EFFECT OF SILICON AND NITRIC OXIDE IN ALLEVIATION OF ARSENIC INDUCED PHYTOTOXICITY IN *RAPHANUS SATIVUS* L.

Thesis Submitted for the Award of the Degree of

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By

Savita Kumari

Registration Number: 11919247

Supervised By

Dr. Shabnam (28725)

Department of Botany (Assistant Professor)

School of Bioengineering and Biosciences,

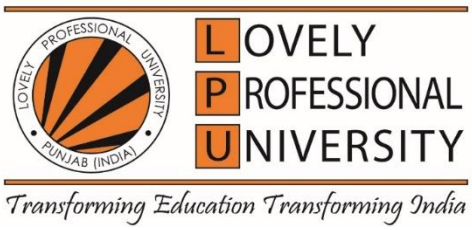
Lovely Professional University, Punjab

Co-supervised by

Dr. Dhriti Kapoor

School of Biological and Environmental Sciences, (Assistant Professor)

Shoolini University, Solan, Himachal Pradesh



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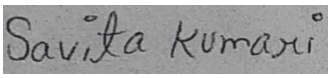
2024

DECLARATION

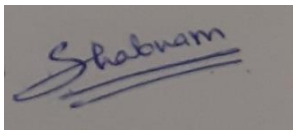
I, hereby declared that the presented work in the thesis entitled "**Effect of Silicon and Nitric Oxide in Alleviation of Arsenic Induced Phytotoxicity in *Raphanus sativus* L.**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr Shabnam, 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.



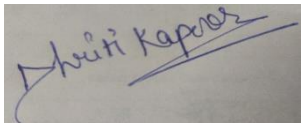
(Signature of Scholar)
Name of the scholar: Savita Kumari
Registration No.: 11919247
Department/school: Department of Botany, School of Bioengineering and Biosciences
Lovely Professional University, Punjab

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Effect of Silicon and Nitric Oxide in Alleviation of Arsenic Induced Phytotoxicity in *Raphanus sativus* L."** 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 Savita Kumari, 11919247, 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. Shabnam
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

Arsenic (As) presence has become a threat to plant productivity globally at both terrestrial and aquatic organisms. Present research work was performed to check the influence of Silicon (Si) and nitric oxide (NO) in ameliorating As toxicity in *Raphanus sativus*. Seeds of radish were pre-treated with sodium nitroprusside (SNP) solution as NO donor for 8 hours. Three quantities of As (0.3, 0.5 and 0.7 mM) in the form of sodium arsenate were used. Seedlings and plants of radish were supplemented with Si as silicic acid in liquid form at 2 mM concentration as foliar spray. Seedlings and plants of radish were then harvested after 7, 30 and 60 days for evaluating various morphological, physicochemical and molecular aspects.

Various morphological attributes (root length, shoot length, fresh weight, dry weight) were measured. Photosynthetic pigments (chlorophyll, carotenoid and xanthophyll content), and gas exchange attributes (total photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration) were evaluated. In addition to this, metabolites content (flavonoid, phenolic and anthocyanin content) and oxidative stress markers (malondialdehyde and hydrogen peroxide) were measured. Membrane and nuclear membrane damage in *R. sativus* seedlings were studied by confocal microscope. Content of osmolytes (proline and glycine betaine), total sugar and protein contents were measured. Moreover, As metalloid content was also evaluated by the application of Si and NO at 0.7 mM concentration of As. Activities of antioxidative enzymes and non-enzymatic antioxidants, and expression of antioxidative genes i.e., *SOD* and *CAT* were also evaluated.

Root and shoot length, biomass and vigor index were diminished under As stress. However, Si and NO treatment improved these attributes in seedlings and plants under As stress. Relative water content was lowered under As stress but increased by Si and NO. Contents of photosynthetic pigments i.e. chlorophyll, carotenoid and xanthophylls were noticed to be declined under As toxicity. The highest reduction in photosynthetic pigments was at 0.7 mM concentration. Further, Si and NO application led to increased contents of these photosynthetic pigments. As stress also caused a reduction in the gas exchange attributes. But, the application of Si and NO enhanced these gas exchange attributes. Contents of metabolites (anthocyanin, flavonoids, and phenolics) were recorded to be decreased in As exposed seedlings and plants of *R. sativus*. However, Si

and NO led to an upsurge in the contents of anthocyanin, flavonoids and phenolics. Supplementation with Si and NO also showed better results in increasing metabolite content as compared to their individual applications. Arsenic stress elevated the level of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Maximum elevation in the MDA and H_2O_2 were at 0.7 mM concentration. However, Si and NO reduced their level to diminish oxidative stress. High membrane and nuclear damages were seen in As treated seedlings, while treatment with Si and NO resulted in reduction of membrane and nuclear damages. High As content was noticed in As-treated seedlings and plants. Roots showed higher As contents as compared to shoot tissues. However, As content was declined in Si and NO-treated seedlings and plants. Contents of osmolytes were diminished in As stressed plants. The highest reduction in the osmolytes contents was at 0.7 mM concentration. Silicon and NO treatment increased their contents under stressed conditions. Contents of total carbohydrates and proteins were noticed to be declined under As stress. However, supplementation of Si and NO led to elevation in total carbohydrates and proteins amount in seedlings and plants of *R. sativus*. Activities of antioxidative enzymes were decreased in As-treated seedlings and plants. However, application of Si and NO under stressed conditions increased their activities. It was observed that contents of non-enzymatic antioxidants were reduced under As toxicity. Whereas, application with Si and NO increased their contents in seedlings and plants under stressed conditions. Gene expression of SOD and CAT genes were upregulated under As stress. However, application of mitigants in individual plus combined manner further increased their expressions under stressed conditions.

It was found from the study that As posed negative effects on Radish but Individual as well as combined application of Si and NO reversed the As induced effects. Overall, it was concluded from the present study that treatment with NO and Si is an useful practice to lessen the As-induced noxious influence in *Raphanus sativus* by improving their morphological, physiological, biochemical, and molecular aspects.

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Abbreviations

DNPH	2,4-Dinitrophenylhydrazine
DAPI	4,6-diamino-2-phenylindole
$AlCl_3$	Aluminum chloride
As^{5+}	Arsenate
As	Arsenic
As^{3+}	Arsenite
APX	Ascorbate peroxidase
AAS	Atomic absorption spectrophotometer
Cd	Cadmium
CO	Carbon monoxide
CAT	Catalase
Co	Cobalt
Cu	Copper
Cm	Centimeter
Cr	Chromium
DHAR	Dehydroascorbate reductase
DMA	Dimethylarsinic acid
DDW	Double distilled water
EDTA	Ethylenediamine tetraacetic acid
POD	Guaiacol peroxidase
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
OH^-	Hydroxyl
IRGA	Infrared gas analyzer
Fe	Iron
Hg	Mercury
µg	Microgram
µM	Micromolar
Mg	Milligram
mM	Millimole
Pb	Lead
Lsi1	Low silicon rice 1
MDA	Malondialdehyde
Mn	Manganese
MDHAR	Monodehydroascorbate reductase
MMA	Monomethylarsonic acid
Ni	Nickel
HNO_3	Nitric acid
NO	Nitric oxide
NBT	Nitroblue tetrazolium
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
$HClO_4$	Perchloric acid
PS II	Photosystem II

PPO	Polyphenol oxidase
PBG	Porphobilinogen
PCA	Principal component analysis
PI	Propidium iodide
P5CS1	Pyrroline-5-carboxylate synthetase
ROS	Reactive oxygen species
H_4SiO_4	Silicic acid
Si	Silicon
NaOH	Sodium hydroxide
$NaNO_2$	Sodium nitrite
SNP	Sodium nitroprusside
O_2^-	Superoxide
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
TMAsO	Trimethylarsine oxide
WUE	Water use efficiency
Zn	Zinc
GABA	γ-aminobutyric acid

List of Legends

Abbreviations	Treatment	Legend
C	Control	
T_1	As I	
T_2	As II	
T_3	As III	
T_4	Si	
T_5	Si + As I	
T_6	Si + As II	
T_7	Si + As III	
T_8	NO	
T_9	NO + As I	
T_{10}	NO + As II	
T_{11}	NO + As III	
T_{12}	Si + NO	
T_{13}	Si + NO + As I	
T_{14}	Si + NO + As II	
T_{15}	Si + NO + As III	

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Chapter 1

Introduction

Food security is currently a major challenge and concern for agronomists and plant physiologists globally (Zahid et al., 2023). However, plants confront one or more abiotic stresses such as salinity, heavy metal, temperature, flooding stress, etc. throughout their growth stages, which can induce adverse consequences on various growth aspects (Dutta et al., 2018; Khan et al., 2019a; Shikari et al., 2022). Crop yield as well as plant biomass declines under these abiotic stress conditions, which leads to reduction in food production worldwide (Kerchev et al., 2020). Among these environmental constraints, heavy metal toxicity became a serious issue worldwide because of rapid development and modernization and high use of agrochemicals (Yang et al., 2022; Zhai et al., 2018). Heavy metal pollution become so frequent nowadays as it also impacts the human health (Arif et al., 2019). Food security is highly threatened by heavy metals through restricting the development of plants (Irshad et al., 2020; Javaid et al., 2020). It is necessary to increase the current crop raising system by 70~100 % as the global population is assumed to expand in near future (Mueller et al., 2012; Zhou et al., 2020). Hence removal of these toxic metals or their immobilization should be practiced to decline their level in polluted agricultural lands. Interference caused by humans for instance, the use of insecticides, toxic waste released by industries, sewage disposals, etc., release toxic metals e.g. lead (Pb), cobalt (Co), cadmium (Cd), manganese (Mn), zinc (Zn), mercury (Hg) and metalloids i.e., arsenic (As) in soils (Aydinalp & Marinova, 2009) and also cause their accumulation or leaching into groundwater (Basheer, 2018a; Dağhan et al., 2015; Hakeem et al., 2015). These pollutants are absorbed by plants, which poses major health risks to people who live in contaminated areas mostly through the food chain (Feng et al., 2021; Khan et al., 2021). These noxious metal/lloids cannot be expelled naturally from the ecosystem, as these are non-biodegradable. Some of the metals are mobile because plants can take up them by root, via various processes whereas other metals cannot move from the place of their accumulation and are termed as immobile (Alharbi et al., 2018b; Burakova et al., 2018). Metals such as copper (Cu), nickel (Ni) and Zn are requisite in minute quantities, therefore are recognized as essential micronutrients, whereas Pb and Cd are toxic above their permissible limit and do not exhibit any beneficial role in plants (Ali et al., 2017; Tabrez et al., 2011). These heavy metals interfere with plant functions like growth, photosynthetic efficiency, and water potential, and have a negative effect on

agricultural productivity, ultimately resulting in crop failure (Choudhary et al., 2012). Plant responses to heavy metal stress are complex processes that depend on a number of factors, including concentration and kind of solute, stage of plant growth, genetic potential, and environmental factors (Nazir et al., 2020). Accumulation and extraction of the toxic metals are performed by the plants through the process of phytoremediation and are considered as natural bio accumulators (Ahmad et al., 2023; Ali et al., 2013a). Among these toxic heavy metals, As is a hazardous metalloid present in the natural habitats (Bukhari & Rehman, 2023; Mandal and Suzuki, 2002; Niazi et al., 2017). Arsenic is in the Group 1 category for causing cancer in humans (Martinez et al., 2011). Arsenic topped the list of the twenty most toxic elements (ATSDR, 2013). Around world, one out of every sixty people reside in those habitats where As content is greater or equal to 50 µg L^{-1} in groundwater (Imran et al., 2013; Tibbetts, 2000). Worldwide, As concentrations in natural soil range from 5 mg kg^{-1}, with variations based on the soil's place of origin (Han et al., 2003; Mitra et al. 2017a). Arsenic has received greater attention from the health perspective of both ecosystem and humans. Exposure of As at a high level emerged as a serious issue. Arsenic is reported to be present in more than 70 nations in groundwater (Bakhat et al., 2017). Arsenic enters either via human activities i.e. mining, industries, high use of insecticides, and irrigation with As-polluted groundwater, or via natural activities like volcanic emissions (Rahman et al., 2014). Arsenic enters the grains and ultimately the food after its absorption by plants which was reported by Liu et al. (2014).

Around two hundred compounds contain As in them which shows its abundance in nature. Arsenates (As^{5+}) are the most abundant type of As (around 60%), while sulfides and sulfo-salts forms constitute 20%, and forms like silicates, oxides, arsenides, arsenites (As^{3+}), and elemental As constitute the rest of the As (Abbas et al., 2018). Forms i.e. As^{-3}, As^{0}, As^{3+} and As^{5+} are the 4 oxidation states of As (Panda et al., 2010). Inorganic and organic are the two categories of As among which arseno-sugars and betaines, and monomethyl arsonic acid, are in the organic category while arsenate and arsenite are the inorganic species. Inorganic species of As occurs in the natural ecosystem and are mobile and noxious. As^{5+} dominates in oxidized environment whereas As^{3+} prevails in reduced conditions (Baig et al., 2010; Khalid et al., 2017; Sadiq, 1997). Contaminated water contributes majorly to the human exposure to As,

while some minor routes of exposure are inhalation and skin absorption (Shi et al., 2004). Phosphate (Pi) transporters are the major route for As^{5+} entry into plant roots whereas aquaporin channels which are involved in the passage of water and small neutral molecules also transports As^{3+} (Li et al., 2014 , Mukhopadhyay et al., 2014). After its uptake from the soil, As accumulates in edible plant parts, and is then consumed by humans (Finnegan and Chen, 2012). Carcinogenesis is caused in humans by As exposure through contaminated drinking water and also causes skin diseases which ultimately cause skin and epithelial tissue cancer (Islam et al., 2022; Rahman et al., 2009). Exposure to As also increase disease and mortality rate (Bhattacharya, 2017; Kapaj et al., 2006; Mazumder, 2008).

Accumulation of As by plant plants mainly depends on its total amount and majorly upon As speciation present in soil. Transporter proteins are helpful for the transport of inorganic forms of As i.e. As^{3+} and As^{5+} in plants (Ghosh et al., 2015; Zhao et al., 2009). The same transporters are shared by As^{5+} and phosphate to penetrate the root's cell membranes as both are analog to each other (Ali et al., 2009; Niazi et al., 2015). Normal cellular metabolic activities of plants are altered by As by binding to the enzymes. Nitrogen (N) assimilation is disrupted by As by interfering with carbon and sulfur metabolism (Finnegan et al., 2012). Moreover, loss of function and cell death is also caused by As because it can alter the functioning of proteins and peptides (Garg and Singla, 2011; Muzaffar et al., 2023).

Nations falling in Asian (especially those of South Asian region) and South American continent have been severely polluted by As in their groundwater resources (Hashim et al., 2019). Bangladesh, India, China, Nepal, Cambodia, Vietnam, Myanmar, Indonesia with that of Argentina, Canada, Chile, Hungary are some of the worst hit countries on account of presence of this heavy metal (Shaji et al., 2021). In India, As contamination encompasses about 25 states (230 districts) and 4 Union territories- West Bengal, Assam, Bihar, Chhattisgarh, Punjab, Haryana, Jharkhand, Karnataka, Uttar Pradesh, New Delhi are to be named as few among them where maximum permissible threshold of 10 ppb is exceeded (The Hindu, 4th December, 2023).

Furthermore, exceedingly high levels of As in ground water of agricultural state like Punjab has emerged as a key contributor to the problem of environmental disasters. Department of Drinking Water and Sanitation, Ministry of Jal Shakti has reported 591

arsenic affected habitations in Punjab in an affidavit filed by them before National Green Tribunal as on 21st of January, 2021 (Source: IMIS, DDWS). Central Ground Water Board has identified 12 major As rich sites in Punjab belonging to 6 districts- Mansa, Amritsar, Kapurthala, Hoshiarpur, Ropar and Gurdaspur, mainly located alongside the riverbanks of Beas and Ravi (CGWB, 2023).

Plant cannot survive under high contents of As (Stoeva et al., 2005). Transpiration rate was significantly lessened by As in plants which might be due to the deterioration of water transport processes (Verbruggen et al., 2009). Reactive oxygen species (ROS) are released in excessive quantities by As at the biochemical level which results in oxidative stress in plants. Arsenic-induced oxidative damage also causes alterations in metabolic pathways and cellular ultrastructure by altering membrane integrity, protein carbonylation, and damage to nucleic acids (Alvarenga et al., 2020; Bibha et al., 2023; Chandrakar et al., 2016). Nutrient uptake, cellular redox homeostasis, chlorophyll metabolism and photosynthesis are also destructed by As in different plant species (Kaya et al., 2020; Mishra et al., 2019). In parent material, content of As metalloid varies between different soil types (Basu et al., 2014; Matschullat, 2000). Higher arsenic concentrations cause the plant to lose its ability to balance toxicity and resistance, which kills the plant (Stoeva et al., 2005). Thus, to maintain safe food production and human health, it is imperative to minimise As uptake and translocation in plants and reduce As contamination in the soil using effective methods.

In this context, several effective methods have been in practice to enhance the tolerance potential of economically imperative crop plants to As stress (Chandrakar et al., 2017), which includes the cultivation of As tolerant genotypes via conventional selection, breeding, genetic engineering, etc. But these strategies are time consuming and high-priced, therefore inexpensive, economical, and eco-friendly strategies are in practice in present-day time to improve plant tolerance to As toxicity (Chandrakar & Keshavkant, 2019). Against this metalloid, exogenous supplementation of mineral nutrient silicon (Si) and signaling molecule nitric oxide (NO) may be a valuable approach to decline As uptake or As absorption from soil to plant roots for better food production. Hence, application with these moieties could be one of the effective strategies to prevent As toxicity in plants.

Silicon, is the second abundant molecule after oxygen on earth's crust with an atomic

number and weight of 14 and 28.085, respectively (Ma, 2003; Pooniyan et al., 2023). In the periodic table, this metalloid belongs to Group 14 and occurs in two allotropic forms with a 2.42 specific gravity and 1420 °C melting point (Sommer et al., 2006). Moreover, -4, +2, and +4 are the three oxidation states of Si in nature among which the most common is the +4 state. In addition, ^{28}Si, ^{29}Si and ^{30}Si are the three naturally occurring isotopes of Si (Richmond & Sussman, 2003; Tubaña & Heckman, 2015). Silica, silicon dioxide (SiO_2), silica gel, and silicate are the major Si compounds that are accumulated in soil (Ali et al., 2023; Sommer et al., 2006). Silica gel is the form of silica that possess a porous composition whereas silicate is a silicic acid salt, found in a wide variety of rocks present in earth (Farooq & Dietz, 2015; Gérard et al., 2008). A variation of 23 to 35% by weight occurs in Si content in soil with a 28.8% average value (Prychid et al., 2003). Around 28% is the average amount of Si in the earth's core pedosphere (Wedepohl, 1995). Silicon concentration in soil typically lies between 0.1 to 0.6 mM which is extremely low (Sommer et al., 2006). Silicon which is found in soil, is not available for plant uptake despite of large Si abundance in soil, because of the insolubility of Si compounds in soil solution (Richmond & Sussman, 2003). Available form of Si for plants is silicic acid which is soluble at basic pH (Currie & Perry, 2007). Due to its many positive effects on plants, Si is receiving more attention in the field of agriculture (Deshmukh et al., 2017).

Plants benefit greatly from Si's stress-alleviating properties since it enhances their defense systems against various biotic and abiotic stresses. To achieve global sustainable agriculture and to improve productivity, the addition of Si might be considered as an useful strategy to protect plants from stress conditions (Guntzer et al., 2012; Souri et al., 2021). Silicon exhibits numerous mechanisms to regulate plant development via regulating morphological attributes, photosynthetic system, antioxidant defense system, nutrient uptake, and also establish plant cell-wall barrier by polymerization of $Si(OH)_4$ (orthosilicic acid) (Mir et al., 2022; Soundararajan et al., 2014). Silicon-mediated stress alleviation can be attributed to several factors such as translocation, complexation and chelation, reduction in heavy metal uptake, regulation of antioxidant compounds biosynthesis, gene expression etc. (Kleiber, 2018; Luyckx et al., 2017). Silicon exhibits multipurpose influence in elevating plant growth, productivity and eventually crop quality by endorsing plant mechanical strength and

tolerance against a variety of stresses and hence, regarded as “quasi-essential” element (Manivannan et al., 2023; Vulavala et al., 2016). Silicon lessens metal toxicity by amending the soil pH, compartmentation of metals in plant tissues, modifying metal speciation, metal co-precipitation, chelation and structural modifications in plants (Adrees et al., 2015; Debona et al., 2017; Xiao et al., 2014).

Silicon performs both external and internal plant mechanisms to successfully tolerate the stressful conditions (Kovács et al., 2022; Sahebi et al., 2015). Formation of metal silicate precipitates to decline the metal phyto-availability by the application of silicates through increasing the pH is the Si mediated external mechanism (Zhang et al., 2008a). Even though Si alleviates metal stress in both monocots and dicots, high accumulators of Si like monocots will generally benefit more from Si, which is attributed to the fact that plant’s potential to accumulate Si can vary (Greger et al., 2018). Silicon-mediated immobilization of toxic metals is another important mechanism to reduce the metal-induced negative effects in plants (Li et al., 2022). Escalation in the values of soil pH or alterations in metal speciation is the reason for this Si-induced immobilization via forming silicate complexes. An increase in the soil pH from 4.0 to 5.0–6.4 was observed in Si applied plants (Gu et al., 2011). Improvement in growth attributes, gas exchange parameters, photosynthetic activity, nutrient balance, and enhanced level of plant hormones and antioxidant defense system, were found in different Si treated plant species to mitigate heavy metal-induced toxicity (Malhotra and Kapoor, 2019). Numerous investigators have discovered that exogenous application of silicon in a variety of plant species can reduce the effects of several metal and metalloid stresses, such as Pb, As, Cd, Hg, aluminium (Al), Mn, Zn, Cu, or antimony (Sb) (Bhat et al., 2019; Vaculík et al., 2020, 2021).

Among the signaling molecules, NO has emerged as an important molecule in plants which regulates various signaling pathways (Aroca et al., 2018; Mariyam et al., 2023; Neill et al., 2003). It is also known as nitric monoxide, and has long been recognized as a component in the nitrogen cycle (Chen et al., 2022; Lamattina et al., 2003). However, this molecule has become the focus in animal and plant system over the last 30 years due to its pleiotropic effects at tissue, cellular and physiological levels under both regular and stressful conditions. Nitric oxide was known only for its involvement in air pollution along with nitrogen dioxide (NO_2) for many years and influenced plant

growth and development (Anderson & Mansfield, 1979; Capron & Mansfield, 1975). Nitric oxide, formerly known as an endothelium-derived relaxing factor, was shown to be involved in the control of blood vessel vasodilation as shown by two scientific publications released in 1987 by two different groups (Ignarro et al., 1987; Palmer et al., 1987). Alderton et al. (2001) described that a class of enzymes known as nitric oxide synthases produced this chemical in mammalian cells from the amino acid L-arginine. Involvement of NO in both the neurological and immunological systems of animal creatures was reported later on. It could be mentioned as a historical curiosity that Fewson and Nicholas released the first study in 1960, revealing that bacteria and higher plants may utilize NO as an intermediary in nitrogen metabolism (Fewson & Nicholas, 1960). Later in 1979, it was found that NO can also be produced in plants that were treated with herbicides (Klepper, 1979). Furthermore, the NO study has also been expanded to plant cells, exhibiting that various plant processes such as various growth aspects, process of communication with useful microbes and resistance in contrast to stressful conditions, are regulated by this molecule (Corpas & Palma, 2018; Puppo et al., 2013; Yoshioka et al., 2011).

Being a small redox signaling molecule in plants, NO is produced in plant cells under both normal and environmental stress conditions (Arasimowicz & Floryszak-Wieczorek 2007; Khan et al., 2023). When a plant's ROS concentration becomes hazardous, NO reverses these effects by acting as a detoxifier (Lipton et al., 1993). Nitric oxide is also well-thought-out as a plant hormone that has imperative role in many plants' physiological processes under stressful conditions. It is recognized as a significant molecule that is related to counteracting stress induced by toxic metals, for instance As. Nitric oxide, as a cell signaling molecule is easily transported in various plant tissues, and appears as a beneficial moiety in signal transduction mechanisms (Khan et al., 2023; Lamattina et al., 2003). Numerous physiological processes were regulated by NO through transcriptional regulation and/or through the induction of post-translational modifications (PTMs) of proteins, which might be attributed to the widespread distribution of NO in different subcellular sections (Fancy et al., 2017).

The respiratory system i.e., electron transport chain in plant’s mitochondria is also regulated by NO where it stimulates defense mechanisms to regulate the ROS level (Kumari et al., 2023; Zottini et al., 2002). Plant growth aspects starting from

germination to cell death are regulated by NO and decline the detrimental effects of stress by inhibiting programmed cell death (PCD) (Nabi et al., 2019). Small size and easily diffusible properties of NO across cell membranes make it a beneficial signaling molecule. NO even under minute concentrations i.e., nM (nano molar) and µM (micro molar), significantly improves stress tolerance (Fancy et al., 2017; Xiong et al., 2010). NO performs as a signal molecule at lower concentration but at higher concentrations, it induces nitro-oxidative stress via causing cellular damage. Hence, its endogenous concentration determines its mode of action like other free radical molecules (Asgher et al., 2017; Frukh et al., 2022). Because of its signalling function, NO is also thought to lessen metal toxicity. It also boosts a plant's antioxidant defence system, which lowers oxidative stress by breaking down hydrogen peroxide (H_2O_2) into molecular oxygen and activating enzymes that suppress H_2O_2 production (Bhat et al. 2021; Emamverdian et al., 2021; Zheng et al., 2009).

Raphanus sativus commonly known as radish, is a member of *Brassicaceae* family and utilized as a root vegetable crop (Jahangir, 2010; Younus et al., 2022). It is grown and consumed all over the globe and is recognized imperative portion of the human diet. Raw, cooked, or preserved radish may be consumed. Consumption of stems, leaves, seed pods and seedlings are also observed in several species of radish, in addition to roots. While the flesh of radishes is normally white, they can have a variety of skin colors, including red, purple, black, yellow and white through pink (Ahmed et al., 2022). The edible radish root also differs globally in terms of flavor, length and size (Banihani, 2017). Several diseases are also treated at the household level by using radish (Jeong et al., 2005; Kopta & Pokluda, 2013; Shukla et al., 2011).

Radish grows well in mild to cool environments and is mostly a winter-season crop. Nearly all of India's states grow radish, to which Gujarat, Punjab, Haryana and West Bengal are the major states that produce radish (Samir et al., 2016). *Raphanus sativus* is a well-known crop for hyperaccumulating heavy metals (Kapoor et al., 2016). Radishes have been an important part of the human diet globally in various forms because of their high nutritional and therapeutic importance (Manivannan et al., 2019). Different agricultural operations, irrigation in particular, are largely dependent upon As rich groundwater reservoirs leading to accumulation of As not only in the soil but also in various plant species inclusive of major cereals plus vegetables. This is substantiated

by the significant transfer of As in the food chain reported from various developing countries due to inadequate water resources and treatment facilities for purifying wastewater (Shahid et al., 2021). Indian Council of Agricultural Research (ICAR) has documented higher levels of As in vegetables like radish, amaranth, lady finger, cauliflower, brinjal and potato in farm rich states of Punjab and Haryana due to enhancement in the usage of pesticides together with employment of 90% of As contaminated water for irrigation over there (ICAR, 2015).

Additionally, members of family *Brassicaceae* such as *Arabidopsis thaliana, Brassica napus*, *Raphanus sativus*, *Brassica juncea*, *Brassica oleracea* var. *botrytis, Brassica oleracea* var. *capitata and Spinacia oleracea* have been found to be affected in terms of their morphological and biochemical attributes due to As toxicity and therefore their consumption can lead to serious health hazards (Niazi et al., 2017; Pita-barbosa et al., 2019; Kumar et al., 2020; Bano et al., 2022). Since fleshy roots and leafy vegetables uptake and accumulate higher levels of As, so their usage can readily put food security at stake (Sarkar et al., 2022). Radish is one such imperative vegetable crop of family *Brassicaceae* exhibiting stocking up of As in its roots which not only affects its normal growth, metabolite production, antioxidant defense system but also lowers down its economic worth (Pavilkova et al., 2023). Arsenic toxicity is increasing in vegetable crops such as radish, hence it is necessary to reduce its negative effects. Therefore, present study is carried out to mitigate the toxic effects of As by the application of Si and NO through studying growth parameters, physiological, biochemical and gene expression in radish.

Chapter 2
Review of Literature

2.1 Bioavailability of arsenic

In India, parts of many states have been identified as As contaminated in the case of soil and sediments (Shrivastava et al., 2015). Groundwater, which is the main source of drinking water in these areas, has been found to have As as high as 300 ppb. Out of 29 states in India, reports of As contamination have emerged from 17 states (Shukla et al., 2020). Higher accumulation of As in soils irrigated with As contaminated irrigation waters was reported by Sidhu et al. (2012) in sub-mountainous region consisted of districts namely Gurdaspur, Hoshiarpur, Nawanshehar, and Rupnagar and the central plain region namely Amritsar, Jalandhar, Kapurthala, and Ludhiana. The Punjab region's soil arsenic content varied greatly, according to two separate studies by Hundal et al. (2007) and Vicky-Singh et al. (2010). The availability of As in terms of the quantity absorbed by plants is a key knowledge of the risk associated with As poisoning. Environmental conditions, alterations in the rhizospheric soil and bioaccumulation kinetics, defines the As distribution in plants. Interconversion of As^{3+} and As^{5+} is facilitated by alterations in the redox potential, pH, and microbes (Nearing et al., 2014). Arsenate reduction in the ecosystem is performed by microbes through two principal mechanisms i.e. dissimilatory reduction and detoxification. During anaerobic respiration, dissimilatory reduction occurs with arsenate being a terminal electron acceptor. Arsenate becomes arsenite and then gets extruded by the As^{3+}-efflux pump during the detoxification mechanism. Manganese oxides mediated biotic oxidation is slower as compared to abiotic AsIII oxidation (Parikh et al., 2010). Manganese oxides act as e^- acceptors during abiotic AsIII oxidation as Mn oxides are abundant in the natural ecosystem. Sulfide is the reductant during the abiotic reduction of arsenate (Rochette et al., 2000). Moreover, strong affinity has been shown by inorganic As for oxides, and aspects like redox potential, change in pH, organic matter, competing ions, soil texture, and mineral constituents, highly influence this affinity (Shrivastava et al., 2015). Strong affinity is shown by arsenate for oxides as compared to arsenite.

Immobilization of As is favored by quick co-precipitation of As^{5+} with Fe^{3+}/Fe^{2+} ions under anaerobic soil environments (Sasaki et al., 2009; Yamaguchi et al., 2014). The influx of As^{3+} may increase in rice by the predominance of As^{3+} in Fe plaque under anoxic environments and elucidates the minor attraction of iron (Fe) plaque for As^{3+} as compared to As^{5+} (Liu et al., 2006). Furthermore, due to As^{5+} conversion into As^{3+} in

water logged soils, As^{3+} is the highly available form (Stroud et al., 2011). Small amount of As was found in water logged soils from China, Bangladesh, and the United Kingdom in which As^{3+} (>95%) predominantly occurs (Khan et al., 2010). Mobilization of As is governed by two processes i) increased As into the solution phase by the conversion of As^{5+} to As^{3+} and (ii) release of associated As.

Mobilization of As is augmented in paddy fields by dissolved organic carbon. Dissolved organic carbon and As bioavailability exhibits close association because the decomposition of biomass and the release of adsorbed As improves the bioavailability of As, in terms of organic matter functional groupings (Williams et al., 2011). Li et al. (2011) and Liu et al. (2004) conveyed in rice and in wetland plants that variations occur in Fe plaque formation. Uptake and transport of As is regulated by Fe plaque by holding its flow towards roots through the sorbing system (Syu et al., 2013).

Methylated As moieties are also present in addition to arsenate and arsenite as reported under both controlled ecological circumstances or under natural conditions (Aposhian, 1997; Zhao et al., 2013). Dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), and trimethylarsine oxide (TMAsO) are formed from microbial methylation of As under oxidizing conditions, as indicated by several reports. The level of As was increased in the past in cultivated lands due to the wide practice of MMA and DMA (Williams et al., 2007). Under anoxic environments, increased As methylation was shown by several studies either by stimulation of soil microorganisms in a more effective way or by functioning of As^{3+} mobility (Mestrot et al., 2009). Sorbed MMA and DMA are also released in the rhizosphere by declined termination of Fe oxides. Change in soil characteristics, microbial communities and ecological surroundings cause alterations in As speciation (Zhao et al., 2013). Bioavailability of As in soil is powerfully influenced by the chemical and physical characteristics of soils together with the character of minerals and clay content, organic matter, texture, pH and cation-exchange capability (CEC), and presence and concentration of oxides and hydroxides of metals, Al, Mn, etc (Shrivastava et al., 2015).

2.2. Accumulation of Arsenic and its speciation in plants

2.2.1 Accumulation

Major pathway through which As is transported to organic bodies is constituted by the roots of plants. Habitats and plant species determine the As uptake and accumulation

by plants. Less As is accumulated from the soil by terrestrial plants as compared to wetland plants. *Pteris vittata* was the first plant which was recognized as As hyperaccumulator (Ma et al., 2001a). In addition to this, 12 other plants were also reported as hyperaccumulators of As from the family i.e. *Pteridaceae* (Zhao et al., 2009). It is mobilized to the upper regions of the plant after its absorption by roots. Arsenic level ranges from 0.01–1.5 mg kg^{-1} DW in edible parts which were cultivated under polluted soils (Anawar et al., 2013; Kabata-Pendias & Pendias, 1992). Submerged plants were reported to accumulate high As concentrations (Robinson et al., 2006). Maximum As was accumulated in the root tissues of *Eriophorum angustifolium* as compared to the surrounding soil (Stoltz & Greger, 2002). Up to 400 mg kg^{-1} of As can be tolerated and accumulated in *Wolffia globosa* (Zhang et al., 2009a), while low levels of As are accumulated in the tissues of trees and shrubs. As is present in high amounts in rice, whereas its amount in cereals is low. Arsenic can be accumulated in higher amounts in stems and needles of some gymnosperms such as *Pseudotsuga menziesie* (Haug et al., 2004).

2.2.2 Speciation

It is important to evaluate the metal metabolism and toxicity, however, sufficient information is not provided. Different methods are used to assess As speciation, and used analysis method determines the speciation reliability. Variations occur in relative proportions of As^{5+} and As^{3+} (Meharg et al., 2009; Mohan & Pittman, 2007). In tobacco, inorganic As majorly constituted major part of the As which was exhibited by AE-HPLC-ICP-MS analysis (Taebunpakul et al., 2011). Xu et al. (2007) found that As^{5+} converted to As^{3+} and escaped to the exterior medium in tomato roots while Su et al. (2008) found that As was in the form of As^{3+} (93-98%) in *P. vittata* xylem sap regardless to the As^{3+} or As^{5+} application. Arsenite (As^{3+}) was the predominant form of As which was found in wheat (D'Amato et al., 2011).

