \frac{change\ in\ Abs\ min^{-1}(blank) - change\ in\ Abs\ min^{-1}(sample)}{change\ in\ Abs\ min^{-1}(blank)} \times 100$$

Where, x (%) of inhibition is produced by 100 µl of the sample.

50 % inhibition is due to

$$\frac{50\times100}{x} = z\ \mu l\ of\ sample$$

5.10.4 Catalase (CAT)

CAT activity was determined by using Aebi (1983) standard protocol. The reaction solution for CAT enzyme contains. The reaction mixrure for CAT enzyme contains H_2O_2 of 300 µl (150 mM) and phosphate buffer1of 2.650 ml (100 Mm) was added to 50 µl of plant sample. 240 nm optical density was used for noticing absorbance of the reaction mixture. CAT activity was determined by

Unit activity (Unit min^{-1} g^{-1} FW)

$$= \frac{change\ in\ Abs\ min^{-1}\times\ total\ volume\ (ml)}{Extinction\ coefficient\times\ volume\ of\ sample\ taken\ (ml)\times wt\ of\ tissue\ (g)}$$

Where, Extinction co-efficient is 43.6 M^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.5 Ascorbate peroxidase (APX)

Ascorbate peroxidase activity calculated by Nakano and Asada (1981). Optical density was taken at 290 nm after adding ascorbate (5 mM) and H_2O_2 (0.5 mM) (0.3 ml each) and phosphate buffer (100 mM, pH 7.0) in 2.370 ml quantity to 50 µl of plant sample.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1}\times\ total\ volume\ (ml)}{Extinction\ coefficient\times\ volume\ of\ sample(ml)\times wt\ of\ tissue\ (g)}$$

Where Extinction co-efficient is 2.8 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.6 Guaiacol peroxidase (POD)

Guaiacol peroxidase (POD) enzymatic activity was determined by method given by Putter (1974). Change in optical density was observed at 436 nm after adding 0.3 ml each of guaiacol (20 mM) and H_2O_2 (12.3 mM) along with phosphate buffer (100

mM, pH 7.0) in 2.370 ml quantity to 50 µl of plant sample. Activity of POD enzyme was calculated as follows:

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 25.5 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.7 Glutathione reductase (GR) activity

The standard protocol of Carlberg and Mannervik (1975) was followed to evaluate the activity of GR enzyme. Briefly, 2 ml of PPB (50 mM, 7.0 pH), 300 µl each of EDTA (3 mM), NADPH (0.1 mM) and oxidized glutathione (1 mM) and 100 µl of plant sample, were included in the reaction mixture. Readings were taken at 340 nm.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where, extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.8 Glutathione peroxidase (GPOX) activity

Glutathione peroxidase enzyme action was estimated by the standard protocol of Flohe and Gunzlar (1984). Reaction mixture contained 1470 µl of PPB (50 mM, pH 7.0), 300 µl each of EDTA (0.5 mM), glutathione reduced (1 mM), NADPH (0.15 mM), sodium azide (1 mM), H_2O_2 (0.15 mM) and 30 µl sample. Optical density was taken at 340 nm.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.9 Dehydroascorbate reductase (DHAR)

Dehydroascorbate reductase activity was estimated by the method of Dalton et al. (1986). Reaction mixture contained 2050 µl phosphate buffer (50 mM, pH 7.0), 300 µl each of EDTA (0.1 mM), GSH (1.5 mM) and dehydroascorbate (0.2 mM) and 50 µl enzyme extract. The absorbance of samples was measured at 265 nm using spectrophotometer.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 14 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.10 Monodehydroascorbate reductase (MDHAR)

Hossain et al. (1984) method was used to measure the activity of enzyme MDHAR. The reaction was initiated by addition of sample of 50 µl followed by addition of 1450 µl phosphate buffer (50 mM, pH 7.5), 300 µl each of EDTA (0.1 mM), ascorbate oxidase (0.25 units), NADH (0.3 mM), Triton X-100 (0.25%), and ascorbate (3 mM). Decrease in the MDHAR enzymatic activity was read at absorbance 340 nm.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where, extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.11 Glutathione-S-transferase (GST)

Habig et al. (1974) method was followed for the evaluation of GST enzyme activity. Briefly, 2330 µl of phosphate buffer (0.2 M, pH 7.5), 300 µl of GSH (20 mM) and 300 µl of CDNB, were added, followed by the incorporation of enzyme extract in 70 µl quantity. Absorbance was taken at 340 nm wavelength.

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 9.6 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.10.12 Polyphenol oxidase (PPO)

Kumar and Khan (1982) protocol was used for polyphenol oxidase enzymatic activity. Change in absorbance was observed at 495 nm after adding 0.5 ml each of 2.5 N H_2SO_4, and catechol (0.1 M) and 1.95 ml of PPB (0.1 M) in 50 µl of plant sample.

Unit activity (Unit min^{-1} g^{-1} FW)

$$= \frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 2.9 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.11 Non-enzymatic antioxidants

About 1 g of seedling and plant samples was crushed in 50 mM tris buffer in 3 ml quantity, centrifugation was carried at speed of 13,000 rpm for time 20 min at 4 ^oC temperature. Collected supernatant was used for antioxidants analysis.

5.11.1 Ascorbic acid

Method of Roe and Kuether (1943) was used for ascorbic acid. Briefly, 0.5 ml of enzyme extract was added to 100 mg charcoal, 4 ml of DDW, and 0.5 ml of 50 % TCA and mixed. After that, 0.4 ml of DNPH and H_2SO_4 of 1.6 ml was used for incubation and left for 30 min. ascorbic acid of about 1 mg 100 ml^{-1} was used as standard and 520 nm was used as absorbance. Content of ascorbic acid was estimated

Ascorbic acid (µg g^{-1} FW) = $\frac{Abs\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$

5.11.2 Glutathione content

Content of glutathione was estimated by Sedlak and Lindsay (1968). In 100 µl of

supernatant prepared from tissue, 4 ml of absolute methanol, 50 µl of 0.01 M DTNB, and 1 ml of Tris buffer (0.2 M, pH 8.2) were added and left for 15 minutes. The mixture was re-centrifuged (3000 rpm; 15 minutes) followed by noting down its absorbance at 412 nm. Glutathione determination (1 mg 100 ml^{-1}) was done using glutathione. The GSH content was calculated from following equation:

Glutathione content (µg g^{-1} FW) = $\frac{Abs\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$

5.11.3 Tocopherol (vitamin E)

Martinek (1964) method was used to evaluate tocopherol content. Briefly, 0.5 ml each of absolute ethanol and 0.5 ml DDW added to plant extract. Shaking was done to divide protein precipitates. After adding, 0.5ml of xylene tube was used and then centrifugation was carried. Top layer of 0.5 ml of xylene with 0.5 ml reagent of TPTZ and 600 nm wavelength was used for determination of tocopherol content. Standard was done using tocopherol

Tocopherol (µg g^{-1} FW) = $\frac{Abs\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$

5.12 Gene expression analysis by qRT-PCR

Isolation of RNA was done using TRIzol method on *B. juncea* plants. Quantification of isolated RNA was done on nanodrop spectrophotometer after that agarose gel electrophoresis using 2% gel was used to check the quality. Synthesis from RNA to cDNA was done by following method of Awasthi et al. (2016). qRT-PCR quantification was done by using ROTOR geneq. RT-PCR system. Gene-specific primer, cDNA and SYBR green was used as reaction mixture, Triplicates and housekeeping gene actin were used for each assay. Ct value was estimated $2^{-\Delta\Delta ct}$ method which helped in determining relative expression of a gene (Livak & Schmittgen, 2001).

5.13 Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) and Tukey's test was applied to check the statistical significant difference ($P < 0.05$ level of significance) between treatments by using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Experimentation was carried using triplicates and was expressed as the means ± SEM.

Chapter-6

Results and Discussion

6.1 Results

6.1.1 *In vitro* grown seedlings

6.1.1.1 Plant growth

Length of root was found to decrease dramatically under salinity (Fig. 6.1; Table 6.1). Highest reduction of 3.41 cm was found at NaCl III concentration. Decrease of 50% occurred in NaCl II seedlings. The result exhibited that TRIA and H_2S alleviated salinity stress by increasing the root length. Among TRIA-treated seedlings under salt stress, the highest root length of 3.41 cm was observed at NaCl III concentration. Foliar application of H_2S under salt stress improved root length of 12.50 cm at NaCl III concentration. Association of TRIA and H_2S under salinity reported rise in root length at NaCl II conc. Root length was found to increase by 9.98 cm at TRIA+H_2S+NaCl II concentration in comparison to NaCl I treated seedlings.

Shoot length was found to decline under salt stress with lowest shoot length of 4.18 cm at NaCl III concentration. TRIA and H_2S treated seedlings showed increased in shoot length (Fig. 6.1; Table 6.1). Application of TRIA improved the shoot length. Under salt stress, it was found that application of TRIA showed highest shoot length of 8.24 cm in TRIA+H_2S I treated seedlings. Shoot length was found to increase in H_2S-treated seedlings under salt stress. Combination of TRIA and H_2S enhanced the shoot length under salinity with the highest shoot length of 8.89 cm at NaCl III concentration. Shoot length was found to be decreased as salt level increased in the case of TRIA + H_2S.

Fresh weight was found to be influenced under salt stress with highest reduction in fresh weight (82.33 mg) in NaCl II treated seedlings (Fig. 6.1; Table 6.1). TRIA application under salinity enhanced the fresh weight from 121.33 mg to 210.66 mg in contrast to NaCl I alone seedlings. Similarly, H_2S pre-treatment resulted improved fresh weight under salt stress in *Brassica* treated seedlings. In H_2S pre-treated seedlings, the highest and lowest fresh weight was found to be 184.66 mg and 123.66 mg at salt stressed I and II seedlings, respectively. Association of TRIA and H_2S was proved to be beneficial in improving fresh weight as compared to individual treatment under stress Maximum increase in fresh weight was found at NaCl I concentration

with 224.00 mg.

Table 6.1 Effect of TRIA and H_2S on morphological parameters of *B. juncea* under salinity stress

Treatment	**Root length (cm)**	**Shoot length (cm)**	**Fresh weight (g)**	**Dry weight (g)**
Control	7.39^{bcd}±0.54	7.28^{bcde} ±0.74	151.33^{d}±5.81	1.95^{cd}±0.04
NaCl I	5.56^{abc}±0.39	5.31^{ab}±0.39	121.33^{bc}±6.33	1.40^{abc}±0.02
NaCl II	4.68^{ab}±0.15	4.41^{a} ±0.24	82.33^{a}±2.02	0.91^{ab}±0.10
NaCl III	3.41^{a}±0.18	4.18^{a} ±0.52	104.66^{ab}±3.17	0.42^{a}±0.82
TRIA	12.57^{fg}±0.88	9.06^{efg} ±0.23	235.33^{gh}±8.74	3.71^{hi}±0.23
TRIA + NaCl I	11.10^{efg}±0.61	8.24^{cdef} ±0.63	210.66^{fg}±6.74	2.92^{fgh}±0.07
TRIA + NaCl II	10.22^{def}±1.13	7.48^{bcde} ±0.29	186.33^{ef}±3.71	2.39^{def}±0.20
TRIA + NaCl III	9.95^{de}±0.57	6.42^{abcd}±0.63	169.00^{de}±5.77	1.82^{bcd}±0.18
H_2S	12.50^{fg}±0.71	9.72^{fg} ±0.57	229.66^{g}±15.05	3.73^{hi}±0.21
H_2S + NaCl I	9.84^{def}±0.72	8.01^{cdef} ±0.33	184.66^{def}±8.45	2.50^{de}±0.15
H_2S + NaCl II	8.08^{cde}±0.79	7.14^{bcde}± 0.48	161.66^{de}±8.19	2.28^{bcde}±0.25
H_2S + NaCl III	9.66^{de}±0.88	5.89^{abc} ±0.56	123.66^{bc}±4.17	1.41^{abcd}±0.07
TRIA + H_2S	13.59^{g}±0.54	11.07^{g}± 0.50	264.66^{h}±3.17	3.97^{i}±0.12
TRIA + H_2S + NaCl I	9.43^{def}±1.06	8.89^{defg}± 0.13	224.00^{g}±4.04	3.50^{ghi}±0.10
TRIA + H_2S + NaCl II	9.98^{de}±0.84	7.41^{bcde}± 0.60	185.66^{def}±3.52	2.65^{efg}±0.29
TRIA + H_2S + NaCl III	8.44^{cde}±0.53	6.12^{abc}± 0.20	1.66^{de} ±3.71	2.41^{def}±0.28

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Dry weight was reported to be (NaCl II) 0.91 mg and (NaCl III) 0.42mg treated seedlings showed higher reduction in dry weight (Fig. 6.2; Table 6.1). Application of triacontanol and hydrogen sulphide alone under stress reported an increase in dry weight with maximum dry weights of 3.71 mg and 3.73 mg at NaCl I concentration. Almost a two-fold increase in dry weight i.e., 2.65 mg was observed in TRIA+H_2S+NaCl II concentration as compared to NaCl II (0.91 mg) seedlings. Combination of TRIA and H_2S showed better results in improving dry weight as compared to individual treatments.

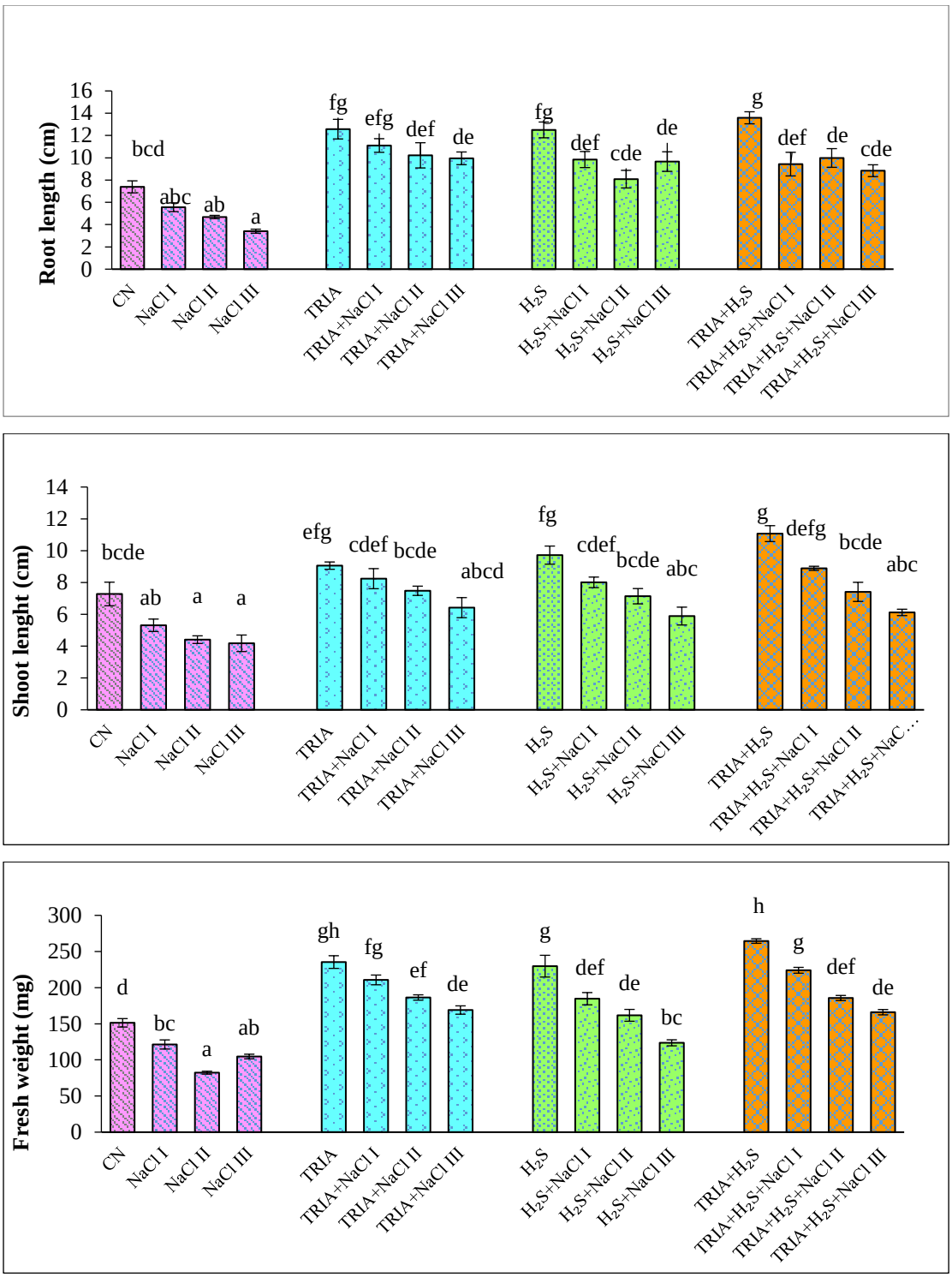


Fig. 6.1 Effect of TRIA and H_2S on root and shoot length and fresh weight in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

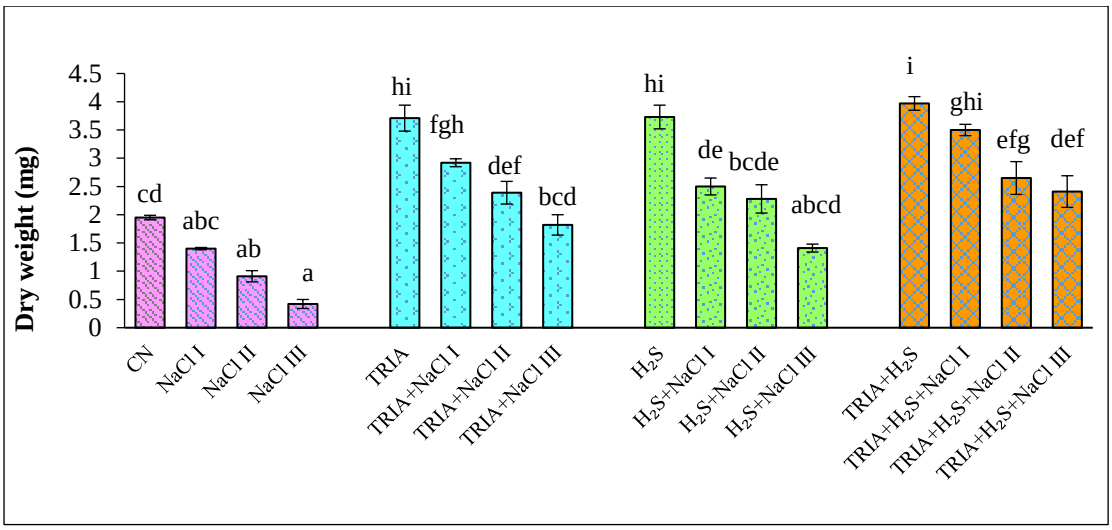


Fig. 6.2 Effect of TRIA and H_2S on dry weight in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Salt stress severely affected the germination percentage of *Brassica* seedlings (Fig. 6.3; Table 6.2). Germination percentage reduced with rise of salt concentration from 67.05% at NaCl I concentration to 55.93% at NaCl III concentration. Control seedlings exhibited germination percentage of 81.51%. Individual application of TRIA under unstressed conditions showed a germination percentage of 89.76% which is greater in comparison to control seedlings. TRIA application at NaCl I and II showed similar results in germination percentage i.e., 72.14% and 75.76%. Similar trend was observed in H_2S treated seedlings, where maximum germination percentage of 79.15% was observed at NaCl III concentration. Synergistic association of TRIA+H_2S reported higher germination percentage of 88.28% at salt stressed stage as compared to their individual treatments.

Vigor Index of *Brassica* seedlings was found to decrease under salt stress (Fig. 6.3; Table 6.3). Salinity reduced vigor index with almost 50% reduction at NaCl I concentration (648.86%), as compared to control seedlings (1247.7%). NaCl III treated seedlings showed greater reduction of 347.56% in vigor index in comparison to (648.46%) NaCl I treated seedlings. TRIA and H_2S alone under salinity increased the vigor index. Highest vigor index of 1354.56% was observed at NaCl I concentration in case of TRIA-treated seedlings among all the three concentrations of NaCl. Seedlings pre-treated with H_2S improved the vigor index against salinity with

maximum vigor index of 1286.2% at NaCl I concentration. According to the data, it was pertained that among all the individual treatments of TRIA + H_2S in case of salinity, Application of triacontanol showed better results contrast to H_2S treatments. Combination of TRIA and H_2S reported rise in the vigor index under salinity. Vigor index decrease reduced from 648.46% to 1439.46% at TRIA+H_2S+NaCl I treated seedlings, as compared to treated seedlings at NaCl I concentration.

Table 6.2 Effect of TRIA and H_2S on germination percentage, vigor index, and relative water content of *B. juncea* under salt stress

Treatment	**Germination percentage (%)**	**Vigor index (%)**	**Relative water content (%)**
Control	81.51^{def}±0.41	1247.4^{c}±66.70	84.80^{bcd}± 1.08
NaCl I	67.05^{a}±0.39	648.46^{b}±55.59	81.27^{bc}±0.42
NaCl II	58.16^{a}±1.29	526.93^{ab}±42.22	77.58^{ab}±1.62
NaCl III	55.93^{b}±1.03	347.56^{a}±2.95	71.25^{a}±0.56
TRIA	89.76^{gh}±0.67	1961.93^{f}±37.02	89.13^{gh}±0.27
TRIA + NaCl I	72.14^{cd}±1.71	1354.56^{cd}±105.03	80.83^{cdef}±6.23
TRIA + NaCl II	75.76^{cd}±2.23	1231.2^{cd}±7.90	76.13^{cd}±3.37
TRIA + NaCl III	78.45^{cde}±1.05	1296.03b^{c}±25.14	79.39^{bcd}±0.51
H_2S	84.63^{efg}±0.37	1796.96^{fg}±58.52	88.61^{fgh}±0.80
H_2S + NaCl I	78.57^{cde}±0.32	1286.2^{cd}±31.33	85.35^{defg}±0.59
H_2S + NaCl II	72.61^{bc}±1.00	1228.5^{cd}±25.95	82.55^{cde}±1.19
H_2S + NaCl III	79.15^{cde}±1.59	1178.8^{bc}±39.26	77.73^{bc}±0.74
TRIA + H_2S	91.62^{h}±0.80	2274.13^{h}±34.74	92.50^{h}±0.17
TRIA + H_2S + NaCl I	83.4^{efg}±1.21	1439.46^{de}±118.09	90.87^{efgh}±0.17
TRIA + H_2S + NaCl II	86.73^{fgh}±0.80	1666.36^{ef}± 65.04	84.28^{cdef}±1.21
TRIA + H_2S + NaCl III	88.28^{fgh}±3.25	1303.16^{cd}±48.10	86.14^{defg}±0.75

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

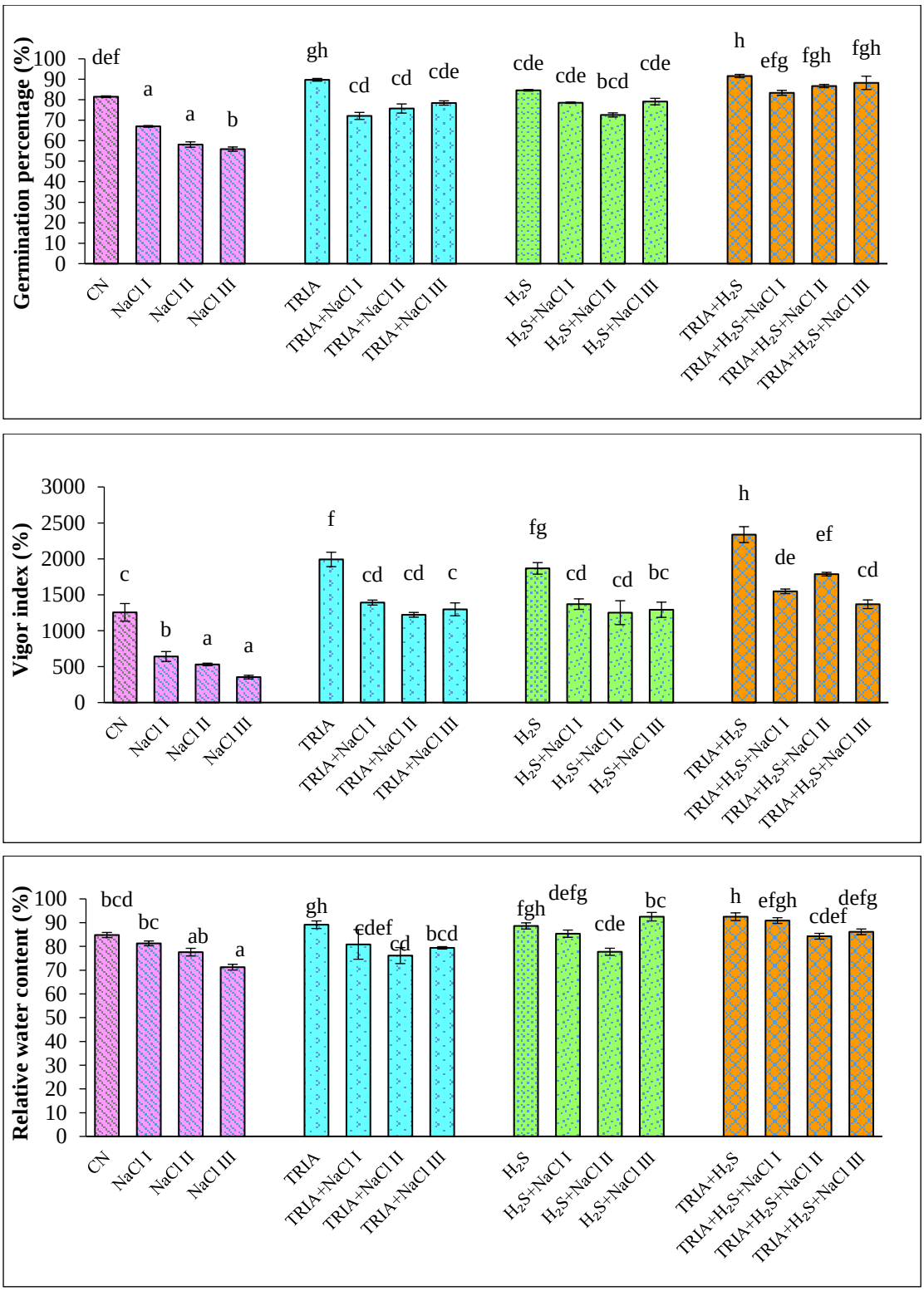


Fig. 6.3 Effect of TRIA and H_2S on germination percentage, vigor index and relative water content in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

RWC was reduced under salinity in case of *Brassica* seedlings (Fig. 6.3; Table 6.3). Decrease with increase in the salt level from 81.27% at NaCl I concentration to 77.58% at NaCl III concentration. Control seedlings exhibited 84.80% relative water content. Individual application of TRIA showed relative water content of 89.13% which is higher in comparison to control seedlings. TRIA application under salt stressed conditions reported relative water content of 80.83% at NaCl II concentration. According to the data, it was found that H_2S treated seedlings showed maximum relative water content of 88.61% at NaCl I concentration. Synergistic association of TRIA and H_2S showed higher relative water content of 90.87% at NaCl I concentration in comparison to their individual treatment which reported highest relative water content.

6.1.1.2 Photosynthetic pigments

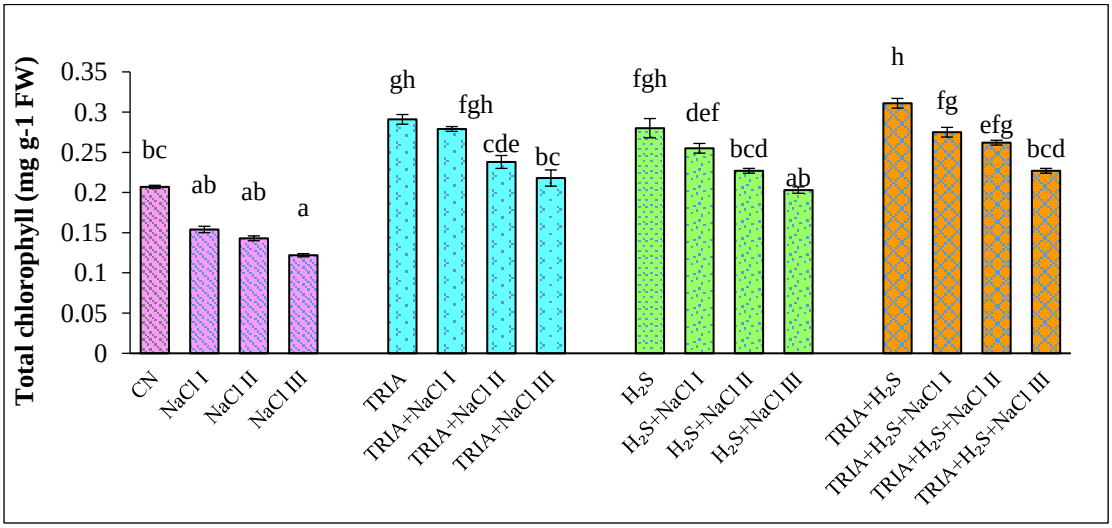
Photosynthetic pigments like total chlorophyll, chl a and chl b was affected by salt stress in *Brassica* seedlings (Fig. 6.4; Table 6.3). Minimum total chlorophyll content was reported in NaCl III stressed seedlings with content of 0.12 mg g^{-1} FW content. Application of TRIA and H_2S individually enhanced the level of chlorophyll content under unstressed conditions in comparison to salt stressed seedlings, among which highest total chlorophyll contents i.e., 0.279 and 0.255 mg g^{-1} FW at NaCl I. Comparison to their individual application, the synergistic association between TRIA and H_2S played beneficial role in improving total chlorophyll content under salt stress. Highest total chlorophyll value of 0.275 mg g^{-1} FW was observed in TRIA+H_2S+ NaCl I treated seedlings.

Likewise, In case of chl a and chl b in *Brassica* seedlings. Lowest content of chl a (0.05 mg g^{-1} FW) and chl b (0.037 mg g^{-1} FW) was found at NaCl III concentration. Furthermore, application of TRIA and H_2S significantly mitigated stress in seedlings of *Brassica* by increasing content of chl a and chl b. TRIA and H_2S association under stressed condition enhanced chl a and chl b level with the highest content of 0.187 and 0.140 mg g^{-1} FW contents at NaCl I concentration.

Table 6.3 Effect of TRIA and H_2S on photosynthetic pigments of *B. juncea* seedlings under salt stress

Treatment	Total chlorophyll (mg g^{-1} FW)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)
Control	0.207^{bc}±0.002	0.12^{bcd}±0.006	0.092^{cd}±0.003
NaCl I	0.154^{ab}±0.004	0.09^{b}±0.003	0.081^{bc}±0.003
NaCl II	0.143^{a}±0.003	0.07^{ab}±0.003	0.066^{b}±0.004
NaCl III	0.122^{a}±0.002	0.05^{a}±0.006	0.037^{a}±0.002
TRIA	0.291^{gh}±0.006	0.19^{ghi}±0.006	0142^{g}±0.001
TRIA + NaCl I	0.279^{fgh}±0.003	0.16^{efg}±0.007	0.113^{def}±0.004
TRIA + NaCl II	0.238^{cde}±0.008	0.13^{cde}±0.001	0.104^{de}±0.003
TRIA + NaCl III	0.218^{bc}±0.010	0.10^{bc}±0.009	0.093^{cd}±0.010
H_2S	0.280^{fgh}±0.012	0.20^{hi}±0.006	0.141^{g}±0.001
H_2S + NaCl I	0.255^{def}±0.006	0.185^{ghi}±0.004	0.121^{efg}±0.005
H_2S + NaCl II	0.227^{bcd}±0.003	0.134^{cdef}±0.005	0.105^{de}±0.002
H_2S + NaCl III	0.203^{bc}±0.004	0.109^{bc}±0.010	0.098^{cd}±0.005
TRIA + H_2S	0.311^{h} ±0.006	0.213^{i}±0.009	0.183^{h}±0.004
TRIA + H_2S + NaCl I	0.275^{fg} ±0.006	0.187^{ghi}±0.006	0.140^{g}±0.004
TRIA + H_2S + NaCl II	0.262^{efg}±0.003	0.167^{fgh}±0.004	0.133^{fg}±0.004
TRIA + H_2S + NaCl III	0.227^{bcd} ±0.003	0.144^{def}±0.005	0.111^{def}±0.003

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments



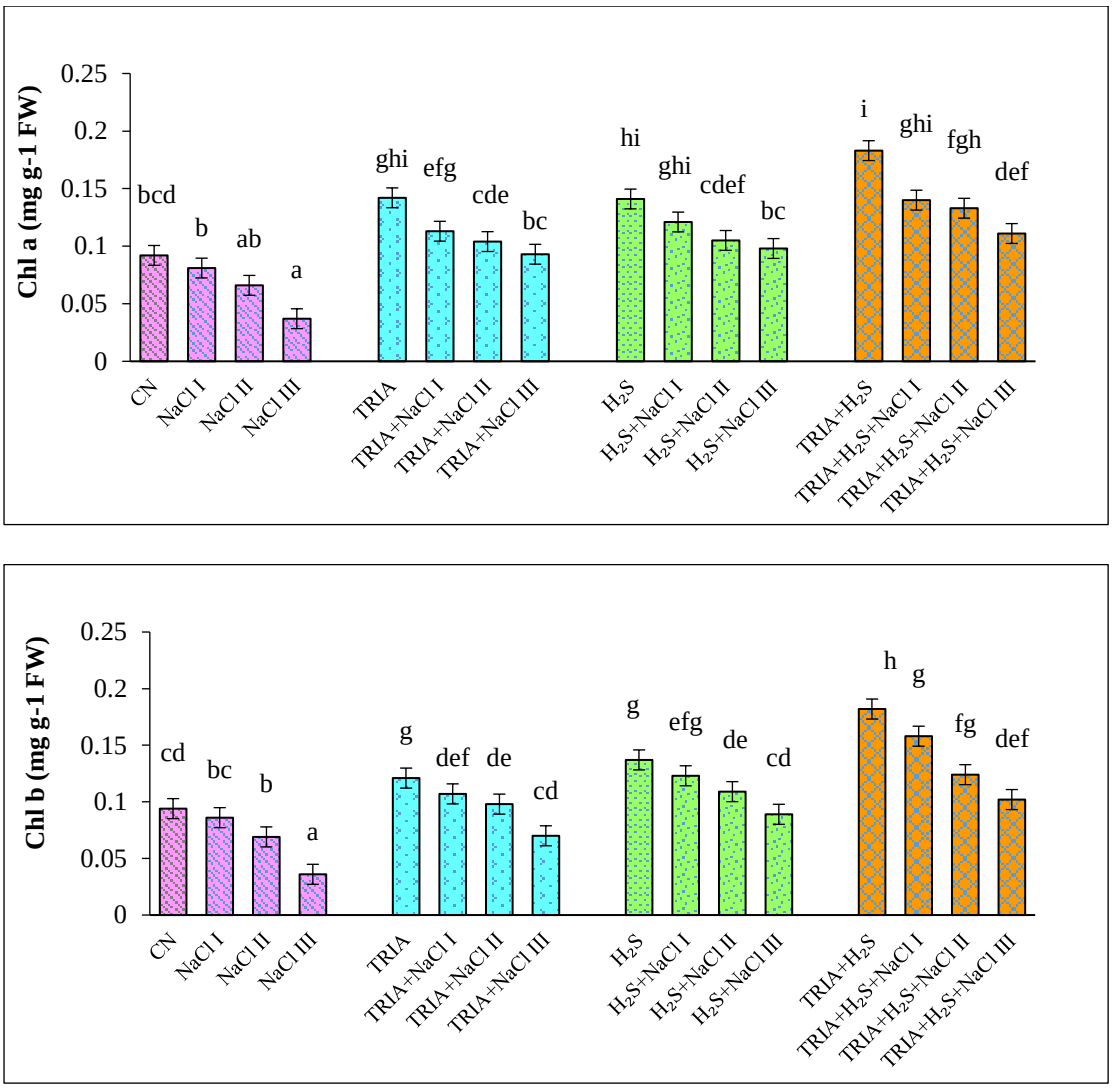


Fig. 6.4 Effect of TRIA and H_2S on total chlorophyll, chl a and chl b in 7-days ld seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Content of carotenoid was found to be reduced with 0.122 mg g^{-1} FW at NaCl III to NaCl I treated seedlings with 0.154 mg g^{-1} FW (Fig. 6.5; Table 6.4). Control seedlings exhibited content of carotenoid of 0.207 mg g^{-1} FW. Supplementation of H_2S against salt stress showed increase in carotenoid content at NaCl II concentration to 0.277 mg g^{-1} FW. Synergistic association of TRIA and H_2S further enhanced content of carotenoid by mitigating salt-induced toxic effects. TRIA+H_2S+NaCl I concentration showed highest carotenoid content of 0.275 mg g^{-1} FW while the minimum 0.227 mg g^{-1} FW was noticed at NaCl III concentration.

Table 6.4 Effect of TRIA and H_2S on photosynthetic pigments of *B. juncea* seedlings under salt stress

Treatment	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
Control	0.207^{bcde}±0.002	3.27^{cde}±0.14
NaCl I	0.154^{abc}±0.004	2.28^{ab}±0.09
NaCl II	0.143^{ab}±0.003	1.72^{ab}±0.07
NaCl III	0.122^{a}±0.002	1.06^{a}±0.02
TRIA	0.291^{def}±0.006	4.24^{fg}±0.07
TRIA + NaCl I	0.279^{bcde}±0.003	3.13^{cde}±0.07
TRIA + NaCl II	0.238^{abcd}±0.008	2.40^{bcd}±0.03
TRIA + NaCl III	0.218^{bcde}±0.01	2.21^{abc}±0.06
H_2S	0.28^{ef}±0.012	4.56^{fg}±0.33
H_2S + NaCl I	0.255^{abcd}±0.006	3.45^{def}±0.03
H_2S + NaCl II	0.277^{abcd}±0.003	3.13^{cde}±0.03
H_2S + NaCl III	0.203^{ab}±0.004	2.75^{bcd}±0.04
TRIA + H_2S	0.311^{f}±0.006	5.21^{g}±0.24
TRIA + H_2S + NaCl I	0.275^{def}±0.006	4.15^{efg}±0.60
TRIA + H_2S + NaCl II	0.262^{cde}±0.003	3.45^{def}±0.50
TRIA + H_2S + NaCl III	0.227^{bcde}±0.003	3.21^{cde}± 0.13

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Salt stress reduced the content of xanthophyll in the seedlings of *B. juncea* (Fig. 6.5; Table 6.4). Reduction in xanthophyll content was observed at NaCl III treated seedlings i.e., 1.06 mg g^{-1} FW. Control reported 3.27 mg g^{-1} FW of xanthophyll content which was higher in comparison to salt stressed seedlings. TRIA and H_2S exhibited elevation in the xanthophyll content. Highest and lowest xanthophyll contents were observed at NaCl I and III, in both TRIA and H_2S applied seedlings i.e., 3.13 mg g^{-1} FW and 2.21 mg g^{-1} FW and 3.45 mg g^{-1} FW and 2.75 mg g^{-1} FW, respectively. Association of TRIA and H_2S boosted the content of xanthophyll in stressed conditions. TRIA +H_2S+NaCl I concentration reported higher xanthophyll content of 4.15 mg g^{-1} FW in comparison to NaCl II and NaCl III concentration.

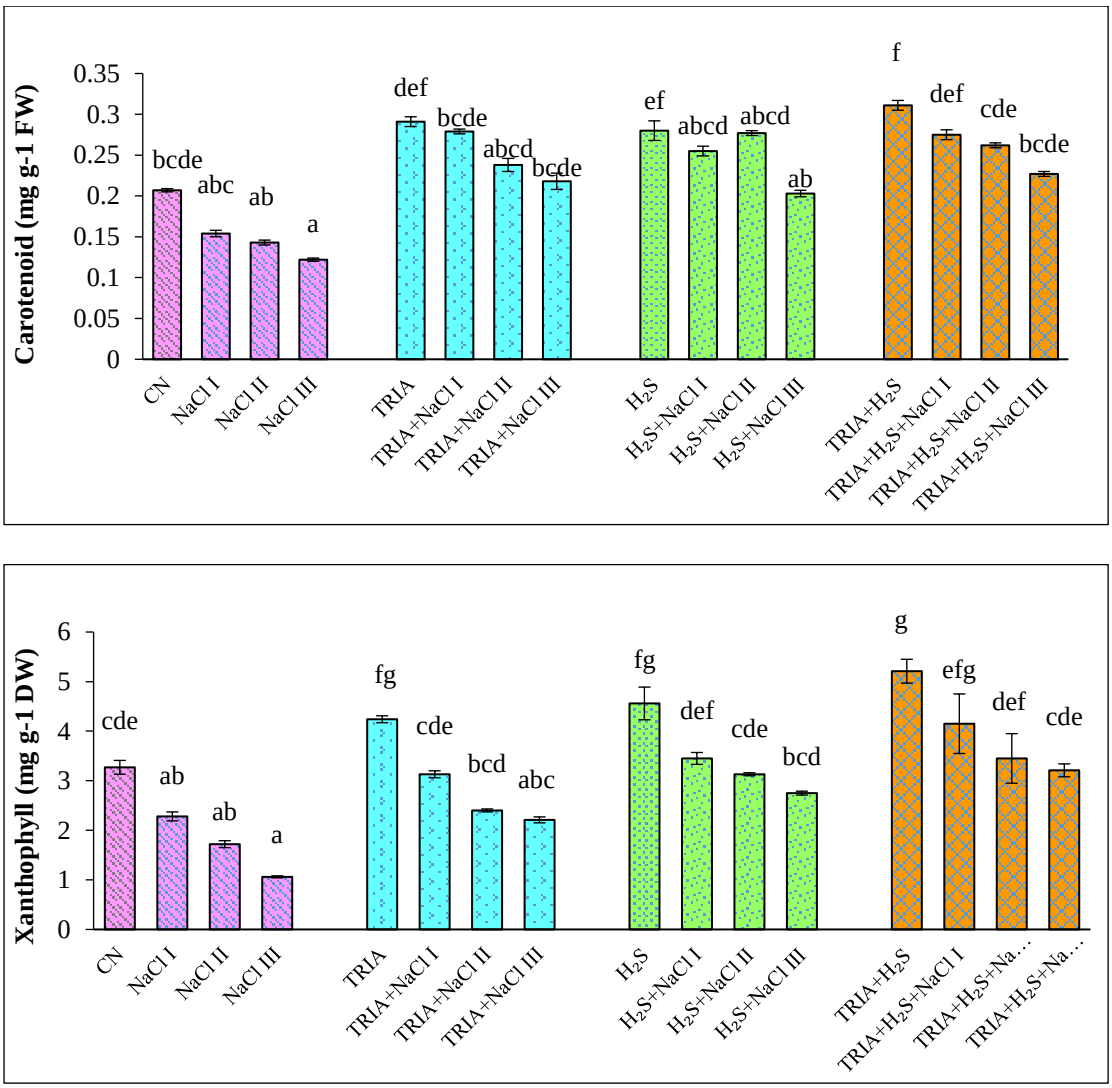


Fig. 6.5 Effect of TRIA and H_2S on carotenoid and xanthophyll content in 7-days old seedlings of *B. juncea* seedlings under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.1.3 Metabolites

Anthocyanin content drastically decreased at different concentrations of NaCl. It was found that content of anthocyanin decrease at NaCl III concentration with 0.85 mg g^{-1} FW (Fig 6.6; Table 6.5). Among which TRIA and H_2S control seedlings when used individually, H_2S treated seedlings showed higher anthocyanin content (2.67 mg g^{-1} FW) than TRIA applied seedlings. TRIA supplementation against salinity resulted in improved anthocyanin content in comparison to NaCl treated seedlings under salt

stress, in contrast to NaCl I concentration. However, in case of H_2S applied seedlings, anthocyanin content was found to increase from 2.14 mg g^{-1} FW to 1.47 mg g^{-1} FW at NaCl II concentration. TRIA application under NaCl was found to be more in increasing anthocyanin content in comparison to H_2S treatment. Combination of TRIA and H_2S under salt stress enhanced the anthocyanin content with 1.84, 1.22 and 1.07 mg g^{-1} FW with the anthocyanin content at NaCl I, II and III treated seedlings, respectively.

Table 6.5 Effect of TRIA and H_2S on metabolites of *B. juncea* seedlings under salt stress

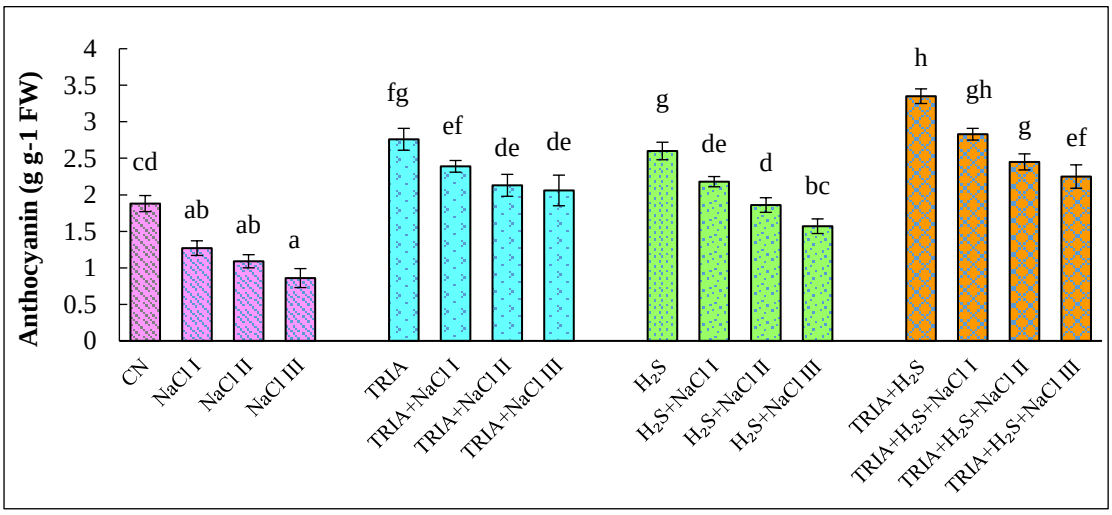
Treatment	**Anthocyanin content (mg g^{-1} FW)**	**Flavonoid content (mg g^{-1} FW)**	**Phenolic content (mg g^{-1} FW)**
Control	1.84^{cd}±0.03	1.36^{de}±0.16	2.13^{fg}±0.02
NaCl I	1.22^{ab}±0.09	0.93^{bc}±0.05	0.93^{cd}±0.05
NaCl II	1.07^{a}±0.02	0.63^{ab}±0.05	0.63^{ab}±0.05
NaCl III	0.85^{a}±0.06	0.45^{a}±0.05	0.45^{a}±0.05
TRIA	2.67^{fg}±0.16	2.43^{hi}±0.05	2.6^{i}±0.08
TRIA + NaCl I	2.31^{ef}±0.06	1.61^{fg}±0.05	1.61^{ef}±0.05
TRIA + NaCl II	2.13^{de}±0.03	1.43^{def}±0.08	1.43^{def}±0.08
TRIA + NaCl III	1.99^{de}±0.06	1.18^{cd}±0.03	1.18^{cd}±0.03
H_2S	2.72^{g}±0.04	2.73^{i}±0.07	2.73^{i}±0.07
H_2S + NaCl I	2.16^{de}±0.02	2.13^{gh}±0.03	2.13^{gh}±0.03
H_2S + NaCl II	1.84^{d}±0.05	1.50^{def}±0.04	1.56^{def}±0.03
H_2S + NaCl III	1.47^{bc}±0.02	1.35^{de}±0.02	1.23^{cde}±0.03
TRIA + H_2S	3.57^{h}±0.14	3.25^{j}±0.15	3.25^{k}±0.15
TRIA + H_2S + NaCl I	2.96^{gh}±0.02	2.31^{h}±0.05	2.37^{hi}±0.14
TRIA + H_2S + NaCl II	2.73^{g}±0.05	1.73^{fg}±0.06	1.73^{fg}±0.06
TRIA + H_2S + NaCl III	2.31^{ef}±0.06	1.69^{ef}±0.05	1.46^{def}±0.07

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Flavonoid content reduced with rise of NaCl concentration (Fig 6.6; Table 6.5). Lowest flavonoid content of 0.45 mg g^{-1} FW was observed in NaCl III treated

seedlings. Flavonoid content was found to decrease in comparison to control seedlings (1.36 mg g^{-1} FW). Application of Triacontanol and Hydrogen sulphide increased the content of flavonoid in seedlings of *B. juncea* than all other treatments. Decrease in flavonoid content was found as the content of NaCl content increased in TRIA treated plants. TRIA application under unstressed conditions enhanced the flavonoid content from 1.18 mg g^{-1} FW at NaCl III concentration to 2.43 mg g^{-1} FW. In case of H_2S under stressed conditions, maximum flavonoid content (2.73 mg g^{-1} FW) was noticed at NaCl I concentration and minimum flavonoid content was noticed at NaCl III concentration (1.35 mg g^{-1} FW). Moreover, response of TRIA and H_2S reported highest (2.31 mg g^{-1} FW) and lowest flavonoid content (1.69 mg g^{-1} FW) at NaCl I and III concentration, respectively.

Phenolic content reduced with NaCl level (Fig. 6.6; Table 6.5). It was found that phenolic content showed decline of 2.13 mg g^{-1} FW phenolic content under NaCl III stressed condition (2.13 mg g^{-1} FW). Exogenous application of TRIA exhibited maximum phenolic content of 2.6 mg g^{-1} FW in NaCl I stressed seedlings. Individual application of TRIA under unstressed conditions reported maximum phenolic content of 2.60 mg g^{-1} FW. Whereas, H_2S application under stress reported maximum phenolic content of 2.73 mg g^{-1} FW which was found to decrease with increase in the concentration of salt. Synergistic association of TRIA and H_2S under salt stressed conditions showed phenolic content of 2.37 mg g^{-1} FW at NaCl I concentration. TRIA+ H_2S control seedlings showed highest phenolic content of 3.25 mg g^{-1} FW in comparison to all other 15 treatments used, respectively.



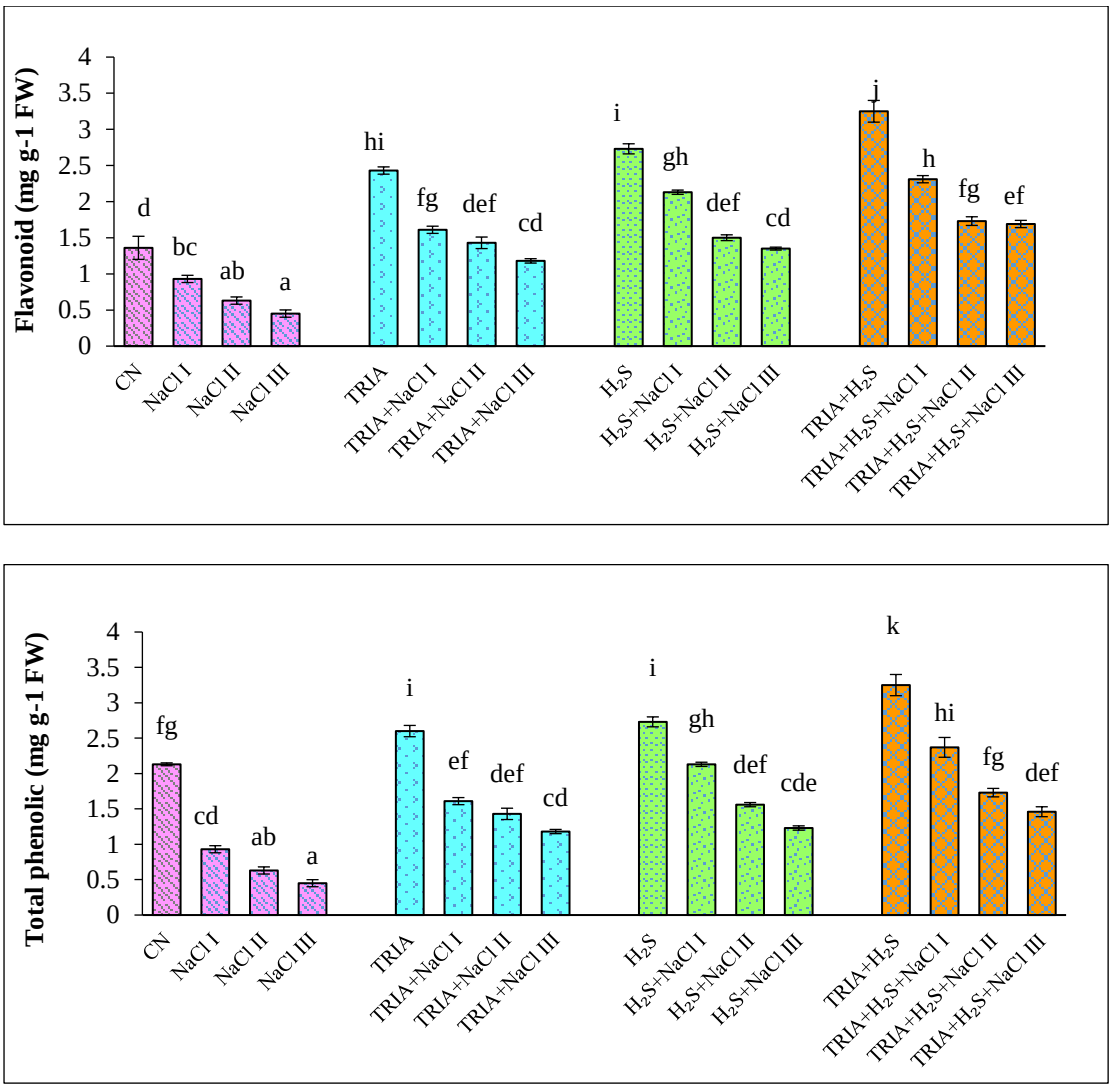


Fig. 6.6 Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.1.4. Oxidative stress markers

Level of lipid peroxidation was found to increase through salt stress rise in MDA content (Fig. 6.7; Table 6.6). Rise of 56% in MDA content was noticed in NaCl III treated seedlings in comparison to control seedlings. Whereas, TRIA and H_2S alone under salt stress reduced the MDA content. In case of TRIA and H_2S alone maximum reduction in MDA level was reported under salt stress was reported at NaCl I and II with 5.02 and 4.78. Furthermore, MDA level was found to decrease from 3.17 to 2.69 µmol g^{-1} FW in TRIA+ H_2S + NaCl II seedlings in comparison to NaCl II seedlings.

H_2O_2 content was found to increase in seedlings of *Brassica* under salt stress (Fig. 6.7;

Table 6.6) A rise of almost two-fold in the content of H_2O_2 was found in NaCl I treated seedlings (4.5028 µmol g^{-1} FW) in comparison to control seedlings (2.28 µmol g^{-1} FW). Likewise, level of H_2O_2 was increased to 4.50, 4.71and 5.19 µmol g^{-1} FW in NaCl I, II and III treated seedlings. Maximum decrease in H_2O_2 content was found at combination of TRIA+H_2S under stressed conditions. Whereas, individual application of TRIA (2.72 µmol g^{-1} FW) and H_2S (3.13 µmol g^{-1} FW) against salt stressed condition reported decrease in H_2O_2 content at NaCl I concentration. Content of H_2O_2 was found to reduce at combined application of TRIA +H_2S (2.14 µmol g^{-1} FW) under NaCl I concentration.