Arsenite may produce composites with different thiol compounds. In *Brassica juncea* and *Arabidopsis thaliana* tissues, As is mostly found as As^{3+} (Bluemlein et al., 2008; Castillo-Michel et al., 2011). Metal speciation is also modified by plant root tissues, as indicated by wheat and rice's rhizodermis tissues due to the high As^{5+} concentrations, while in the cortex and stele, AsIII–thiol was present in high amounts (Kopittke et al., 2013). Further, DMA and MMA, have been found in minor amounts in plant tissues

which are released into the soils by the high use of As-containing fertilizers and also by soil microbes mediated As biomethylation (Huang, 2014; Zangi & Filella, 2012). Higher amounts of methylated As forms can be present in some plants, however, less efficiency is shown by plants to absorb methylated species as compared to inorganic As (Raab et al., 2005; Xu et al., 2007; Zhao et al., 2009).

Uptake of methylated As can be enhanced by the imbalance in nutrient level. Uptake of DMA was observed to be increased by 90% in maize by phosphorus starvation (Abbas & Meharg, 2008). In rice, methylated As content was found to be enhanced in the southern states of the USA. Methylated As were found in minor amounts in tissues of sunflower (Raab et al., 2007a) and *Holcus lanatus* (Quaghebeur and Rengel, 2003). Arsenic forms i.e. MMA and DMA were found as major forms with 35% and 24% respectively, in *Trifolium pretense* which were grown on As-polluted sites (Geiszinger et al., 2002). Under redox conditions, arsenite can be formed by the reduction of arsenate, and microorganism activity metabolizes As to methylated species (Raab et al., 2007b).

2.3 Absorption and transport of As in plants

Arsenate, arsenite, along with methylated As (MMA and DMA) are the major forms of As which are accessible for crops to accumulate in soil solutions (Virk et al., 2023).

2.3.1 Arsenate (As^{5+}) uptake

As^{5+} is the major form of As in oxygenic conditions. Arsenate uses phosphate (Pi) transporters to enter plant root tissue (Fig. 2.1). For instance, in *Arabidopsis thaliana*, uptake of As^{5+} and Pi is facilitated by genes expressing for Pi transporters in conditions of both excessive and minute phosphorus concentrations (Shin et al., 2004). High affinity was shown by transporter genes for the Pi as compared to As^{5+} in barley and *Holcus lanatus*, however As/Pi uptake takes place via the same plasma membrane (Meharg & Macnair, 1990). The addition of genes over-expressing the Pi transporter enhanced the acquisition and transport of Pi and As in rice plants. Strong relation for the *Pht1* associated with As^{5+} accumulation, was shown by *OsPht1;1* found in the cell organization.

AtPht1;1 and *AtPht1;7*, two Pi transporter family members, are hypersensitive to arsenate but insensitive to arsenite in thale cress (LeBlanc et al., 2013). The influx of As^{5+} is also modulated by some WRKY transcription factors (TFs). Expression of

Atpht1;1 which modulates uptake of As^{5+} is regulated by WRKY TFs i.e. WRKY6, and WRKY45 (Wang et al., 2014a). High affinity was shown by *PvPht1;3* for arsenate as compared to phosphate in *P. vittata* but similar affinities were exhibited by *PvPht1;3* and *AtPht1;5* for phosphate.

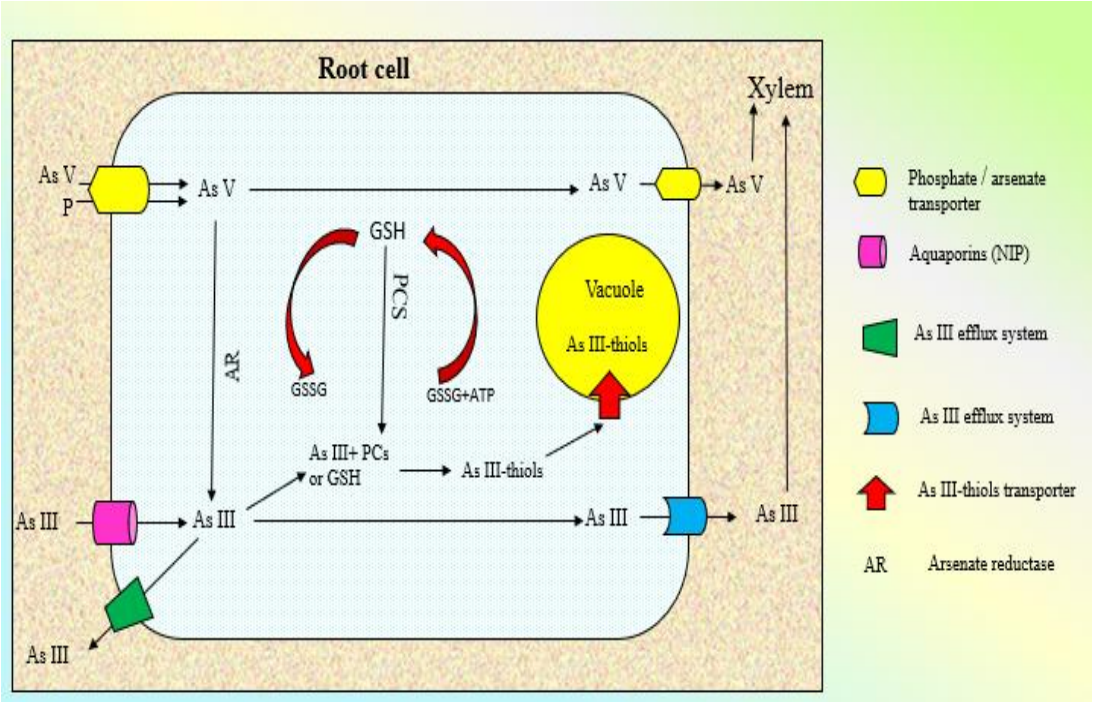

Fig. 2.1 Diagrammatic representation of arsenic uptake and transport in plants (modified after Marmiroli et al., 2014).

2.3.2 Arsenite (As^{3+}) uptake

Arsenite is transported across the cell membranes by plant aquaporins that are complex network channels for the passage of H_2O and essential compounds (Mukhopadhyay et al., 2000; Zhang et al., 2022). In roots of rice, *Arabidopsis* and lotza, various transporters performed the arsenite influx (Bienert et al., 2008). Moreover, arsenite-permeable NIPs also include OsNIP3;3 and HvNIP1;2. In Arabidopsis roots, transport of arsenite was also carried out by NIP3;1 (Xu et al., 2015b). Similarly, in rice roots, arsenite influx is also carried out by *Lsi1*. But no effect was detected on the uptake of arsenate by the increased functioning of *Lsi1* in yeast or *Xenopus laevis*.

In rice, adsorption and tolerance to As^{3+} are also performed by some membranal intrinsic proteins (Mosa et al., 2012). In yeast cells, arsenite transport is facilitated by *PvTIP4;1*, an aquaporin gene, whereas increased As accumulation and sensitivity was observed by its excessive functioning in transgenic thale cress. Arsenite is transferred

to phloem by an inositol transporter which is used by plants as a sugar alcohol (Duan et al., 2016). Furthermore, *AtINT2* or *AtINT4* caused an increase in As^{3+} accumulation when expressed in *Saccharomyces cerevisiae*.

2.3.3 Methylated arsenic species uptake

Uptake of As in its methylated forms are less efficient as contrast to inorganic one (Li et al., 2009; Preetha et al., 2023). Raab et al. (2007b) noticed that arsenate uptake was larger than methylated species. Uptake of these forms is carried out by Lsi1 in rice roots, the lower influx of MMA and DMA i.e. 80% and 50% was observed in rice Lsi deficient mutant (Li et al., 2009). The intake of several moieties in rice is carried out by NIP2;1 (Ma and Yamaji, 2006). In castor bean and rice, DMA was easily transported (Carey et al., 2011; Ye et al., 2010). It was transferred to the grain at a higher rate via xylem and phloem as compared to arsenate which was transferred through the phloem (Carey et al., 2010). The glycerol transport pathway is followed by dimethylarsinic acid (DMAA) and monomethylarsonic acid (MMAA). Transport of DMAA and MMAA is mediated by the aquaglyceroporins in rice roots, signifying the contribution of aquaglyceroporins in the transport (Rahman et al., 2011; Upadhyaya & Roychoudhury, 2022).

2.4 Effect of As in plants

Presence of As in plants disturbs various plant developmental processes including growth attributes, physiological aspects i.e. photosynthetic pigments and gas exchange attributes, biochemical processes and promote oxidative stress by excessive release of ROS, etc., (Fig. 2.2) which are described as follows

2.4.1 Plant growth

Growth attributes of the plants is adversely affected by the accumulation of As in them which ultimately declines food superiority globally (Cozzolino et al., 2010; Ulhassan et al., 2022). Arsenic occurrence in the H_2O reduced the plant's morphological parameters (Ahmed et al., 2006). Plant growth and metabolic processes can be altered by As which often the reason for senescence (Jiang & Singh, 1994). Plants grown in As polluted soils caused alterations in morphological parameters, and plant biomass, as reported by numerous studies (Mokgalaka-Matlala et al., 2008; Shaibur et al., 2008; Srivastava et al., 2009). High concentrations of As affected the vegetative and root system in tomato plants (Miteva, 2002). A decrease in plant's dry matter yield was

observed in *Sorghum bicolor* (Shaibur et al., 2008). Inhibition of growth parameters and decline in germination rate in *Brassica* species at higher concentrations of both As^{5+} and As^{3+} were reported by Srivastava et al. in 2009. High concentrations of As^{5+} and As^{3+} caused a decline in root organic matter formation in *Triticum aestivum* (Liu et al., 2005). Pigna et al. (2008) found reduced plant biomass in As polluted water.

2.4.2 Photosynthetic system

The photosynthetic rate in plants is recognized to be inhibited by As (Gusman et al., 2013). Chlorophyll content and photosystem II (PS II) activity are reduced after As absorption by plants, which ultimately impacts the light-harvesting apparatus (Anjum et al., 2011). Excessive concentration of As results in significant decline in chlorophyll pigment synthesis which could be attributed to a lack of regulations of PS I and -II. Arsenic stress also caused a reduction in chl formation in maize, red clover and lettuce (Abbas et al., 2018). Fundamental photosynthetic functioning and structure of chloroplast membrane are altered by As (Pandey et al., 2015; Nahar et al., 2022). Rate of CO_2 fixation and functionality of PS II was also altered under As stress (Stoeva & Bineva, 2003). Arsenic stress also causes alterations in the gaseous exchange rate and fluorescence emission by negatively impacting the photochemical efficiency and heat dissipation capacity (Debona et al., 2017). In bean, oat and red clover exposed to As, decreased carotenoid content was observed (Mascher et al., 2002), whereas its content was increased in *P. Vittata* and declined in *Pteris ensiformis* (Singh & Ma, 2006).

2.4.3 Carbohydrates

Arsenic stress negatively affects the carbohydrates content, and in response to this, soluble sugars accumulate in plants (Gramss, 2012). *Oryza sativa* under As toxicity exhibited diminished ratio of sugars (Jha & Dubey, 2004). Arsenic stress caused the conversion of sugars and also suppressed the synthesis of sucrose with respect to available measures of hexose monophosphate. Furthermore, α- and β-amylase and starch phosphorylase (SP) activities were also inhibited by As (Jha & Dubey, 2004). The release of soluble sugars was increased as a result of an upsurge in SP performance in rice and bean (Jha & Dubey, 2004). Arsenic stress also upregulated the functioning of acid invertase and sucrose synthase. Glucose and fructose from sucrose are majorly produced by acid invertases and in this regard, it was determined that its activity and the amount of hexose were directly correlated as reported by Roitsch and González

(2004) and Kaur et al. (2012). Oxidation of hexoses takes place through a glycolytic pathway which is synthesized from acid invertase or sucrose synthase (Baud & Lepiniec, 2009).

Fig. 2.2 Effect of As on various developmental aspects of plants.

2.4.4 Oxidative stress

Oxidative stress, an imbalance between ROS concentration and ROS scavenging, is caused due to As accumulation in plant tissue, which subsequently disturbs the ETC (Pourrut et al., 2013). Conversion of arsenate to arsenite cause ROS overaccumulation (Talukdar, 2013a). ROS are also generated in plants through the suppression of key enzymes and by transformation of arsenate to arsenite (Sharma, 2012; Zulfiqar & Ashraf, 2022). ROS formation is favored by redox-driven reactions via conversion of arsenate to arsenite which is then followed by a methylation progression (Singh et al., 2007). ROS is produced in cellular compartments. These moieties were highly made in wheat (Li et al., 2007). The level of $O_2^{\cdot-}$ and H_2O_2 increased by 15–28% and 23–82% after *Phaseolus aureus* exposure to 50 µM As (Kaur et al., 2012). Exposure of 50 µM As to rice, a hyperaccumulating species, resulted in an increased level of H_2O_2 and thiobarbituric acid reactive substance (TBARS) (Asgher et al., 2021). Arsenate application at 100 µM concentration in a hydroponic system also caused elevation in

the H_2O_2 and TBARS content in maize seedlings (Arikan et al., 2022). Electrolyte leakage (69%) was caused in pea under As pollution (Alsahli et al., 2021). It also caused escalated generation of $O_2^{\cdot-}$ in annual and perennial ryegrass (Li et al., 2019). Similarly, the level of ROS was remarkably increased in soybean under As pollution due to which lipoxygenase performance increased (Chandrakar et al., 2016).

2.4.5 Antioxidant defense system

Arsenic exposure to peanut plants caused an increase in antioxidative enzymes activities (Bianucci et al., 2017). Activities of superoxide dismutase (SOD), peroxidase (POD) and glutathione reductase (GR) enzymes were increased while catalase (CAT) and ascorbate peroxidase (APX) activities were decreased in *Lemna gibba* under As contamination (Leão et al., 2013). Similarly in rice, activities of antioxidative enzymes were observed to be increased (Mishra et al., 2011). An upsurge in SOD and glutathione peroxidase (GPOX) performance in roots of castor tolerant genotypes was observed, while activities of these enzymes were decreased in castor sensitive genotypes in an As concentration-dependent manner. Sensitive castor plants exhibited augmented CAT functioning while unaffected in tolerant plants (Singh et al., 2021a). The consequence of As on the antioxidant defense system in various plants is presented in table 2.1.

Table 2.1 Effect of As on antioxidant defense system in various plant species

Plant	**As species**	**As concentration**	**Effect**		**References**
			Antioxidative enzyme	Effect	
Oryza sativa	----------	70 µM	CAT	Increased	Faizan et al. (2021)
			POD	Increased	
			SOD	Increased	
Oryza sativa	----------	50 µM	SOD	Decreased	Mridha et al. (2021)
			APX	Increased	
			CAT	Decreased	
			GR	Decreased	
Oryza sativa	Na_2HAsO_4	50 µM	SOD	Decreased	Dixit et al. (2015)
			APX	Increased	
			CAT	Increased	

Brassica juncea	Na_3AsO_4	5 and 25 µM	SOD	Increased	Khan et al. (2009)
			CAT	Increased	
			APX	Increased	
			GR	Increased	
Triticum aestivum	Na_2HAsO_4	25, 50, and 100 mg As kg^{-1} soil	SOD	Decreased	Sharma et al. (2017)
			CAT	Decreased	
			GPOX	Decreased	
			APX	Decreased	
Camellia sinensis	Na_2HAsO_4	25 µM	APX	Increased	Li et al. (2021)
			CAT	Increased	
			SOD	Increased	
			POD	Increased	
Brassica napus	$NaAsO_2$	50 and 200 µM	SOD	Increased	Farooq et al. (2016)
			APX	Increased	
			CAT	Increased	
			POD	Decreased	
Oryza sativa	$NaAsO_2$	50 µM	CAT	Increased	Ghorbani et al. (2022)
			SOD	Increased	
			APX	Increased	
			GR	Increased	
Oryza sativa	Na_2HAsO_4	0.5 and 1 mM	SOD	Escalated	Rahman et al. (2015)
			CAT	Escalated	
			GPX	Reduced	
			Dehydro ascorbate reductase (DHAR)	Reduced	
			Mono-dehydro ascorbate	Escalated	

			reductase (MDHAR)		
			GR	Escalated	
			GST	Decreased	
			APX	Escalated	
Pennisetum typhoides and *Pisum sativum*	Na_2HAsO_4	10, 25, 50, 100, 200µM	SOD	Increased	Iti (2013)
			APX	Decreased	
			CAT	Decreased	
Lactuca sativa	As III, As V, and DMA	50, 100, or 200 µg L−1	SOD	Decreased	Song et al. (2020)
			CAT	Increased	
			POD	No change	
			MDHAR	Decreased	
			DHAR	Decreased	
Pisum sativum	$NaAsO_2$	20 µM	SOD	Increased	Alsahli et al. (2021)
			CAT	Increased	
			GST	Increased	
			APX	Increased	
			GR	Increased	
			MDHAR	Decreased	
			DHAR	Decreased	
Solanum lycopersicum	$NaAsO_2$	53.6 µ mol L^{-1}	SOD	Increased	Singh and Upadhyay (2014)
			CAT	Increased	
			APX	Increased	
Artemisia annua	Na_2HAsO_4	45 mg As kg^{-1}	CAT	Increased	Naeem et al. (2020)
			POX	Increased	
			SOD	Increased	
			APX	Increased	

2.5 Silicon induced metal stress alleviation in plants

2.5.1 Uptake, translocation, and accumulation in plants

Silicon is present in almost all plants. Silicon content in plants may be highly similar to macro elements in some plants, termed Si-accumulator plants. In Si-accumulator plants, the highest Si accumulation takes place in leaves with greater than 1.5% of leaf dry weight (Hu et al., 2020). Plants uptake and transport Si as silicic acid (H_4SiO_4) (Feng et al., 2011; Puppe et al., 2023). Silicic acid is transported to cell walls and extracellular spaces by transpirational flow via its irreversible precipitation as amorphous silica. A plant's Si absorption and loading potential from roots to xylem determines the content of Si in various plant species (Ma et al., 2001b). The only form of silicon that plants can receive is the neutral monomeric molecule, H_4SiO_4 which is deposited as amorphous silica ($SiO_2.nH_2O$) in soils (Coskun et al., 2019; Epstein, 2001). Distribution of Si inside the plants is carried out by numerous influx and efflux Si transporters (Chaiwong et al., 2020; Ma et al., 2007; Ma & Yamaji, 2015). *Lsi1* (*Low silicon 1*) and *Lsi2* were the 1st influx and efflux Si-transporters respectively, which were firstly discovered and characterized in rice (Hassan et al., 2023). *Lsi1* is a Nodulin 26-like intrinsic proteins (NIP) protein, while *Lsi2* is a putative anion transporter and Si transfer from rhizosphere into rice roots occurs by the cooperative action of these two proteins (Ma et al., 2006).

Passage of Si in the cellular membrane carried out by *Lsi1* along the concentration gradient from apoplast to symplast as it is a passive transporter. Through an active mechanism paired with a proton anti-port, *Lsi2* facilitates the its passage in opposite manner. *Lsi1* transfers Si towards the living tissues of the endodermis at the bottom end, and then *Lsi2* extrudes Si to the stele at the proximal side (Fig. 2.3). Polar localization of these *Lsi1* and *Lsi2* allows the unidirectional Si uptake system in plants (Ma et al., 2006). For the absorption of Si in various parts, each plant has a unique set of transporters such as *OsLsi*, *AtLsi*, *ZmLsi*, etc. In rice, cucumber, and tomato, Si passage occurs in the following order: rhizosphere < root < cortical cells < xylem vessels (Mitani & Ma, 2005). Symplastic regions contain more Si concentration as compared to the soil region. Silicon uptake and transport differ in tomato, cucumber, and rice and it was found that rice has the highest Si concentration than tomato and cucumber (Ma & Yamaji, 2006, 2008). In these three species, a transporter mediates

radial transport of Si in the order of rice > cucumber > tomato as reported by Mitani and Ma (2005) and Ma and Yamaji (2006).

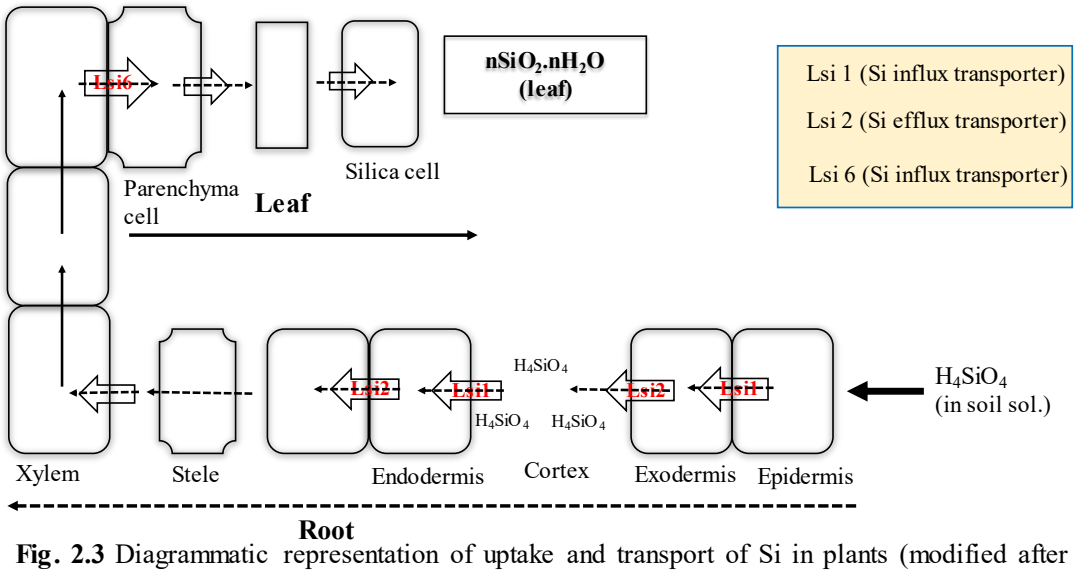

Fig. 2.3 Diagrammatic representation of uptake and transport of Si in plants (modified after Khan et al., 2019b).

Even at low temperature conditions, the energy-dependent Si absorption mechanism continues to function (Mitani & Ma, 2005). Silicon is transported by diffusion in cucumber and tomato, while in rice, it is mediated by transporter genes (Ma & Yamaji, 2006). Silicon accumulation at a high level in rice is due to the presence of Si transporter in its stem. Lack of or a malfunctioning transport that favors xylem loading may be the cause of the lower amount of Si accumulation in cucumbers and tomatoes (Ma et al., 2004; Ma & Yamaji, 2006). Silicon is delivered to the xylem from the cortical cells after being absorbed from the external media, through a process known as xylem loading and then transferred to shoot from roots. Additionally, silicic acid is transformed into silica gel ($SiO_2.nH_2O$) at concentrations higher than 2 mM. Silicon occurs as silica gel in rice and wheat as the concentration of Si is higher than 2 mM (Ma & Yamaji, 2006). After being transported from the root system, it gets accumulated in the hydrated polymeric kind in upper parts of the plant (Ma et al., 2006). Through transpiration, silicic acid becomes increasingly concentrated in aerial tissues, where it then polymerizes to create silica gel (Ma & Takahashi, 2002).

The stem of rice contained more than 90% silica gel. Transpiration rate determines the Si distribution and accumulation in shoots. Si forms a double-layered structure via its deposition in a cuticle layer below 0.1 mm (Ma & Takahashi, 2002). Rice leaf has two

types of silicified cells that are silica motor cells and silica cells. However, after gathering in particular cells, they take distinct shapes. For example, bulliform cells are silicified cells which are named according to their outer organization structure. Silica cells assemble into dumbbell-shaped structures in the vascular tissues (Ma & Yamaji, 2008).

2.5.2 Plant growth

Silicon supplementation augmented the root elongation and biomass in Cd stressed maize seedlings (Dresler et al., 2015). Plant growth parameters were enhanced by Si addition in Cd stressed rice (Huang et al., 2021). Silicon addition to maize plants under Zn contamination improved the plant biomass, which were cultivated hydroponically (Bokor et al., 2014a, 2014b). Seedling biomass was also upsurged in Si treated rice seedlings against As exposure and Zn content was diminished. Moreover, Si significantly suppressed the Zn accumulation in cotton and maize (Anwaar et al., 2015; Patrícia et al., 2008). In *Oryza sativa*, Cd absorption and translocation from root to stem were minimized by Si (Shi et al., 2005). Table 2.2 represents the effect of Si on growth parameters in different plants.

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

Sr No	Metal	Plant name	Si concentration	Effect	Reference
1.	Pb	*Gossypium hirsutum*	1 Mm	Exogenous supplementation of Si increased the plant growth attributes and biomass of cotton.	Bharwana et al. (2013)
2.	Pb	*Pleioblastus pygmaeus*	100 and 500 µM	Application of Si NPs increased the dry weight of plant shoot against Pb toxicity.	Emamverdian et al. (2020)

3.	Pb	*Coriandrum sativum*	1.5 mM	Application of Si NPs augmented the plant resistance to Pb toxicity by improving the length of roots, plant biomass, plant height and leaf area in combination with Pb resistant microbes.	Fatemi et al. (2020)
4.	Cd	*Oryza sativa*	10 µmol L^{-1}	Cadmium stress caused chlorosis and curling in newly emerged leaves. But Si treatment as foliar spray boosted the growth attributes of rice.	Tripathi et al. (2012)
5.	Cd	*Gossypium hirsutum*	1 mM	Supplementation of Si in cotton plants enhanced the various morphological parameters.	Farooq et al. (2013)
6.	Ni	*Zea mays*	2.5 mM	Increased biomass and root length was noticed in Ni stressed maize plants.	Fiala et al. (2021)

7.	Cd	*Triticum aestivum*	25, 50, and 100 mg kg^{-1}	Cadmium exposure to wheat with the addition of Si resulted in better plant and spike length and biomass.	Khan et al. (2020b)
8.	As	*Brassica juncea*	1.5 mM	Growth parameters related to root morphological characters were adversely affected by As stress. However, Si increased the lateral root formation, root length ratio, root mass ratio, root tissue density and root fineness.	Pandey et al. (2016)
9.	As	*Oryza sativa*	0.25, 1, 2, and 3 mM	Silicon improvised the growth parameters as well as biomass of rice in response to As stress.	Zia et al. (2017)
10	As	*Oryza sativa*	0.5 and 1.0 mM	Shoot length and biomass were found to be enhanced under As stress by the addition of Si in both tolerant and	Tripathi et al. (2013)

				sensitive varieties of rice.	
11	Al	*Zea mays*	2 mM	Seedling length, germination percentage and vigor index were augmented in Si treated plants.	Delavar et al. (2017)

2.5.3 Photosynthetic system

Silicon has been shown to have beneficial effects on photosynthetic activity and chlorophyll production under metal toxicity (Vieira-Filho & Monteiro, 2022). Exogenous application of Si in maize resulted in increased chlorophyll level in response to zinc toxicity which is attributed to Si-mediated stimulation of chlorophyll synthesis via enhancing the iron content in maize (Kaya et al., 2009). The content of photosynthetic pigments was found to be increased in Si treated plants of wheat (Hussain et al., 2015; Rizwan et al., 2012), and rice (Nwugo & Huerta, 2008) when exposed to Cd. In Si applied *Oryza sativa* under As (Sanglard et al., 2014), *Triticum aestivum* under chromium (Cr) (Tripathi et al., 2015), *Oryza sativa* under Al (Singh et al., 2011) and barley under Cr (Ali et al., 2013b) stress, chlorophyll content was improved.

Nwugo and Huerta (2008) and Feng et al. (2010) described that gas exchange parameters were enhanced in cotton, and rice by Si application in a hydroponics system when exposed to Cd. Gas exchange parameters were also enhanced in *Arachis hypogaea* and *Hordeum vulgare* under Al and Cr stress, respectively (Ali et al., 2013b; Shen et al., 2014), and also in *Gossypium hirsutum* under Pb contamination (Bharwana et al., 2013) by Si supplementation. Arsenic induced toxicity in *Oryza sativa* was alleviated by the application of Si by improving the photosynthetic rate and also decreasing the stomatal limitation (Hu et al., 2013). Arsenic stress in rice plants negatively affected the gas exchange characteristics and photosynthetic performance. However, Si addition significantly mitigated the As-induced noxiousness by improving the carbon fixation (Sanglard et al., 2014). In barley and wheat, photosynthetic activity

and chlorophyll fluorescence under Cr stress were improved by Si (Ali et al., 2013b; Tripathi et al., 2015). Table 2.3 represents the influence of Si on the photosynthetic system of various plant species.

Table 2.3 Effect of Si on the photosynthetic system of various plant species

Sr No	Metal	Plant name	Si concentration	Effect	Reference
1.	Cr	*Hordeum vulgare*	1 and 2 mM	Exogenous application of Si in barley significantly alleviated the Cr-induced toxicity by improving the photosynthetic parameters, such as SPAD value, gas exchange attributes and chlorophyll fluorescence efficiency (Fv/Fm).	Ali et al. (2013b)
2.	Cr	*Brassica juncea*	500 and 700 μM	Chlorophyll and carotenoid level and the net photosynthetic rate was observed to be upsurged in Si treated brassica plants exposed to Cr which ultimately resulted in improved photosynthetic activity.	Ashfaque et al. (2017)
3.	Zn	*Oryza sativa*	1.5 Mm	Photosynthetic parameters, i.e. chlorophyll content, and gas exchange attributes were found to be	Song et al. (2014)

				increased in Si applied rice.	
4.	Cd	*Gossypium hirsutum*	1 mM	Water use efficiency, stomatal conductance, photosynthetic rate and transpiration rate were enhanced by Si addition in cotton plants.	Farooq et al. (2013)
5.	Mn	*Cucumis sativus*	1 mM	Silicon improved the cucumber tolerance to Mn toxicity by augmenting the photosynthetic related parameters.	Feng et al. (2009)
6.	Cd	*Oryza sativa*	0.6 mM	Water use efficiency, carboxylation efficiency of Rubisco and light use efficiency were enhanced by Si in rice leaves under Cd stress.	Nwugo and Huerta (2011)
7.	Cd	*Zea mays*	5 mM	Silicon application remarkably alleviated the Cd-induced negative effects in maize by escalating the chlorophyll and carotenoid levels.	Malčovská et al. (2014)
8.	Ni	*Gossypium hirsutum*	1 mM	Silicon addition in cotton plants increased the content of Chlorophyll a and b, total chlorophyll and carotenoid and also	Khaliq et al. (2016)

				improved the gas exchange attributes under Ni stress.	
9.	Mn	*Oryza sativa*	1.5 mM	Pigments level and gas exchange attributes were significantly increased under Mn stress in Si treated rice.	Li et al. (2015)
10.	Cd	*Cucumis sativus*	1 mM	Silicon significantly augmented the total chlorophyll and carotenoid content and also improved the gas exchange parameters in cucumber leaves under Cd pollution.	Feng et al. (2010)
11.	Cd, Zn	*Oryza sativa*	42 $mg \cdot kg^{-1}$	Silicon enhanced the fluorescence parameters under stressed conditions.	Huang et al. (2018a)
12.	Cd	*Zea mays*	5 mM	The rate of net photosynthesis, the effective photochemical quantum yield of photosystem II (ΦPSII), chlorophyll and carotenoid content were found to be enhanced in Si supplemented maize plants.	Vaculík et al. (2015)
13.	Al	*Eucalyptus platyphylla*	2 mmol L^{-1}	Total chlorophyll, and gas exchange parameters	Lima et al. (2016)

				were improved in Si applied plants under Al toxicity.	

2.5.4 Oxidative stress

The application of Si was shown to be efficient in lowering metal-induced oxidative stress. The effect of Si on oxidative stress has been presented in table 2.4.

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

Sr No	**Metal**	**Plant name**	**Si concentration**	**Effect**	**Reference**
1.	Cd	*Gladiolus grandiflora*	-------	Silicon addition lowered the ROS production.	Zaheer et al. (2018)
2.	Cd	*Ocimum basilicum*	1 and 2 mM	Levels of MDA and extent of electrolyte leakage were elevated by Cd but the application of Si reduced their level to reduce Cd-induced phytotoxicity.	Gheshlaghpour et al. (2021)
3.	As	*Triticum aestivum*	6 and 12 mM	Application of Si under As stress reduced the level of ROS burst.	Maghsoudi et al. (2020)
4.	As	*Zea mays*	50, and 100 mg kg^{-1}	The level of MDA and H_2O_2 was increased by As however	Kashif et al. (2021)

				exogenous application of Si reduced their level.	
5.	As	*Brassica juncea*	1.5 mM	Silicon diminished the level of MDA and H_2O_2 under As stress.	Praveen et al. (2020)
6.	Al	*Borago officinalis*	0.5, 1, 1.5 and 2 mM	MDA content declined in Si supplemented plants under stressed conditions.	Gagoonani et al. (2011)
7.	Cd	*Gossypium hirsutum*	1 mM	A decline in MDA and H_2O_2 levels was observed by the addition of Si.	Farooq et al. (2013)

2.5.5 Metal(loids) uptake

One of the straightforward mechanisms of silicon's beneficial appearance by its treatment is the immobilization of noxious metal in the soil (Mousavi 2022). Supplementation with high amounts of Si (sodium metasilicate) triggers soil pH to high level via the production of silicate compounds which ultimately diminish heavy metal bioavailability (Rizwan et al., 2012). Silicon supplementation decreased the absorption of Pb in banana, when sown in Pb polluted soil and this reduced absorption of Pb was related to considerably escalated soil pH and declined a fraction of exchangeable Pb (Li et al., 2012). Silicon also reduced the heavy metal detoxification by modifying the form of metals in soil solution by converting toxic metals to nontoxic forms via making silicate molecules (Putwattana et al., 2010). Silicon significantly lessened the exchangeable Cr level by escalating the Cr precipitation which is bound with organic matter (Ding et al., 2013). Silicon decreased Pb metal mobility in soil by converting Pb into non-toxic insoluble Pb-silicate form (Shim et al., 2014) and reduced Cr availability

in soil by declining the proportion of commutable Cr (Zhang et al., 2013). Table 2.5 represents the influence of Si on metal(loids) uptake in various plant plants.

Table 2.5 Effect of Si on metal(loids) uptake in different plant species

Sr. NO	**Metal**	**Plant species**	**Effect**	**Reference**
1.	Cd	*Oryza sativa*	Cadmium content was noticed to be diminished in rice.	Tripathi et al. (2012)
2.	Cr	*Oryza sativa*	Chromium content was noticed to be reduced in Si applied rice plants.	Ashfaque et al. (2017)
3.	Cr	Barley	Silicon addition markedly decreased the Cr level in barley.	Ali et al. (2013b)
4.	Cd	*Zea mays*	Cadmium content was found to be declined in Si applied plants while in the shoot tissues, no significant influence on Cd content was observed.	Dresler et al. (2015)
5.	Pb	Cotton	Silicon addition lowered the Pb level in various parts of cotton plants.	Bharwana et al. (2013)
6.	Cd	*Solanum nigrum*	Cadmium concentration was decreased in Si supplemented plants.	Liu et al. (2013)
7.	Fe	*Oryza sativa*	Content of Fe was diminished in Si applied plants under 250 mg L^{-1} Fe stress.	Chalmardi et al. (2014)
8.	As	*Brassica juncea*	Silicon supplementation in Brassica plants increased the concentration of As.	Pandey et al. (2016)

9.	Sb	Maize	At higher doses of Sb, Si enhanced the Sb accumulation in maize plants.	Vaculíková et al. (2014)
10.	Zn	*Oryza sativa*	Silicon application increased the content of Zn in vacuoles of rice plants.	Song et al. (2011)
11.	Ni	Cotton	The application of Si suppressed the Ni uptake in cotton plants.	Khaliq et al. (2016)
12.	Cu	Flax	Silicon treatment decreased the Cu accumulation in roots and shoot.	El-Beltagi et al. (2020)
13.	Cu	*Nicotiana tabacum*	Silicon application caused a two-fold decrease in Cu concentration in the roots.	Flora et al. (2019)
14.	Cd	*Triticum aestivum*	Individual together with combined supplementation of Si and NO significantly lowered the Cd content.	Singh et al. (2020)
15.	Cd	*Zea mays*	Uptake and accumulation of Cd was decreased via treatment with Si and NO in individual and combined form.	Liu et al. (2020)

2.5.6 Carbohydrates and protein content

Table 2.6 represents the influence of Si supplementation on total carbohydrates and protein content in various plant species.