Table 6.6 Effect of TRIA and H_2S on oxidative stress markers of *B. juncea* seedlings under salt stress

Treatment	**MDA (µmol g^{-1} FW)**	**H_2O_2 (µmol g^{-1} FW)**
Control	3.01^{de}±0.01	2.28^{abcd}±0.08
NaCl I	5.02^{k}±0.02	4.50^{g}±0.22
NaCl II	4.78^{ij}±0.09	4.71^{gh}±0.13
NaCl III	4.13^{j}±0.09	5.19^{h}±0.07
TRIA	2.33^{ab}±0.18	2.07^{abc}±0.04
TRIA + NaCl I	3.33^{efg}±0.06	2.72^{de}±0.13
TRIA + NaCl II	3.61^{ghi}±0.08	3.13^{ef}±0.03
TRIA + NaCl III	3.96^{hi}±0.04	2.62^{bcde}±0.15
H_2S	2.44^{abc}±0.08	2.06^{ab}±0.09
H_2S + NaCl I	3.28^{defg}±0.05	3.13^{ef}±0.02
H_2S + NaCl II	2.85^{bcde}±0.06	2.50^{bcde}±0.12
H_2S + NaCl III	3.73^{ghi}±0.05	3.41^{e}±0.17
TRIA + H_2S	2.10^{a}±0.06	1.65^{a}±0.07
TRIA + H_2S + NaCl I	2.69^{bc}±0.18	2.14^{abc}±0.16
TRIA + H_2S + NaCl II	2.76^{bcd}±0.12	2.32^{abcd}±0.07
TRIA + H_2S + NaCl III	3.17^{def}±0.05	2.69^{cde}±0.03

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

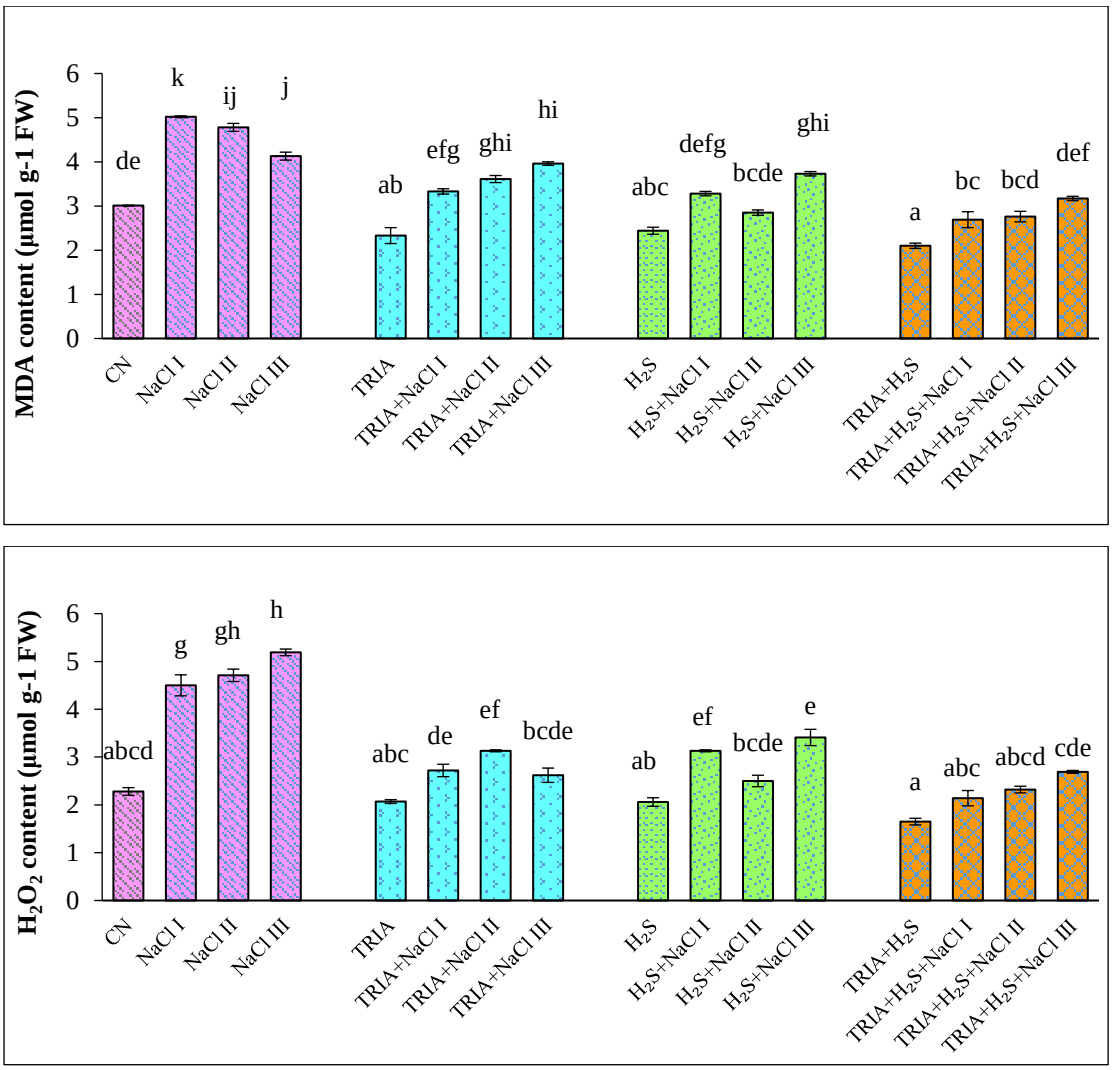
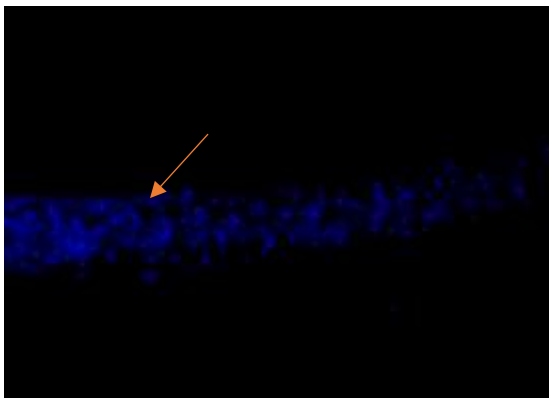


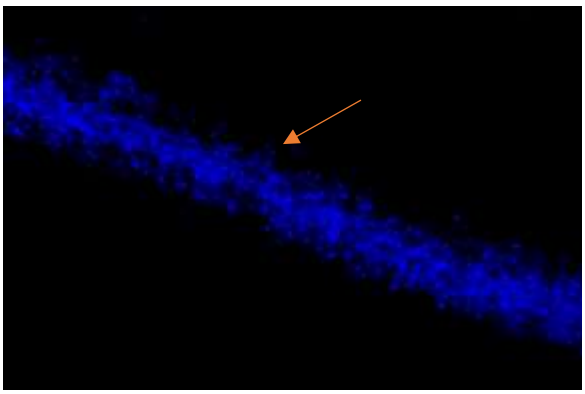
Fig. 6.6 Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.1.5 Histochemical studies

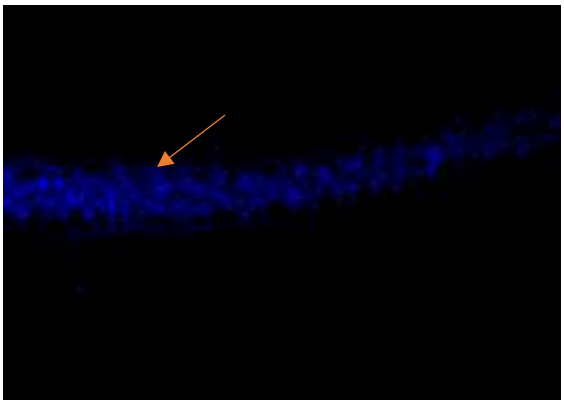
Histochemical studies were performed by using confocal microscope on 7 day old seedlings of *B. juncea* (Fig. 6.8) According to studies it was found that, membrane damage was found to increase with increase in salt stress. Salt stressed seedlings exhibit more obvious root cell membrane damage in comparison to control seedlings, as shown by vivid blue fluorescence. TRIA and H_2S applied seedlings when used alone or in combination under salt stress resulted in lesser membrane damage, as seen by less intense blue color.



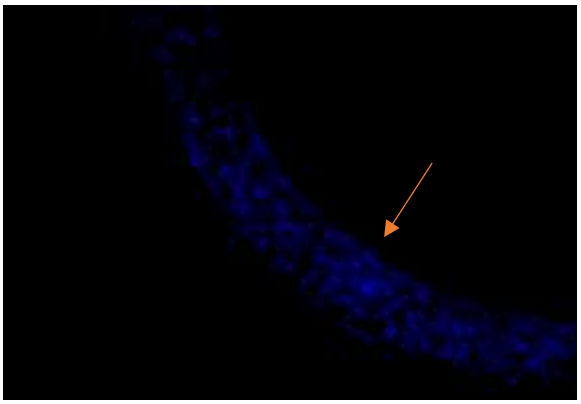
CN



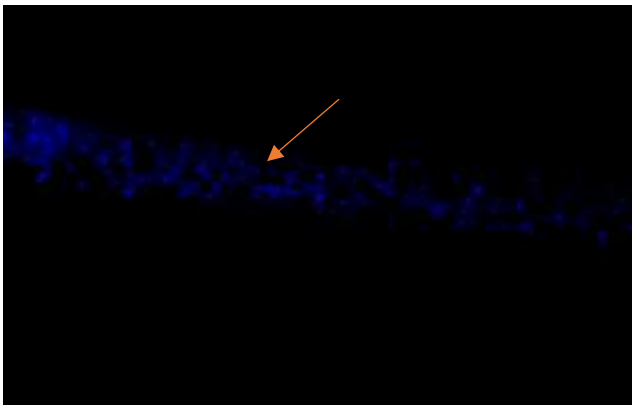
NaCl III



TRIA+NaCl III

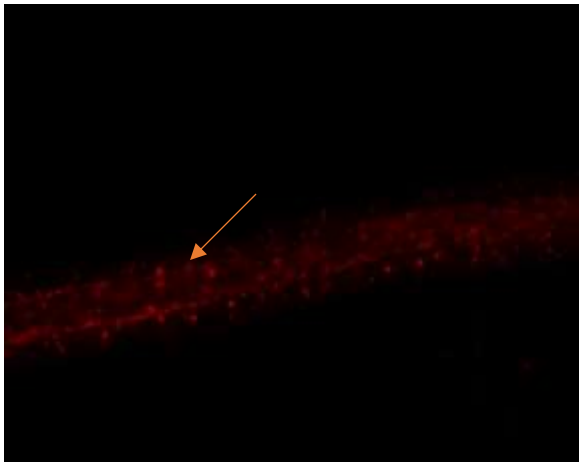


H_2S+NaCl III

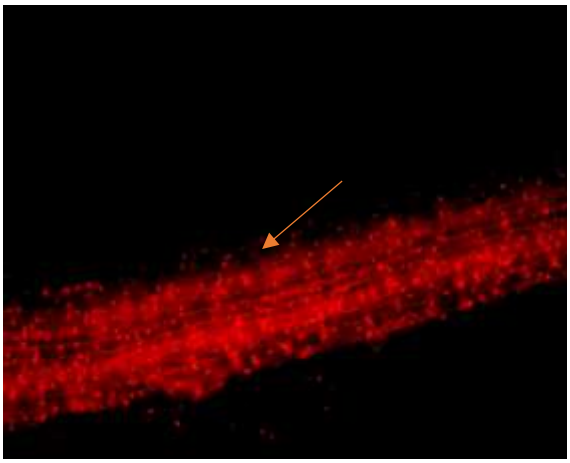


TRIA+H_2S+NaCl III

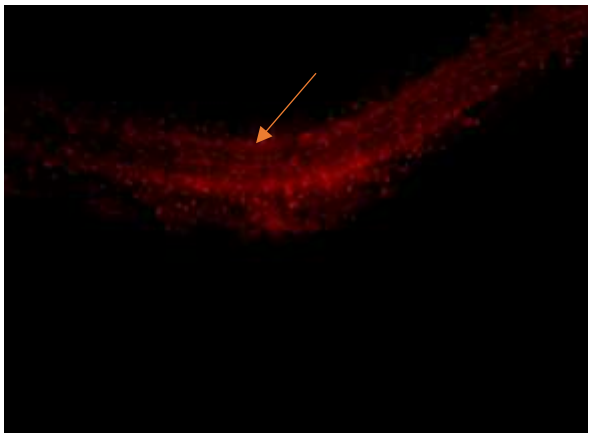
Fig. 6.8 Effect of TRIA and H_2S on membrane damage in *B. juncea* seedlings under salt stress by confocal microscope.



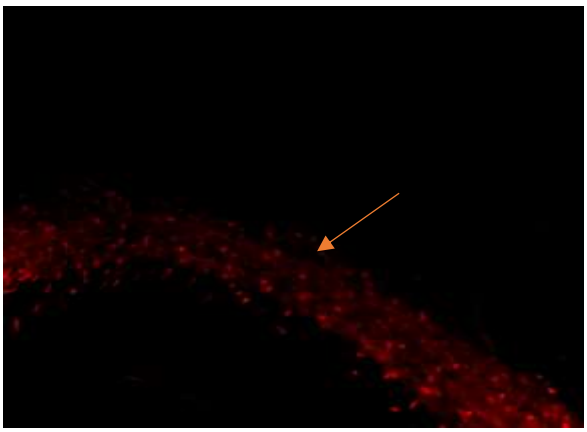
CN



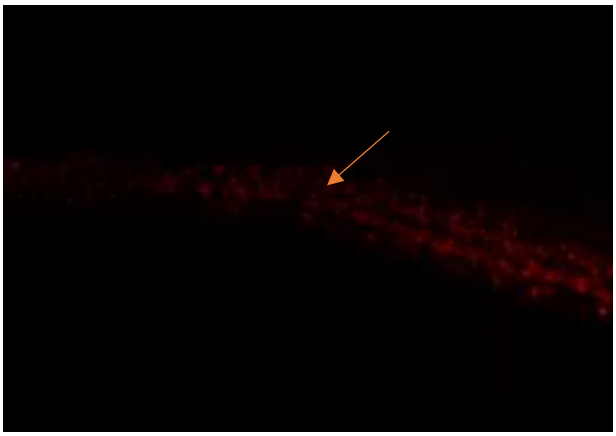
NaCl III



TRIA+NaCl III



H_2S+NaCl III



TRIA+H_2S+NaCl III

Fig. 6.9 Effect of TRIA and H_2S on nuclear damage in *B. juncea* seedlings under salt stress by using confocal microscope.

Salt stressed seedlings more strained with propidium iodide dye forms fluorescent complex and intercalates with nucleic acids (Fig. 6.9). It was found that this PI dye stains the nuclei by passing the nuclear damage to the dead and damaged cells as indicated by high red color intensity. NaCl III stressed seedlings resulted in higher nuclear damage. In contrast, seedlings treated with TRIA and H_2S when used alone or in combination under salt stress displayed lesser nuclear damage as evidenced by decrease in the intensity of red color.

6.1.1.6 Osmolytes

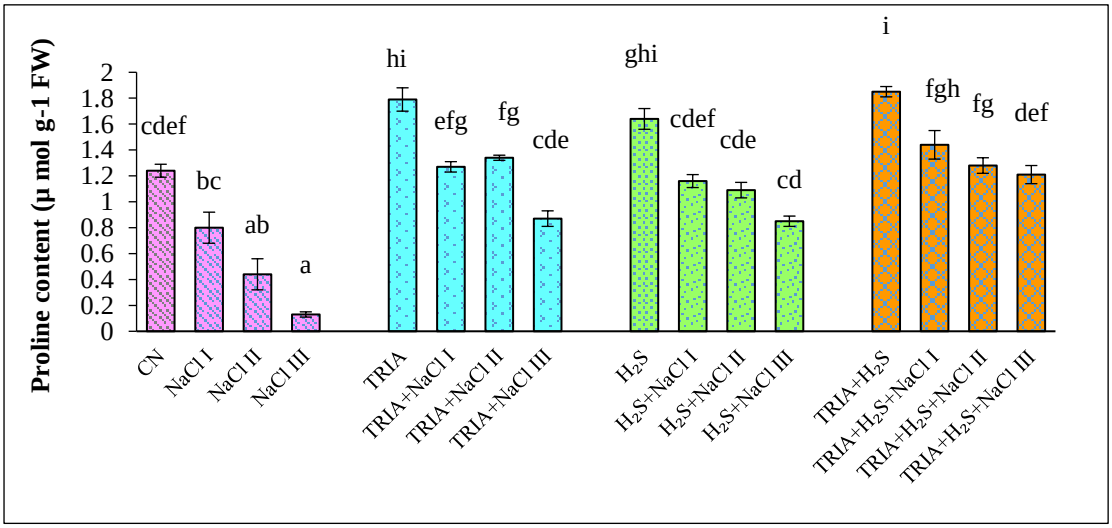
Proline content reduced under stress in seedlings of *B. juncea* (Fig.6.10; Table 6.7). Minimum proline content was reported at NaCl III concentration (0.13 mg g^{-1} FW), TRIA treated seedlings enhanced the proline content under stressed conditions. TRIA+NaCl I treated seedlings reported decrease in proline content from 1.79 mg g^{-1} FW to 1.27 mg g^{-1} FW in comparison to its individual application. Likewise, H_2S treatment reduced proline content to 1.45 mg g^{-1} FW at NaCl III concentration. Maximum proline content was reported at association of TRIA and H_2S under unstressed situations (1.85 mg g^{-1} FW). Under salt stressed conditions, highest proline content was reported in TRIA+H_2S (1.44 mg g^{-1} FW) at NaCl I concentration.

Glycine-betaine content was found to decrease significantly under salt stressed seedlings (Fig. 6.10; Table 6.7). Conc of NaCl I, II and III, glycine betaine level found to decrease were 1.30, 1.24 and 0.80 µ mol g^{-1} FW, respectively. TRIA treatment raised the content glycine betaine when used individually (4.32 µ mol g^{-1} FW) in comparison to control seedlings (3.15 µ mol g^{-1} FW). Glycine betaine content were 2.77, 2.51 and 1.78 µ mol g^{-1} FW at NaCl I, II and III concentration, when applied with TRIA. Likewise, H_2S foliar spray showed reduction in glycine-betaine levels of 2.58, 1.67 and 1.45 µ mol g^{-1} FW at NaCl I, II and III concentrations, respectively. Combined application of TRIA and H_2S under unstressed conditions showed highest glycine betaine content (4.72 µ mol g^{-1} FW) among all the 16 types of treatment used. Content of glycine-betaine was found to decrease as the concentration of NaCl elevated in case of combined TRIA and H_2S with maximum content at NaCl I (3.76 µ mol g^{-1} FW).

Table 6.7 Effect of TRIA and H_2S on osmolytes of *B. juncea* seedlings under salt stress

Treatment	Proline (µ mol g^{-1} FW)	Glycine betaine (µ mol g^{-1} FW)
Control	1.24^{cdef}±0.05	3.15^{de}±0.03
NaCl I	0.80^{bc}±0.12	1.30^{ab}±0.05
NaCl II	0.44^{ab}±0.12	1.24^{ab}±0.10
NaCl III	0.13^{a}±0.02	0.83^{a}±0.05
TRIA	1.79^{hi}±0.09	4.32^{gh}±0.13
TRIA + NaCl I	1.27^{efg}±0.04	2.77^{cd}±0.05
TRIA + NaCl II	1.34^{fg}±0.02	2.51^{c}±0.14
TRIA + NaCl III	0.87^{cde}±0.06	1.78^{b}±0.05
H_2S	1.64^{ghi}±0.08	3.74^{fg}±0.07
H_2S + NaCl I	1.16^{cdef}±0.05	2.58^{cd}±0.13
H_2S + NaCl II	1.09^{cde}±0.06	1.67^{b}±0.04
H_2S + NaCl III	0.85^{cd}±0.04	1.45^{b}±0.11
TRIA + H_2S	1.85^{i}±0.04	4.72^{h}±0.20
TRIA + H_2S + NaCl I	1.44^{fgh}±0.11	3.76^{fg}±0.17
TRIA + H_2S + NaCl II	1.28^{fg}±0.06	3.09^{ef}±0.10
TRIA + H_2S + NaCl III	1.21^{def}±0.07	2.69^{cd}±0.03

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments



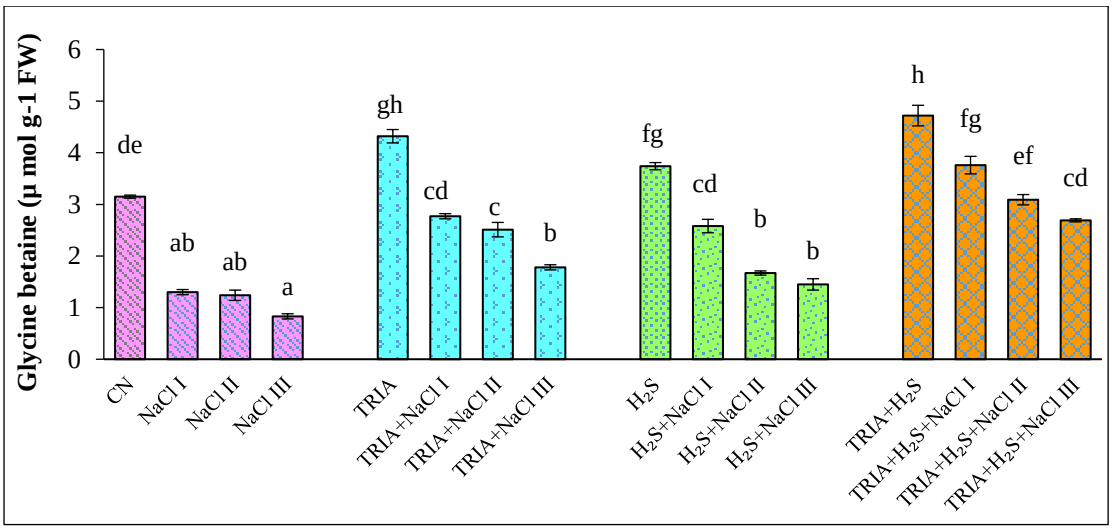


Fig. 6.10 Effect of TRIA and H_2S on proline and glycine betaine content in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.1.7 Total carbohydrates content

Total carbohydrates content was found to decrease when exposed to salt stress in *Brassica* seedlings (Fig. 6.11; Table 6.8). Minimum carbohydrate content was found to be 1.12 mg g^{-1} FW at NaCl III. Individual application of TRIA under unstressed condition showed carbohydrate content of 6.27 mg g^{-1} FW. Whereas, in case of stressed condition application of TRIA reduced carbohydrate content from 4.98 mg g^{-1} FW (TRIA+NaCl I) to 2.34 mg g^{-1} FW (TRIA+NaCl II). Co-application of TRIA+H_2S reported higher carbohydrate content of 7.99 mg g^{-1} FW in comparison to all other 16 treatments. H_2S pre-treated seedlings reported reduction in carbohydrate content under NaCl III concentration (2.84 mg g^{-1} FW). Treatment of triacontanol and hydrogen sulphide under salt stressed condition improved carbohydrates content with the highest content of 5.17 mg g^{-1} FW at NaCl III concentration.

Table 6.8 Effect of TRIA and H_2S on total carbohydrates of *B. juncea* seedlings under salt stress

Treatment	Total carbohydrates (mg g^{-1} FW)
Control	3.31^{bcde}±0.17
NaCl I	1.87^{abc}±0.06
NaCl II	1.66^{ab}±0.05
NaCl III	1.12^{c}±0.09
TRIA	6.27^{fg}±0.62
TRIA + NaCl I	4.98^{def}±0.06
TRIA + NaCl II	3.81^{cde}±0.55
TRIA + NaCl III	2.34^{abc}±0.32
H_2S	6.19^{fg}±0.46
H_2S + NaCl I	5.05^{def}±0.57
H_2S + NaCl II	3.02^{abcd}±0.22
H_2S + NaCl III	2.84^{abc}±0.16
TRIA + H_2S	7.99^{g}±0.56
TRIA + H_2S + NaCl I	6.11^{efg}±0.55
TRIA + H_2S + NaCl II	6.82^{fg}±0.17
TRIA + H_2S + NaCl III	5.17^{ef}±0.50

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

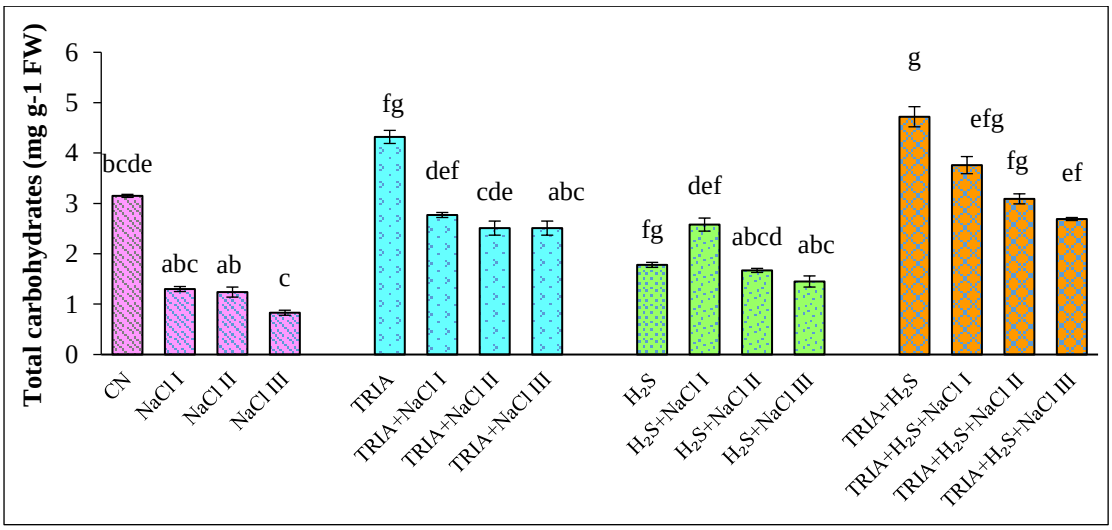


Fig. 6.11 Effect of TRIA and H_2S on total carbohydrates in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

6.1.1.8 Protein content and Antioxidative Enzymes

Reduction in protein content in the seedlings of B. *juncea* (Fig 6.12; Table 6.9) In NaCl I treated seedlings content was found to be 0.35 mg g^{-1} FW. Minimum protein content of 0.27 mg g^{-1} FW was noticed at NaCl III concentration. TRIA treatment under stressed and unstressed conditions enhanced the protein content. Individual application of TRIA enhanced 1.92 mg g^{-1} FW protein content. However, under salt stress, maximum protein content of 1.60 mg g^{-1} FW was reported at NaCl III concentration. Similarly, seedlings pre-treated with H_2S increased the protein content under stress and unstressed conditions. H_2S control seedlings reported 2.03 mg g^{-1} FW of protein content. In case of salt stress, seedlings pre-treated with H_2S reported highest (1.31 mg g^{-1} FW) and lowest protein content (1.13 mg g^{-1} FW) at salt stressed condition. Application of TRIA and H_2S reported highest protein content of 2.61 mg g^{-1} FW among all other treatments. Treatment of seedlings with TRIA+H_2S+NaCl I exhibited highest protein content of 1.77 mg g^{-1} FW.

SOD enzymatic activity was found to be considerably reduced when exposed to salt stress (Fig. 6.12; Table 6.9). In NaCl III stressed seedlings, minimum SOD activity was found to be 2.38 UA mg^{-1} protein. Maximum SOD activity was found to be 5.39 UA mg^{-1} protein at NaCl I concentration in case of TRIA applied seedlings under salt stress. H_2S foliar spray improved the enzymatic activity of SOD enzyme under salt stress, in which highest (4.31 UA mg^{-1} protein) and lowest SOD activity (3.42 UA mg^{-1} protein) was found at salt stress. TRIA+H_2S application enhanced the activity of SOD enzyme under stressed condition with maximum content of 6.55 UA mg^{-1} protein at NaCl I concentration.

Catalase activity significantly reduced under stress (Fig. 6.12; Table 6.9). Minimum catalase enzymatic activity was found to be 1.51 UA mg^{-1} protein at NaCl III concentration. Maximum CAT activity was observed at combination of TRIA+H_2S (4.77 UA mg^{-1} protein). TRIA treatment under salinity showed minimum CAT activity of 2.39 UA mg^{-1} protein at NaCl III concentration. Supplementation of H_2S under salt stress caused rise in the activity of CAT with maximum content of 2.84 UA mg^{-1} protein at NaCl I concentration. Its activity was found to be reduced as the level of salinity stress increased. Synergistic treatment using TRIA and H_2S reported

maximum activity of 2.36 UA mg^{-1} protein at NaCl I concentration.

Table 6.9 Effect of TRIA and H_2S on protein content and antioxidative enzymes of *B. juncea* seedlings under salt stress

Treatment	**Protein content (mg g^{-1} FW)**	**SOD (UA mg^{-1} protein)**	**CAT (UA mg^{-1} protein)**	**APX (UA mg^{-1} protein)**
Control	1.40^{bcde}±0.31	5.21^{e}±0.05	3.16^{def}±0.15	4.65^{ef}±0.06
NaCl I	0.35^{a}±0.05	3.38^{b}±0.08	2.36^{bc}±0.12	3.75^{bc}±0.03
NaCl II	0.80^{ab}±0.05	3.26^{ab}±0.06	1.95^{ab}±0.04	3.50^{b}±0.19
NaCl III	0.27^{a}±0.06	2.38^{a}±0.13	1.51^{a}±0.12	2.80^{ab}±0.06
TRIA	1.92^{ef}±0.03	6.92^{g}±0.02	3.84^{g}±0.10	5.85^{h}±0.06
TRIA + NaCl I	1.60^{cdef}±0.08	5.39^{e}±0.14	3.53^{efg}±0.17	5.35^{g}±0.15
TRIA + NaCl II	1.13^{def}±0.03	4.50^{d}±0.19	2.76^{cde}±0.06	4.74^{ef}±0.06
TRIA + NaCl III	1.06^{bcde}±0.30	3.78^{bc}±0.09	2.39^{bc}±0.09	4.13^{cd}±0.03
H_2S	2.03^{fg}±0.03	6.38^{fg}±0.08	3.69^{fg}±0.15	5.83^{h}±0.04
H_2S + NaCl I	1.31^{bcde}±0.05	4.31^{cd}±0.11	2.84^{cde}±0.09	4.78^{ef}±0.10
H_2S + NaCl II	1.42^{cdef}±0.05	3.61^{b}±0.13	2.64^{cd}±0.14	4.39^{de}±0.09
H_2S + NaCl III	1.13^{bcd}±0.07	3.42^{b}±0.08	2.48^{bc}±0.11	4.06^{cd}±0.04
TRIA + H_2S	2.61^{g}±0.20	7.83^{h}±0.09	4.77^{h}±0.12	6.85^{i}±0.07
TRIA + H_2S + NaCl I	1.54^{cdef}±0.12	6.55^{g}±0.22	3.86^{g}±0.06	5.83^{h}±0.07
TRIA + H_2S + NaCl II	1.77^{def}±0.07	5.77^{ef}±0.10	3.64^{fg}±0.06	5.41^{gh}±0.10
TRIA + H_2S + NaCl III	1.35^{bcde}±0.03	5.35^{e}±0.14	3.34^{efg}±0.05	5.06^{fg}±0.02

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

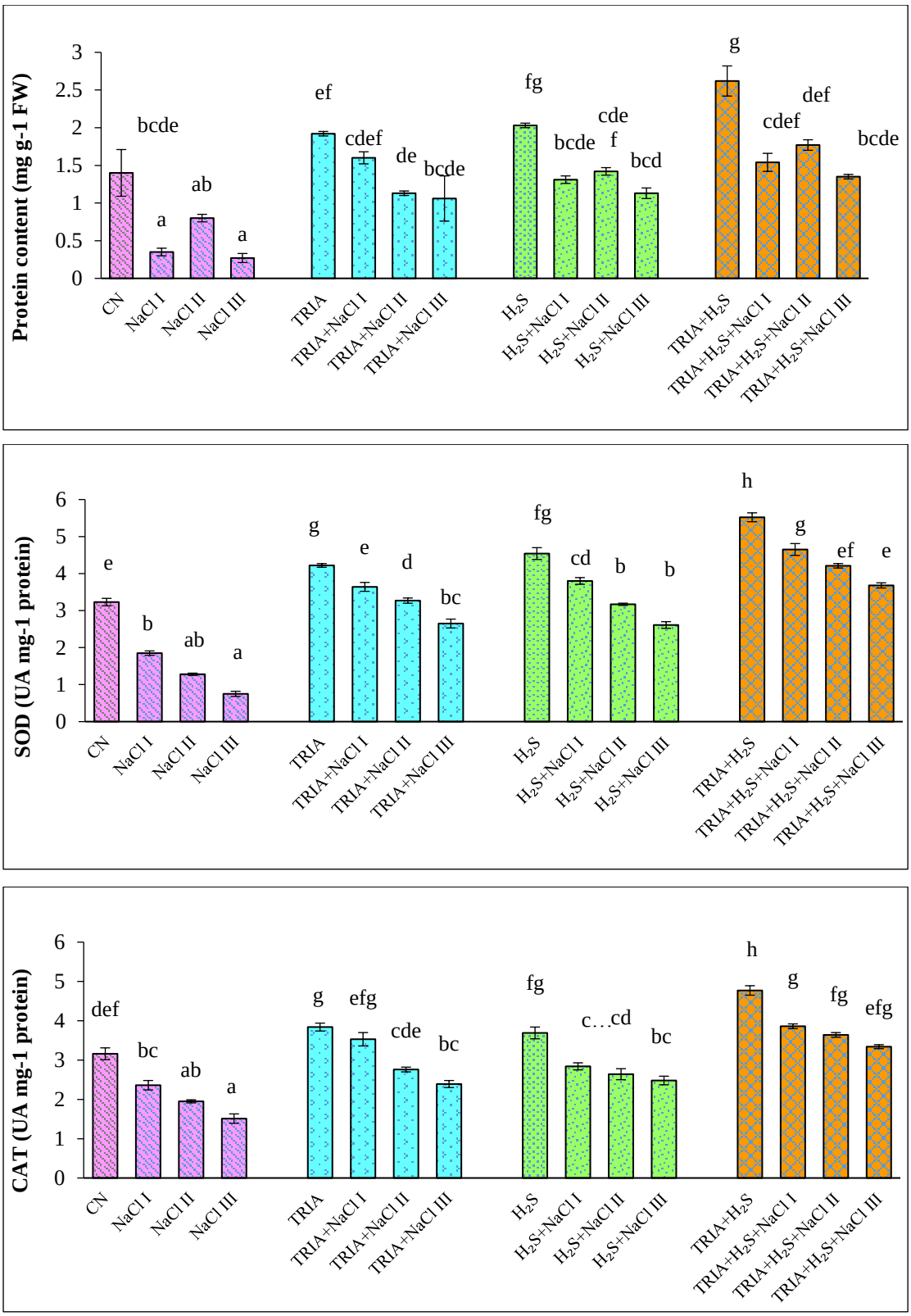


Fig. 6.12 Effect of TRIA and H_2S on protein, SOD and CAT enzyme activity in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

APX enzyme activity was found to get reduced under salt stress (Fig. 6.13; Table 6.10). Highest reduction in the APX activity was found at NaCl III concentration (2.80 UA mg^{-1} protein). Application of TRIA caused elevation of 5.85 UA mg^{-1} protein while decrease of 4.74 UA mg^{-1} protein and 4.13 UA mg^{-1} protein was observed at salinity in response to control seedlings. Likewise, H_2S treatment markedly increased the activity of enzyme APX. Maximum content of 4.78 UA mg^{-1} protein in case of H_2S application at NaCl I concentration. Combined treatment of TRIA and H_2S under unstressed condition significantly enhanced enzymatic activity of APX (6.85 UA mg^{-1} protein). Under salt stress, level of APX was found to be highest at 5.83 UA mg^{-1} protein at combination of TRIA+H_2S +NaCl I.

Table 6.10 Effect of TRIA and H_2S on antioxidative enzymes of *B. juncea* seedlings under salt stress

Treatment	**POD (UA mg^{-1} protein)**	**GR (UA mg^{-1} protein)**	**GPOX (UA mg^{-1} protein)**	**DHAR (UA mg^{-1} protein)**
Control	5.29^{cdef}±0.13	4.13^{de}± 0.03	3.23^{f}±0.10	5.14^{fg}±0.03
NaCl I	4.36^{b}±0.10	2.74^{b} ±0.06	1.85^{c}±0.06	3.41^{bc}±0.15
NaCl II	3.65^{a}±0.18	2.16^{b} ±0.02	1.28^{b}±0.03	3.06^{b}±0.04
NaCl III	3.20^{a}±0.12	1.47^{a}± 0.15	0.75^{a}±0.07	2.54^{a}±0.06
TRIA	6.67^{h}±0.09	4.75^{ef}±0.08	4.22^{hi}±0.05	5.74^{h}±0.03
TRIA + NaCl I	5.69^{gh}±0.19	3.74^{cd}±0.06	3.64^{fg}±0.12	4.35^{d}±0.04
TRIA + NaCl II	5.22^{ef}±0.06	3.62^{bcd}± 0.13	3.27^{f}±0.07	3.70^{cd}±0.03
TRIA + NaCl III	4.66^{def}±0.16	2.87^{bc}± 0.27	2.65^{de}±0.12	4.87^{ef}±0.06
H_2S	5.98^{fg}±0.05	4.43^{de}± 0.39	4.54^{i}±0.16	5.69^{gh}±0.13
H_2S + NaCl I	5.46^{ef}±0.10	3.62^{bcd}± 0.22	3.80^{gh}±0.09	4.75^{def}±0.06
H_2S + NaCl II	4.76^{bcd}±0.05	3.16^{bc} ±0.06	3.17^{ef}±0.03	4.34^{d}±0.15
H_2S + NaCl III	4.37^{b}±0.10	2.81^{b}±0.08	2.61^{ef}±0.09	3.77^{cd}±0.11
TRIA + H_2S	7.78^{i}±0.05	5.37^{f}±0.12	5.52^{j}±0.12	6.41^{i}±0.05
TRIA + H_2S + NaCl I	6.36^{gh}±0.03	4.30^{de}± 0.34	4.65^{i}±0.16	5.57^{fg}±0.07
TRIA + H_2S + NaCl II	5.65^{ef}±0.26	3.58^{bcd}±0.08	4.21^{hi}±0.06	4.43^{def}±0.17
TRIA + H_2S + NaCl III	5.45^{def}±0.12	3.80^{cd}±0.03	3.68^{fg}±0.07	5.07^{de}±0.03

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

POD activity was found to be reduced significantly under salt stressed conditions (Fig. 6.13; Table 6.10) Minimum activity of POD enzyme was observed at NaCl III concentration with the content of 3.20 UA mg^{-1} protein in comparison to control seedlings. Enzymatic activity of POD was found to be enhanced by application of TRIA by 6.69 UA mg^{-1} protein under unstressed conditions. However, it was found to get reduced in TRIA+NaCl I treated seedlings from 5.69 UA mg^{-1} protein to 4.66 UA mg^{-1} protein in contrast to seedlings treated with TRIA+ NaCl III concentration. Pre-treatment of seedlings with H_2S reported decrease in POD activity at NaCl I concentration (5.46 UA mg^{-1} protein). Co-application of TRIA+H_2S alone performed better role in boosting POD activity in comparison to their individual application. Highest POD activity of 5.45 UA mg^{-1} protein was observed in case of combined applications of TRIA and H_2S at NaCl I concentrations.

Recent studies revealed that salt stress led to the highest reduction of 1.47 UA mg^{-1} protein was observed at NaCl III concentration (Fig. 6.13; Table 6.10). Seedlings at control showed 4.13 UA mg^{-1} protein GR activity. TRIA application significantly enhanced the level of GR with a maximum of 2.13 UA mg^{-1} protein at NaCl II concentration. H_2S application augmented activity with a minimum of 2.75 UA mg^{-1} protein at NaCl III concentration. The highest 4.51 UA mg^{-1} protein GR activity was observed at the combination of TRIA and H_2S under NaCl I treatment.

Activity of enzyme GPOX was found to be diminished in NaCl treated seedlings of *B. juncea* with 1.82, 1.38, and 0.67 UA mg^{-1} protein at NaCl I, II, and III concentrations, respectively (Fig. 6.14; Table 6.10). Individual treatment of TRIA enlarged the GPOX activity in comparison to NaCl alone treated seedlings. Exogenous application of TRIA under salt stress showed minimum GPOX activity of 2.65 UA mg^{-1} protein at NaCl III concentration. Foliar application of H_2S under salt stress raised the level of GPOX with the highest activity of 3.61 UA mg^{-1} protein in NaCl I treated seedlings. Activity of GPOX was observed to be decreased as the level of NaCl elevated. combined application of TRIA and H_2S with the minimum activity of 5.07 UA mg^{-1} protein at NaCl III concentration.

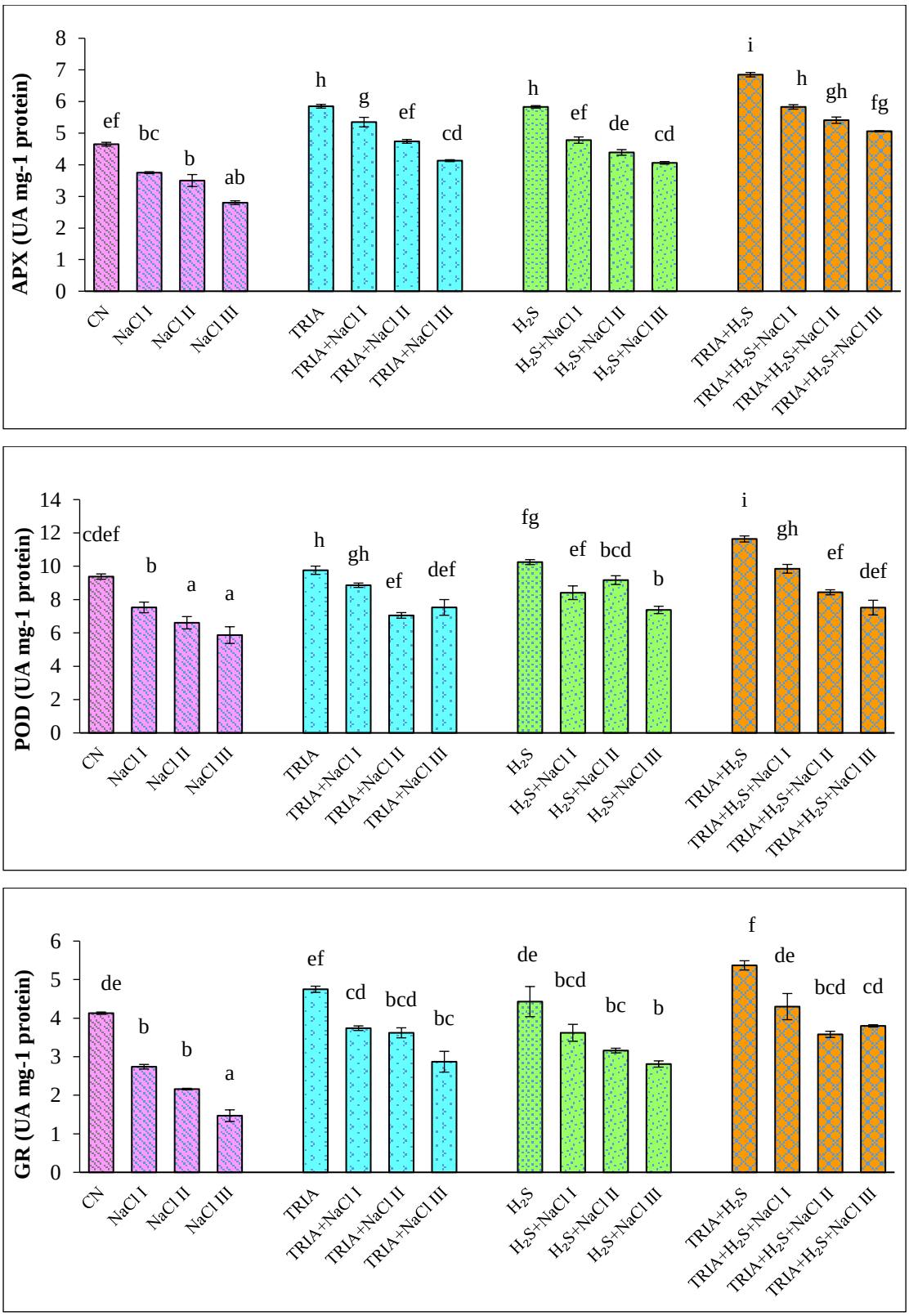


Fig. 6.13 Effect of TRIA and H_2S on APX, POD and GR enzyme activity in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Enzymatic activity of enzyme DHAR was found to decline under salt stress in NaCl treated plants (Fig. 6.14; Table 6.11). Maximum activity of enzyme DHAR was found to be 3.07 UA mg^{-1} protein in NaCl I treated seedlings. In case of TRIA-applied seedlings under salt stress highest DHAR activity of 4.72 UA mg^{-1} protein was at NaCl III concentration. H_2S application increased the DHAR activity under stressed conditions, in which the lowest activity of 3.77 UA mg^{-1} protein at NaCl III concentration. TRIA + H_2S further enhanced the activity of DHAR under stressed conditions with maximum activity of 5.57 UA mg^{-1} protein at NaCl I concentration.

MDHAR enzyme activity decreased drastically under salt stress (Fig. 6.14; Table 6.11). Minimum MDHAR activity of 3.07 UA mg^{-1} protein was found in NaCl III Activity of MDHAR was found to be increased from 4.53 to 3.22 UA mg^{-1} protein in TRIA-treated seedlings under salt stress. H_2S also increased the MDHAR activity under salt stress with the highest activity 5.13 UA mg^{-1} protein in NaCl I stressed seedlings. Among all 16 treatments highest MDHAR activity of 6.11 UA mg^{-1} protein was noticed in the case of combined application of TRIA and H_2S at salt stress.

GST enzymatic activity was found to be reduced with rise in the of concentration of NaCl. Different concentrations of NaCl reported decrease in GST enzymatic activities with 5.65, 4.65 and 3.45 UA mg^{-1} protein at NaCl I, II, and III concentrations, respectively (Fig. 6.15; Table 6.11). Treatment with TRIA and H_2S significantly increased the GST activity under salt stress. Highest GST activity in case of TRIA applied seedlings was reported at NaCl I concentration (7.32 UA mg^{-1} protein) and lowest GST activities was reported at NaCl III concentration (6.01 UA mg^{-1} protein). H_2S applied seedlings under stressed conditions reported higher GST activity of 5.13 UA mg^{-1} protein and lowest of 6.32 UA mg^{-1} protein was reported at NaCl III concentration. Combined application of TRIA+H_2S enhanced the GST activity under salt stressed conditions with the highest 8.95 UA mg^{-1} protein GST activity in case of NaCl I treated seedlings.

Salt stress significantly reduced the activity of enzyme PPO with the minimum content of 0.86 UA mg^{-1} protein at salt stressed stage in contrast to control seedlings with the content of 2.91 UA mg^{-1} protein (Fig. 6.15; Table 6.11). Individual application of TRIA and H_2S showed highest activity of 3.72 UA mg^{-1} protein and

3.75 UA mg^{-1} protein. However, TRIA application under salt stress showed highest of 2.64 UA mg^{-1} protein at NaCl I concentration. Whereas in the case of H_2S treated seedlings, maximum PPO activity of 3.75 UA mg^{-1} protein was reported. In case of combined application of TRIA and H_2S, highest PPO activity of 364 UA mg^{-1} protein was reported in NaCl I stressed seedlings.

Table 6.11 Effect of TRIA and H_2S on antioxidative enzymes of *B. juncea* seedlings under salt stress

Treatment	**MDHAR (UA mg^{-1} protein)**	**GST (UA mg^{-1} protein)**	**PPO (UA mg^{-1} protein)**
Control	4.75^{a} ±0.12	7.38^{ef}±0.16	2.91^{cdefg}±0.15
NaCl I	3.13^{ab}±0.04	5.65^{c}±0.12	1.59^{ab}±0.17
NaCl II	2.61^{a} ±0.16	4.65^{b}±0.16	1.09^{b}±0.09
NaCl III	3.07^{ab} ±0.02	3.45^{c}±0.11	0.86^{a}±0.07
TRIA	5.42^{efg}± 0.06	9.07^{h}±0.06	3.72^{gh}±0.10
TRIA + NaCl I	4.53^{c}± 0.17	7.32^{ef}±0.06	2.65^{cde}±0.12
TRIA + NaCl II	3.76^{b} ±0.27	6.68^{de}±0.13	2.44^{bcd}±0.16
TRIA + NaCl III	3.22^{ab} ±0.06	6.01^{cd}±0.09	2.14^{bc}±0.07
H_2S	5.27^{def}± 0.19	9.51^{hi}±0.18	3.75^{gh}±0.07
H_2S + NaCl I	5.13^{cdef} ±0.09	7.71^{fg}±0.19	3.33^{defg}±0.05
H_2S + NaCl II	4.59^{cd} ±0.13	7.04^{ef}±0.10	3.13^{efgh}±0.03
H_2S + NaCl III	3.70^{b}± 0.16	6.32^{cd}±0.15	2.77^{cdef}±0.19
TRIA + H_2S	7.10^{h} ±0.06	9.98^{i}±0.13	3.85^{h}±0.06
TRIA + H_2S + NaCl I	6.11^{g}± 0.16	8.95^{h}±0.03	3.64^{fgh}±0.07
TRIA + H_2S + NaCl II	5.76^{fg} ±0.11	8.11^{g}±0.05	3.52^{efgh}±0.12
TRIA + H_2S + NaCl III	4.85^{cde}± 0.10	7.33^{ef}±0.18	3.10^{defgh}±0.07

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

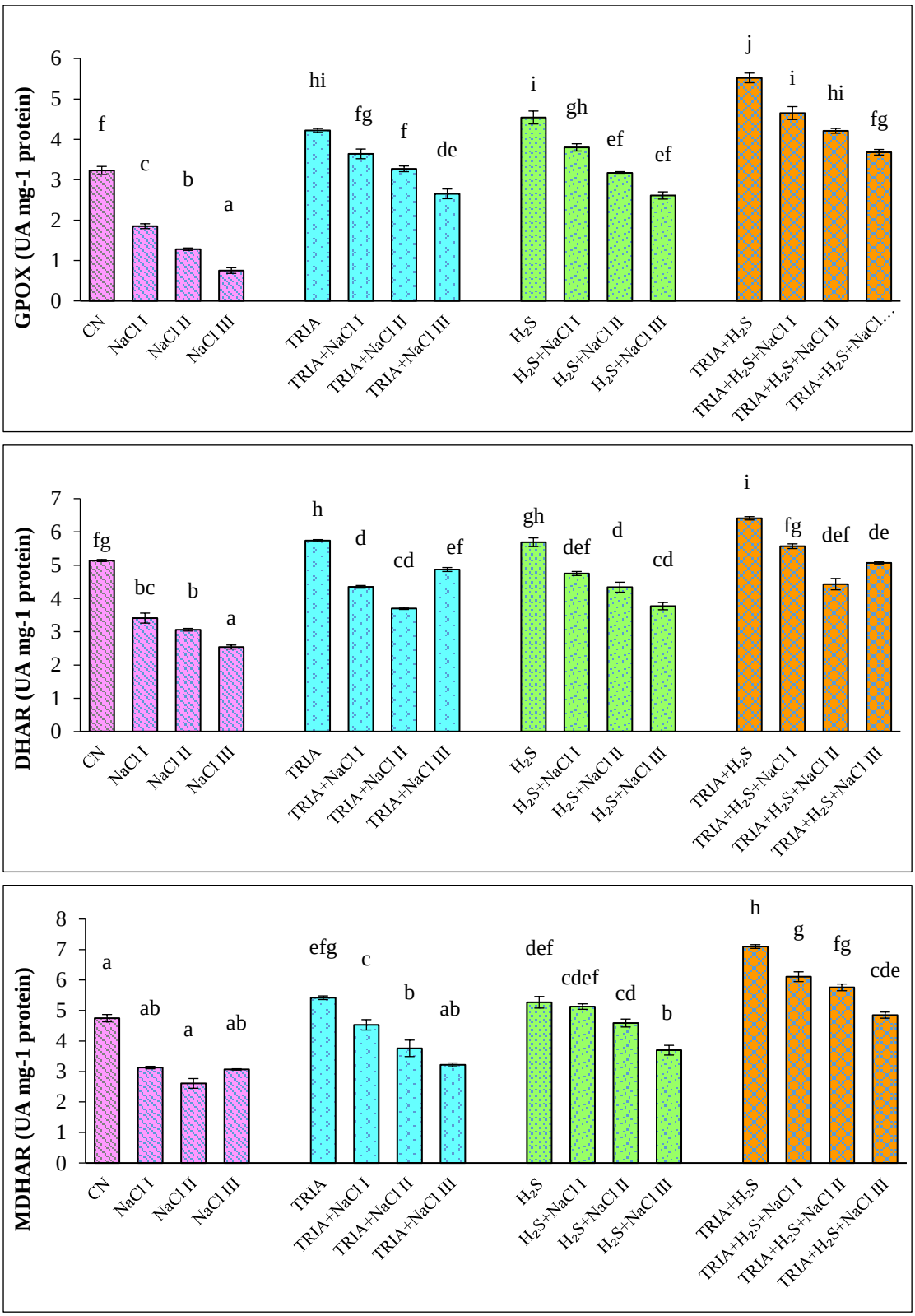


Fig. 6.14 Effect of TRIA and H_2S on GPOX, DHAR and MDHAR enzyme activities in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

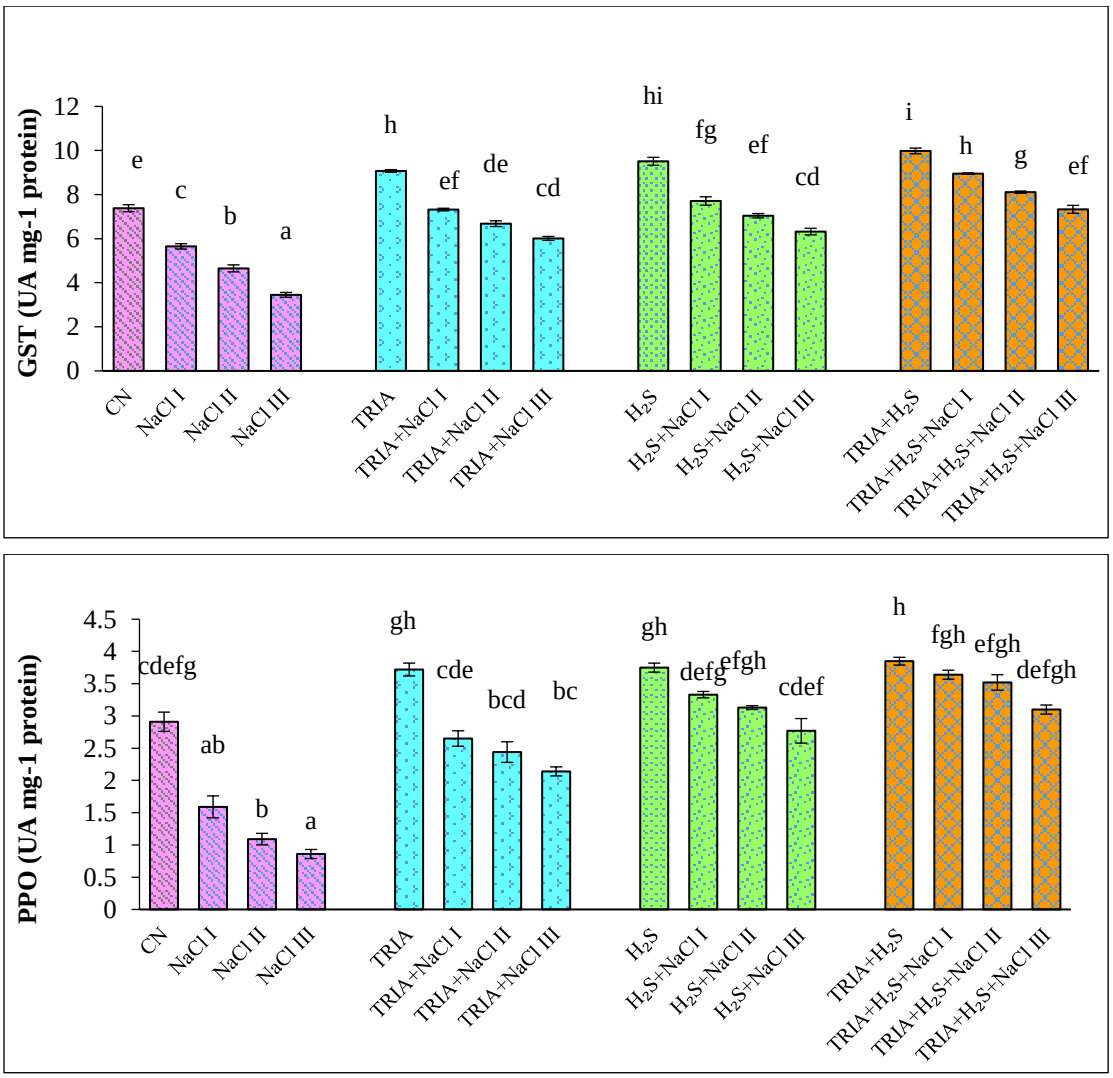


Fig. 6.15 Effect of TRIA and H_2S on GST and PPO enzyme activities in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.1.9 Non-enzymatic antioxidants

Content of ascorbic acid was found to be reduced in plants of *Brassica* under salt stress (Fig. 6.16; Table 6.12). Minimum content of 0.95 µg g^{-1} FW was detected at NaCl III concentration. Control plants showed 2.36 µg g^{-1} FW ascorbic acid content. TRIA and H_2S control plants reported 14.36 and 3.03 µg g^{-1} FW ascorbic acid content, respectively. Treatment with TRIA under stressed conditions reported maximum ascorbic acid content of 2.36 µg g^{-1} FW at NaCl I concentration. Application with H_2S improved the ascorbic acid content under salinity with highest of 2.57 µg g^{-1} FW at NaCl I concentration. In the combination of TRIA and H_2S,

content was found to be increased from 2.75 to 2.37 µg g^{-1} FW at NaCl I

Glutathione content reduced with increase in the concentration of NaCl from 0.79 to 0.40 µg g^{-1} FW, when concentration got reduced from NaCl I to NaCl III concentration (Fig. 6.16; Table 6.13). Glutathione content increased from 15.42 µg g^{-1} FW to 11.72 µg g^{-1} FW in TRIA + NaCl I concentration. Control seedlings reported 14.63 µg g^{-1} FW increase in the content of glutathione. H_2S treated seedlings under salt stress showed maximum glutathione content of 2.21 µg g^{-1} FW at NaCl I concentration. TRIA + H_2S boosted glutathione content in seedlings of *B. juncea* under salt stressed conditions. Synergistic association of seedlings treated with TRIA + H_2S showed 2.53, 2.32, and 2.24 µg g^{-1} FW amount at NaCl I, II, and III concentrations, respectively.

Table 6.12 Effect of TRIA and H_2S on non-enzymatic antioxidants of *B. juncea* seedlings under salt stress

Treatment	**Ascorbic acid (µg g^{-1} FW)**	**Glutathione (µg g^{-1} FW)**	**Tocopherol (µg g^{-1} FW)**
Control	2.36^{cdef}±0.22	1.41^{bc}± 0.12	1.20^{cdef}±0.01
NaCl I	1.78^{bc}±0.04	0.79^{b}± 0.10	0.86^{bc}± 0.05
NaCl II	1.58^{b}±0.07	0.54^{a} ±0.05	0.62^{ab}±0.10
NaCl III	0.95^{a}±0.04	0.40^{a}± 0.13	0.27^{a}± 0.06
TRIA	2.88^{fg}±0.05	2.89^{ij}± 0.08	1.80^{ghi}± 0.04
TRIA + NaCl I	2.36^{efg}±0.20	1.91^{defg}± 0.06	1.36^{defg}± 0.09
TRIA + NaCl II	2.16^{defg}±0.15	1.72^{cde}± 0.07	1.20^{cdef}±0.05
TRIA + NaCl III	1.92^{cdef}±0.03	1.50^{cd}± 0.04	1.13^{cd}±0.09
H_2S	2.93^{fg}±0.04	2.76^{ij}± 0.17	1.88^{hi}±0.05
H_2S + NaCl I	2.57^{defg}±0.26	2.21^{fgh}±0.05	1.42^{efg}±0.17
H_2S + NaCl II	2.06^{bcd}±0.03	2.04^{efg}±0.04	1.25^{defg}±0.04
H_2S + NaCl III	1.88^{bc}±0.07	1.82^{cdef}± 0.07	1.14^{cde}±0.03
TRIA + H_2S	3.03^{g}±0.04	3.33^{j}±0.05	2.18^{i}±0.06
TRIA + H_2S + NaCl I	2.75^{efg}±0.07	2.53^{hij}± 0.06	1.72^{gh}±0.13
TRIA + H_2S + NaCl II	2.56^{defg}±0.08	2.32^{ghi}± 0.06	1.60^{fgh}±0.11
TRIA + H_2S + NaCl III	2.37^{cdef}±0.02	2.24^{fgh}± 0.09	1.58^{efgh}±0.05

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

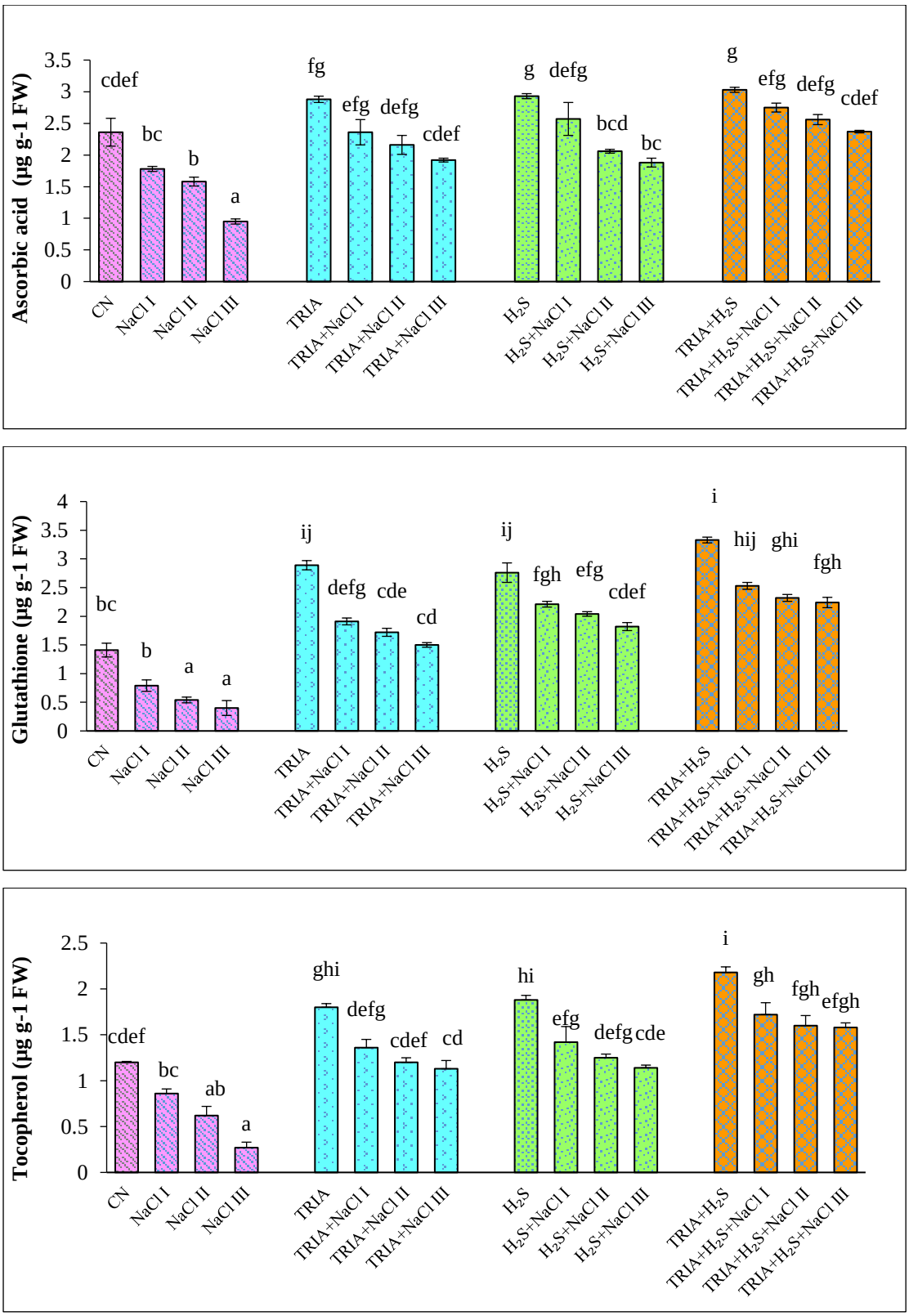


Fig.6.16 Effect of TRIA and H_2S on ascorbic acid, glutathione and tocopherol content in 7-days old seedlings of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Tocopherol content showed decrease in the content with increase in the concentrations of NaCl. Tocopherol content decreased from 0.86 µg g^{-1} FW to 0.27 µg g^{-1} FW in case of stressed situation (Fig. 6.16; Table 6.12). TRIA treated seedlings under salt stress showed tocopherol content of 1.36 at NaCl II concentration. However, in case of H_2S treated seedlings maximum tocopherol content was found to be 1.42 µg g^{-1} FW at NaCl I concentration and minimum tocopherol content was found to be 1.14 µg g^{-1} FW at NaCl I concentration. Synergistic association of TRIA and H_2S in case of stress showed maximum tocopherol content of 1.72 µg g^{-1} FW at NaCl I concentration.

6.1.2 30-days old plants

6.1.2.1 Plant growth

Salt stress significantly reported minimum root length of 2.43 cm in NaCl III stressed plants (Fig. 6.17; Table 6.13). TRIA reported highest root length of 6.56 cm in NaCl I treated plants. Application of H_2S reported highest root length of 5.43 cm at NaCl I and lowest of 4.70 cm at NaCl III concentrations. Pre-treatment with TRIA showed better results in increasing root lengths in response to H_2S application. TRIA + H_2S application further improved the root length to 6.40 cm at NaCl I.

Shoot length decreased with increase of NaCl (Fig. 6.17; Table 6.13). Highest shoot length was found to be 6.60 cm at NaCl I concentration and lowest shoot length was found 5.83 at NaCl III concentration. TRIA application reported highest shoot length of 12.00 cm at NaCl I stressed plants. Individual application of H_2S reported higher shoot length of 11.80 cm in comparison to salt stressed plants. Minimum shoot length of 8.73 cm was noticed in NaCl III stressed seedlings. Synergistic treatment of TRIA and H_2S reported higher shoot length of 12.43 cm under unstressed conditions. TRIA+H_2S treatment under salt stressed condition reported higher shoot length of 12.10 cm at NaCl I concentration.

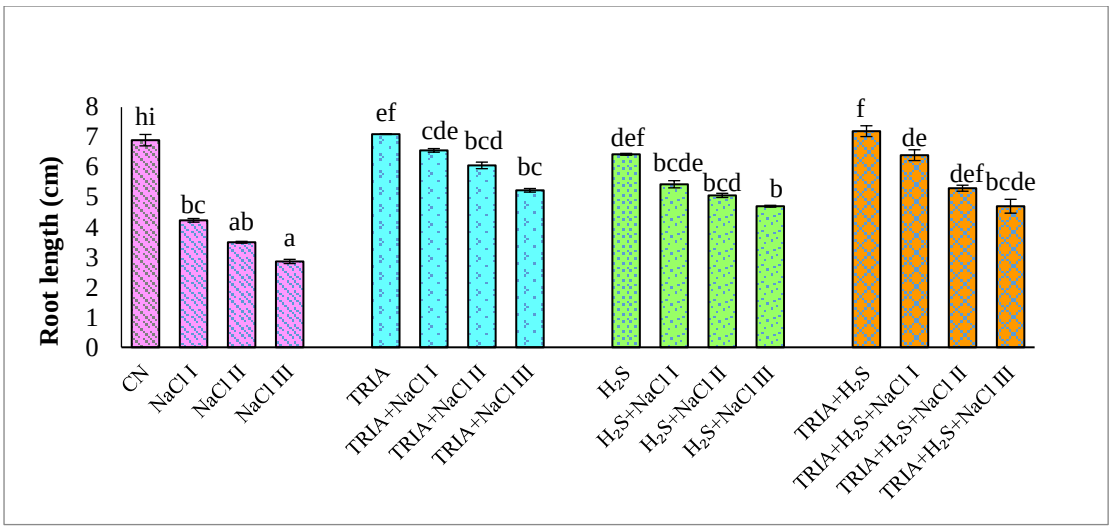
Fresh weight decreased drastically under salt stress to 0.53 g in NaCl III stressed plants in comparison to control plants (Fig. 6.17; Table 6.13). Application of TRIA under NaCl stress increased the fresh weight from 2.19 g to 2.55 g, in contrast to NaCl stressed plants alone. H_2S treated plants reported highest and lowest fresh weights of 2.57 g and 2.19 g at different salt concentration. Combination significantly

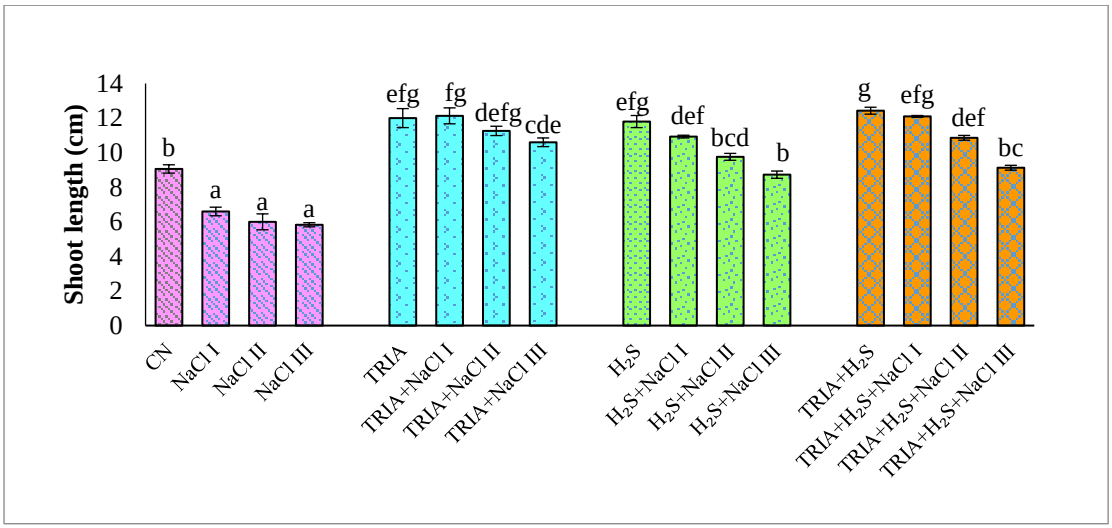
improved fresh weights as compared to their individual treatments. Maximum weight of 2.37g was noticed at NaCl I concentration.

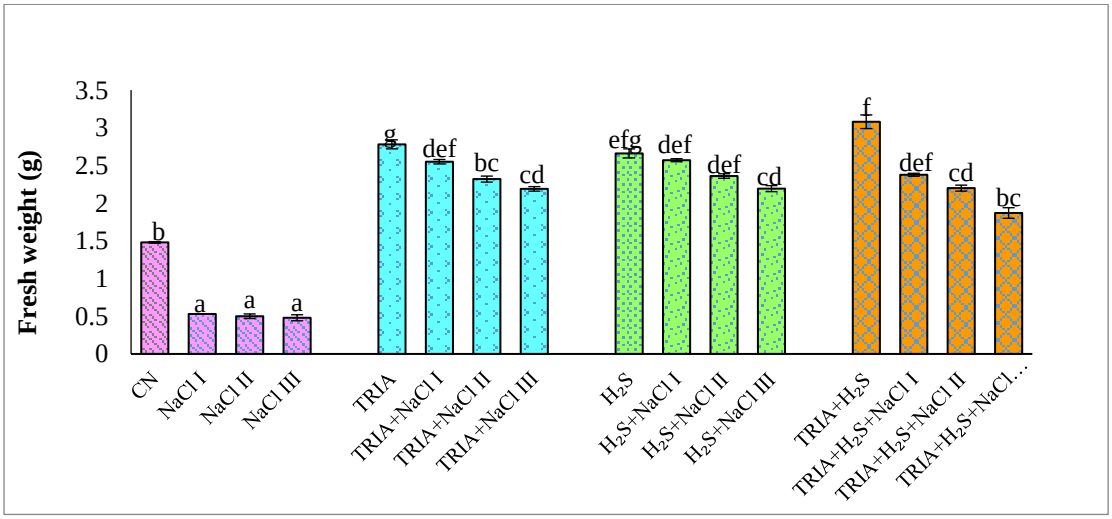
Table 6.13 Effect of TRIA and H_2S on morphological parameters of 30-days old plants of *B. juncea* under salt stress

Treatment	**Root length (cm)**	**Shoot length (cm)**	**Fresh weight (g)**	**Dry weight (g)**
Control	6.9^{hi}±0.20	9.06^{b} ±0.24	1.48^{b}±0.01	0.72^{bc}±0.15
NaCl I	4.23^{bc}±0.20	6.60^{a}±0.25	0.53^{a}±0.02	0.30^{ab}±0.06
NaCl II	3.50^{ab}±0.26	6.00^{a} ±0.46	0.50^{a}±0.03	0.28^{ab}±0.02
NaCl III	2.86^{a}±0.08	5.83^{a} ±0.12	0.48^{a}±0.04	0.23^{a}±0.03
TRIA	7.10^{ef}±0.17	12.00^{efg} ±0.55	2.78^{g}±0.06	1.70^{efgh}±0.01
TRIA + NaCl I	6.56^{cde}±0.24	12.13^{fg} ±0.46	2.55^{def}±0.03	1.54^{efg}±0.02
TRIA + NaCl II	6.06^{bcd}±0.08	11.26^{defg} ±0.27	2.32^{bc}±0.04	1.77^{fgh}±0.20
TRIA + NaCl III	5.23^{bc}±0.23	10.60^{cde}±0.25	2.19^{cd}±0.03	1.71^{efgh}±0.03
H_2S	6.43^{def}±0.43	11.80^{efg} ±0.35	2.66^{efg}±0.06	1.74^{efgh}±0.06
H_2S + NaCl I	5.43^{bcde}±0.25	10.93^{def} ±0.08	2.57^{def}±0.02	1.42^{de}±0.11
H_2S + NaCl II	5.06^{bcd}±0.22	9.76^{bcd}± 0.20	2.36^{def}±0.03	1.28^{de}±0.19
H_2S + NaCl III	4.70^{bc}±0.12	8.73^{b} ±0.21	2.19^{cd}±0.04	0.93^{cd}±0.03
TRIA + H_2S	7.20^{f}±0.31	12.43^{g}± 0.20	3.08^{f}±0.09	2.14^{h}±0.01
TRIA + H_2S + NaCl I	6.40^{de}±0.65	12.10^{efg}± 0.05	2.37^{def}±0.02	1.97^{gh}±0.07
TRIA + H_2S + NaCl II	5.30^{def}±0.48	10.86^{def}± 0.14	2.20^{cd}±0.04	1.82^{gh}±0.08
TRIA + H_2S + NaCl III	4.70^{bcde}±0.16	9.13^{bc}± 0.14	1.87^{bc} ±0.07	1.49^{efg}±0.18

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments


Root length (cm)
0
1
2
3
4
5
6
7
8
hi
bc
ab
a
ef
cde
bcd
bc
def
bcde
bcd
b
f
de
def
bcde
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Shoot length (cm)
0
2
4
6
8
10
12
14
b
a
a
a
efg
fg
defg
cde
efg
def
bcd
b
g
efg
def
bc
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Fresh weight (g)
0
0.5
1
1.5
2
2.5
3
3.5
b
a
a
a
g
def
bc
cd
efg
def
def
cd
f
def
cd
bc
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl…

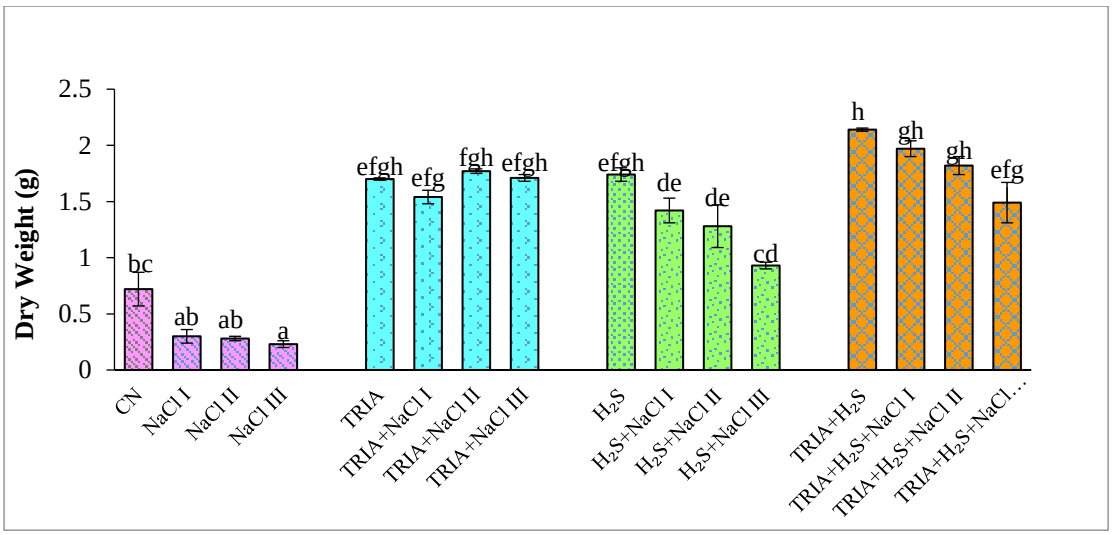


Fig. 6.17 Effect of TRIA and H_2S on root length, fresh and dry weight in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

NaCl I, II and III treated plants showed reduction in dry weight in contrast to control plants which reported dry weight of 0.72g (Fig. 6.17; Table 6.13). Dry weight decreased at different concentrations from 0.30g to 0.23g at salt stressed stages Treatment of TRIA and H_2S under unstressed condition reported maximum dry weight of 1.70g and 1.74g at NaCl I concentrations, respectively. TRIA + H_2S combined treatment showed better results in increasing dry weights as compared to their individual treatments. TRIA+H_2S+NaCl I concentration reported maximum dry weight of 1.97g and minimum dry weight of 1.49 g at NaCl III concentration.

Germination percentage decreased significantly under salt stress. It was found that it reduced to 53.65% in case of NaCl III stressed plants (Fig. 6.18; Table 6.14). Germination percentage was found to be higher in control plants (74.21%) as compared to salt stressed plants. TRIA application caused an escalation in the germination percentage of plants of *B. juncea* with the highest of 75.65% at NaCl I concentration. H_2S treatment enhanced germination of seeds under salt stress. In H_2S treated plants germination percentage raised from 68.91% to 79.17% under NaCl I stress. TRIA and H_2S control plants reported nearly equal germination percentages of 83.69% and 83.88% under unstressed condition. Combination of triacontanol and hydrogen sulphide reported highest germination percentage of 90.22% in comparison to all other treatments.

Relative water content declined significantly under different concentration of salt (Fig. 6.18; Table 6.14). Elevation in salt stress caused decrease in the level from 68.36% at NaCl I concentration to 65.28% at NaCl III concentration. Control seedlings exhibited 79.06% relative water content. TRIA treatment alone showed relative water content of 85.97% was higher in contrast to its control. TRIA application exhibited a minimum relative water content of 73.31% at NaCl III concentration. Out of all 3 NaCl treatments, H_2S-treated seedlings, the maximum relative water content of 81.35% at NaCl I concentration. Combined treatment of TRIA and H_2S showed higher relative water content as compared to their individual treatments with the highest relative water content of 82.06% at NaCl I concentration.

Table 6.14 Effect of TRIA and H_2S on germination percentage and relative water content of 30-days old plants of *B. juncea* under salt stress

Treatment	**Germination percentage**	**Relative water content (%)**
Control	$74.21^{cdef} \pm 2.94$	$79.06^{def} \pm 2.64$
NaCl I	$67.83^{bc} \pm 3.64$	$68.36^{bc} \pm 1.99$
NaCl II	$58.43^{ab} \pm 2.07$	$70.07^{ab} \pm 5.77$
NaCl III	$53.65^{a} \pm 2.32$	$65.28^{a} \pm 3.80$
TRIA	$83.69^{efg} \pm 1.85$	$85.79^{gh} \pm 3.43$
TRIA + NaCl I	$75.65^{cdef} \pm 2.88$	$83.13^{efgh} \pm 4.09$
TRIA + NaCl II	$71.88^{cd} \pm 3.52$	$76.36^{cdef} \pm 3.15$
TRIA + NaCl III	$69.61^{cd} \pm 0.37$	$73.31^{cd} \pm 3.76$
H_2S	$83.88^{fg} \pm 0.80$	$87.10^{gh} \pm 1.00$
H_2S + NaCl I	$79.17^{cde} \pm 0.86$	$81.35^{defg} \pm 1.43$
H_2S + NaCl II	$73.29^{cde} \pm 1.82$	$77.73^{cde} \pm 1.76$
H_2S + NaCl III	$68.91^{bcd} \pm 3.44$	$74.03^{cd} \pm 1.46$
TRIA + H_2S	$90.22^{g} \pm 0.85$	$89.33^{h} \pm 0.84$
TRIA + H_2S + NaCl I	$82.70^{efg} \pm 0.63$	$82.06^{fgh} \pm 0.64$
TRIA + H_2S + NaCl II	$78.84^{def} \pm 0.63$	$80.50^{defg} \pm 0.93$
TRIA + H_2S + NaCl III	$70.24^{cd} \pm 0.61$	$77.83^{cdef} \pm 1.33$

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

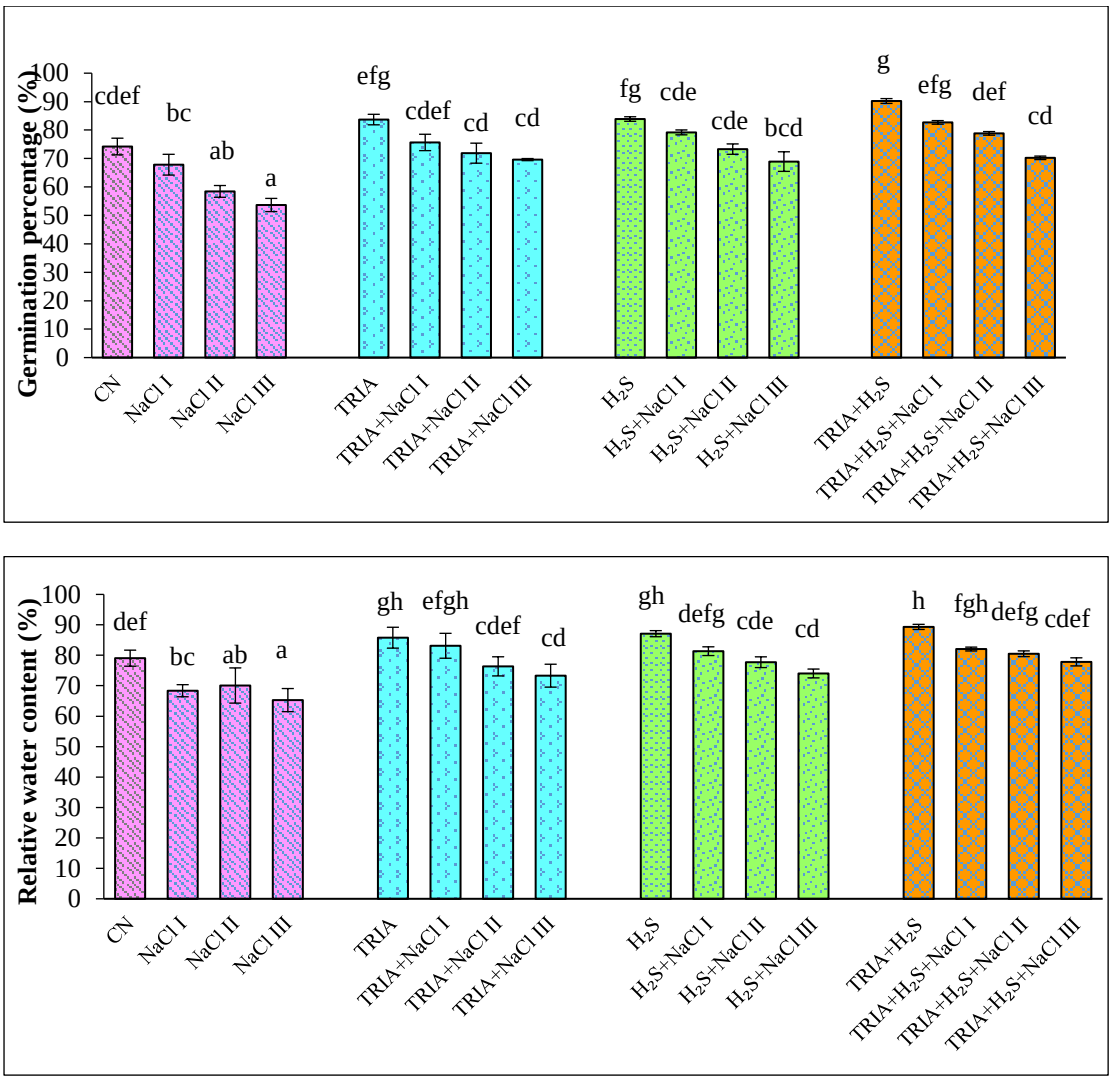


Fig 6.18 Effect of TRIA and H_2S on germination percentage and relative water content of 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

6.1.2.2 Photosynthetic activity

6.1.2.2.1 Photosynthetic pigments

Content of photosynthetic pigments was found to reduce under NaCl stress in plants of *B. juncea* (Fig. 6.19; Table 6.15). Total chlorophyll dropped at different concentration of NaCl. Content of chlorophyll decreased from 0.586 mg g^{-1} FW,0.532 mg g^{-1} FW to 0.460 mg g^{-1} FW at NaCl I, II and III concentration. TRIA and H_2S application enhanced the total chlorophyll content out of which the highest total chlorophyll contents i.e., 0.775 and 0.729 FW was noticed at NaCl I concentration

when treated individually with TRIA and H_2S, respectively. TRIA and H_2S supplementation enhanced total chlorophyll contents under unstressed conditions in comparison to their individual treatments. Highest total chlorophyll content of 0.818 mg g^{-1} FW was found at TRIA + H_2S+ NaCl I treated plants. Likewise, it was found that content of chl decreased in B. *juncea*. NaCl III concentration reported lowest chlorophyll content of 0.462 mg g^{-1} FW (Chl a) and 0.272 mg g^{-1} FW (Chl b). TRIA and H_2S treatment enhanced the content of chl a and b. Treatment of TRIA and H_2S elevated chl in stress conditions with the highest 0.860 and 0.710 mg g^{-1} FW contents, at NaCl I concentration.

Table 6.15 Effect of TRIA and H_2S on photosynthetic pigments of 30-days old plants of *B. juncea* under salt stress

Treatment	**Total chlorophyll (mg g^{-1} FW)**	**Chl a (mg g^{-1} FW)**	**Chl b (mg g^{-1} FW)**
Control	0.721^{def}±0.005	0.600^{bc}±0. 048	0.576^{cdef}±0. 041
NaCl I	0.586^{bc}±0.011	0.539^{ab}±0. 015	0.414^{abc}±0. 024
NaCl II	0.532^{ab}±0.016	0.517^{ab}±0. 013	0.374^{a}±0. 062
NaCl III	0.460^{a}±0.031	0.462^{a}±0. 012	0.272^{a}±0. 037
TRIA	0.865^{hi}±0.026	0.870^{ij}±0. 012	0.737^{fg}±0. 028
TRIA + NaCl I	0.775^{efg}±0.006	0.746^{fghi}±0. 019	0.674^{efg}±0. 027
TRIA + NaCl II	0.703^{de}±0.004	0.664^{efgh}±0. 019	0.533^{bcde}±0. 027
TRIA + NaCl III	0.673^{d}±0.010	0.589^{defg}±0. 012	0.441^{abcd}±0. 084
H_2S	0.800^{fgh}±0.011	0.865^{h}±0. 017	0.735^{fg}±0. 037
H_2S + NaCl I	0.729^{def}±0.009	0.747^{de}±0. 013	0.658^{efg}±0. 053
H_2S + NaCl II	0.672^{d}±0. 015	0.661^{cde}±0. 020	0.569^{bcde}±0. 015
H_2S + NaCl III	0.647^{bc}±0. 031	0.577^{bc}±0. 012	0.431^{abc}±0. 019
TRIA + H_2S	0.911^{i}±0. 016	0.948^{h}±0. 015	0.857^{g}±0. 030
TRIA + H_2S + NaCl I	0.818^{gh}±0. 007	0.860^{fg}±0. 014	0.710^{efg}±0. 020
TRIA + H_2S + NaCl II	0.795^{fgh}±0. 004	0.758^{ef}±0. 012	0.634^{def}±0. 011
TRIA + H_2S + NaCl III	0.701^{de}±0. 006	0.644^{cd}±0. 027	0.527^{bcde}±0. 020

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

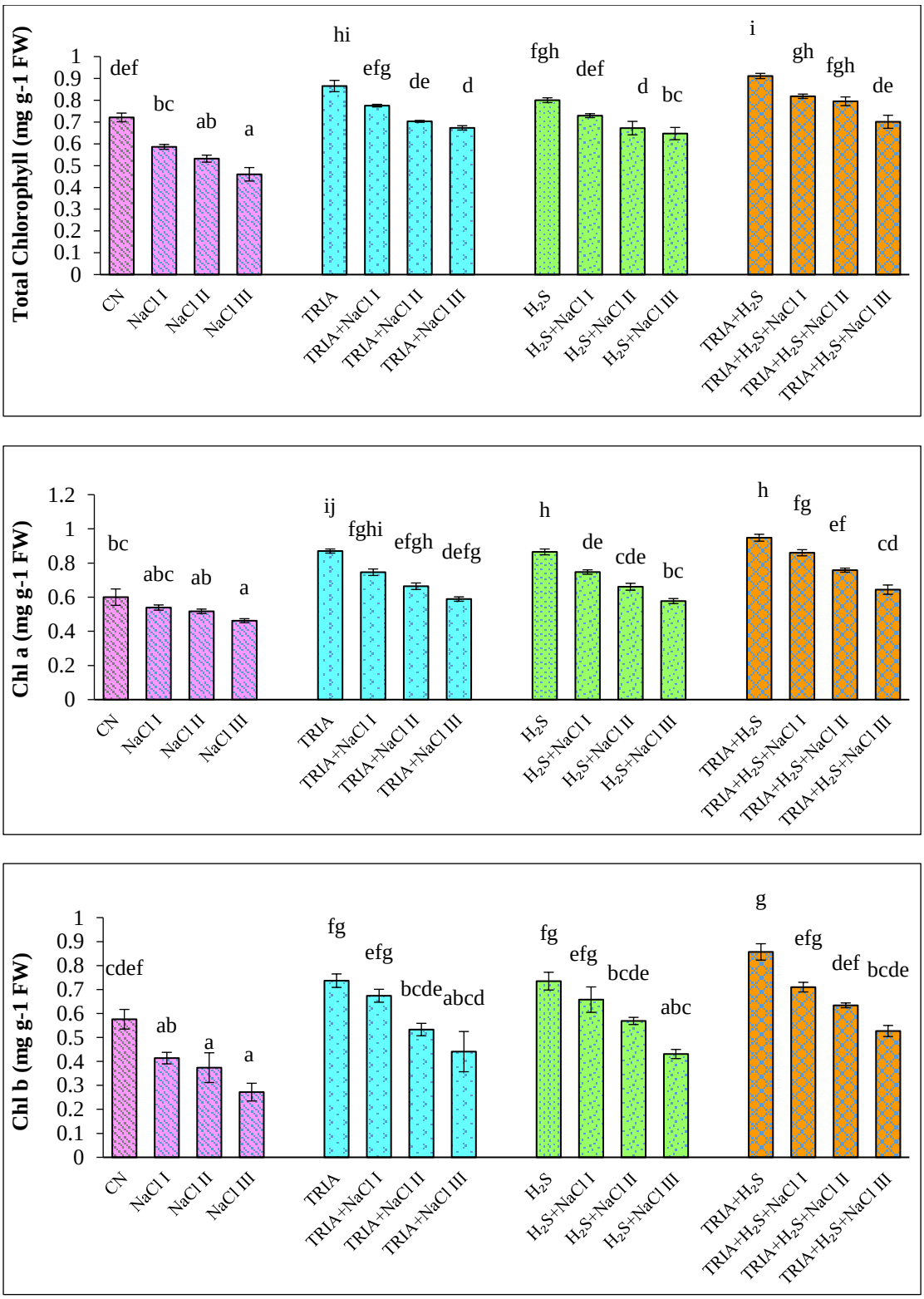


Fig. 6.19 Effect of TRIA and H_2S on total chlorophyll, chl a and chla b content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

Carotenoid was found to be decreased with increase in the concentration of NaCl (Fig. 6.20; Table 6.16). Among different concentration of NaCl, highest reduction of 0.359 mg g^{-1} FW was found at NaCl III concentration. NaCl I stressed plants showed decline in content of carotenoid in comparison to TRIA+NaCl I plants from 0.503 mg g^{-1} FW to 0.633 mg g^{-1} FW. H_2S pre-treatment under salt stress raised the carotenoid content with the highest carotenoid content of 0.725 mg g^{-1} FW at NaCl I concentration. TRIA+H_2S+NaCl I concentration reported highest carotenoid content 0.647 mg g^{-1} FW whereas minimum of 0.517 mg g^{-1} FW was at NaCl III concentration.

Table 6.16 Effect of TRIA and H_2S on photosynthetic pigments of 30-days old plants of *B. juncea* under salt stress

Treatment	**Carotenoid content (mg g^{-1} FW)**	**Xanthophyll content (mg g^{-1} FW)**
Control	0.595^{bcd}±0. 02	7.50^{cdef}±0.26
NaCl I	0.476^{ab}±0. 01	5.96^{abc}±0.24
NaCl II	0.400^{a}±0. 02	5.01^{ab}±0.01
NaCl III	0.359^{a}±0. 04	4.50^{a}±0.26
TRIA	0.729^{de}±0. 02	8.80^{fg}±0.49
TRIA + NaCl I	0.633^{bcd}±0. 02	7.28^{e}±0.43
TRIA + NaCl II	0.585^{bcd}±0. 03	6.43^{d}±0.23
TRIA + NaCl III	0.503^{abc}±0. 01	5.97^{cd}±0.05
H_2S	0.725^{d}±0. 02	8.32^{efg}±0.38
H_2S + NaCl I	0.644^{ef}±0.03	7.04^{cdef}±0.59
H_2S + NaCl II	0.599^{def}±0. 05	6.36^{cdef}±0.13
H_2S + NaCl III	0.546^{bcd}±0. 03	6.83^{cde}±0.39
TRIA + H_2S	0.892^{j}±0. 01	9.28^{g}±0.29
TRIA + H_2S + NaCl I	0.647^{ghi}±0. 02	8.12^{defg}±0.35
TRIA + H_2S + NaCl II	0.610^{fgh}±0.03	7.64^{cdef}±0.33
TRIA + H_2S + NaCl III	0.517^{ef}±0. 01	6.66^{bcde}±0.32

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Salt stress caused decreased in xanthophyll content (Fig. 6.20; Table 6.16). Minimum xanthophyll content of 4.50 mg g-1 FW was noticed at NaCl III stressed plants. TRIA and H_2S treatment elevated xanthophyll content under salt stress. TRIA and H2S treatment alone under salt stress reported highest content of 7.28 and 7.04 mg g-1 FW at NaCl I. Xanthophyll content was noticed to be diminished as salt level increased in the case of TRIA and H_2S alone. Combination of TRIA and H_2S caused xanthophyll content of 8.12 mg g-1 FW at NaCl I concentration.

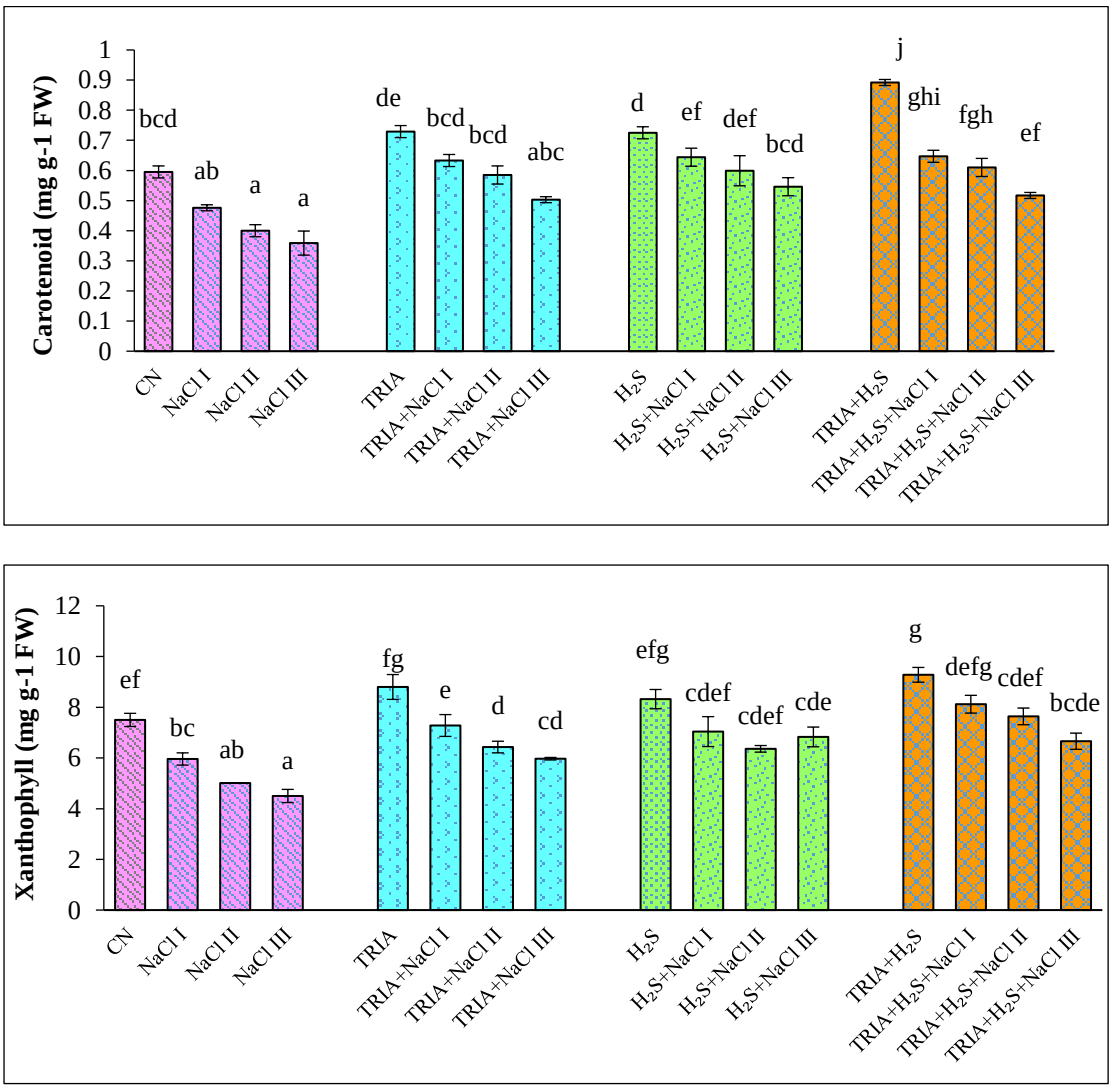


Fig. 6.20 Effect of TRIA and H_2S on carotenoid and xanthophyll content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

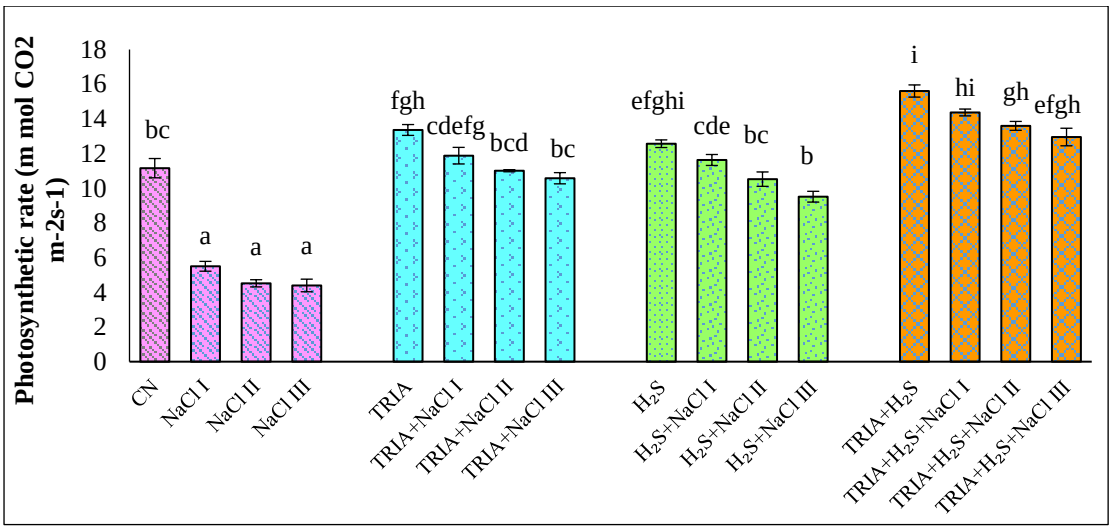
6.1.2.2.2 Gas exchange parameters

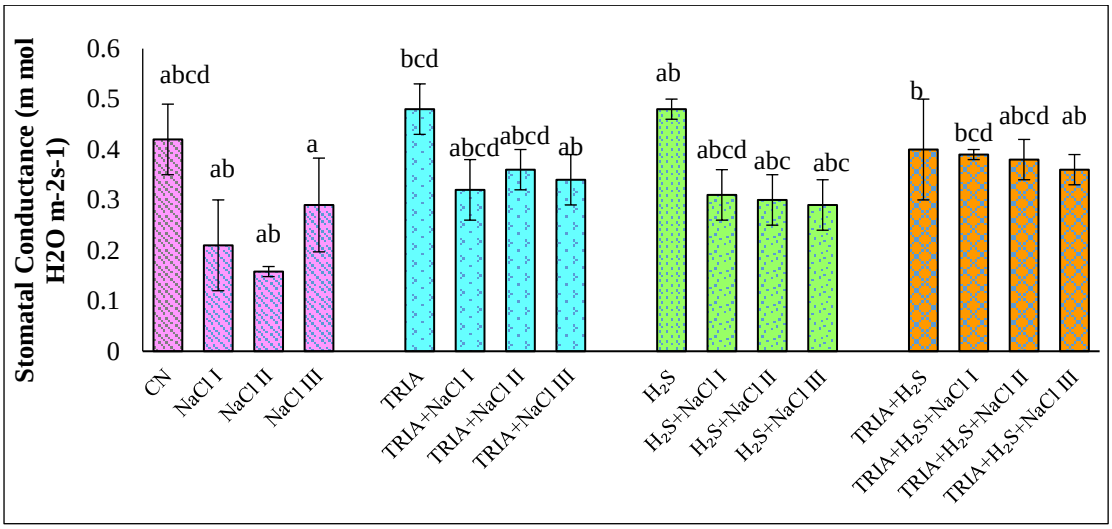
Photosynthetic rate declined under salt stress with a minimum 4.39 m mol CO_2 m^{-2} S^{-1} at NaCl III concentration (Fig. 6.21; Table 6.17). TRIA control plants noticed in contrast TRIA at salt stressed conditions from 10.57 m mol CO_2 m^{-2} S^{-1} to 13.36 m mol CO_2 m^{-2} S^{-1}. H_2S application enhanced the photosynthetic rate with the highest photosynthetic rate of 11.63 m mol CO_2 m^{-2} S^{-1} at NaCl I concentration. TRIA application exhibited higher photosynthetic rate than H_2S application. TRIA + H_2S treatment increased the photosynthetic rate with highest photosynthetic rate of 14.37 m mol CO_2 m^{-2} S^{-1} at NaCl I concentration.

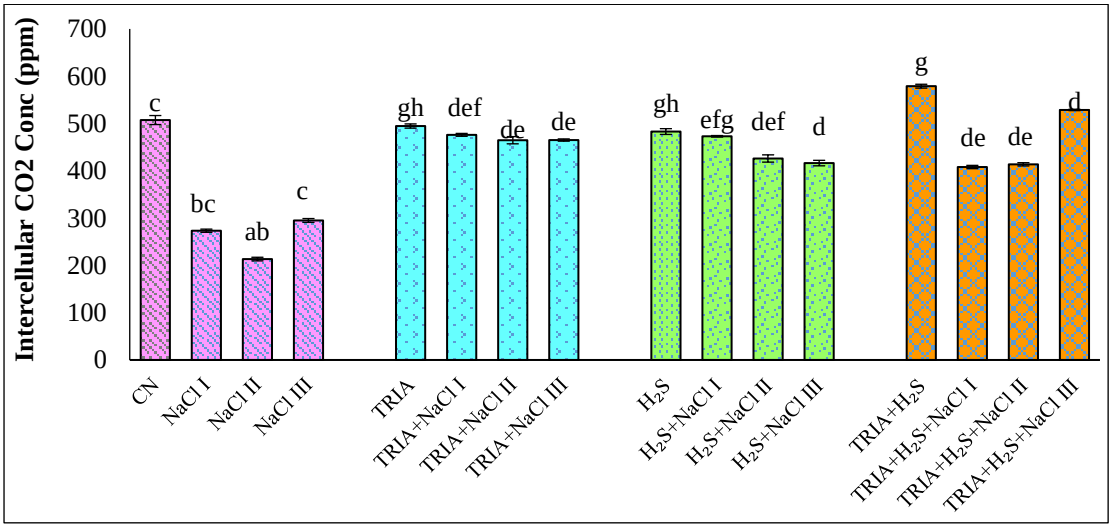
Table 6.17 Effect of TRIA and H_2S on gas exchange parameters of 30-days old plants of *B. juncea* under salt stress

Treatment	Photosynthetic rate (m mol CO_2 m^{-2} S^{-1})	Stomatal conductance (m mol H_2O m^{-2} S^{-1})	Intercellular CO_2 concentration (ppm)	Transpiration rate (m mol H_2O m^{-2} S^{-1})
Control	11.15^{bc} ±0.55	0.32^{abcd} ±0.01	321^{c} ±1.45	0.425^{d} ±0.02
NaCl I	5.50^{a} ±0.28	0.24^{ab} ±0.03	273.3^{b} ±3.38	0.371^{bc} ±0.06
NaCl II	4.51^{a} ±0.20	0.21^{ab} ±0.02	213^{a} ±3.84	0.242^{ab} ±0.02
NaCl III	4.39^{a} ±0.36	0.16^{a} ±0.01	295^{ab} ±4.04	0.196^{a} ±0.01
TRIA	13.36^{fgh} ±0.31	0.40^{bcd} ±0.02	494.66^{f} ±4.37	0.899^{gh} ±0.01
TRIA + NaCl I	11.87^{cdefg} ±0.47	0.32^{abcd} ±0.06	476^{ef} ±3.05	0.766^{def} ±0.02
TRIA + NaCl II	11.01^{bcd} ±0.52	0.30^{abcd} ±0.05	464.66^{ef} ±7.62	0.688^{de} ±0.04
TRIA + NaCl III	10.57^{bc} ±0.32	0.27^{ab} ±0.02	465de ±2.30	0.628^{de} ±0.01
H_2S	12.56^{efghi} ±0.21	0.48^{ab} ±0.02	483^{f} ±6.11	0.898^{gh} ±0.01
H_2S + NaCl I	11.63^{cde} ±0.31	0.31^{abcd} ±0.05	473^{ef} ±1.52	0.729^{efg} ±0.02
H_2S + NaCl II	10.52^{bc} ±0.41	0.30^{abc} ±0.05	426^{ef} ±7.63	0.627^{def} ±0.03
H_2S + NaCl III	9.51^{b} ±0.30	0.29^{abc} ±0.05	416.33^{de} ±5.84	0.591^{d} ±0.01
TRIA + H_2S	15.60^{i} ±0.34	0.52^{b} ±0.03	579^{k} ±4.04	0.954^{h} ±0.01
TRIA + H_2S + NaCl I	14.37^{hi} ±0.19	0.40^{bcd} ±0.04	408de ±3.51	0.814^{fgh} ±0.03
TRIA + H_2S + NaCl II	13.59^{gh} ±0.25	0.35^{abcd} ±0.04	413.66^{de} ±3.48	0.765^{efg} ±0.01
TRIA + H_2S + NaCl III	12.95^{efgh} ±0.50	0.24^{ab} ±0.02	388^{d} ±2.33	0.721^{def} ±0.04

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments


Photosynthetic rate (m mol CO2 m-2s-1)
0
2
4
6
8
10
12
14
16
18
bc
a
a
a
fgh
cdefg
bcd
bc
efghi
cde
bc
b
i
hi
gh
efgh
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Stomatal Conductance (m mol H2O m-2s-1)
0
0.1
0.2
0.3
0.4
0.5
0.6
abcd
ab
ab
a
bcd
abcd
abcd
ab
ab
abcd
abc
abc
b
bcd
abcd
ab
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Intercellular CO2 Conc (ppm)
0
100
200
300
400
500
600
700
c
bc
ab
c
gh
def
de
de
gh
efg
def
d
g
de
de
d
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III

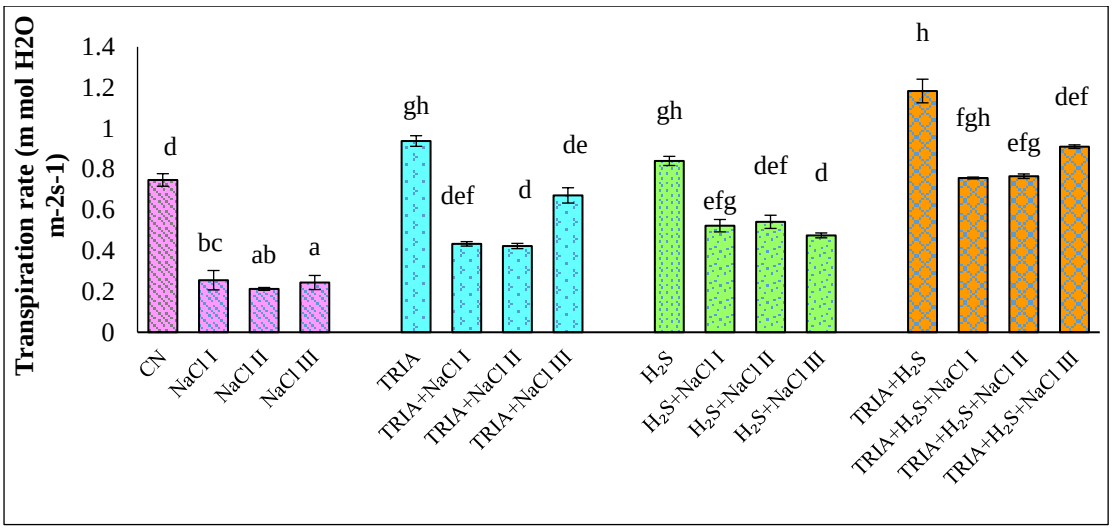


Fig. 6.21 Effect of TRIA and H_2S on photosynthetic rate, stomatal conductance and intercellular CO_2 concentration in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

Elevated level of NaCl decreased stomatal conductance in plants of *Brassica*. Minimum stomatal conductance of 0.16 m mol H_2O m^{-2} s^{-1} was noticed at NaCl III concentration (Fig. 6.21; Table 6.17). NaCl I stressed plants caused reduction of 0.24 m mol H_2O m^{-2} s^{-1} in stomatal conductance. TRIA and H_2S control plants showed higher stomatal conductance of 0.40 and 0.48 m mol H_2O m^{-2} s^{-1}, respectively. TRIA and H_2S application alone under salt stress reported greater stomatal conductance of 0.32 and 0.31 m mol H_2O m^{-2} s^{-1} at NaCl I and II, respectively.

Control plants showed 321 ppm intercellular CO_2 concentration (Fig. 6.21; Table 6.17). NaCl II concentration reported decrease of 213 ppm intercellular CO_2 concentration. H2S pre-treatment increased the intercellular CO_2 concentration with the highest of 473 ppm at NaCl I concentration. Synergistic association of triacontanol and hydrogen sulphide under salt stressed conditions raised the intercellular CO_2 concentration with the highest of 413 ppm at NaCl II concentration.

Transpiration rate was found to be decreased in 30 day old plants of *Brassica* (Fig. 6.21; Table 6.17). Salt stress reported decline of 0.371, 0.242, and 0. 196 m mol H_2O m^{-2} S-1. TRIA application under stressed conditions showed 0.688 m mol H_2O m^{-2} S^{-1} of transpiration at NaCl II concentration, whereas H_2S showed the higher rate of transpiration of 0.729 m mol H_2O m^{-2} S^{-1} at NaCl I concentration. Combination of

TRIA and H_2S reported highest and lowest transpiration rate of 0.814 and 0.721 m mol H_2O m^{-2} S^{-1} at NaCl respectively.

6.1.2.3 Metabolites

Current study stated that salt stress significantly affected anthocyanin content. Maximum reduction in anthocyanin content of 4.85 mg g^{-1} FW at NaCl III concentration and minimum reduction was found at NaCl I concentration (Fig. 6.22; Table 6.18). Triacontanol and hydrogen sulphide in salt stress increased anthocyanin content with maximum content of 8.60 mg g^{-1} FW and 7.17 mg g^{-1} FW in NaCl I treated plants. Maximum anthocyanin content of 9.32 mg g^{-1} FW was noticed at TRIA+H_2S+NaCl I in contrast to all other treatments used.

TRIA and H_2S application alone under salt stressed condition had improved flavonoid content in *plants* of *B. juncea* in comparison to all other treatments (Fig. 6.22; Table 6.18). Maximum decline of 5.55 mg g^{-1} FW in flavonoid at NaCl III concentration. Control plants reported flavonoid content of 7.71 mg g^{-1} FW. TRIA plants reported flavonoid content of 8.60 mg g^{-1} FW at NaCl I concentration and minimum of 7.58 8.06 mg g^{-1} FW at NaCl III. In H_2S treated plants, minimum flavonoid content of 7.63 mg g^{-1} FW was noticed in NaCl III stress. However, TRIA and H_2S treatment significantly alleviated salinity in plants of *B. juncea* with maximum content of 9.30 mg g^{-1} FW at TRIA + H_2S + NaCl I.

Exposure to salt stress reduced phenolic content in contrast to control plants. NaCl I concentration reported maximum reduction of 5.28 mg g^{-1} FW and minimum reduction of 3.87 mg g^{-1} FW at NaCl III concentration (Fig. 6.22; Table 6.19). However, TRIA and H_2S application under salt stress significantly declined phenolic content than salt stressed alone. It was found that combination of TRIA+H_2S was found to be effective in increasing the phenolic content in plants of *B. juncea*. Phenolic content of 7.47 mg g^{-1} FW was highes at TRIA+H_2S+NaCl II treatment in comparison to all other 16 treatments.