Table 2.6 Effect of Si on total carbohydrates and protein content in different plant species

Sr No	Metal name	Plant species	Effect	Reference
1.	As	Wheat	Content of soluble sugars and proteins were noticed to be up-regulated under As stress.	Maghsoudi et al. (2020)
2.	As/ Cd	*Isatis cappadocica*	Content of total soluble protein was enhanced by Si addition exposed to As and Cd toxicity.	Azam et al. (2021)
3.	Cd	*Gladiolus grandiflora*	Improved production of protein was detected in Si-applied plants under stressed environments.	Zaheer et al. (2018)
4.	Pb	*Pleioblastus pygmaeus*	Silicon efficiently augmented the protein amount under stressed conditions.	Emamverdian et al. (2020)
5.	As	*Triticum aestivum*	Silicon addition to wheat improved the content of reducing and non-reducing sugars.	Sil et al. (2019)
6.	Cd	*Zea mays*	Total soluble protein content was increased in Si treated plants.	Singh et al. (2019)
7.	Cd	*Triticum aestivum*	Individual and combined treatment with Si and NO up-regulated the total	Singh et al. (2020)

			soluble protein amount in wheat seedlings.	

2.5.7 Osmolytes

Plants' osmotic potential is diminished by various environmental stresses which in turn declines cell turgor to inhibit plants' growth. Plants synthesize various osmolytes to cope with these stresses. Due to their hydrophilic nature, osmolytes readily stabilize various cell components under adverse circumstances without changing their composition or functionality. Osmotic stress is declined by proline via removing the $OH^{\cdot}$ ions. Osmotic adjustment and leaf turgor were increased in Si applied plants through osmolyte accumulation (Kafi et al., 2021). Table 2.7 represents the influence of Si supplementation on osmolytes in different plant species.

Table 2.7 Effect of Si on osmolytes in different plant species

Sr No	**Metal**	**Plant name**	**Si concentration**	**Effect**	**Reference**
1.	Cd	*Zea mays*	50, 100, and 150 mM	Proline content was increased by Si under stressed environments.	Mohsenzadeh et al. (2012)
2.	Cd and Zn	*Cajanus cajan*	300 mg kg^{-1}	The addition of Si enhanced the proline level under stressed conditions.	Garg and Singh (2018)
3.	Pb	*Coriandrum sativum*	1.5 and 3 mM	The combined treatment with Si NPs and biological treatments significantly decreased the proline content under Pb stress.	Fatemi and Esmaielpour (2020)

4.	As	*Cajanus cajan*	300 mg kg^{-1}	Application of Si effectively increased the proline level under stressed environments.	Bhalla and Garg (2021)
5.	As	*Oryza sativa*	5 mM	Silicon application decreased the proline content in the shoot at 7 days growth stage but in the root, its content was found to be increased under As stress.	Khan and Gupta (2018)
6.	Cd	*Triticum aestivum*	2, 4 and 6 mM	Proline level was up-regulated in Si treated plants under stressed circumstances.	Alzahrani et al. (2018)
7.	Cd	*Pisum sativum*	2 mM	Silicon increased the compatible solutes level under Cd toxicity.	Jan et al. (2018)
8.	Al	*Borago officinalis*	0.5, 1, 1.5 and 2 mM	Proline amount was augmented in Si treated plants under stressed conditions.	Gagoonani et al. (2011)

2.5.8 Antioxidative enzymes and non-enzymatic antioxidants

Functioning of SOD, CAT, GPOX and APX enzymes was stimulated by the application of Si in wheat (Naeem et al., 2018). Additionally, when exposed to organoarsenic stress,

Si variably induced SOD, POD and CAT enzymes in three distinct rice plants (Geng et al., 2018). Several reports have revealed that Si mediated mechanism of the antioxidant defense system varies within and between plant species. Silicon nanoparticles (NPs) treatment under Cd stress in wheat exhibited an increase in the functioning of CAT, POD and SOD enzymes (Hussain et al., 2019). A decrease in APX, POD, CAT and SOD levels was reported in the leaves of *Solanum nigrum* (Liu et al., 2013) and decline in SOD and POD levels in sorghum plants (Masarovič et al., 2012) under Cd and Zn stress, respectively via Si addition. Similarly, APX, CAT and GPOX enzyme activities were declined with the addition of Si in Sb exposed maize roots (Vaculíková et al., 2014).

Content of ascorbate and glutathione was reported to be declined by the application of Si against As stress in rice, resulting in inhibited functioning of APX, GR and GPX enzymes (Das et al., 2018). The CAT functioning was observed to be up-regulated in cucumber plants by Si under Cd toxicity in Negin plants (Khodarahmi et al., 2012). Cadmium stress increased the POD and CAT contents, while Si application decreased their levels in rice (Wang et al., 2014b). Further, CAT, SOD and POD contents were decreased against Al and Zn contamination in the roots of peanut and maize (Bokor et al., 2014a; Shen et al., 2014). Activity of POD was found to be decreased in Mn stressed cucumber plants (Dragišić Maksimović et al., 2007). The impact of Si application on the plant's antioxidant defense system is depicted in table 2.8.

Table 2.8 Effect of Si application on plant's antioxidant defense system in different plant species

Sr No	**Metal**	**Plant name**	**Si concentration**	**Effect**	**Reference**
1.	Al	*Arachis hypogaea*	80 mg L^{-1}	Silicon application significantly augmented the SOD level while lessened the CAT and POD	Shen et al. (2014)

				enzyme levels under Al stress.	
2.	Al	*Zea mays*	4 mg kg^{-1}	Co application of Si NPs with Al upsurged the SOD, APX, POD, CAT and GPX functioning and tocopherol, ascorbic acid and glutathione levels were also enhanced.	De Sousa et al. (2019)
3.	Cd	*Brassica chinensis*	1.5 mM	Silicon addition in pakchoi enhanced the SOD, CAT and APX activities.	Song et al. (2009)
4.	Cd	*Pisum sativum*	2 mM	Activities of SOD, CAT, GST, APX, GR, DHAR, MDHAR and ascorbic acid and glutathione level were improved in Si treated pea seedlings.	Jan et al. (2018)
5.	Boron	*Pisum sativum*	2 mmol L^{-1}	Functioning of SOD, CAT, APX, GPOX and GR enzymes were significantly amplified by Si in combination with B stress.	Oliveira et al. (2020)
6.	Al	*Lolium perenne*	0.5, and 2.0 mM	Activity of SOD enzyme was	Pontigo et al. (2017)

				significantly reduced, while CAT, APX and POD contents were up-regulated when Al and Si were simultaneously applied.	
7.	Ni	*Brassica juncea*	10^{-5} M	Escalation in SOD, CAT, GST, APX, GR, DHAR and MDHAR enzymes functioning and ascorbic acid and glutathione contents were noticed by the addition of Si against Ni toxicity.	Abd_Allah et al. (2019)
8.	Ni	*Glycyrrhiza glabra*	0.5, and 1.50 mM	Levels of GPOX and POD enzymes were amplified while SOD activity was reduced by the application of Si.	Yazdani et al. (2021)
9.	Ni	*Oryza sativa*	0.50 mM	Exogenous application of Si markedly escalated the functioning of SOD, CAT, APX, DHAR, GR, GST and GPX enzymes.	Hasanuzzaman et al. (2019)
10.	Al	*Fagopyrum esculentum*	0.5 and 1 mM	Si improved the Al-induced toxicity in	Dar et al. (2022)

				buckwheat by increasing the polyphenol oxidase (PPO) enzyme activity.	
11.	Cd	*Brassica napus*	1.0 mM	Silicon treatment to rapeseed plants under Cd stress resulted in increased stimulation of CAT, APX, MDHAR, DHAR and GR enzymes.	Hasanuzzaman et al. (2017)
12.	Al	*Phoenix dactylifera*	1.0 mM	Enzymes of antioxidative system i.e. APX, CAT, PPO and POD were stimulated by Si.	Bilal et al. (2022)

2.5.9 Gene expression

The Si-mediated alteration in gene expression is the key mechanism for lessening the metal toxicity. The influence of Si application on gene expression in different plant species is presented in table 2.9.

Table 2.9 Effect of Si application on gene expression in different plant species

Sr No	**Metal**	**Plant species**	**Effect**	**Reference**
1.	Cd/Cu	*Oryza sativa*	*OsLsi1* and *OsLsi2* genes were stimulated in Si treated plants under stressed environments.	Kim et al. (2014)
2.	Zn	*Oryza sativa*	Silicon addition in response to Zn toxicity upregulated	Song et al. (2014)

			the transcript levels of photosynthesis-associated genes.	
3.	Cu	*Arabidopsis thaliana*	*PCS1* and metallothionein gene expressions were stimulated and inhibited respectively, in Si-applied plants under stressed conditions.	Khandekar and Leisner (2011)
4.	Cd	*Oryza sativa*	Exogenous application of Si exhibited over-expression of *OsLsi1* aquaporin under Cd stress.	Ma et al. (2015)
5.	Cd	*Triticum aestivum*	The expression of spermine (Spm) and spermidine (Spd) biosynthetic genes i.e., *SPMS* and *SPDS* were upregulated in Si treated plants under Cd toxicity.	Howladar et al. (2018)
6.	Cu	*Nicotiana tabacum*	Silicon treatment augmented the *PCS1* and ethylene biosynthetic genes such as *SAMS2, ACS* and *ACO* under Cu stress.	Flora et al. (2019)
7.	Mn	*Oryza sativa*	Photosynthesis-related genes such as *HemD, Lhcb3* and *PsbP* were reported to increase in Si-applied plants under Mn stress.	Li et al. (2015)
8.	Cu	*Salvia officinalis*	Silicon and NO supplementation increased	Pirooz et al. (2022)

			the expression of *PAL, TAT* and *RAS* genes in *Salvia officinalis* against stressed environments.	

2.6 Nitric oxide mediated alleviation of heavy metal stress in plants

2.6.1 Plant growth

Exogenous application of NO improved germination, length and biomass of seedlings under As stress in rice seedlings (Praveen & Gupta, 2018). Decrease in Ni accumulation, and an increase in seedlings length, biomass and mineral concentration were observed in NO-supplemented finger millet seedlings to ameliorate Ni-induced toxicity (Kotapati et al., 2017). Wheat's plant growth was improved by NO application via reducing the Zn content (Duan et al., 2015). Root and shoot length and biomass were augmented by the application of NO in ryegrass seedlings (Chen et al., 2018). Table 2.10 represents the influence of NO supplementation on growth parameters in different plant species.

Table 2.10 Effect of NO application on growth parameters in different plant species

Sr No	Metal	Plant name	NO concentration	Effect	Reference
1.	Cd	*Oryza sativa*	30 µM	Nitric oxide application in response to Cd toxicity increased the seed germination index, vigor index, root and stem lengths and fresh weight.	He et al. (2014)
2.	Cd	*Brassica juncea*	100 µM	Leaf area and plant dry mass were found to be	Per et al. (2017)

				enhanced in NO pretreated mustard plants.	
3.	Cd	*Oryza sativa*	50 µM	SNP upsurged the length and weight of rice plants.	Singh and Shah (2014)
4.	As	*Phaseolus vulgaris*	100 µM	The length and dry weight of *Phaseolus vulgaris* were increased by the addition of SNP under As stress.	Talukdar (2013a)
5.	As	*Vigna radiata*	75 µM	SNP addition augmented the seed germination and biomass under As contamination.	Ismail (2012)
6.	Al	*Citrus grandis*	10 µM	Application of SNP significantly alleviated the Al-induced toxicity by refining the growth attributes.	Yang et al. (2012)
7.	As	*Vicia faba*	100 µM	Plant length, and biomass of *Vicia faba* plants were observed to be increased in NO applied plants.	Mohamed et al. (2016)
8.	As	*Oryza sativa*	100 µM	Supplementation with NO improved	Singh et al. (2016)

				the plant length, biomass and root hairs growth against stressed environments.	
9.	Cd	*Oryza sativa*	30 μM	Nitric oxide improved the plant growth under Cd stress.	Yang et al. (2016)
10.	Ni	*Brassica napus*	0.2 mM	The dry weight of roots and shoots was increased and Ni-induced noxious effects such as chlorosis and necrosis were also diminished in NO applied leaves.	Kazemi et al. (2010)

2.6.2 Photosynthetic system

Chromium toxicity was mitigated in tall fescue plants by the application of NO through improving the plant metabolism and photosynthetic attributes (Huang et al., 2018b). Photosystem II photochemistry was maintained by the addition of calcium or sulfur by retaining endogenous NO levels to alleviate metal toxicity. Moreover, photosynthetic activity was improved after exogenous supplementation of NO in combination with calcium and sulfur in response to Cr toxicity (Singh & Prasad, 2019). Photosynthetic activity was significantly regulated by NO by enhancing Fv/Fm and photochemical quenching under Zn stress in wheat (Tripathi et al., 2017). Further, 100 μM SNP application caused the increase in the PS II efficiency through ROS scavenging and by stimulating antioxidant machinery against Cd stress (Per et al., 2017). Chloroplast injury, respiration rates and non-photochemical quenching were recovered upon NO treatment against Cd. Moreover, NO also up-regulated the functioning of Rubisco

activase and Rubisco content against metal stress conditions (Khairy et al., 2016, Per et al., 2017). Table 2.11 represents the effect of NO application on the photosynthetic system of different plant species.

Table 2.11 Effect of NO application on the photosynthetic system in different plant species

Sr No	**Metal**	**Plant name**	**NO concentration**	**Effect**	**Reference**
1.	Cd	*Brassica napus*	200 μg ml^{-1}	Photosynthetic parameters i.e., chl a, chl b, total chlorophyll, chl a/b ratio and Hill reaction activity of chloroplasts were observed to be enhanced in *Brassica napus* by the application of SNP.	Jhanji et al. (2012)
2.	Cd	*Helianthus annuus*	100 μM	Cadmium-induced chlorophyll decay was markedly reduced by SNP in response to stressed conditions.	Laspina et al. (2005)
3.	Pb	*Lolium perenne*	50, 100, and 200 μM	Exogenous application in the form of SNP augmented the net photosynthetic rate, transpiration rate, chlorophyll and carotenoid levels.	Bai et al. (2015)

4.	Cu	*Lolium perenne*	50, 100, 200 µM	Exogenous application of NO intensified the chlorophyll content and gas exchange parameters under Cu toxicity.	Dong et al. (2014)
5.	Cd	*Lolium perenne*	0.1 mM	Nitric oxide application significantly ameliorated the Cd-induced toxicity by improving the pigments content and also increased the gas exchange attributes.	Wang et al. (2013a)
6.	Cd	*Arachis hypogaea*	0.25 mM	Chlorophyll content and photosynthetic rate were increased in NO applied plants.	Xu et al. (2015a)
7.	Cd	*Arachis hypogaea*	250 µM	Content of pigments as well as photosynthetic rate, and transpiration rate were found to be enhanced under Cd stress with the application of SNP.	Dong et al. (2016)
8.	Cu	*Brassica juncea*	25, 50, 100, 200, and 250 µM	NO increased the PSII photochemical	Rather et al. (2020)

				efficiency and Rubisco activity.	
9.	Pb and Cd	*Capsicum annuum*	0.1 Mm	Pigments content, and Fv/Fm were augmented by the application of NO under stressed environments.	Kaya et al. (2019)
10	Cd	*Oryza sativa*	200 µM	Supplementation of SNP improved the pigments content under Cd stress.	Mostofa et al. (2019)
11	As	*Vicia faba*	100 µM	Application of NO increased the chl a, chl b and carotenoid contents under As toxicity.	Mohamed et al. (2016)
12	Cu	*Hordeum vulgare*	500 µM	Chlorophyll a, chl b, total carotenoid and Fv/Fm ratio were increased in NO treated plants against Cu toxicity.	Ben Massoud et al. (2022)
13	Cd	*Satureja hortensis*	50, 100 and 200 µM	Exogenous application of NO effectively upsurged the photosynthetic pigment contents against stressed environments.	Azizi et al. (2021)

14	Mn	*Phaseolus vulgaris*	100, 200, 300, 400, 500, 600, and 700 µM	Nitric oxide application alleviated the Mn-induced toxic effects by improving the total chlorophyll and carotenoid levels in bean plants.	Mahjoubi et al. (2022)
15	Cu	*Panicum miliaceum*	150 µM	Photosynthetic pigment contents and photosynthetic rate were noticed to be improved by NO under Cu stress.	Saman and Sepehri (2021)

2.6.3 Oxidative stress

Nitric oxide has been recognized to alleviate metal-induced toxicity by suppressing the level of oxidative stress markers (Singh et al., 2022). Level of H_2O_2 and MDA were elevated in lettuce by their exposure to As, however, the application of NO diminished the As-triggered ROS burst by decreasing their level (Silveira et al., 2015). Table 2.12 represents the effect of NO application on oxidative stress in different plant species.

Table 2.12 Effect of NO application on oxidative stress in different plant species

Sr No	**Metal**	**Plant name**	**NO concentration**	**Effect**	**Reference**
1.	Zn	*Triticum aestivum*	10 µM	The level of ROS and MDA was reduced by NO application under Zn stress.	Chen et al. (2015)
2.	Zn	*Plantago major*	100 and 200 µM	Application of NO declined the H_2O_2 level and lipid	Nasiri-Savadkoohi et al. (2017)

				peroxidation under Zn stress.	
3.	Zn	*Triticum aestivum*	100 μM	Zinc stress caused an upsurge in the H_2O_2 and lipid peroxidation level but the application of NO decreased their level under Zn pollution.	Tripathi et al. (2017)
4.	Cu	*Triticum aestivum*	100 μM	Supplementation with NO in wheat plants under Cu stress reduced the level of MDA and H_2O_2.	Hu et al. (2007)
5.	Cu	*Hordeum Vulgare*	200 μM	Level of MDA, H_2O_2, and $O_2^{\cdot -}$ were found to be reduced in NO-supplemented plants in response to stressed conditions.	Hu et al. (2015)
6.	Cu	*Solanum lycopersicum*	100 μM	MDA and H_2O_2, were noticed to be decreased in NO-treated plants under Cu toxicity.	Wang et al. (2010a)
7.	As	*Oryza sativa*	100 μM	A reduction in MDA and H_2O_2 contents was observed when	Praveen et al. (2018)

				supplemented with NO.	
8.	As	*Phaseolus Vulgaris*	100 μM	The application of NO lowered the level of MDA and H_2O_2.	Talukdar (2013a)
9.	Cd	*Oryza sativa*	100 μM	The levels of MDA and H_2O_2 were reported to be reduced by NO addition to improve plant tolerance.	Hsu and Kao (2004)
10.	Pb	*Lolium Perenne*	50, 100, 200, and 400 μM	Levels of MDA, H_2O_2, and $O_2^{\cdot-}$ were diminished in NO-treated plants against Pb toxicity.	Bai et al. (2015)

2.6.4 Metal(loids) uptake

Table 2.13 represents the effect of NO application on metal(loids) uptake in different plant species.

Table 2.13 Effect of NO application on metal(loids) uptake in different plant species

Sr. NO	**Metal**	**Plant species**	**Effect**	**Reference**
1.	Cd	*Oryza sativa*	Application of SNP inhibited the Cd uptake.	Singh and Shah (2014)
2.	Cd	*Oryza sativa*	A reduction in Cd content was observed in rice.	Xiong et al. (2009)
3.	Cd	*Medicago truncatula*	Nitric oxide application decreased Cd uptake in Cd-	Xu et al. (2010)

			stressed *M. truncatula* seedlings.	
4.	As	*Oryza sativa*	Arsenic content was diminished in NO treated rice plants.	Praveen and Gupta (2018)
5.	As	*Oryza sativa*	Nitric oxide addition declined the As content in rice.	Kushwaha et al. (2019)
6.	Pb	*Triticum aestivum*	Arsenic content was not detected in NO applied wheat plants.	Kaur et al. (2015)
7.	Ni	*Brassica napus*	Nickel content was observed to be increased in the roots while decreased in the shoots by NO application.	Kazemi et al. (2010)
8.	Ni	*Eleusine coracana*	Nitric oxide application decreased Ni level in *E. coracana* plants.	Kotapati et al. (2017)
9.	Ni	*Oryza sativa*	Nickel content was diminished in rice by the application of NO.	Rizwan et al. (2018)
10.	Cd	*Solanum lycopersicum*	Content of Cd was reduced in all parts of tomato when applied with NO against Cd toxicity.	Ahmad et al. (2018)
11.	Cd	*Vigna radiata*	Content of Cd was lowered in the roots and shoot by NO addition.	Nahar et al. (2016)
12.	Cd	*Trifolium repens*	Nitric oxide application decreased the content of Cd.	Liu et al. (2015)
13.	Cd	*Triticum aestivum*	Content of Cd was declined in wheat by NO.	Basalah et al. (2013)

14.	Cd	*Lycopersicon esculentum*	Cadmium content was not affected in NO-treated plants against Cd contamination.	Wang et al. (2016)
15.	Cu	*Lycopersicon esculentum*	A decline in the content of Cu was noticed in the roots and shoot of NO-supplemented plants.	Wang et al. (2016)
16.	Cu	*Oryza sativa*	Nitric oxide decreased the content of Cu in rice.	Mostofa et al. (2014)
17.	Cu	*Triticum aestivum*	Copper uptake was unaffected in the seeds of wheat.	Hu et al. (2007)

2.6.5 Carbohydrates and protein content

Table 2.14 represents the effect of NO application on total carbohydrates and protein content in different plant species.

Table 2.14 Effect of NO application on total carbohydrates and protein content in different plant species

Sr No	Metal name	Plant species	Effect	Reference
1.	Cu	*Portulaca oleracea*	Nitric oxide increased the soluble carbohydrate content in *Portulaca oleracea* under Cu stress.	Fendereski et al. (2015)
2.	Cd	*Satureja Hortensis*	Carbohydrate content was upsurged in NO supplemented plants.	Azizi et al. (2021)
3.	Cd	*Hordeum vulgare*	Carbohydrate and protein metabolism were significantly regulated by NO application to enhance barley tolerance to Cd.	Alp et al. (2022)

4.	Cr	*Solanum lycopersicum*	The content of total soluble carbohydrates was increased in NO-treated seedlings under Cr stress.	Alamri et al. (2020)
5.	Cd	*Triticum aestivum*	Individual and combined treatment of NO with Si increased the total soluble protein content in wheat seedlings.	Singh et al. (2020)
6.	Cu	*Vigna radiata*	Application of NO along with Si improved the content of total soluble protein under Cu stress.	Gaur et al. (2021)
7.	Cd	*Nostoc muscorum* and *Anabaena* sp.	Application of NO in combination with H_2O_2 improved the carbohydrate content under Cd stress.	Verma and Prasad (2021)

2.6.6 Osmolytes

Nitric oxide application increased the proline content to improve the proline assimilation in tomato seedlings against Cr toxicity (Khan et al., 2021). Proline level was also increased in NO-treated peanut in response to Cd contamination (Dong et al., 2016). SNP addition upsurged the proline accumulation in NO pretreated algal cells under Cu stress indicating the positive interaction between endogenous NO and proline level (Zhang et al., 2008b). Table 2.15 represents the effect of NO treatment on osmolytes in different plant species.

Table 2.15 Effect of NO application on osmolytes in different plant species

Sr No	**Metal**	**Plant name**	**NO concentration**	**Effect**	**Reference**
1.	Cd	*Solanum Lycopersicum*	100 µM	An enhancement in proline and glycine	Ahmad et al. (2018)

				betaine content was recorded by NO supplementation under stressed conditions.	
2.	Cd	*Medicago Truncatula*	100 µM	Proline level was enhanced by NO application under Cd stress.	Xu et al. (2010)
3.	Pb	*Sesamum Indicum*	200 µM	Application of NO increased the proline level against Pb stress.	Amooaghaie and Enteshari (2017)
4.	Ni	*Lupinus Termis*	0.4 and 0.6 mM	Proline content was found to be enhanced by NO supplementation.	Hashem et al. (2018)
5.	As	*Vicia faba*	100 and 200 µM	Proline and glycine betaine contents were increased by NO treatment against As toxicity.	Ahmad et al. (2020b)
6.	Ni	*Solanum melongena*	100 and 150 µM	Osmo-protectants amount was boosted by NO addition.	Soliman et al. (2019)
7.	As	*Isatis cappadocica*	200 µM	Nitric oxide application improved the	Souri et al. (2020)

				proline content against As stress.	
8.	Pb and Cd	*Arundinaria pygmaea*	200 µM	Contents of osmolytes were significantly amplified by SNP under Pb and Cd stress.	Emamverdian et al. (2021)
9.	Cd and Pb	*Capsicum annuum*	0.1 mM	Proline content was decreased in NO-applied plants against individual stresses.	Kaya et al. (2019)

2.6.7 Antioxidant defense system

Functioning of different antioxidative enzymes were stimulated in roots upon NO application in bean seedlings (Talukdar, 2013a). Addition of SNP under As stress stimulated the functioning of CAT, POX and APX enzymes in lettuce (Farnese et al., 2013). Antioxidative enzymes were also stimulated in SNP-treated *Triticum aestivum* against Ni stress (Wang et al., 2010b). Further, CAT activity was lowered while GR and POX activities were improved in NO applied plants (Panda et al., 2011). Nitric oxide application mitigated the Pb-induced noxiousness by stimulating the SOD, CAT, APX, and POD enzymes in *Triticum aestivum* plants (Kaur et al., 2015). Similarly, antioxidant enzymes functioning was increased in NO-treated cowpea to alleviate Pb toxicity (Sadeghipour, 2016).

Farnese et al. (2013) described the defensive character of NO against As stress as NO stimulates the accumulation of phytochelatins which are involved in As detoxification. Glutathione content was found to be increased by the application of NO (Hasanuzzaman & Fujita, 2013). Detoxification of methylglyoxal is carried out by glutathione homeostasis (Mustafiz et al., 2010). Sodium nitroprusside (SNP) treatment in *Triticum aestivum* seedlings under As stress resulted in increased performance of glyoxalase enzymes in order to detoxify methylglyoxal (Hasanuzzaman & Fujita,

2013). Nitric oxide also amended the levels of Asc and glutathione under metal stresses (Ahmad et al., 2018; Hsu & Kao, 2004; Panda et al., 2011). Thiol and glutathione metabolism was regulated by the exogenous application of NO to ameliorate As stress and moreover, As content was reduced in rice (Singh et al., 2016). Further, it was observed that As accumulation might by decreased and the glutathione cycle was modulated by NO to counterbalance As stress. Glutathione and ascorbic acid levels in periwinkle roots were also amplified by SNP under Cu stress (Liu et al., 2016). Table 2.16 represents the effect of NO application on the antioxidant defense system in different plant species.

Table 2.16 Effect of NO application on antioxidant defense system in different plant species

Sr No	**Metal**	**Plant name**	**NO concentration**	**Effect**	**Reference**
1.	Cu	*Solanum lycopersicum*	100 μM	Application of NO elevated antioxidative enzymes activities against Cu toxicity.	Cui et al. (2009)
2.	Cu	*Panax ginseng*	50 μM	Activities of SOD, CAT, POD, APX and GR enzymes and ascorbic acid content were reported to be enhanced under Cu toxicity in NO treated plants.	Tewari et al. (2008)
3.	Cu	*Solanum lycopersicum*	100 μM	Nitric oxide addition to tomato increased the antioxidative enzymes	Zhang et al. (2009b)

				activities under Cu pollution.	
4.	As	*Oryza sativa*	50 μM	Activities of antioxidative enzymes were noticed to be boosted in NO applied plants.	Singh et al. (2009)
5.	Ni	*Brassica Napus*	0.2 mM	Functioning of different antioxidative enzymes were noticed to be enhanced by NO application under Ni stress.	Kazemi et al. (2010)
6.	As	*Brassica Juncea*	100 μM	Functioning of different antioxidative enzymes were reduced by NO addition against As toxicity.	Praveen et al. (2019)
7.	Ni	*Oryza sativa*	100 and 200 μM	Activities of antioxidative enzymes, and ascorbic acid and glutathione were improved under Ni stress by the addition of NO.	Rizwan et al. (2018)

8.	As	*Triticum Aestivum*	0.25 mM	Nitric oxide boosted the performance of APX, GSH, GR enzymes and ascorbic acid.	Hasanuzzaman and Fujita (2013)
9.	Pb	*Arabidopsis Thaliana*	0.5 mM	Functioning of antioxidative enzymes were noticed to be down-regulated by NO supplementation under Pb stress.	Phang et al. (2011)
10.	Pb	*Triticum Aestivum*	100 μM	The functioning of antioxidative enzymes was reduced against Pb by NO.	Kaur et al. (2015)
11.	Pb	*Sesamum Indicum*	200 μM	In NO-treated plants under Pb stress, the functioning of POD, APX, SOD and CAT enzymes were accelerated.	Amooaghaie and Enteshari (2017)
12.	Pb	*Melissa Officinalis*	100, and 200 μM	Functioning of APX, CAT and POD enzymes were increased in NO-applied plants against Pb stress.	Jafarnezhad-Moziraji et al. (2017)
13.	Pb	*Lupinus luteus*	10 μM	Activity of SOD enzyme was boosted	Kopyra and

				by the application of NO in response to Pb toxicity.	Gwóźdź (2003)
14.	Cd	*Cucumis sativus*	100 μM	Functioning of CAT, GPX, SOD, APX, POD and GST enzymes were upregulated in NO treated plants.	Gong et al. (2017)
15.	Cd	*Oryza sativa*	30 μM	SOD, GR, APX activities and glutathione content were augmented in NO applied plants.	Yang et al. (2016)
16.	Cd	*Typha Angustifolia*	100 μM	Activities of SOD and CAT enzymes were reduced in NO-supplemented plants.	Zhao et al. (2016)
17.	Cd	*Lolium perenne*	50, 100, 200, and 400 μM	In NO-treated plants against Cd stress, the functioning of antioxidative enzymes were enhanced.	Wang et al. (2013a)

2.6.8 Gene expression

Table 2.17 represents the effect of NO application on gene expression in different plant species.

Table 2.17 Effect of NO application on gene expression in different plant species

Sr No	Metal stress	Plant species	Effect	Reference
1.	Ni	*Oryza sativa*	Expression of antioxidative genes were up-regulated by NO addition under Ni stress	Rizwan et al. (2018)
2.	As	*Oryza sativa*	Expression of PIN genes were up-regulated by the application of NO.	Praveen and Gupta (2018)
3.	As	*Oryza sativa*	Expression of IAA metabolism gene i.e., *OsPIN9*, IAA synthesis and transport gene i.e., *OsSAUR39* and CTK biosynthesis gene (*Isopentenyl transferase*: *IPT7*) were upregulated in NO applied plants.	Singh et al. (2017)
4.	As	*Oryza sativa*	*OsLsi1* and *OsLsi2* gene expressions were down-regulated by NO treatment in rice.	Singh et al. (2016)
5.	Cd	Tobacco	Expression of *Hsr203J* gene was improved under Cd stress,	Ma et al. (2010)
6.	Cd	*Hordeum vulgare*	Nitric oxide boosted the expression of *HvAOX* gene under stressed conditions.	He et al. (2019)

7.	Al	Wheat	Gene expression of DHAR and GR enzymes was noticed to be enhanced by NO application under Al stress.	Sun et al. (2015)

Applying NO or/and Si to plants growing in soil that has been spiked with As could be a useful way to prevent As toxicity in plants. Both Si and NO alleviates metal toxicity by performing various mechanisms such as metal chelation, compartmentation, stimulation of defense system, and synthesis of phytochelatins to reduce uptake and translocation of As from soil to various plant parts. Arsenic induced overaccumulation of ROS causing the photo-oxidative injury of photosynthetic apparatus can be averted by Si and NO application. Moreover, deposition of Si creates an amorphous silica barrier in cell walls and NO causes the walls to lignify. The formation of NO and Si complex with metalloid may reduce uptake and translocation of As from soil to various plant parts. Nevertheless, not enough research has been done to describe how NO and Si might work together to reduce As uptake and reduce stress in crop plants. In this way, Si and NO in combination can help to significantly alleviate the As toxicity in radish.

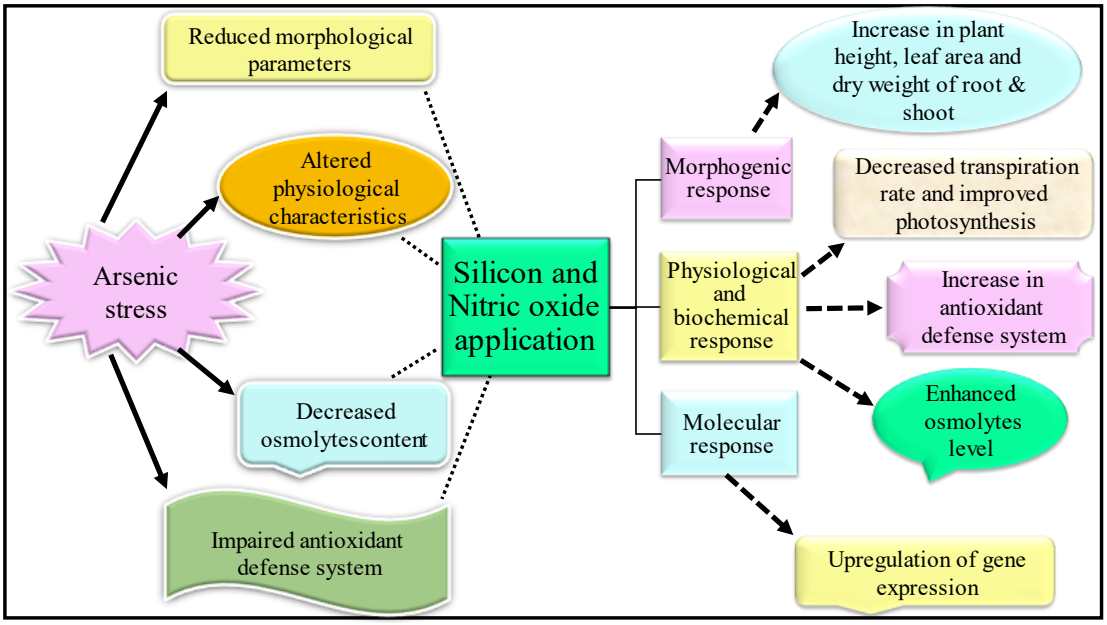

Fig. 2.4 Silicon mediated mechanism for alleviation of metal/loid stress.