Table 6.18 Effect of TRIA and H_2S on metabolites of 30-days old plants of *B. juncea* under salt stress

Treatment	**Anthocyanin content (mg g^{-1} FW)**	**Flavonoid content (mg g^{-1} FW)**	**Phenolic content (mg g^{-1} FW)**
Control	$8.34^{defg} \pm 0.31$	$7.71^{b} \pm 0.24$	$8.51^{efg} \pm 0.18$
NaCl I	$5.76^{ab} \pm 0.20$	$5.55^{a} \pm 0.34$	$5.28^{a} \pm 0.19$
NaCl II	$5.56^{ab} \pm 0.18$	$5.37^{a} \pm 0.15$	$4.83^{a} \pm 0.15$
NaCl III	$5.05^{a} \pm 0.14$	$4.98^{a} \pm 0.12$	$3.87^{a} \pm 0.21$
TRIA	$9.25^{gh} \pm 0.08$	$10.34^{ef} \pm 0.32$	$9.14^{g} \pm 0.06$
TRIA + NaCl I	$8.60^{efgh} \pm 0.24$	$8.56^{bcd} \pm 0.18$	$7.78^{cd} \pm 0.19$
TRIA + NaCl II	$7.70^{cde} \pm 0.23$	$8.14^{bc} \pm 0.08$	$8.11^{def} \pm 0.25$
TRIA + NaCl III	$7.40^{cde} \pm 0.21$	$7.58^{b} \pm 0.19$	$7.10^{c} \pm 0.10$
H_2S	$7.90^{def} \pm 0.13$	$9.45^{de} \pm 0.24$	$9.29^{g} \pm 0.10$
H_2S + NaCl I	$7.17^{cd} \pm 0.10$	$8.60^{bcd} \pm 0.26$	$7.47^{cd} \pm 0.09$
H_2S + NaCl II	$6.61^{bc} \pm 0.12$	$7.89^{b} \pm 0.30$	$7.45^{cd} \pm 0.11$
H_2S + NaCl III	$6.57^{bc} \pm 0.22$	$7.63^{b} \pm 0.21$	$8.13^{def} \pm 0.05$
TRIA + H_2S	$9.99^{h} \pm 0.41$	$11.28^{f} \pm 0.19$	$9.29^{h} \pm 0.23$
TRIA + H_2S + NaCl I	$9.32^{fgh} \pm 0.20$	$9.30^{cde} \pm 0.24$	$7.47^{fg} \pm 0.15$
TRIA + H_2S + NaCl II	$8.47^{defgh} \pm 0.22$	$8.44^{bcd} \pm 0.34$	$7.45^{fg} \pm 0.14$
TRIA + H_2S + NaCl III	$7.20^{cd} \pm 0.56$	$7.66^{b} \pm 0.25$	$8.13^{def} \pm 0.16$

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

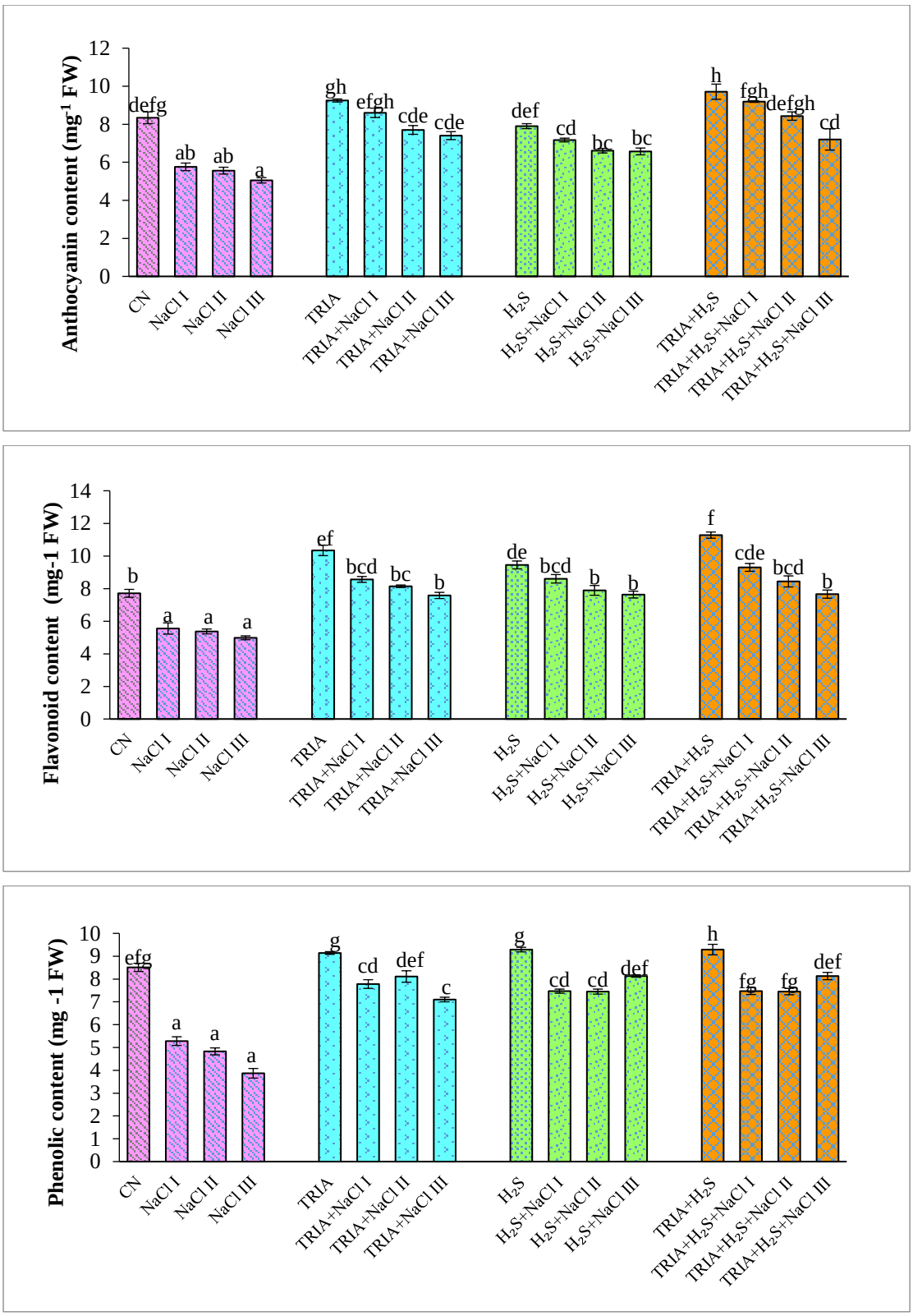


Fig. 6.22 Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

6.1.2.4 Oxidative stress markers

Level of MDA elevated under salt stress. Highest level of MDA was noticed at 9.77 and 9.55 µmol g^{-1} FW at NaCl III and II concentrations, respectively (Fig. 6.23; Table 6.19). TRIA and H_2S alone or in combination significantly reduced the content of MDA under salt stress. Minimum level of MDA of 6.57, 5.44, and 4.69 µmol g^{-1} FW was noticed in TRIA, H_2S, and TRIA + H_2S applied plant at NaCl III concentration

Table 6.19 Effect of TRIA and H_2S on oxidative stress markers of 30-days old plants of *B. juncea* under salt stress

Treatment	**MDA content (µmol g^{-1} FW)**	**H_2O_2 content (µmol g^{-1} FW)**
Control	$7.21^{c} \pm 0.27$	$7.84^{e} \pm 0.31$
NaCl I	$9.55^{d} \pm 0.13$	$11.32^{f} \pm 0.17$
NaCl II	$9.30^{d} \pm 0.37$	$12.12^{f} \pm 0.34$
NaCl III	$9.77^{d} \pm 0.61$	$11.72^{f} \pm 0.31$
TRIA	$6.57^{bc} \pm 0.26$	$7.89^{e} \pm 0.15$
TRIA + NaCl I	$5.86^{abc} \pm 0.22$	$6.91^{cde} \pm 0.31$
TRIA + NaCl II	$6.04^{abc} \pm 0.08$	$6.24^{bcd} \pm 0.30$
TRIA + NaCl III	$5.44^{ab} \pm 0.21$	$5.95^{bcd} \pm 0.26$
H_2S	$6.16^{abc} \pm 0.25$	$7.71^{e} \pm 0.23$
H_2S + NaCl I	$5.85^{abc} \pm 0.53$	$7.78^{e} \pm 0.20$
H_2S + NaCl II	$5.72^{abc} \pm 0.26$	$7.24^{de} \pm 0.12$
H_2S + NaCl III	$5.20^{ab} \pm 0.22$	$6.99^{cde} \pm 0.14$
TRIA + H_2S	$5.55^{ab} \pm 0.19$	$5.86^{abc} \pm 0.31$
TRIA + H_2S + NaCl I	$5.38^{ab} \pm 0.24$	$5.46^{ab} \pm 0.39$
TRIA + H_2S + NaCl II	$5.29^{ab} \pm 0.17$	$5.65^{ab} \pm 0.39$
TRIA + H_2S + NaCl III	$4.88^{a} \pm 0.15$	$4.58^{a} \pm 0.12$

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

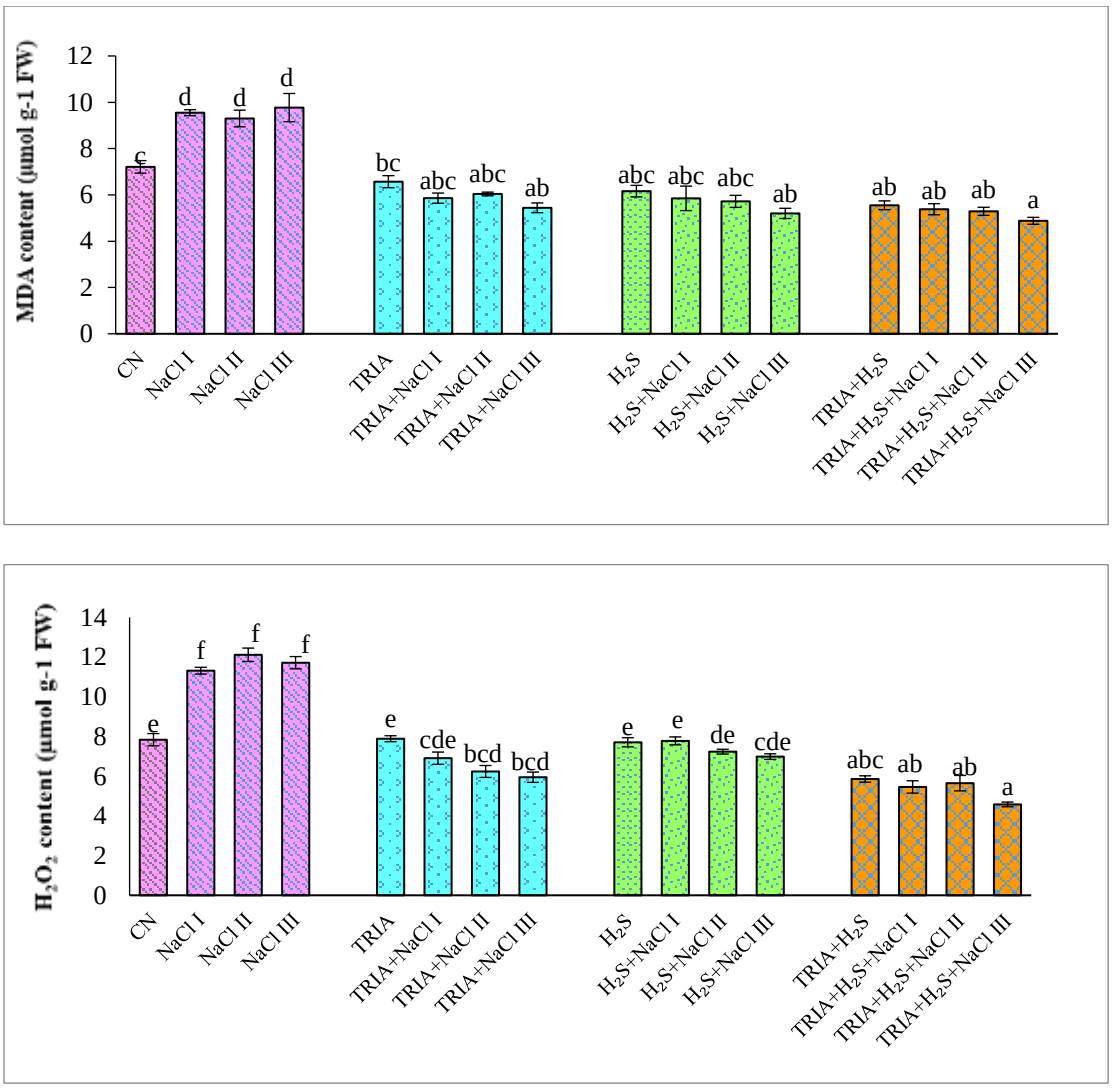


Fig. 6.23 Effect of TRIA and H_2S on MDA and H_2O_2 content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

Likewise, TRIA application the H_2O_2 content was found to be increased with increase in the concentration of NaCl (Fig. 6.23; Table 6.19). Highest content of MDA was found at NaCl III concentration (9.77 µmol g^{-1} FW). Likewise, H_2O_2 content decreased as the level of NaCl increased in case of TRIA and H_2S when used individually. Minimum H_2O_2 content of 4.58 µmol g^{-1} FW was found at NaCl III concentration when TRIA and H_2S were used in combination to salt stress.

6.1.2.5 Osmolytes

Proline content was found to get reduced in NaCl (Fig. 6.24; Table 6.20). Proline contents declined to 3.58, 3.37 and 2.82 µ mol g^{-1} FW were found at salt stress. which

exhibited proline content of 4.33 µ mol g^{-1}. Treatment in case of unstressed of TRIA and H_2S reported higher proline content in comparison to plants under salt stressed conditions. Content of proline increased from 2.82 to 3.65 µ mol g^{-1} FW in TRIA supplied plants under NaCl III. H_2S treatment enhanced proline content to 5.44µ mol g^{-1} FW at NaCl I concentration. TRIA and H_2S treatment at NaCl I concentration elevated proline content with the greatest content of 6.25 µ mol g^{-1} FW

Table 6.20 Effect of TRIA and H_2S on osmolytes of 30-days old plants of *B. juncea* under salt stress

Treatment	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
Control	4.33^{abcd}±0.16	5.91^{bcd}±0.57
NaCl I	3.58^{abc}±0.39	5.11^{abc}±0.07
NaCl II	3.37^{ab}±0.46	4.39^{ab}±0.32
NaCl III	2.82^{a}±0.32	3.43^{a}±0.25
TRIA	6.52^{ef}±0.80	7.47^{de}±0.28
TRIA + NaCl I	5.25^{bcde}±0.25	6.50^{cde}±0.42
TRIA + NaCl II	4.73^{abcd}±0.25	6.21^{bcde}±0.29
TRIA + NaCl III	3.65^{abc}±0.38	5.17^{abc}±0.35
H_2S	6.46^{ef}±0.28	7.37^{de}±0.30
H_2S + NaCl I	5.44^{cde}±0.30	6.35^{bcde}±0.28
H_2S + NaCl II	4.63^{abcd}±0.29	6.08^{bcd}±0.52
H_2S + NaCl III	4.26^{abcd}±0.36	5.80^{bcd}±0.23
TRIA + H_2S	7.78^{f}±0.21	$8.16.^{e}$±0.42
TRIA + H_2S + NaCl I	6.25^{def}±0.42	7.61^{de}±0.64
TRIA + H_2S + NaCl II	5.56^{cde}±0.30	6.21^{bcde}±0.50
TRIA + H_2S + NaCl III	5.25^{bcde}±0.51	5.71^{bcd}±0.29

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Content of glycine betaine reduced salt stress in *B. juncea* (Fig. 6.24; Table 6.20). NaCl I stressed plants reported content of 5.11 µ mol g^{-1} FW. TRIA + H_2S application significantly mitigated by increasing the content of glycine betaine. TRIA and H_2S individual treatment reported highest glycine betaine content of 6.50 and 6.35 µ mol g^{-1} FW at NaCl I concentration, respectively. TRIA and H_2S combination reported

8.16 mol g^{-1} FW of glycine betaine content under unstressed condition. Under stressed conditions, combination of triacontanol and hydrogen sulphide when applied at NaCl I conc reported highest glycine betaine content of 7.61 µ mol g^{-1} FW.

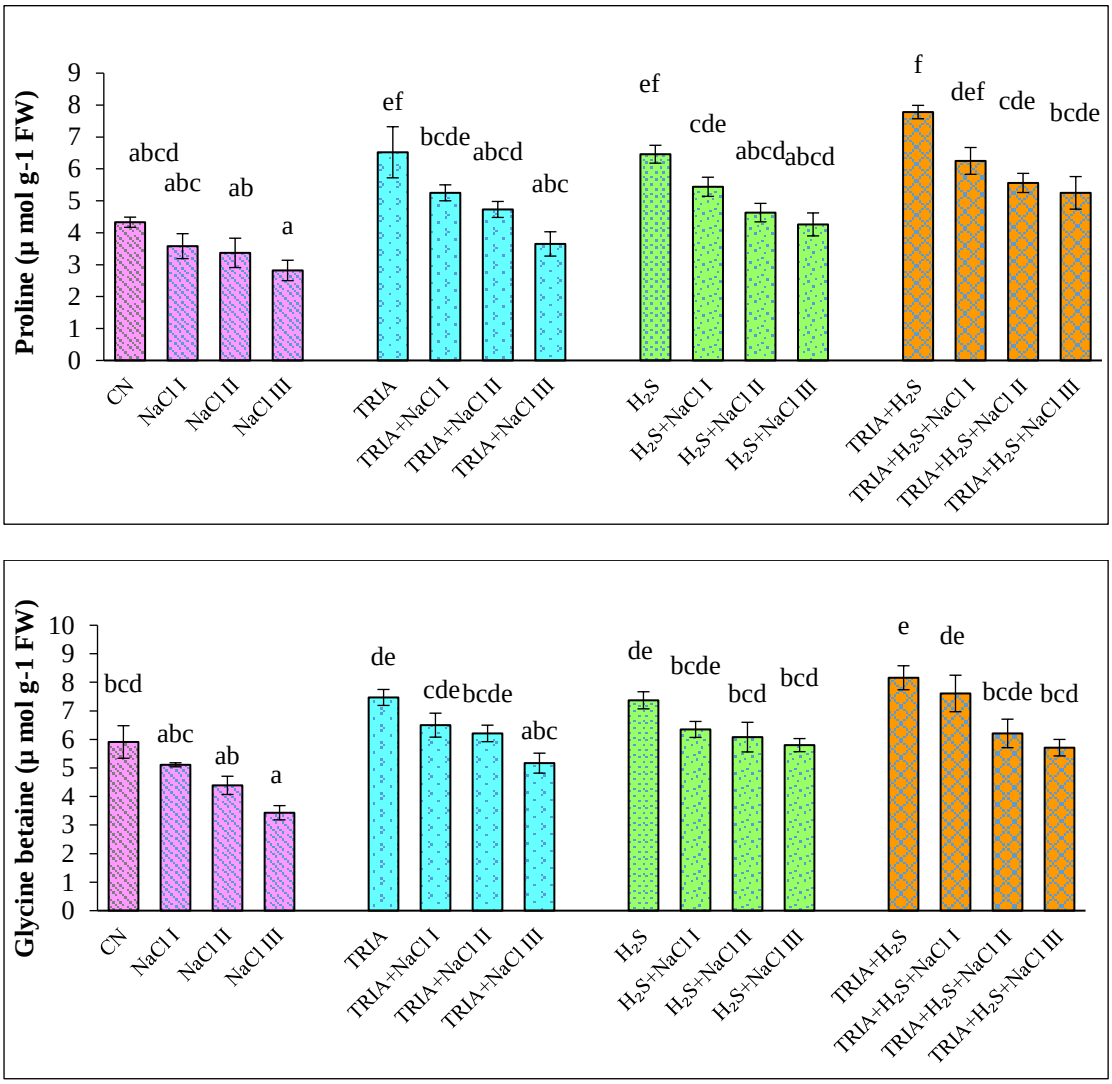


Fig. 6.24 Effect of TRIA and H_2S on proline and glycine betaine content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.2.6 Total carbohydrates

Salt stress reduced the content of carbohydrates content with a maximum content of 3.77 mg g^{-1} FW at NaCl I and content of 2.43 mg g^{-1} FW at NaCl III stressed conditions (Fig. 6.25; Table 6.21). Salt stressed plants showed maximum reduction in total carbohydrates as compared to control plants. H_2S foliar treatment under stressed conditions showed maximum and minimum carbohydrates content of 6.07 and 4.44

mg g^{-1} FW content at NaCl I and NaCl III concentration. Pre-treatment with H_2S under salt stressed conditions reported highest carbohydrate content of 6.01 mg g^{-1} FW under NaCl II stressed plants. TRIA+H_2S+NaCl I showed a maximum carbohydrate content of 7.10 mg g^{-1} FW and minimum of 5.71 mg g^{-1} FW at NaCl III concentration.

Table 6.21 Effect of TRIA and H_2S on total carbohydrates of 30-days old plants of *B. juncea* under salt stress

Treatment	**Total carbohydrates (mg g^{-1} FW)**
Control	5.79^{def}±0.18
NaCl I	3.77^{abc}±0.17
NaCl II	3.06^{ab}±0.16
NaCl III	2.43^{a}±0.29
TRIA	7.47^{fg}±0.28
TRIA + NaCl I	6.47^{efg}±0.40
TRIA + NaCl II	5.66^{cdef}±0.29
TRIA + NaCl III	4.92^{bcde}±0.10
H_2S	7.61^{gh}±0.31
H_2S + NaCl I	6.07^{def}±0.13
H_2S + NaCl II	5.75^{cdef}±0.37
H_2S + NaCl III	4.44^{bcd}±0.34
TRIA + H_2S	8.16^{g}±0.42
TRIA + H_2S + NaCl I	7.10^{fg}±0.71
TRIA + H_2S + NaCl II	6.21^{defg}±0.50
TRIA + H_2S + NaCl III	5.71^{cdef}±0.29

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

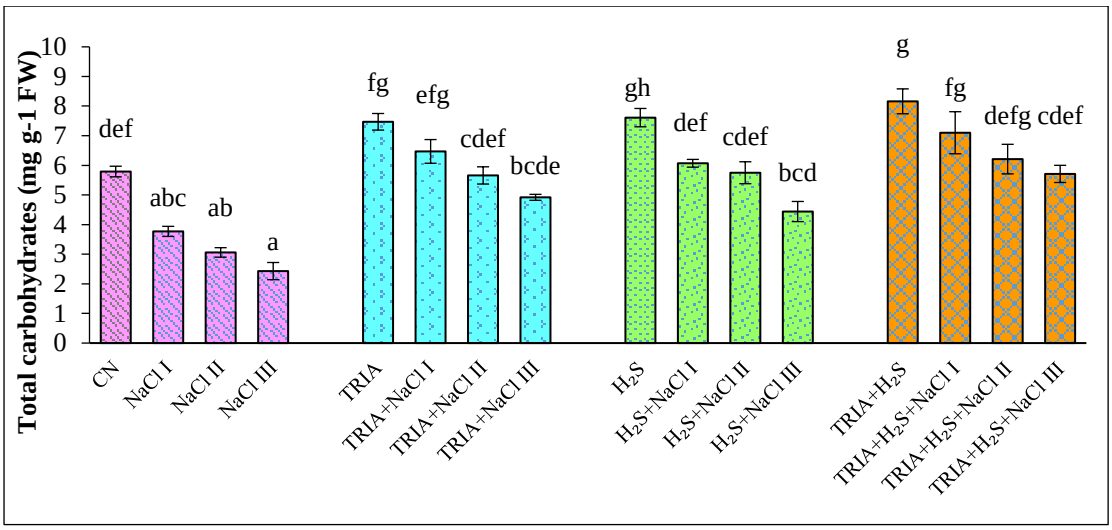


Fig. 6.25 Effect of TRIA and H_2S on carbohydrates content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

6.1.2.7 Protein content and antioxidant defense system

6.1.2.7.1 Protein content and antioxidative enzymes

Content of protein decreased *B. juncea* plants under salt stress (Fig. 6.26; Table 6.22). Protein contents of 7.18, 7.22, and 6.90 mg g^{-1} FW was noticed at salt stress. TRIA showed content of 10.10 mg g^{-1} FW under unstressed condition. Under salt stress, TRIA application reported content of 8.52 mg g^{-1} FW at NaCl I concentration. Pre-treatment of radish plants under stressed conditions also increased the protein content with the highest protein of 8.89 at NaCl I concentration. TRIA and H_2S association showed 9.62, 8.36, and 8.58 mg g^{-1} FW protein contents at salt stress.

Salt stress declined the enzymatic activity of enzyme SOD to 5.63, 5.28, and 4.46 UA mg^{-1} protein (Fig. 6.26; Table 6.22). Control plants reported SOD activity of 7.07 UA mg^{-1} protein. Individual application of TRIA and H_2S under unstressed condition reported maximum SOD activity of 7.28 UA mg^{-1} protein and 7.66 UA mg^{-1} protein at NaCl I concentration. H_2S pre-treatment improved SOD activity to 7.66 UA mg^{-1} protein at NaCl I concentration. TRIA+H_2S+NaCl I treated plants reported highest SOD activity of 8.23 UA mg^{-1} protein and lowest of 6.92 UA mg^{-1} protein at NaCl III concentration.

Salt stressed plants showed decline of 6.71, 6.33, and 6.12 UA mg^{-1} protein (Fig. 6.26;

Table 6.22). in CAT activity at salt stress. TRIA under stressed conditions reported 7.04, 7.22, and 7.08 UA mg^{-1} protein. Likewise, H_2S treated plants reported 7.90, 7.59 and 7.76 UA mg^{-1} protein. TRIA and H_2S application under unstressed condition reported CAT activity of 10.50 UA mg^{-1} protein. Under salt stressed condition, TRIA and H_2S combination showed CAT activity of 10.00 UA mg^{-1} protein in NaCl I treated plants.

Table 6.22 Effect of TRIA and H_2S on protein content and antioxidative enzymes of 30-days old plants of *B. juncea* under salt stress

Treatment	**Protein content (mg g^{-1} FW)**	**SOD (UA mg^{-1} protein)**	**CAT (UA mg^{-1} protein)**	**APX (UA mg^{-1} protein)**
Control	$8.44^{bcd} \pm 0.24$	$7.07^{bcde} \pm 0.57$	$7.54^{abcd} \pm 0.24$	$19.06^{abcd} \pm 0.60$
NaCl I	$7.18^{ab} \pm 0.25$	$5.63^{abc} \pm 0.27$	$6.71^{ab} \pm 0.20$	$17.14^{ab} \pm 0.16$
NaCl II	$7.22^{abc} \pm 0.13$	$5.28^{ab} \pm 0.30$	$6.33^{ab} \pm 0.23$	$17.25^{ab} \pm 0.20$
NaCl III	$6.90^{a} \pm 0.06$	$4.46^{a} \pm 0.30$	$6.12^{a} \pm 0.28$	$14.91^{a} \pm 0.55$
TRIA	$10.10^{fg} \pm 0.11$	$8.80^{fg} \pm 0.49$	$9.14^{def} \pm 0.15$	$20.32^{bcde} \pm 1.09$
TRIA + NaCl I	$8.51^{bcde} \pm 0.29$	$7.28^{cdef} \pm 0.43$	$7.24^{abc} \pm 0.60$	$19.07^{bcd} \pm 0.92$
TRIA + NaCl II	$8.48^{bcde} \pm 0.28$	$6.43^{bcde} \pm 0.23$	$7.32^{abc} \pm 0.29$	$17.58^{abc} \pm 0.46$
TRIA + NaCl III	$7.55^{abcd} \pm 0.23$	$5.97^{abcd} \pm 0.05$	$7.17^{abc} \pm 0.18$	$18.56^{abcd} \pm 0.55$
H_2S	$11.32^{gh} \pm 0.27$	$8.32^{efg} \pm 0.38$	$8.62^{cdef} \pm 0.33$	$22.14^{def} \pm 1.27$
H_2S + NaCl I	$8.69^{de} \pm 0.17$	$7.66^{def} \pm 0.34$	$7.90^{bcde} \pm 0.26$	$21.53^{cdef} \pm 0.75$
H_2S + NaCl II	$8.54^{bcde} \pm 0.24$	$6.42^{bcd} \pm 0.30$	$7.59^{abcd} \pm 0.26$	$20.99^{bcdef} \pm 0.54$
H_2S + NaCl III	$7.19^{ab} \pm 0.41$	$6.13^{abcd} \pm 0.07$	$7.76^{abcd} \pm 0.19$	$24.80^{f} \pm 0.71$
TRIA + H_2S	$11.62^{h} \pm 0.33$	$9.87^{g} \pm 0.60$	$10.50^{g} \pm 0.32$	$24.06^{ef} \pm 1.22$
TRIA + H_2S + NaCl I	$9.62^{ef} \pm 0.22$	$8.23^{efg} \pm 0.26$	$10.00^{fg} \pm 0.57$	$20.03^{bcd} \pm 0.74$
TRIA + H_2S + NaCl II	$8.36^{bcde} \pm 0.30$	$7.76^{def} \pm 0.22$	$9.58^{ef} \pm 0.32$	$22.77^{def} \pm 0.61$
TRIA + H_2S + NaCl III	$8.58^{cde} \pm 0.33$	$6.92^{bcde} \pm 0.53$	$7.63^{abcd} \pm 0.28$	$19.32^{bcd} \pm 1.16$

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

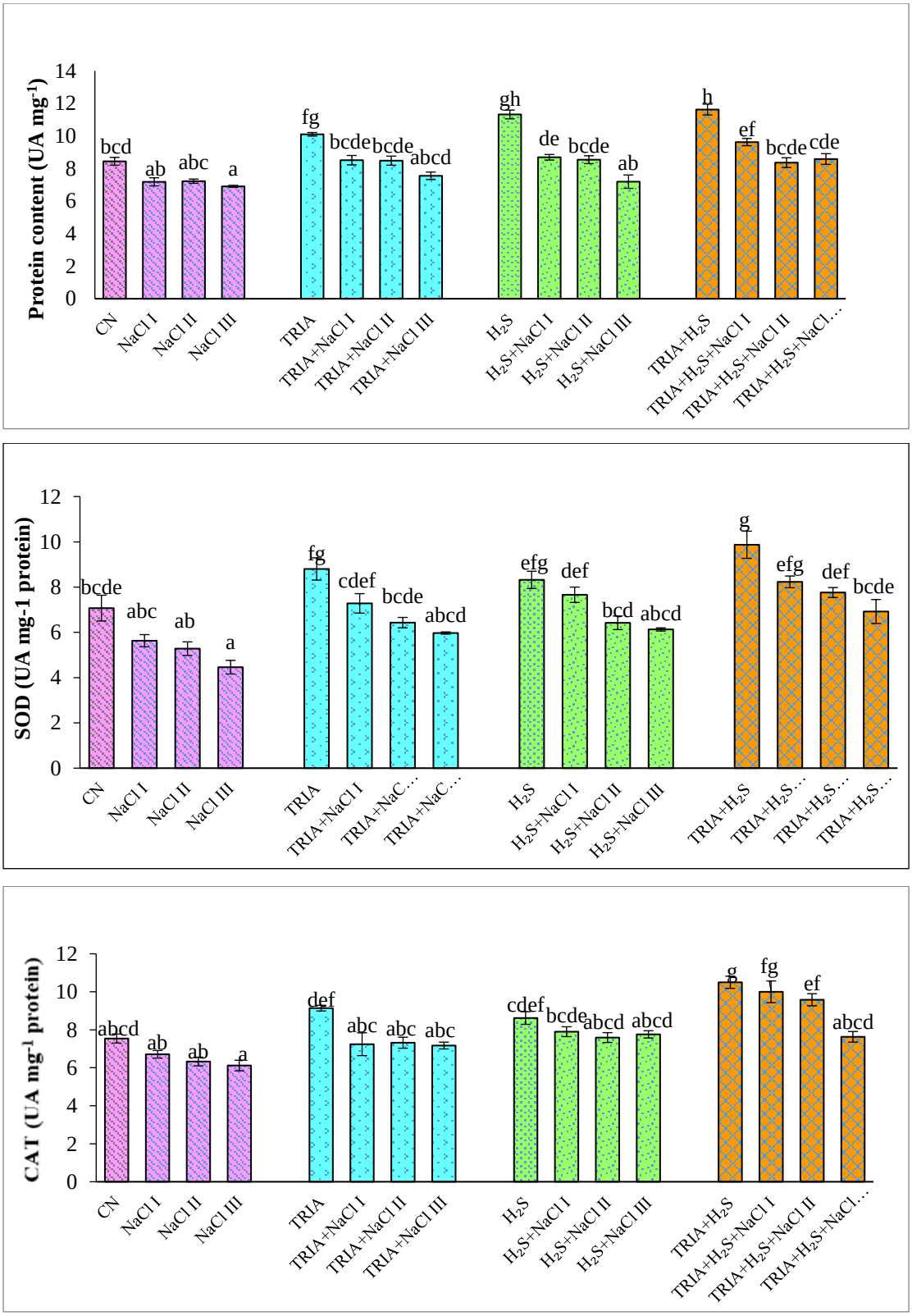


Fig. 6.26 Effect of TRIA and H_2S on protein content, SOD and CAT enzyme activity in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Salt stress reduced the enzymatic activity of enzyme APX and reported minimum APX activity of 14.91 UA mg^{-1} protein at NaCl III concentration (Fig. 6.26; Table 6.22) Both the treatments when used alone under unstressed conditions raised the level of enzyme APX. Maximum APX activity of 19.07 UA mg^{-1} protein was observed in case of TRIA at NaCl I concentration while H_2S treated plants alone reported higher APX activity of 21.53 UA mg^{-1} protein in NaCl I stressed plants. Under unstressed condition, combination of TRIA and H_2S reported highest APX activity of 24.06 UA mg^{-1} protein among all other treatment. In case of salt stressed condition, highest APX enzyme activity of 22.77 UA mg^{-1} protein was noticed at combined treatment in case of salt stressed condition when both were used individually.

Activity of enzyme POD was found to be decreased under different concentration of NaCl. Enzymatic activity of enzyme POD was reduced to 13.65, 12.65 and 12.02 UA mg^{-1} protein (Fig. 6.27; Table 6.23). TRIA and H_2S control plants reported higher POD activities of 20.98 and 21.85 UA mg^{-1} protein which was found to be greater than control plants. Under salt stressed conditions highest POD activities were observed in case of triacontanol and hydrogen sulphide was found at NaCl I concentrations with 18.28 and 18.17 UA mg^{-1} protein POD activities. POD activity of 19.72 UA mg^{-1} protein at NaCl I conc by using triacontanol and hydrogen sulphide among all treatments.

GR activity was found to be reduced under salt stress. Minimum GR activity of 4.77 UA mg^{-1} protein was found at NaCl III concentration (Fig. 6.27 Table 6.23). GR enzymatic activity was found to be reduced as the level of NaCl increased. H_2S foliar spray stressed condition increased the activity of GR. Activity of GR enzyme was raised from 4.77 to 8.08 UA mg^{-1} protein in TRIA treated plants at NaCl I concentration. H_2S treated plants reported highest and lowest GR at NaCl with 7.21 and 5.68 UA mg^{-1} protein, respectively. TRIA and H_2S combination under salt stress reported GR activity 9.25 UA mg^{-1} protein at NaCl I concentration.

GPOX enzyme activity was found to be declined in salt stressed plants. Maximum GPOX activity of 14.17 UA mg^{-1} protein was reported at NaCl I concentration (Fig. 6.28; Table 6.23). Exogenous application of TRIA and H_2S under unstressed condition

reported maximum GPOX activity of 8.41 UA mg^{-1} protein. In case of stressed condition, it was found that TRIA regulated GPOX activity of 18.11 UA mg^{-1} protein at NaCl I concentration.

Table 6.23 Effect of TRIA and H_2S on antioxidative enzymes of 30 days old plants of *B. juncea* under salt stress

Treatment	**POD (UA mg^{-1} protein)**	**GR (UA mg^{-1} protein)**	**GPOX (UA mg^{-1} protein)**	**DHAR (UA mg^{-1} protein)**
Control	15.71^{bcd} ±0.29	6.06^{cde}±0.52	13.36^{bcd}±0.36	8.75^{cde}±0.18
NaCl I	13.57^{abc} ±0.26	4.77^{abc}±0.22	10.46^{abc}±0.31	6.94^{abc}±0.22
NaCl II	12.65^{ab} ±0.84	3.77^{ab}±0.26	8.68^{ab}±0.40	6.24^{ab} ±0.16
NaCl III	12.02^{a} ±0.58	2.98^{a}±0.12	7.78^{a}±0.17	5.39^{a}±0.22
TRIA	20.76^{ghi} ±0.83	9.17^{gh}±0.24	15.29^{hi}±0.43	11.64^{gh}±0.67
TRIA + NaCl I	18.28^{defgh} ±1.76	8.08^{efgh}±0.27	13.86^{efgh}±0.54	9.72^{efg}±0.26
TRIA + NaCl II	18.06^{defg} ±0.04	7.07^{def}±0.12	12.02^{bcde}±0.54	8.28^{bcde}±0.13
TRIA + NaCl III	17.10^{cdef} ±0.50	654^{cde}±0.28	11.11^{bc}±0.52	7.72^{bcde}±0.18
H_2S	20.39^{hi} ±0.71	9.31^{hi}±0.84	14.78^{efgh}±0.50	11.24^{fgh}±0.31
H_2S + NaCl I	18.17^{fghi} ±0.34	7.21^{efghi}±0.12	13.00^{bcdef}±0.58	9.31^{def}±0.28
H_2S + NaCl II	17.20±0.17	6.41^{defg}±0.30	11.32^{bcd}±0.60	8.67^{cde}±0.40
H_2S + NaCl III	16.39^{bcd} ±0.49	5.68^{bcd}±0.35	10.86^{bc}±0.49	7.39^{abcd}±0.30
TRIA + H_2S	23.28^{i} ±0.62	11.36^{i}±0.32	16.93^{i}±0.54	13.02^{h}±0.54
TRIA + H_2S + NaCl I	19.72^{efgh} ±0.73	9.25^{gh}±0.56	14.53^{ghi}±0.58	11.25^{fgh}±0.37
TRIA + H_2S + NaCl II	17.58^{defg} ±0.76	8.66^{fgh}±0.32	13.33^{fgh}±0.38	9.29^{def}±0.83
TRIA + H_2S + NaCl III	16.32^{cde} ±0.32	7.40^{defgh}±0.59	11.30^{cdef}±0.73	8.59^{cde}±0.30

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

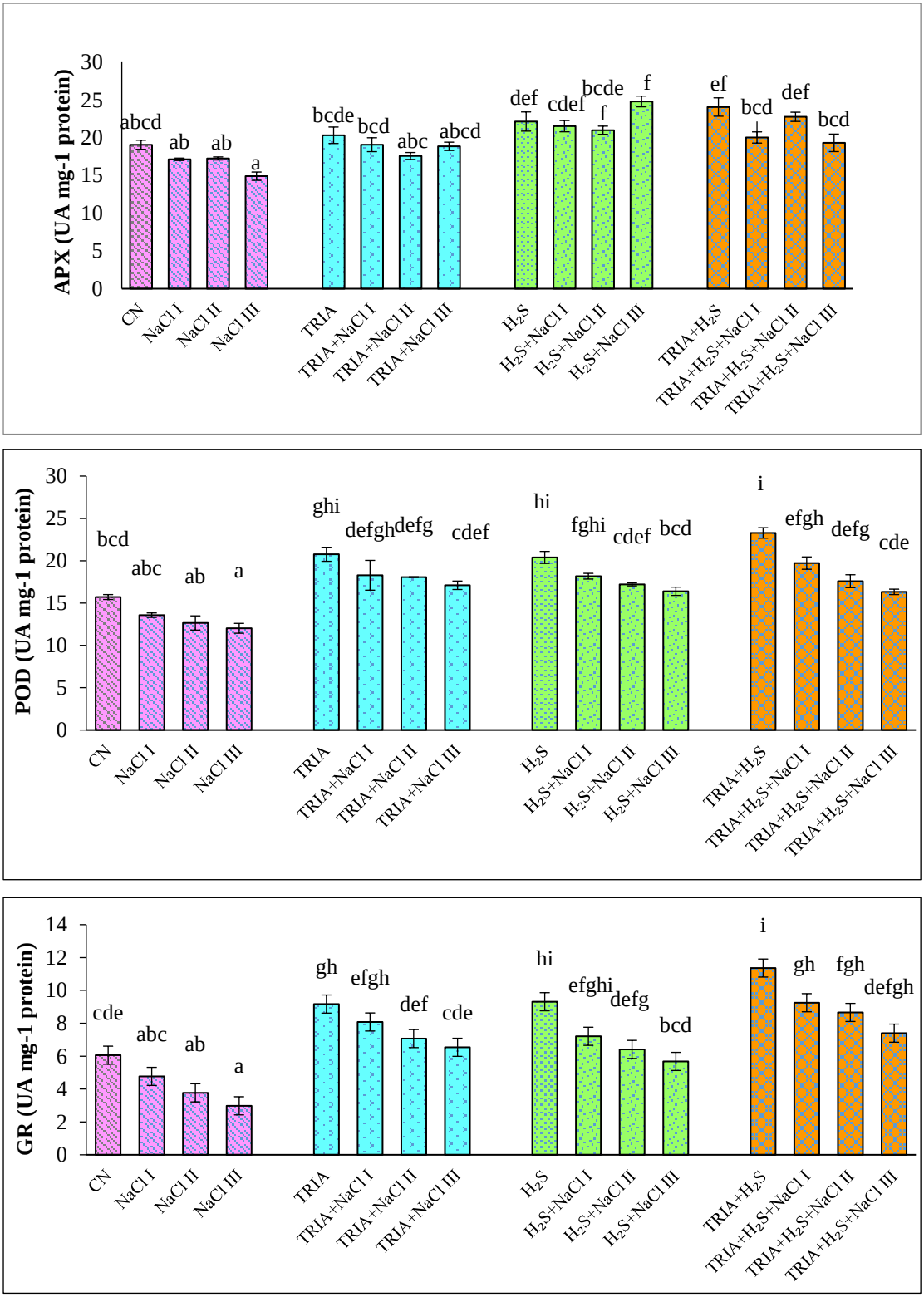
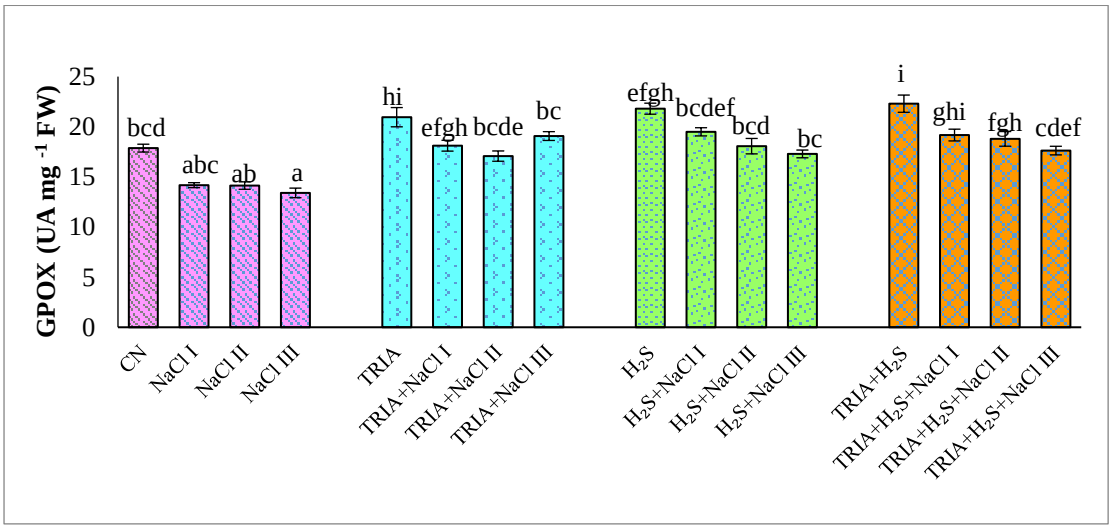
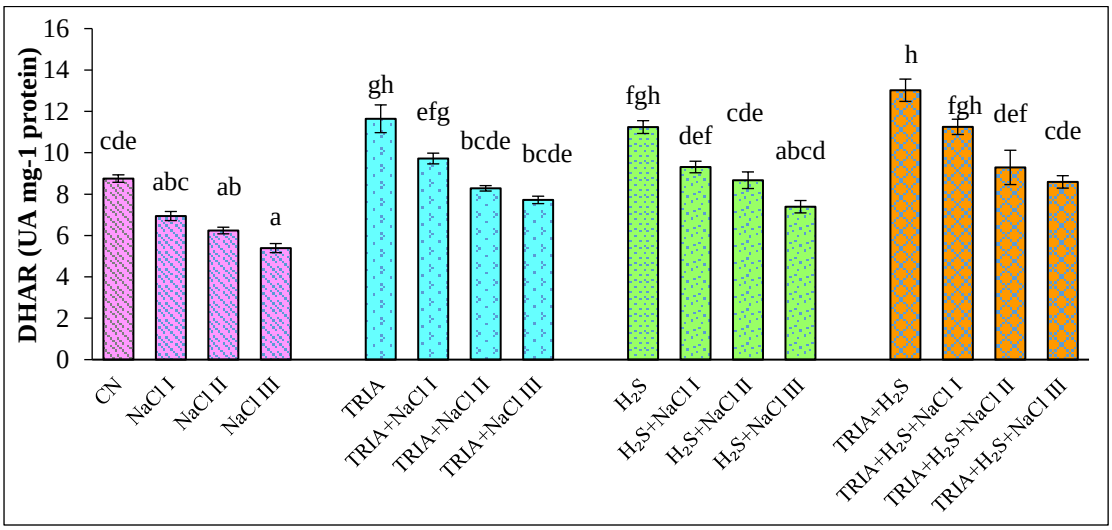


Fig. 6.27 Effect of TRIA and H_2S on APX, POD and GR activity in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Upregulation reported the activity of GPOX to 9.31 UA mg^{-1} protein by using H_2S in NaCl II salt stess conditions. TRIA and H_2S treatment reported maximum and minimum activity of 7.42 and 6.19 UA mg^{-1} protein.

DHAR enzyme level was reduced in plants of *Brassica* under salt stress (Fig. 6.28; Table 6.23). In case of TRIA treated plants, maximum enzymatic activity of 9.72 UA mg^{-1} protein was noticed at NaCl I stressed plants. H_2S under salt stress reported highest DHAR activity of 9.31 UA mg^{-1} protein at NaCl I concentration. Application of TRIA+H_2S alone enhanced the activity of enzyme DHAR to 13.02 UA mg^{-1} protein. Under stressed condition, application of TRIA+H_2S reported maximum DHAR activity of 11.25 UA mg^{-1} protein at NaCl I concentration and minimum of 13.75 UA mg^{-1} protein at NaCl III concentration.





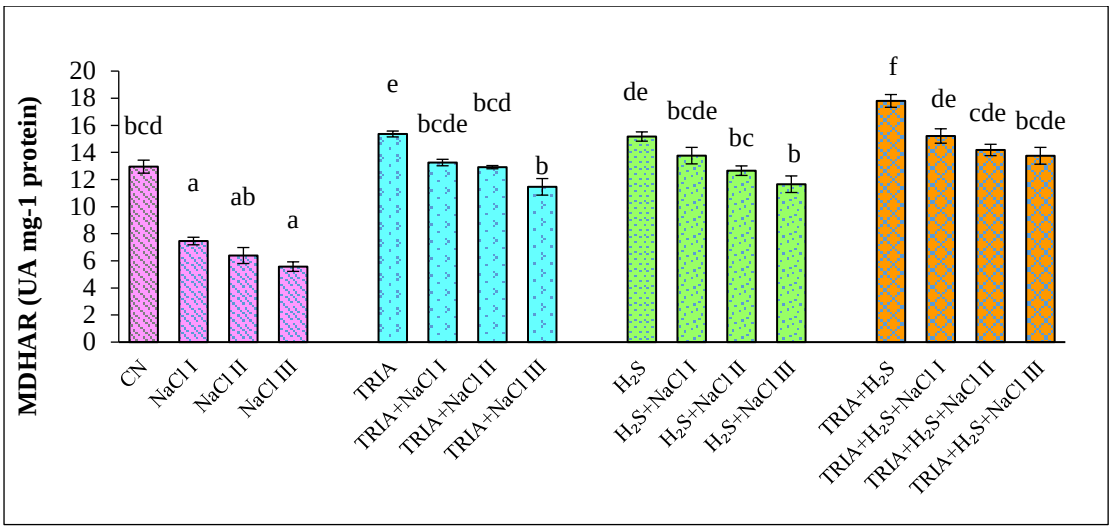


Fig. 6.28 Effect of TRIA and H_2S on GPOX, DHAR, and MDHAR enzyme activity in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

MDHAR activity was found to reduce by elevated level of salt stress (Fig. 6.28; Table 6.24). TRIA treatment under unstressed conditions reported activity of 13.25, 12.91 and 11.46 UA mg^{-1} protein activity. However, H_2S application under salt stressed conditions reported 13.76, 12.65 and 11.65 UA mg^{-1} protein activity. Among all the treatments, TRIA+H_2S under unstressed conditions reported maximum MDHAR activity of 17.80 UA mg^{-1} protein. TRIA+H_2S+NaCl I concentration reported highest MDHAR activity of 15.21UA mg^{-1} protein.

GST activity was found to be reduced in 30-days old plants due to elevated level of NaCl. Maximum GST activity of 3.47 UA mg^{-1} protein was noticed in NaCl III stressed plants (Fig. 6.29; Table 6.24). Control exhibited GST activity of 12.95 UA mg^{-1} protein. TRIA and H_2S individual application under stressed conditions reported maximum GST activity of 7.24 and 7.72 UA mg^{-1} protein under NaCl I stress. Combination of TRIA and H_2S control plants exhibited maximum GST activity of 11.43 UA mg^{-1} protein activity. However, under salt stress association of TRIA and H_2S reported 10.36, 9.50, and 8.47 UA mg^{-1} protein activities at salt stress.

Table 6.24 Effect of TRIA and H_2S on antioxidative enzymes of 30-days old plants of *B. juncea* under salt stress

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
Control	12.95^{bcd}±0.48	7.95^{cde}±0.46	11.43^{c}±0.42
NaCl I	7.46^{a}±0.28	6.03^{abc}±0.07	8.50^{b}±0.28
NaCl II	6.39^{a}±0.59	5.80^{ab}±0.41	6.02^{ab}±0.48
NaCl III	5.57^{a}±0.35	4.50^{a}±0.22	4.95^{a}±0.10
TRIA	15.36^{e}±0.22	10.35^{de}±0.63	14.73^{ef}±0.44
TRIA + NaCl I	13.25^{bcde}±0.24	8.09^{cde}±0.29	13.09^{cde}±0.23
TRIA + NaCl II	12.91^{bcd}±0.12	7.17^{cd}±0.18	12.24^{cd}±0.58
TRIA + NaCl III	11.46^{b}±0.61	6.55^{bcd}±0.31	11.46^{c}±0.61
H_2S	15.17^{de}±0.34	10.46^{fg}±0.26	15.21^{ef}±0.38
H_2S + NaCl I	13.76^{bcde}±0.61	8.21^{de}±0.16	13.73^{cde}±0.62
H_2S + NaCl II	12.65^{bc}±0.35	7.61^{bcde}±0.63	13.65^{cde}±0.67
H_2S + NaCl III	11.65^{b}±0.61	7.02^{bcd}±0.48	12.36^{cd}±0.57
TRIA + H_2S	17.80^{f}±0.47	12.20^{g}±0.54	16.84^{f}±0.84
TRIA + H_2S + NaCl I	15.21^{de}±0.53	10.36^{fg}±0.26	15.62^{ef}±0.32
TRIA + H_2S + NaCl II	14.18^{cde}±0.42	9.50^{ef}±0.37	14.77^{def}±0.39
TRIA + H_2S + NaCl III	13.75^{bcde}±0.62	8.47^{def}±0.25	13.39^{cde}±0.33

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

PPO activity was found to be diminished in salt stressed plants. Minimum activity of 8.50 UA mg^{-1} protein was noticed in NaCl III stressed plants (Fig. 6.29; Table 6.24). However, TRIA application under salinity at PPO enzymatic activity at 13.09 in comparison to salt stressed condition. In case of H_2S-treated plants maximum PPO activity of 13.73 UA mg^{-1} protein was noticed at NaCl I concentration. Synergistic association of TRIA and H_2S reported maximum PPO activities of 15.62 UA mg^{-1} protein at NaCl I concentration and 13.39 UA mg^{-1} protein at NaCl III concentration.

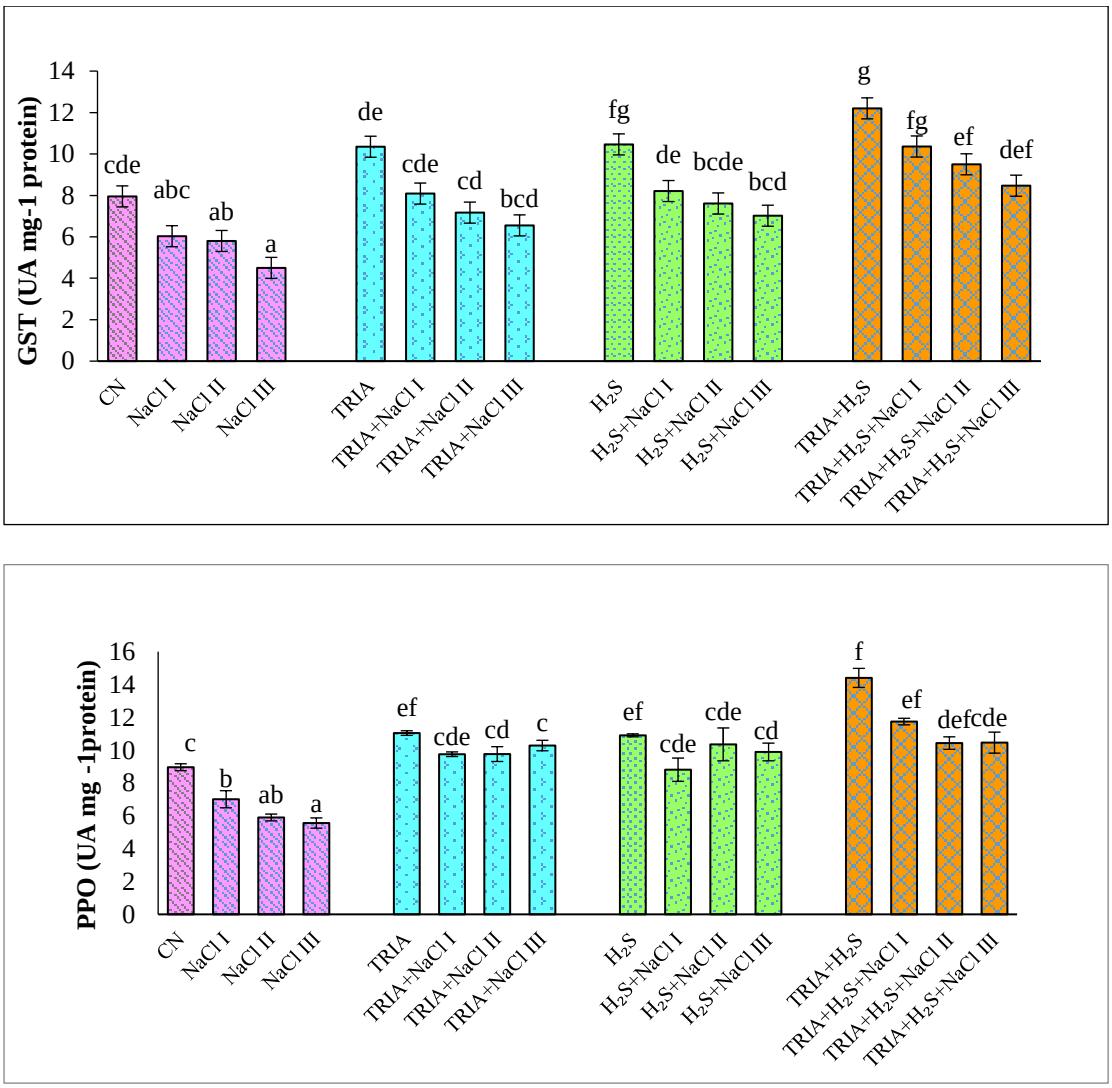


Fig. 6.29 Effect of TRIA and H_2S on GST and PPO enzyme activity in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.2.7.2 Non-enzymatic antioxidants

Ascorbic acid content was found to be lowered in salt stressed plants (Fig. 6.30; Table 6.25). Minimum ascorbic acid content of 3.61 was found at NaCl III concentration. Whereas, TRIA and H_2S application reduced the content of ascorbic acid amount at salinity. Highest ascorbic content of 10.69 µg g^{-1} FW was found at TRIA + H_2S treated plants. Reduction of 10.69, 8.55, and 7.76 µg g^{-1} FW was observed at salinity in the case of combination TRIA and H_2S.

Glutathione content reduced as the level of NaCl increased (Fig. 6.30; Table 6.25). Minimum glutathione content of 4.82 µg g^{-1} FW was found at NaCl II concentration.

Syngergistic response of TRIA and H_2S increased the content of glutathione to 7.62 and 7.44 µg g^{-1} FW. Treated plants reported content of 6.59, 6.11, and 5.53 µg g^{-1} FW at salinity. Triacontanol and hydrogen sulphide raised the content of glutathione to 8.99, 8.83, and 8.58 µg g^{-1} FW in salt stressed conditions.

Table 6.25 Effect of TRIA and H_2S on non-enzymatic antioxidants of 30-days old plants of *B. juncea* under salt stress

Treatment	**Ascorbic acid (µg g^{-1} FW)**	**Glutathione (µg g^{-1} FW)**	**Tocopherol content (µg g^{-1} FW)**
Control	6.06^{bcde} ±0.42	6.93^{def} ±0.46	7.39^{cd}±0.34
NaCl I	4.80^{abc} ±0.35	5.82^{bcd} ±0.08	5.92^{bc}±0.26
NaCl II	4.46^{ab} ±0.31	4.82^{a} ±0.14	4.95^{ab}±0.47
NaCl III	3.61^{a} ±0.31	5.85^{a} ±0.34	3.98^{a}±0.06
TRIA	8.53^{f} ±0.35	7.62^{ef} ±0.28	8.83^{de}±0.64
TRIA + NaCl I	7.01^{def} ±0.57	6.59^{cdef} ±0.09	7.43^{cde}±0.29
TRIA + NaCl II	6.21^{bcde} ±0.17	6.11^{bcd} ±0.06	6.43^{cde}±0.23
TRIA + NaCl III	5.05^{abc} ±0.40	5.53^{bc} ±0.28	6.08^{bc}±0.51
H_2S	8.65^{f} ±0.33	7.44^{ef} ±0.27	8.32^{e}±0.38
H_2S + NaCl I	7.21^{def} ±0.11	6.90^{def} ±0.09	7.66^{de}±0.34
H_2S + NaCl II	6.29^{cde} ±0.28	6.71^{cdef} ±0.32	6.42^{bcd}±0.30
H_2S + NaCl III	5.54^{bcd} ±0.39	6.36^{cdef} ±0.27	6.16^{bc}±0.09
TRIA + H_2S	10.69^{g} ±0.18	10.14^{h} ±0.14	10.29^{f}±0.35
TRIA + H_2S + NaCl I	8.55^{f} ±0.34	8.99^{def} ±0.27	8.93^{e}±0.26
TRIA + H_2S + NaCl II	7.76^{ef} ±0.22	8.83^{cde} ±0.25	7.11^{cde}±0.39
TRIA + H_2S + NaCl III	6.44^{cde} ±0.53	8.58^{bc} ±0.30	6.43^{bcd}±0.26

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

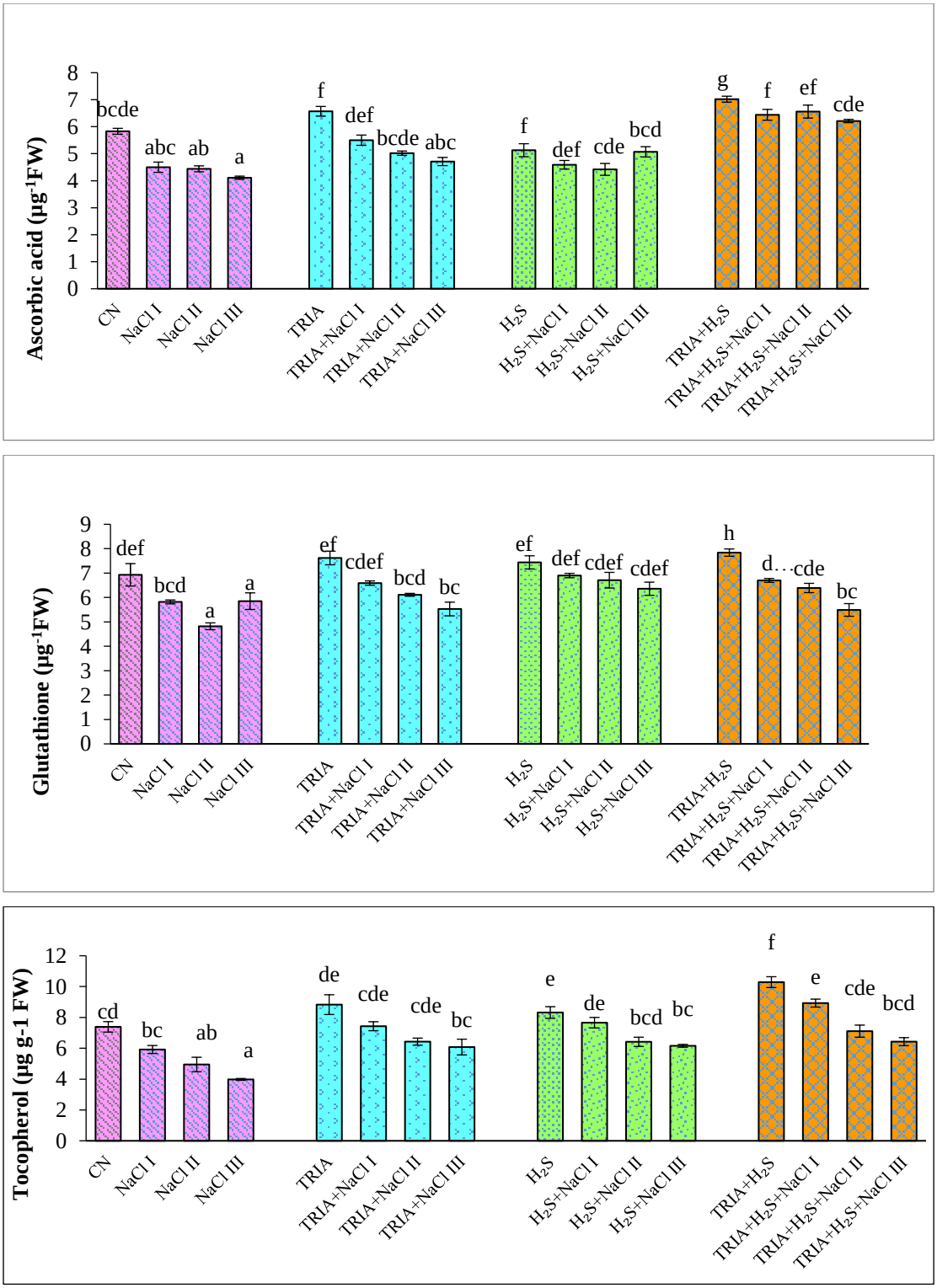


Fig. 6.30 Effect of TRIA and H_2S on ascorbic acid, glutathione and tocopherol content in 30-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Tocopherol content was found to be significantly reduced as the level of NaCl increased (Fig. 6.30; Table 6.25). NaCl III stressed plants showed minimum tocopherol content of 3.98 µg g^{-1} FW which reduced the content among all three concentrations of NaCl used in the study. TRIA and H_2S treated control plants under salt stress reported maximum tocopherol content of 8.83 and 8.32 µg g^{-1} FW at NaCl II concentration. Highest tocopherol content of 8.93 µg g^{-1} FW was found in TRIA+ H_2S + NaCl I treated plants.

6.1.2.8 Gene expression

Among all the treatments, lowest relative gene expression with 0.90-fold change was noticed in NaCl III stressed plants (Fig. 6.31; Table 6.26). Control reported relative gene expression of 1.25-fold change. TRIA and H_2S alone reported 4.26 and 4.92-fold changemin case of salinity. Whereas, synergistic association of triacontanol and hydrogen sulphide reported gene expression of SOD gene with 8.62-fold change under salt-stressed conditions.

Table 6.26 Effect of TRIA and H_2S on relative gene expression of SOD and CAT in *B. juncea* plants under salt stress

Treatment	**Relative gene expression**	
	SOD	**CAT**
Control	1.25^{a}±0.12	2.00^{a}±0.34
NaCl III	0.90^{a}±0.24	1.50^{a}±0.19
TRIA + NaCl III	4.26^{b}±0.37	5.62^{b}±0.40
H_2S + NaCl III	4.92^{b}±0.67	6.37^{bc}±0.31
TRIA + H_2S + NaCl III	8.01^{c}±0.27	7.62^{c}±0.29

*Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments**.**

Minimum expression of CAT gene was showed in control plants with 2.00-fold change (Fig. 6.31; Table 6.26). NaCl III stressed plants reported maximum relative gene expression of 1.50-fold change. Individual application under stressed condition increased the expression of CAT gene with 5.62 and 6.37-fold change, respectively. In case of NaCl III, association TRIA and H_2S showed 7.62-fold change in expression of gene CAT which was found to be highest among all the treatments. Synergistic

treatment reported 7.62-fold change in CAT gene was found to be highest among all treatments.

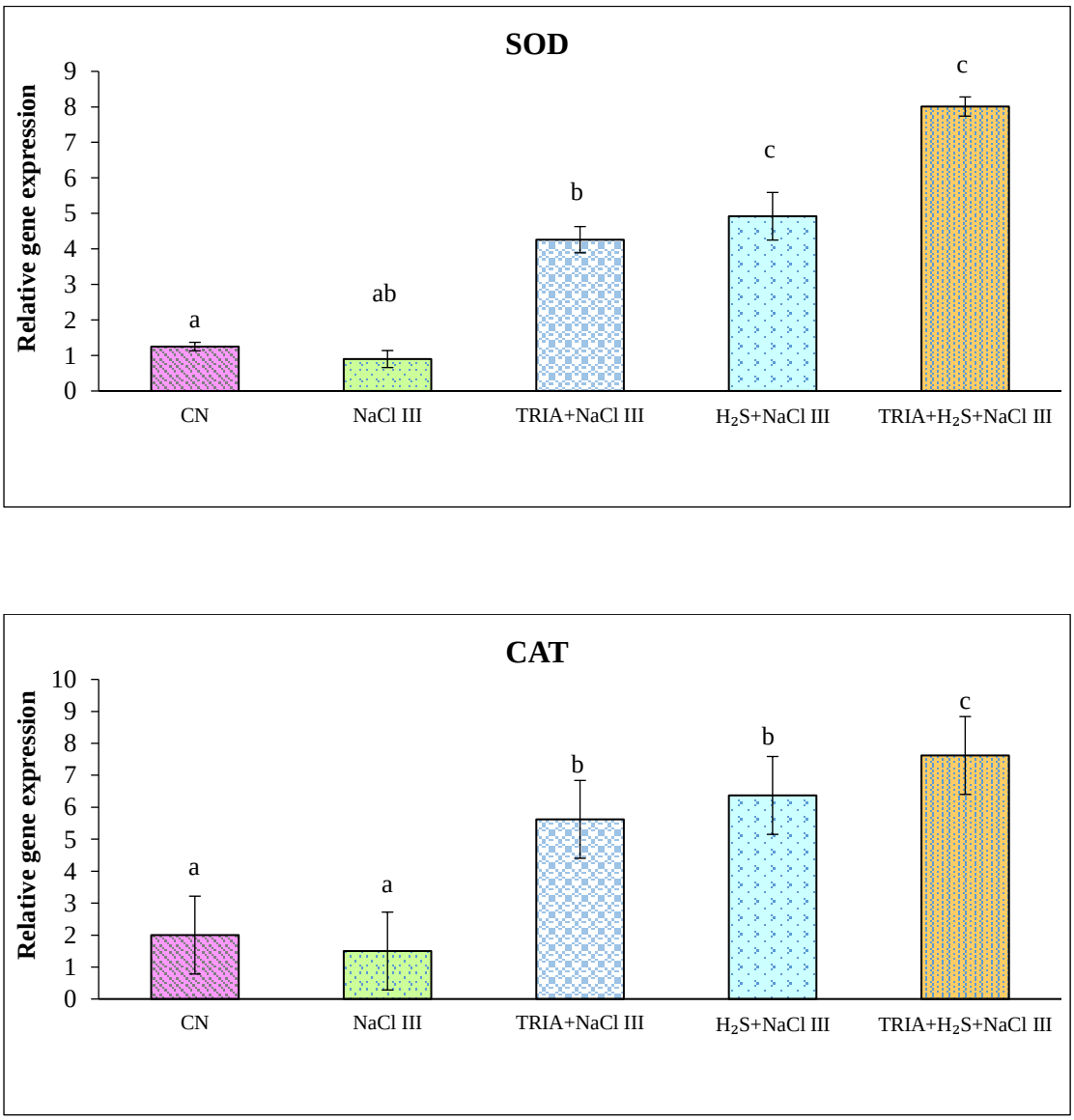


Fig. 6.31 Effect of TRIA and H_2S on relative gene expression of SOD and CAT genes in *B. juncea* plants under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Fig. 6.32 Indiviual and combined effect of TRIA, H_2S and NaCl in 7-days seedlings and 30 and 60 days old plants of *B. juncea*.

6.1.3 60-days old plants

6.1.3.1 Plant growth

Length of root reduced under stressed condition (Fig. 3.2; Table 6.27) Minimum root length was found to be 5.78cm at NaCl III concentration. Control seedlings reported root length of 8.14 cm in stressed plants. TRIA treated plants reported decrease of 7.96 cm in root length at NaCl I concentration. However, TRIA treated plants under unstressed conditions showed significantly higher root length of 10 cm. Likewise, application of H_2S under stressed condition noticed root length of 7.99 cm at NaCl III concentration. Whereas, H_2S under unstressed conditions reported significantly higher root length of 9.93cm. Results depicted that triacontanol and hydrogen sulphide mitigated by increasing root length. Triacontanol and hydrogen sulphide co-application of alleviated salinity stress by increasing root length in plants of *Brassica*. TRIA+H_2S application under stressed condition reported decrease in root length of 9.66 cm

Shoot length was found to be significantly reduced in plants of *B. juncea* under salinity stress (Fig. 32; Table 6.27). NaCl conc reported reduced shoot length from 9.29 cm to 7.25 cm in contrast to control plants (10.66 cm). TRIA+NaCl I treated plants reported shoot length of 13.51cm in comparison to plants treated alone with TRIA (14.85 cm). H_2S treatment reported increase in shoot length of 14.55cm when treated alone. However, shoot length showed significant reduction of 11.77 cm at NaCl I concentration when treated with H_2S. Synergistic association of TRIA and H_2S improved the shoot length to 15.33 cm.

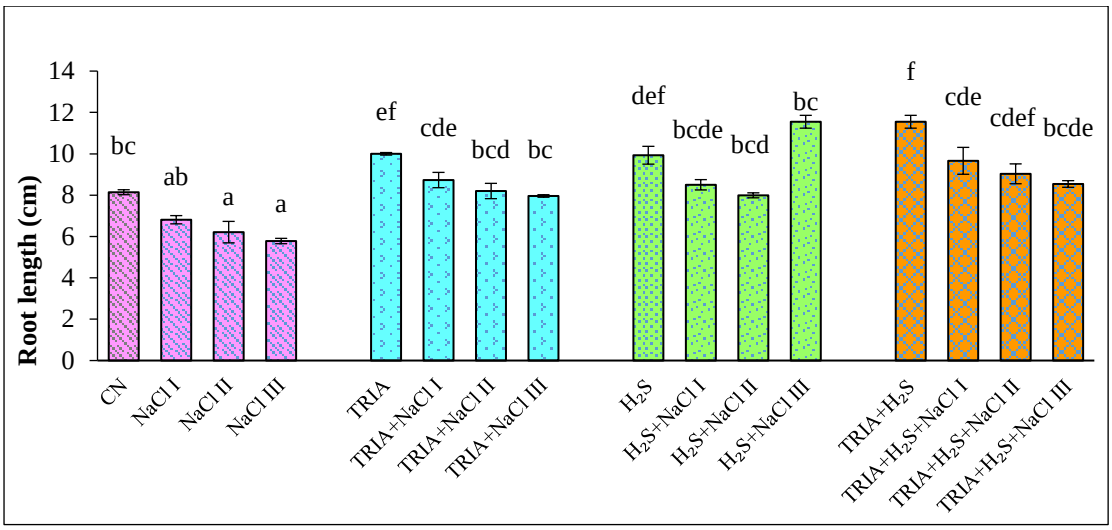
Fresh weight of plants *Brassica* was found to be drastically reduce under salinity stress (Fig. 6. 32; Table 6.27). In NaCl III treated plants fresh weight reported maximum reduction of 1.78g in contrast to control seedlings (4.33g). TRIA Application under unstressed condition increased the fresh weight by 6.09g. However maximum and minimum fresh weights under TRIA application i.e., 5.56g and 4.15g were observed at NaCl I and NaCl III concentration, respectively. Likewise, H_2S treatment reported maximum decrease of 3.66g in fresh weight at NaCl III concentration. Combined treatment of TRIA and H_2S significantly enhanced the fresh weight under unstressed conditions in comparison to their individual application.

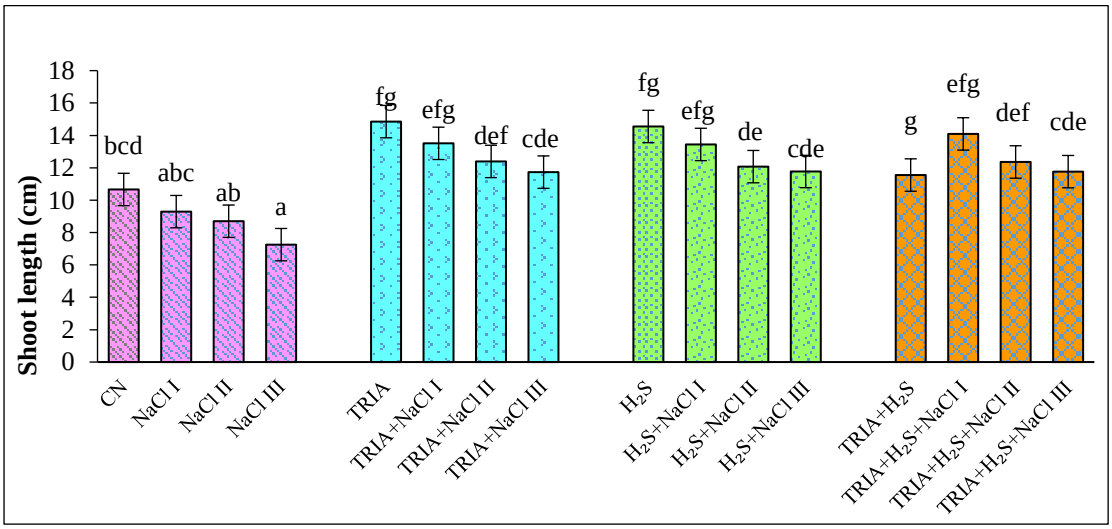
Table 6.27 Effect of TRIA and H_2S on morphological parameters and 60 days old plants of *B. juncea* under salt stress

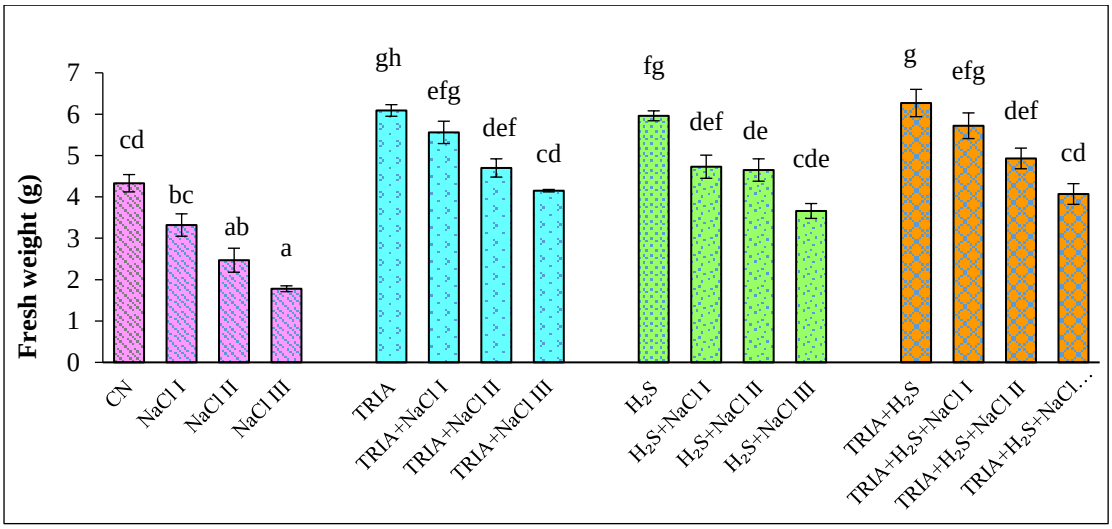
Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Relative water content (%)
Control	8.14^{bc}±0.12	10.66^{bcd}±0.32	4.33^{cd}±3.85	1.95^{cd}±0.04	79.06^{def}± 2.64
NaCl I	6.81^{ab}±0.20	9.29^{abc}±0.23	3.32^{bc} ±0.27	1.40^{abc}±0.02	68.36^{bc}± 1.99
NaCl II	6.21^{a}±0.52	8.70^{ab}±0.34	2.47^{ab}±0.29	0.91^{ab}± 0.10	70.07^{ab}± 5.77
NaCl III	5.78^{a}±0.13	7.25^{a}±0.12	1.78^{a} ±0.07	0.82^{a}± 0.22	65.28^{a}± 3.80
TRIA	10.00^{ef}±0.06	14.85^{fg}±0.32	6.09^{gh}±0.14	3.71^{gh}±0.23	85.79^{gh}± 3.43
TRIA + NaCl I	8.73^{cde}±0.37	13.51^{efg}±0.75	5.56^{efg}±0.27	2.92^{efg}±0.07	83.13^{efgh}±4.09
TRIA + NaCl II	8.20^{bcd}±0.37	12.39^{def}±0.69	4.70^{def}±0.22	2.39^{def}±0.20	76.36^{cdef}±3.15
TRIA + NaCl III	7.96^{bc}±0.06	11.73^{cde}±0.26	4.15^{cd}±3.89	1.81^{bcd}±0.18	73.31^{cd}± 3.76
H_2S	9.93^{def}±0.43	14.55^{fg}±0.33	5.96^{fg}±0.12	3.73^{gh}± 0.21	87.10^{gh}± 1.00
H_2S + NaCl I	8.50^{bcde}±0.25	13.44^{efg}±0.85	4.73^{def}±0.28	2.50^{de}± 0.15	81.35^{defg}± 1.43
H_2S + NaCl II	8.32^{bcd}±0.22	12.07^{de}±0.63	4.65^{de}±0.27	2.28^{cde}±0.25	77.73^{cde}±1.76
H_2S + NaCl III	7.99^{bc}±0.12	11.77^{cde}±0.45	3.66^{cde}±0.18	1.41^{abc}±0.07	74.03^{cd}± 1.46
TRIA + H_2S	11.55^{f}±0.31	15.33^{g}±0.33	6.27^{g}±0.33	3.97^{h}±0.12	89.33^{h}± 0.84
TRIA + H_2S + NaCl I	9.66^{cde}±0.65	14.09^{efg}±0.63	5.72^{efg}±0.31	3.50^{fgh}±0.10	82.06^{fgh}± 0.64
TRIA + H_2S + NaCl II	9.03^{cdef}±0.48	12.46^{def}±0.25	4.93^{def}±0.25	2.65^{def}±0.29	80.50^{defg}± 0.93
TRIA + H_2S + NaCl III	8.54^{bcde}±0.16	11.76^{cde}±0.23	4.07^{cd}±0.25	2.41^{de}±0.28	77.83^{cdef}± 1.33

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

At different concentrations of NaCl I, II and III, dry weight was found to decrease by 1.40g, 0.91g and 0.82g in comparison to control plants (Fig. 6.32; Table 6.27). At NaCl I concentration, supplementation of TRIA and H_2S alone under salt stress produced maximum dry weights of 3.71g and 3.73g at NaCl I concentration. TRIA+H_2S treatment showed better results in increasing dry weight as compared to their individual treatment. TRIA+H_2S+NaCl I stress reported highest dry weight of 3.50g at NaCl I concentration


Root length (cm)
0
2
4
6
8
10
12
14
bc
ab
a
a
ef
cde
bcd
bc
def
bcde
bcd
bc
f
cde
cdef
bcde
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Shoot length (cm)
0
2
4
6
8
10
12
14
16
18
bcd
abc
ab
a
fg
efg
def
cde
fg
efg
de
cde
g
efg
def
cde
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl III


Fresh weight (g)
0
1
2
3
4
5
6
7
cd
bc
ab
a
gh
efg
def
cd
fg
def
de
cde
g
efg
def
cd
CN
NaCl I
NaCl II
NaCl III
TRIA
TRIA+NaCl I
TRIA+NaCl II
TRIA+NaCl III
H2S
H2S+NaCl I
H2S+NaCl II
H2S+NaCl III
TRIA+H2S
TRIA+H2S+NaCl I
TRIA+H2S+NaCl II
TRIA+H2S+NaCl…

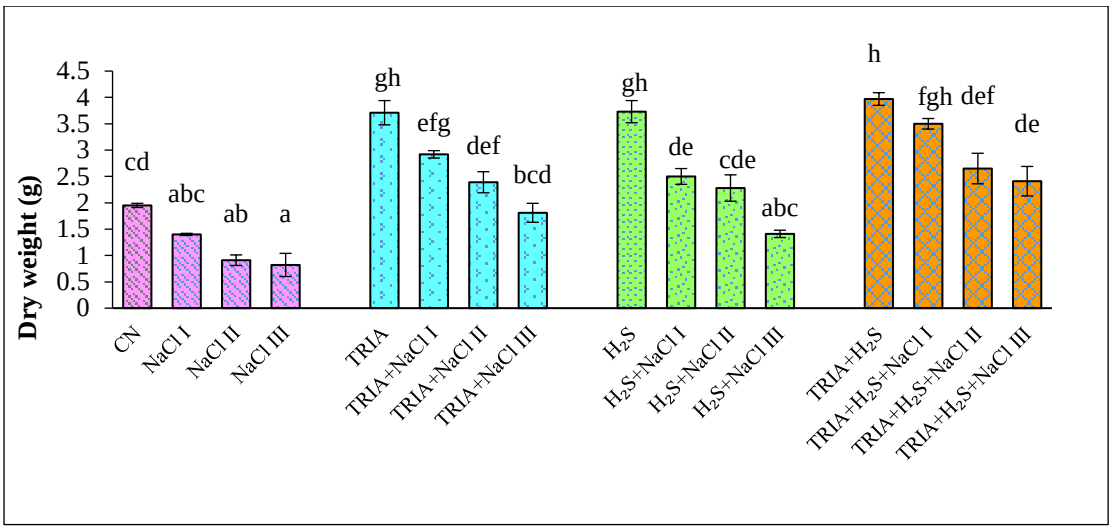


Fig. 6.33 Effect of TRIA and H_2S on root length, fresh and dry weight in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

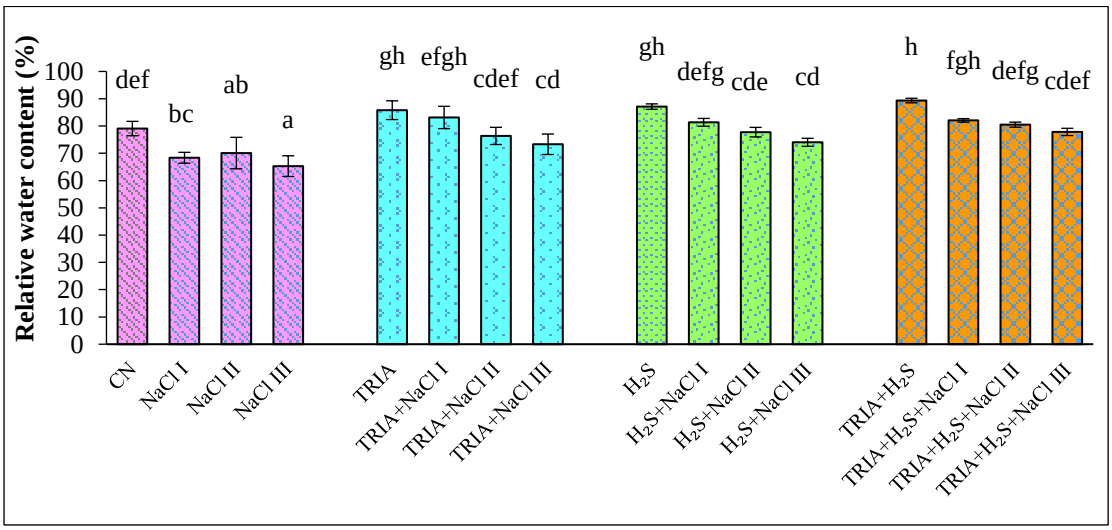


Fig. 6.33 Effect of TRIA and H_2S on relative water content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

Salt stress exposure decreased relative water content in *Brassica* plants (Fig. 6.33; Table 6.28). Lowest relative water content of 65.28% was observed at NaCl III concentration. TRIA application under unstressed condition showed 85.79% relative water content. However, treatment of TRIA under stressed condition showed maximum relative water content of 83.13% at NaCl I concentration. H_2S applied plants showed that relative water content was found to decrease 81.35%, 77.73% and 74.03% at NaCl I, II and III concentration in comparison to H_2S control seedlings.

Relative water content was found to be 82.06% when applied together with TRIA+H_2S at NaCl I concentration.

6.1.3.2 Photosynthetic activity

6.1.3.2 Photosynthetic pigments

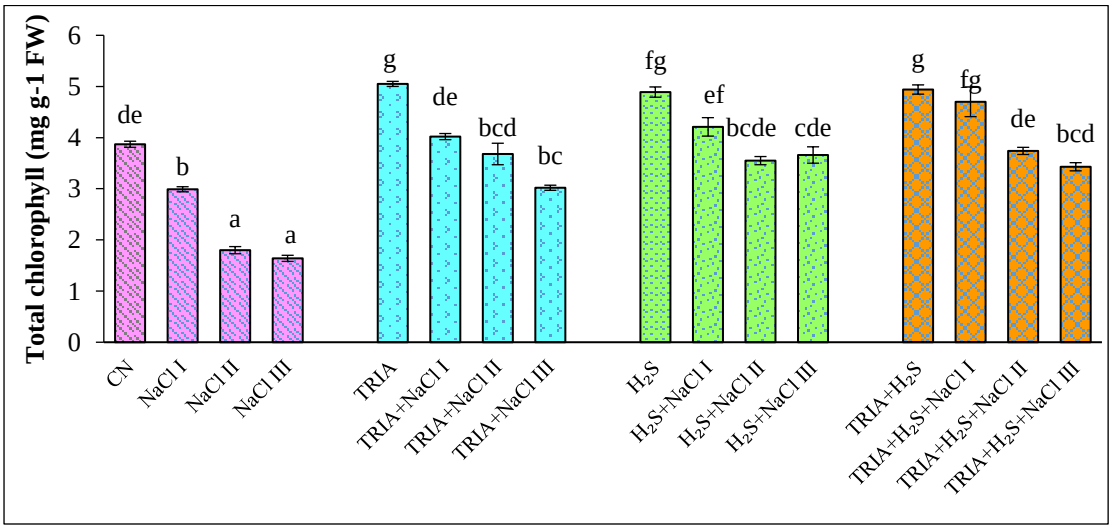
Due to salinity stress, a significant decrease in photosynthetic content was recorded in 60 day old plants (Fig. 6.28; Table 6.29). Lowest value of total chlorophyll (1.64 mg g^{-1} FW) was observed in plants exposed to NaCl III concentration than control plants (3.87 mg g^{-1} FW). Application of TRIA and H_2S alone or in combination enhanced the total chlorophyll content. Under salinity stress, highest total chlorophyll content i.e., 4.02 and 4.21 mg g^{-1} FW was observed at NaCl I concentration when treated individually with TRIA and H_2S respectively. Combined application of TRIA and H_2S under stress condition showed better results in improving total chlorophyll content in comparison to individual treatment. Highest total chlorophyll content of 4.70 mg g^{-1} FW was noticed in TRIA+H_2S+NaCl I treated plants. Similar results were observed in case of chl a and chl b contents in *Brassica* plants. Content of chl a (1.27 mg g^{-1} FW) and chl b (0.21 mg g^{-1} FW) was found to decrease at NaCl III concentration. Synergistic association of TRIA and H_2S significantly alleviated salinity stress with the highest 2.95 and 1.50 mg g^{-1} FW contents, respectively at NaCl I concentration. The data revealed that combination of TRIA and H_2S mitigated negative impact of salinity stress by increasing the amount of pigment formed.

Salinity stress drastically decreased carotenoid content in plants (Fig. 6.35; Table 6.29). It was found that carotenoid content decreased with increase in the concentration of NaCl. Reduction of 1.32 mg g^{-1} FW was observed in NaCl III treated plants in comparison to control (4.22 mg g^{-1} FW). TRIA treatment under salinity stress reported increase in carotenoid content from 5.05 to 3.0 mg g^{-1} FW in TRIA + NaCl I plants. Likewise, H_2S under salinity stress showed increased carotenoid content with highest content (4.21 mg g^{-1} FW) at NaCl I concentration. Combined TRIA with H_2S mitigated salinity stress by increasing carotenoid content in plants.

Table 6.28 Effect of TRIA and H_2S on morphological parameters and of 60-days old plants of *B. juncea* under salt stress

Treatment	Total chlorophyll (mg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)
Control	3.87^{de} ±0.06	1.66^{abc}± 0.03	0.81^{cde} ±0.03
NaCl I	2.99^{b}± 0.05	1.41^{ab}±0.04	0.67^{ab} ±0.02
NaCl II	1.80^{a}± 0.07	1.26^{a}±0.03	0.48^{b}± 0.04
NaCl III	1.64^{a}± 0.06	1.27^{a}±0.01	0.21^{a} ±0.01
TRIA	5.05^{g}± 0.05	3.29^{f}±0.29	1.54^{ij}± 0.04
TRIA + NaCl I	4.02^{de} ±0.06	2.97^{ef} ±0.10	1.23^{gh} ±0.03
TRIA + NaCl II	3.68^{bcd}±0.21	2.34^{cde}± 0.26	1.01^{defg}± 0.09
TRIA + NaCl III	3.02^{bc} ±0.05	1.92^{abc}±0.02	0.76^{cd} ±0.07
H_2S	4.89^{fg}± 0.10	2.98^{ef}±0.06	1.60^{ij} ±0.04
H_2S + NaCl I	4.21^{ef} ±0.18	2.74±0.20	1.24^{gh} ±0.02
H_2S + NaCl II	3.55^{bcde}± 0.08	2.18^{def}± 0.19	1.03^{efg} ±0.03
H_2S + NaCl III	3.66^{cde}± 0.16	1.58^{ab}±0.04	0.76^{cd}±0.06
TRIA + H_2S	4.94^{g}± 0.09	4.07^{g}± 0.03	1.79^{j}± 0.03
TRIA + H_2S + NaCl I	4.70^{fg} ±0.29	2.95^{ef}±0.09	1.50^{hi} ±0.03
TRIA + H_2S + NaCl II	3.74^{de}±0.07	2.29^{cde}±0.09	1.09^{fg}± 0.04
TRIA + H_2S + NaCl III	3.43^{bcd} ±0.08	2.16^{bcd}± 0.06	0.84^{cdef} ±0.08

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments



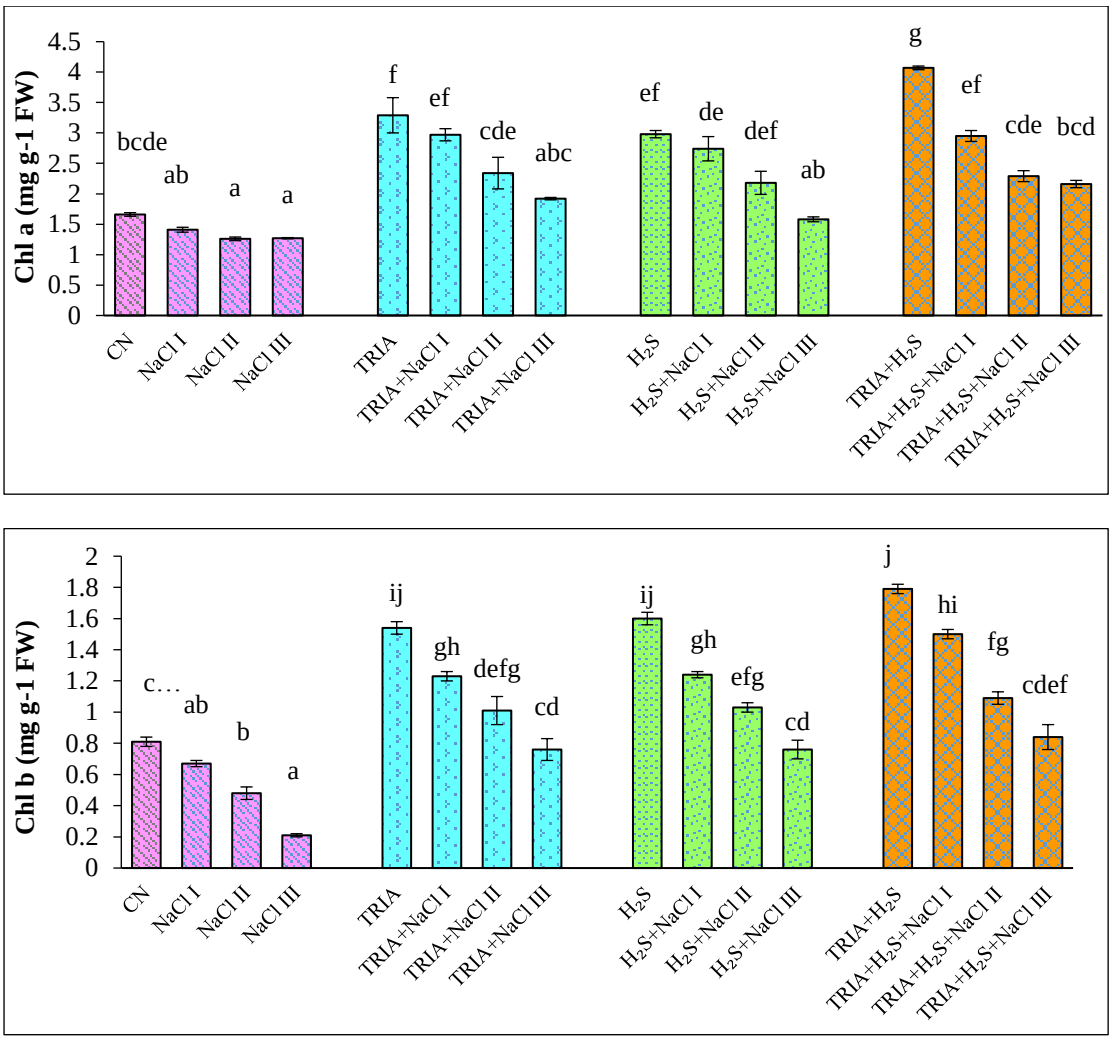


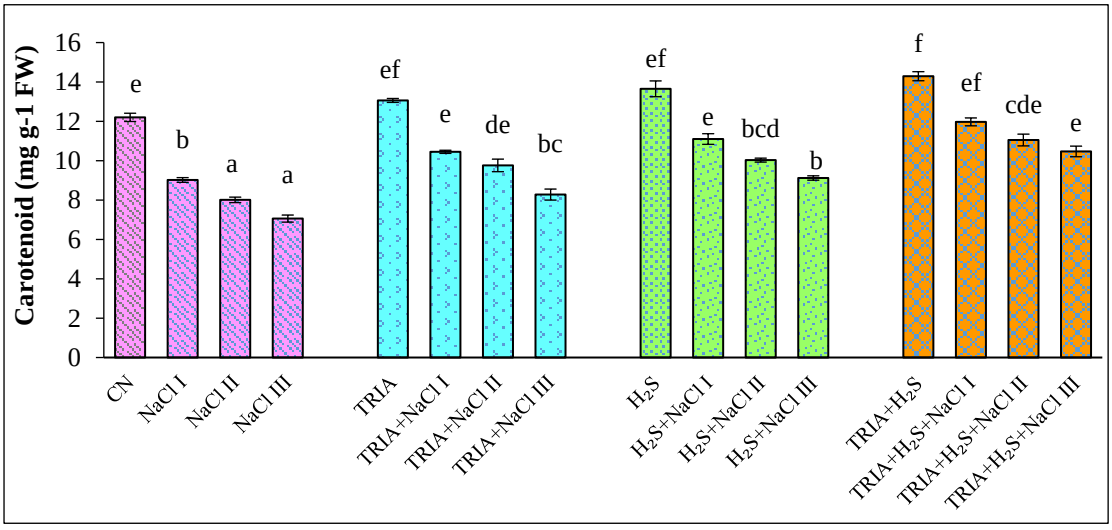
Fig. 6.34 Effect of TRIA and H_2S on total chlorophyll, chl a and chl b in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

Xanthophyll content was reduced in *Brassica* plants under salinity stress (Fig. 6.29; Table 6.29). Increased Concentration of NaCl significantly decreased xanthophyll content from 9.02 mg g^{-1} FW to 7.06 mg g^{-1} FW. TRIA, H_2S and TRIA+H_2S treatments significantly enhanced xanthophyll content in plants. Control reported xanthophyll content of 12.20 mg g^{-1} FW which was significantly higher to salt stressed plants. Combination of triacontanol and hydrogen sulphide decreased xanthophyll content under salt-stressed conditions. Highest xanthophyll content of 13.06 and 13.65 mg g^{-1} FW was observed in case TRIA and H_2S.

Table 6.29 Effect of TRIA and H_2S on photosynthetic pigments of 60-days old plants of *B. juncea* under salt stress

Treatment	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
Control	4.22^{e}± 0.21	12.20^{fg}± 0.12
NaCl I	3.01^{b}± 0.12	9.02^{bc} ±0.11
NaCl II	1.85^{a}± 0.14	8.01^{ab}± 0.01
NaCl III	1.32^{a} ±0.18	7.06^{a} ±0.09
TRIA	5.05^{ef}± 0.10	13.06^{gh}±0.08
TRIA + NaCl I	3.99^{e}± 0.08	10.45^{de}±0.19
TRIA + NaCl II	3.84^{de} ±0.32	9.76^{cd}±0.06
TRIA + NaCl III	3.23^{bc} ±0.28	8.28^{ab} ±0.43
H_2S	5.10^{ef}± 0.04	13.65^{h} ±0.22
H_2S + NaCl I	4.03^{e}± 0.27	11.10^{ef}±0.12
H_2S + NaCl II	3.34^{bcd} ±0.10	10.03±0.37
H_2S + NaCl III	2.95^{b} ±0.11	9.12^{cde}±0.04
TRIA + H_2S	5.54^{f} ±0.23	14.29^{i}±0.11
TRIA + H_2S + NaCl I	5.00^{ef} ±0.20	11.97^{fg}±0.05
TRIA + H_2S + NaCl II	4.08^{cde} ±0.30	11.05^{ef}±0.49
TRIA + H_2S + NaCl III	3.75^{e} ±0.27	10.47^{de}±0.43

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments



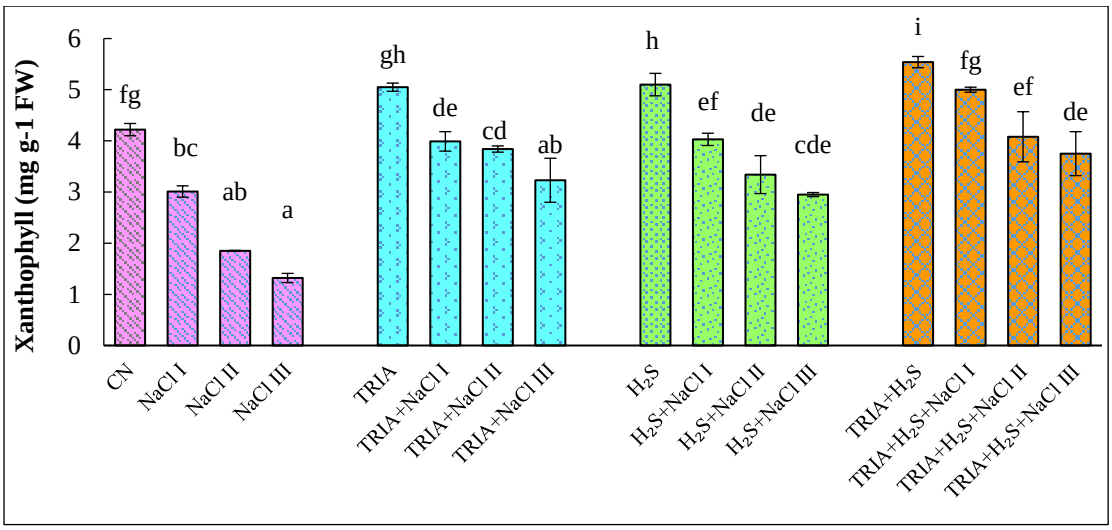


Fig. 6.35 Effect of TRIA and H_2S on carotenoid and xanthophyll content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.3.3 Gas exchange parameters

Salinity stress significantly decreased photosynthetic rate with lowest concentration of 9.07 m mol CO_2 m^{-2} S^{-1} at NaCl III concentration. (Fig. 6.36; Table 6.30). Pre-treatment of TRIA increased the photosynthetic rate from 14.70 to 16.50 m mol CO_2 m^{-2} S^{-1} at NaCl I concentration. H_2S enhanced the photosynthetic rate under salinity stress with highest photosynthetic rate at 15.52 m mol CO_2 m^{-2} S^{-1} at NaCl I concentration. TRIA application recorded higher photosynthetic rate in comparison to H_2S application. TRIA+H_2S treatment increased the photosynthetic rate from 15.61 to 17.06 m mol CO_2 m^{-2} S^{-1} at NaCl I concentration.

Stomatal conductance with elevation of NaCl concentration decreased drastically. Lowest stomatal conductance of 0.44 m mol H_2O m^{-2} s^{-1} was found at NaCl III concentration (Fig. 6.36; Table 6.30). Alone treatment of TRIA + H_2S under salinity caused rise in the level of stomatal conductance. TRIA and H_2S control exhibited higher stomatal conductance of 1.30 and 1.45 m mol H_2O m-2 s-1. Together TRIA + H_2S exhibited elevation in stomatal conductance from 17.06, 16.31 to 15.61 m mol H_2O m-2 s-1in salt treated plants, respectively.

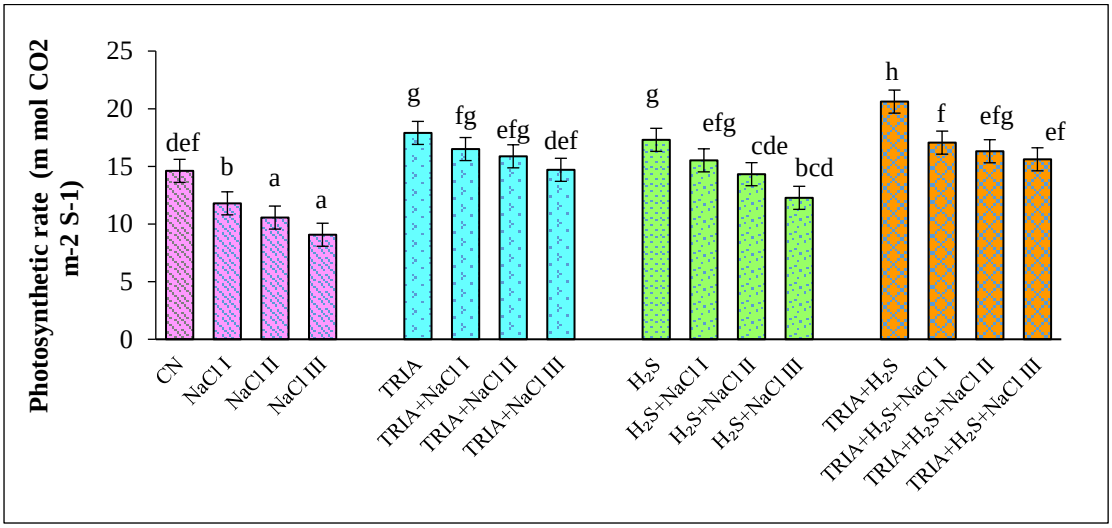
Table 6.30 Effect of TRIA and H_2S on gas exchange parameters of 60-days old plants of *B. juncea* under salt stress

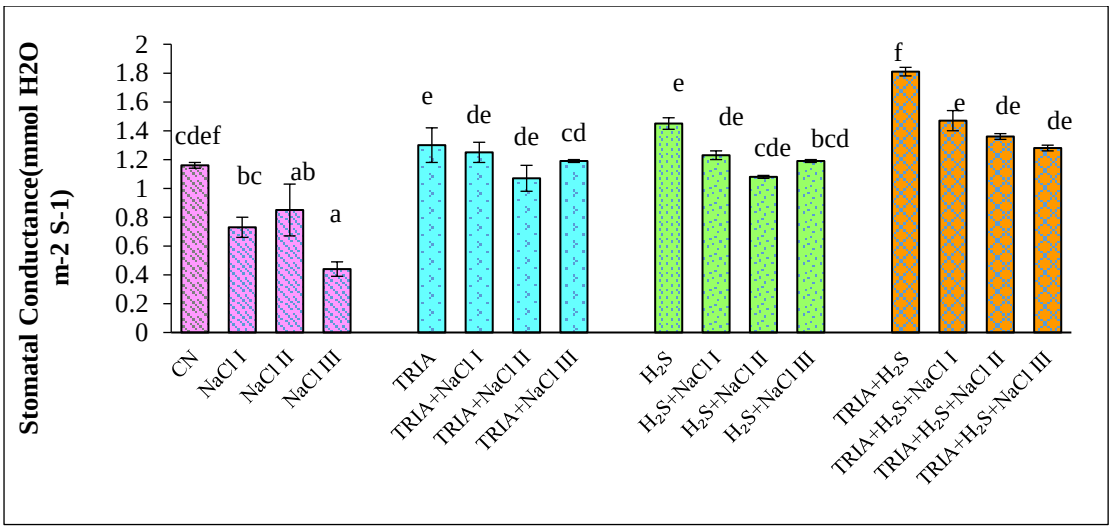
Treatment	**Photosynthetic rate (m mol CO_2 m^{-2} S^{-1})**	**Stomatal conductance (m mol H_2O m^{-2} S^{-1})**	**Intercellular CO_2 concentration (ppm)**	**Transpiration rate (m mol H_2O m^{-2} S^{-1})**
Control	14.61^{def} ±0.72	1.16^{cdef}± 0.02	462.33^{cde}±25.31	1.38^{bcde}±0.05
NaCl I	11.79^{bc} ±0.59	0.73^{bc}± 0.07	367.66^{ab}±17.07	0.94^{abc}±0.32
NaCl II	10.56^{ab}± 0.07	0.85^{ab}±0.10	337^{a} ±15.37	0.81^{ab}±0.03
NaCl III	9.07^{a}±0.34	0.44^{a} ±0.05	301.33^{a}± 12.91	0.57^{a}±0.05
TRIA	17.90^{g}±0.39	1.30^{e} ±0.12	580.33^{g}± 5.60	1.53^{e}±0.17
TRIA + NaCl I	16.50^{fg}± 0.28	1.25^{de}±0.07	536.66^{efg} ±3.38	1.10^{abcd}±0.09
TRIA + NaCl II	15.87^{efg}± 0.26	1.07^{de}±.09	514.66^{defg}±3.17	1.08^{abcd}±0.05
TRIA + NaCl III	14.70^{def}± 0.92	1.19^{cd}±0.01	472.66^{cde}±20.07	0.97^{abc}±0.06
H_2S	17.30^{g} ±0.51	1.45^{e}±0.04	572^{g} ± 5.50	1.73^{e}±0.27
H_2S + NaCl I	15.52^{efg}± 0.39	1.23^{de}±0.03	490.66^{cdef} ±3.52	1.21^{bcde}±0.04
H_2S + NaCl II	14.32^{cde}± 0.48	1.08^{cde}±0.01	440.66^{c}± 6.96	1.23^{bcde}±0.14
H_2S + NaCl III	12.27^{bcd}± 0.25	1.19^{bcd}±0.01	433^{bc}± 5.85	1.14^{abcd}±0.03
TRIA + H_2S	20.62^{h}± 0.63	1.81^{f} ±0.03	655.33^{h}± 11.31	2.97^{f}±0.07
TRIA + H_2S + NaCl I	17.06^{fg} ±0.46	1.47^{e}±0.07	548.33^{fg}±9.49	1.61^{de}±0.02
TRIA + H_2S + NaCl II	16.31^{efg}±0.17	1.36^{de}± 0.02	525.33^{defg}±5.36	1.50^{cde}±0.04
TRIA + H_2S + NaCl III	15.61^{ef}± 0.49	1.28^{de}±0.02	464.33^{cde}±24.34	1.38^{bcde}±0.01

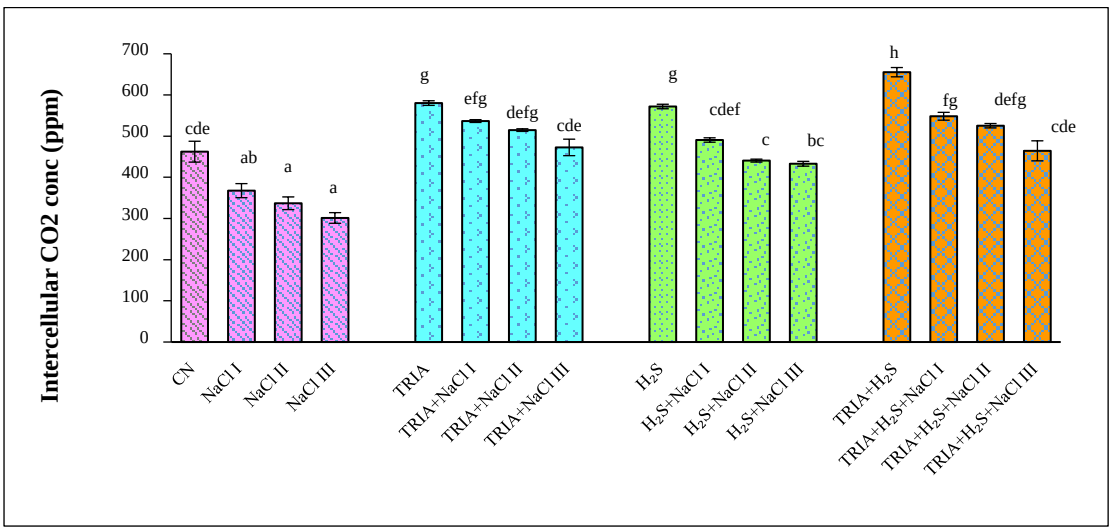
* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Intercellular CO_2 concentration decreased in 60 day old plants of Brassica under salinity stress (Fig. 6.36; Table 6.30). Control plants showed better results in improving intercellular CO_2 concentration of 462.33 ppm in comparison to plants under salt-stressed condition. Salt stressed plants showed 367.66, 337 and 301.33 ppm intercellular CO_2 concentrations, respectively. Application of TRIA and H_2S increased CO_2 concentration of 580.33 and 572.00 ppm, respectively under NaCl I concentration. Individual control plants of TRIA and H_2S was recorded to be 580.00 ppm and 572.00 ppm highest among all the 16 treatments used. Under salt stress,

maximum intercellular CO_2 concentration of 548.33 ppm was found in TRIA+H_2S+NaCl I concentration.







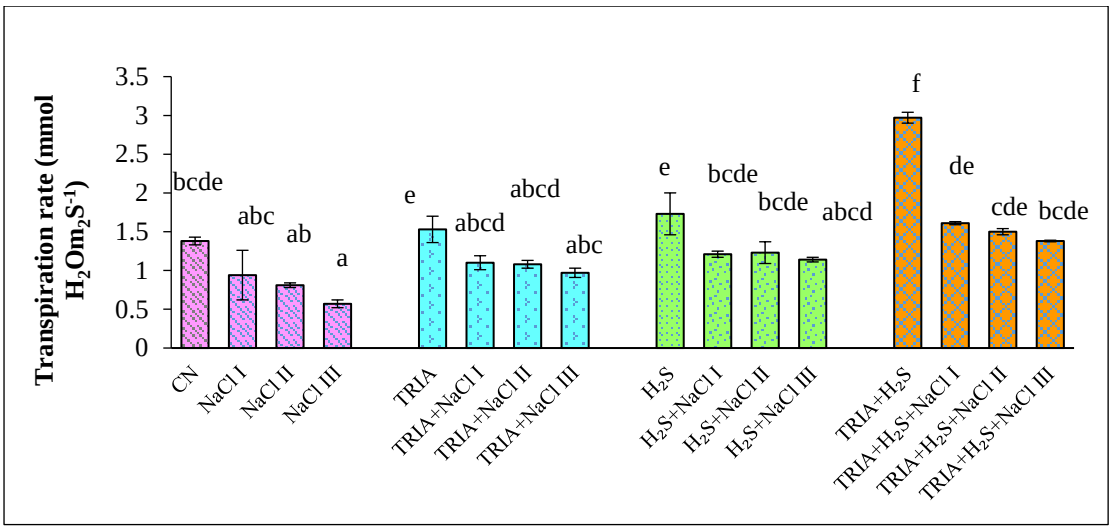


Fig. 6.36 Effect of TRIA and H_2S on photosynthetic rate, stomatal conductance and intercellular CO_2 concentration in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

60 day plants of *Brassica* under control showed 1.38 m mol H_2O m^{-2} S^{-1} transpiration rate whereas plants treated with NaCl I, II and III exhibited transpiration rate of 0.94, 0.81, and 0.57 m mol H_2O m^{-2} S^{-1} rates respectively. (Fig. 6.36; Table 6.30). Likewise, H_2S displayed the highest transpiration rate of 1.23 m mol H_2O m^{-2} S^{-1} at NaCl II concentration, application of TRIA under stressful conditions revealed maximum transpiration rate of 1.10 m mol H_2O m^{-2} S^{-1}at NaCl I concentration. At salt stress combined treatment of TRIA and H_2S showed highest and lowest transpiration rate of 1.61 and 1.38 m mol H_2O m^{-2} S^{-1}

6.1.3.4. Metabolites

Under salt stress, anthocyanin content reduced in plants (Fig. 6.37; Table 6.31). NaCl III concentration reported lowest anthocyanin concentration of 6.88 mg g-1 FW in comparison to all other three concentrations. Under stressful conditions, TRIA application raised the amount with 10.15, 9.41, and 8.70 mg g^{-1} FW at salt stress. Higher anthocyanin content of 10.92 mg g^{-1} FW in H_2S-treated plants under stressful conditions, respectively at NaCl I concentration. Combined treatment of TRIA+ H_2S control plants recorded highest anthocyanin content in comparison to all other 16 treatments. TRIA + H_2S + NaCl I treated plants showed highest level (11.7 mg g^{-1} FW) under salt stress.

Flavonoid content is negatively connected with salt stress (Fig. 6.37; Table 6.31). The tocopherol content was found to decrease by increase in salinity stress. NaCl III concentration showed minimum flavonoid content of 6.88 mg g^{-1} FW which was minimum among all three treatments used in the study. Application of triacontanol and hydrogen sulphide significantly increased the flavonoid content under all conditions. In case of individual triacontanol and hydrogen sulphide, maximum of 9.65 mg g^{-1} FW and 9.37 mg g^{-1} FW was noticed at NaCl I concentration with respectively. Maximum flavonoid content of 9.24 mg g^{-1} FW was found at TRIA+H_2S+NaCl I treatment, in comparison to all other treatments used in the study under stressed conditions.

Table 6.31 Effect of TRIA and H_2S on metabolites of 60-days old plants of *B. juncea* under salinity stress

Treatment	**Anthocyanin (mg g^{-1} FW)**	**Flavonoid (mg g^{-1} FW)**	**Phenolic content (mg g^{-1} FW)**
Control	11.00^{fg}± 0.13	8.71^{efgh} ±0.32	11.95^{ef}± 0.20
NaCl I	8.83^{bc}± 0.14	6.82^{bc} ±0.24	9.94^{bcd} ±0.49
NaCl II	7.42^{ab} ±0.30	6.16^{ab}± 0.16	8.74^{ab}± 0.31
NaCl III	6.88^{a} ±0.50	5.49^{a}± 0.15	7.86^{a}±0.10
TRIA	12.20^{gh} ±0.22	9.65^{hi}± 0.18	13.01^{fg}± 0.05
TRIA + NaCl I	10.15^{de} ±0.21	8.16^{def} ±0.21	11.03^{cde}±0.13
TRIA + NaCl II	9.74^{cd}±0.08	7.51^{cd} ±0.22	10.46^{bcde}±0.35
TRIA + NaCl III	8.70^{ab}± 0.23	6.70^{bc}± 0.25	9.33^{abc}± 0.32
H_2S	13.16^{gh}± 0.36	9.37^{gh} ±0.18	13.22^{fg}± 0.10
H_2S + NaCl I	10.91^{ef} ±01.9	8.75^{efgh}± 0.21	11.04^{cde}± 0.34
H_2S + NaCl II	10.33^{cde}± 0.53	8.28^{defg} ±0.18	10.37^{bcde}±0.38
H_2S + NaCl III	9.37^{bc}±0.11	7.62^{cde} ±0.27	10.17^{bcd} ±0.41
TRIA + H_2S	13.94^{h}±0.16	10.52^{i} ±0.29	14.00^{g}± 0.50
TRIA + H_2S + NaCl I	11.42^{fg}± 0.34	9.24^{fgh}±0.08	11.61^{def}±0.20
TRIA + H_2S + NaCl II	10.12^{ef} ±0.31	8.97^{fgh}± 0.09	11.11^{de}±0.47
TRIA + H_2S + NaCl III	9.52^{de} ±0.73	8.35^{defg} ±0.13	10.13^{bcd}± 0.44

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

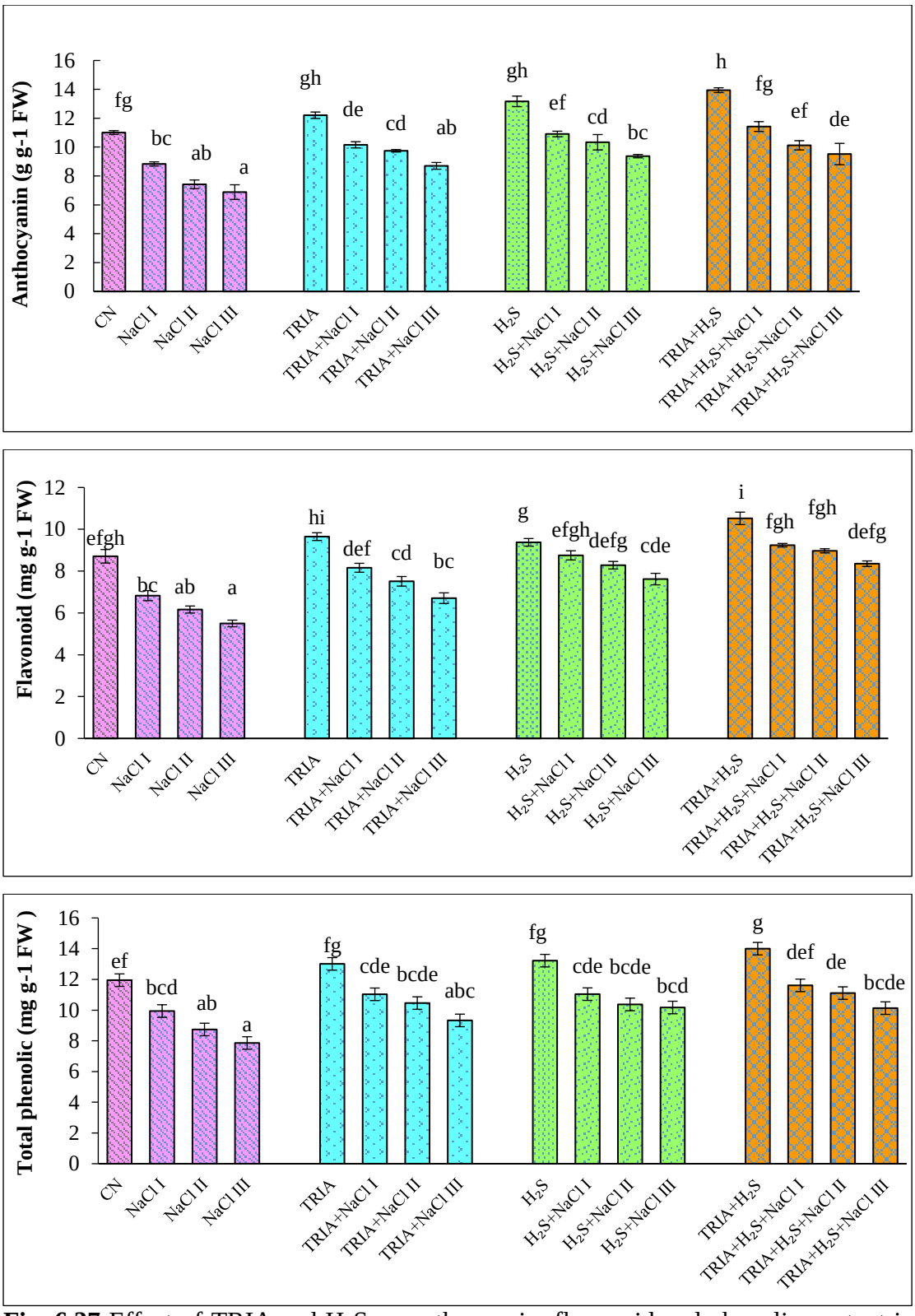


Fig. 6.37 Effect of TRIA and H_2S on anthocyanin, flavonoid and phenolic content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Plants treated with NaCl showed lower level of phenolics (7.86 mg g-1 FW) to the control (11.95 mg g-1 FW) (Fig. 6.37; Table 6.31). NaCl III stressed plants showed minimum phenolic content of 7.86 mg g-1 FW. TRIA and H_2S application significantly enhanced NaCl reduced content of phenol. Likewise, NaCl III using TRIA + H_2S reported minimum phenolic content of 9.33 mg g-1 FW and 10.17 mg g-1 FW in comparison to all other concentrations. TRIA +H_2S under salt combination of 11.61, 11.11, and 10.13 mg g-1 FW phenolic contents, respectively.

6.1.3.5 Oxidative stress markers

Rise of 14.97 µmol g^{-1} in MDA content was noticed in NaCl III stressed seedlings in comparison to control plants (Fig. 6.38; Table 6.32). Whereas, salt stress reduced the MDA content using triacontanol and hydrogen sulphide. TRIA and H_2S application reported the highest decline in content of MDA under salt stress at NaCl I with content of 7.82 and 7.72 µmol g^{-1} FW contents. TRIA + H_2S + NaCl I treatment reported MDA content of 6.86 µmol g^{-1} FW.