Chapter 3
Hypothesis

Environmental pollution with As in soil has become an acute hazard to plant productivity globally. Arsenic is the detrimental hazardous metalloid which accumulates in soil and water and is extremely noxious to plants, animals as well as to humans. Arsenic is ubiquitously present detrimental toxic metalloid which dispersed within the atmosphere by both natural progressions and via man-made actions for instance, mining, smelting, and the practice of As-containing fertilizers, and feeds additives (Sadee et al., 2016; Smedley & Kinniburgh, 2002). Irrigation of crop fields with excessive As polluted water has gained great consideration in the present day time because of the translocation of As to the food chain (Rahman et al., 2008). Furthermore, exceedingly high levels of As in ground water of agricultural state like Punjab has emerged as a key contributor to the problem of environmental contamination. Radish is an important edible and commercial crop of states like Punjab, due to its nutritional and medicinal properties. Higher levels of As in vegetables like radish, lady finger, cauliflower, brinjal and potato in farm rich states of Punjab and Haryana occurs due to enhancement in the usage of pesticides together with employment of 90% of As contaminated water for irrigation over there (ICAR, 2015). Uptake of As metalloid in radish negatively affects all the vital parameters of importance ranging from morpho-anatomical, biochemical, physiological to molecular aspects by altering plant growth, photosynthesis process, mineral nutrients, gene expression, etc. Hence, it is essential to reduce As toxicity in plants.

Inexpensive, economical, and eco-friendly strategies are needed in the present-day time to improve plants' tolerance to As toxicity. Against this metalloid, exogenous supplementation of mineral nutrient Si and signaling molecule NO may be a valuable approach to decline As uptake or As absorption from soil to plant roots for better food production. Hence, application with these moieties could be one of the effective strategies to prevent As toxicity in plants. There are many studies which showed the individual effects of Si (Gregar et al., 2015; and NO (Andrade et al., 2016; Farnese et al., 2017; Singh et al., 2016) in different plant species under As stress. However, the combined effect of Si and NO has never been tested yet to mitigate the negative effects of As in terms of growth, physiological and biochemical aspects in *Raphanus sativus* therefore, this study could be a novel approach for evaluating the Si and NO mediated alleviation of As toxicity. Hence, the current research work investigates about Si and

NO effect on morphological, biochemical, physiological and molecular characteristics of *Raphanus sativus* under As toxicity.

Chapter 4

Objectives

4.1 Background

Reducing the absorption and translocation together with accumulation of As from soil to radish plants have lately emerged as a worldwide concern for enhancing their development and productivity as well as meeting the rising global population's need for food. To increase As tolerance in plants, a range of techniques may be utilized to restrict or lower As uptake through the roots and to decrease its passage to the aerial parts (Bali et al., 2021; Mitra et al., 2017b). Physical methods such as remediation by chemical or electrokinetic means and thermal desorption, however, have not yet proven successful for As removal because of concerns about their cost, efficacy, and environmental friendliness. That is why, mitigation of As toxicity is much needed with the application of secure and cost-effecient substances. Hence, use of Si and NO could be an advantageous strategy for this.

Plants deviate prominently in their competence to absorb Si and due to frequent chemical transformations and alterations in the absorption and passage strategies within plants, various plant tissues exhibit varied concentrations of Si in them. Silicon has been shown to diminish As induced toxicity in genotypes. Nitric oxide is acquiring great consideration as a growth regulator in regulating several biological progressions in plants, including stimulation of plant strategies to counteract As-triggered toxicity. The positive appearance of NO depends on its capability to maintain the optimal amount and mitigation of toxicity caused by overaccumulation of ROS (He et al., 2014). NO serves as a signaling moiety in several cellular tissues and at the molecular level (Misra et al. 2011).

4.2 Research objectives

1. *In vitro* and *in vivo* monitoring of growth of radish exposed to As stress along with the supplementation of Si and NO.
2. Assessment of Si and NO induced physicochemical aspects of radish *in vitro* and *in vivo* under As stress.
3. Comparative study of gene expression of As stress related genes in radish plants in response to Si and NO.

Chapter 5

Materials and Methods

5.1 Study material

Seeds of *Raphanus sativus* var Punjab Safed Mooli 2 were availed from PAU, Ludhiana, Punjab. Experiment was conducted in the agricultural field of Lovely Professional University, Punjab in the year 2021-2022.

5.2.1 Combination of treatments

Different treatments used in this study are mentioned in table 5.1.

Table 5.1 Different treatments selected for the experiment

S. No.	Treatment	As (mM)	Si (mM)	NO (µM)
1.	CN	0	0	0
2.	As I	0.3	0	0
3.	As II	0.5	0	0
4.	As III	0.7	0	0
5.	Si	0	2	0
6.	Si + As I	0.3	2	0
7.	Si + As II	0.5	2	0
8.	Si + As III	0.7	2	0
9.	NO	0	0	100
10.	NO + As I	0.3	0	100
11.	NO + As II	0.5	0	100
12.	NO + As III	0.7	0	100
13.	Si + NO	0	2	100
14.	Si + NO + As I	0.3	2	100
15.	Si + NO + As II	0.5	2	100
16.	Si + NO + As III	0.7	2	100

5.2.2 Preparation of solutions

Sodium arsenate:

0.3 mM: 6.23 mg per 100 ml of water

0.5 mM: 10.39 mg per 100 ml of water

0.7 mM: 14.55 mg per 100 ml of water

Silicic acid:

2 mM: 87.76 mg per 100 ml of water

Sodium nitroprusside (SNP):

100 µM: 2.97 mg per 100 ml of water

5.3 Raising of seedlings and plants

5.3.1 Surface sterilization

1% sodium hypochlorite was taken for surface sterilizing the seeds of *R. sativus* for 10 minutes followed by rinsing them 4 times in distilled water.

5.3.2 Raising of seedlings *In vitro*

Surface sterilized seeds of *R. sativus* were pre-soaked in 100 µM solution of SNP (which acts as NO donor), for 8 hours. Other seeds were dipped in distilled water for the same time duration. As solution of different concentrations i.e. 0.3, 0.5 and 0.7 mM was supplied in petri-dishes which were lined with *Whatmann* no.1 filter paper. SNP-treated seeds were firstly made clean with distilled water and then placed in petri-dishes containing As solutions. Silicon (in the form of silicic acid) was applied as a foliar spray. Control seeds were supplied with distilled water. Petri plates were placed inside a seed germinator (Thermotech NSW 191-192) under controlled environments with a temperature of 25 ± 0.5 ^{o}C, 16 hr photoperiod, the light intensity of 175 µmol m^{-2} s^{-1}, and 68-70 % relative humidity. Then 7 days old seedlings were harvested for further analysis.

5.3.3 Raising of plants in *In-vivo*

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.

5.4 Growth analysis

5.4.1 Germination percentage

It was calculated by using the below mentioned formula

$$= \frac{Total\ seeds\ germinated}{Number\ of\ initial\ seeds\ used} \times 100$$

5.4.2 Growth parameters

In radish seedlings, root and shoot length were measured in cm. Measurement of fresh weight was taken in grams using a weighing balance (Danwer DW500NANO). Dry weight was taken by drying the samples at 80 ^{0}C for 24 h.

Similarly, root length and fresh and dry weights were measured in 30- and 60-days old plants.

5.4.3 Vigor index

Following formula was used to calculate vigor index

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

5.4.4 Relative water content

It was calculated both in seedlings and plants by using the following formula

Relative water content = $\frac{Fresh\ weight - dry\ weight}{Turger\ weight - dry\ weight} \times 100$

5.5 Photosynthetic pigments and gas exchange parameters

5.5.1 Pigments

5.5.1.1 Chlorophyll content (mg g^{-1} FW)

Arnon (1949) method was followed for the evaluation of chlorophyll contents. Briefly, 0.5 g of fresh plant tissue was crushed in a pestle and mortar (chilled) in 80% acetone (4 ml) and centrifuged for 20 minutes (13,000 rpm; 4 ^{0}C). Chlorophyll values were evaluated in the supernatant by taking the absorbance at 645 and 663 nm.

Calculations

Total chlorophyll, chl a and chl b contents were measured in mg g^{-1} FW by using the below-mentioned equations

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

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

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

Where, v = volume of plant extract

w = weight of plant sample

5.5.1.2 Total carotenoid content (mg g^{-1} FW)

Maclachlan and Zalik (1963) method was followed for the evaluation of total carotenoid content. Briefly, 0.5 g of fresh plant tissue was crushed in a chilled pestle and mortar in 80% acetone (4 ml) and centrifuged for 20 minutes at 13,000 rpm at 4 ^{0}C. Supernatant was collected for the evaluation of total carotenoid content by taking the absorbance at 480 and 510 nm.

Calculations

It was measured in mg g^{-1} FW by using the below-mentioned equation

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

Where, v = volume of plant extract

w = weight of plant sample

5.5.1.3 Total xanthophyll content (mg g^{-1} FW)

Method given by Lawrence (1990) was followed for the evaluation of xanthophyll content. Dried plant tissue was homogenized into a fine powder. After this, 100 ml

flask was taken to which 50 mg of dried plant tissue powder was transferred. Extractant made up of hexane (10 ml) + acetone (7 ml) + absolute alcohol (6 ml) + toluene (7 ml), was added to the flask in 30 ml quantity containing the plant sample followed by shaking for 10-15 min. Then, 2 ml of 40% methanolic KOH was added to the flask after which refluxing of the flask was done at 56 ^{o}C for 20 min. The sample was then placed for 1 hour in dark to which an addition of hexane (30 ml) was made. Shaking of the flask was done for 1 minute followed by the addition of 10% sodium sulphate solution to make a final volume of 100 ml. Flask was again kept in dark for 1 hour after shaking the flask for 1 minute. In a 50 ml volumetric flask, upper phase was mixed with hexane. After mixing, absorbance was noted down at 474 nm wavelength.

Calculations

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

Where D= final dilution

W= weight of sample taken

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

5.5.2 Gaseous exchange parameters

LI-COR LI-6400XT portable open photosynthesis system was used to measure the following gaseous exchange parameters

- Photosynthetic rate
- Stomatal conductance
- Inter-cellular CO_2
- Transpiration rate

Procedure

In an open system infrared gas analyzer (IRGA) at a constant CO_2 level, air from the same source is allowed to enter into analysis and reference lines. Differences in the levels of CO_2 and H_2O in the air flowing into the reference chamber in comparison to air coming out of the sample chamber determine above mentioned parameters. The measurements of these parameters were taken in the sunlight from 11:00 am to 1:00 pm. Instrument was set on the following conditions:

Air temperature = 25 ^{o}C

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 (mg g^{-1} FW)

For measuring the anthocyanin content, method of Mancinelli (1984) was put to use. Then, homogenization of 0.5 g of plant tissue (fresh) was performed in 3 ml of extraction mixture (methanol: H_2O: HCl in a ratio of 79:20:1), and then it was subjected to centrifugation at 13,000 rpm. At 530 and 657 nm wavelengths, optical density was taken. Calculations were done by using the following equations:

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

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

Where; MW = mol weight of cyanidin-3-glucoside (449.2)

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

5.6.2 Flavonoid content (mg g^{-1} FW)

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 4 ml double distilled water (DDW), 0.3 ml of sodium nitrite ($NaNO_2$) and 0.3 ml of aluminum chloride ($AlCl_3$) were 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 (mg g^{-1} FW)

Phenolic content was analyzed by Malick and Singh (1980) protocol. Briefly, 0.5 g of plant sample was crushed in 80% ethanol, followed by centrifugation. In the supernatant, 0.5 ml of FC reagent and 2 ml of 20% Na_2CO_3 were mixed. Then 2 hours incubation was done and absorbance was read at 650 nm. Reference standard used to measure phenolic content was gallic acid.

5.7 Oxidative damage

5.7.1 Oxidative stress markers

5.7.1.1 Malondialdehyde (MDA) content (μ mole g^{-1} FW)

MDA content was measured by Heath and Packer (1968) method. Briefly, 1 g of plant sample was crushed in 0.1% TCA. After centrifugation, supernatant was collected to

which 20% TCA containing 0.5% thiobarbituric acid (TBA) was added followed by incubation at 95 ^{0}C. At 532 and 600 nm, optical density was taken.

Calculations

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

Where extinction coefficient =155 mM^{-1} cm^{-1}

5.7.1.2 Hydrogen peroxide (H_2O_2) content (µ mole g^{-1} FW)

Velikova et al. (2000) was followed to evaluate H_2O_2 content. Briefly, 100 mg of plant sample was crushed in 0.1% of TCA followed by centrifugation. In 0.5 ml of supernatant, 0.4 ml of potassium phosphate buffer (PPB) and 0.8 ml of potassium iodide were added. Reference standard used was H_2O_2 and optical density was read at 390 nm.

5.7.2 Histochemical studies by confocal microscope

Roots of radish seedlings were used to study the membrane and nuclear damage by the method of Callard et al. (1996) and Gutierrez-Alcala et al. (2000) with the help of a confocal microscope (Nikon AIR). Briefly, 1 cm roots were cut from each sample followed by washing with water. To evaluate the membrane and nuclear damage, 4,6-diamino-2-phenylindole (DAPI, 0.1 mg in 100 mL phosphate buffer saline (PBS) and propidium iodide (50 µM), respectively were used to stain the roots of *R. sativus* seedlings. After 30 minutes of incubation in the dark, PBS washing was performed. Stained slides mounted with water were observed under a confocal microscope.

5.8 Arsenic metalloid uptake (mg g^{-1} FW)

Uptake level of As metalloid was calculated at high As stress treatment i.e., As III concentration via using Atomic Absorption Spectrophotometer (AAS) (Shimadzu model 6200). Briefly, 1 g of oven-dried plant samples (roots, shoot and leaves) were digested in a 5 ml mixture of HNO_3 and $HClO_4$ which were mixed in a 2:1 ratio. After digestion, cooling and filtering, the final volume of the sample was made up to 15 ml with DDW to measure their contents.

5.9 Estimation of Osmolytes

5.9.1 Proline content (µ mol g^{-1} FW)

Bates et al. (1973) protocol was followed to check proline content. Briefly, 0.25 g of plant tissue was extracted in 3% sulfosalicylic acid and then separated at 10,000 rpm for 15 minutes. Then, 2 ml of the upper layer of plant sample was taken in test tube and

given an addition of ninhydrin and glacial acetic acid (2 ml each). Further, toluene was used as an extractant of this mixture and checked at 520 nm wavelength. As a standard, L-proline was used.

5.9.2 Glycine betaine content (µ mol g^{-1} FW)

Glycine betaine was detected by Grieve and Grattan (1983) method. Briefly, 1 g of dried plant sample was homogenized. After filtration, 1 ml of 2M HCl and 0.2 ml of potassium tri-iodide solution was mixed to the 1 ml of supernatant. Shaking and cooling was done in an ice bath for 90 min with occasional shaking. Then, 2.0 ml and 20 ml of ice-cooled distilled water and 1-2 dichloromethane, respectively were added to it. Wavelength was set at 365 nm and the upper aqueous layer was discarded. The content of glycine betaine was calculated from the standard curve.

5.10 Total carbohydrates content (mg g^{-1} FW)

Total carbohydrates were calculated by following the protocol of Scott and Melvin (1953). Briefly, 1.25 ml of HCl (2.5 N) was added to 25 mg of plant sample, followed by cooling. The final volume was made to 25 ml after adding Na_2CO_3 to neutralize it. In 1 ml of supernatant, anthrone reagent in 4 ml quantity was added, with subsequent heating for 8 minutes. After cooling, optical density was taken at 630 nm when the dark green color appeared. Glucose was used as standard and plotted in the graph. Total carbohydrates content was calculated from the standard graph.

5.11 Protein content and Antioxidant defense system

5.11.1 Protein content (mg g^{-1} FW)

Lowry et al. (1951) protocol was used for evaluating protein content. Briefly, in 3 ml of potassium phosphate buffer ((50 mM, pH = 7.0), 500 mg plant tissue was crushed in pestle and mortar. 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) in supernatant (0.1 ml). Then 5 ml of reagent C which was prepared by mixing of reagent A (100 ml) and B (2 ml). Blue color appeared after addition of reagent D (0.5 ml) i.e. FC reagent. Optical density was recorded at 660 nm. Protein content was determined from the standard curve of bovine serum albumin (BSA).

Sodium carbonate (2%) in sodium hydroxide (0.1 N) = Reagent A

Copper sulphate (0.5%) in potassium sodium tartarate (1%) = Reagent B.

5.11.2 Enzymatic antioxidants

Extract preparation

For SOD activity, enzyme was extracted by homogenizing 1 g of plant tissue in sodium carbonate buffer (50 mM, pH 10.2) of 3 ml quantity and then centrifuged at 5,000 rpm for 20 minutes. Whereas, for other enzymes i.e., CAT, APX, POD, GPOX, GR, DHAR, MDHAR, GST and PPO, extraction was carried out by homogenizing 1 g of plant tissue in 3 ml phosphate buffer (100 mM, pH 7.0) and centrifuged. Supernatants were used as the sample for further analysis.

5.11.2.1 Superoxide dismutase (SOD; UA mg^{-1} protein)

Superoxide dismutase performance was evaluated by the standard procedure of Kono (1978). Briefly, 300 µl nitro blue tetrazolium (NBT; 96 µM) and 300 µl Triton X-100 (0.6 %) and 1700 µl of Na_2CO_3 buffer (50 mM, pH 10), were added to the test cuvettes. Then, 300 µl of hydroxylamine hydrochloride (20 mM, pH 6.0) and 300 µl of ethyl diamine tetra acetic acid (EDTA; 0.1 mM), were added thereafter to start the reaction. The addition of a 100 µl plant sample was done after 2 minutes. Absorbance was noted down at 540 nm.

The percent inhibition of NBT reduction was computed as following

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

where x is the percent inhibition caused by 100 µl of the sample.

50 % inhibition is caused by = $\frac{50 \times 100}{x} = y\ \mu l\ of\ sample$

5.11.2.2 Catalase (CAT; UA mg^{-1} protein)

Aebi (1983) protocol was followed to evaluate CAT activity. Briefly, in 50 µl of plant sample, 300 µl of H_2O_2 (150 mM) and 2.650 ml of 100 mM phosphate buffer were added. Optical density was read at 240 nm and calculated from the following equation:

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

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

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

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

5.11.2.3 Ascorbate peroxidase (APX; UA mg^{-1} protein)

Ascorbate peroxidase enzyme performance was estimated by Nakano and Asada (1981) method. Change in 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) =

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

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

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

5.11.2.4 Guaiacol peroxidase (POD; UA mg^{-1} protein)

Estimation of POD enzyme activity was carried out by Putter (1974) method. 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.

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

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

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

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

5.11.2.5 Glutathione reductase (GR; UA mg^{-1} protein)

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

5.11.2.6 Glutathione peroxidase (GPOX; UA mg^{-1} protein)

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 of plant sample. Absorbance was taken at 340 nm.

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

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

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

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

5.11.2.7 Dehydroascorbate reductase (DHAR; UA mg^{-1} protein)

Dalton et al. (1986) method was used to check the DHAR activity. 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. Absorbance was taken at 265 nm.

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

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

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

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

5.11.2.8 Monodehydroascorbate reductase (MDHAR; UA mg^{-1} protein)

Hossain et al. (1984) method was used for MDHAR analysis. Reaction mixture contained 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), followed by addition of 50 µl enzyme extract. The decline in absorbance was read at 340 nm.

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

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

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

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

5.11.2.9 Glutathione-S-transferase (GST; UA mg^{-1} protein)

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 1-chloro-2,4-dinitrobenzene (CDNB, 20 mM) 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) =

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

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

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

5.11.2.10 Polyphenol oxidase (PPO; UA mg^{-1} protein)

Activity of PPO enzyme was calculated by Kumar and Khan (1982) method. 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) =

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

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

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

5.11.3 Non-enzymatic antioxidants

About 1 g of seedling and leaf samples were extracted in tris buffer (50 mM, pH 10.0) taken in 3 ml quantity, followed by centrifugation (13,000 rpm; 20 min; 4 ^{o}C). The collected supernatant was used for non-enzymatic antioxidants analysis.

5.11.3.1 Ascorbic acid (µg g^{-1} FW)

Content of ascorbic acid was measured by Roe and Kuether (1943) method. Briefly, 0.5 ml of 50% TCA, 4 ml of DDW and 100 mg charcoal were mixed with 0.5 ml of plant extract and filtered. Then 0.4 ml of 2,4-Dinitrophenylhydrazine (DNPH) and 1.6 ml of cold H_2SO_4 (65%) were added followed by incubation for 3 h at 37 ^oC and left for 30 minutes. Optical density was observed at 520 nm. Ascorbic acid (1 mg 100 ml^{-1}) was used as standard and calculated from the following equation:

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

5.11.3.2 Glutathione content (µg g^{-1} FW)

Sedlak and Lindsay (1968) method was used to evaluate glutathione content. In 100 µl of supernatant, 4 ml of absolute methanol, 50 µl of 0.01 M DTNB [(5,5'-dithiobis-(2-nitrobenzoic acid)], 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 (1 mg 100 ml^{-1}) was used as standard and calculated from the following equation:

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

5.11.3.3 Tocopherol (vitamin E; (µg g^{-1} FW))

Tocopherol content was evaluated by the method of Martinek (1964). Briefly, 0.5 ml each of absolute ethanol and DDW were added to 0.5 ml of plant extract. Shaking was done to divide protein precipitates. After adding 0.5 ml xylene to it, centrifugation was done for 10 minutes at 3,000 rpm. Absorbance was recorded at 600 nm after mixing 0.5 ml xylene (top) with 0.5 ml of 2,4,6-tripyridyl-S-triazine (TPTZ) reagent. Then, tocopherol (1 mg 100 ml^{-1}) was taken as a standard for the estimation of tocopherol content.

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

5.12 Gene expression analysis by qRT-PCR

RNA was isolated from the 30 days old plants of radish by TRIzol method. Isolated RNA was quantified by a nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and then subjected to a quality check on 2% agarose gel electrophoresis. Awasthi et al. (2016) was followed to synthesize RNA to cDNA. ROTOR geneq RT-PCR system was used for qRT-PCR quantification. Reaction mixture had SYBR green, gene-specific primer and cDNA. Actin was utilized as a housekeeping gene and

triplicates were used for each assay. Ct value was used for estimating the relative expression of a gene by making use of $2^{-\Delta\Delta ct}$ method (Livak & Schmittgen, 2001).

5.13 Statistical analysis

One-way analysis of variance (ANOVA) was performed with the help of Tukey's test to check the statistically significant difference ($P < 0.05$ level of significance) between the treatments using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Triplicates were used for each experiment and data is depicted as the means ± SEM in the figures.

Chapter 6
Results and Discussion

List of legends

Abbreviations	Treatment	Legend
C	Control	
T_1	As I	
T_2	As II	
T_3	As III	
T_4	Si	
T_5	Si + As I	
T_6	Si + As II	
T_7	Si + As III	
T_8	NO	
T_9	NO + As I	
T_{10}	NO + As II	
T_{11}	NO + As III	
T_{12}	Si + NO	
T_{13}	Si + NO + As I	
T_{14}	Si + NO + As II	
T_{15}	Si + NO + As III	

6.1 Results

6.1.1 *In vitro* grown seedlings

6.1.1.1 Plant growth

Root length was observed to be declined under As stress (Fig. 6.1; Table 6.1). The highest reduction of 2.36 cm was found at T_3. Almost a 50% reduction occurred in root length in T_2 seedlings. The results also exhibited that Si and NO alleviated the As noxiousness by increasing the root length. Among the Si-treated seedlings under As stress, the highest root length of 11.33 cm was observed at T_7 concentration. Pretreatment with NO under As stress also showed improved root length under As toxicity with a maximum root length of 9.83 cm at T_{11} concentration. Under stress conditions, Si application showed better root length as compared to NO pre-treatment. The synergistic treatment of Si + NO also augmented the root length in As stressed seedlings with a maximum root length of 12.3 cm in T_{14}. A nearly 2-fold increase in root length was observed at T_{13} seedlings when contrasted with T_1 treated seedlings. A 25% increase was observed in T_{14} seedlings than T_9. Furthermore, a 45% increase was found in T_{14} seedlings when compared with T_{10}. T_{14} showed 51.29% increase than control seedlings.

Shoot length was reduced under As stress with the lowest shoot length of 4.26 cm at T_3 concentration. Si and NO control seedlings showed increased shoot length (Fig. 6.1; Table 6.1). Under As stress, Si application improved the shoot length with the highest shoot length of 7.26 cm in T_5 treated seedlings. Shoot length was also noticed to be increased in NO-treated seedlings under As stress. It was increased to 7.26 cm at T_9 treated seedlings in contrast to As I alone treated seedlings at which shoot length was just 4.33 cm. Collective application of Si and NO in As toxicity proved beneficial with the uppermost 8.23 cm at T_{15} concentration. Shoot length was found to be decreased as As level increased in the case of combined application of Si and NO. T_5 seedlings showed 67.66% increase in shoot length than T_2. Similarly, 44.60% increase was in noticed in T_{11} than T_3. Shoot length was increased by 13.36% in T_{13} than T_5 and T_9. T_{13} showed 18.24% increase than control seedlings.

Fresh weight was markedly influenced under metal stress with the highest reduction in fresh weight (91.6 mg) in T_2 treated seedlings (Fig. 6.1; Table 6.1). T_5 showed increase

in the fresh weight from 119.56 to 211.26 mg in contrast to T_1 alone seedlings. Similarly, pretreatment with NO also resulted in improved fresh weight in radish seedlings under As stress. In NO pre-treated seedlings, the highest and lowest fresh weights i.e., 200.3 and 125.03 mg were noticed at T_9 and T_{11} treated seedlings, respectively. The maximum increase in fresh weight in the case of combined treated seedlings was reported at T_{13} with 231.23 mg fresh weight. Highest increase of 93% was found in T_{13} than T_2 seedlings. Fresh weight was also elevated by 9.45% in T_{13} than T_5. T_{13} showed 40.69% increase in fresh weight than control seedlings.

Table 6.1 Effect of Si and NO on morphological parameters of 7 days old *R. sativus* seedlings under As stress

Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
C (Control)	$8.13^{c}\pm0.43$	$6.96^{cd}\pm0.38$	$164.33^{c}\pm5.6$	$14.36^{bcd}\pm0.27$
T_1 (AsI)	$5.23^{b}\pm0.21$	$4.33^{a}\pm0.20$	$119.56^{ab}\pm5.95$	$12.16^{ab}\pm0.21$
T_2 (AsII)	$4.73^{b}\pm0.18$	$4.93^{ab}\pm0.26$	$91.60^{a}\pm5.48$	$9.73^{a}\pm0.29$
T_3 (AsIII)	$2.36^{a}\pm0.24$	$4.26^{a}\pm0.14$	$106.23^{ab}\pm6.09$	$9.53^{a}\pm0.17$
T_4 (Si)	$13.5^{hi}\pm0.30$	$8.6^{f}\pm0.20$	$249.53^{g}\pm5.49$	$22.30^{i}\pm0.4$
T_5 (Si+AsI)	$11.33^{fg}\pm0.07$	$7.26^{cde}\pm0.23$	$211.26^{ef}\pm5.97$	$18.56^{fg}\pm0.14$
T_6 (Si+AsII)	$9.33^{cde}\pm0.17$	$6.96^{cd}\pm0.21$	$185.16^{cde}\pm6.00$	$14.53^{bcd}\pm0.28$
T_7 (Si+AsIII)	$10.26^{ef}\pm0.23$	$6.4^{c}\pm0.26$	$173.97^{cd}\pm1.86$	$13.93^{bc}\pm0.35$
T_8 (NO)	$13.15^{hi}\pm0.36$	$8.8^{f}\pm0.17$	$247.53^{g}\pm4.55$	$21.66^{hi}\pm1.7$
T_9 (NO+AsI)	$9.83^{def}\pm0.26$	$7.26^{cde}\pm0.12$	$200.3^{de}\pm9.83$	$19.00^{fgh}\pm0.51$
T_{10} (NO+AsII)	$8.46^{cd}\pm0.17$	$6.76^{cd}\pm0.23$	$164.8^{c}\pm4.33$	$16.76^{def}\pm0.12$
T_{11} (NO+AsIII)	$9.6^{cde}\pm0.26$	$6.16^{bc}\pm0.29$	$125.03^{b}\pm6.12$	$15.66^{cde}\pm0.2$
T_{12} (Si+NO)	$14.51^{i}\pm0.80$	$10.07^{g}\pm0.28$	$282.68^{h}\pm4.82$	$23.43^{i}\pm0.31$
T_{13} (Si+NO+AsI)	$10^{def}\pm0.28$	$8.23^{ef}\pm0.27$	$231.23^{fg}\pm3.64$	$20.76^{ghi}\pm0.37$
T_{14} (Si+NO+AsII)	$12.3^{gh}\pm0.15$	$7.93^{def}\pm0.20$	$199.90^{de}\pm5.34$	$18.23^{efg}\pm0.34$
T_{15} (Si+NO+AsIII)	$8.36^{cd}\pm0.27$	$6.83^{cd}\pm0.20$	$170.06^{c}\pm6.03$	$14.43^{bcd}\pm0.2$

In the case of dry weight, T_2 (9.73 mg) and T_3 (9.53 mg) treated seedlings did not show much difference in reducing the dry weight while As I treatment showed a dry weight of 12.16 mg (Fig. 6.2; Table 6.1). Individual dosage of Si and NO in case of As stress showed an escalation in dry weight with a maximum of 18.56 and 19 mg dry

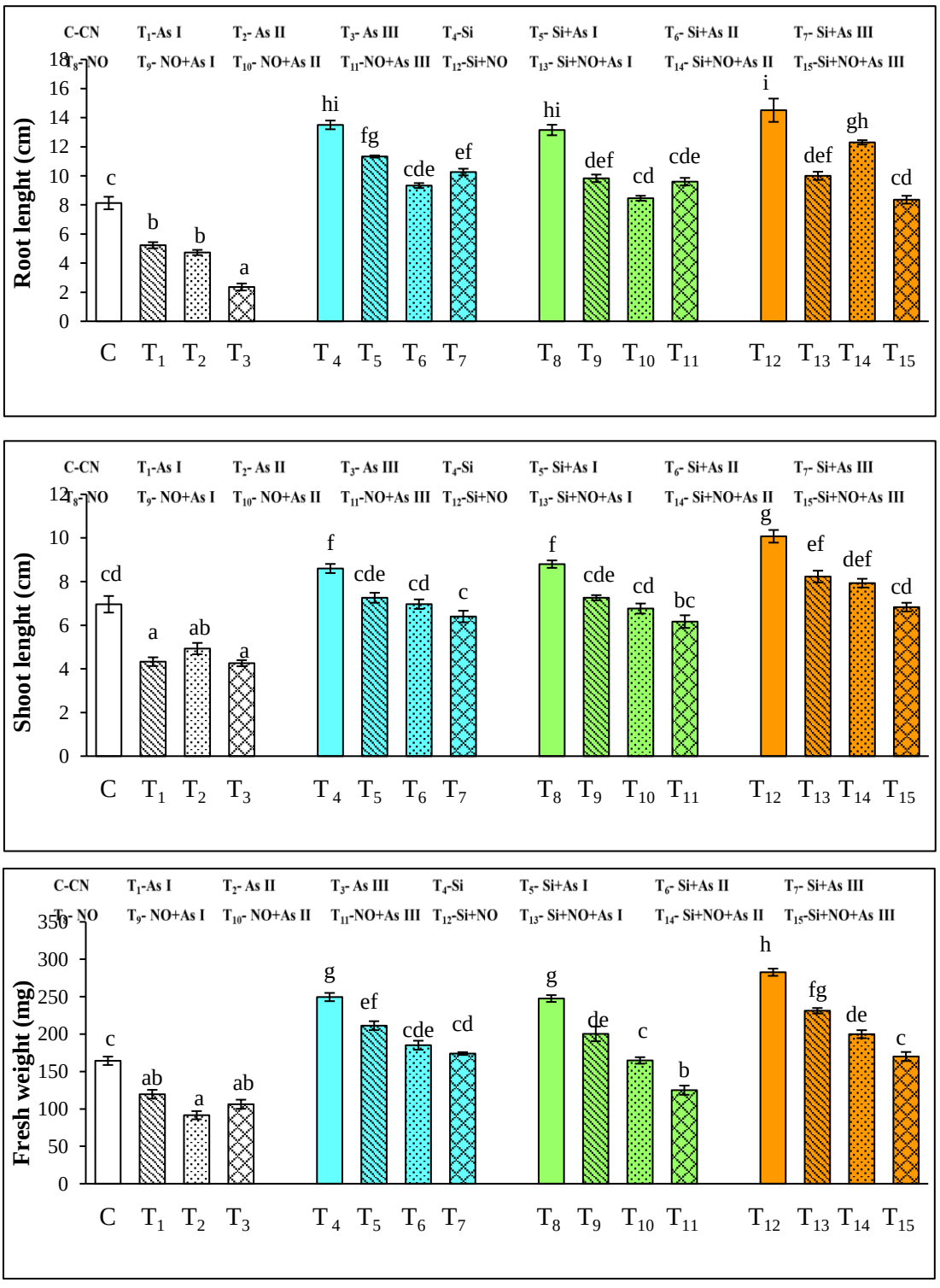

Fig. 6.1 Effect of Si and NO on root and shoot length and fresh weight in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

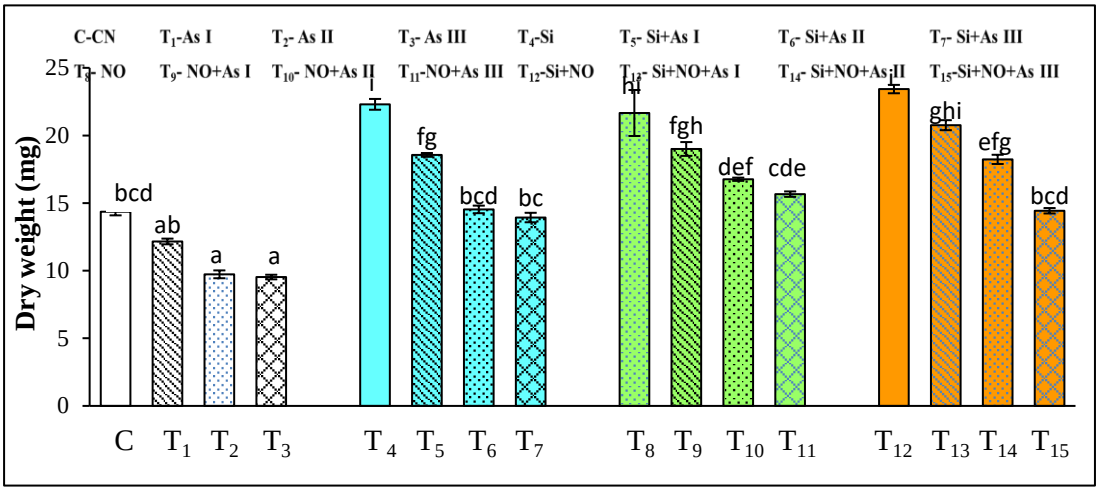

Fig. 6.2 Effect of Si and NO on dry weight in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

weights, respectively at T_5 and T_9 concentration. Almost a two-fold increase in dry weight i.e., 18.23 mg was observed in T_{14} concentration when compared with T_2 alone treated seedlings (9.73 mg). Si and NO in combination showed better results in the case of dry weights when compared with their individual treatments. Dry weight was augmented by 46.16% in T_7 as compared to T_3. T_9 exhibited 56.25% increase in dry weight when compared to T_2. T_{13} showed 44.56% increase than control seedlings.

Germination percentage was adversely affected by As stress in radish seedlings (Fig. 6.3; Table 6.2). Germination percentage was lowered down with increasing concentrations of As i.e. 66.66% at T_1 concentration to 53.33% at T_2 concentration. Control seedlings exhibited 82.77% germination percentage. In unstressed conditions, application of Si under exhibited a germination percentage of 90% which is greater in comparison to control seedlings. Silicon application at T_5 and T_6 showed similar germination percentages i.e., 75%. Among all three As treatments in NO-pre-treated seedlings, a maximum germination percentage of 81.66% was noticed at T_{11} concentration. Si + NO treatments showed a higher germination percentage with the highest germination percentage of 90% at T_{15} concentration. T_5 showed 12.51% increase than T_1. An elevation of 20.01% was noticed in T_9 than T_1. Combination of Si and NO i.e., T_{13} exhibited 6.25% increase than T_9. T_{15} showed 8.73% increase than control seedlings.