Table 6.32 Effect of TRIA and H_2S on oxidative stress markers of 60-days old plants of *B. juncea* under salt stress

Treatment	**MDA (µmol g^{-1} FW)**	**H_2O_2 (µmol g^{-1} FW)**
Control	10.21^{fg}±0.35	9.65^{e}± 0.44
NaCl I	12.83^{h}±0.09	13.48^{d} ±0.42
NaCl II	13.7^{hi}±0.48	14.74^{ef}± 0.45
NaCl III	14.97^{h}±0.16	15.84^{f}±0.55
TRIA	7.82^{bc}±0.11	6.29^{ab}±0.29
TRIA + NaCl I	8.28^{bcde}±0.20	7.01^{ab}±0.06
TRIA + NaCl II	9.00^{cdef}±0.38	7.68^{bc}±0.23
TRIA + NaCl III	9.54^{defg}±0.37	8.94^{abc}±0.15
H_2S	7.21^{ab}±0.22	6.85^{ab}±0.30
H_2S + NaCl I	8.26^{bcde}±0.14	7.48^{bc}±0.30
H_2S + NaCl II	10.57^{g}±0.30	8.40^{bc}±0.28
H_2S + NaCl III	9.67^{efg}±0.23	8.85^{cd}±0.17
TRIA + H_2S	5.85^{a}±0.06	5.54^{a}±0.37
TRIA + H_2S + NaCl I	6.86^{ab}± 0.34	6.17^{ab}±0.53
TRIA + H_2S + NaCl II	7.40^{b}±0.13	6.28^{ab}±0.21
TRIA + H_2S + NaCl III	8.20^{bcd}±0.39	7.26^{abc}±0.18

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

H_2O_2 level was found to be significantly reduced in seedlings of *B. juncea* upon exposure to salt stress (Fig. 6.38; Table 6.32). H_2O_2 content was found to be improved to 13.48, 14.74, and 15.84 µmol g^{-1} FW in NaCl I, II, and III treated stage. All the three treatments which included triacontanol and hydrogen sulphide reduced the H_2O_2 level. Maximum decrease of 6.17 µmol g^{-1} FW was noticed in H_2O_2 level at TRIA + H_2S + NaCl I treatment.

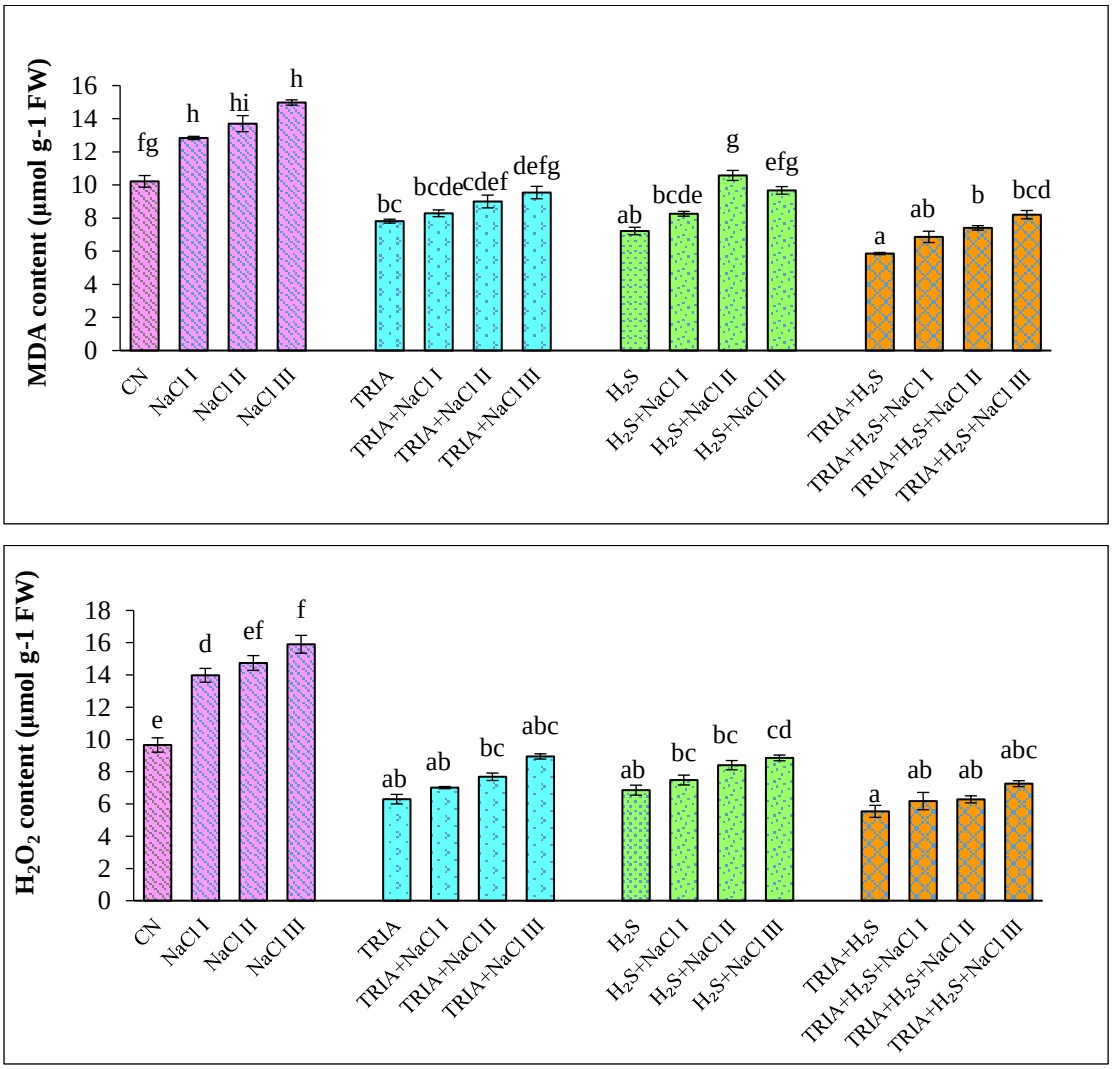


Fig. 6.38 Effect of TRIA and H_2S on MDA and H_2O content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.3.6 Osmolytes

Content of proline was found to be reduced when *Brassica* exposed to salt stress (Fig. 6.39; Table 6.33). Proline level was found to reduce in NaCl conc Proline content of 4.80, 4.11 and 3.66 µ mol g^{-1} FW was reported at salt stress voncentrations. Control plants showed proline content of 6.05 µ mol g^{-1} FW. TRIA control plants reported proline content of 8.39 µ mol g^{-1} FW, which was higher in comparison to TRIA treated plants under stressed conditions. Individual application of H_2S under unstressed conditions (8.35 µ mol g^{-1} FW) raised the level of proline in comparison to plants under stressed conditions with maximum content of 7.54 µ mol g^{-1} FW at NaCl I concentration.

Table 6.33 Effect of TRIA and H_2S on osmolytes of 60-days old plants of *B. juncea* under salinity stress

Treatment	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
Control	6.05^{cde}±0.04	12.92^{def} ±0.51
NaCl I	4.80^{abc}±0.12	9.82^{bc}± 0.03
NaCl II	4.11^{ab}±0.02	8.58^{ab}± 0.33
NaCl III	3.66^{a}±0.16	7.44^{a}± 0.29
TRIA	8.39^{g}±0.29	16.01^{hi}± 0.18
TRIA + NaCl I	6.29^{def}±0.35	13.20^{ef}± 0.56
TRIA + NaCl II	5.77^{cde}±0.32	11.87^{de}± 0.11
TRIA + NaCl III	5.03^{abcd}±0.28	11.40^{cd} ±0.26
H_2S	8.35^{g}±0.43	15.74^{gh} ±0.25
H_2S + NaCl I	7.54^{fgh}±0.28	14.21^{fg}± 0.33
H_2S + NaCl II	6.34^{defg}±0.10	13.16^{ef}± 0.26
H_2S + NaCl III	5.40^{bcde}±0.25	12.11^{de}± 0.12
TRIA + H_2S	10.46^{h}±0.30	17.65^{i}± 0.33
TRIA + H_2S + NaCl I	7.71^{gh}±0.29	14.50^{gh} ±0.42
TRIA + H_2S + NaCl II	6.61^{efg}±0.31	14.22^{fg}± 0.28
TRIA + H_2S + NaCl III	5.80^{cde}±032	12.27^{de}± 0.36

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

Glycinebetaine content was found to decline to 9.82, 8.58, and 7.44 µ mol g^{-1} in NaCl conc. (Fig. 6.39; Table 6.33). Both types of application mitigated salt stress by raising the level of glycinebetaine. TRIA application increased content of glycine betaine to 13.20, 11.87, and 11.40 µ mol g^{-1} FW at salt stressed condition. H_2S treated plants raised glycine betaine contents of 14.21, 13.16, and 12.11 µ mol g^{-1} FW under salt stress. Furthermore, treated plants with TRIA and H_2S showed the highest glycine betaine content of 17.65 µ mol g^{-1} FW among all the 16 treatments used in the present study. Glycine betaine amount was found to be lessened as the level of NaCl increased application of triacontnaol and hydrogen sulphide with minimum activity of 12.27 µ mol g^{-1} FW) at NaCl III.

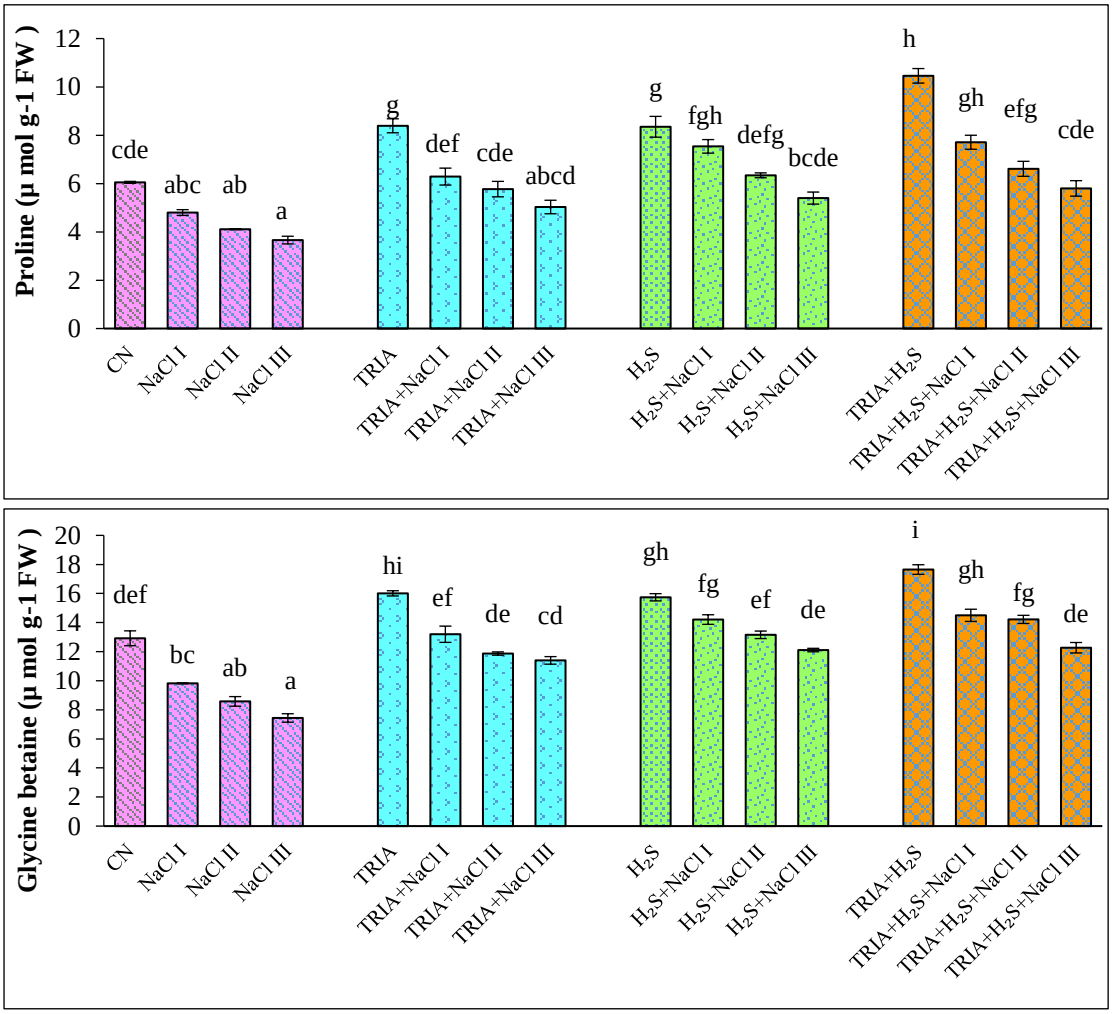


Fig. 6.39 Effect of TRIA and H_2S on proline, and glycine betaine in 60 days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.3.6 Total carbohydrates

Total carbohydrate content decreased in plants of *Brassica* lowest of 6.23 mg g^{-1} FW at NaCl III concentration (Fig. 6.40; Table 6.34). TRIA application under unstressed conditions exhibited 12.07 mg g^{-1} FW carbohydrate amount. It decreased from 10.17 mg g^{-1} FW at NaCl I concentration to 8.44 mg g^{-1} FW at NaCl III concentration. TRIA+H_2S reported maximum carbohydrate content of 10.16 mg g^{-1} FW at NaCl I and minimum carbohydrate content of 8.39 mg g^{-1} FW at NaCl III. TRIA +H_2S treatment under unstressed condition reported increase in total carbohydrate content. However, TRIA+H_2S treatment under unstressed condition reported maximum carbohydrate content of 11.41 mg g^{-1} FW at NaCl I concentration.

Table 6.34 Effect of TRIA and H_2S on total carbohydrates of 60-days old plants of *B. juncea* under salt stress

Treatment	**Total carbohydrates (mg g^{-1} FW)**
Control	9.05^{bcd}±0.02
NaCl I	8.25^{bc}±0.25
NaCl II	7.90^{ab}±040
NaCl III	6.23^{a}±0.46
TRIA	12.07^{f}±0.04
TRIA + NaCl I	10.17^{de}±0.25
TRIA + NaCl II	9.95^{cde}±0.53
TRIA + NaCl III	8.44^{bcd}±0.26
H_2S	12.27^{f}±0.42
H_2S + NaCl I	10.16^{de}±0.65
H_2S + NaCl II	9.32^{cde}±0.23
H_2S + NaCl III	8.39^{bcd}±0.29
TRIA + H_2S	15.76^{g}±0.16
TRIA + H_2S + NaCl I	11.41^{ef}±0.29
TRIA + H_2S + NaCl II	9.79^{cde}±0.25
TRIA + H_2S + NaCl III	8.56^{bcd}±0.26

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

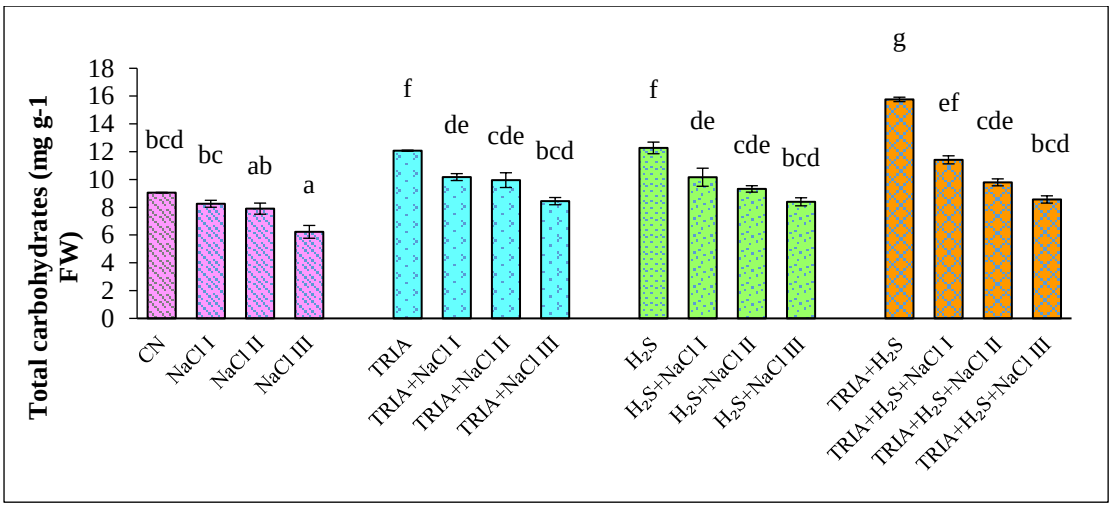


Fig. 6.40 Effect of TRIA and H_2S on carbohydrates content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.3.8 Protein content and antioxidant defense system

6.1.3.8.1 Protein content and antioxidative enzymes

Plants of *Brassica* under control showed content of 10.68 mg g^{-1} FW (Fig. 6.41; Table 6.35) NaCl III concentration reported decline in content of 6.68 mg g^{-1} FW. Whereas NaCl I stressed plants reported protein content of 8.77 mg g^{-1} FW. In the case of TRIA under stressed conditions, the maximum protein content of 12.97 mg g^{-1} FW was found at NaCl I . Pre-treatment with H_2S enhanced the protein content under salt stress and reported highest protein content of 12.32 mg g^{-1} FW at NaCl I. Out all 16 treatments, TRIA + H_2S control plants reported highest protein content of 20.28 mg g^{-1} FW while under stress, the highest protein content of 14.37 mg g^{-1} FW was in TRIA+ H_2S + NaCl I

Enzyme action was found to be decreased under salt stress and reported lowest SOD activity of 10.56 UA mg^{-1} protein at NaCl I (Fig. 6.41; Table 6.35). SOD activity was found to be reduced to 5.93 UA mg^{-1} protein in NaCl III stressed plants than control. Escalation in the SOD level was found in TRIA control plants. TRIA application increased the SOD from 9.60 cm to 13.73 UA mg^{-1} protein at NaCl I concentration. H_2S treated plants increased the SOD activity with the highest of 11.99 UA mg^{-1} protein at NaCl I concentration. TRIA + H_2S further improved the SOD level from 11.28 to 15.33 UA mg^{-1} protein at NaCl II concentration.

Salt stress is decreased due to CAT activity with the lowest (6.96 UA mg^{-1} protein) at NaCl III concentration (Fig. 6.41; Table 6.35). Triacontanol and hydrogen sulphide increased CAT activity. TRIA under salinity resulted in the highest of 11.42 UA mg^{-1} protein at NaCl I. Likewise, H_2S application showed the highest CAT activity of 10.44 UA mg^{-1} protein at NaCl I concentration and lowest CAT activity of 9.09 UA mg^{-1} protein was reported at NaCl III concentration. Triacontanol and hydrogen sulphide in case of salinity reported higher and lower activities of 12.36 and 9.05 UA mg^{-1} protein in NaCl I and III stressed plants.

Table 6.35 Effect of TRIA and H_2S on protein content and antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress

Treatments	**Protein content (mg g^{-1} FW)**	**SOD (UA mg^{-1} protein)**	**CAT (UA mg^{-1} protein)**	**APX (UA mg^{-1} protein)**
Control	10.68^{bcde}±1.18	10.56^{cd}±0.62	11.18^{bcde}±0.94	21.0^{abc}±2 0.64
NaCl I	8.77^{abc} ±0.44	8.07^{bc}±0.13	8.72^{ab} ±0.53	19.20^{ab}± 0.96
NaCl II	8.02^{ab}± 0.49	6.70^{a}±0.29	7.72^{b}± 0.49	17.87^{ab}± 0.35
NaCl III	6.68^{a}± 0.34	5.93^{a}±0.51	6.96^{b} ±0.55	15.11^{a}± 0.61
TRIA	16.22^{g}± 0.72	13.73^{ef}±0.64	12.30^{e}± 0.46	24.94^{ef}± 0.91
TRIA + NaCl I	12.97^{ef}± 0.51	12.26^{de}±0.31	11.42^{cde} ±0.30	23.09^{bcd} ±0.65
TRIA + NaCl II	11.98^{cdef}± 0.66	11.33^{cde}±0.34	9.13^{abcd}± 0.23	21.58^{abc} ±0.89
TRIA + NaCl III	9.46^{bcde}± 0.43	9.60^{bc}±0.47	8.81^{abc} ±0.10	20.27^{abc} ±0.35
H_2S	16.50^{g}± 0.47	13.66^{ef}±0.39	13.04^{e}± 0.57	30.16^{ef}± 1.30
H_2S + NaCl I	12.32^{def}± 0.66	11.99^{cde}±0.26	11.72^{de}± 0.32	27.95^{def}± 0.58
H_2S + NaCl II	9.53^{bcde}± 0.79	10.56^{cd}±0.48	10.77^{bcde}±0.45	25.18^{bcde}±1.66
H_2S + NaCl III	8.94^{abc}± 0.54	9.83^{bcd}±0.31	9.09^{abcd}±0.12	23.94^{bcde}±3.38
TRIA + H_2S	20.28^{h}± 0.93	15.33^{f}±0.52	16.32^{f}±0.25	32.28^{f}±0.60
TRIA + H_2S + NaCl I	14.37^{fg}± 0.57	13.36^{ef}±0.33	12.36^{e}±0.15	28.56^{def}± 0.78
TRIA + H_2S + NaCl II	11.38^{cdef}± 0.73	12.14^{de}±0.36	10.71^{bcde}±0.27	26.93^{cdef}± 0.93
TRIA + H_2S + NaCl III	10.65^{bcde}±0.45	11.28^{cde}±0.91	9.05^{abcd} ±0.17	24.55^{bcde}±1.43

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

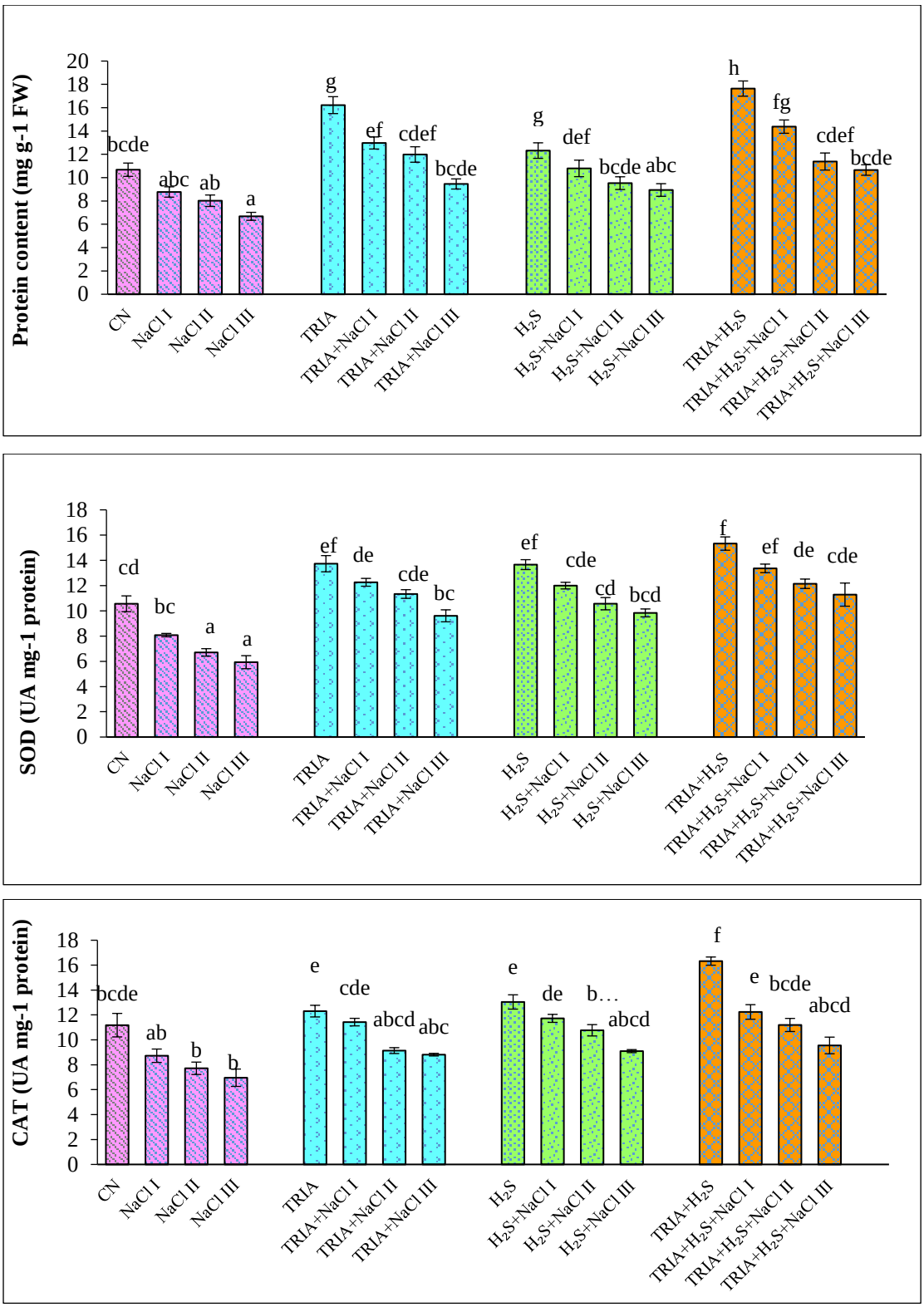


Fig. 6.41 Effect of TRIA and H_2S on protein content, SOD and CAT enzyme activities in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

APX enzymatic activity was found to be reduced under salt stress in 60 day plants of *Brassica* (Fig. 6.41; Table 6.35). NaCl I treated plants showed a decline in APX activity (19.20 UA mg^{-1} protein). TRIA application reported maximum APX activity of 23.09 UA mg^{-1} protein in NaCl I treated plants. APX activity was enhanced from 23.94 to 27.95 UA mg^{-1} protein at NaCl I concentration when treated with H_2S, in comparison to NaCl I stressed plants. TRIA + H_2S further upregulated the APX activity under stressed conditions with the highest of 28.56 UA mg^{-1} protein activity under NaCl I.

POD action declined with the rise in NaCl (Fig. 6.42; Table 6.36). Highest decline in POD activity (5.73 UA mg^{-1} protein) was noticed at NaCl III concentration. Activity of enzyme POD was found to be raised from 7.81 to 9.24 UA mg^{-1} protein in TRIA + NaCl I treated plant, in contrast to NaCl I stressed plants. H_2S treatment against salt stress also improved the POD activity with the highest activity (9.91 UA mg^{-1} protein) at NaCl I concentration. TRIA + H_2S raised the level of enzyme POD and mitigated salinity stress. Maximum POD activity of 9.92 UA mg^{-1} protein was at NaCl I concentration whereas minimum 7.95 UA mg^{-1} protein POD activity at NaCl III concentration.

TRIA and H_2S application caused mitigation in salinity stress by causing highest improvement in the GR activity in comparison to all other treatments (Fig. 6.42; Table 6.36). Highest decline of 8.68 mg^{-1} protein in GR observed at NaCl III concentration. Individual application of TRIA under stressed conditions increased the GR of 13.53 UA mg^{-1} protein in NaCl I treated plants. Treatment of plants with H_2S against salt stress reduced the GR activity (11.18 UA mg^{-1} protein) under NaCl III. Furthermore, combination of TRIA, H_2S and NaCl significantly mitigated salinity stress by increasing GR activity of 14.14 UA mg^{-1} protein at TRIA + H_2S + NaCl I.

GPOX enzymatic activity was found to be reduced under salinity in 60-days old plants of *Brassica* (Fig. 6.42; Table 6.36). Minimum GPOX activity of 6.62 UA mg^{-1} protein was observed at salinity. Application of TRIA under salt stress caused reduction of 11.32, 10.06 and 9.48 UA mg^{-1} protein in activity of GPOX to at different NaCl concentration to their respective plants. H_2S reported highest. Activity of GPOX was found to be 11.43 UA mg^{-1} protein at NaCl I concentration. TRIA and

H_2S under unstressed conditions showed 16.69 UA mg^{-1} protein GPOX activity. Highest GPOX level under stressed conditions was found to be 10.26 UA mg^{-1} when TRIA and H_2S were applied together at NaCl I concentration.

Salt stress decreased the DHAR activity with highest reduction of 7.78 UA mg^{-1} protein at NaCl III concentration (Fig. 6.42; Table 6.36). Individual TRIA and H_2S significantly enhanced the DHAR activity to 13.86 UA mg^{-1} protein and 13.00 UA mg^{-1} protein at NaCl I concentration. Under stressed conditions, highest DHAR activity of 14.19 UA mg^{-1} protein was found at combination of TRIA+H_2S+ NaCl II.

Table 6.36 Effect of TRIA and H_2S on antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress

Treatments	**POD (UA mg^{-1} protein)**	**GR (UA mg^{-1} protein)**	**GPOX (UA mg^{-1} protein)**	**DHAR (UA mg^{-1} protein)**
Control	9.08^{bcd} ±0.79	13.48^{cdef}±0.29	10.42^{bcde}±0.66	13.36^{cdef}±0.36
NaCl I	7.05^{abc}±0.54	10.30^{ab}±0.34	8.41^{ab}±0.48	10.46^{bc}±0.31
NaCl II	7.28^{ab}±0.10	9.87^{ab}±0.64	7.66^{ab}±0.33	8.68^{ab}±0.40
NaCl III	5.73^{a}±0.21	8.68^{a}±0.66	6.62^{a}±0.32	7.78^{a}±0.17
TRIA	10.12^{de}±0.49	15.88^{fg}±0.66	13.7^{fg} ±0.86	15.29^{gh}±0.43
TRIA + NaCl I	9.24^{cde}±0.32	13.53^{cdef}±0.32	11.32^{cdef}±0.75	13.86^{efg}±0.54
TRIA + NaCl II	8.13^{bcd}±0.57	11.79^{bcd}±0.70	10.06^{bcd}±0.20	12.02^{cdef}±0.54
TRIA + NaCl III	7.81^{abcd}±0.27	11.02^{abc}± 0.51	9.48^{bc} ±0.37	11.11^{bcd}±0.52
H_2S	11.28^{ef}±0.41	15.66^{efg}±0.34	14.42^{gh}±0.75	14.78^{gh}±0.50
H_2S + NaCl I	9.91^{de}±0.41	13.44^{cdef}±033	11.43^{cdef}± 0.38	13.00^{defg}±0.58
H_2S + NaCl II	9.04^{bcde}±0.31	12.22^{bcd}±0.42	10.21^{bcd}±0.43	11.32^{cde}±0.60
H_2S + NaCl III	7.81^{abcd}±0.41	11.18^{abcd}±1.05	9.65^{bc}±0.34	10.86^{bcd}±0.49
TRIA + H_2S	13.24^{f}±0.88	17.80^{g}±0.73	16.69^{h}±0.65	16.93^{h}±0.54
TRIA + H_2S + NaCl I	9.92^{de}±0.28	14.14^{def}±0.50	13.03^{efg}±0.58	14.53^{fgh}±0.58
TRIA + H_2S + NaCl II	8.57^{bcd}±0.27	12.67^{bcde}±0.80	12.52^{defg}±0.43	13.33^{defg}±0.38
TRIA + H_2S + NaCl III	7.95^{abcd}±0.40	11.72^{abcd}±0.58	10.04^{bcd}±0.57	11.30^{bcde}±0.73

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

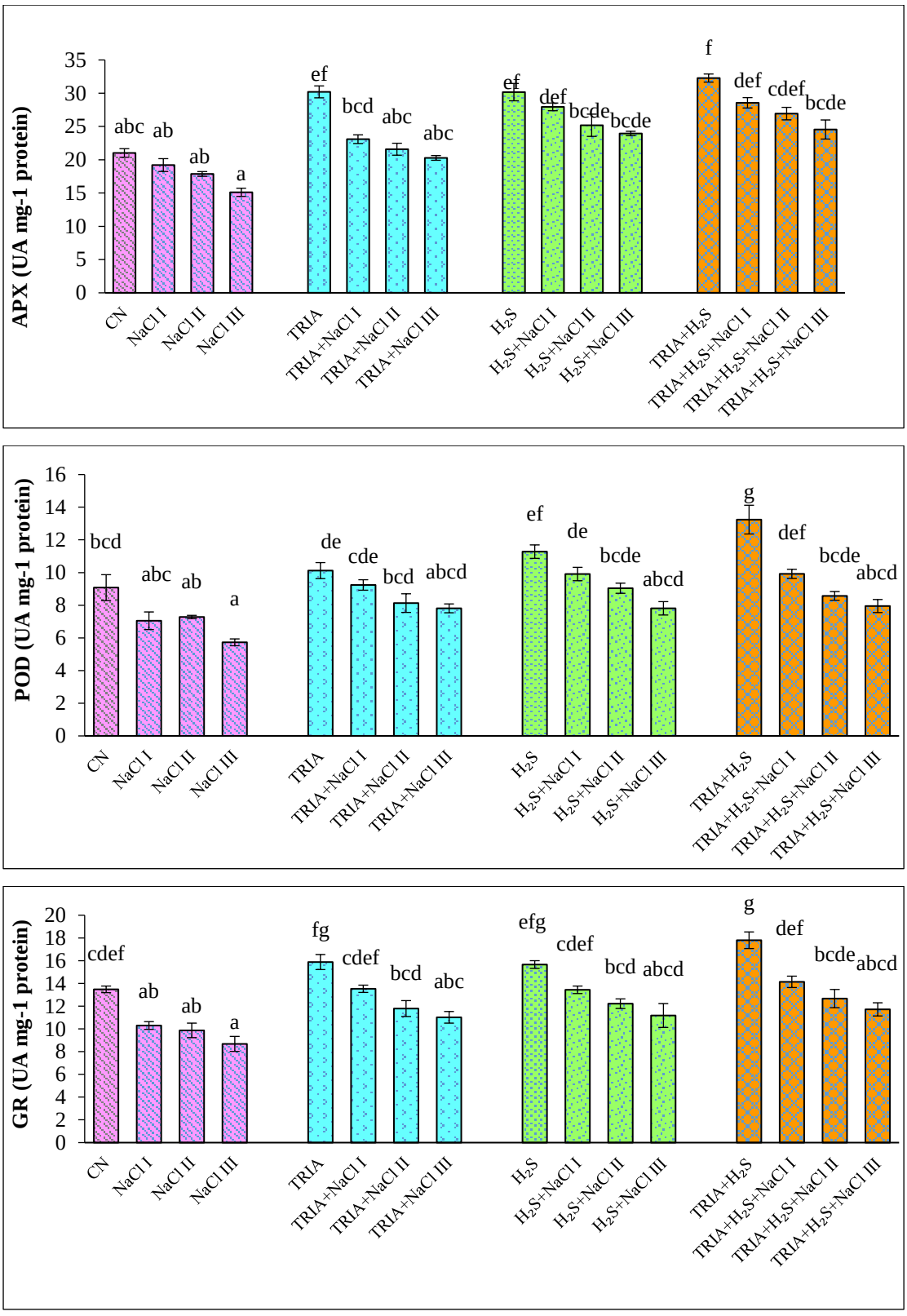


Fig. 6.42 Effect of TRIA and H_2S on APX, POD and GR enzyme activities in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

Salt stressed plants showed decreased MDHAR activity to 11.18 UA, mg^{-1} protein at NaCl I (Fig. 6.43; Table 6.37). TRIA application under stressed conditions mitigated salinity stress by improving the activity of enzyme MDHAR. Highest MDHAR was found to be 16.03 UA mg^{-1} protein in TRIA under NaCl stress. MDHAR activity was enhanced from 11.65 to 15.45 UA mg^{-1} protein at NaCl concentration when treated with H_2S. TRIA + H_2S further augmented the MDHAR activity under stressed conditions with a maximum of 16.65 UA mg^{-1} protein activity under NaCl I.

Table 6.37 Effect of TRIA and H_2S on antioxidative enzymes of 60-days old plants of *B. juncea* under salt stress

Treatment	**MDHAR (UA mg^{-1} protein)**	**GST (UA mg^{-1} protein)**	**PPO (UA mg^{-1} protein)**
Control	16.24^{efg}± 0.79	12.02^{defg}± 0.03	8.96^{bc}±0.21
NaCl I	11.18^{abc}± 0.45	8.32^{abc} ±0.67	7.01^{ab}± 0.52
NaCl II	10.05^{ab}± 0.42	7.76^{ab}±0.52	5.90^{a}±0.21
NaCl III	9.32^{a} ±0.27	6.79^{a}±0.35	5.56^{a}±0.31
TRIA	18.13^{gh} ±0.32	14.04^{gh}±0.84	11.04^{gh}±0.14
TRIA + NaCl I	16.03^{efg} ±0.58	11.80^{defg}±0.74	9.75^{defg}±0.14
TRIA + NaCl II	14.80^{def}± 0.63	10.71^{cdef}±0.30	9.76^{cde}±0.45
TRIA + NaCl III	13.63^{cde}± 0.69	9.38^{abcd}±0.31	10.28^{abcd}±0.32
H_2S	17.62^{fgh} ±0.36	14.32^{gh}±1.20	10.90^{fgh}±0.09
H_2S + NaCl I	15.45^{efg}±0.82	12.40^{efg}±0.32	8.81^{bcde}±0.71
H_2S + NaCl II	12.02^{bcd}± 0.58	11.36^{defg}±0.33	10.35^{cdef}±1.00
H_2S + NaCl III	11.65^{abcd} ±0.34	10.57^{bcde}±0.38	9.89^{abcd}±0.54
TRIA + H_2S	19.33^{h} ±0.84	16.01^{h}±0.57	14.40^{e}±0.58
TRIA + H_2S + NaCl I	16.65^{fgh} ±0.34	13.70^{gh}±0.85	11.74^{bc}±0.20
TRIA + H_2S + NaCl II	15.35^{efg} ±0.31	12.69^{fgh} ±0.86	10.43^{d}±0.64
TRIA + H_2S + NaCl III	13.68^{cde}±0.34	11.66^{efg}± 0.66	120.46^{cd}±0.64

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

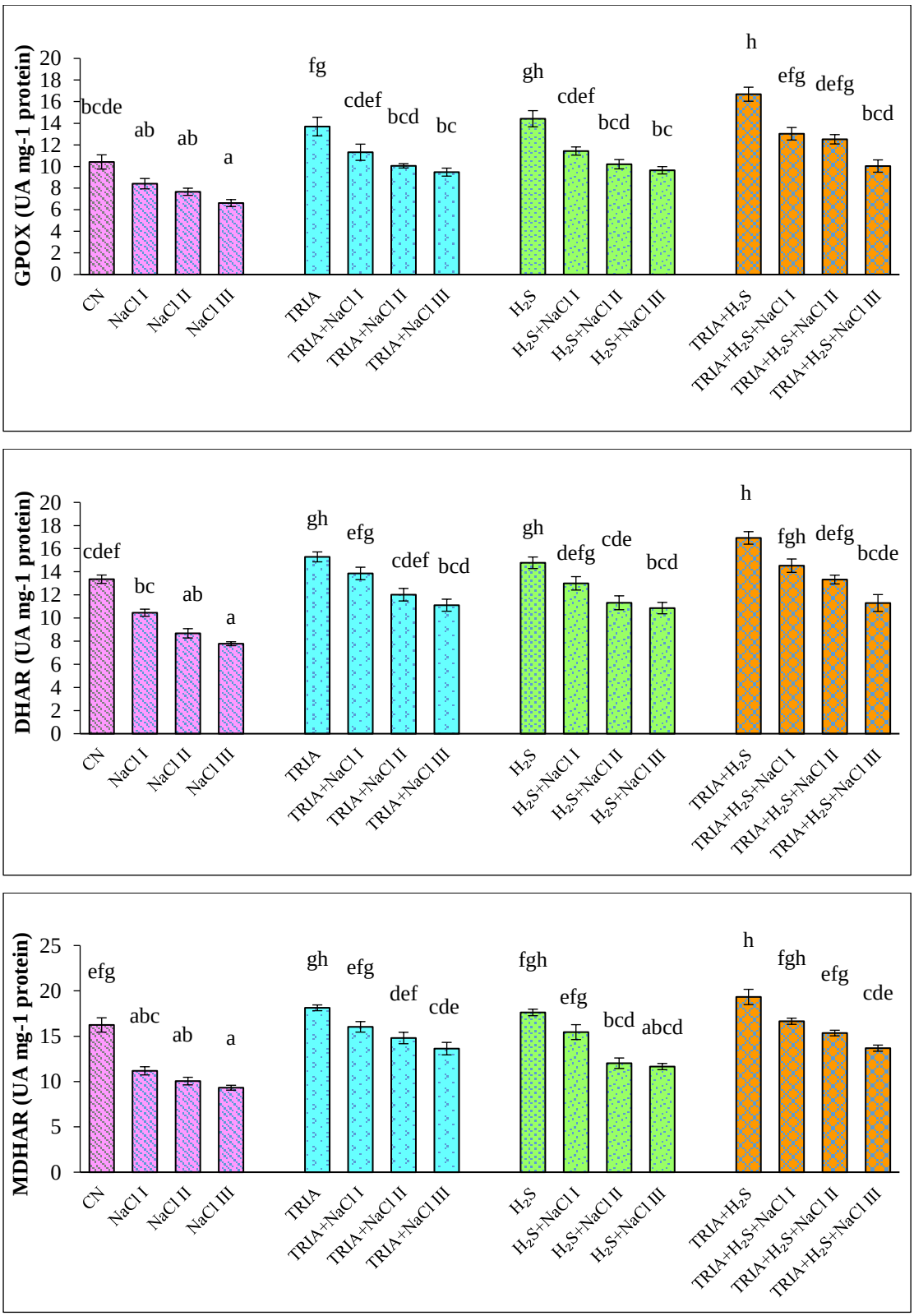
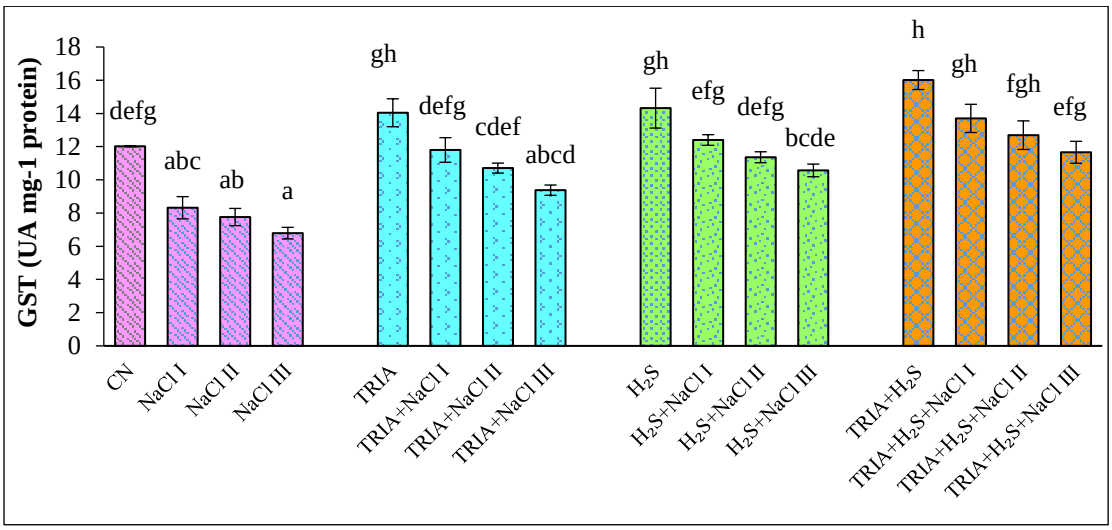


Fig. 6.43 Effect of TRIA and H_2S on GPOX, DHAR and MDHAR enzyme activities in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

GST activity decreased in plants of *Brassica* under salinity (Fig. 6.44; Table 6.37). Lowest GST activity of 6.79 UA mg^{-1} protein was found at NaCl III concentration. NaCl I stressed plants showed 8.32 UA mg^{-1} protein GST activity. TRIA reduced the content of GST to 11.80, 10.71 and 9.38 UA mg^{-1} protein at NaCl I, II, and III concentrations. Individually triacontanol and hydrogen sulphide reported GST activity of 14.04 and 14.32 UA mg^{-1} protein at NaCl I concentration. As per data, it was found that individual application of TRIA, H_2S showed better results in improving GST activity as compared to plants under stressed conditions. Treatment of TRIA and H_2S under unstressed conditions reported GST activity 16.01 UA mg^{-1} protein which was found to be highest out of all 16 treatments. TRIA and H_2S treatment when applied together under stressed condition reported highest and lowest GST activity i.e., 13.70 and 11.66 UA mg^{-1} protein was at NaCl I and III concentration.

PPO enzymatic activity was found to be declined in *B. juncea* under salinity. Minimum PPO activity of 9.19 UA mg^{-1} protein at NaCl III concentration. Triacontaol and Hydrogen sulphide treatment improved proformnace of enzyme PPO in 60 day plants of *Brassica* under salt stress (Fig. 6.44; Table 6.37). Application when used individually or in combination reported highest PPO activity of 15.91 and 15.39 UA mg^{-1} protein at NaCl I. TRIA+ H_2S +NaCl I reported highest PPO activity of 14.36 UA mg^{-1} protein.



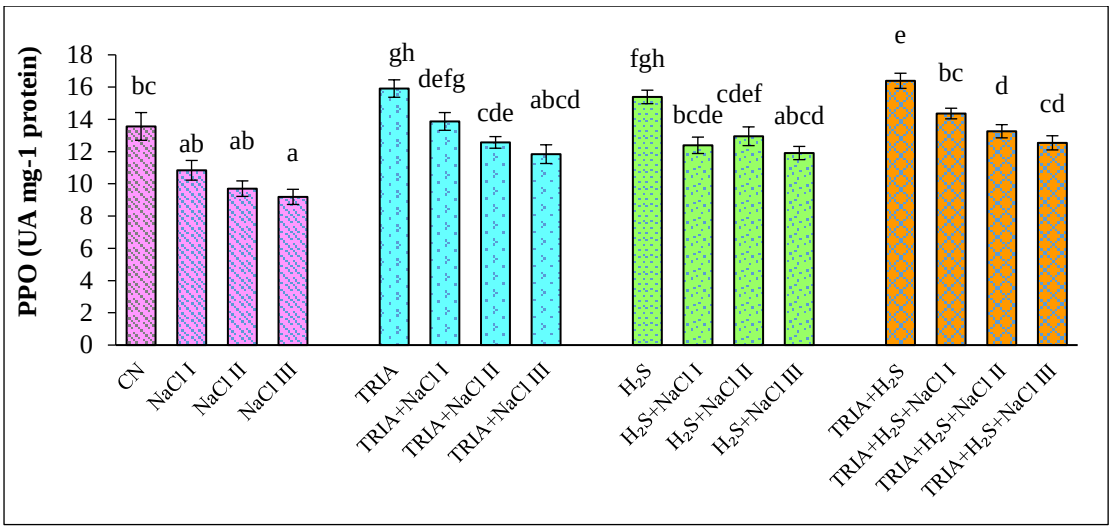


Fig. 6.44 Effect of TRIA and H_2S on GST and PPO enzyme activites in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at P<0.05.

6.1.3.8.2 Non-enzymatic antioxidants

Content of ascorbic acid was found to be declined under salt stress in plants of *Brassica* (Fig. 6.45; Table 6.38). NaCl III concentration reported lowest amount of 8.62 µg g^{-1} FW. TRIA + H_2S treated plants under unstressed conditions reported ascorbic acid content of 17.62 µg g^{-1} FW. Application of TRIA under stressed conditions showed maximum ascorbic acid content of 13.25 µg g^{-1} FW at NaCl I concentration. H_2S treated plants improved the content of ascorbic acid under NaCl with a maximum content of 13.16 µg g^{-1} FW at NaCl I concentration. Combination increased ascorbic acid content to 14.18 µg g^{-1} FW at NaCl I.

Glutathione content significantly declined under concentration of salt. Increase in concentration from NaCl I to NaCl III reported decline from 10.22 to 11.72 µg g^{-1} FW (Fig. 6.45; Table 6.38). Content of glutathione increased to 15.42 05 µg g^{-1} FW in TRIA+NaCl I concentration in contrast to NaCl III stressed plants. H_2S treated plants showed maximum glutathione content of 14.24 µg g^{-1} FW at NaCl I concentration. TRIA+ H_2S under stressed condition increased the content of glutathione. Combination of TRIA+H_2S treated plants under stressed conditions showed increased in content of glutathione from 15.87, 14.56, and 11.95 µg g^{-1} FW

Tocopherol content reduced in NaCl in comparison to CN plants which showed

tocopherol content of 12.79 µg g^{-1} FW (Fig. 6.45; Table 6.38). Lowest tocopherol content of 6.53 µ g^{-1} FW was found at NaCl III concentration. Synergistic association of TRIA and H_2S under unstressed condition reported maximum tocopherol content of 18.88 µg g^{-1} FW in comparison to plants under unstressed conditions.

Table 6.38 Effect of TRIA and H_2S on non-enzymatic antioxidants of 60-days old plants of *B. juncea* under salt stress

Treatment	**Ascorbic acid (µg g^{-1} FW)**	**Glutathione (µg g^{-1} FW)**	**Tocopherol content (µg g^{-1} FW)**
Control	12.80^{def}±0.30	14.63^{de} ±0.18	12.79^{ef}± 0.34
NaCl I	10.66^{bc}±0.23	11.72^{ab}± 0.26	8.93^{bc}±0.09
NaCl II	10.02^{ab}±0.43	10.88^{a}± 0.18	7.55^{ab}±0.30
NaCl III	8.62^{a}±0.41	10.22^{a}± 0.32	6.53^{a}±0.40
TRIA	14.99^{gh}±0.14	16.96^{f}± 0.42	16.35^{g}±0.81
TRIA + NaCl I	13.25^{efgh}±0.35	15.42^{ef}± 0.68	12.72^{def}±0.41
TRIA + NaCl II	11.54^{bcde}±0.35	13.92^{cde}± 0.15	10.50^{cd}±0.42
TRIA + NaCl III	9.34^{bcd}±0.39	12.05^{abc} ±0.21	8.83^{bc}± 0.52
H_2S	15.10^{h}±0.20	17.25^{f} ± 0.35	14.46^{fg}±0.23
H_2S + NaCl I	13.16^{efg}±0.25	14.24^{de}± 0.53	10.38^{c}±0.38
H_2S + NaCl II	11.64^{bcde}±0.27	13.18^{bcd}± 0.20	9.50^{bc} ±0.42
H_2S + NaCl III	11.70^{bcde}±0.25	12.91^{bcd}± 0.15	9.62^{bc} ±0.24
TRIA + H_2S	17.62^{i}±0.71	19.97^{g}± 0.61	18.88^{h}±0.11
TRIA + H_2S + NaCl I	14.18^{fgh}±0.29	15.87^{ef}± 0.57	13.91^{f}±0.51
TRIA + H_2S + NaCl II	12.24^{cde}±0.31	14.56^{de}± 0.20	12.96^{ef}±0.30
TRIA + H_2S + NaCl III	10.87^{bc}±0.30	11.95^{abc}± 0.34	10.63^{cde}±0.44

* Values presented as means ± standard error. Different letters in lowercase represent the significant difference between treatments

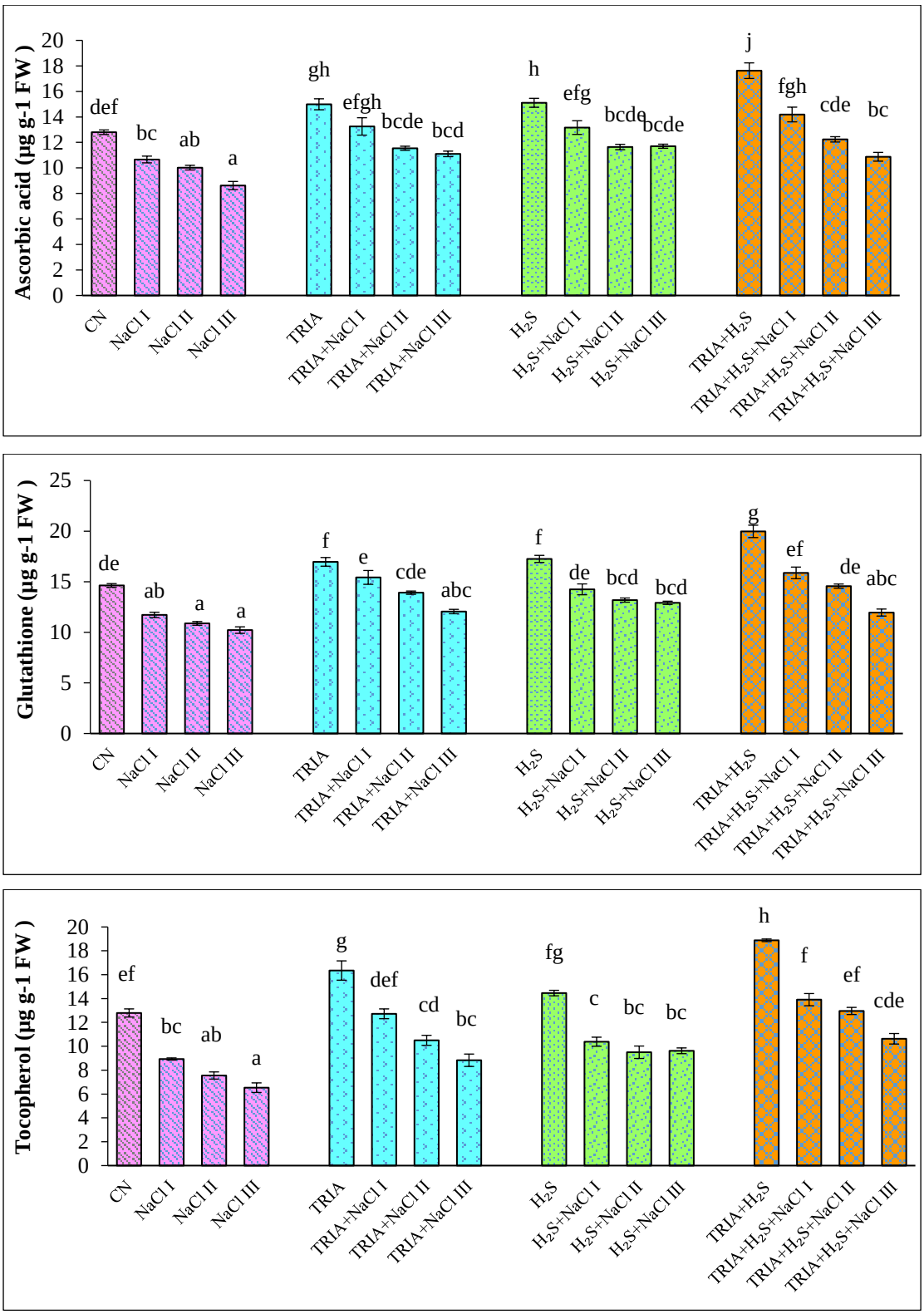


Fig. 6.45 Effect of TRIA and H_2S on ascorbic acid, glutathione and tocopherol content in 60-days old plants of *B. juncea* under salt stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by dissmiliar letter are significantly different at $P<0.05$.

6.2 Discussion

6.2.1 Plant growth

In our investigation, it was found that salt stress significantly affect germination percentage of *Brassica juncea* at different concentrations of NaCl promoted seed germination in seedlings and plants of *Brassica juncea* using triacontanol and hydrohen sulphide by exogenous application. Salt stress significantly inhibited major morphological parameters like plant length, biomass and gaseous exchange attributes. Furthermore, application of TRIA enhanced gas exchange characterstics. Ali et al. (2017) in wheat and Alzahrani et al., (2019) in *Vicia faba*. These studies depicted that salinity stress activates osmotic stress and ionic stress which in turn leads to generation of ROS (Reactive Oxygen species). The generation of these ROS cause excessive leakage of ions, membrane peroxidation, damage to cell structure and nucleic acid. Salinity is known to effect all morphological traits in plants at different stages (Mbinda and kimtai, 2019). Generally, increasing salinity affect plant growth characters like length, weight, (RWC) relative water content and germination percentage of the exposed plants.

Salt based inhibition declined in photosynthetic uptake, rise in stomatal closing and CO_2 assimilation (Odjegba and Chukwunwike, 2012; Menezes et al., 2017; Sarker and Oba, 2020b). These findings are in accordance of Belaqziz et al. (2009) who found that seed germination is affected by salinity as it destroys embryo by decreasing potential of soil as it hampers soil water uptake. Apart from these reasons reduction in carbon gains and energy diversion from growth to homeostatsis are another major factors which are responsible for reduction in growth. It has been found that during germination, salinity disrupts the nutrient and hormonal balances of major plant hormones such as gibberellin, abscisic acid etc. salt stress is the prime reason caused dynamic balance by using ROS. Several scientists have investigated reduction in fresh weight due to salinity stress in crop such as tomato and *Ocimum basilcum*. Biomass reduction was also found to be increased with increase in salinity which is due to disturbances caused in physiological and biochemical activities under salt stressed conditions (Shahid et al., 2020). Similar result in dry matter reduction was observed in sugar beet cultivars (Dadkhah and Grrifiths, 2006).

Growth regulators have significant regulatory effect for maintaining development and growth (Lucas et al., 2004). Triacontanol application is an effective strategy which mitigate impacts of salt stress on different agricultural crops. These plant growth regulators are highly involved in enhancing biological functioning of plant. It was reported from various studies that TRIA play an essential role in improving different growth parameters by maintaining water balance, uptake of essential nutrient and regulation of different metabolic compounds (Naeem et al., 2012). Productivity, yield and growth of plant is improved by antioxidant defense system which in turn increased plant height, weight and yield (Dhall et al., 2004; Khan et al., 2009). It plays essential role in division of cell and enlargement by activating secondary messenger called 9-β-L (+) adenosine which influence plant water relations and growth (Islam and Mohammad, 2020).

Exogenous application of TRIA increased absorption and uptake of mineral nutrients under stressed environmental conditions (Kilic et al., 2010). Triacontanol increased plant biomass and and growth attributes in ginger (Singh et al., 2010). Result was quite similar to our study where TRIA significantly enhanced root length, shoot length, biomass under different conditions in plants and seedlings of *Brassica juncea.* Various findings have reported improvement in number, weight and yield of plants by application of TRIA, which improved permeability of membranes, division of cell, cell elongation (Hangarter et al., 1978). Application of TRIA and H_2S upregulated growth attributes in plants under abiotic stress conditions. Our findings states that H_2S play beneficial role in alleviating different kind of environmental stresses (Zhang et al. 2011; Zhang et al., 2009; Fotopoulos et al., 2013). H_2S is known to regulate generation of ROS and control inhibition of ETC (Mancardi et al., 2009). Treatment with H_2S modify target genes which alleviate negative effect of salinity stress and improve root development in plant by modifying functioning of miRNA (Li et al. 2021). Seed germination was found to be influenced by application of H_2S in *Arabidopsis thaliana* by activating different AOX mediated cyanide-resistant respiration pathway (Fang et al., 2021). Likewise, in barley showed supplementation of H_2S improved root morphology by increasing activity of enzyme APX under Al-stress (Dawood et al., 2012). Qian et al. (2014) reported that H_2S application improved growth of plant by raising length of embryo and content photosynthetic

pigment like chlorophyll.

6.2.2 Photosynthetic system

6.2.2.1 Photosynthetic pigments

Photosynthetic activity was severely affected under salt stress which had major impact on its activity. Photosynthesis activity severely impacted yield and productivity of crop (Chaum et al., 2009; Shelke et al., 2017). Salt stress reduced activity of pigments, ETC, enzymes and photosystems (Rahman et al., 2021). It was exhibited that content of photosynthetic pigments reduced under stressed conditions. Decline in level of porphobilinogen (PBG) molecule caused reduction in pigment molecule by affecting synthesis of chlorophyll content (Cencki et al., 2010). Perveen et al. (2010) stated that salinity stress decreased photosynthetic pigments in *Triticum aestivum*. These findings go consistent with our studies where salinity stress severely affected activity of photosynthetic enzymes. Foliar application of TRIA improved content of pigments like Chl a and Chl b. Apart from its beneficiary role on photosynthetic pigment, TRIA was found to improve efficiency of PS II (Aziz et al., 2013). Similar findings were reported in *Brassica napus* where application of TRIA increased the activity of photosynthetic pigments. Therefore, increase in photosynthetic pigment could be increased CO_2 assimilation and rubisco performance (Erikson et al., 1991).