Arsenic stress also decreased the vigor index of radish seedlings with an almost 50% reduction at T_1 concentration (641.16%), as compared to control seedlings (1254.87%). T_2 treated seedlings showed a 354% vigor index which was almost half of the vigor index at T_1 treated seedlings (Fig. 6.3; Table 6.2). Individual supplementation of Si and NO under As toxicity increased the vigor index. The highest vigor index of 1391.5% was recorded at As I concentration in the case of T_5 seedlings among all three As concentrations used. Pretreatment of NO also improved the vigor index against As toxicity with a maximum vigor index of 1369.33% at T_9 concentration. Among individual treatments of Si and NO in response to As stress, Si application showed better results as compared to NO treatments. Si and NO application in combination showed elevation in the vigor index under As toxicity. Vigor index was found to be increased from 641.16% to 1547.83% at T_{13} treated seedlings, when contrasted with T_1 alone treated seedlings. T_{13} showed 11.23% increase than T_5. Furthermore, T_{13} exhibited 13.03% increase than T_9. T_{14} showed 42.36% increase than control seedlings.

Relative water content was abridged in response to As in radish seedlings (Fig. 6.3; Table 6.2). It was decreased with the elevation in the As level from 83.48% at T_1 concentration to 73.28% at T_3 concentration. Control seedlings exhibited 85.55% relative water content. Si application under unstressed conditions showed relative water content of 90.23% which is greater when compared with control seedlings. Si application against As stress exhibited a minimum relative water content of 75.12% at T_6 concentration. Among all three As treatments in NO-pre-treated seedlings, the maximum relative water content of 87.55% was noticed at T_9 concentration. Si + NO treatments showed higher relative water content with the highest of 92.73% at T_{13} concentration. T_{13} showed 14.25% increase than T_5. T_{13} exhibited 5.91% increase than T_9. T_{13} showed 8.39% increase in relative water content than control seedlings.

6.1.1.2 Photosynthetic pigments

Total chlorophyll, chl a and chl b levels were affected by As stress in radish seedlings (Fig. 6.4; Table 6.3). The lowest total chlorophyll content was in T_3 stressed seedlings with 0.12 mg g^{-1} FW content. Individual treatments of Si and NO exhibited upregulation in the total chlorophyll amount under As in comparison to As treated seedlings, among which the highest total chlorophyll contents i.e., 0.222 and 0.220 mg g^{-1} FW,

respectively got noticed at T_5 and T_9 concentration. Synergistic application of Si and NO showed better results in improving total chlorophyll content under stress conditions, as compared to their individual treatments. Under stressed conditions, the highest level (0.251 mg g^{-1} FW) was noticed in T_{13} treated seedlings. An increase of 14.09% was noticed in T_{13} seedlings in contrast to T_5. T_{15} showed an elevation of 37.41% in total chlorophyll content as compared to T_{11}. T_{13} showed 20.09% increase than control seedlings. Similar findings were observed in the case of chl a and chl b in radish seedlings. The lowest content of chl a (0.058 mg g^{-1} FW) and chl b (0.036 mg g^{-1} FW) were found at T_3 concentration. Further, Si and NO supplementation significantly alleviated the As-induced harmfulness by increasing their contents. The combination of Si and NO further boosted their amounts under stressed conditions with the highest 0.212 and 0.158 mg g^{-1} FW contents, respectively at T_{13}. An increase of 39.47 and 28.45% in chl a and chl b was found in T_{13} in contrast to T_9, respectively.

Table 6.2 Effect of Si and NO on germination percentage, vigor index and relative water content of 7-days old seedlings of *R. sativus* under As stress

Treatment	Germination percentage (%)	Vigor index (%)	Relative water content (%)
C (Control)	82.77^{cde}±5.47	1254.87^{b}±122.8	85.55^{cdef}±1.4
T_1 (AsI)	66.66^{abc}±4.40	641.16^{a}±68.62	83.48^{bcdef}±1.26
T_2 (AsII)	55^{ab}±2.88	530.33^{a}±17.7	79.66^{abcd}±2.79
T_3 (AsIII)	53.33^{a}±3.33	354^{a}±24.68	73.28^{a}±1.68
T_4 (Si)	90^{de}±2.88	1991.93^{ef}±100.17	90.23^{efg}±0.93
T_5 (Si+AsI)	75^{cd}±2.88	1391.5^{bc}±34.71	81.16^{abcde}±3.26
T_6 (Si+AsII)	75^{cd}±2.88	1221^{b}±33.53	75.12^{ab}±1.8
T_7 (Si+AsIII)	78.33^{cde}±4.40	1297^{b}±89.71	80.63^{abcde}±1.84
T_8 (NO)	85^{cde}±2.88	1867.43^{de}±80.37	89.74^{efg}±1.65
T_9 (NO+AsI)	80^{cde}±2.88	1369.33^{bc}±72.99	87.55^{cdefg}±1.97
T_{10} (NO+AsII)	73.33^{bcd}±4.40	1250.83^{b}±166.46	86.47^{cdefg}±1.52
T_{11} (NO+AsIII)	81.66^{cde}±4.40	1291.33^{b}±105.66	78.5^{abc}±2.49
T_{12} (Si+NO)	95^{e}±2.88	2336.91^{f}±110.37	95.53^{g}±1.661
T_{13} (Si+NO+AsI)	85^{cde}±2.88	1547.83^{bcd}±32.3	92.73^{fg}±.05
T_{14} (Si+NO+AsII)	88.33^{de}±1.66	1786.5^{cde}±24.78	86.72^{cdefg}±1.79
T_{15} (Si+NO+AsIII)	90^{de}±4.40	1368.16^{bc}±60.61	89.29^{defg}±0.64

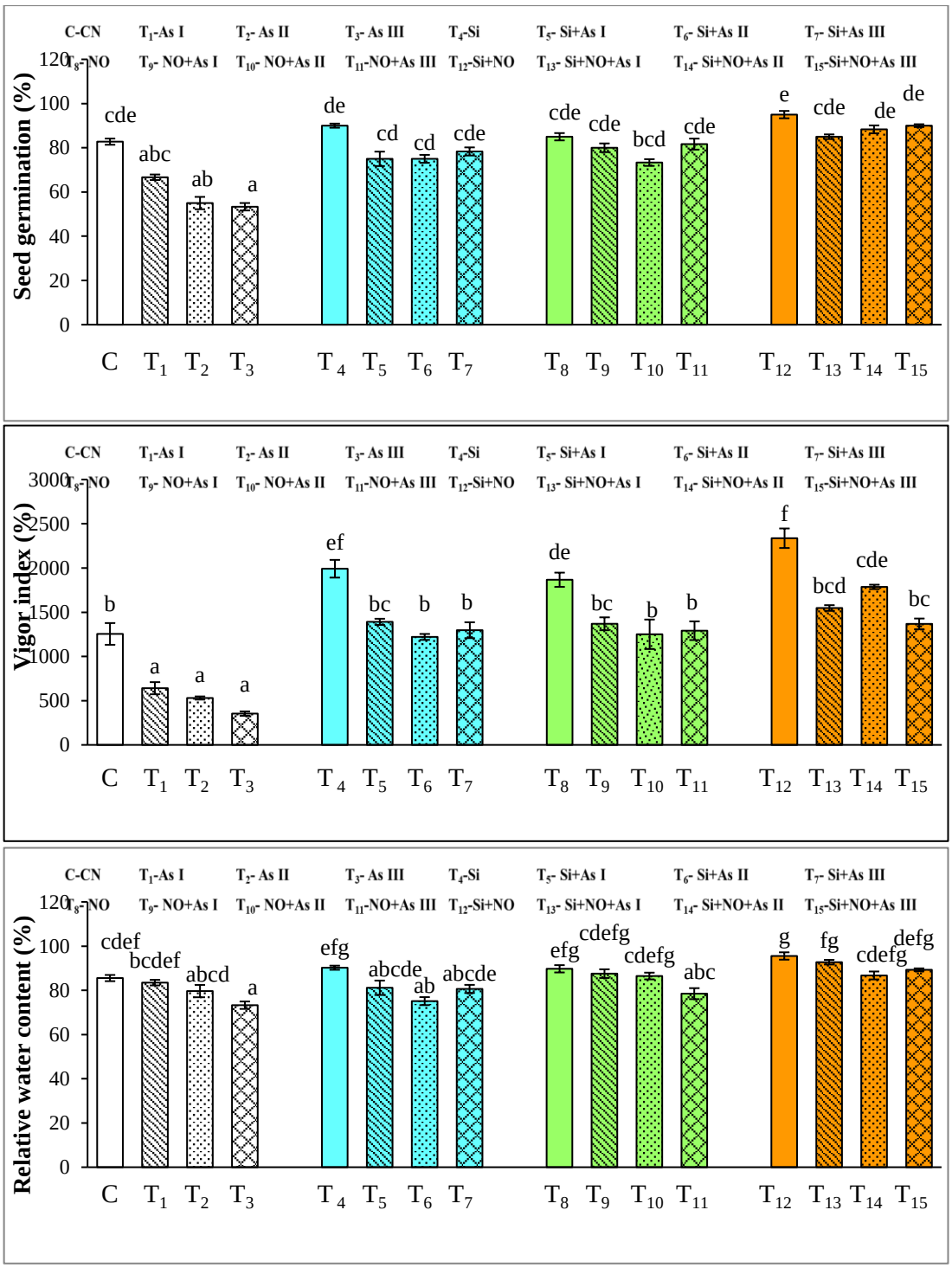

Fig. 6.3 Effect of Si and NO on gemination percentage, vigor index and relative water content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

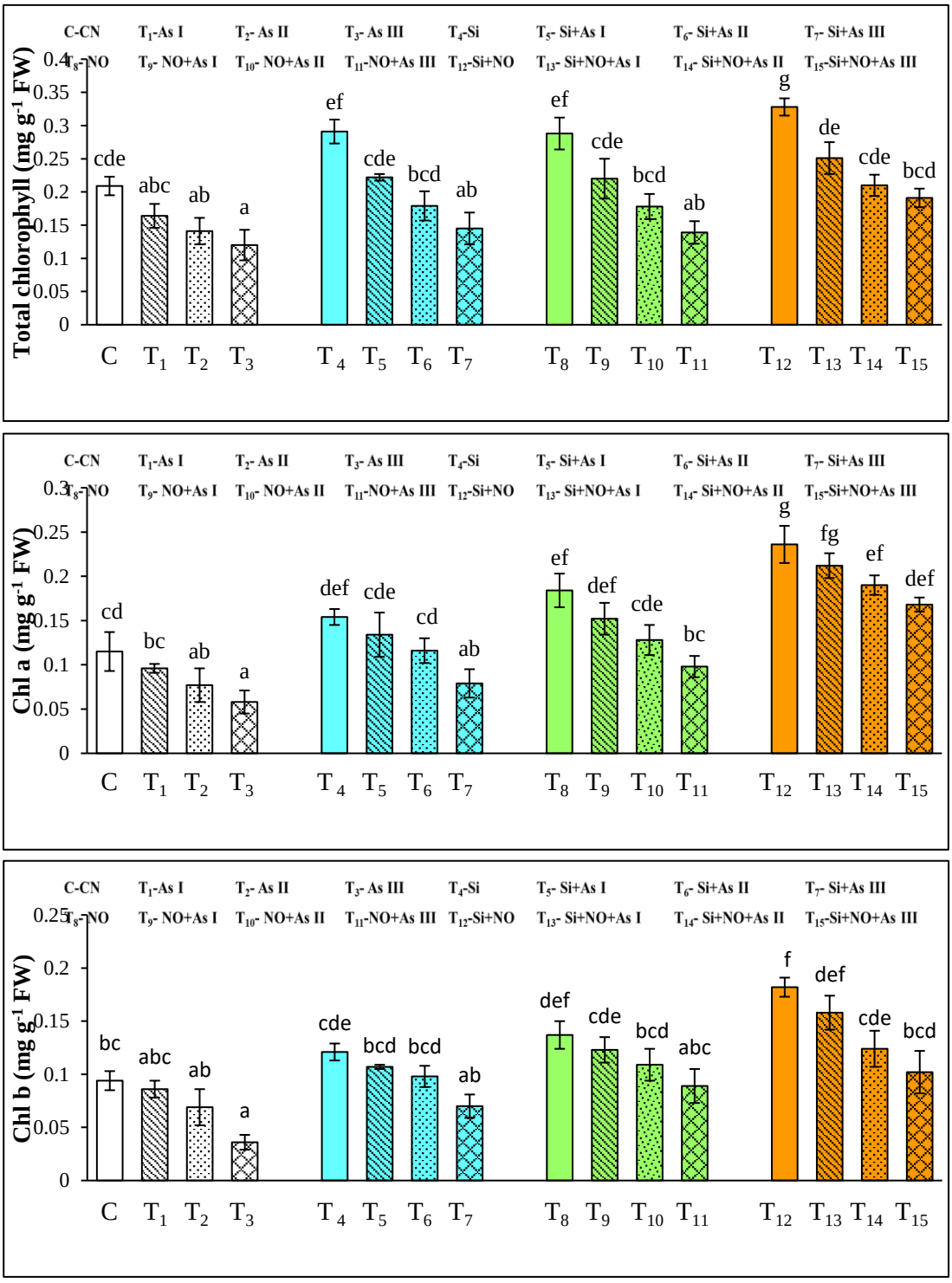

Fig. 6.4 Effect of Si and NO on total chlorophyll, chl a and chl b in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Table 6.3 Effect of Si and NO on photosynthetic pigments of 7-days old *R. sativus* seedlings under As stress

Treatment	Total chlorophyll (mg g^{-1} FW)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)
C (Control)	0.209^{cde}±0.014	0.115^{cd}±0.022	0.094^{bc}±0.009
T_1 (AsI)	0.164^{abc}±0.018	0.096^{bc}±0.005	0.086^{abc}±0.008
T_2 (AsII)	0.141^{ab}±0.020	0.077^{ab}±0.019	0.069^{ab}±0.017
T_3 (AsIII)	0.120^{a}±0.23	0.058^{a}±0.013	0.036^{a}±0.007
T_4 (Si)	0.291^{ef}±0.018	0.154^{def}±0.009	0.121^{cde}±0.008
T_5 (Si+AsI)	0.222^{cde}±0.005	0.134^{cde}±0.025	0.107^{bcd}±0.002
T_6 (Si+AsII)	0.179^{bcd}±0.022	0.116^{cd}±0.014	0.098^{bcd}±0.010
T_7 (Si+AsIII)	0.145^{ab}±0.024	0.079^{ab}±0.016	0.07^{ab}±0.011
T_8 (NO)	0.288^{ef}±0.024	0.184^{ef}±0.019	0.137^{def}±0.013
T_9 (NO+AsI)	0.220^{cde}±0.030	0.152^{def}±0.018	0.123^{cde}±0.012
T_{10} (NO+AsII)	0.178^{bcd}±0.019	0.128^{cde}±0.017	0.109^{bcd}±0.015
T_{11} (NO+AsIII)	0.139^{ab}±0.017	0.098^{bc}±0.012	0.089^{abc}±0.016
T_{12} (Si+NO)	0.328^{g}±0.013	0.236^{g}±0.021	0.182^{f}±0.009
T_{13} (Si+NO+AsI)	0.251^{de}±0.024	0.212^{fg}±0.014	0.158^{def}±0.016
T_{14} (Si+NO+AsII)	0.210^{cde}±0.016	0.190^{ef}±0.011	0.124^{cde}±0.017
T_{15} (Si+NO+AsIII)	0.191^{bcd}±0.014	0.168^{def}±0.008	0.102^{bcd}±0.020

It was found that As stress decreased the carotenoid content (Fig. 6.5; Table 6.4). Carotenoid content was lowest at T_3 concentration with 0.133 mg g^{-1} FW content. Carotenoid level got increased from 0.221 mg g^{-1} FW to 0.291 mg g^{-1} FW in T_5 seedlings, in contrast to As I seedlings. Pre-treatment with NO against As stress also increased the carotenoid content with maximum value recorded at 0.251 mg g^{-1} FW in case of T_9 concentration. Synergistic supplementation with Si and NO further boosted the carotenoid content in order to mitigate the As-induced toxic effects. The highest 0.349 mg g^{-1} FW level was noticed at T_{13} concentration while the minimum 0.253 mg g^{-1} FW was at T_{15} concentration. Its content was increased by 31.67% in T_5 than T_1 seedlings. Carotenoids content was increased by 39.04% in T_{13} plants than T_9. T_{13} showed 42.44% increase than control seedlings.

Arsenic stress diminished the xanthophyll content in 7-days old radish seedlings (Fig. 6.5; Table 6.4). The highest decline in the xanthophyll content i.e., 1.09 mg g^{-1} FW was noticed in T_3 treated seedlings. A 34% reduction in xanthophyll content was reported in T_2 applied seedlings. Elevation in the xanthophylls was exhibited by the supplication of Si and NO under As toxicity. Highest and lowest xanthophyll contents were noticed at T_5, T_7 and T_9, T_{11} i.e., 3.23 and 2.28 and 3.68 and 2.72 mg g^{-1} FW, respectively.

Furthermore, Si and NO together boosted the xanthophylls level in response to stressed conditions. Xanthophyll content was elevated from 2.35 to 4.23 mg g^{-1} FW at T_{13} concentration when contrasted with T_2 concentration. Xanthophyll content was augmented by 14.94% in T_{13} than T_9. An increase of 12.54% was noticed in T_{15} as compared to T_{11}. T_{13} showed 18.15% increase in xanthophyll content than control seedlings.

6.1.1.3 Metabolites

Anthocyanin content was found to be reduced under As stress with minimum anthocyanin content (0.86 mg g^{-1} FW) at T_3 concentration which was 54% decrease (Fig. 6.6; Table 6.5). Among individual Si and NO control seedlings, T_4 seedlings showed higher anthocyanin (2.76 mg g^{-1} FW) than T_8 seedlings. Supplementation of Si against As toxicity resulted in improved anthocyanin content, as compared to As alone treated seedlings. Anthocyanin content was enhanced from 1.27 to 2.39 mg g^{-1} FW in T_5, in contrast to T_1 concentration. Whereas, in the case of NO applied seedlings, anthocyanin content was observed to be increased from 1.09 to 1.57 mg g^{-1} FW at T_{10} concentration. Under As stress, Si application was proved to be more significant in increasing anthocyanin content than NO treatment. Combined Si and NO under As stress further enhanced the anthocyanin content with 2.83, 2.45 and 2.25 mg g^{-1} FW anthocyanin contents at T_{13}, T_{14} and T_{15} treated seedlings, respectively. Its content was augmented by 88.18% in T_5 when compared with T_1. T_7 showed an elevation of 31.59% as compared with T_{11}. T_{13} showed 50.53% increase than control seedlings.

The lowest flavonoid content was observed in T_3 stressed seedlings (1.21 mg g^{-1} FW; Fig. 6.6; Table 6.5). In T_2 stressed seedlings, a 39% decrease in its content was found when compared with control seedlings. A 47% upsurge in the flavonoid content from 1.80 to 2.65 mg g^{-1} FW at T_5 when compared with T_1 stressed seedlings. Nitric oxide also enhanced the flavonoid content, in comparison to As stressed seedlings. Flavonoid contents of 2.45, 2.16 and 2.05 mg g^{-1} FW were observed in T_9, T_{10} and T_{11} treated seedlings. With regard to combined application, the highest (2.89 mg g^{-1} FW) and lowest (2.56 mg g^{-1} FW) flavonoid contents were noticed at T_{13} and T_{14} concentration,

respectively. Its content was augmented by 47.22% in T_5 when compared with T_1. T_7 showed an elevation of 9.73% as compared with T_{11}. T_{13} showed 22.45% increase than control seedlings.

Table 6.4 Effect of Si and NO on photosynthetic pigments of 7-days old *R. sativus* seedlings under As stress

Treatment	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
C (Control)	0.245^{cde}±0.019	3.58^{ef}±0.05
T_1 (AsI)	0.221^{bc}±0.027	2.35^{c}±0.04
T_2 (AsII)	0.185^{ab}±0.025	1.74^{b}±0.06
T_3 (AsIII)	0.133^{a}±0.029	1.09^{a}±0.1
T_4 (Si)	0.332^{ef}±0.025	4.38^{g}±0.09
T_5 (Si+AsI)	0.291^{def}±0.016	3.23^{def}±0.08
T_6 (Si+AsII)	0.236^{cde}±0.020	2.55^{c}±0.17
T_7 (Si+AsIII)	0.191^{ab}±0.034	2.28^{c}±0.11
T_8 (NO)	0.302^{ef}±0.032	4.7^{g}±0.1
T_9 (NO+AsI)	0.251^{cde}±0.023	3.68^{f}±0.06
T_{10} (NO+AsII)	0.217^{bc}±0.022	3.28^{ef}±0.07
T_{11} (NO+AsIII)	0.188^{ab}±0.015	2.72^{cd}±0.07
T_{12} (Si+NO)	0.439^{f}±0.037	5.36^{h}±0.08
T_{13} (Si+NO+AsI)	0.349^{ef}±0.017	4.23^{g}±0.1
T_{14} (Si+NO+AsII)	0.300^{def}±0.034	3.58^{ef}±0.11
T_{15} (Si+NO+AsIII)	0.253^{cde}±0.029	3.11^{de}±0.14

With the increase in the As level, phenolic content was declined (Fig. 6.6; Table 6.5). It was reduced to 0.55 mg g^{-1} FW (T_3) than control seedlings which showed 2.17 mg g^{-1} FW phenolic content. Exogenous application of Si exhibited maximum phenolic content of 1.75 mg g^{-1} FW in T_5 seedlings. Individual application of NO under unstressed conditions showed maximum phenolic content of 2.86 mg g^{-1} FW. However, under As stress, T_9 treatment exhibited maximum phenolic content of 2.18 mg g^{-1} FW which was subsequently lessened with the rise in As concentration. T_{13} exhibited greatest phenolic content of 2.25 mg g^{-1} FW. Si + NO control seedlings showed the highest phenolic content of 3.29 mg g^{-1} FW amongst the 16 treatments used in the study. An increase of 90.21% was noticed in T_5 than T_1. T_{13} seedlings showed 28.57% increase

as compared to T_5. T_{13} showed 3.68% increase than control seedlings.

Table 6.5 Effect of Si and NO on metabolites of 7-days old *R. sativus* seedlings under As stress

Treatment	Anthocyanin (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)
C (Control)	1.88^{cde}±0.11	2.36^{def}±0.043	2.17^{f}±0.04
T_1 (AsI)	1.27^{abc}±0.1	1.80^{bc}±0.089	0.92^{ab}±0.02
T_2 (AsII)	1.09^{ab}±0.09	1.43^{ab}±0.104	0.67^{a}±0.04
T_3 (AsIII)	0.86^{a}±0.13	1.21^{a}±0.08	0.55^{a}±0.06
T_4 (Si)	2.76^{ghi}±0.15	3.24^{i}±0.05	2.65^{gh}±0.05
T_5 (Si+AsI)	2.39^{efgh}±0.08	2.65^{efgh}±0.109	1.75^{de}±0.05
T_6 (Si+AsII)	2.13^{defg}±0.15	2.38^{def}±0.075	1.54^{cde}±0.1
T_7 (Si+AsIII)	2.06^{def}±0.21	2.25^{cde}±0.118	1.17^{bc}±0.09
T_8 (NO)	2.6^{fgh}±0.12	2.96^{hi}±0.099	2.86^{h}±0.05
T_9 (NO+AsI)	2.18^{defgh}±0.07	2.45^{defg}±0.098	2.18^{f}±0.08
T_{10} (NO+AsII)	1.86^{cde}±0.1	2.16^{cde}±0.124	1.67^{de}±0.09
T_{11} (NO+AsIII)	1.57^{bcd}±0.1	2.05^{cd}±0.118	1.44^{cd}±0.08
T_{12} (Si+NO)	3.35^{i}±0.1	3.96^{j}±0.052	3.29^{i}±0.14
T_{13} (Si+NO+AsI)	2.83^{hi}±0.08	2.89^{ghi}±0.121	2.25^{fg}±0.04
T_{14} (Si+NO+AsII)	2.45^{efgh}±0.11	2.77^{fghi}±0.076	1.85^{ef}±0.07
T_{15} (Si+NO+AsIII)	2.25^{efgh}±0.16	2.56^{efgh}±0.08	1.57^{de}±0.05

6.1.1.4 Oxidative damage

6.1.1.4.1 Oxidative stress markers

Lipid peroxidation level was reported to be increased through As-induced enhancement in the MDA content, compared to the control seedlings (Fig. 6.7; Table 6.6). An elevation of 56% in MDA content was noticed in T_3 stressed seedlings when contrasted with control seedlings. However, treatment with Si and NO under As stress declined the level of MDA. With regard to individual application of Si and NO, maximum reduction in MDA content under As stress was noticed at T_5 and T_9 with 3.25 and 2.87 µmol g^{-1} FW contents, respectively. In addition, MDA content was decreased from 4.12 to 2.55 µmol g^{-1} FW in T_{14} seedlings, in contrast to T_2 seedlings. A decrease of 28.25% was noticed in T_5 than T_1. T_9 seedlings exhibited 28.91% reduction than T_1. T_{13} seedlings showed 16.30% decline as compared to T_5. T_{14} showed 16.93% decrease than control seedlings.

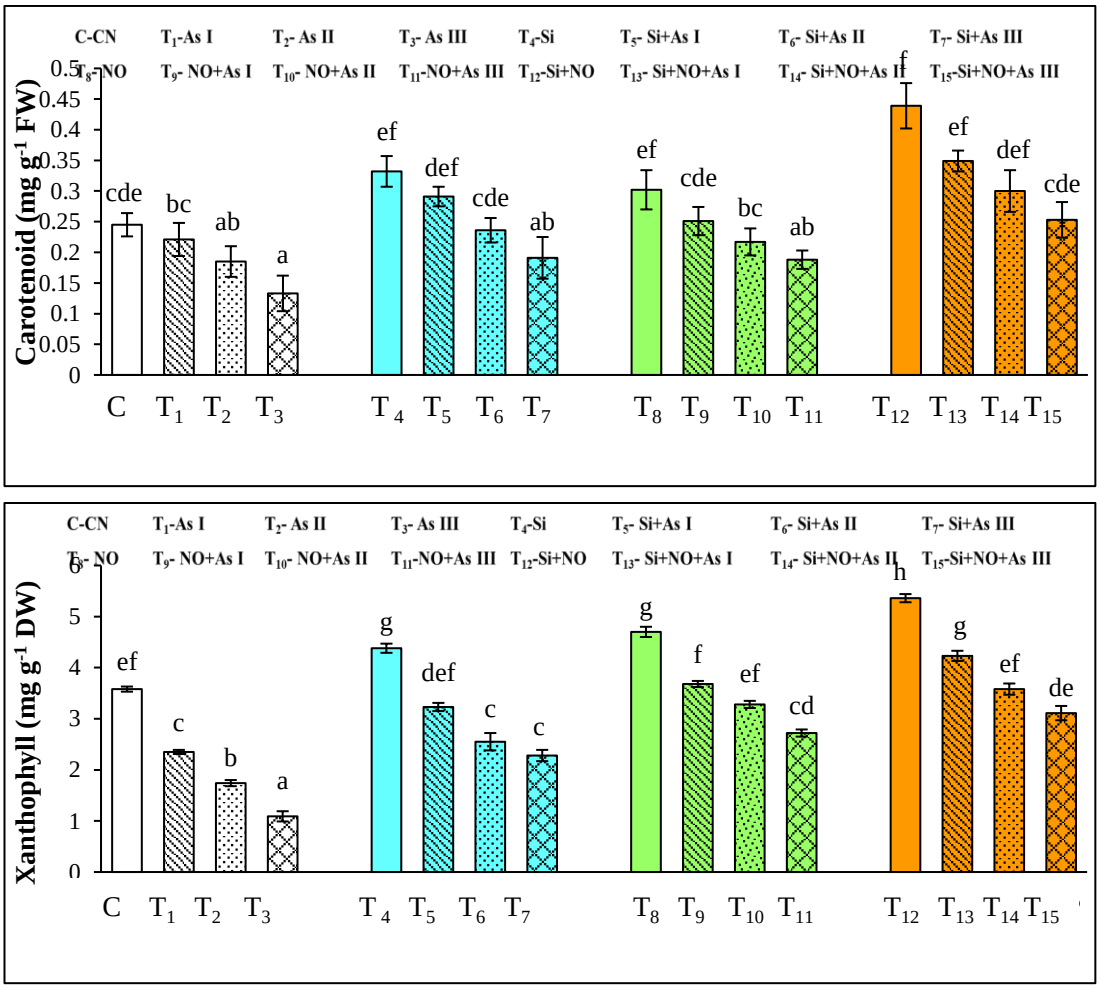

Fig. 6.5 Effect of Si and NO on carotenoid and xanthophyll content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

The level of H_2O_2 was significantly enhanced in radish seedlings upon their exposure to As stress (Fig. 6.7; Table 6.6). An almost two-fold increase in the H_2O_2 level was found in T_3 stressed seedlings when compared with control seedlings. Moreover, H_2O_2 levels were increased to 4.25, 4.76 and 5.14 µmol g^{-1} FW in T_1, T_2 and T_3 treated seedlings. However, the application of Si, NO and NO + Si against As stress diminished the H_2O_2 level. The maximum decrease in the H_2O_2 level (2.12 µmol g^{-1} FW) was at the combination of T_{13} concentration. A decrease of 39.52% was noticed in T_5 than T_1. T_9 seedlings exhibited 28.47% decline than T_1. T_{13} seedlings showed 17.50% reduction as compared to T_5. T_{13} showed 16.53% decrease than control seedlings.

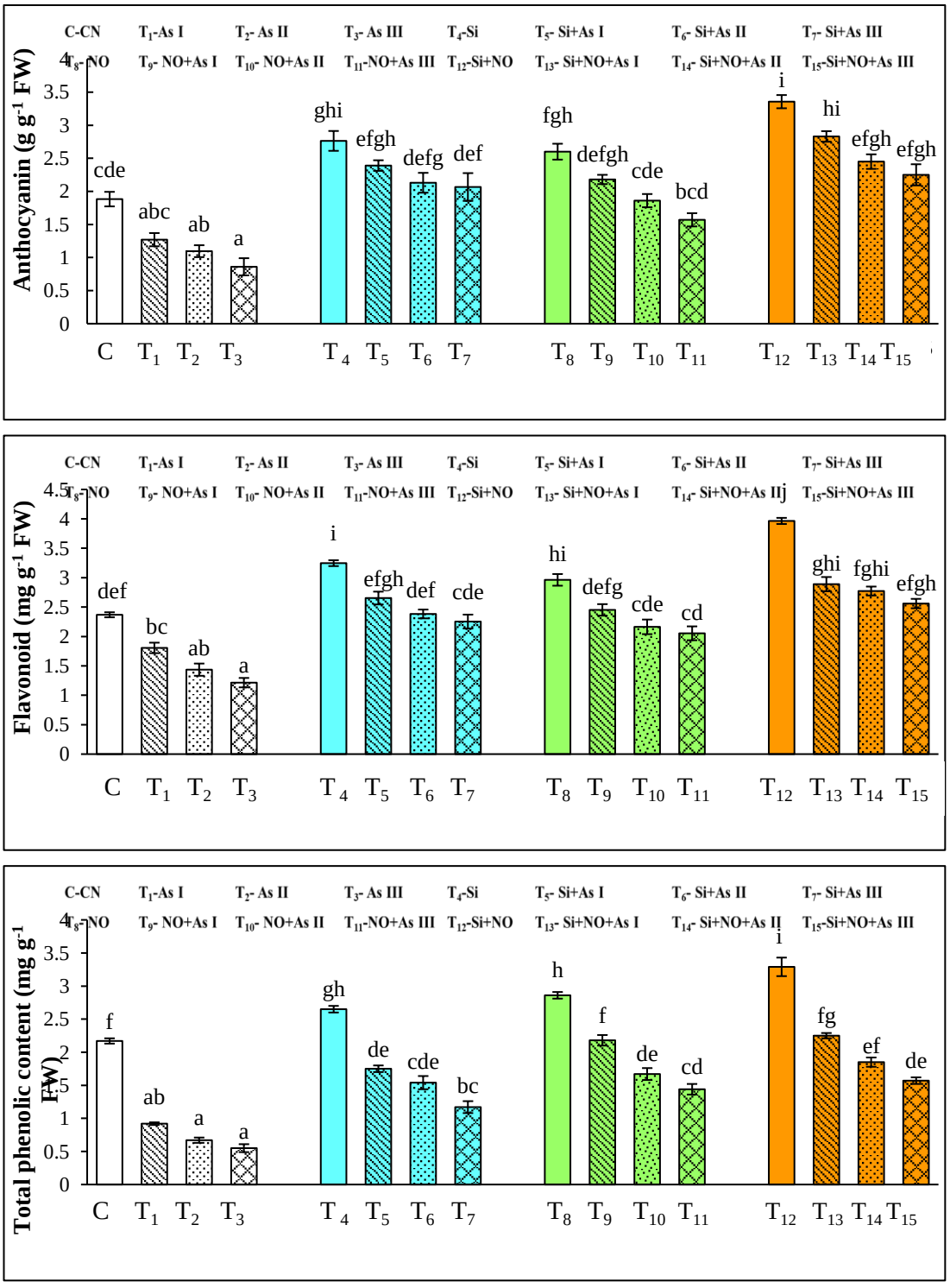

Fig. 6.6 Effect of Si and NO on anthocyanin, flavonoid and phenolic content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.6 Effect of Si and NO on oxidative stress markers of 7-days old *R. sativus* seedlings under As stress

Treatment	MDA (µmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)
C (Control)	3.07^{bcd}±0.11	2.54^{bcd}±0.08
T_1 (AsI)	4.53^{gh}±0.07	4.25^{f}±0.11
T_2 (AsII)	4.12^{fg}±0.08	4.76^{gh}±0.07
T_3 (AsIII)	4.8^{h}±0.08	5.14^{h}±0.1
T_4 (Si)	2.79^{bc}±0.07	2.38^{bc}±0.15
T_5 (Si+AsI)	3.25^{cde}±0.04	2.57^{bcd}±0.1
T_6 (Si+AsII)	3.54^{def}±0.08	3.03^{de}±0.11
T_7 (Si+AsIII)	3.91^{f}±0.18	2.77^{cde}±0.11
T_8 (NO)	2.6^{ab}±0.08	2.1^{ab}±0.09
T_9 (NO+AsI)	3.22^{cde}±0.19	3.04^{de}±0.13
T_{10} (NO+AsII)	2.87^{bc}±0.1	2.44^{bc}±0.11
T_{11} (NO+AsIII)	3.8^{ef}±0.13	3.16^{e}±0.06
T_{12} (Si+NO)	2.18^{a}±0.13	1.75^{a}±0.09
T_{13} (Si+NO+AsI)	2.72^{abc}±0.06	2.12^{ab}±0.11
T_{14} (Si+NO+AsII)	2.55^{ab}±0.12	2.23^{ab}±0.07
T_{15} (Si+NO+AsIII)	3.25^{cde}±0.09	2.46^{bc}±0.06

6.1.1.4.2 Histochemical studies

Membrane damage in the root cells of radish seedlings was seen using DAPI dye (Fig. 6.8). Arsenic III has caused more membrane damage to the roots cells as indicated by bright blue fluorescence, as compared to control seedlings. However, less membrane damage has been observed in Si and NO applied seedlings under As stress when used alone or in combination which was evident by reduced blue color intensity.