Application of TRIA alleviated salinity stress by enhancing photosynthetic pigments, photosystem efficiency, photochemical quenching and electron transport chain (Zaid et al., 2020). H_2S is known to be one of the most important signaling molecule which regulate functioning of photosynthetic apparatus by improving photosynthesis and content of chloroplast under different kind of stresses Fluorescence of chlorophyll is the main reason for causing primary reactions mandatory for photosynthesis (Tang et al., 2020). In our study H_2S maintain the membrane integrity and chloroplast functioning under salt stress. Treatment with H_2S increase the synthesis and development of chloroplast and alleviate degradation rate under stressed environmental conditions (Chen et al., 2015). Similar reports of alleviation of Cd-stress was studied by Ali et al. (2014) in *Brassica napus* by elevation of different photosynthetic attributes, enzymatic activities of different antioxidants and enzyme,

net photosynthetic rate, carboxylation efficiency. It was found that higher rate of A_{sat} improved photosynthesis (Strasser et al., 2018).

6.2.2.2 Gaseous exchange characteristics

Stress like conditions caused due to environmental factors alter gaseous characteristics (Ahmad et al. 2011; Asgher et al., 2014). Salinity caused disruption in gas exchange characteristics (Munns and Tester, 2008). Different agricultural crops have reported effect of salt stress (Hatami et al. 2010), wheat (Ashraf and Bashir, 2003), rice (Cha-um et al., 2010). Reduction in growth is caused due to closure of stomata and sub-stomatal CO_2 concentration under salinity stress (Netondo et al., 2004). This kind of stomatal variation in concentration of CO_2 is caused due to direct and indirect influence of salinity stress. TRIA tends to have positive response on photosynthetic apparatus (Khan et al., 2010). It was found that Treatment of H_2S and TRIA improved gaseous characteristics in seedlings and plants of *Brassica juncea.* It had been found that increase in gaseous characteristics is due to improvement in photosynthetic rate, transpiration rate, electron transport rate, q, P, K^+ mineral content in root and shoot. Similar results were reported by Habib (2020) in wheat under arsenic stress, where foliar application of TRIA positively influenced stomatal conductance, transpiration rate and photosynthetic rate and internal CO_2. Pre-sowing with TRIA was found to be enhanced due photochemical quenching, improvement in PSII, electron transport chain in different environmental conditions (Perveen et al., 2013). Studies by Duan et al. (2015) in H_2S treated rice plants. It was found that gaseous exchange characteristics in plants were found be enhanced by regulation of enzyme Rubisco (Chen et al., 2011). Reduction of photosynthesis was due to causing stomatal closure and reduction in intercellular $CO_,$ carbon assimilation. Gaseous exchange characteristics were found to be upsurged due to application of H_2S under cadmium stress (Kaur et al., 2022). Net photosynthetic rate was found to be increased in *Artemisia annua* due to stomatal limitations under copper stress (Nomani et al., 2021).

6.2.3 Metabolites

In case of stressful environmental conditions, different kinds of pathway like shikimic acid and phenylpropanoid show antioxidant nature (Ren and Sun, 2014). The

antioxidant nature of the OH group provides H to the ROS in the termination reaction and disrupt the formation of new radicals. Higher activity of phenylpropanoid pathway in case of stressed like conditions activates PAL enzyme which cause higher phenols accumulation due to ROS scavenging (Siboza et al., 2014). Recent studies stated that content of different metabolites like flavonoid, anthocyanin and phenols was found to be reduced under different concentrations of NaCl. Further, phytohormone and signaling molecule increased the content of different metabolites under salt stressed conditions. Similar studies were conducted in pepper (Diaz et al., 2001) and maize (Winkely-Shirley, 2002) under different environmental conditions.

Exogenous application of TRIA increase the content of phenol by stimulating gene like PAL due to generation of ROS (De Pinto et al., 2002). Likewise, activity of gene PAL was found to enhanced due to exogenous application of TRIA in green gram (Kumaravelu et al., 2000). Flavonoids are well known to cause homeostasis of ROS due to its antioxidant nature under stressed environmental conditions. A rise in the content of flavonoid and anthocyanin was noticed in *Triticum aestivum* TRIA in As toxicity (Ali et al., 2020). H_2S mediated increase in the content of different metabolites at drought stressed (Kolupaev et al., 2018). TRIA and H_2S application caused rise in the phenolic compounds in *Brassica juncea* under salt stress by reducing generation of ROS.

6.2.4 Oxidative stress

Excessive accumulation of salt in the plants cause ROS generation which results in peroxidation of proteins, lipids, enzymes. Higher generation of ROS results in increase production rate of MDA and H_2O_2 which are to damage cell (Wani et al., 2018; Farooq et al., 2019) and its constituents (Foyer and Noctor 2009; Groß et al., 2013). This ROS generation effect the membrane integrity by raising the MDA and H_2O_2 level in *Brassica juncea*. Studies conducted on different cultivars of wheat like (MH-97 and S-24). 10 and 20 µM Triacontanol reduces membrane damage caused due improved enzymatic system by strong oxidizing agent responsible for reducing negative impact of stress. TRIA application is responsible malonaldehyde and hydrogen peroxidae content by increasing the activity of enzyme Peroxidase (POD) (Perveen et al., 2012). Under drought stress, level of H_2O_2 was found to be reduced

whereas the level of MDA was found to be unaffected. Similar findings were reported in case of maize where foliar spray of TRIA reduced H_2O_2 and MDA content under osmotic stress (Perveen et al., 2016). H_2S also declined the level of MDA and H_2O_2 in seeds of wheat under osmotic stress conditions activity of enzyme lipoxygenase (Zhang et al., 2010b). H_2S tends to alleviate oxidative stress in plants by improving APX, SOD, and CAT enzyme response by using ROS (Dixit et al., 2002, Yu et al., 2007) by acting as signaling molecule which stimulate different cellular defense mechanism (Clijsters et al., 1999, Matés 2000).

6.2.5 Osmolytes

Content of osmolytes in plants is found to be reduced in different kind of environmental stresses due to hindered growth rate and electron leakage. In order deal with these kinds of environmental stresses plants produce different kind of compatible solutes like osmolytes which stabilize the integrity of membrane (Ashraf and Foolad, 2007; Hayat et al., 2010; Murmu et al., 2017). In response to NaCl stress, proline content was found to be increased due to activation of proline biosynthesis and decrease in protein turnover (Misra and Saxena, 2009). Levels of osmolytes were noticed to be declined in the present study. However, TRIA and H_2S treatment escalated their content in *Brassica juncea*. Foliar treatment of TRIA decreased proline content under abiotic stress condition (Krishnan and Kumari, 2008). The decrease in content proline was found due to uptake of atmospheric CO_2 (Lawlor and Cornic, 2002) and decrease in availability of NADP+ (Hossain et al. 2011). Likewise, TRIA applied on the seeds of *Brassica napus* L. with different concentrations of TRIA by abiotic stress conditions in plants of *Brassica* by increasing glycinebetaine content (Shahbaz et al., 2013) and maize showed increase in proline content (Perveen et al., 2017). Rise in the content of osmolytes maintain redox homeostasis and functioning of enzyme through ROS and protect key processes by maintain cellular osmolarity (Ahanger & Ahmad, 2019). H_2S raised the content of proline in spinach by synthesis of polyamine (Chen et al., 2016). The key enzyme which is responsible for the synthesis of glycine betaine is betaine aldehyde dehydrogenase. The modulations caused in the biosynthetic pathways is responsible for causing variation in producton of osmolytes (Ahmad et al., 2013). The production of these biosynthetic pathways is

responsible for increasing content of different osmolytes and securing membrane level under abiotic conditions. Plants antioxidant as well as glyoxalase system regulate content of osmolytes by enhancing stress endurance (Hasanuzzaman et al., 2014).

6.2.6 Carbohydrates

Starch build in the chloroplast is distributed to other component and supply energy to the cells and needs fixation of carbon during photosynthesis for the production (Wahid et al., 2007). Carbohydrates is known to be the main source of energy required for performing metabolic activities (Muller et al., 2011). In investigation it was stated that contents of carbohydrates increased under salinity stress. However, application TRIA and H_2S mitigated salinity stress by increasing content of carbohydrates. Source of carbohydrates which provide energy for cellular metabolism and protect cellular components is photosynthesis (Munns & Jermaat, 1986). Decrease in starch was observed in poor enviornmental factors (Sadak et al., 2012; Hassanein et al. 2009). Salinity impairs the accumulation of carbohydrates in growing and expanded tissues of plant (Munns et al., 1993). Accumulation of carbohydrates is responsible for regulating pentose phosphate pathway due to formation of ROS scavenger which act as free radical scavenger (Ende and Peshev, 2013; Hu et al., 2012; Van den Ende & Valluru, 2009). Content of carbohydrates was found to be enhanced by application of TRIA on exposed to *Zingiber officinale* (Singh et al., 2011), *Arachis hypogea* (Azizi et al., 2011), and *Zea mays* (El-shahfey et al., 2018) under salt stress. Hydrogen sulfide (H_2S) mediated content of carbohydrate in plants *of Brassica juncea*. It has been found that organic molecules like carbohydrates are synthesized under stressed environmental conditions (Siddiqui et al., 2019, 2020).

6.2.7 Antioxidant defense system

6.2.7.1 Antioxidative enzymes

Salt stress is known to generate oxidative stress which damage electron transport chain in different cellular organelles. In order to develop tolerance towards these oxidative stress plants develop well developed enzymatic system to deal with these kind of stressful enviornments (Parida and Das, 2005). Enzymatic activities of

different enzymes like CAT, SOD, APX, GR, DHAR, MDHAR, GST and POD cause Scavenging of ROS (Herbette et al., 2011). Enzymatic system plays major part in ROS detoxification by maintaining a balance in production and scavenging of ROS under adverse effect of salinity. Different antioxidant performs different function to maintain level of ROS in plants like SOD causes O^{2-} scavenging, CAT convert H_2O_2 into water and oxygen, GSH is responsible for oxidizing glutathione, GPX cause reduction of H_2O_2 and HO_2 to H_2O and lipid alcohols (Rajput et al., 2021). In our reaserch, Level of different enzymes like SOD, CAT, POD GST, GPOX, GR APX, PPO, DHAR and MDHAR was found to be enhanced in different conditions. Similar case of enzymatic activity of antioxidants was found to be enhanced in different crop like tomato and maize under salt stress (Mursheed et al., 2014; AbdElgawad et al., 2016).

In our present study, it was found that treatment of TRIA and H_2S increase the functioning of different antioxidative enzymes. Similarly, it was found that TRIA improved the antioxidant defense mechanism in *Mentha piperita* L. under different salinity stress. Antioxidant enzymatic activity of the plant is directly linked with ROS concentration (Apel and Hirt, 2014). Application of TRIA increase antioxidant enzymatic activity of enzymes like SOD, CAT and POD by reducing production of ROS (Raza et al., 2022). Generation of ROS affect growth of plant by causing damage on the cellular components. Excessive production of these ROS cause damage on biomolecular structure like lipid, protein (Mittler et al. 2017; Singh et al., 2019). Therefore, ROS production manage defense system (Mitller et al., 2011, Gill et al., 2015). Functioning of enzyme POD was enhanced by TRIA application which mitigated salinity stress (Perveen et al., 2011).

Likewise, activity of enzyme SOD and CAT was found to be increased under TRIA application which mitigated salinity stress in coriander plants (Karam et al. 2016). Application of TRIA played a beneficial role in alleviating chilling stress in plants of *Ocimum basilicum* L. by increasing the activity of enzyme catalase (Borowski and Blamowski, 2009). Likewise, activity of enzyme POX increased in pea plants in comparison to control (Henry and Gordon, 1980). GPX enzyme was found to be improved by triacontanol application under Cd-induced stressed in *Zea mays* (Ahmed

et al., 2012). It has been found that ROS detoxification and scavenging of peroxides protects the components of cell by increasing activity of enzyme GST and GPX under oxidative stress (Gill and Tuteja, 2010a, 2010b). Likewise, it was found that content of GSH and ASA increased in rice plants by application of TRIA, which regulated level of ROS under stressed conditions (Li et al., 2016).

TRIA treatment improve antioxidant enzyme activity under stressed situations by inhibiting the production of MDA and H_2O_2 and increasing the levels of antioxidants. TRIA modulates the activity of different defense related genes which play important role in increasing antioxidant enzymatic and non-enzymatic actvities (Hernández &Almansa, 2002; Aghaleh et al., 2009). It was investigated that H_2S as a signaling moiety mitigate drought and metal stress by modifying the antioxidant enzymatic activities (Zhang et al. 2010). H_2S modulate the antioxidant enzymatic activities in plants by regulating ROS level (Chongchatuporn et al., 2013). Shan et al. (2014) reported that H_2S mitigate the salinity in maize by maintaining enzymatic performance of CAT, SOD, APX and POD as well as ASA and GSH under salinity stress. Increased level of ascorbic acid and glutathione content which cause homeostastis of reactive oxygen species by managing redox metabolism in maize plants (Tiwari et al., 2019). Similar reports found that H_2S alleviate stresseful conditions by ascorbic acid and glutathione content (Shan et al., 2011).

6.2.7.2 Non-enzymatic antioxidants

Glutathione and ascorbic acid like non-enzymtaic antioxidants show a significant effect in improving plant-stressby regulating reactive oxygen species. These kinds of non-enzymatic antioxidants stabilize membrane structure within in the cells by stabilizing level of glutathione and ascorbic acid due its reductant properties which directly scavenge OH^- radicals (Foyer & Noctor, 2011). It has been found that application of TRIA protect membrane from damage due to presence of antioxidant compound which inhibit membrane peroxidation (Ramanaryan et. 2000; Khan et al. 2009). Furthermore, improvement in the contents of non-enzymatic antioxidants was reported by using TRIA-treatment by Karam and Keramat in *coriandrum sativum*, Maresca et al. (2017) in *Brassica napus*, and Zaid et al. (2020) in *Mentha arvensis* L. Similarly, Lin et al. (2023) in rice seedlings under chromium stress, Silva et al. (2017)

in tobacco, and Zhou et al. (2020b) in *Zea mays* also reported H_2S-regulated upsurge in the levels of antioxidants under temperature stress.

6.2.9 Gene Expression

SOD and CAT gene was stimulated under stressed environmental conditions. Treatment with TRIA and H_2S improved the expression of different types of genes. Recently a study conducted on TRIA found that it up-regulated the level of rbc gene due to activity of enzyme Rubisco in seedlings of Rice (Houtz et al., 1985). Likewise, genes like SOD and CAT was expressed by application of TRIA in salt stressed conditions in *Zea mays* L. plants (Rizwan et al., 2018). Furthermore, H_2S application improved SOD and CAT gene expression under salt stress of eggplant (Ekinci et al. 2021). H_2S-mediated salinity stress by facilitate the gene expression due defense mechanism of antioxidant enzymes. Present research states that TRIA and H_2S played essential role in mitigating salinity stress by escalation in performance of genes and enzymes.

`

Chapter 7

Summary and Conclusions

Salt stress is main factor that affect growth and production of crop. World's agriculture is suffering major setback as it is known to effect different physiological and yield aspects in plants and this situation is getting worse day by day. Among all the major crops, *Brassica juncea* is potential crop in the country's economic growth in agriculture. This is a major cost-effective crop and is known as backbone of the agriculture. Yield of this crop is severely affected due to salinity. Salinity causes change in plant characteristic from time of salt imposition until it matures (Munns, 2002). Thus, it is of vital importance to determine the resistance of salt in order to improve crop yield and production. This may be done through the successful management of salt stress crop. Therefore, there is need of reducing salinity in *Brassica juncea* by the use of cost effective and environment-friendly strategies.

The present research work entitled as "Effect of exogenous application on triacontanol and hydrogen sulphide on *Brassica juncea* L. exposed to salinity stress" aimed in mitigation of salinity in *Brassica juncea* under influence of TRIA and H_2S on different physiochemical aspects and biochemical parameters. Therefore, this study was undertaken to meet following objectives:

1. Analysis of Triacontanol and H_2S in mitigating malicious effect of salinity stress on *Brassica juncea*
2. Assessment of Triacontanol and H_2S induced growth attributes and physiochemical aspect of *Brassica juncea* in vitro and in vivo under salinity stress.
3. Comparative study of gene expression of salt stress related genes in *Brassica juncea* in response to Triacontanol and H_2S.

In order to achieve above-mentioned objectives, Pre-treatment of seeds was carried out in 150 µM TRIA solution for 8 hr left out seeds placed in distilled water served as control. Sterilized seedlings were germinated in petriplates lined with *Whatman* No-1 filter paper lined glass. Salt stress was given in the form of NaCl solution. NaCl was applied at different concentrations to the soil i.e., 50, 100 and 150 mM respectively. Seedlings were exogenously supplied with H_2S in the form of NaHS donor (Sodium hydrosulfide) as foliar spray at 25 µM concentration. Petriplate contained 3ml of test

solution on day 1 followed by 2 ml of test solution on day 2 and this process was followed till 7 days.

Experimentation using raised plants was carried out in agro-bags in Botanical garden, Lovely Professional University. Where uniform sized seeds were surface sterilized 0.01% sodium hypochlorite and rinsed 3-4 times. After pre-treatment of seeds with TRIA (150 µM) they were sown in agro-bags filled with soil and manure

Different treatments used for carrying out experimentation were:

- NaCl solution (50,100 and 150 mM)
- 25 µM H_2S

Seedlings and plants of *Brassica juncea* were harvested after 7, 30 and 60 days for further analysis. Different physiological, molecular, morphological and biochemical parameters were analyzed.

- Morphological traits like length, biomass and germination percentage were recorded in seedlings and plants.
- Photosynthetic pigments i.e., chlorophylls, carotenoids and xanthophylls were measured under As stress. Gas exchange attributes were measured by IRGA in 30 and 60 days old plants.
- Metabolites i.e., flavonoids, anthocyanin and phenolic were measured
- MDA and H_2O_2 contents were measured in plants and 7-days old seedlings
- Membrane and nuclear damage in 7 old seedlings of *Brassica juncea* was noted using confocal microscope.
- Proline and glycine betaine contents were evaluated
- Protein content and total sugar were estimated in seedlings and plants of *B. juncea*.
- Antioxidative enzymes namely SOD, CAT, APX, POD, GR, GPOX, DHAR, MDHAR, GST and PPO were measured.
- Ascorbic acid, glutathione and **α-** tocopherol contents were measured among

antioxidants.

- Gene expression was analyzed by using qRT-PCR for different stress related genes.

Statistical significant difference using SPSS 16.0 was measured. The current studies done on salinity stress in *Brassica juncea* seedlings or plants showed following important observations:

Length, Biomass, vigor index and germination percentage were found to be severely affected under salt stress. Among all three different concentrations of NaCl. Maximum reduction was observed at saline condiions in contrast to all other concentrations. However, combined application TRIA and H_2S improved all these attributes in seedlings and plants under salt stress in comparison to plants under control. Application of TRIA and H_2S improved different characterstics of plant growth, development, increase in germination and biomass after treatment, which played beneficial role in enhancing the productivity of the crop.

Different photosynthetic pigment i.e., chlorophyll, carotenoid and xanthophyll was found to decline under salt stress. Maximum reduction in photosynthetic pigment was found at 150 mM concentration. Whereas, minimum reduction in photosynthetic pigment was observed in control in seedlings and plants. Salinity stress decreased gaseous exchange characteristics in comparison to control. But, the application of TRIA and H_2S significantly enhanced these gaseous exchange characteristics.

Metabolites i.e., flavonoids, anthocyanin and phenolic content was found to get decreased when seedlings and plants of *B. juncea* were exposed to salinity stress. Application of TRIA and H_2S enhanced content of metabolites in seedlings and plants of *B. juncea*. Indiviual application of TRIA and H_2S led to the upsurge in the content of anthocyanin, flavonoid and phenolics. Combination of TRIA and H_2S showed better results in enhancing metabolic content as compared to their individual application.

Elevated of MDA and H_2O_2 content due to salinity. Maximum elevation in MDA and H_2O_2 content was observed at 50mM as compared to other two concentrations. Control seedlings and plants showed maximum elevation in MDA and H_2O_2 content

as compared to all other treatments. Higher rate of membrane and nuclear damage was seen NaCl treated seedlings,

Content of osmolytes was found to increase in seedlings and plants subjected to salinity. Proline and glycine-betaine was found to enhance with increasing concentration of salt. Co-application enhanced the osmolyte content as compared to their individual application under unstressed conditions.

Antioxidant enzymes like CAT, SOD, POD, APX, POD, GPOX, DHAR, GR, MDHAR, GST, and PPO was found to be increased by defense system. Seedlings and plants showed maximum reduction in antioxidant enzymatic activity. Content of ascorbic acid, glutathione and tocopherol get reduced under salinity stress by exogenous application

SOD and CAT gene expression improved in case of stress. Salinity severely affected expression of genes. Individual and combined application further enhanced gene expression under stressed condition. Different functional groups were observed in the region of lipds, carbohydrates, proteins and cell wall components.

Therefore, it was found that combined application of TRIA and H_2S is an effective strategy that alleviate salinity stress in seedlings and plants of *B. juncea* by enhancing their morphological, physiological, biochemical and molecular aspects in comparison to their individual applications under stressed conditions.

Future Prospects

- Salinity has an adverse impact on growth, physiological and biochemical processs on *B. juncea*. Synergistic association of triacontanol and hydrogen sulphide ameliorated salinity by improving growth, yield, photosynthetic pigments and gaseous exchange at different growth stages in this study.
- Mechanism of action of TRIA and H_2S in mitigating stress could be helpful to draw conclusion about its growth promoting properties on plants.
- Ameliorating role of TRIA and H_2S in mitigating different abiotic stresses like metal, drought, heat and cold stress in different crops can be studied.
- Synergistic association between TRIA and H_2S can serve as economical and eco-friendly approach for the farmers.

Bibliography

Abbasi, G. H., Ijaz, M., Akhtar, J., Anwar-Ul-Haq, M., Jamil, M., Ali, S., ... & Khan, H. N. (2016). Profiling of anti-oxidative enzymes and lipid peroxidation in leaves of salt tolerant and salt sensitive maize hybrids under NaCl and Cd stress. *Sains Malaysiana*, *45*(2), 177-184.

Abdel Latef, A. (2010). Changes of antioxidative enzymes in salinity tolerance among different wheat cultivars. *Cereal Research Communications*, *38*(1), 43-55.

Aebi, H. (1983). Catalase. In Bergmeyer, H. U. (Ed.), *Methods of enzymatic analysis* (Vol. 2, pp.673-684). Verlag Chemie: Weinhan.

Aftab T, Khan MMA, Idrees M, Naeem M, Singh M, Ram M. Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. *J Plant Interact.* 2010; 5:273–281.

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., & Razavi, K. (2009). Salt stress effects on growth, pigments, proteins and lipid peroxidation in Salicornia persica and S. europaea. *Biologia plantarum*, *53*, 243-248.

Aghdam, M. S., Mahmoudi, R., Razavi, F., Rabiei, V., & Soleimani, A. (2018). Hydrogen sulfide treatment confers chilling tolerance in hawthorn fruit during cold storage by triggering endogenous H2S accumulation, enhancing antioxidant enzymes activity and promoting phenols accumulation. *Scientia Horticulturae*, *238*, 264-271.

Ahanger, M. A., Mir, R. A., Alyemeni, M. N., & Ahmad, P. (2020). Combined effects of brassinosteroid and kinetin mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism. *Plant Physiology and Biochemistry*, *147*, 31-42.

Ahmad, J., Ali, A. A., Al-Huqail, A. A., & Qureshi, M. I. (2021). Triacontanol attenuates drought-induced oxidative stress in Brassica juncea L. by regulating lignification genes, calcium metabolism and the antioxidant system. *Plant Physiology and Biochemistry*, *166*, 985-998.

Ahmad, P., Ahanger, M. A., Alam, P., Alyemeni, M. N., Wijaya, L., Ali, S., & Ashraf, M. (2019). Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [Vigna radiata (L.) Wilczek] through the modifications of physio-biochemical

attributes and key antioxidant enzymes. *Journal of Plant Growth Regulation*, *38*, 70-82.

Ahmad, P., Azooz, M. M., & Prasad, M. N. V. (Eds.). (2013). *Ecophysiology and responses of plants under salt stress*. New York: Springer.

Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., & Gucel, S. (2015). Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (Brassica juncea L) through antioxidative defense system. *Frontiers in plant science*, *6*, 868.

Ahmad, P., Nabi, G., & Ashraf, M. (2011). Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *South African Journal of Botany*, *77*(1), 36-44.

Ahmad, R., Ali, S., Rizwan, M., Dawood, M., Farid, M., Hussain, A., ... & Ahmad, P. (2020). Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. *Physiologia plantarum*, *168*(2), 289-300.

Ahmed, S., Ahmad, M., Sardar, R., & Ismail, M. A. (2023). Triacontanol priming as a smart strategy to attenuate lead toxicity in Brassica oleracea L. *International Journal of Phytoremediation*, *25*(9), 1173-1188.

Ahmed, S., Amjad, M., Sardar, R., Siddiqui, M. H., & Irfan, M. (2023). Seed Priming with Triacontanol Alleviates Lead Stress in Phaseolus vulgaris L. (Common Bean) through Improving Nutritional Orchestration and Morpho-Physiological Characteristics. *Plants*, *12*(8), 1672.

Ajibade, F. O., Adelodun, B., Lasisi, K. H., Fadare, O. O., Ajibade, T. F., Nwogwu, N. A., ... & Wang, A. (2021). Environmental pollution and their socioeconomic impacts. In *Microbe mediated remediation of environmental contaminants* (pp. 321-354). Woodhead Publishing.

Akbarimoghaddam, H., Galavi, M., Ghanbari, A., & Panjehkeh, N. (2011). Salinity effects on seed germination and seedling growth of bread wheat cultivars. *Trakia journal of Sciences*, *9*(1), 43-50.

Alharbi, B. M., Abdulmajeed, A. M., & Hassan, H. (2021). Biochemical and

molecular effects induced by triacontanol in acquired tolerance of rice to drought stress. *Genes*, *12*(8), 1119.

Ali, B., Gill, R. A., Yang, S., Gill, M. B., Ali, S., Rafiq, M. T., & Zhou, W. (2014). Hydrogen sulfide alleviates cadmium-induced morpho-physiological and ultrastructural changes in Brassica napus. *Ecotoxicology and Environmental Safety*, *110*, 197-207.

Ali, H. M. M., & Perveen, S. (2020). Effect of foliar applied triacontanol on wheat (Triticum aestivum L.) under arsenic stress: a study of changes in growth, yield and photosynthetic characteristics. *Physiology and Molecular Biology of Plants*, *26*, 1215-1224.

Ali, H. M. M., & Perveen, S. (2020). Effect of foliar applied triacontanol on wheat (Triticum aestivum L.) under arsenic stress: a study of changes in growth, yield and photosynthetic characteristics. *Physiology and Molecular Biology of Plants*, *26*, 1215-1224.

Alp, K., Terzi, H., & Yildiz, M. (2022). Proteomic and physiological analyses to elucidate nitric oxide-mediated adaptive responses of barley under cadmium stress. *Physiology and Molecular Biology of Plants*, *28*(7), 1467-1476.

Alsahli, A. A., Bhat, J. A., Alyemeni, M. N., Ashraf, M., & Ahmad, P. (2021). Hydrogen sulfide (H 2 S) mitigates arsenic (As)-induced toxicity in pea (Pisum sativum L.) plants by regulating osmoregulation, antioxidant defense system, ascorbate glutathione cycle and glyoxalase system. *Journal of Plant Growth Regulation*, *40*, 2515-2531.

Alzahrani, S. M., Alaraidh, I. A., Migdadi, H., Alghamdi, S., Khan, M. A., & Ahmad, P. (2019). Physiological, biochemical, and antioxidant properties of two genotypes of Vicia faba grown under salinity stress. *Pak. J. Bot*, *51*(3), 786-798.

Amooaghaie, R., & Enteshari, S. (2017). Role of two-sided crosstalk between NO and H_2S on improvement of mineral homeostasis and antioxidative defense in Sesamum indicum under lead stress. *Ecotoxicology and Environmental Safety*, *139*, 210-218.

Antoniou, C., Xenofontos, R., Chatzimichail, G., Christou, A., Kashfi, K., &

Fotopoulos, V. (2020). Exploring the potential of nitric oxide and hydrogen sulfide (NOSH)-releasing synthetic compounds as novel priming agents against drought stress in Medicago sativa plants. *Biomolecules*, *10*(1), 120.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, *55*, 373-399.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Photophenoloxidase in *Beta vulgaris. Plant Physiology*, *24*, 1-15.

Aroca, A., Gotor, C., & Romero, L. C. (2018). Hydrogen sulfide signaling in plants: emerging roles of protein persulfidation. *Frontiers in Plant Science*, *9*, 1369.

Asadi Karam, E., & Keramat, B. (2017). Foliar spray of triacontanol improves growth by alleviating oxidative damage in coriander under salinity. *Indian Journal of Plant Physiology*, *22*, 120-124.

Asgher, M., Khan, N. A., Khan, M. I. R., Fatma, M., & Masood, A. (2014). Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. *Ecotoxicology and Environmental Safety*, *106*, 54-61.

Ashraf, M. A., Riaz, M., Arif, M. S., Rasheed, R., Iqbal, M., Hussain, I., & Mubarik, M. S. (2019). The role of non-enzymatic antioxidants in improving abiotic stress tolerance in plants. In *Plant tolerance to environmental stress* (pp. 129-144). CRC Press.

Ashraf, M. F. M. R., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), 206-216.

Ashraf, M., & Harris, P. (Eds.). (2005). *Abiotic stresses: plant resistance through breeding and molecular approaches*. CRC press.

Ashraf, M., & McNeilly, T. (2004). Salinity tolerance in Brassica oilseeds. *Critical reviews in plant Sciences*, *23*(2), 157-174.

Ashraf, M., Akram, N. A., Al-Qurainy, F., & Foolad, M. R. (2011). Drought tolerance: roles of organic osmolytes, growth regulators, and mineral

nutrients. *Advances in agronomy*, *111*, 249-296.

Ashraf, M., Athar, H. R., Harris, P. J. C., & Kwon, T. R. (2008). Some prospective strategies for improving crop salt tolerance. *Advances in agronomy*, *97*, 45-110.

Awasthi, P., Mahajan, V., Jamwal, V. L., Kapoor, N., Rasool, S., Bedi, Y. S., & Gandhi, S. G. (2016). Cloning and expression analysis of chalcone synthase gene from *Coleus forskohlii*. *Journal of Genetics*, *95*(3), 647-657.

Ayers, R. S., & Westcot, D. W. (1985). *Water quality for agriculture* (Vol. 29, p. 174). Rome: Food and Agriculture Organization of the United Nations.

Aziz, R., Shahbaz, M., & Ashraf, M. (2013). Influence of foliar application of triacontanol on growth attributes, gas exchange and chlorophyll fluorescence in sunflower (Helianthus annuus L.) under saline stress. *Pak. J. Bot*, *45*(6), 1913-1918.

Aziz, R., Shahbaz, M., & Ashraf, M. (2013). Influence of foliar application of triacontanol on growth attributes, gas exchange and chlorophyll fluorescence in sunflower (Helianthus annuus L.) under saline stress. *Pak. J. Bot*, *45*(6), 1913-1918.

Babu, N. N., Krishnan, S. G., Vinod, K. K., Krishnamurthy, S. L., Singh, V. K., Singh, M. P., ... & Singh, A. K. (2017). Marker aided incorporation of Saltol, a major QTL associated with seedling stage salt tolerance, into Oryza sativa 'Pusa basmati 1121'. *Frontiers in plant science*, *8*, 41.

Bacilio, M., Rodriguez, H., Moreno, M., Hernandez, J. P., & Bashan, Y. (2004). Mitigation of salt stress in wheat seedlings by a gfp-tagged Azospirillum lipoferum. *Biology and Fertility of Soils*, *40*(3), 188-193.

Banu, M. S. A., Huda, K. M. K., Sahoo, R. K., Garg, B., Tula, S., Islam, S. S., ... & Tuteja, N. (2015). Pea p68 imparts salinity stress tolerance in rice by scavenging of ROS-mediated H 2 O 2 and interacts with argonaute. *Plant Molecular Biology Reporter*, *33*, 221-238.

Bates, L. S., Waldren, R. P., & Tear, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, *39*, 205-207.

Belaqziz R, Romane A, Abbad A. Salt stress effects on germination, growth and essential oil content of an endemic thyme species in Morocco (Thymus maroccanus

Ball.). Journal of Applied Sciences Research. 2009; 5:858-863.

Bertuzzi, S., & Tretiach, M. (2013). Hydrogen sulphide inhibits PSII of lichen photobionts. *The Lichenologist*, *45*(1), 101-113.

Bhandari, S., Bhandari, A., & Shrestha, J. (2021). Effect of different doses of triacontanol on growth and yield of kohlrabi (Brassica oleracea L. var. gongylodes). *Heliyon*, *7*(10).

Bhardwaj, R., Kaur, R., Bali, S., Kaur, P., Sirhindi, G., K Thukral, A., ... & P Vig, A. (2015). Role of various hormones in photosynthetic responses of green plants under environmental stresses. *Current Protein and Peptide Science*, *16*(5), 435-449.

Bharwana S.A., Ali S., Farooq M.A., Iqbal N., Abbas F., Ahmad M.S.A. (2013): Alleviation of lead toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. Journal of Bioremediation and Biodegradation, 4: 2–11.

Bharwana, S. A., Ali, S., Farooq, M. A., Ali, B., Iqbal, N., Abbas, F., & Ahmad, M. S. A. (2014). Hydrogen sulfide ameliorates lead-induced morphological, photosynthetic, oxidative damages and biochemical changes in cotton. *Environmental Science and Pollution Research*, *21*, 717-731.

Boros, M., & Keppler, F. (2018). Production and Signaling of Methane. *Gasotransmitters, edited by: Wang, R., Royal Society of Chemistry, London*, 192-234.

Borowski, E., & Blamowski, Z. K. (2009). The effects of triacontanol 'TRIA'and Asahi SL on the development and metabolic activity of sweet basil (L.) plants treated with chilling. *Folia Horticulturae*, *21*(1), 39-48.

Busch, F. A. (2014). Opinion: the red-light response of stomatal movement is sensed by the redox state of the photosynthetic electron transport chain. *Photosynthesis research*, *119*, 131-140.

Caines, A. M., & Shennan, C. (1999). Interactive effects of Ca2+ and NaCl salinity on the growth of two tomato genotypes differing in Ca2+ use efficiency. *Plant Physiology and Biochemistry*, *37*(7-8), 569-576.

Callard, D., Axelos, M., & Mazzolini, L. (1996). Novel molecular markers for late phases of the growth cycle of *Arabidopsis thaliana* cell-suspension cultures are expressed during organ senescence. *Plant Physiology*, *112*(2), 705-715.

Carlberg, I., & Mannervik, B. (1975). Purification and characterization of the flavoenzyme glutathione reductase from Rat liver. *Journal of Biological Chemistry*, *250*, 5475-5480.

Carpici, E. B., Celik, N., & Bayram, G. (2010). The effects of salt stress on the growth, biochemical parameter and mineral element content of some maize (Zea mays L.) cultivars. *African Journal of Biotechnology*, *9*(41), 6937-6942.

Cenkci S, Cigerci IH, Yildiz M, Ozay C, Bozdag A, Terzi H (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ Exp Bot 67:467–473.

Chattha, M. U., Khan, M. A., Khan, I., Mahmood, A., Chattha, M. B., Hassan, M. U., ... & Elsabagh, A. (2023). Comparison Of Physio-Biochemical And Antioxidant Enzymes In Maize During Early Growth Stage In Response To Salt Stress. *Pak. J. Bot*, *55*(6), 1991-1997.

Chaum, S., & Kirdmanee, C. (2010). Effect of glycinebetaine on proline, water use, and photosynthetic efficiencies, and growth of rice seedlings under salt stress. *Turkish Journal of Agriculture and Forestry*, *34*(6), 517-527.

Cha-Um, S., Yooyongwech, S., & Supaibulwatana, K. (2010). Water deficit stress in the reproductive stage of four indica rice (Oryza sativa L.) genotypes.

Cha-Um, S., Yooyongwech, S., & Supaibulwatana, K. (2010). Water deficit stress in the reproductive stage of four indica rice (Oryza sativa L.) genotypes.

Chen X, Yuan H, Chen R, Zhu L, Du B, Weng Q, He G (2002) Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): possible role of triacontanol as a plant growth stimulator. Plant Cell Physiol 43:869–876.

Chen X, Yuan H, Chen R, Zhu L, Du B, Weng Q, He G. Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): possible role of triacontanol as a plant growth stimulator. *Plant Cell Physiol*. 2002;43:869–876.

Chen X, Yuan H, Chen R, Zhu L, He G. Biochemical and photochemical changes in response to triacontanol in rice (*Oryza sativa* L.) *Plant Growth Regul.* 2003;40:249–256.

Chen, J., Shang, Y. T., Wang, W. H., Chen, X. Y., He, E. M., Zheng, H. L., & Shangguan, Z. (2016). Hydrogen sulfide-mediated polyamines and sugar changes are involved in hydrogen sulfide-induced drought tolerance in Spinacia oleracea seedlings. *Frontiers in Plant Science*, *7*, 1173.

Chen, J., Wu, F. H., Wang, W. H., Zheng, C. J., Lin, G. H., Dong, X. J., ... & Zheng, H. L. (2011). Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. *Journal of experimental botany*, *62*(13), 4481-4493.

Chen, X., Chen, Q., Zhang, X., Li, R., Jia, Y., Ef, A., ... & Hu, X. (2016). Hydrogen sulfide mediates nicotine biosynthesis in tobacco (Nicotiana tabacum) under high temperature conditions. *Plant Physiology and Biochemistry*, *104*, 174-179.

Chen, X., Yuan, H., Chen, R., Zhu, L., Du, B., Weng, Q., & He, G. (2002). Isolation and characterization of triacontanol-regulated genes in rice (Oryza sativa L.): possible role of triacontanol as a plant growth stimulator. *Plant and Cell Physiology*, *43*(8), 869-876.

Chen, Y. E., Liu, W. J., Su, Y. Q., Cui, J. M., Zhang, Z. W., Yuan, M., ... & Yuan, S. (2016). Different response of photosystem II to short and long- term drought stress in Arabidopsis thaliana. *Physiologia Plantarum*, *158*(2), 225-235.

Chen, Y., Wang, X. M., Zhou, L., He, Y., Wang, D., Qi, Y. H., & Jiang, D. A. (2015). Rubisco activase is also a multiple responder to abiotic stresses in rice. *PLoS one*, *10*(10), e0140934.

Chen, Z., Yang, B., Hao, Z., Zhu, J., Zhang, Y., & Xu, T. (2018). Exogenous hydrogen sulfide ameliorates seed germination and seedling growth of cauliflower under lead stress and its antioxidant role. *Journal of Plant Growth Regulation*, *37*, 5-15.

Chibnall, A. C., Williams, E. F., Latner, A. L., & Piper, S. H. (1933). The isolation of n-triacontanol from lucerne wax. *Biochemical Journal*, *27*(6), 1885.

Chongchatuporn U, Ketsa S, van Doorn WG (2013) Chilling injury in mango (Mangifera indica) fruit peel: Relationship with ascorbic acid concentrations and antioxidant enzyme activities. Postharvest Biol Technol 86:409–417.

Christou, A., Filippou, P., Manganaris, G. A., & Fotopoulos, V. (2014). Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. *BMC Plant Biology*, *14*(1), 1-11.

Christou, A., Manganaris, G. A., & Fotopoulos, V. (2014). Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants via transcriptional regulation of enzymatic and non-enzymatic antioxidants. *Environmental and Experimental Botany*, *107*, 46-54.

Corpas, F. J., González-Gordo, S., & Palma, J. M. (2021). Nitric oxide and hydrogen sulfide modulate the NADPH-generating enzymatic system in higher plants. *Journal of Experimental Botany*, *72*(3), 830-847.

Çulha, Ş., & ÇAKIRLAR, H. (2011). Effect of Salt Stress Induced by NaCl on Safflower Carthamus tinctorius L. Cultivars at Early Seedling Stages. *Hacettepe Journal of Biology and Chemistry*, *39*(1), 61-64.

Dadkhah, A. R., & Grrifiths, H. (2006). The effect of salinity on growth, inorganic ions and dry matter partitioning in sugar beet cultivars.

Dalton, D. A., Russell, S. A., Hanus, F. J., Pascoe, G. A., & Evans, H. J. (1986). Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proceedings of National Academy of Sciences*, *83*, 3811-3815.

Dawood, M. F., Sofy, M. R., Mohamed, H. I., Sofy, A. R., & Abdel-kader, H. A. (2022). Hydrogen sulfide modulates salinity stress in common bean plants by maintaining osmolytes and regulating nitric oxide levels and antioxidant enzyme expression. *Journal of Soil Science and Plant Nutrition*, *22*(3), 3708-3726.

Dawood, M. G., Abdelhamid, M. T., & Schmidhalter, U. (2014). Potassium fertiliser

enhances the salt-tolerance of common bean (Phaseolus vulgaris L.). *The Journal of Horticultural Science and Biotechnology*, *89*(2), 185-192.

Dawood, M., Cao, F., Jahangir, M. M., Zhang, G., & Wu, F. (2012). Alleviation of aluminum toxicity by hydrogen sulfide is related to elevated ATPase, and suppressed aluminum uptake and oxidative stress in barley. *Journal of hazardous materials*, *209*, 121-128.

de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, *56*(1), 87-94.

De la Torre-González, A., Montesinos-Pereira, D., Blasco, B., & Ruiz, J. M. (2018). Influence of the proline metabolism and glycine betaine on tolerance to salt stress in tomato (Solanum lycopersicum L.) commercial genotypes. *Journal of plant physiology*, *231*, 329-336.

de Pinto, M. C., Tommasi, F., & De Gara, L. (2002). Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. *Plant physiology*, *130*(2), 698-708.

Delledonne, M.: NO news is good news for plants. — Curr. Opin. Plant Biol. **8**: 390–396, 2005.

Demetriou, G., C. Neonaki, E. Navakoudis, and K. Kotzabasis, 2007. Salt stress impact on the molecular structure and function of the photosynthetic apparatus—the protective role of polyamines. Biochim Biophys Acta-Bioenerget, 1767:272–280.

Dhall RK, Sanjeev A, Ahuja S. Effect of triacontanol (vipul) on yield and yield attributing characters of tomato (*Lycopersicon esculentum* Mill.) *Environ Ecol.* 2004;22:64–66

Digruber, T., Sass, L., Cseri, A., Paul, K., Nagy, A. V., Remenyik, J., ... & Dudits, D. (2018). Stimulation of energy willow biomass with triacontanol and seaweed extract. *Industrial Crops and Products*, *120*, 104-112.

Ding, H., Ma, D., Huang, X., Hou, J., Wang, C., Xie, Y., ... & Guo, T. (2019). Exogenous hydrogen sulfide alleviates salt stress by improving antioxidant defenses and the salt overly sensitive pathway in wheat seedlings. *Acta Physiologiae Plantarum*, *41*, 1-11.

Doganlar, Z. B., Demir, K., Basak, H., & Gul, I. (2010). Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars.

Duan, B., Ma, Y., Jiang, M., Yang, F., Ni, L., & Lu, W. (2015). Improvement of photosynthesis in rice (Oryza sativa L.) as a result of an increase in stomatal aperture and density by exogenous hydrogen sulfide treatment. *Plant growth regulation*, *75*, 33-44.

Ekinci, M., Yildirim, E., & Turan, M. (2021). Ameliorating effects of hydrogen sulfide on growth, physiological and biochemical characteristics of eggplant seedlings under salt stress. *South African Journal of Botany*, *143*, 79-89.

Ende, W. V. D., & Peshev, D. (2013). Sugars as antioxidants in plants. *Crop improvement under adverse conditions*, 285-307.

Erikson, R., Hooker, E., & Meija, M. (1991). Underwater light penetration, phytoplankton biomass and photosynthetic activity in Lake Xolotlán (Managua). *Hydrobiological Bulletin*, *25*(2), 137-144.

Ertani, A., Schiavon, M., Muscolo, A., & Nardi, S. (2013). Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed Zea mays L. plants. *Plant and soil*, *364*, 145-158.

Esfandiari, E., & Abdoli, M. (2017). Variations of grain yield and agro-morphological traits of some promising durum wheat lines (Triticum turgidum L. var. durum) at zinc sufficient and deficient conditions. *Journal of Genetic Resources*, *3*(2), 68-79.

Faisal, M., Faizan, M., Tonny, S. H., Rajput, V. D., Minkina, T., Alatar, A. A., & Pathirana, R. (2023). Strigolactone-Mediated Mitigation of Negative Effects of Salinity Stress in Solanum lycopersicum through Reducing the Oxidative Damage. *Sustainability*, *15*(7), 5805.

Faisal, S., Mujtaba, S. M., Asma, & Mahboob, W. (2019). Polyethylene Glycol

mediated osmotic stress impacts on growth and biochemical aspects of wheat (Triticum aestivum L.). *Journal of Crop Science and Biotechnology*, *22*, 213-223.

Faiz, H., Khan, O., Ali, I., Hussain, T., Haider, S. T., Siddique, T., ... & Anjum, Q. S. (2022). Foliar application of triacontanol ameliorates heat stress through regulation of the antioxidant defense system and improves yield of eggplant. *Brazilian Journal of Biology*, *84*, e253696.

Fang, H., Liu, R., Yu, Z., Shao, Y., Wu, G., & Pei, Y. (2022). Gasotransmitter H2S accelerates seed germination via activating AOX mediated cyanide-resistant respiration pathway. *Plant Physiology and Biochemistry*, *190*, 193-202.

Fatma, M., Rahman, Z., & Khan, I. (2016). Measuring consumer perception of CSR in tourism industry: Scale development and validation. *Journal of Hospitality and Tourism Management*, *27*, 39-48.

Fichman, Y., Gerdes, S. Y., Kovács, H., Szabados, L., Zilberstein, A., & Csonka, L. N. (2015). Evolution of proline biosynthesis: enzymology, bioinformatics, genetics, and transcriptional regulation. *Biological Reviews*, *90*(4), 1065-1099.

Flohe, L., & Gunzler, W.A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology*, *105*, 114-121.

Fotopoulos, V., Christou, A., & Manganaris, G. A. (2013). Hydrogen sulfide as a potent regulator of plant responses to abiotic stress factors. *Molecular approaches in plant abiotic stress*, 353-373.

Foyer, C. H., & Noctor, G. (2011). Ascorbate and glutathione: the heart of the redoxhub. Plant Physiology, 155(1), 2-18.

Fu, P., Wang, W., Hou, L., & Liu, X. (2013). Hydrogen sulfide is involved in the chilling stress response in Vitis vinifera L. *Acta Societatis Botanicorum Poloniae*, *82*(4).

Gama, P. B. S., Inanaga, S., Tanaka, K., & Nakazawa, R. (2007). Physiological response of common bean (Phaseolus vulgaris L.) seedlings to salinity stress. *African Journal of biotechnology*, *6*(2).

Ganie, S. A., Molla, K. A., Henry, R. J., Bhat, K. V., & Mondal, T. K. (2019).

Advances in understanding salt tolerance in rice. *Theoretical and Applied Genetics*, *132*, 851-870.

Gao, J., Cao, X., Shi, S., Ma, Y., Wang, K., Liu, S., ... & Ma, H. (2016). Genome-wide survey of Aux/IAA gene family members in potato (Solanum tuberosum): Identification, expression analysis, and evaluation of their roles in tuber development. *Biochemical and biophysical research communications*, *471*(2), 320-327.

Gao, X., Hong, H., Li, W. C., Yang, L., Huang, J., Xiao, Y. L., ... & Chen, G. Y. (2016). Downregulation of rubisco activity by non-enzymatic acetylation of RbcL. *Molecular plant*, *9*(7), 1018-1027.

García- Mata, C., & Lamattina, L. (2010). Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. *New phytologist*, *188*(4), 977-984.

Gautam, A., Kumar, N., Dubey, A. K., Ranjan, R., Sahu, N., Behera, S. K., ... & Mallick, S. (2020). Sucrose plays key role in amelioration of arsenic induced phytotoxicity through modulating phosphate and silicon transporters, physiological and biochemical responses in C3 (Oryza sativa L.) and C4 (Zea mays L.). *Environmental and Experimental Botany*, *171*, 103930.

Gharsallah, C., Fakhfakh, H., Grubb, D., & Gorsane, F. (2016). Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants*, *8*, plw055.

Ghassemi, F., Jakeman, A. J., & Nix, H. A. (1995). *Salinisation of land and water resources: human causes, extent, management and case studies*. CAB international.

Ghose, B. (2014). Food security and food self- sufficiency in China: from past to 2050. *Food and Energy Security*, *3*(2), 86-95.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

Gill, S. S., & Tuteja, N. (2010b). Polyamines and abiotic stress tolerance in plants. *Plant signaling & behavior*, *5*(1), 26-33.

Giridhar, P., Rajasekaran, T., & Ravishankar, G. A. (2005). Improvement of growth and root specific flavour compound 2-hydroxy-4-methoxy benzaldehyde of micropropagated plants of Decalepis hamiltonii Wight & Arn., under triacontanol treatment. *Scientia horticulturae*, *106*(2), 228-236.

Glick B.R., Cheng Z., Czarny J., Duan J. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.* 2007; 119:329–339.

Goharrizi, K. J., Baghizadeh, A., Afroushteh, M., Amirmahani, F., & Kermani, S. G. (2020). Effects of salinity stress on proline content and expression of Δ1-pyrroline-5-carboxylate synthase and vacuolar-type H+ subunit E genes in wheat. *Plant Genetic Resources*, *18*(5), 334-342.

Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil 70*(2), 303-307.

Gul, H., Ali, R., Rauf, M., Hamayun, M., Arif, M., Khan, S. A., ... & Lee, I. J. (2023). Aspergillus welwitschiae BK Isolate Ameliorates the Physicochemical Characteristics and Mineral Profile of Maize under Salt Stress. *Plants*, *12*(8), 1703.

Guo, J., Li, G., & Yang, L. (2020). Role of H2S in pain: Growing evidences of mystification. *European Journal of Pharmacology*, *883*, 173322.

Gutiérrez-Alcalá, G., Gotor, C., Meyer, A. J., Fricker, M., Vega, J. M., & Romero, L. C. (2000). Glutathione biosynthesis in Arabidopsis trichome cells. *Proceedings of the National Academy of Sciences*, *97*(20), 11108-11113.

Habib, N., Ali, Q., Ali, S., Javed, M. T., Zulqurnain Haider, M., Perveen, R., ... & Bin-Jumah, M. (2020). Use of nitric oxide and hydrogen peroxide for better yield of wheat (Triticum aestivum L.) under water deficit conditions: growth, osmoregulation, and antioxidative defense mechanism. *Plants*, *9*(2), 285.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *246*, 7130-7139.

Hammam, A. A., & Mohamed, E. S. (2020). Mapping soil salinity in the East Nile Delta using several methodological approaches of salinity assessment. *The Egyptian*

Journal of Remote Sensing and Space Science, *23*(2), 125-131.

Hancock, J. T. (2019). Hydrogen sulfide and environmental stresses. *Environmental and Experimental Botany*, *161*, 50-56.

Hangarter, R., Ries, S. K., & Carlson, P. (1978). Effect of triacontanol on plant cell cultures in vitro. *Plant physiology*, *61*(5), 855-857.

Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., & Fujita, M. (2014). Exogenous Proline and Glycine Betaine Mediated Upregulation of Antioxidant Defense and Glyoxalase Systems Provides Better Protection against Salt-Induced Oxidative Stress in Two Rice (*Oryza sativa* L.) Varieties. *BioMed research international*, *2014*, 1-17.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences*, *14*(5), 9643-9684.

Hashmi, N., Khan, M. M. A., Naeem, M., Idrees, M., Aftab, T., & Moinuddin, T. (2010). Ameliorative effect of triacontanol on the growth, photosynthetic pigments, enzyme activities and active constituents of essential oil of Ocimum basilicum L. *Med Arom Plant Sci Biotechnol*, *5*, 20-24.

Hassanein, R. A., Hashem, H. A., & Khalil, R. R. (2012). Stigmasterol treatment increases salt stress tolerance of faba bean plants by enhancing antioxidant systems. *Plant Omics*, *5*(5), 476-485.

Hatami, E., Esna-Ashari, M., & Javadi, T. (2010). Effect of salinity on some gas exchange characteristics of grape (Vitis vinifera) cultivars. *Int. J. Agric. Biol*, *12*, 308-310.

Hatami, E., Esna-Ashari, M., & Javadi, T. (2012). Effect of salinity on some growth characteristics and concentration of elements in two grape (Vitis vinifera L.) cultivars,'Rishbaba'and 'Sahebi'. *Plant Stress*, *6*(1), 77-80.

Hayat, S., Hasan, S. A., Hayat, Q., Irfan, M., & Ahmad, A. (2010). Effect of salicylic acid on net photosynthetic rate, chlorophyll fluorescence, and antioxidant enzymes in Vigna radiata plants exposed to temperature and salinity stresses. *Plant Stress*, *4*, 62-

71.

He, J., Zhang, L., He, S. Y., Ryser, E. T., Li, H., & Zhang, W. (2022). Stomata facilitate foliar sorption of silver nanoparticles by Arabidopsis thaliana. *Environmental Pollution*, *292*, 118448.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*, 189-198.

Herbette, S., de Labrouhe, D. T., Drevet, J. R., & Roeckel-Drevet, P. (2011). Transgenic tomatoes showing higher glutathione peroxydase antioxidant activity are more resistant to an abiotic stress but more susceptible to biotic stresses. *Plant Science*, *180*(3), 548-553.

Hernandez, J.A. and M.S. Almansa. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant., 115: 251–257.

Honda, K., Yamada, N., Yoshida, R., Ihara, H., Sawa, T., Akaike, T., & Iwai, S. (2015). 8-Mercapto-cyclic GMP mediates hydrogen sulfide-induced stomatal closure in Arabidopsis. *Plant and Cell Physiology*, *56*(8), 1481-1489.

Hopmans, J. W., Qureshi, A. S., Kisekka, I., Munns, R., Grattan, S. R., Rengasamy, P., ... & Taleisnik, E. (2021). Critical knowledge gaps and research priorities in global soil salinity. *Advances in agronomy*, *169*, 1-191.

Hossain, M. A., Nakano, Y., & Asada, K. (1984). Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiology*, *25*, 385-395.

Hossain, M. A., Uddin, M. K., Ismail, M. R., & Ashrafuzzaman, M. (2012). Responses of glutamine synthetase-glutamate synthase cycle enzymes in tomato leaves under salinity stress. *International Journal of Agriculture and Biology*, *14*(4).

Houtz, R. L., Ries, S. K., & Tolbert, N. E. (1985). Effect of triacontanol on Chlamydomonas: I. Stimulation of growth and photosynthetic CO2 assimilation. *Plant physiology*, *79*(2), 357-364.

Hu, L. Y., Hu, S. L., Wu, J., Li, Y. H., Zheng, J. L., Wei, Z. J., ... & Zhang, H. (2012). Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *Journal of agricultural and food chemistry*, *60*(35), 8684-8693.

Hu, P., & Tirelli, N. (2012). Scavenging ROS: superoxide dismutase/catalase mimetics by the use of an oxidation-sensitive nanocarrier/enzyme conjugate. *Bioconjugate chemistry*, *23*(3), 438-449.

Hu, Y., & Schmidhalter, U. (2005). Drought and salinity: a comparison of their effects on mineral nutrition of plants. *Journal of plant nutrition and soil science*, *168*(4), 541-549.

Iqbal, J., Perveen, S., Parveen, A., Saeed, M., Zafar, S., & Iqbal, N. (2023). Interactive effect of salicylic acid and triacontanol to improve lead stress tolerance in maize (Zea mays L.). *Arabian Journal of Geosciences*, *16*(5), 314.

Iqbal, N., Umar, S., Khan, N. A., & Khan, M. I. R. (2014). A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism. *Environmental and Experimental Botany*, *100*, 34-42.

Islam, S., & Mohammad, F. (2020). Triacontanol as a dynamic growth regulator for plants under diverse environmental conditions. *Physiology and molecular biology of plants*, *26*, 871-883.

Ismail, H. A., & Younis, A. A. (2021). Triacontanol Foliar Spray Alleviated Drought Stress Effects by Maintaining Photosynthesis and Cellular Redox Balance in Sunflower Seedlings. *Egyptian Academic Journal of Biological Sciences, H. Botany*, *12*(2), 103-118.

Ivits, E., & Cherlet, M. (2013). Land-Productivity Dynamics: Towards integrated assessment of land degradation at global scales. *Jt. Res. Cent. Eur. Comm*, *10*, 59315.

Ivushkin, K., Bartholomeus, H., Bregt, A. K., Pulatov, A., Kempen, B., & De Sousa, L. (2019). Global mapping of soil salinity change. *Remote sensing of environment*, *231*, 111260.

Jahan, M. S., Guo, S., Baloch, A. R., Sun, J., Shu, S., Wang, Y., ... & Roy, R. (2020).

Melatonin alleviates nickel phytotoxicity by improving photosynthesis, secondary metabolism and oxidative stress tolerance in tomato seedlings. *Ecotoxicology and environmental safety*, *197*, 110593.

James, R. A., Blake, C., Byrt, C. S., & Munns, R. (2011). Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1; 4 and HKT1; 5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. *Journal of experimental botany*, *62*(8), 2939-2947.

Jamil, M., Bashir, S. A. M. I. N. A., Anwar, S., Bibi, S., Bangash, A., Ullah, F., & Rha, E. S. (2012). Effect of salinity on physiological and biochemical characteristics of different varieties of rice. *Pakistan Journal of Botany*, *44*(2), 7-13.

Jat, N. K., Shamim, M., Kumar, S., Ravisankar, N., Yadav, R. S., Babu, S., & Panwar, A. S. (2022). Agrometeorological evaluation of mustard (Brassica juncea) under organic production in North Western Indo-Gangetic Plains. *The Indian Journal of Agricultural Sciences*, *92*(3).

Javaid, T., Farooq, M. A., Akhtar, J., Saqib, Z. A., & Anwar-ul-Haq, M. (2019). Silicon nutrition improves growth of salt-stressed wheat by modulating flows and partitioning of Na+, Cl− and mineral ions. *Plant physiology and biochemistry*, *141*, 291-299.

Jia, H., Wang, X., Dou, Y., Liu, D., Si, W., Fang, H., ... & Li, J. (2016). Hydrogen sulfide-cysteine cycle system enhances cadmium tolerance through alleviating cadmium-induced oxidative stress and ion toxicity in Arabidopsis roots. *Scientific Reports*, *6*(1), 39702.

Jia, J., Liang, Y., Gou, T., Hu, Y., Zhu, Y., Huo, H., ... & Gong, H. (2020). The expression response of plasma membrane aquaporins to salt stress in tomato plants. *Environmental and Experimental Botany*, *178*, 104190.