PI dye forms a fluorescent complex and intercalates with the nucleic acids (Fig. 6.9). This dye stains the nuclei by passing the nuclear membrane in damaged and dead cells. It was observed that As III treated seedlings caused high nuclear damage as indicated by bright red color. However, less nuclear damage has been observed in Si and NO applied seedlings under As stress when used alone or in combination which was evident by reduced red color intensity.

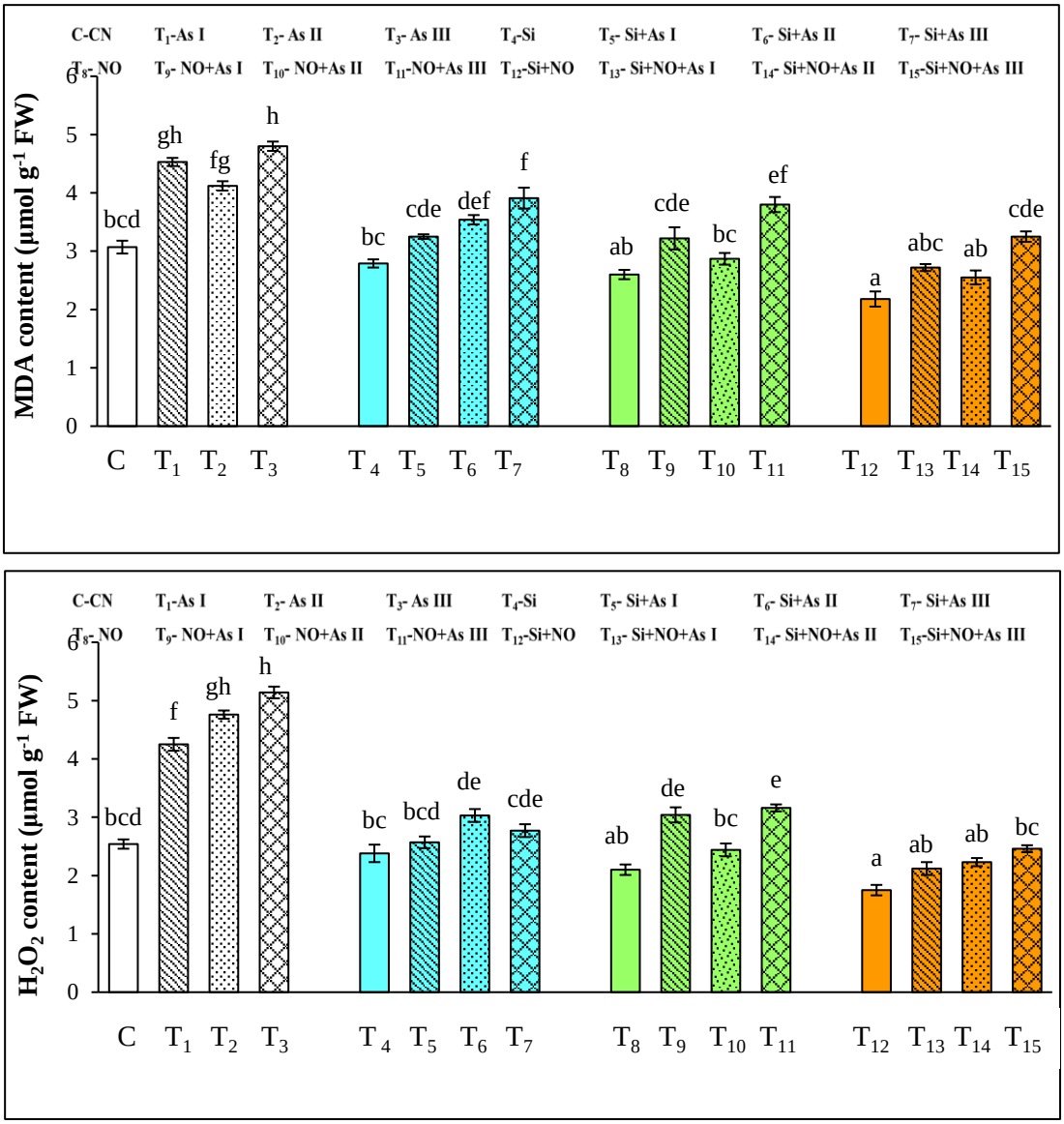

Fig. 6.7 Effect of Si and NO on MDA and H_2O_2 content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

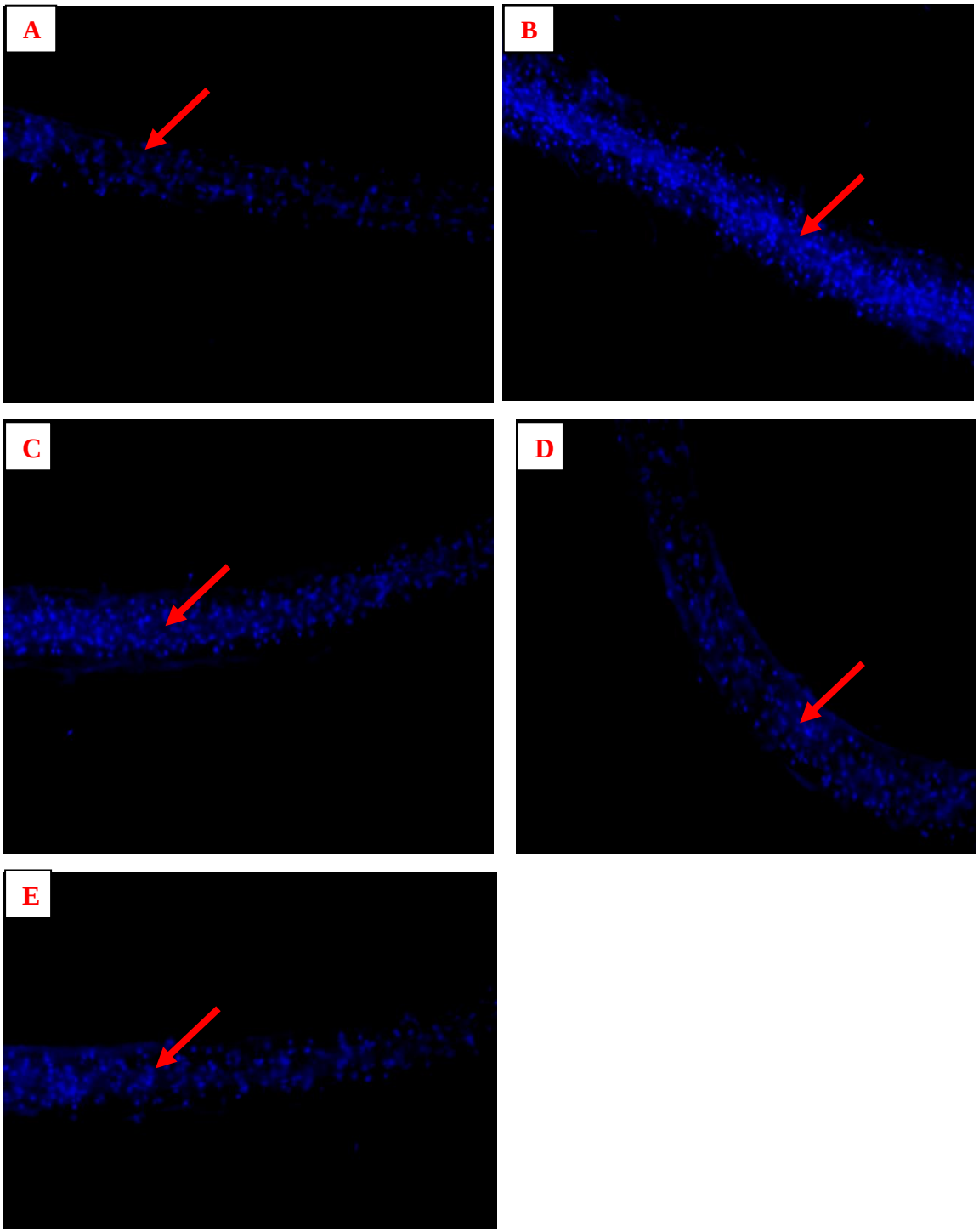

Fig. 6.8 Effect of Si and NO on membrane damage in roots of 7-days old seedlings of *R. sativus* seedlings under As stress by confocal microscope. (A) CN; (B) As III; (C) Si + As III; (D) NO + As III and (E) Si + NO + As III.

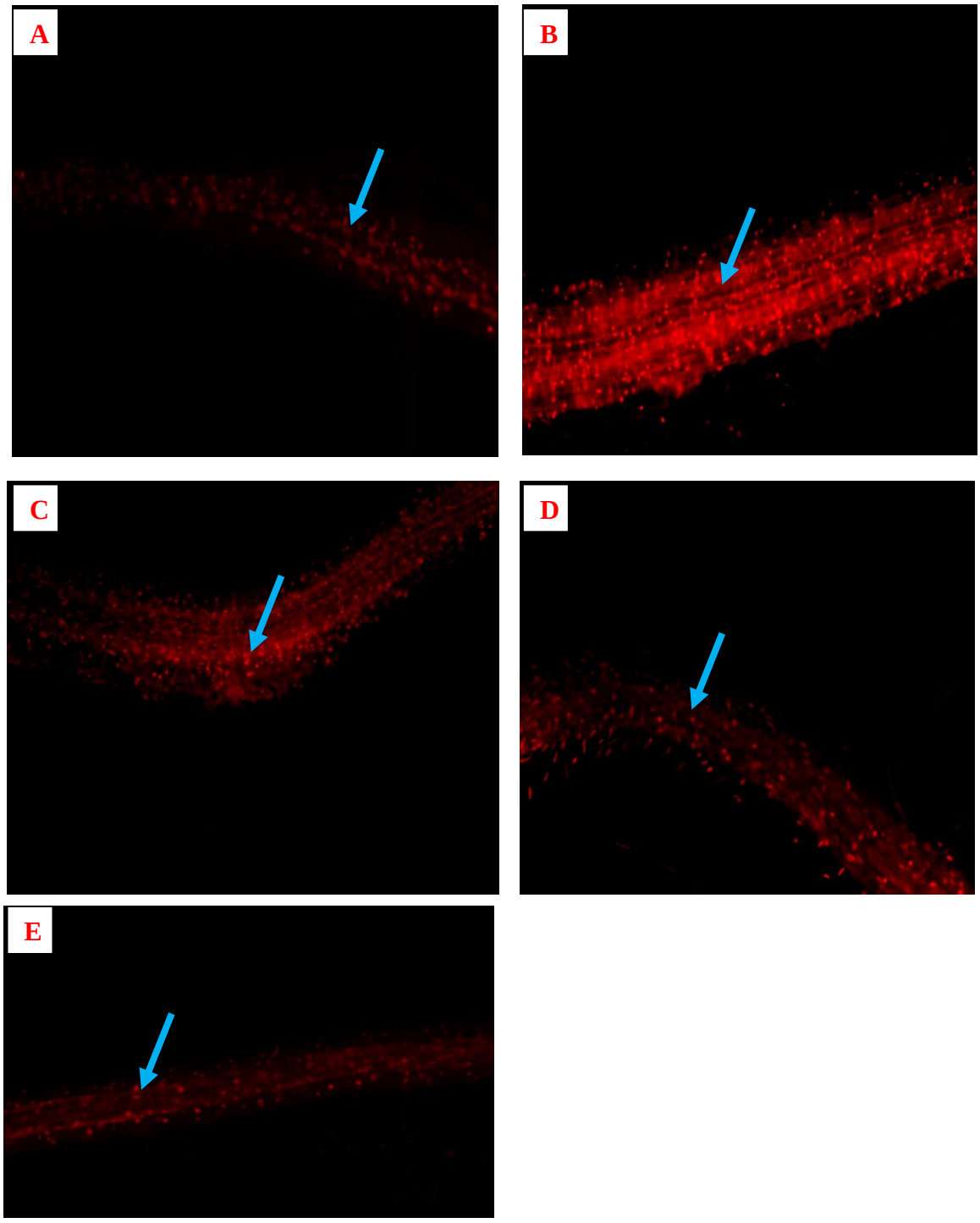

Fig. 6.9 Effect of Si and NO on nuclear damage in roots of 7-days old seedlings of *R. sativus* seedlings under As stress by confocal microscope. (A) CN; (B) As III; (C) Si + As III; (D) NO + As III and (E) Si + NO + As III.

6.1.1.5 Arsenic metalloid uptake

No As content was found in control samples of 7-days old seedlings of radish (Fig 6.11; Table 6.7). Under As alone stress, 0.520 and 0.344 mg g^{-1} DW As content in roots and shoot of radish seedlings, respectively was observed. Exogenous application of Si under stressed conditions reduced the As content with 0.417 and 0.344 mg g^{-1} DW in roots and shoot, respectively. Pre-treatment of radish seedlings with NO also lessened the As in roots and shoot with 0.433 and 0.247 mg g^{-1} DW As content, respectively. Combined supplementation of Si and NO further declined the As content in roots and shoot of radish seedlings with 0.346 and 0.144 mg g^{-1} DW As content.

Table 6.7 Effect of Si and NO on As uptake of 7-days old *R. sativus* seedlings under As stress

Treatment	Root (mg g^{-1} FW)	Shoot (mg g^{-1} FW)
C (Control)	ND	ND
T_3 (AsIII)	0.520^{b}±0.010	0.344^{c}±0.012
T_7 (Si+AsIII)	0.417^{a}±0.029	0.270^{b}±0.013
T_{11} (NO+AsIII)	0.433^{ab}±0.025	0.247^{b}±0.021
T_{15} (Si+NO+AsIII)	0.346^{a}±0.012	0.144^{a}±0.011

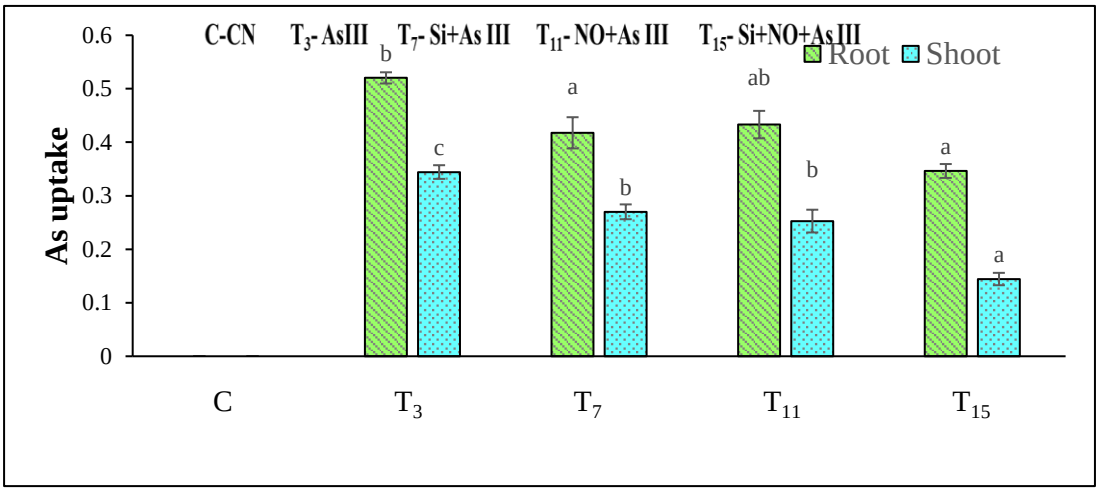

Fig. 6.10 Effect of Si and NO on As uptake in root and shoot of 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.1.6 Osmolytes

Proline content was significantly inhibited by As in radish seedlings (Fig. 6.11; Table 6.8). At all the three concentrations of As i.e. T_1, T_2 and T_3, proline contents were 0.7, 0.49, and 0.38 µ mol g^{-1} FW, respectively. Individual application of Si raised the proline level under stress conditions. Proline content was increased from 0.7 to 1.7 µ mol g^{-1}

FW in T_5 treated seedlings. It was further decreased to 0.92 µ mol g^{-1} FW at T_7 concentration when applied with Si. Proline amount was also elevated by the application of NO under As stress with maximum content (1.15 µ mol g^{-1} FW) at T_9 concentration. Under As stress, the highest proline level (1.37 µ mol g^{-1} FW) was in T_{13} treatment. T_{13} seedlings showed 28.03% increase as compared to T_5. T_{13} exhibited 17.09% increase than control seedlings.

A significant decrease in glycine-betaine content was noticed in As stressed seedlings (Fig. 6.11; Table 6.8). A decrease of 1.69, 1.13 and 0.77 µ mol g^{-1} FW in glycine-betaine was noticed in T_1, T_2 and T_3 seedlings. Glycine betaine contents were 2.76, 2.53 and 1.87 µ mol g^{-1} FW at T_5, T_6 and T_7, respectively when treated with Si. Nitric oxide application showed glycine-betaine levels of 2.48, 1.84 and 1.15 µ mol g^{-1} FW in T_9, T_{10} and T_{11} concentrations, respectively. Furthermore, Si + NO combined treated seedlings under unstressed conditions showed the highest glycine betaine quantities (4.64 µ mol g^{-1} FW) among all the 16 treatments used in the study. Glycine-betaine content was found to be decreased as the level of As elevated as observed in combined Si and NO dosage with maximum content (3.55 µ mol g^{-1} FW) at T_{13}. An increase of 63.31% was noticed in T_5 than T_1. T_9 seedlings exhibited 46.74% elevation than T_1. T_{13} seedlings showed % increase as compared to T_5. T_{13} exhibited 8.23% increase than control seedlings.

6.1.1.7 Total carbohydrates content

Total carbohydrates content was declined by As stress in radish seedlings with the minimum of 0.97 mg g^{-1} FW at T_3 concentration (Fig. 6.12; Table 6.9). Application of Si under unstressed conditions showed 6.02 mg g^{-1} FW carbohydrates content. It was found to be reduced from 4.85 mg g^{-1} FW (T_5) to 2.12 mg g^{-1} FW (T_6). T_{10} showed minimum total carbohydrate content (2.41 mg g^{-1} FW) when pre-treated with NO. Co-application of Si and NO under stressed conditions further improved the total carbohydrates content with the highest content (7.15 mg g^{-1} FW) at T_{14}. T_{13} seedlings showed 30.10% increase as compared to T_5. T_{15} exhibited 59.20% increase than control seedlings.

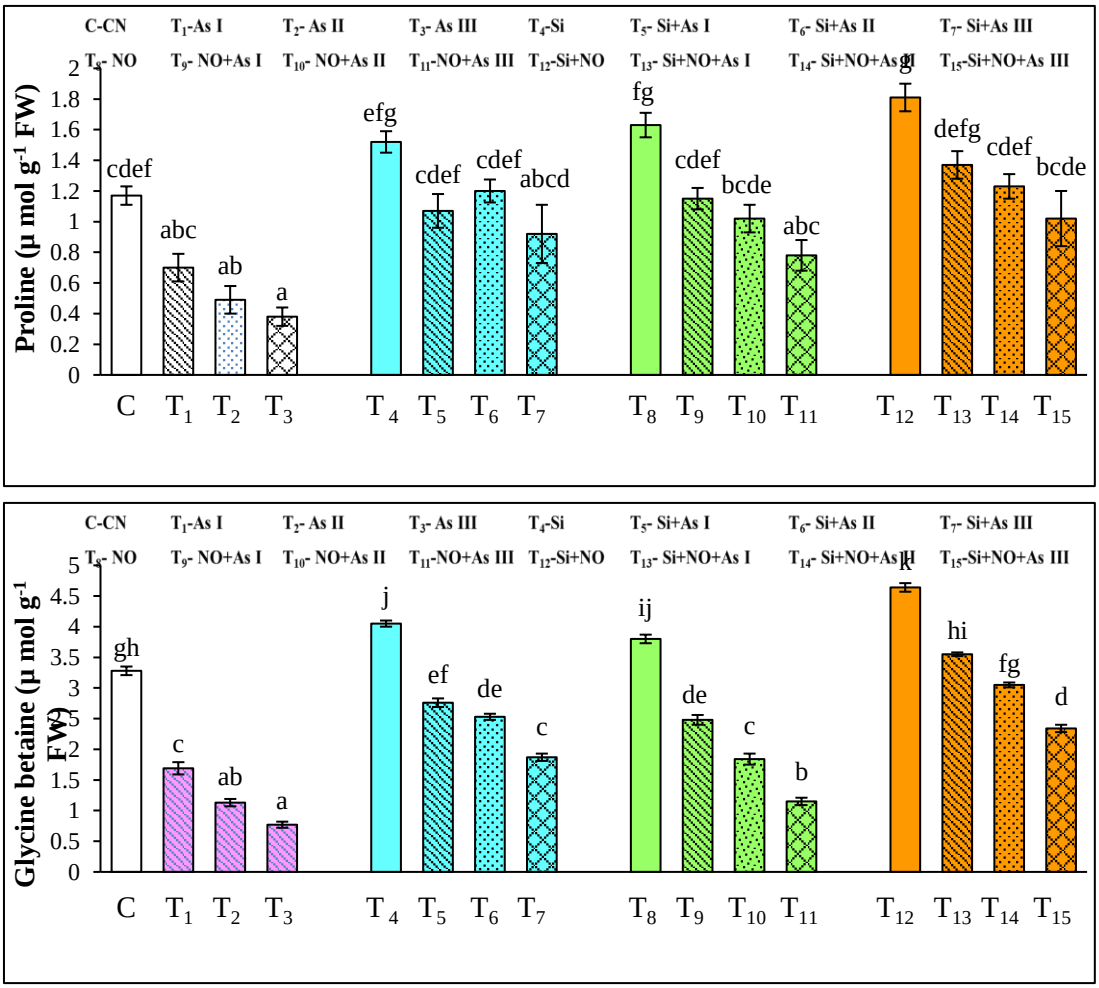

Fig. 6.11 Effect of Si and NO on proline and glycine betaine content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.1.8 Protein content and Antioxidant defense system

6.1.1.8.1 Protein content and antioxidative enzymes

Arsenic toxicity resulted in a reduction in protein content (Fig. 6.13; Table 6.10). In T_1 seedlings, protein content was 0.73 mg g^{-1} FW, which was 50% to the control seedlings. The lowest protein content of 0.22 mg g^{-1} FW was at T_3 treatment. Supplementation of Si escalated the protein content under stressed and unstressed conditions. Silicon control seedlings exhibited 1.87 mg g^{-1} FW protein content. Under As stress, maximum protein content i.e., 1.23 mg g^{-1} FW was reported T_6. Nitric oxide pre-treated seedlings showed high protein content under As stress, as compared to Si-treated seedlings. The highest (1.56 mg g^{-1} FW) and lowest (0.89 mg g^{-1} FW) protein content was noticed at

T_{10} and T_{11} concentrations, respectively when pre-treated with NO. In the synergistic application of Si and NO, the highest (1.96 mg g^{-1} FW) content was in T_{14} seedlings. T_{13} seedlings showed 48.62% increase as compared to T_5. T_{14} exhibited 33.33% increase than control seedlings.

The activity of the SOD enzyme was diminished under As stress in 7-days old seedlings of radish (Fig. 6.13; Table 6.10). Minimum SOD activity of 2.47 UA mg^{-1} protein was found in T_3 stressed seedlings. In the case of Si-applied seedlings under As stress, the highest SOD activity (5.16 UA mg^{-1} protein) was at T_5. Nitric oxide application also increased its performance, in which the highest (4.3 UA mg^{-1} protein) and lowest (3.28 UA mg^{-1} protein) SOD activity were at T_9 and T_{11}, respectively. Si + NO further increased the SOD activity under stressed conditions with maximum (6.11 UA mg^{-1} protein) at T_{13} concentration. An increase of 39.45% was noticed in T_5 than T_1. T_9 seedlings exhibited 16.21% elevation than T_1. T_{13} seedlings showed 18.41% increase as compared to T_5. T_{13} exhibited 10.09% increase than control seedlings.

Table 6.8 Effect of Si and NO on osmolytes of 7-days old *R. sativus* seedlings under As stress

Treatment	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
C (Control)	1.17^{cdef}±0.06	3.28^{gh}±0.07
T_1 (AsI)	0.7^{abc}±0.09	1.69^{c}±0.1
T_2 (AsII)	0.49^{ab}±0.09	1.13^{ab}±0.06
T_3 (AsIII)	0.38^{a}±0.06	0.77^{a}±0.05
T_4 (Si)	1.52^{efg}±0.07	4.05^{j}±0.05
T_5 (Si+AsI)	1.07^{cdef}±0.11	2.76^{ef}±0.07
T_6 (Si+AsII)	1.2^{cdef}±0.075	2.53^{de}±0.05
T_7 (Si+AsIII)	0.92^{abcd}±0.19	1.87^{c}±0.06
T_8 (NO)	1.63^{fg}±0.08	3.8^{ij}±0.07
T_9 (NO+AsI)	1.15^{cdef}±0.07	2.48^{de}±0.08
T_{10} (NO+AsII)	1.02^{bcde}±0.09	1.84^{c}±0.09
T_{11} (NO+AsIII)	0.78^{abc}±0.1	1.15^{b}±0.06
T_{12} (Si+NO)	1.81^{g}±0.09	4.64^{k}±0.07
T_{13} (Si+NO+AsI)	1.37^{defg}±0.09	3.55^{hi}±0.03
T_{14} (Si+NO+AsII)	1.23^{cdef}±0.08	3.05^{fg}±0.04
T_{15} (Si+NO+AsIII)	1.02^{bcde}±0.18	2.34^{d}±0.06

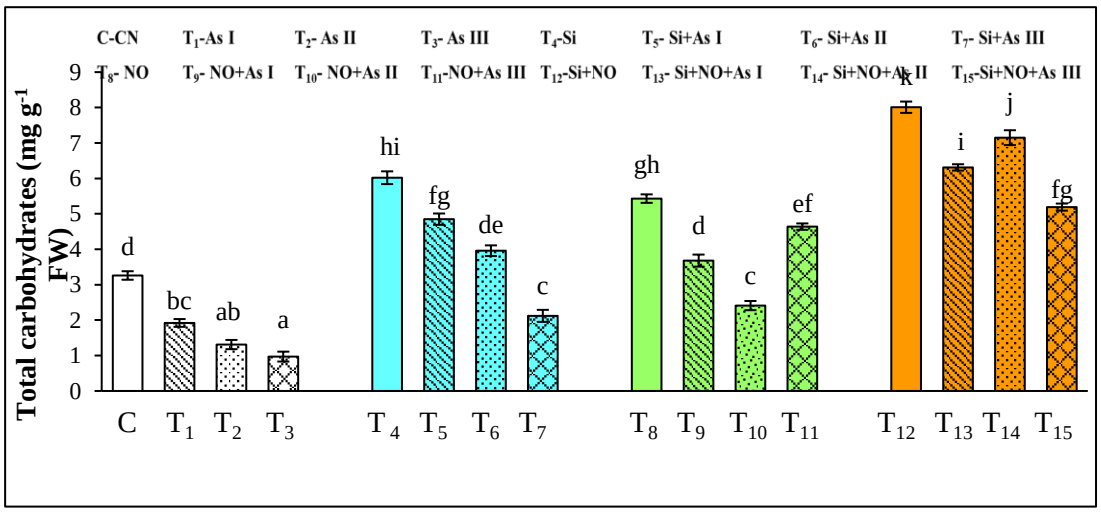

Fig. 6.12 Effect of Si and NO on total carbohydrates in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.9 Effect of Si and NO on total carbohydrates content of 7-days old *R. sativus* seedlings under As stress

Treatment	**Total carbohydrates (mg g^{-1} FW)**
C (Control)	3.26^{d}±0.12
T_1 (AsI)	1.92^{bc}±0.11
T_2 (AsII)	1.31^{ab}±0.13
T_3 (AsIII)	0.97^{a}±0.14
T_4 (Si)	6.02^{hi}±0.18
T_5 (Si+AsI)	4.85^{fg}±0.16
T_6 (Si+AsII)	3.96^{de}±0.15
T_7 (Si+AsIII)	2.12^{c}±0.17
T_8 (NO)	5.43^{gh}±0.12
T_9 (NO+AsI)	3.68^{d}±0.17
T_{10} (NO+AsII)	2.41c±0.13
T_{11} (NO+AsIII)	4.64^{ef}±0.09
T_{12} (Si+NO)	8.01^{k}±0.16
T_{13} (Si+NO+AsI)	6.31^{i}±0.09
T_{14} (Si+NO+AsII)	7.15^{j}±0.21
T_{15} (Si+NO+AsIII)	5.19^{fg}±0.1

Catalase activity was diminished under As stress with minimum of 1.35 UA mg^{-1} protein at T_3 concentration (Fig. 6.13; Table 6.10). Individual Si boosted the CAT activity than As alone treated seedlings. Exogenous Si application under As toxicity showed minimum CAT activity of 2.31 UA mg^{-1} protein at T_7 concentration. Applying the mitigant NO under As stress caused an escalation in the CAT content with the

highest activity of 2.99 UA mg^{-1} protein in T_9 seedlings. Its activity was observed to be lessened as As level increased in the case of Si + NO with a maximum activity of 3.8 UA mg^{-1} protein at T_{13}. An increase of 51.94% was noticed in T_5 than T_1. T_9 seedlings exhibited 45.14% elevation than T_1. T_{13} seedlings showed 21.40% increase as compared to T_5. T_{13} exhibited 22.58% increase than control seedlings.

Arsenic stress led to the maximum reduction in the APX enzyme activity with the highest reduction of 2.82 UA mg^{-1} protein at T_3 concentration (Fig. 6.14; Table 6.10). Treating the radish plants with Si and NO alone under As stress markedly upsurged the APX level as compared to the As alone. The maximum content of 5.13 UA mg^{-1} protein in the case of Si treatment was noticed at T_5 while in NO treated seedlings, higher APX activity (4.83 UA mg^{-1} protein) was also in T_9 stressed seedlings. Under As stress, the highest APX enzyme activity of 5.88 UA mg^{-1} protein got observed at combined treatments of Si and NO at T_{13} concentration, among all the treatments used. An increase of 33.94% was noticed in T_5 than T_1. T_9 seedlings exhibited 26.10% elevation than T_1. T_{13} seedlings showed 14.61% increase as compared to T_5. T_{13} exhibited 33.63% increase than control seedlings.

Table 6.10 Effect of Si and NO on protein content and antioxidative enzymes of 7-days old *R. sativus* seedlings under As stress

Treatment	**Protein content (mg g^{-1} FW)**	**SOD (UA mg^{-1} protein)**	**CAT (UA mg^{-1} protein)**	**APX (UA mg^{-1} protein)**
C (Control)	$1.47^{efg}\pm0.09$	$5.55^{ef}\pm0.14$	$3.1^{efg}\pm0.08$	$4.4^{de}\pm0.08$
T_1 (AsI)	$0.43^{ab}\pm0.05$	$3.7^{bc}\pm0.18$	$2.06^{bc}\pm0.06$	$3.83^{bc}\pm0.1$
T_2 (AsII)	$0.73^{bc}\pm0.09$	$3.15^{ab}\pm0.25$	$1.73^{ab}\pm0.09$	$3.37^{b}\pm0.08$
T_3 (AsIII)	$0.22^{a}\pm0.09$	$2.47^{a}\pm0.15$	$1.35^{a}\pm0.04$	$2.82^{a}\pm0.1$
T_4 (Si)	$1.87^{ghi}\pm0.13$	$6.66^{gh}\pm0.18$	$3.85^{h}\pm0.06$	$5.64^{hi}\pm0.09$
T_5 (Si+AsI)	$1.09^{cde}\pm0.04$	$5.16^{de}\pm0.17$	$3.13^{fg}\pm0.14$	$5.13^{fg}\pm0.1$
T_6 (Si+AsII)	$1.23^{def}\pm0.03$	$4.54^{cd}\pm0.18$	$2.65^{de}\pm0.07$	$4.81^{ef}\pm0.09$
T_7 (Si+AsIII)	$0.86^{bcd}\pm0.07$	$3.74^{bc}\pm0.14$	$2.31^{cd}\pm0.07$	$4.1^{cd}\pm0.13$
T_8 (NO)	$2.15^{i}\pm0.03$	$6.37^{fg}\pm0.15$	$3.64^{h}\pm0.06$	$5.34^{gh}\pm0.06$
T_9 (NO+AsI)	$1.26^{def}\pm0.06$	$4.3^{cd}\pm0.3$	$2.99^{ef}\pm0.08$	$4.83^{ef}\pm0.06$
T_{10} (NO+AsII)	$1.56^{fgh}\pm0.06$	$3.64^{bc}\pm0.09$	$2.68^{def}\pm0.07$	$4.53^{de}\pm0.11$
T_{11} (NO+AsIII)	$0.89^{cd}\pm0.08$	$3.28^{ab}\pm0.08$	$2.46^{cd}\pm0.08$	$4.16^{cd}\pm0.06$
T_{12} (Si+NO)	$2.22^{i}\pm0.13$	$7.38^{h}\pm0.13$	$4.38^{i}\pm0.11$	$6.68^{j}\pm0.09$
T_{13} (Si+NO+AsI)	$1.62^{fgh}\pm0.07$	$6.11^{fg}\pm0.14$	$3.8^{h}\pm0.09$	$5.88^{i}\pm0.1$
T_{14} (Si+NO+AsII)	$1.96^{hi}\pm0.08$	$5.63^{ef}\pm0.13$	$3.54^{gh}\pm0.09$	$5.54^{ghi}\pm0.05$
T_{15} (Si+NO+AsIII)	$1.35^{ef}\pm0.06$	$5.08^{de}\pm0.17$	$3.04^{ef}\pm0.1$	$5.08^{fg}\pm0.1$

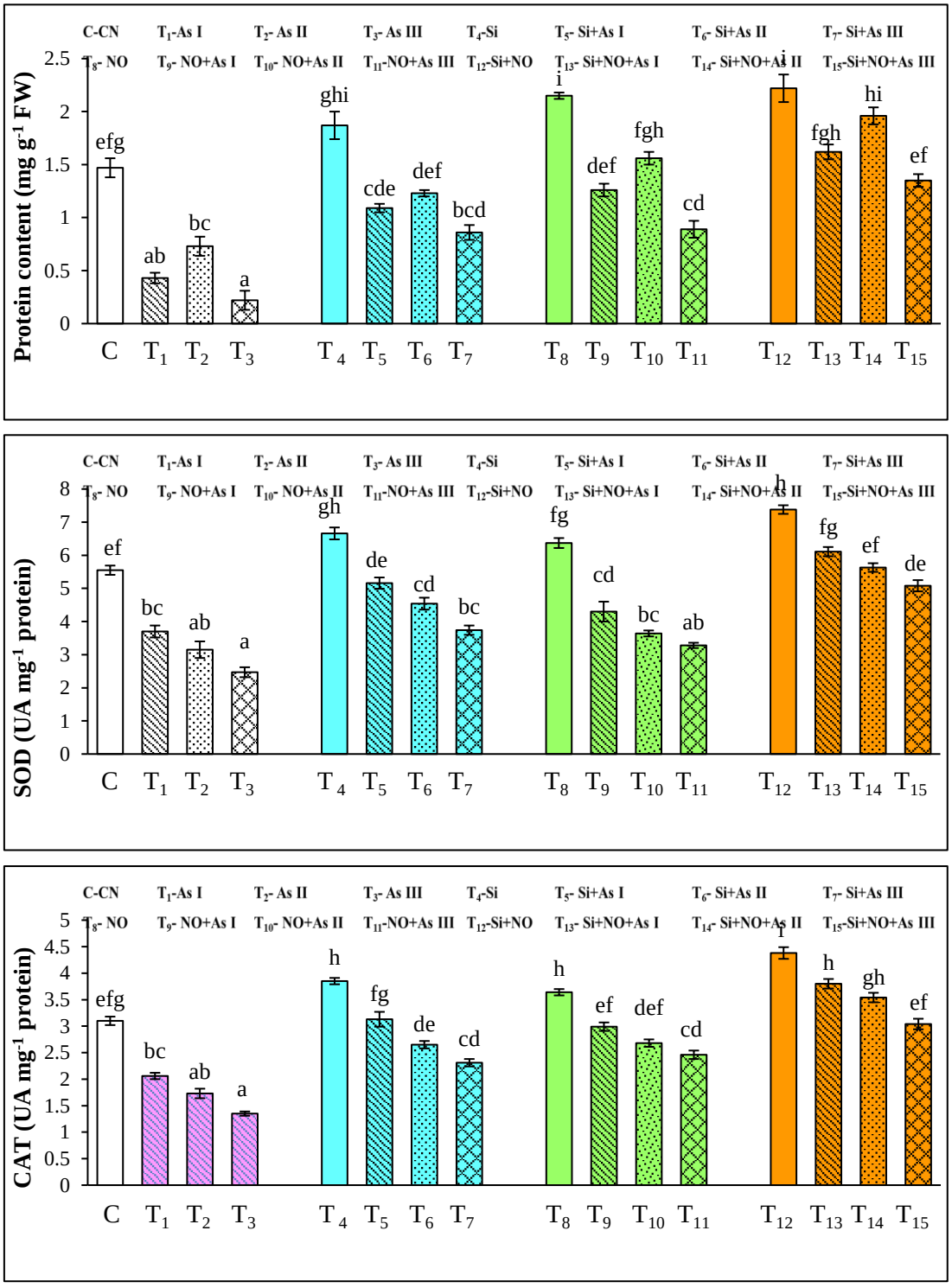

Fig. 6.13 Effect of Si and NO on protein, SOD and CAT enzyme activity in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Arsenic toxicity significantly declined the POD activity, as compared to control (Fig. 6.14; Table 6.11). Under As stress conditions, the activity of the POD enzyme was minimum at T_3 concentration with a content of 3.34 UA mg^{-1} protein. Maximum (5.35 UA mg^{-1} protein) and minimum (4.64 UA mg^{-1} protein) POD activity in the case of Si supplementation were reported in T_5 and T_7 stressed seedlings. NO treatment under stressed conditions showed the highest POD activity (5.34 UA mg^{-1} protein) at T_9. In combination, Si + NO were more efficient in escalating the POD activity under As stress as compared to their individual applications. It can be substantiated by the highest POD activity of 6.28 UA mg^{-1} protein at T_{13} concentration when plants were treated with Si + NO. An increase of 25.58% was noticed in T_5 than T_1. T_9 seedlings exhibited 25.35% elevation than T_1. T_{13} seedlings showed 17.38% increase as compared to T_5. T_{13} exhibited 17.38% increase than control seedlings.

Arsenic stress led to the maximum reduction in the GR activity with the highest reduction of 1.55 UA mg^{-1} protein at T_3 concentration (Fig. 6.14; Table 6.11). Control seedlings showed 4.01 UA mg^{-1} protein GR activity. Application of Si markedly augmented the GR level with a maximum of 3.86 UA mg^{-1} protein at T_6 plants. Application of NO also boosted its activity with a minimum of 2.75 UA mg^{-1} protein at T_{11}. The highest 4.51 UA mg^{-1} protein GR activity was observed at T_{13} treatment. An increase of 20.21% was noticed in T_5 than T_1. T_9 seedlings exhibited 32.12% elevation than T_1. T_{13} seedlings showed 35.43% increase as compared to T_5. T_{13} exhibited 12.46% increase than control seedlings.

GPOX activity was diminished in As treated radish with 1.82, 1.38, and 0.67 UA mg^{-1} protein at As concentrations namely T_1, T_2 and T_3, respectively (Fig. 6.15; Table 6.11). Individual Si treatment enlarged the GPOX activity than As alone treated seedlings. Exogenously applied Si under As stress showed minimum GPOX activity of 2.7 UA mg^{-1} protein at T_7. Nitric oxide application under As stress boosted the GPOX level with the highest activity of 3.61 UA mg^{-1} protein in T_9 seedlings. It was observed to be decreased as As level elevated as observed in coupled treatment of Si + NO with the maximum activity of 4.46 UA mg^{-1} protein at T_{13}. An increase of 88.46% was noticed in T_5 than T_1. T_{13} seedlings showed 30.02% increase as compared to T_5. A 42.03% increase was found in T_{13} than control seedlings.

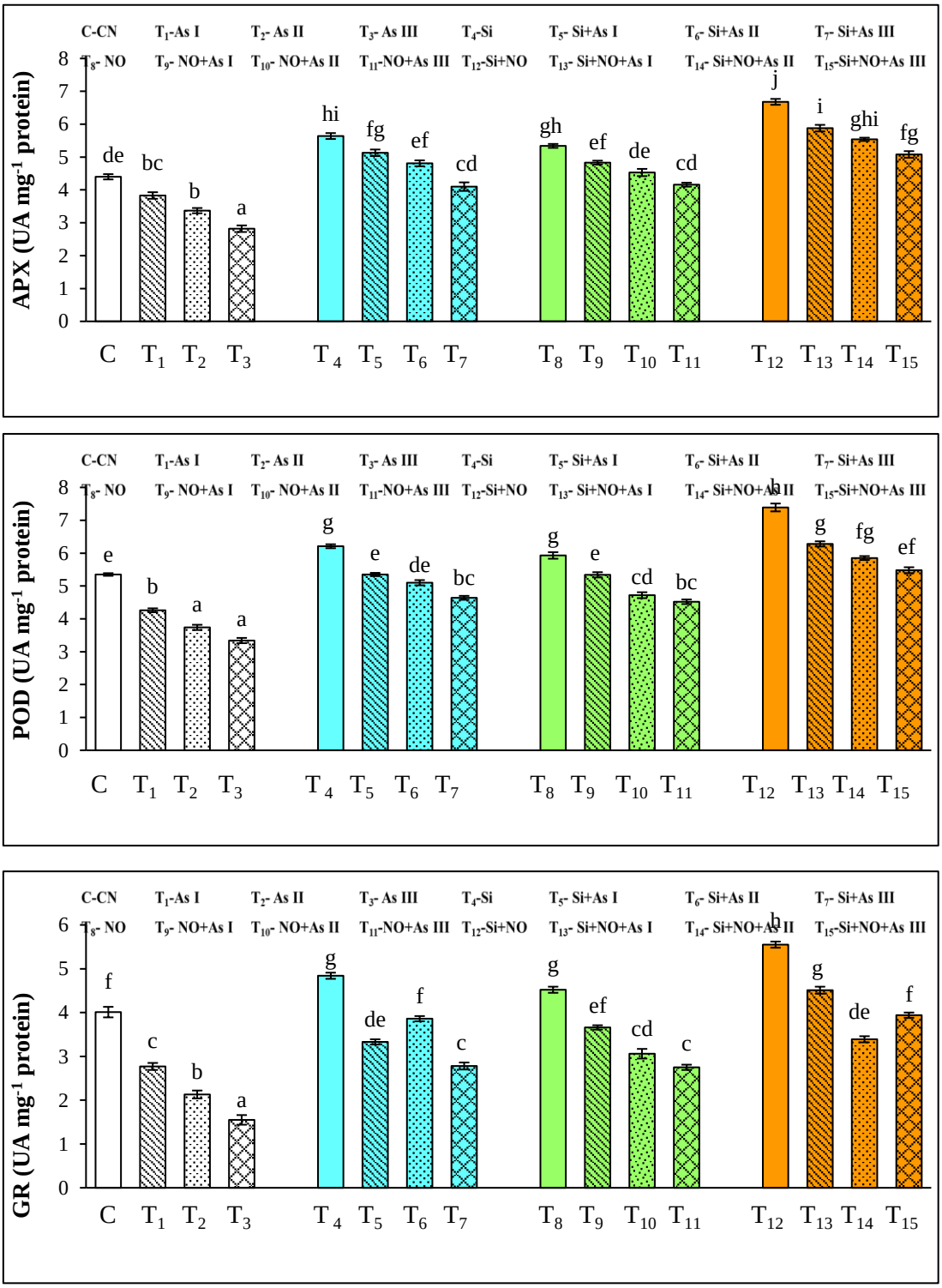

Fig. 6.14 Effect of Si and NO on APX, POD and GR enzyme activity in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

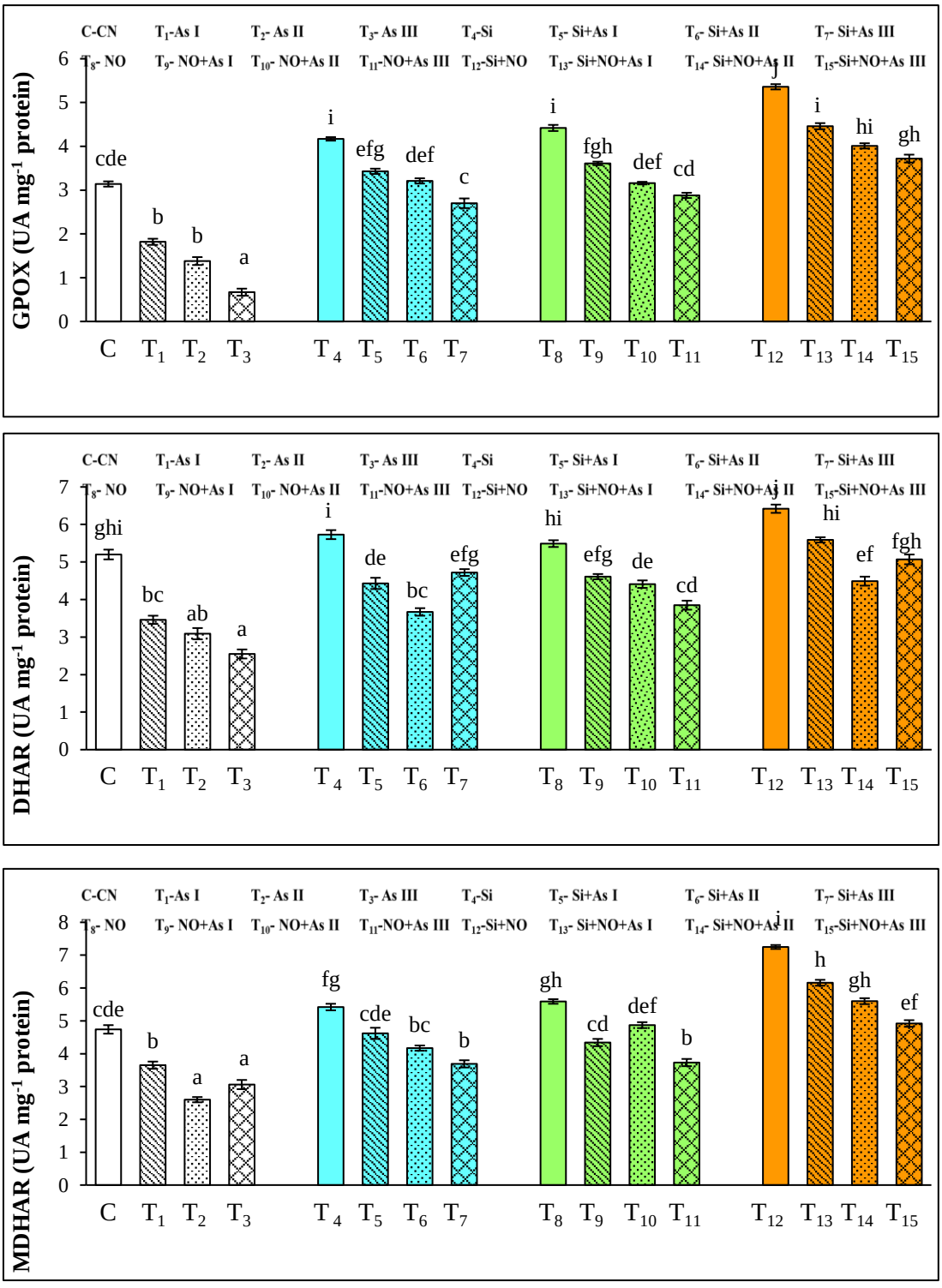

Fig. 6.15 Effect of Si and NO on GPOX, DHAR and MDHAR enzyme activity in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.11 Effect of Si and NO on antioxidative enzymes of 7-days old *R. sativus* seedlings under As stress

Treatment	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
C (Control)	$5.35^{e}\pm0.04$	$4.01^{f}\pm0.12$	$3.14^{cde}\pm0.06$	$5.2^{ghi}\pm0.13$
T_1 (AsI)	$4.26^{b}\pm0.06$	$2.77^{c}\pm0.08$	$1.82^{b}\pm0.13$	$3.46^{bc}\pm0.11$
T_2 (AsII)	$3.74^{a}\pm0.08$	$2.13^{b}\pm0.09$	$1.38^{b}\pm0.09$	$3.09^{ab}\pm0.15$
T_3 (AsIII)	$3.34^{a}\pm0.08$	$1.55^{a}\pm0.11$	$0.67^{a}\pm0.1$	$2.55^{a}\pm0.12$
T_4 (Si)	$6.21^{g}\pm0.06$	$4.84^{g}\pm0.07$	$4.17^{i}\pm0.08$	$5.73^{i}\pm0.12$
T_5 (Si+AsI)	$5.35^{e}\pm0.05$	$3.33^{de}\pm0.06$	$3.43^{efg}\pm0.07$	$4.43^{de}\pm0.15$
T_6 (Si+AsII)	$5.1^{de}\pm0.08$	$3.86^{f}\pm0.06$	$3.21^{def}\pm0.1$	$3.67^{bc}\pm0.1$
T_7 (Si+AsIII)	$4.64^{bc}\pm0.06$	$2.78^{c}\pm0.08$	$2.7^{c}\pm0.12$	$4.72^{efg}\pm0.09$
T_8 (NO)	$5.93^{g}\pm0.1$	$4.52^{g}\pm0.07$	$4.42^{i}\pm0.1$	$5.49^{hi}\pm0.09$
T_9 (NO+AsI)	$5.34^{e}\pm0.08$	$3.66^{ef}\pm0.05$	$3.61^{fgh}\pm0.07$	$4.61^{efg}\pm0.07$
T_{10} (NO+AsII)	$4.72^{cd}\pm0.09$	$3.06^{cd}\pm0.11$	$3.16^{def}\pm0.06$	$4.41^{de}\pm0.1$
T_{11} (NO+AsIII)	$4.52^{bc}\pm0.07$	$2.75^{c}\pm0.06$	$2.88^{cd}\pm0.04$	$3.85^{cd}\pm0.12$
T_{12} (Si+NO)	$7.39^{h}\pm0.12$	$5.55^{h}\pm0.07$	$5.36^{j}\pm0.5$	$6.42^{j}\pm0.11$
T_{13} (Si+NO+AsI)	$6.28^{g}\pm0.08$	$4.51^{g}\pm0.08$	$4.46^{i}\pm0.07$	$5.59^{hi}\pm0.07$
T_{14} (Si+NO+AsII)	$5.85^{fg}\pm0.06$	$3.39^{de}\pm0.07$	$4.01^{hi}\pm0.08$	$4.49^{ef}\pm0.12$
T_{15} (Si+NO+AsIII)	$5.48^{ef}\pm0.09$	$3.94^{f}\pm0.06$	$3.72^{gh}\pm0.04$	$5.07^{fgh}\pm0.13$

DHAR enzyme activity was diminished under As stress in 7-days old seedlings of radish (Fig. 6.15; Table 6.11). Maximum DHAR activity of 3.46 UA mg^{-1} protein was found in T_1 treated seedlings. In the case of Si-applied seedlings under As stress, the highest DHAR activity (4.72 UA mg^{-1} protein) was at T_7. NO application also increased the DHAR activity under stress conditions, in which the lowest (3.85 UA mg^{-1} protein) DHAR activity was at T_{11} concentration. Si + NO further augmented the DHAR activity under stressed conditions with maximum activity (5.59 UA mg^{-1} protein) at T_{13} treatment. An increase of 28.03% was noticed in T_5 than T_1. T_9 seedlings exhibited 33.23% elevation than T_1. T_{13} seedlings showed 26.18% increase as compared to T_5. T_{13} showed 7.5% increase than control seedlings.

MDHAR enzyme action was noticed to be negatively hampered under As stress (Fig. 6.15; Table 6.12). The minimum MDHAR activity of 2.6 UA mg^{-1} protein was in T_2 treated seedlings. MDHAR activity was found to be increased from 3.65 to 4.62 UA mg^{-1} protein in Si-treated T_5 seedlings. Pre-treatment with NO also increased the MDHAR activity under As stress with the highest 4.87 UA mg^{-1} protein activity in

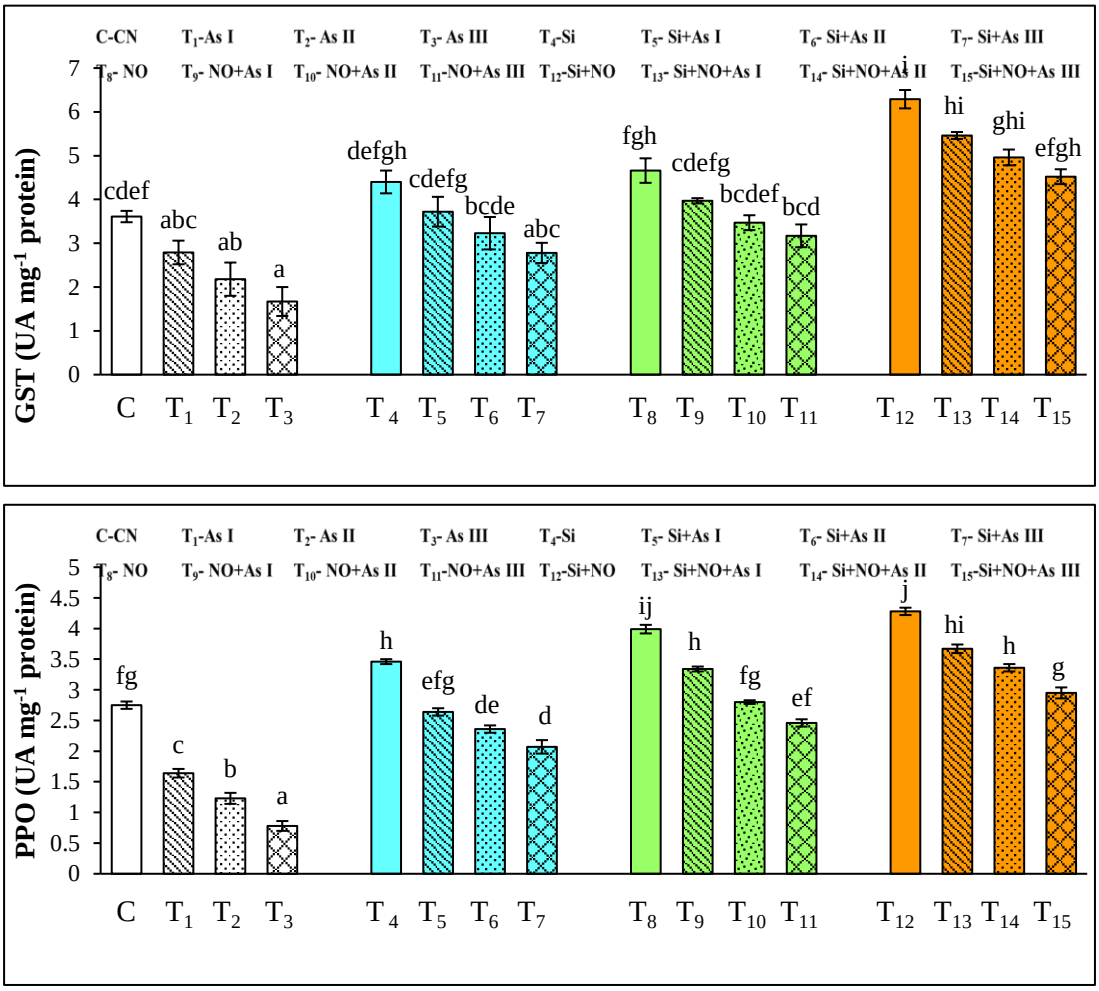

Fig. 6.16 Effect of Si and NO on GST and PPO enzyme activity in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

T_{10} seedlings. The highest MDHAR activity (6.16 UA mg^{-1} protein) got noticed in the case of Si and NO combined application at T_{13}. An increase of 26.57% was noticed in T_5 than T_1. T_9 seedlings exhibited 18.90% elevation than T_1. T_{13} seedlings showed 33.33% increase as compared to T_5. T_{13} showed 29.95% increase than control seedlings.

GST activity was 2.79, 2.18 and 1.67 UA mg^{-1} protein at As concentrations namely T_1, T_2 and T_3, respectively (Fig. 6.16; Table 6.12). Highest and lowest GST activities were found at T_5 and T_7 concentrations with 3.72 and 2.78 UA mg^{-1} protein, respectively, in Si applied seedlings. In NO-pre-treated seedlings under stressed conditions, maximum GST activity (3.97 UA mg^{-1} protein) was in T_9 stressed seedlings. Si + NO further boosted the GST activity under stressed conditions with the highest 5.46 UA mg^{-1}

protein GST activity in T_{13} treated seedlings. An increase of 33.33% was noticed in T_5 than T_1. T_9 seedlings exhibited 42.29% elevation than T_1. T_{13} seedlings showed 46.77% increase as compared to T_5. T_{13} showed 51.24% increase than control seedlings.

PPO enzyme was lowest at the highest concentration of As i.e. T_3 (0.78 UA mg^{-1} protein) in comparison to control plants (Fig. 6.16; Table 6.12). However, treatment with Si under As stress showed maximum PPO activity of 2.64 UA mg^{-1} protein in T_5 plants. Whereas in the case of NO treated seedlings under unstressed conditions, maximum PPO activity of 3.99 UA mg^{-1} protein (T_8) was observed. Application of NO in As stressed radish plants showed minimum PPO activity of 2.46 UA mg^{-1} protein at T_{11}. In Si and NO (combined) seedlings, the highest PPO activity (3.67 UA mg^{-1} protein) got recorded in T_{13} stressed seedlings. An increase of 60.97% was noticed in T_5 than T_1. T_{13} seedlings showed 39.01% and 33.45% increase as compared to T_5 and control seedlings, respectively.

Table 6.12 Effect of Si and NO on antioxidative enzymes of 7-days old *R. sativus* seedlings under As stress

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
C (Control)	$4.74^{cde}\pm0.13$	$3.61^{cdef}\pm0.13$	$2.75^{fg}\pm0.06$
T_1 (AsI)	$3.65^{b}\pm0.11$	$2.79^{abc}\pm0.27$	$1.64^{c}\pm0.07$
T_2 (AsII)	$2.6^{a}\pm0.08$	$2.18^{ab}\pm0.38$	$1.23^{b}\pm0.09$
T_3 (AsIII)	$3.06^{a}\pm0.14$	$1.67^{a}\pm0.33$	$0.78^{a}\pm0.08$
T_4 (Si)	$5.42^{fg}\pm0.1$	$4.4^{defgh}\pm0.26$	$3.46^{h}\pm0.04$
T_5 (Si+AsI)	$4.62^{cde}\pm0.17$	$3.72^{cdefg}\pm0.34$	$2.64^{efg}\pm0.06$
T_6 (Si+AsII)	$4.17^{bc}\pm0.08$	$3.23^{bcde}\pm0.37$	$2.36^{de}\pm0.06$
T_7 (Si+AsIII)	$3.69^{b}\pm0.11$	$2.78^{abc}\pm0.23$	$2.07^{d}\pm0.11$
T_8 (NO)	$5.59^{gh}\pm0.07$	$4.66^{fgh}\pm0.28$	$3.99^{ij}\pm0.07$
T_9 (NO+AsI)	$4.34^{cd}\pm0.11$	$3.97^{cdefg}\pm0.06$	$3.34^{h}\pm0.04$
T_{10} (NO+AsII)	$4.87^{def}\pm0.09$	$3.47^{bcdef}\pm0.17$	$2.8^{fg}\pm0.03$
T_{11} (NO+AsIII)	$3.73^{b}\pm0.11$	$3.17^{bcd}\pm0.26$	$2.46^{ef}\pm0.06$
T_{12} (Si+NO)	$7.25^{i}\pm0.6$	$6.29^{i}\pm0.21$	$4.28^{j}\pm0.06$
T_{13} (Si+NO+AsI)	$6.16^{h}\pm0.09$	$5.46^{hi}\pm0.08$	$3.67^{hi}\pm0.07$
T_{14} (Si+NO+AsII)	$5.6^{gh}\pm0.09$	$4.96^{ghi}\pm0.18$	$3.36^{h}\pm0.06$
T_{15} (Si+NO+AsIII)	$4.92^{ef}\pm0.1$	$4.52^{efgh}\pm0.17$	$2.95^{g}\pm0.09$

6.1.1.8.2 Non-enzymatic antioxidants

Ascorbic acid amount was reduced under all three concentrations of As stress (Fig. 6.17; Table 6.13). The maximum content of 1.75 µg g^{-1} FW was noticed at T_2 concentration among all three As concentrations used. In the case of Si applied

seedlings under As stress, the minimum ascorbic acid content of 1.71 µg g^{-1} FW was noticed at T_7 whereas in NO pre-treated seedlings, minimum ascorbic acid content (1.99 µg g^{-1} FW) was also in T_{11} seedlings. However, coupled application of Si and NO in case of As stress showed maximum ascorbic acid content of 2.47 µg g^{-1} FW at T_{14}. An increase of 35.25% was noticed in T_5 than T_1. T_9 seedlings exhibited 63.46% elevation than T_1. T_{13} seedlings showed 28.43% and 7.11 % increase as compared to T_5 and control, respectively.

Arsenic stress significantly decreased the content of glutathione with the minimum content of 0.65 µg g^{-1} FW at T_2 in comparison to control seedlings (Fig. 6.17; Table 6.13). However, treatment with Si under As stress exhibited maximum glutathione content of 1.6 µg g^{-1} FW in T_5. Whereas in the case of NO-treated seedlings under unstressed conditions (T_8), maximum glutathione content of 2.36 µg g^{-1} FW was observed. Application of NO under As stress showed minimum glutathione content of 1.58 µg g^{-1} FW in T_{11}. In Si and NO combination, the highest glutathione content (2.67 µg g^{-1} FW) was in T_{14} stressed seedlings. T_{13} seedlings showed 53.12% and 6.01% increase as compared to T_5 and control, respectively.

Table 6.13 Effect of Si and NO on non-enzymatic antioxidants of *R. sativus* under As stress

Treatment	**Ascorbic acid (µg g^{-1} FW)**	**Glutathione (µg g^{-1} FW)**	**Tocopherol (µg g^{-1} FW)**
C (Control)	2.53^{defg}±0.08	1.64^{cde}±0.09	1.25^{ef}±0.08
T_1 (AsI)	1.56^{b}±0.11	0.74^{ab}±0.06	0.65^{abc}±0.04
T_2 (AsII)	1.75^{bc}±0.06	0.65^{a}±0.07	0.4^{ab}±0.05
T_3 (AsIII)	0.89^{a}±0.18	1.15^{abc}±0.15	0.21^{a}±0.02
T_4 (Si)	2.8^{fg}±0.03	2.3^{fg}±0.12	1.76^{ghi}±0.04
T_5 (Si+AsI)	2.11^{bcde}±0.17	1.6^{cd}±0.1	0.96^{cde}±0.05
T_6 (Si+AsII)	2.34^{cdef}±0.07	1.55^{cd}±0.09	1.16^{def}±0.06
T_7 (Si+AsIII)	1.71^{bc}±0.1	1.25^{bc}±0.07	0.75^{bcd}±0.07
T_8 (NO)	3.07^{g}±0.17	2.36^{fg}±0.13	1.88^{ij}±0.13
T_9 (NO+AsI)	2.55^{defg}±0.09	1.61^{cd}±0.15	1.4^{efgh}±0.09
T_{10} (NO+AsII)	2.18^{bcdef}±0.13	2^{def}±0.08	1.08^{cde}±0.06
T_{11} (NO+AsIII)	1.99^{bcd}±0.16	1.58^{cd}±0.07	0.71^{bcd}±0.07
T_{12} (Si+NO)	3.74^{h}±0.12	3.15^{h}±0.12	2.33^{j}±0.1
T_{13} (Si+NO+AsI)	2.71^{efg}±0.08	2.45^{fg}±0.09	1.33^{efg}±0.11
T_{14} (Si+NO+AsII)	2.47^{defg}±0.11	2.67^{gh}±0.06	1.79^{hi}±0.13
T_{15} (Si+NO+AsIII)	2.58^{defg}±0.06	2.16^{efg}±0.08	1.55^{fghi}±0.1

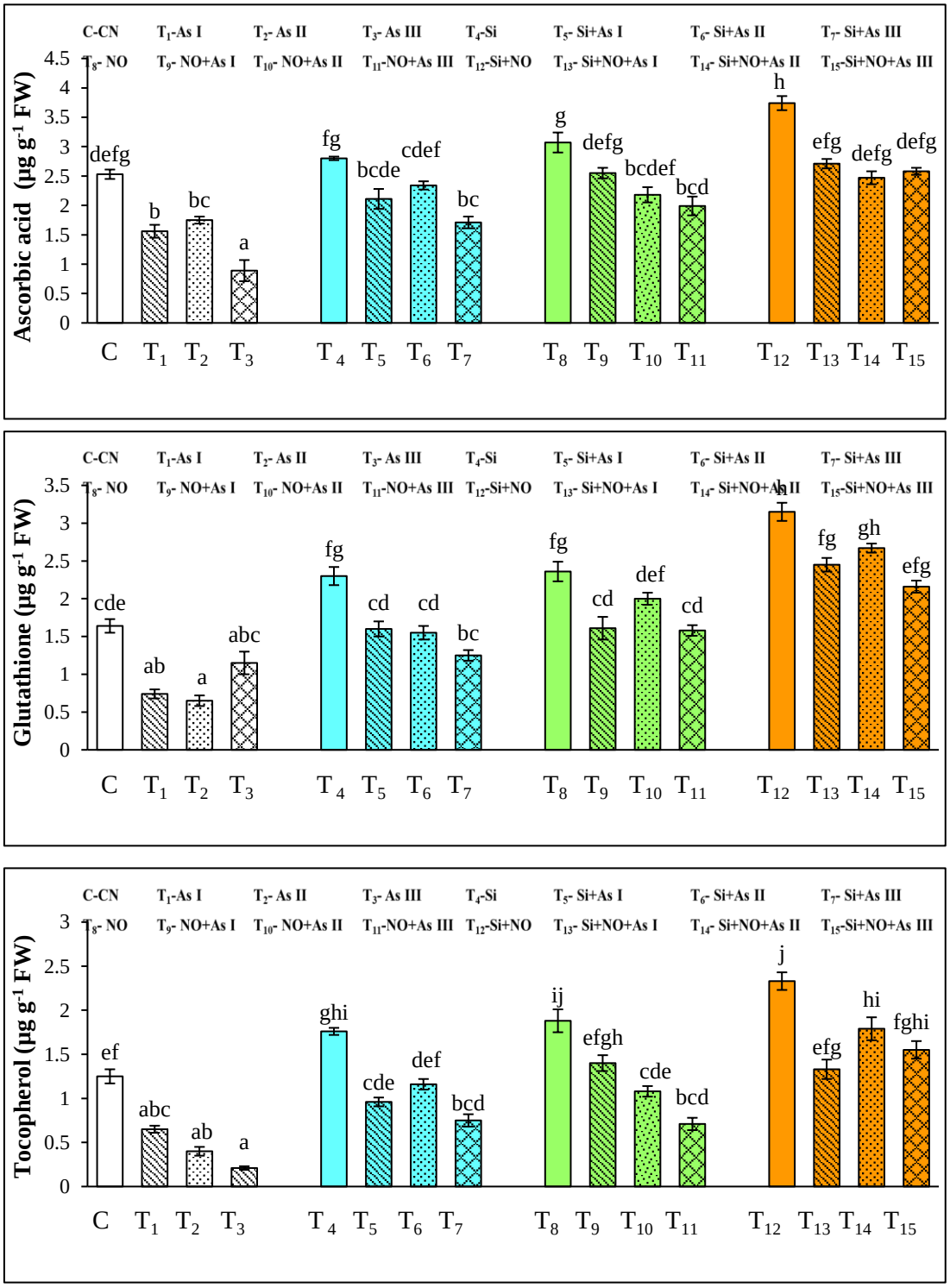

Fig. 6.17 Effect of Si and NO on ascorbic acid, glutathione and tocopherol content in 7-days old seedlings of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Tocopherol content was observed to be declined under all three concentrations of As stress (Fig. 6.17; Table 6.13). Minimum tocopherol content of 0.21 µg g^{-1} FW was observed at T_3 among all three As concentrations used. In the case of Si applied seedlings under As stress, maximum tocopherol content of 1.16 µg g^{-1} FW was

observed at T_6 concentration whereas in NO pre-treated seedlings, maximum tocopherol content (1.4 µg g^{-1} FW) was in T_9 seedlings. However, Si + NO displayed highest tocopherol content of 1.79 µg g^{-1} FW in T_{14}. An increase of 47.69% was noticed in T_5 than T_1. T_{13} seedlings showed 27.81% increase as compared to T_5. T_{14} showed 43.20% increase than control seedlings.

6.1.2 30-days old plants

6.1.2.1 Plant growth

Arsenic stress lowered the root length with a minimum of 2.43 cm in T_3 stressed plants (Fig. 6.18; Table 6.14). The highest 5.3 cm root length was observed in T_5 plants when applied with Si. Pre-treatment with NO also enhanced the root length with the highest (6.53 cm) and lowest (6.16 cm) root length at T_{10} and T_{11} concentrations. NO application showed better results in increasing root lengths in response to As than Si application. Si + NO further improved the root length in As stressed plants with a maximum of 6.76 cm at T_{13}. T_4 showed 4% decline in root length than T_4 plants. An increase of 4.64% was found in T_{13} than T_9 plants. T_{13} exhibited 22.24% increase than control plants.

Fresh weight was markedly influenced under metalloid stress with the highest reduction in fresh weight (0.53 g) in T_3 treated plants (Fig. 6.18; Table 6.14). T_6 increased the fresh weight from 0.71 g to 2.57 g, in contrast to T_2 treated plants. In NO pre-treated plants, the maximum and minimum i.e., 1.85 g and 1.5 g fresh weights were noticed at T_9 and T_{10} treated plants, respectively. The maximum increase in fresh weight in case of combined treated plants was reported at T_{13} with 2.59 g fresh weight. T_5 exhibited 38.37% increase in contrast to T_9. An augmentation of 1.17% was noticed in T_{13} than T_5. T_{13} exhibited 52.35% increase than control plants.