Jiang, J. L., Tian, Y., Li, L., Yu, M., Hou, R. P., & Ren, X. M. (2019). H2S alleviates salinity stress in cucumber by maintaining the Na+/K+ balance and regulating H2S metabolism and oxidative stress response. *Frontiers in plant science*, *10*, 678.

Jin, Z., & Pei, Y. (2015). Physiological implications of hydrogen sulfide in plants:

pleasant exploration behind its unpleasant odour. *Oxidative Medicine and Cellular Longevity*, *2015*.

Jungklang, J. (2004). Physiological and biochemical mechanisms of salt tolerance in Sesbania rostrata Brem. & Oberm.

Karam, E. A., Keramat, B., Asrar, Z., & Mozafari, H. (2016). Triacontanol-induced changes in growth, oxidative defense system in Coriander (Coriandrum sativum) under arsenic toxicity. *Indian Journal of Plant Physiology*, *21*, 137-142.

Kaur, H., Hussain, S. J., Al- Huqail, A. A., Siddiqui, M. H., Al- Huqail, A. A., & Khan, M. I. R. (2022). Hydrogen sulphide and salicylic acid regulate antioxidant pathway and nutrient balance in mustard plants under cadmium stress. *Plant Biology*, *24*(4), 660-669.

Kaya, C., Akram, N. A., Sürücü, A., & Ashraf, M. (2019). Alleviating effect of nitric oxide on oxidative stress and antioxidant defence system in pepper (Capsicum annuum L.) plants exposed to cadmium and lead toxicity applied separately or in combination. *Scientia Horticulturae*, *255*, 52-60.

Kaya, C., Higgs, D., Ashraf, M., Alyemeni, M. N., & Ahmad, P. (2020). Integrative roles of nitric oxide and hydrogen sulfide in melatonin- induced tolerance of pepper (Capsicum annuum L.) plants to iron deficiency and salt stress alone or in combination. *Physiologia plantarum*, *168*(2), 256-277.

Keramat, B., Sorbo, S., Maresca, V., Asrar, Z., Mozafari, H., & Basile, A. (2017). Interaction of triacontanol and arsenic on the ascorbate-glutathione cycle and their effects on the ultrastructure in Coriandrum sativum L. *Environmental and Experimental Botany*, *141*, 161-169.

Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C. V., Van Breusegem, F., & Gechev, T. (2020). Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology advances*, *40*, 107503.

Keutgen, A. J., & Pawelzik, E. (2009). Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. *Environmental and*

experimental botany, *65*(2-3), 170-176.

Khan MMA, Bhardwaj G, Naeem M, Mohammad F, Singh M, Nasir S, Idrees M. (2009). Response of tomato (*Solanum lycopersicum* L.) to application of potassium and triacontanol. *Acta Hort.*; 823:199–208.

Khan, M. A., Lee, H. J., Lee, W. S., Kim, H. S., Ki, K. S., Hur, T. Y., ... & Choi, Y. J. (2007). Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *Journal of dairy science*, *90*(7), 3376-3387.

Khan, M. H., & Panda, S. K. (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum*, *30*, 81-89.

Khan, M. H., & Panda, S. K. (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum*, *30*, 81-89.

Khan, M. I. R., Nazir, F., Asgher, M., Per, T. S., & Khan, N. A. (2015). Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *Journal of plant physiology*, *173*, 9-18.

Khan, M. N., AlZuaibr, F. M., Al-Huqail, A. A., Siddiqui, M. H., M. Ali, H., Al-Muwayhi, M. A., & Al-Haque, H. N. (2018). Hydrogen sulfide-mediated activation of O-acetylserine (thiol) lyase and L/D-cysteine desulfhydrase enhance dehydration tolerance in Eruca sativa Mill. *International Journal of Molecular Sciences*, *19*(12), 3981.

Khan, M. N., Zhang, J., Luo, T., Liu, J., Ni, F., Rizwan, M., ... & Hu, L. (2019). Morpho-physiological and biochemical responses of tolerant and sensitive rapeseed cultivars to drought stress during early seedling growth stage. *Acta Physiologiae Plantarum*, *41*, 1-13.

Khan, M.M.A., Bhardwaj, G., Naeem, M., Moinuddin, Mohammad, F., Singh, M., Nasir, S., Idrees, M. (2009). Response of tomato (Solanum lycopersicum L.) to

application of potassium and triacontanol. – ISHS Acta Hort. 823: 199-208.

Khan, N., Zandi, P., Ali, S., Mehmood, A., Adnan Shahid, M., & Yang, J. (2018). Impact of salicylic acid and PGPR on the drought tolerance and phytoremediation potential of Helianthus annus. *Frontiers in microbiology*, *9*, 2507.

Khan, Z. H., Mohammad, F., & Khan, M. M. A. (2014). Enhancing the growth, yield and production of essential oil and citral in lemongrass by the application of triacontanol. *Int J Agric Res*, *4*, 113-122.

Khanam, D., & Mohammad, F. (2018). Plant growth regulators ameliorate the ill effect of salt stress through improved growth, photosynthesis, antioxidant system, yield and quality attributes in Mentha piperita L. *Acta physiologiae plantarum*, *40*, 1-13.

Kılıç, N. K., Karatay, S. E., Duygu, E., & Dönmez, G. (2011). Potential of Gonium spp. in synthetic reactive dye removal, possible role of laccases and stimulation by triacontanol hormone. *Water, Air, & Soil Pollution*, *222*, 297-303.

Kim, D. G., Grieco, E., Bombelli, A., Hickman, J. E., & Sanz-Cobena, A. (2021). Challenges and opportunities for enhancing food security and greenhouse gas mitigation in smallholder farming in sub-Saharan Africa. A review. *Food Security*, *13*, 457-476.

Kim, M. S., Kim, C, Jo, D. H., & Ryu, Y. W. (1999). Effect of fungal elicitor and heavy metals on the production of flavonol glycosides in the cell cultures of *Ginko biloba*. *Journal of Microbiology and Biotechnology*, *9*, 661-667.

Klein, A., Hüsselmann, L., Keyster, M., & Ludidi, N. (2018). Exogenous nitric oxide limits salt-induced oxidative damage in maize by altering superoxide dismutase activity. *South African journal of botany*, *115*, 44-49.

Kolupaev, Y. E., Yemets, A. I., Yastreb, T. O., & Blume, Y. B. (2023). The role of nitric oxide and hydrogen sulfide in regulation of redox homeostasis at extreme temperatures in plants. *Frontiers in Plant Science*, *14*, 1128439.

Kono, Y. (1978). Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of Biochemistry and*

Biophysics, *186*, 189-195.

Koohafkan, P. (2012). *Water and cereals in drylands*. Routledge.

Krishnan, R. R., & Kumari, B. D. (2008). Effect of N-triacontanol on the growth of salt stressed soybean plants. *Journal of Bioscience*, *19*(2), 53-62.

Krishnan, R.R. and Kumari, B.D. (2008). Effect of N-triacontanol on the growth of salt stressed soybean plants. *Journal of Biosciences* **19** (2): 53–62.

Kumar, K. B., & Khan, P. A. (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. *Indian Journal of Experimental Botany*, *20*, 412–416.

Kumar, P., & Sharma, P. K. (2020). Soil salinity and food security in India. *Frontiers in Sustainable Food Systems*, *4*, 533781.

Kumar, K. B., & amp; Khan, P. A. (1982). Peroxidase and polyphenol oxidase in excised ragi (Eleusine coracana cv. PR 202) leaves during senescence. Indian Journal of Experimental Botany, 20, 412–416.

Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Liu, Z., ... & Hou, H. (2021). Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (Oenanthe javanica) cultivars. *Frontiers in plant science*, *12*, 660409.

Kumaravelu, G., Livingstone, V. D., & Ramanujam, M. P. (2000). Triacontanol-induced changes in the growth, photosynthetic pigments, cell metabolites, flowering and yield of green gram. *Biologia plantarum*, *43*, 287-290.

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, cell & environment*, *25*(2), 275-294.

Lawrence, J. F. (1990). Determination of total xanthophyll and marigold oleoresin. *Journal of Association of Official Analytical Chemists*, *2*, 970-975.

Li, H., Shi, J., Wang, Z., Zhang, W., & Yang, H. (2020). H2S pretreatment mitigates the alkaline salt stress on Malus hupehensis roots by regulating Na+/K+ homeostasis and oxidative stress. *Plant Physiology and Biochemistry*, *156*, 233-241.

Li, H., Yu, T. T., Ning, Y. S., Li, H., Zhang, W. W., & Yang, H. Q. (2021). Hydrogen sulfide alleviates alkaline salt stress by regulating the expression of microRNAs in Malus hupehensis Rehd. roots. *Frontiers in Plant Science*, *12*, 663519.

Li, J., Shi, C., Wang, X., Liu, C., Ding, X., Ma, P., ... & Jia, H. (2020). Hydrogen sulfide regulates the activity of antioxidant enzymes through persulfidation and improves the resistance of tomato seedling to copper oxide nanoparticles (CuO NPs)-induced oxidative stress. *Plant Physiology and Biochemistry*, *156*, 257-266.

Li, X., Wang, Y., Dong, J., & Wu, M. (2022). Physiological and Biochemical Responses of Kandelia obovata to Upwelling Stress. *Water*, *14*(6), 899.

Li, X., Zhong, Q., Li, Y., Li, G., Ding, Y., Wang, S., ... & Chen, L. (2016). Triacontanol reduces transplanting shock in machine-transplanted rice by improving the growth and antioxidant systems. *Frontiers in plant science*, *7*, 872.

Li, Z. G. (2015). Synergistic effect of antioxidant system and osmolyte in hydrogen sulfide and salicylic acid crosstalk-induced heat tolerance in maize (Zea mays L.) seedlings. *Plant signaling & behavior*, *10*(9), e1051278.

Li, Z. G., Long, W. B., Yang, S. Z., Wang, Y. C., Tang, J. H., Wen, L., ... & Min, X. (2015). Endogenous hydrogen sulfide regulated by calcium is involved in thermotolerance in tobacco Nicotiana tabacum L. suspension cell cultures. *Acta Physiologiae Plantarum*, *37*, 1-11.

Li, Z. G., Xie, L. R., & Li, X. J. (2015). Hydrogen sulfide acts as a downstream signal molecule in salicylic acid-induced heat tolerance in maize (Zea mays L.) seedlings. *Journal of Plant Physiology*, *177*, 121-127.

Li, Z. G., Yang, S. Z., Long, W. B., Yang, G. X., & Shen, Z. Z. (2013). Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide- induced heat tolerance of maize (Z ea mays L.) seedlings. *Plant, Cell & Environment*, *36*(8), 1564-1572.

Li, Z. G., Yi, X. Y., & Li, Y. T. (2014). Effect of pretreatment with hydrogen sulfide donor sodium hydrosulfide on heat tolerance in relation to antioxidant system in maize (Zea mays) seedlings. *Biologia*, *69*(8), 1001-1009.

Lin, Y. J., Feng, X. H., & Feng, Y. X. (2023). Regulation of enzymatic and non-enzymatic antioxidants in rice seedlings against chromium stress through sodium hydrosulfide and sodium nitroprusside. *Environmental Science and Pollution Research*, *30*(10), 25851-25862.

Litalien, A., & Zeeb, B. (2020). Curing the earth: A review of anthropogenic soil salinization and plant-based strategies for sustainable mitigation. *Science of the Total Environment*, *698*, 134235.

Liu, H., Wang, J., Liu, J., Liu, T., & Xue, S. (2021). Hydrogen sulfide (H_2S) signaling in plant development and stress responses. *Abiotech*, *2*, 32-63.

Liu, J., Mesfin, F. M., Hunter, C. E., Olson, K. R., Shelley, W. C., Brokaw, J. P., ... & Markel, T. A. (2022). Recent development of the molecular and cellular mechanisms of hydrogen sulfide gasotransmitter. *Antioxidants*, *11*(9), 1788.

Liu, J., Shabala, S., Zhang, J., Ma, G., Chen, D., Shabala, L., ... & Zhao, Q. (2020). Melatonin improves rice salinity stress tolerance by NADPH oxidase- dependent control of the plasma membrane K+ transporters and K+ homeostasis. *Plant, cell & environment*, *43*(11), 2591-2605.

Liu, Y., Wei, L., Feng, L., Zhang, M., Hu, D., Tie, J., & Liao, W. (2022). Hydrogen sulfide promotes adventitious root development in cucumber under salt stress by enhancing antioxidant ability. *Plants*, *11*(7), 935.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402-408.

López-Climent, M. F., Arbona, V., Pérez-Clemente, R. M., & Gómez-Cadenas, A. (2008). Relationship between salt tolerance and photosynthetic machinery performance in citrus. *Environmental and experimental botany*, *62*(2), 176-184.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin- phenol reagent. *Journal of Biological Chemistry*, *193*, 265-275.

Lucas Garcia, J. A., Probanza, A., Ramos, B., Barriuso, J., & Gutierrez Manero, F. J. (2004). Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and

Sinorhizobium fredii on biological nitrogen fixation, nodulation and growth of Glycine max cv. Osumi. *Plant and Soil*, *267*, 143-153.

Luo, Z., Li, D., Du, R., & Mou, W. (2015). Hydrogen sulfide alleviates chilling injury of banana fruit by enhanced antioxidant system and proline content. *Scientia Horticulturae*, *183*, 144-151.

Lycoskoufis, I. H., Savvas, D., & Mavrogianopoulos, G. (2005). Growth, gas exchange, and nutrient status in pepper (Capsicum annuum L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. *Scientia Horticulturae*, *106*(2), 147-161.

Ma, D., Ding, H., Wang, C., Qin, H., Han, Q., Hou, J., ... & Guo, T. (2016). Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pathway in wheat. *PloS one*, *11*(9), e0163082.

MacLachlan, S., & Zalik, S. (1963). Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany*, *41*, 1053-1060.

Mahdavi, B., S. Sanavy, 2007. Germination and seedling growth in grass pea (Lathyrus sativus) cultivars under salinity conditions. Pakistan Journal of Biological Sciences, 10(2), 273-279.

Malabadi, R. B., Mulgund, G. S., & Nataraja, K. (2005). Effect of triacontanol on the micropropagation of Costus speciosus (Koen.) Sm. Using rhizome thin sections. *In Vitro Cellular & Developmental Biology-Plant*, *41*, 129-132.

Malick, C. P., & Singh, M. B. (1980). Phenolics. In Malick, C. P., & Singh, M. B. (Ed.), *Plant Enzymology and Histoenzymology* (pp. 286). Kalyani Publishers: New Delhi.

Manai, J., Kalai, T., Gouia, H., & Corpas, F. J. (2014). Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (Solanum lycopersicum) plants. *Journal of soil science and plant nutrition*, *14*(2), 433-446.

Mancardi, D., Penna, C., Merlino, A., Del Soldato, P., Wink, D. A., & Pagliaro, P. (2009). Physiological and pharmacological features of the novel gasotransmitter:

hydrogen sulfide. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1787*(7), 864-872.

Manchanda, G., & Garg, N. (2008). Salinity and its effects on the functional biology of legumes. *Acta Physiologiae Plantarum*, *30*, 595-618.

Mancinelli, A. L. (1984). Photoregulation of anthocyanin synthesis: VIII. Effect of light pretreatments. *Plant Physiology*, *75*, 447–453.

Mane, A. V., Karadge, B. A., & Samant, J. S. (2010). Salinity induced changes in photosynthetic pigments and polyphenols of Cymbopogon nardus (L.) Rendle. *J. Chem. Pharm. Res*, *2*(3), 338-347.

Mane, A. V., Karadge, B. A., & Samant, J. S. (2011). Salt stress induced alteration in growth characteristics of a grass Pennisetum alopecuroides. *Journal of Environmental Biology*, *32*(6), 753.

Maresca, V., Sorbo, S., Keramat, B., & Basile, A. (2017). Effects of triacontanol on ascorbate-glutathione cycle in Brassica napus L. exposed to cadmium-induced oxidative stress. *Ecotoxicology and environmental safety*, *144*, 268-274.

Martinek, R. G. (1964). Method for the determination of vitamin E (total tocopherols) in serum. *Clinical Chemistry*, *10*, 1078-1086.

Mbinda, W., & Kimtai, M. (2019). Physiological and biochemical analyses of sorghum varieties reveal differential responses to salinity stress. *BioRxiv*, 720789.

Meena SK, Jat NL, Sharma B, Meena VS (2014) Effect of plant growth regulators and sulphur on productivity of coriander (*Coriandrum sativum* L.) in Rajasthan. Ecoscan 6:69–73

Meena SK, Jat NL, Sharma B, Meena VS (2015) Effect of plant growth regulators and sulfur on productivity and nutrient concentration of coriander (*Coriandrum sativum* L.). Environ Ecol 33:1249–1253.

Mehta, P., Jajoo, A., Mathur, S., & Bharti, S. (2010). Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. *Plant physiology and biochemistry*, *48*(1), 16-20.

Memon, S. A., Hou, X., & Wang, L. J. (2010). MORPHLOGICAL ANALYSIS OF

SALT STRESS RESPONSE OF PAK CHOI. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, *9*(1).

Menezes, R. V., Azevedo, A. D. D., Ribeiro, M. D. O., & Cova, A. M. W. (2017). Growth and contents of organic and inorganic solutes in amaranth under salt stress. *Pesquisa Agropecuária Tropical*, *47*, 22-30.

Metternicht, G. I., & Zinck, J. A. (2003). Remote sensing of soil salinity: potentials and constraints. *Remote sensing of Environment*, *85*(1), 1-20.

Metternicht, G., Zinck, J. A., Blanco, P. D., & del Valle, H. F. (2010). Remote sensing of land degradation: Experiences from Latin America and the Caribbean. *Journal of environmental quality*, *39*(1), 42-61.

Misra N., Saxena P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.* 177, 181–189.

Mittler, R. (2017). ROS Are Good. Trends in Plant Science, 22, 11-19.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G. A. D., Tognetti, V. B., Vandepoele, K., ... & Van Breusegem, F. (2011). ROS signaling: the new wave? *Trends in plant science*, *16*(6), 300-309.

Mittova, V., Guy, M., Tal, M., & Volokita, M. (2004). Salinity up- regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt- tolerant tomato species Lycopersicon pennellii. *Journal of experimental botany*, *55*(399), 1105-1113.

Mittova, V., Tal, M., Volokita, M., & Guy, M. (2002). Salt stress induces up- regulation of an efficient chloroplast antioxidant system in the salt- tolerant wild tomato species Lycopersicon pennellii but not in the cultivated species. *Physiologia Plantarum*, *115*(3), 393-400.

Mostofa, M. G., Rahman, A., Ansary, M. M. U., Watanabe, A., Fujita, M., & Tran, L. S. P. (2015). Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. *Scientific reports*, *5*(1), 14078.

Mostofa, M. G., Saegusa, D., Fujita, M., & Tran, L. S. P. (2015). Hydrogen sulfide

regulates salt tolerance in rice by maintaining Na+/K+ balance, mineral homeostasis and oxidative metabolism under excessive salt stress. *Frontiers in plant science*, *6*, 1055.

Mozafari, H. (2017). Effect of interaction Triacontanol and arsenic on the growth and some biochemical and physiological properties of soybean (Glycine max L). *Journal of Plant Research (Iranian Journal of Biology)*, *30*(3), 477-487.

Muller, B., Pantin, F., Génard, M., Turc, O., Freixes, S., Piques, M., & Gibon, Y. (2011). Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany*, *62*(6), 1715-1729.

Munns R and Termaat A (1986) Whole plant responses to salinity. Aust. J. Plant Physiol. 13, 143–160.

Munns, R. (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell & Environment*, *16*(1), 15-24.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, cell & environment*, *25*(2), 239-250.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, *59*, 651-681.

Murshed, R., Lopez-Lauri, F., & Sallanon, H. (2014). Effect of salt stress on tomato fruit antioxidant systems depends on fruit development stage. *Physiology and Molecular Biology of Plants*, *20*, 15-29.

Nabi, A., Aftab, T., Masroor, M., Khan, A., & Naeem, M. (2022). Exogenous triacontanol provides tolerance against arsenic-induced toxicity by scavenging ROS and improving morphology and physiological activities of Mentha arvensis L. *Environmental Pollution*, *295*, 118609.

Naeem, M., Khan, M. M. A., & Moinuddin. (2012). Triacontanol: a potent plant growth regulator in agriculture. *Journal of Plant Interactions*, *7*(2), 129-142.

Naeem, M., Khan, M. M. A., Idrees, M., & Aftab, T. (2010). Phosphorus ameliorates crop productivity, photosynthetic efficiency, nitrogen-fixation, activities of the

enzymes and content of nutraceuticals of Lablab purpureus L. *Scientia horticulturae*, *126*(2), 205-214.

Nagesh Babu, R., & Devaraj, V. R. (2008). High temperature and salt stress response in French bean (Phaseolus vulgaris). *Australian Journal of Crop Science*, *2*(2), 40-48.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific-peroxidase in spinach chloroplasts. *Plant Cell Physiology*, *22*, 867-880.

Nasibi, F., Manouchehri Kalantari, K., & Manzari Tavakoli, Z. (2020). Effects of Hydrogen Sulfide on Cold-Induced Oxidative Damage in Cucumis sativus L. *International Journal of Horticultural Science and Technology*, *7*(3), 199-211.

Negrão, S., Schmöckel, S. M., & Tester, M. J. A. O. B. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of botany*, *119*(1), 1-11.

Netondo, G.W., J.C. Onyango, and E. Beck. 2004b. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci. 44: 806-811.

Nomani, L., Zehra, A., Choudhary, S., Wani, K. I., Naeem, M., Siddiqui, M. H., ... & Aftab, T. (2022). Exogenous hydrogen sulphide alleviates copper stress impacts in Artemisia annua L.: Growth, antioxidant metabolism, glandular trichome development and artemisinin biosynthesis. *Plant Biology*, *24*(4), 642-651.

Obul Reddy, B., Giridhar, P., & Ravishankar, G. A. (2002). The effect of triacontanol on micropropagation of Capsicum frutescens and Decalepis hamiltonii W & A. *Plant cell, tissue and organ culture*, *71*, 253-258.

Odjegba, J.V. and C.I. Chukwunwike. 2012. Physiological responses of Amaranthus hybrids L. under salinity stress. Indian J. Innovations Dev., 1(10): 742-748.

Ozfidan-Konakci, C., Yildiztugay, E., Arikan, B., Elbasan, F., Alp, F. N., & Kucukoduk, M. (2023). Hydrogen Sulfide Protects Damage From Methyl Viologen-Mediated Oxidative Stress by Improving Gas Exchange, Fluorescence Kinetics of Photosystem II, and Antioxidant System in Arabidopsis thaliana. *Journal of Plant Growth Regulation*, *42*(2), 1031-1050.

Ozfidan-Konakci, C., Yildiztugay, E., Elbasan, F., Kucukoduk, M., & Turkan, I.

(2020). Hydrogen sulfide (H_2S) and nitric oxide (NO) alleviate cobalt toxicity in wheat (Triticum aestivum L.) by modulating photosynthesis, chloroplastic redox and antioxidant capacity. *Journal of hazardous materials*, *388*, 122061.

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and environmental safety*, *60*(3), 324-349.

Parida, A. K., & Jha, B. (2010). Antioxidative defense potential to salinity in the euhalophyte Salicornia brachiata. *Journal of Plant Growth Regulation*, *29*, 137-148.

Peleg, Z., & Blumwald, E. (2011). Hormone balance and abiotic stress tolerance in crop plants. *Current opinion in plant biology*, *14*(3), 290-295.

Penella, C., Landi, M., Guidi, L., Nebauer, S. G., Pellegrini, E., San Bautista, A., ... & Calatayud, A. (2016). Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength. *Journal of plant physiology*, *193*, 1-11.

Perveen, R., Jamil, Y., Ashraf, M., Ali, Q., Iqbal, M., & Ahmad, M. R. (2011). He-Ne laser- induced improvement in biochemical, physiological, growth and yield characteristics in sunflower (Helianthus annuus L.). *Photochemistry and photobiology*, *87*(6), 1453-1463.

Perveen, S. H. A. G. U. F. T. A., & Nazir, M. (2018). Proline treatment induces salt stress tolerance in maize (Zea Mays L. CV. Safaid Afgoi). *Pakistan Journal of Botany*, *50*(4), 1265-1271.

Perveen, S. H. A. G. U. F. T. A., Parvaiz, M., Shahbaz, M., Saeed, M. U. H. A. M. M. A. D., & Zafar, S. A. R. A. (2022). Triacontanol positively influences growth, yield, biochemical attributes and antioxidant enzymes of two linseed (Linum usitatissimum L.) accessions differing in drought tolerance. *Pak. J. Bot*, *54*(3), 843-853.

Perveen, S., Iqbal, M., Nawaz, A., Parveen, A., & Mahmood, S. (2016). Induction of drought tolerance in Zea mays L. by foliar application of triacontanol. *Pak J Bot*, *48*(3), 907-915.

Perveen, S., Iqbal, N., Saeed, M., Zafar, S., & Arshad, Z. (2018). Role of foliar application of sulfur-containing compounds on maize (Zea mays L. var. Malka and

hybrid DTC) under salt stress. *Brazilian Journal of Botany*, *41*, 805-815.

Perveen, S., Samad, A. B. D. U. S., Nazif, W., & Shah, S. (2012). Impact of sewage water on vegetables quality with respect to heavy metals in Peshawar, Pakistan. *Pakistan Journal of Botany*, *44*(6), 1923-1931.

Perveen, S., Shahbaz, M., & Ashraf, M. (2010). Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pak. J. Bot*, *42*(5), 3073-3081.

Perveen, S., Shahbaz, M., & Ashraf, M. (2013). Influence of foliar-applied triacontanol on growth, gas exchange characteristics, and chlorophyll fluorescence at different growth stages in wheat under saline conditions. *Photosynthetica*, *51*, 541-551.

Perveen, S., Shahbaz, M., & Ashraf, M. (2014). Triacontanol-induced changes in growth, yield, leaf water relations, oxidative defense system, minerals, and some key osmoprotectants in Triticum aestivum under saline conditions. *Turkish Journal of Botany*, *38*(5), 896-913.

Polash, M. A. S., Sakil, M. A., & Hossain, M. A. (2019). Plants responses and their physiological and biochemical defense mechanisms against salinity: A review. *Trop. Plant Res*, *6*, 250-274.

Poss, J. A., Russell, W. B., Bonos, S. A., & Grieve, C. M. (2010). Salt tolerance and canopy reflectance of Kentucky bluegrass cultivars. *HortScience*, *45*(6), 952-960.

Prittesh, P., Avnika, P., Kinjal, P., Jinal, H. N., Sakthivel, K., & Amaresan, N. (2020). Amelioration effect of salt-tolerant plant growth-promoting bacteria on growth and physiological properties of rice (Oryza sativa) under salt-stressed conditions. *Archives of Microbiology*, *202*, 2419-2428.

Putter, J. (1974). Peroxidase. In Bergmeyer, H. U. (Ed.), *Methods of enzymatic analysis* (Vol. 2, pp. 685-690). Verlag Chemie: Weinhan.

Qian, P., Sun, R., Ali, B., Gill, R. A., Xu, L., & Zhou, W. (2014). Effects of hydrogen sulfide on growth, antioxidative capacity, and ultrastructural changes in oilseed rape seedlings under aluminum toxicity. *Journal of plant growth regulation*, *33*, 526-538.

Rahman, M., Rahman, K., Sathi, K. S., Alam, M. M., Nahar, K., Fujita, M., & Hasanuzzaman, M. (2021). Supplemental selenium and boron mitigate salt-induced oxidative damages in Glycine max L. *Plants*, *10*(10), 2224.

Rahneshan, Z., Nasibi, F., & Moghadam, A. A. (2018). Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (Pistacia vera L.) rootstocks. *Journal of plant interactions*, *13*(1), 73-82.

Rai, P. K., Yadav, P., Kumar, A., Sharma, A., Kumar, V., & Rai, P. (2022). Brassica juncea: A Crop for Food and Health. In *The Brassica juncea Genome* (pp. 1-13). Cham: Springer International Publishing.

Rai, P., Singh, V. P., Peralta-Videa, J., Tripathi, D. K., Sharma, S., & Corpas, F. J. (2021). Hydrogen sulfide (H2S) underpins the beneficial silicon effects against the copper oxide nanoparticles (CuO NPs) phytotoxicity in Oryza sativa seedlings. *Journal of Hazardous Materials*, *415*, 124907.

Ramanarayan, K., Bhat, A., Shripathi, V., Swamy, G. S., & Rao, K. S. (2000). Triacontanol inhibits both enzymatic and nonenzymatic lipid peroxidation. *Phytochemistry*, *55*(1), 59-66.

Ramos-Zambrano, E., Juárez-Yáñez, T. E., Tapia-Maruri, D., Camacho-Díaz, B. H., Jiménez-Aparicio, A. R., & Martínez-Ayala, A. L. (2021). Effects of Triacontanol and Light on Stomatal and Photochemical Responses in Solanum lycopersicum L. *Journal of Plant Growth Regulation*, *40*(5), 2208-2220.

Redondo- Gómez, S., Mateos- Naranjo, E., Figueroa, M. E., & Davy, A. J. (2010). Salt stimulation of growth and photosynthesis in an extreme halophyte, Arthrocnemum macrostachyum. *Plant biology*, *12*(1), 79-87.

Ren, S. C., & Sun, J. T. (2014). Changes in phenolic content, phenylalanine ammonia-lyase (PAL) activity, and antioxidant capacity of two buckwheat sprouts in relation to germination. *Journal of Functional Foods*, *7*, 298-304.

Rengasamy, P. (2006). World salinization with emphasis on Australia. *Journal of experimental botany*, *57*(5), 1017-1023.

Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected

soils. *Functional Plant Biology*, *37*(7), 613-620.

Ries S, Wert V, O'Leary NFD, Nair M. 9-β-L (+) Adenosine: a new naturally occurring plant growth substance elicited by triacontanol in rice. *Plant Growth Regul.* 1990;9:263–273.

Ries, S. (1991). Triacontanol and its second messenger 9-β-L (+)-adenosine as plant growth substances. *Plant Physiology*, *95*(4), 986-989.

Roe, J. H., & Kuether, C. A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. *Journal of Biological Chemistry*, *147*, 399-407.

Rogers, M. E., Colmer, T. D., Frost, K., Henry, D., Cornwall, D., Hulm, E., ... & Craig, A. D. (2008). Diversity in the genus Melilotus for tolerance to salinity and waterlogging. *Plant and Soil*, *304*, 89-101.

Rogers, M. E., Colmer, T. D., Nichols, P. G. H., Hughes, S. J., Frost, K., Cornwall, D., ... & Craig, A. D. (2011). Salinity and waterlogging tolerance amongst accessions of messina (Melilotus siculus). *Crop and Pasture Science*, *62*(3), 225-235.

Rozeff N (1995) Sugarcane and salinity—a review paper. Sugarcane 5:8–19.

Sabagh, A. E., Mbarki, S., Hossain, A., Iqbal, M. A., Islam, M. S., Raza, A., ... & Farooq, M. (2021). Potential role of plant growth regulators in administering crucial processes against abiotic stresses. *Frontiers in Agronomy*, *3*, 648694.

Sadak, M. S., & Mostafa, H. A. (2015). Physiological role of pre-sowing seed with proline on some growth, biochemical aspects, yield quantity and quality of two sunflower cultivars grown under seawater salinity stress. *Scientia Agriculturae*, *9*(1), 60-69.

Sadak, M. S., Hanafy, R. S., Elkady, F. M., Mogazy, A. M., & Abdelhamid, M. T. (2023). Exogenous Calcium Reinforces Photosynthetic Pigment Content and Osmolyte, Enzymatic, and Non-Enzymatic Antioxidants Abundance and Alleviates Salt Stress in Bread Wheat. *Plants*, *12*(7), 1532.

Saha, P., Chatterjee, P., & Biswas, A. K. (2010). NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in

mungbean (Vigna radiata L. Wilczek).

Said-Al Ahl, H. A. H., & Omer, E. A. (2011). Medicinal and aromatic plants production under salt stress. A review. *Herba polonica*, *57*(2).

Sairam, R. K., Rao, K. V., & Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant science*, *163*(5), 1037-1046.

Sánchez-Viveros, G., Ferrera-Cerrato, R., & Alarcón, A. (2011). Short-term effects of arsenate-induced toxicity on growth, chlorophyll and carotenoid contents, and total content of phenolic compounds of Azolla filiculoides. *Water, Air, & Soil Pollution*, *217*, 455-462.

Sardar, R., Ahmed, S., Akbar, M., Yasin, N. A., & Li, G. (2022). Alleviation of cadmium phytotoxicity in triacontanol treated Coriandrum sativum L. by modulation of physiochemical attributes, oxidative stress biomarkers and antioxidative system. *Chemosphere*, *295*, 133924.

Sarker, U., & Oba, S. (2020). The response of salinity stress-induced A. tricolor to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. *Frontiers in Plant Science*, *11*, 559876.

Sarwar, M., Ahmad, S., Chattha, M. B., Chattha, M. U., Alam, M. W., Anjum, S., ... & Mannan, A. (2019). ASSESMENT OF GROWTH AND PRODUCTIVITY OF CUCUMBER (CUCUMIS SATIVUS L.) GENOTYPES UNDER SALT STRESS REGIME. *Applied Ecology & Environmental Research*, *17*(5).

Scott, T. A., & Melvin, E. H. (1953). Determination of dextran with anthrone. *Analytical Chemistry*, *25*, 1656–1661.

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, proteinbound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Chemistry*, *25*, 192-205.

Sehar, Z., Gautam, H., Masood, A., & Khan, N. A. (2023). Ethylene-and proline-dependent regulation of antioxidant enzymes to mitigate heat stress and boost photosynthetic efficacy in wheat plants. *Journal of Plant Growth Regulation*, *42*(5), 2683-2697.

Seifi, M., Ahmadi, A., Neyshabouri, M. R., Taghizadeh-Mehrjardi, R., & Bahrami, H. A. (2020). Remote and Vis-NIR spectra sensing potential for soil salinization estimation in the eastern coast of Urmia hyper saline lake, Iran. *Remote Sensing Applications: Society and Environment*, *20*, 100398.

Selvakumar, G., Kim, K., Hu, S., & Sa, T. (2014). Effect of salinity on plants and the role of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria in alleviation of salt stress. *Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment: Volume 1*, 115-144.

Shahbaz, M., Ashraf, M., Akram, N. A., Hanif, A., Hameed, S., Joham, S., & Rehman, R. (2011). Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (Helianthus annuus L.). *Acta Physiologiae Plantarum*, *33*, 1113-1122.

Shahbaz, M., Ashraf, M., Al-Qurainy, F., & Harris, P. J. (2012). Salt tolerance in selected vegetable crops. *Critical reviews in plant sciences*, *31*(4), 303-320.

Shahbaz, M., Mushtaq, Z., Andaz, F., & Masood, A. (2013). Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (Solanum melongena L.)?. *Scientia Horticulturae*, *164*, 507-511.

Shahbaz, M., Noreen, N., & Perveen, S. (2013). Triacontanol modulates photosynthesis and osmoprotectants in canola (Brassica napus L.) under saline stress. *Journal of Plant Interactions*, *8*(4), 350-359.

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., ... & Garcia-Sanchez, F. (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy*, *10*(7), 938.

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., ... & Garcia-Sanchez, F. (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy*, *10*(7), 938.

Shahzad, H., Ullah, S., Iqbal, M., Bilal, H. M., Shah, G. M., Ahmad, S., ... & Ahmad, I. (2019). Salinity types and level-based effects on the growth, physiology and nutrient contents of maize (Zea mays). *Italian journal of agronomy*, *14*(4), 199-207.

Shahzad, K., Hussain, S., Arfan, M., Hussain, S., Waraich, E. A., Zamir, S., ... & El-Esawi, M. A. (2021). Exogenously applied gibberellic acid enhances growth and salinity stress tolerance of maize through modulating the morpho-physiological, biochemical and molecular attributes. *Biomolecules*, *11*(7), 1005.

Shalaby, O. A. E. S., Farag, R., & Ibrahim, M. F. (2023). Effect of hydrogen sulfide and hydrogen peroxide on growth, yield and nutrient content of broccoli plants grown under saline conditions. *Scientia Horticulturae*, *316*, 112035.

Shan, C., Liu, H., Zhao, L., & Wang, X. (2014). Effects of exogenous hydrogen sulfide on the redox states of ascorbate and glutathione in maize leaves under salt stress. *Biologia plantarum*, *58*(1), 169-173.

Shan, C., Wang, B., Sun, H., Gao, S., & Li, H. (2020). H_2S induces NO in the regulation of AsA-GSH cycle in wheat seedlings by water stress. *Protoplasma*, *257*, 1487-1493.

Shan, C., Zhang, S., & Ou, X. (2018). The roles of H 2 S and H 2 O 2 in regulating AsA-GSH cycle in the leaves of wheat seedlings under drought stress. *Protoplasma*, *255*, 1257-1262.

Shan, H., Qiu, J., Chang, P., Chu, Y., Gao, C., Wang, H., ... & Tao, L. (2019). Exogenous hydrogen sulfide offers neuroprotection on intracerebral hemorrhage injury through modulating endogenous H2S metabolism in mice. *Frontiers in Cellular Neuroscience*, *13*, 349.

Sharma, D. A., Rishi, M. S., & Keesari, T. (2017). Evaluation of groundwater quality and suitability for irrigation and drinking purposes in southwest Punjab, India using hydrochemical approach. *Applied Water Science*, *7*, 3137-3150.

Sharma, D. K., & Chaudhari, S. K. (2012). Agronomic research in salt affected soils of India: an overview. *Indian Journal of Agronomy*, *57*(3s), 175-185.

Sharma, H., & Kumar, A. (2011). Effect of plant growth regulators and chemical fertilizers on growth and productivity of Chlorophytum tuberosum and Pergularia daemia. *Journal of Medicinal Plants Research*, *5*(13), 2647-2651.

Shelke, D. B., Pandey, M., Nikalje, G. C., Zaware, B. N., Suprasanna, P., & Nikam,

T. D. (2017). Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes. *Plant Physiology and Biochemistry*, *118*, 519-528.

Shumilina, J., Kusnetsova, A., Tsarev, A., Janse van Rensburg, H. C., Medvedev, S., Demidchik, V., ... & Frolov, A. (2019). Glycation of plant proteins: regulatory roles and interplay with sugar signalling?. *International journal of molecular sciences*, *20*(9), 2366.

Siboza, X. I., Bertling, I., & Odindo, A. O. (2014). Salicylic acid and methyl jasmonate improve chilling tolerance in cold-stored lemon fruit (Citrus limon). *Journal of Plant Physiology*, *171*(18), 1722-1731.

Siddiqui, H., Ahmed, K. B. M., Sami, F., & Hayat, S. (2020). Phytoremediation of cadmium contaminated soil using Brassica juncea: influence on PSII activity, leaf gaseous exchange, carbohydrate metabolism, redox and elemental status. *Bulletin of Environmental Contamination and Toxicology*, *105*, 411-421.

Siddiqui, M. H., Alamri, S., Al-Khaishany, M. Y., Khan, M. N., Al-Amri, A., Ali, H. M., ... & Alsahli, A. A. (2019). Exogenous melatonin counteracts NaCl-induced damage by regulating the antioxidant system, proline and carbohydrates metabolism in tomato seedlings. *International Journal of Molecular Sciences*, *20*(2), 353.

Silva, S. C., Silva, A. B., & Gomes, J. P. (2021). Hydrogen embrittlement of API 5L X65 pipeline steel in CO2 containing low H2S concentration environment. *Engineering Failure Analysis*, *120*, 105081.

Singh, B. P., & Raghava, R. P. (2020). Effect of triacontanol on biochemical attributes and yield of sesame (Sesamum indicum L.). *Journal of Medicinal Plants*, *8*(4), 33-35.

Singh, M., Khan, M. M. A., Moinuddin, & Naeem, M. (2012). Augmentation of nutraceuticals, productivity and quality of ginger (Zingiber officinale Rosc.) through triacontanol application. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *146*(1), 106-113.

Singh, R., Mehta, A., Mehta, P., & Shukla, K. (2011). Anthelmintic activity of

rhizome extracts of Curcuma longa and Zingiber officinale (Zingiberaceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(2), 236-237.

Skogen, D., Eriksen, A. B., & Nilsen, S. (1982). Effect of triacontanol on production and quality of flowers of Chrysanthemum morifolium Ramat. *Scientia Horticulturae*, *18*(1), 87-92.

Song, Y., Luo, G., Shen, L., Yu, K., Yang, W., Li, X., ... & Zhang, A. (2020). TubZIP28, a novel bZIP family transcription factor from Triticum urartu, and TabZIP28, its homologue from Triticum aestivum, enhance starch synthesis in wheat. *new phytologist*, *226*(5), 1384-1398.

Strasser, R. J., Krüger, G. H., Berner, J. M., & Scheepers, C. C. (2018). Differential response of photosynthetic electron transport and CO2 assimilation in sensitive (S156) and resistant (R123) Phaseolus vulgaris L. (bush bean) genotypes to chronic ozone exposure. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, *37*(1), 1-12.

Su, Q., Zheng, X., Tian, Y., & Wang, C. (2020). Exogenous brassinolide alleviates salt stress in Malus hupehensis Rehd. by regulating the transcription of NHX-Type Na+ (K+)/H+ antiporters. *Frontiers in plant science*, *11*, 38.

Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., ... & Goodell, M. A. (2014). Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell stem cell*, *14*(5), 673-688.

Sun, H., Wang, P., Chen, Q., Zhang, D., & Xing, Y. (2022). Coupling the water use of Populus euphratica and Tamarix ramosissima and evapotranspiration partitioning in a desert riparian forest ecosystem. *Agricultural and Forest Meteorology*, *323*, 109064.

Sun, Y. D., & Luo, W. R. (2014). Effects of exogenous hydrogen sulphide on seed germination and seedling growth of cucumber (Cucumis sativus) under sodium bicarbonate stress. *Seed Science and Technology*, *42*(2), 126-131.

Sytar, O., Kumari, P., Yadav, S., Brestic, M., & Rastogi, A. (2019). Phytohormone priming: regulator for heavy metal stress in plants. *Journal of Plant Growth*

Regulation, *38*, 739-752.

Talaat, N. B., Mostafa, A. A., & El-Rahman, S. N. A. (2023). A novel plant growth–promoting agent mitigates salt toxicity in barley (Hordeum vulgare L.) by activating photosynthetic, antioxidant defense, and methylglyoxal detoxification machineries. *Journal of Soil Science and Plant Nutrition*, *23*(1), 308-324.

Tang, C. S., Cui, Y. J., Tang, A. M., & Shi, B. (2010). Experiment evidence on the temperature dependence of desiccation cracking behavior of clayey soils. *Engineering Geology*, *114*(3-4), 261-266.

Tang, X., An, B., Cao, D., Xu, R., Wang, S., Zhang, Z., ... & Sun, X. (2020). Improving photosynthetic capacity, alleviating photosynthetic inhibition and oxidative stress under low temperature stress with exogenous hydrogen sulfide in blueberry seedlings. *Frontiers in Plant Science*, *11*, 108.

Tanveer, M., Shahzad, B., Sharma, A., & Khan, E. A. (2019). 24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry*, *135*, 295-303.

Taştan, A., Acar, E., Güler, M. A., & Kılınçkaya, Ü. (2016). Optimum crashworthiness design of tapered thin-walled tubes with lateral circular cutouts. *Thin-Walled Structures*, *107*, 543-553.

Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P., & McDonald, G. K. (2011). Additive effects of Na+ and Cl–ions on barley growth under salinity stress. *Journal of Experimental Botany*, *62*(6), 2189-2203.

Tavakkoli, E., Rengasamy, P., & McDonald, G. K. (2010). High concentrations of Na+ and Cl–ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of experimental botany*, *61*(15), 4449-4459.

Tester, M., & Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. *Annals of botany*, *91*(5), 503-527.

Tiwari S, Verma N, Singh VP, Prasad SM (2019) Nitric oxide ameliorates aluminium toxicity in *Anabaena* PCC 7120: Regulation of aluminium accumulation, exopolysaccharides secretion, photosynthesis and oxidative stress markers. Environ

Exp Bot 161:218–227.

Tiwari, P., & Goel, A. (2017). An overview of impact of subsurface drainage project studies on salinity management in developing countries. *Applied Water Science*, *7*(2), 569-580.

Tompa, B., Balint, J., & Fodorpataki, L. (2022). Enhancement of biomass production, salinity tolerance and nutraceutical content of spinach (Spinacia oleracea L.) with the cuticular wax constituent triacontanol. *J Appl Bot Food Qual*, *95*, 121-128.

Valivand, M., & Amooaghaie, R. (2021). Calcium signaling confers nickel tolerance in Cucurbita pepo L. *International Journal of Phytoremediation*, *23*(4), 362-373.

Valivand, M., Amooaghaie, R., & Ahadi, A. (2019). Seed priming with H2S and Ca2+ trigger signal memory that induces cross-adaptation against nickel stress in zucchini seedlings. *Plant Physiology and Biochemistry*, *143*, 286-298.

Van den Ende, W., & Valluru, R. (2009). Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging?. *Journal of experimental botany*, *60*(1), 9-18.

Vandiver, M. S., & Snyder, S. H. (2012). Hydrogen sulfide: a gasotransmitter of clinical relevance. *Journal of molecular medicine*, *90*, 255-263.

Vardhini, B. V., & Anjum, N. A. (2015). Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Frontiers in Environmental Science*, *2*, 67.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. *Plant Science, 151*, 59-66.

Venkat, A., Bae, D. W., & Muneer, S. (2023). Circadian Clock Contributes to Modulate Salinity Stress-Responsive Antioxidative Mechanisms and Chloroplast Proteome in Spinacia oleracea. *Agriculture*, *13*(2), 429.

Verma, A., Malik, C. P., Gupta, V. K., & Bajaj, B. K. (2011). Effects of in vitro triacontanol on growth, antioxidant enzymes, and photosynthetic characteristics in Arachis hypogaea L. *Brazilian Journal of Plant Physiology*, *23*, 271-277.

Verma, S., Nizam, S., & Verma, P. K. (2013). Biotic and abiotic stress signaling in plants. *Stress Signaling in Plants: Genomics and Proteomics Perspective, Volume 1*, 25-49.

Verma, T., Bhardwaj, S., Singh, J., Kapoor, D., & Prasad, R. (2022). Triacontanol as a versatile plant growth regulator in overcoming negative effects of salt stress. *Journal of Agriculture and Food Research*, 100351.

Villa-Rivera, M. G., & Ochoa-Alejo, N. (2021). Transcriptional regulation of ripening in chili pepper fruits (Capsicum spp.). *International Journal of Molecular Sciences*, *22*(22), 12151.

Vimal, S. R., Patel, V. K., & Singh, J. S. (2019). Plant growth promoting Curtobacterium albidum strain SRV4: an agriculturally important microbe to alleviate salinity stress in paddy plants. *Ecological Indicators*, *105*, 553-562.

Wang, C., Deng, Y., Liu, Z., & Liao, W. (2021). Hydrogen sulfide in plants: Crosstalk with other signal molecules in response to abiotic stresses. *International Journal of Molecular Sciences*, *22*(21), 12068.

Wang, J., Ding, J., Yu, D., Teng, D., He, B., Chen, X., ... & Su, F. (2020). Machine learning-based detection of soil salinity in an arid desert region, Northwest China: A comparison between Landsat-8 OLI and Sentinel-2 MSI. *Science of the Total Environment*, *707*, 136092.

Wang, W., Ni, Z. J., Song, C. B., Ma, W. P., Cao, S. Q., & Wei, Z. J. (2023). Hydrogen sulfide treatment improves quality attributes via regulating the antioxidant system in goji berry (Lycium barbarum L.). *Food chemistry*, *405*, 134858.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, *218*, 1-14.

Wang, Y., Dimkpa, C., Deng, C., Elmer, W. H., Gardea-Torresdey, J., & White, J. C. (2022). Impact of engineered nanomaterials on rice (Oryza sativa L.): a critical review of current knowledge. *Environmental Pollution*, *297*, 118738.

Wang, Z., Tan, W., Yang, D., Zhang, K., Zhao, L., Xie, Z., ... & Zhang, D. (2021).

Mitigation of soil salinization and alkalization by bacterium-induced inhibition of evaporation and salt crystallization. *Science of the Total Environment*, *755*, 142511.

Waqas, M. A., Kaya, C., Riaz, A., Farooq, M., Nawaz, I., Wilkes, A., & Li, Y. (2019). Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. *Frontiers in plant science*, *10*, 1336.

Wei, G. Q., Zhang, W. W., Cao, H., Yue, S. S., Li, P., & Yang, H. Q. (2019). Effects hydrogen sulfide on the antioxidant system and membrane stability in mitochondria of Malus hupehensis under NaCl stress. *Biol Plant*, *63*, 228-236.

Weremczuk-Jeżyna, I., Hnatuszko-Konka, K., Lebelt, L., Piotrowska, D. G., & Grzegorczyk-Karolak, I. (2022). The Effect of the Stress-Signalling Mediator Triacontanol on Biochemical and Physiological Modifications in Dracocephalum forrestii Culture. *International Journal of Molecular Sciences*, *23*(23), 15147.

Yan, Z. H. O. U., Ming, D. I. A. O., CUI, J. X., CHEN, X. J., WEN, Z. L., ZHANG, J. W., & LIU, H. Y. (2018). Exogenous GSH protects tomatoes against salt stress by modulating photosystem II efficiency, absorbed light allocation and H2O2-scavenging system in chloroplasts. *Journal of Integrative Agriculture*, *17*(10), 2257-2272.

Yang, D., Li, Y., Shi, Y., Cui, Z., Luo, Y., Zheng, M., ... & Wang, Z. (2016). Exogenous cytokinins increase grain yield of winter wheat cultivars by improving stay-green characteristics under heat stress. *PLoS One*, *11*(5), e0155437.

Yang, X., Tian, Z., Sun, L., Chen, B., Tubiello, F. N., & Xu, Y. (2017). The impacts of increased heat stress events on wheat yield under climate change in China. *Climatic Change*, *140*, 605-620.

Yassin, M., El Sabagh, A., Mekawy, A. M. M., Islam, M. S., Hossain, A., Barutcular, C., ... & Saneoka, H. (2019). Comparative performance of two bread wheat (Triticum aestivum L.) genotypes under salinity stress. *Applied Ecology & Environmental Research*, *17*(2).

Yazdani F, Allahdadi I, Akbari GAB. Impact of super absorbent polymer on yield and growth of Soybean under drought stress condition. Pakistan Journal of Biological

Science. 2007;10(23):4190-4196.

Yihdego, Y., Webb, J., & Leahy, P. (2016). Modelling water and salt balances in a deep, groundwater-throughflow lake—Lake Purrumbete, southeastern Australia. *Hydrological Sciences Journal*, *61*(1), 186-199.

Younis, A. A., & Ismail, H. A. (2019). Triacontanol alleviated nickel toxicity in maize seedling by controlling its uptake and enhancing antioxidant system. *Journal of Advances in Plant Biology*, *1*(3), 1-15.

Younis, A., & Ismail, H. (2022). Alleviation of Zea mays L. Nickel Toxicity by Triacontanol Foliar Spray. *Egyptian Journal of Pure and Applied Science*, *60*(1), 17-33.

Zaid, A., Asgher, M., Wani, I. A., & Wani, S. H. (2020). Role of triacontanol in overcoming environmental stresses. *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives*, 491-509.

Zaid, A., Mohammad, F., & Siddique, K. H. (2022). Salicylic acid priming regulates stomatal conductance, trichome density and improves cadmium stress tolerance in Mentha arvensis L. *Frontiers in Plant Science*, *13*, 895427.

Zekri, S., Al-Rawahy, S. A., & Naifer, A. (2010). Socio-economic considerations of salinity: descriptive statistics of the Batinah sampled farms. *Published in the Monograph on Management of Salt-Affected Soils and Water for Sustainable Agriculture (Mushtaque A, Al-Rawahi SA, Hussain N (eds)). Sultan Qaboos University, Oman*, 99-113.

Zhang, F., Xiao, X., & Wu, X. (2020). Physiological and molecular mechanism of cadmium (Cd) tolerance at initial growth stage in rapeseed (Brassica napus L.). *Ecotoxicology and Environmental Safety*, *197*, 110613.

Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y., & Dai, S. (2012). Mechanisms of plant salt response: insights from proteomics. *Journal of proteome research*, *11*(1), 49-67.

Zhang, H., Hu, L.Y., Hu, K.D., He, Y.D., Wang, S.H., Luo, J.P. (2008). Hydrogen sulfide promotes wheat seed germination and alleviates the oxidative damage against

copper stress. — J. Integr. Plant Biol. **50**: 1518–1529.

Zhang, H., Hu, S. L., Zhang, Z. J., Hu, L. Y., Jiang, C. X., Wei, Z. J., ... & Jiang, S. T. (2011). Hydrogen sulfide acts as a regulator of flower senescence in plants. *Postharvest Biology and Technology*, *60*(3), 251-257.

Zhang, H., Irving, L. J., McGill, C., Matthew, C., Zhou, D., & Kemp, P. (2010). The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Annals of Botany*, *106*(6), 1027-1035.

Zhang, H., Mao, X., Jing, R., Chang, X., & Xie, H. (2011). Characterization of a common wheat (Triticum aestivum L.) TaSnRK2. 7 gene involved in abiotic stress responses. *Journal of experimental botany*, *62*(3), 975-988.

Zhang, H., Ye, Y.K., Wang, S.H., Luo, J.P., Tang, J., Ma, D.F. (2009). Hydrogen sulfide counteracts chlorophyll loss in sweetpotato seedling leaves and alleviates oxidative damage against osmotic stress. — *Plant Growth Regul*. 58: 243–250, 2009.

Zhang, J., Yuan, H., Fei, Z., Pogson, B. J., Zhang, L., & Li, L. (2015). Molecular characterization and transcriptome analysis of orange head Chinese cabbage (Brassica rapa L. ssp. pekinensis). *Planta*, *241*, 1381-1394.

Zhang, J., Zhou, M., Zhou, H., Zhao, D., Gotor, C., Romero, L. C., ... & Xie, Y. (2021). Hydrogen sulfide, a signaling molecule in plant stress responses. *Journal of Integrative Plant Biology*, *63*(1), 146-160.

Zhang, Q., Zhao, M., Meng, F., Xiao, Y., Dai, W., & Luan, Y. (2021). Effect of polystyrene microplastics on rice seed germination and antioxidant enzyme activity. *Toxics*, *9*(8), 179.

Zhang, Z. H., Palta, J. A., Lu, P., Ren, M. J., Zhu, X. T., He, J., & Shabala, S. (2021). Traditional soybean (Glycine max) breeding increases seed yield but reduces yield stability under non-phosphorus supply. *Functional Plant Biology*, *49*(2), 132-144.

Zheng, Q., Liu, Z., Chen, G., Gao, Y., Li, Q., & Wang, J. (2010). Comparison of osmotic regulation in dehydration-and salinity-stressed sunflower seedlings. *Journal of Plant Nutrition*, *33*(7), 966-981.

Zhou, J., Zhao, Y., Hansen, C. S., Yang, J., Chang, Y., Yu, Y., ... & Yang, X. (2020).

Ultraviolet photolysis of H2S and its implications for SH radical production in the interstellar medium. *Nature Communications*, *11*(1), 1547.

Zhu, Y. J., Sun, Z. C., Niu, X. J., Ying, J. Z., Fan, Y. Y., Mou, T. M., ... & Zhuang, J. Y. (2019). Dissection of three quantitative trait loci for grain size on the long arm of chromosome 10 in rice (Oryza sativa L.). *PeerJ*, *7*, e6966.

Zhu, Z., Wei, G., Li, J., Qian, Q., & Yu, J. (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (Cucumis sativus L.). *Plant Science*, *167*(3), 527-533.

Zhu, Z., Zhang, R., Liu, T., & Zheng, H. (2011). Solute accumulation and osmotic adjustment characteristics of the mangrove Avicennia marina under NaCl-induced salinity stress.

Zulfiqar, F., & Ashraf, M. (2021). Nanoparticles potentially mediate salt stress tolerance in plants. *Plant Physiology and Biochemistry*, *160*, 257-268.

Appendices

LIST OF PUBLICATIONS

Research/Review Paper(s)

1. Bhardwaj, S., **Verma, T**., Raza, A., & Kapoor, D. (2022). Silicon and nitric oxide mediated regulation of growth attributes, metabolites and antioxidant defense system of radish (*Raphanus sativus* L.) under arsenic stress. *Phyton: Journal of Experimental Botany*. (Accepted).
2. **Verma, T**., Bhardwaj, S., Singh, J., Kapoor, D., & Prasad, R. (2022). Triacontanol as a versatile plant growth regulator in overcoming negative effects of salt stress. *Journal of Agriculture and Food Research, 10,* 100351.

Book Chapter(s)

1. **Verma T.,** Tabasum S., Bhardwaj S., Gautam V., Kapoor B., and Kapoor D. (2022). Global food security and effects of various environmental constraints on food crops. In Environmental Sustainability in Food Industry: A green perspective. Publisher CRC press, Taylor and Francis.
2. Jan S., Bhardwaj S., **Verma T.,** Bhardwaj R., Kapoor D., and Singh R. (2022). Novel and Innovative strategies for food packaging processes. In Environmental Sustainability in Food Industry: A green perspective. Publisher CRC press, Taylor and Francis.
3. Angurana R., Katoch V., **Verma T**., and Bhardwaj S. (2022). Functional Properties of Food Processing as a novel technology for human health and nutrition. In Environmental Sustainability in Food Industry: A green perspective. Publisher CRC press, Taylor and Francis.
4. Sharma D., Bhardwaj S., **Verma T**., Pujari M., Singh R., and Gautam V. (2022). Food Processing Potential for Energy Efficiency and Use. In Environmental Sustainability in Food Industry: A green perspective. Publisher CRC press, Taylor and Francis.
5. Bhardwaj, S., **Verma, T**., and Kapoor, D. (2022). Ethylene and regulation of metabolites in plants. In *Ethylene in Plant Biology*. Pp. 32-48. John Wiley & Sons.
6. Bhardwaj, S., **Verma, T**., Kapoor, B., & Kapoor, D. (2022). Cereal Physiology, Flowering, and Grain Yield Under Salinity and Drought Stress. In *Omics Approach to Manage Abiotic Stress in Cereals* (pp. 21-36). Springer, Singapore.

7. Pujari M, Jan S, Bhardwaj S**.,** **Verma T,** Sharma D, and Singh R. (2021). Salinity stress in terrestrial as well as aquatic ecosystems: Effects and Biochemical & molecular adaptions in plants. In Environmental contamination and climate change: Effects on plants and remedial strategies. Publisher: Nova Science Publishers, New York.

List of Conferences/ Workshops Attended

1. Oral presentation on the title "Alleviation of salinity stress in Brassica juncea by application of triacontanol and hydrogen sulphide" at **"International conference on Advances and Innovations in Biotechnology and Allied Sciences",** 24-25 March 2022, organized by Chandigarh University, Mohali, Punjab.
2. Oral presentation on the title "Application of Triacontanol and hydrogen sulphide in mitigation of salinity stress in Brassica juncea" at "**International Conference on Sustainability: Life on Earth 2021"**, 17-18 December 2021, organized by Lovely Professional University, Phagwara, Punjab