T_1, T_2 and T_3 plants did not show much difference in reducing the dry weights (Fig. 6.18; Table 6.14) with only 0.28, 0.27 and 0.25 g dry weights were observed, respectively. Individual treatment with Si and NO under As showed an escalation in dry weights with maximum of 1.93 and 1.46 g dry weights at T_6 and T_9 concentrations, respectively. Si + NO showed better results in the case of dry weights when compared with their individual treatments. Individual Si showed high dry weight (1.93 g) at T_6 as compared to T_{14} treatment which exhibited only 1.72 g dry weight. T_5 exhibited 8.90% increase in contrast to T_9. An augmentation of 25.78% was noticed in T_{13} than T_5.

A decrease in germination percentage was noticed in As treated plants (Fig. 6.19; Table 6.15). Germination percentage was 45% reduced in T_3 concentration as revealed by the comparison with control plants. Applying Si to the radish plants caused an escalation in the germination percentage with the highest of 76.66% in T_6 treated plants. Pre-

treatment with NO also improved seed germination under As stress. Germination percentage was increased from 54.55% to 72.21% in NO-treated plants under T_{10}. Si and NO control plants (T_4 and T_8) showed equal germination percentages i.e., 85.55%. Under As stress, NO exhibited a better germination percentage under As stress as compared to Si. In combination of Si and NO, the highest germination percentage was shown by T_{14} and T_{15} i.e., 79.99%. T_5 exhibited 17.85% increase in contrast to T_1 whereas T_9 showed 10.70% elevation than T_1. An augmentation of 7.56% was noticed in T_{13} than T_5. T_{13} exhibited 24.46% decrease than control plants.

Table 6.14 Effect of Si and NO on morphological parameters of 30 days old plants of *R. sativus* under As stress

Treatment	Root length (cm)	Fresh weight (g)	Dry weight (g)
C (Control)	$5.53^{cde} \pm 0.26$	$1.70^{bcde} \pm 0.09$	$0.68^{ab} \pm 0.19$
T_1 (AsI)	$3.7^{b} \pm 0.15$	$0.58^{a} \pm 0.05$	$0.28^{a} \pm 0.06$
T_2 (AsII)	$3.16^{ab} \pm 0.17$	$0.71^{ab} \pm .007$	$0.27^{a} \pm 0.03$
T_3 (AsIII)	$2.43^{a} \pm 0.17$	$0.53^{a} \pm .03$	$0.25^{a} \pm 0.07$
T_4 (Si)	$6.36^{ef} \pm 0.23$	$2.66^{ef} \pm 0.10$	$1.71^{def} \pm 0.01$
T_5 (Si+AsI)	$5.3^{cd} \pm 0.17$	$2.56^{ef} \pm 0.05$	$1.59^{de} \pm 0.06$
T_6 (Si+AsII)	$5.03^{c} \pm 0.12$	$2.57^{ef} \pm 0.03$	$1.93^{ef} \pm 0.11$
T_7 (Si+AsIII)	$5.1^{c} \pm 0.15$	$2.40^{cdef} \pm 0.15$	$1.77^{ef} \pm 0.06$
T_8 (NO)	$6.96^{fg} \pm 0.25$	$2.52^{def} \pm 0.19$	$1.79^{ef} \pm 0.03$
T_9 (NO+AsI)	$6.46^{efg} \pm 0.12$	$1.85^{cde} \pm 0.05$	$1.46^{cde} \pm 0.12$
T_{10} (NO+AsII)	$6.53^{fg} \pm 0.17$	$1.50^{abc} \pm 0.12$	$1.12^{bcd} \pm 0.07$
T_{11} (NO+AsIII)	$6.16^{def} \pm 0.12$	$1.53^{abcd} \pm 0.17$	$0.94^{bc} \pm 0.03$
T_{12} (Si+NO)	$7.33^{g} \pm 0.17$	$3.19^{f} \pm 0.23$	$2.33^{f} \pm 0.18$
T_{13} (Si+NO+AsI)	$6.76^{fg} \pm 0.20$	$2.59^{ef} \pm 0.17$	$2^{ef} \pm 0.18$
T_{14} (Si+NO+AsII)	$6.23^{def} \pm 0.14$	$2.49^{cdef} \pm 0.19$	$1.72^{def} \pm 0.10$
T_{15} (Si+NO+AsIII)	$5.56^{cde} \pm 0.29$	$2.32^{cdef} \pm 0.56$	$1.63^{de} \pm 0.23$

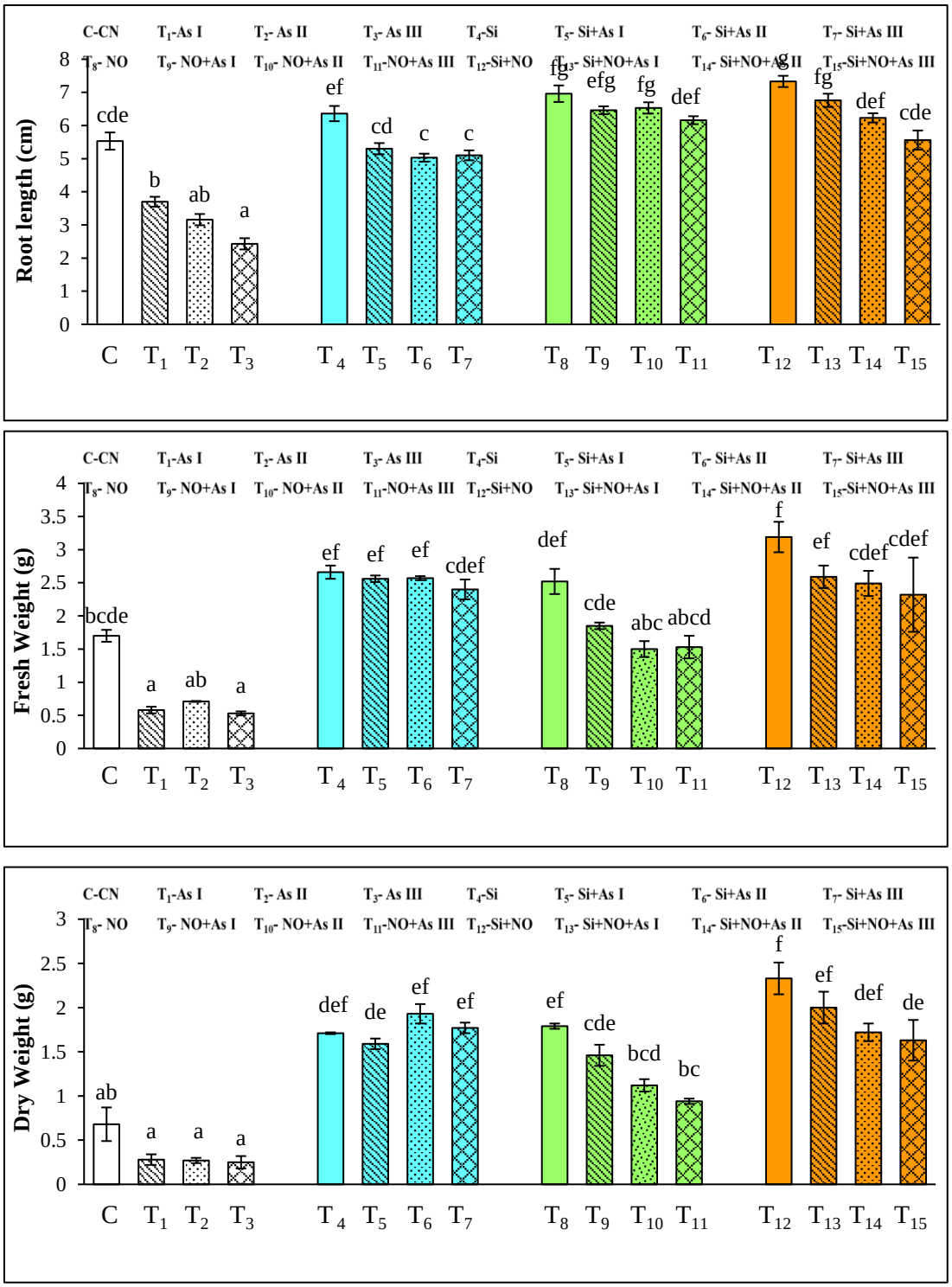

Fig. 6.18 Effect of Si and NO on root length, fresh and dry weight in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

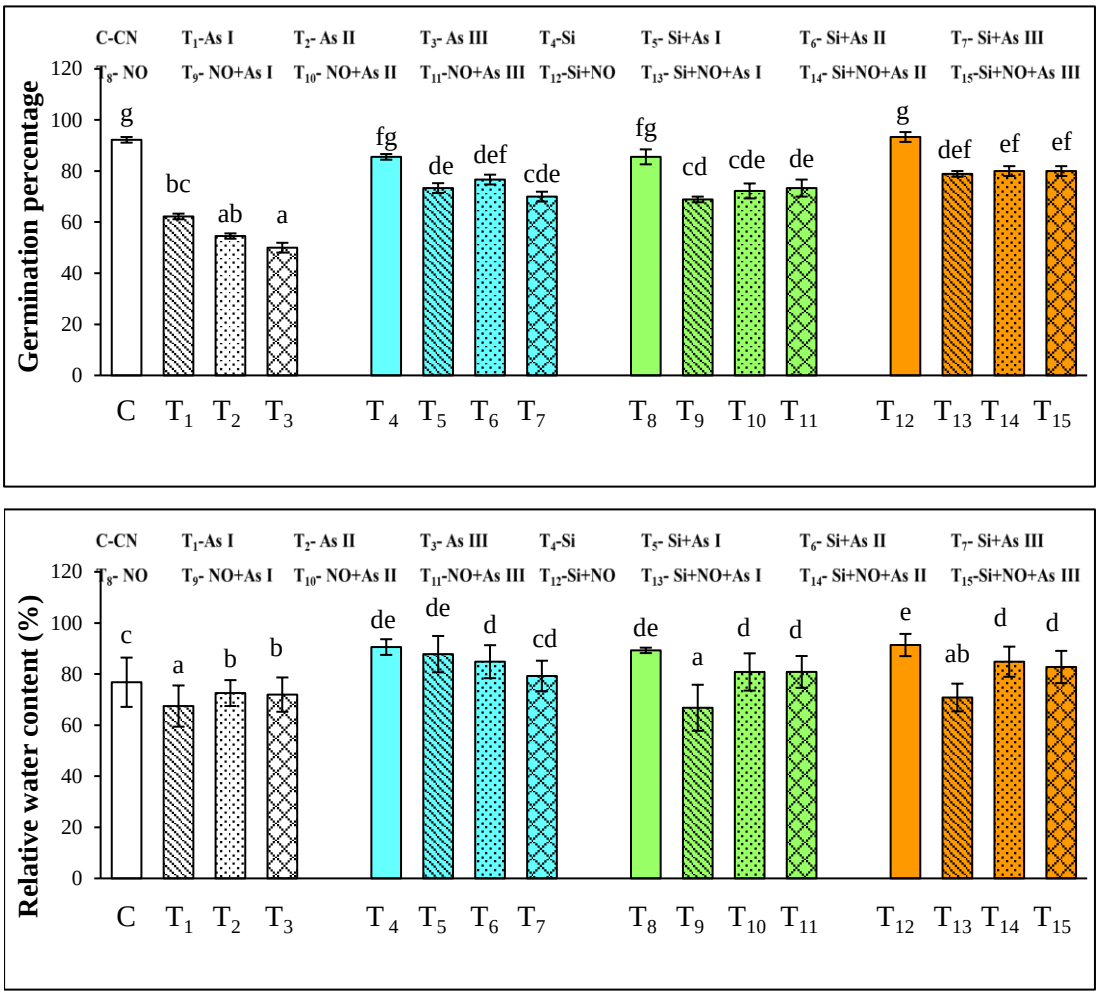

Fig. 6.19 Effect of Si and NO on gemination percentage and relative water content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Relative water content was decreased under As stress with minimum relative water content (67.46%) in T_1 plants (Fig. 6.19; Table 6.15). Silicon application under unstressed conditions showed relative water content of 90.56% (T_4) which is greater when compared with control plants. Silicon application against As stress exhibited a minimum relative water content of 79.24% in T_7 plants. Among all three As treatments in NO-pre-treated seedlings, the maximum relative water content of 80.83% was noticed in T_{11}. T_{14} showed higher relative water content with the highest of 84.83%.

Table 6.15 Effect of Si and NO on germination percentage and relative water content of 30 days old plants of *R. sativus* under As stress

Treatment	Germination percentage	Relative water content (%)
C (Control)	92.22^{g} ±1.11	76.79^{c}±9.65
T_1 (AsI)	62.22^{bc} ±1.11	67.46^{a}±8.07
T_2 (AsII)	54.55^{ab} ±1.05	72.55^{b}±5.07
T_3 (AsIII)	49.99^{a} ±1.92	71.95^{b}±6.72
T_4 (Si)	85.55^{fg} ±1.11	90.56^{de}±3.07
T_5 (Si+AsI)	73.33^{de} ±1.92	87.79^{de}±7.12
T_6 (Si+AsII)	76.66^{def} ±1.92	84.84^{d}±6.45
T_7 (Si+AsIII)	69.99^{cde} ±1.92	79.24^{cd}±5.97
T_8 (NO)	85.55^{fg} ±2.93	89.26^{de}±1.06
T_9 (NO+AsI)	68.88^{cd} ±1.11	66.79^{a}±9.02
T_{10} (NO+AsII)	72.21^{cde} ±2.93	80.81^{d}±7.3
T_{11} (NO+AsIII)	73.33^{de} ±3.33	80.83^{d}±6.23
T_{12} (Si+NO)	93.33^{g} ±1.92	91.36^{e}±4.35
T_{13} (Si+NO+AsI)	78.88^{def} ±1.11	70.81^{ab}±5.43
T_{14} (Si+NO+AsII)	79.99^{ef} ±1.92	84.83^{d}±5.9
T_{15} (Si+NO+AsIII)	79.99^{ef} ±1.92	82.76^{d}±6.31

6.1.2.2 Photosynthetic activity

6.1.2.2.1 Photosynthetic pigments

Photosynthetic pigments were noticed to be declined by As stress, as compared to radish plants (Fig. 6.20; Table 6.16). Almost 35% drop in total chlorophyll was found at T_3 than control plants i.e., from 0.811 to 0.525 mg g^{-1} FW. Individual application of Si and NO boosted its content under As, among which the highest contents i.e., 0.747 and 0.719 mg g^{-1} FW were at T_5 and T_9 when treated individually with Si and NO, respectively. Synergistic application of Si and NO showed better results in improving total chlorophyll contents under stress conditions, as compared to their individual treatments. The highest content of 0.809 mg g^{-1} FW was noticed in T_{13} plants. T_5 exhibited 21.46% increase in contrast to T_1 whereas T_9 showed 16.91% elevation than T_1. An augmentation of 7.49% was noticed in T_{13} than T_5. Similar findings were

observed in the case of chl a and chl b contents in radish plants. The lowest content of chl a (0.303 mg g^{-1} FW) and chl b (0.215 mg g^{-1} FW) were found at T_3 concentration. Si and NO significantly alleviated the As-induced noxiousness by increasing their levels. The combination of Si and NO further upsurged their contents under stress conditions with the highest of 0.533 mg g^{-1} FW contents in T_{13} plants. T_5 exhibited 45.06% and 54.02% increase in contrast to T_1 whereas T_9 showed 21.86% and 60.73% elevation than T_1 in case of chl a and chl b, respectively.

Table 6.16 Effect of Si and NO on photosynthetic pigments of 30 days old plants of *R. sativus* under As stress

Treatment	**Total chlorophyll (mg g^{-1} FW)**	**Chl a (mg g^{-1} FW)**	**Chl b (mg g^{-1} FW)**
C (Control)	0.811^{efgh}±0.019	0.542^{fghi}±0. 016	0.479^{cde}±0. 022
T_1 (AsI)	0.615^{abc}±0.012	0.375^{abc}±0. 01	0.298^{ab}±0. 017
T_2 (AsII)	0.551^{ab}±0.019	0.317^{ab}±0. 021	0.253^{a}±0. 015
T_3 (AsIII)	0.525^{a}±0.014	0.303^{a}±0. 026	0.215^{a}±0. 02
T_4 (Si)	0.884^{gh}±0.015	0.618^{ij}±0. 022	0.572^{ef}±0. 027
T_5 (Si+AsI)	0.747^{cdef}±0.016	0.544^{fghi}±0. 018	0.459^{cde}±0. 032
T_6 (Si+AsII)	0.717^{cde}±0.026	0.512^{efgh}±0. 014	0.394^{bc}±0. 014
T_7 (Si+AsIII)	0.667^{bcd}±0.038	0.487^{defg}±0. 015	0.38^{bc}±0. 025
T_8 (NO)	0.857^{fgh}±0.031	0.596^{hij}±0. 02	0.546^{def}±0. 029
T_9 (NO+AsI)	0.719^{cde}±0.014	0.457^{cdef}±0. 011	0.479^{cde}±0. 03
T_{10} (NO+AsII)	0.687^{bcde}±0. 034	0.414^{bcde}±0. 016	0.449^{cd}±0. 033
T_{11} (NO+AsIII)	0.629^{abc}±0. 044	0.388^{abcd}±0. 012	0.394^{bc}±0. 009
T_{12} (Si+NO)	0.915^{h}±0. 033	0.685^{j}±0. 016	0.649^{f}±0. 014
T_{13} (Si+NO+AsI)	0.803^{defgh}±0. 018	0.57^{ghi}±0. 032	0.533^{def}±0. 011
T_{14} (Si+NO+AsII)	0.777^{defg}±0. 021	0.541^{fghi}±0. 025	0.494^{cde}±0. 005
T_{15} (Si+NO+AsIII)	0.69^{cde}±0. 028	0.511^{efgh}±0. 014	0.475^{cde}±0. 025

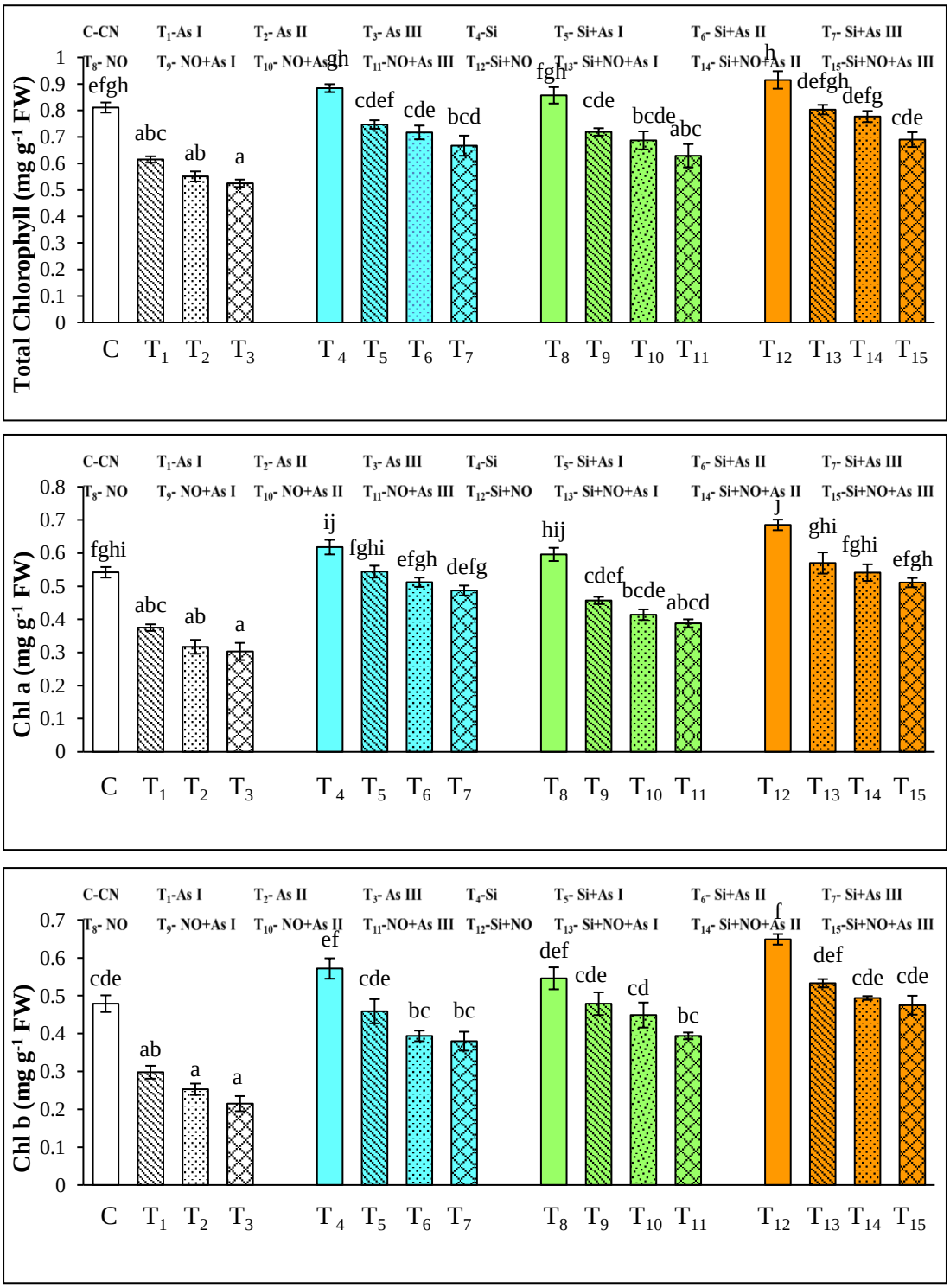

Fig. 6.20 Effect of Si and NO on total chlorophyll, chl a and chl b in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

It was found that As stress decreased the carotenoid content (Fig. 6.21; Table 6.17). Carotenoid content was found to be decreased as the As level increased. The highest reduction in carotenoid (0.368 mg g^{-1} FW) was at T_3. Carotenoid content was increased from 0.462 to 6.3 mg g^{-1} FW in T_5 plants in contrast to T_1 stressed plants. Pre-treatment with NO against As stress also increased the carotenoid content with the highest (0.61 mg g^{-1} FW) at T_9 plants. The highest 0.712 mg g^{-1} FW carotenoid content was noticed at T_{13} while minimum 0.613 mg g^{-1} FW was at T_{15}. An increase of 13.01% was noticed in T_{13} than T_5. T_{13} exhibited 16.33% increase than control plants.

Arsenic stress caused a decrease of 4.38 mg g^{-1} FW in xanthophylls in T_3 plants (Fig. 6.21; Table 6.17). Si and NO treatments upgraded the xanthophyll content under As stress. Individual treatment with Si and NO under As stress caused the highest content of 7.35 and 7.3 mg g^{-1} FW at T_5 and T_9 concentration. Xanthophyll content was noticed to be diminished as As level increased in the case of Si and NO treatments alone. The highest xanthophyll content of 8.11 mg g^{-1} FW was at T_{14} concentration. T_5 exhibited 32.91% increase in contrast to T_1 whereas T_9 showed 32% elevation than T_1. An augmentation of 24.76% was noticed in T_{14} than T_6. T_{14} exhibited 4.64% increase than control plants.

6.1.2.2.2 Gas exchange parameters

The photosynthetic rate was found to be decreased under As stress with a minimum 4.41 m mol CO_2 m^{-2} S^{-1} at T_3 (Fig. 6.22; Table 6.18). Individual application of Si increased the photosynthetic rate under As stress. Silicon application increased the photosynthetic rate to 11.59 m mol CO_2 m^{-2} S^{-1} at T_5. Pre-treatment with NO also improved the photosynthetic rate under As stress with the highest photosynthetic rate of 12.15 m mol CO_2 m^{-2} S^{-1} at T_9. Application of NO exhibited a higher photosynthetic rate under As toxicity than Si application. Si + NO further increased the photosynthetic rate against As toxicity with the maximum photosynthetic rate of 14.18 m mol CO_2 m^{-2} S^{-1} at T_{13}. T_9 exhibited 4.83% increase in contrast to T_5. An augmentation of 16.70% was noticed in T_{13} than T_9. T_{13} exhibited 33.27% increase than control plants.

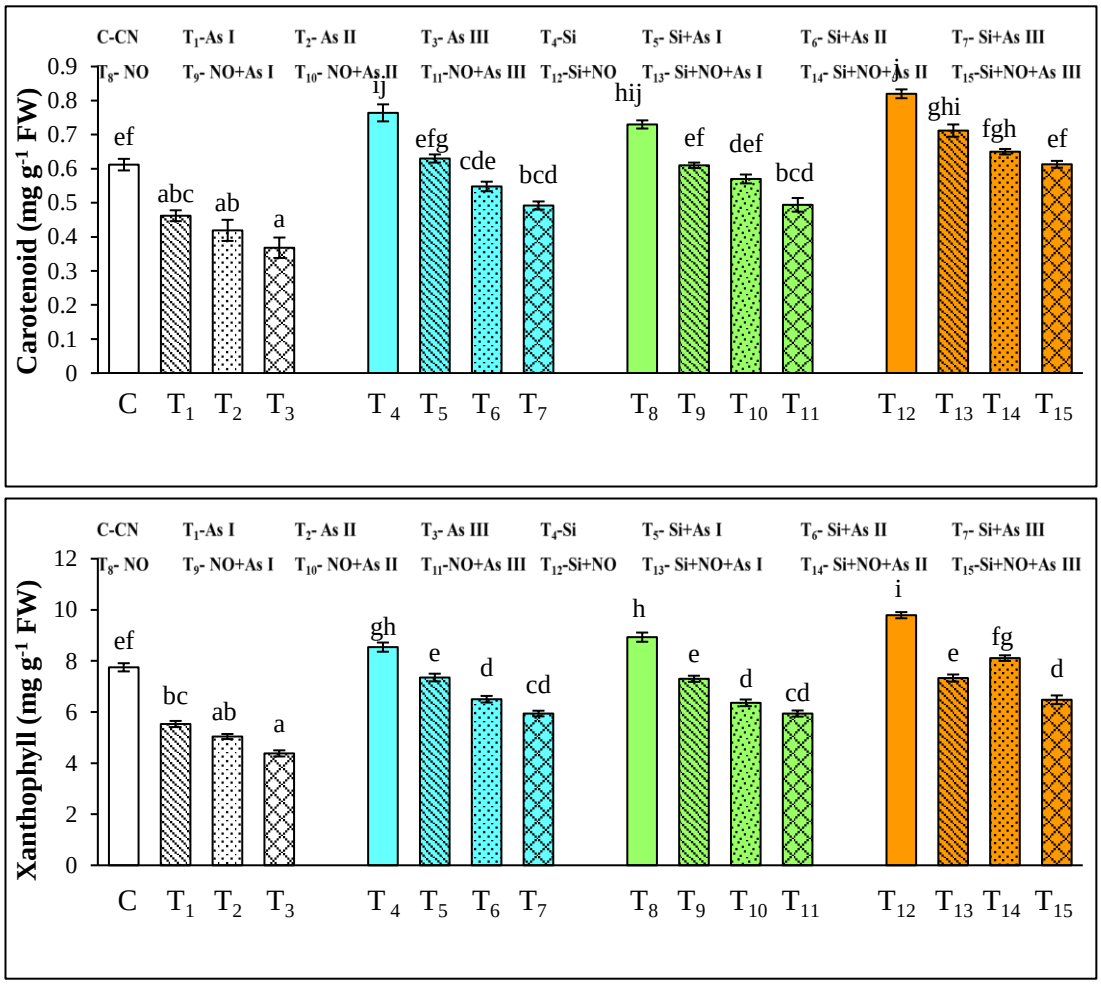

Fig. 6.21 Effect of Si and NO on carotenoid and xanthophyll content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Arsenic exposure to radish plants caused a decrease in stomatal conductance with minimum conductance of 0.15 m mol H_2O m^{-2} s^{-1} at T_2 concentration (Fig. 6.22; Table 6.18). T_3 caused 39.5% decline in stomatal conductance in contrast to control plants. When Si and NO were applied alone in radish plants, it resulted in an elevation in the stomatal conductance under As stress. Control plants in case of silicon and NO i.e., T_4 and T_5 showed higher stomatal conductance of 0.49 and 0.47 m mol H_2O m^{-2} s^{-1}, respectively. With regard to individual Si and NO application under As stress, greater stomatal conductance of 0.34 and 0.39 m mol H_2O m^{-2} s^{-1} were noticed at T_5 and T_{10}, respectively. Coupled application of Si and NO under stressed conditions exhibited the highest stomatal conductance (0.41 m mol H_2O m^{-2} s^{-1}) at T_{13}. T_5 exhibited 78.94% increase in contrast to T_1 whereas T_9 showed 63.15% elevation than T_1. An augmentation of 20.58% was noticed in T_{13} than T_5. T_{13} exhibited 4.65% decrease than control plants.

Table 6.17 Effect of Si and NO on photosynthetic pigments of 30 days old plants of *R. sativus* under As stress

Treatment	**Carotenoid content (mg g^{-1} FW)**	**Xanthophyll content (mg g^{-1} FW)**
C (Control)	0.612^{ef}±0. 017	7.75^{ef}±0.16
T_1 (AsI)	0.462^{abc}±0. 016	5.53^{bc}±0.12
T_2 (AsII)	0.419^{ab}±0. 031	5.04^{ab}±0.1
T_3 (AsIII)	0.368^{a}±0. 03	4.38^{a}±0.12
T_4 (Si)	0.764^{ij}±0. 025	8.54^{gh}±0.18
T_5 (Si+AsI)	0.63^{efg}±0. 012	7.35^{e}±0.15
T_6 (Si+AsII)	0.548^{cde}±0. 014	6.5^{d}±0.13
T_7 (Si+AsIII)	0.492^{bcd}±0. 012	5.94^{cd}±0.11
T_8 (NO)	0.73^{hij}±0. 012	8.93^{h}±0.18
T_9 (NO+AsI)	0.61^{ef}±0.008	7.3^{e}±0.12
T_{10} (NO+AsII)	0.57^{def}±0. 013	6.36^{d}±0.13
T_{11} (NO+AsIII)	0.494^{bcd}±0. 02	5.94^{cd}±0.12
T_{12} (Si+NO)	0.82^{j}±0. 013	9.79^{i}±0.12
T_{13} (Si+NO+AsI)	0.712^{ghi}±0. 018	7.33^{e}±0.14
T_{14} (Si+NO+AsII)	0.65^{fgh}±0.008	8.11^{fg}±0.11
T_{15} (Si+NO+AsIII)	0.613^{ef}±0. 01	6.48^{d}±0.17

Control plants showed 468 ppm intercellular CO_2 concentration (Fig. 6.22; Table 6.18). Furthermore, As stress at T_1, T_2 and T_3 caused a 41, 56 and 37% decrease in intercellular CO_2 concentration, as compared to control plants. Intercellular CO_2 concentration was found to be increased from 275 to 474.66 ppm in case of Si application in T_5 plants than T_1. Pre-treatment with NO also increased the intercellular CO_2 concentration with the highest 469.66 ppm at T_9. Coupled application of Si and NO under stressed conditions further increased the intercellular CO_2 concentration with the highest of 555 ppm at T_{13}. T_5 exhibited 72.60% increase in contrast to T_1 whereas T_9 showed 70.78% elevation than T_1. An augmentation of 16.92% was noticed in T_{13} than T_5. T_{13} exhibited 18.58% increase than control plants.

Transpiration rate was found to be diminished in 30-days old radish plants by their exposure to As (Fig. 6.23; Table 6.18). Control plants showed 0.68 m mol H_2O m^{-2} S^{-1} whereas and T_1, T_2 and T_3 plants exhibited 0.24, 0.21 and 0.25 m mol H_2O m^{-2} S^{-1}, respectively. Application of Si under stressed conditions showed a maximum transpiration rate of 0.67 m mol H_2O m^{-2} S^{-1} at T_6 concentration, whereas NO also showed the highest transpiration rate of 0.71 m mol H_2O m^{-2} S^{-1} at T_{10} concentration. Coupled application of Si and NO showed highest and lowest transpiration at T_{13} and T_{14} with 0.95 and 0.76 m mol H_2O m^{-2} S^{-1} transpiration rates, respectively. T_5 exhibited 83.33% increase in contrast to T_1 whereas T_9 showed 75% elevation than T_1. An augmentation of 7.04% was noticed in T_{15} than T_{10}. T_{13} exhibited 39.70% increase than control plants.

6.1.2.3 Metabolites

Arsenic stress led to the reduction in the anthocyanin content with the largest reduction of 4.85 mg g^{-1} FW at T_3 (Fig. 6.24; Table 6.19). Individual supply of Si and NO under As stress markedly increased the content of anthocyanin with maximum of 8.46 mg g^{-1} FW in T_9 plants. Nitric oxide showed higher anthocyanin content under As toxicity as compared to Si application. The highest anthocyanin content of 9.32 mg g^{-1} FW was noticed in the combination of all three treatments i.e. T_{13}, among all the treatments used in the study. T_5 exhibited 24.51% increase in contrast to T_1 whereas T_9 showed 49.20% elevation than T_1. An augmentation of 10.16% was noticed in T_{13} than T_9.

Table 6.18 Effect of Si and NO on gas exchange parameters of 30 days old plants of *R. sativus* under As 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})
C (Control)	10.64^{bc} ±0.24	0.43^{ab} ±0.08	468^{fg} ±3.78	0.68^{f} ±0.05
T_1 (AsI)	5.46^{a} ±0.25	0.19^{ab} ±0.07	275^{b} ±2.08	0.24^{ab} ±0.04
T_2 (AsII)	4.55^{a} ±0.26	0.15^{a} ±0.01	207^{a} ±1.15	0.21^{a} ±0.01
T_3 (AsIII)	4.41^{a} ±0.30	0.26^{ab} ±0.11	293.66^{c} ±1.45	0.25^{abc} ±0.02
T_4 (Si)	12.75^{def} ±0.15	0.49^{b} ±0.02	481.33^{gh} ±1.45	0.79^{fg} ±0.03
T_5 (Si+AsI)	11.59^{bcd} ±0.30	0.34^{ab} ±0.04	474.66^{fg} ±2.60	0.44^{cd} ±0.08
T_6 (Si+AsII)	10.46^{bc} ±0.52	0.30^{ab} ±0.05	421^{de} ±3.60	0.67^{f} ±0.008
T_7 (Si+AsIII)	9.81^{b} ±0.57	0.22^{ab} ±0.05	434.33^{e} ±5.23	0.46^{de} ±0.009
T_8 (NO)	13.19^{def} ±0.20	0.47^{ab} ±0.05	492.66^{h} ±3.75	0.92^{g} ±0.01
T_9 (NO+AsI)	12.15^{cde} ±0.16	0.31^{ab} ±0.06	469.66^{fg} ±3.28	0.42^{bcd} ±0.01
T_{10} (NO+AsII)	11.72^{cd} ±0.42	0.39^{ab} ±0.07	468^{fg} ±3.78	0.71^{f} ±0.01
T_{11} (NO+AsIII)	10.64^{bc} ±0.31	0.30^{ab} ±0.06	462.33^{f} ±1.45	0.65^{ef} ±0.04
T_{12} (Si+NO)	15.71^{g} ±0.46	0.52^{ab} ±0.03	578^{k} ±3.21	1.14^{h} ±0.04
T_{13} (Si+NO+AsI)	14.18^{fg} ±0.35	0.41^{ab} ±0.07	555^{j} ±2.88	0.95^{gh} ±0.01
T_{14} (Si+NO+AsII)	13.63^{ef} ±0.29	0.38^{ab} ±0.04	410.33^{d} ±4.33	0.76^{fg} ±0.007
T_{15} (Si+NO+AsIII)	12.68^{def} ±0.40	0.37^{ab} ±0.03	531.33^{i} ±2.40	0.91^{g} ±0.01

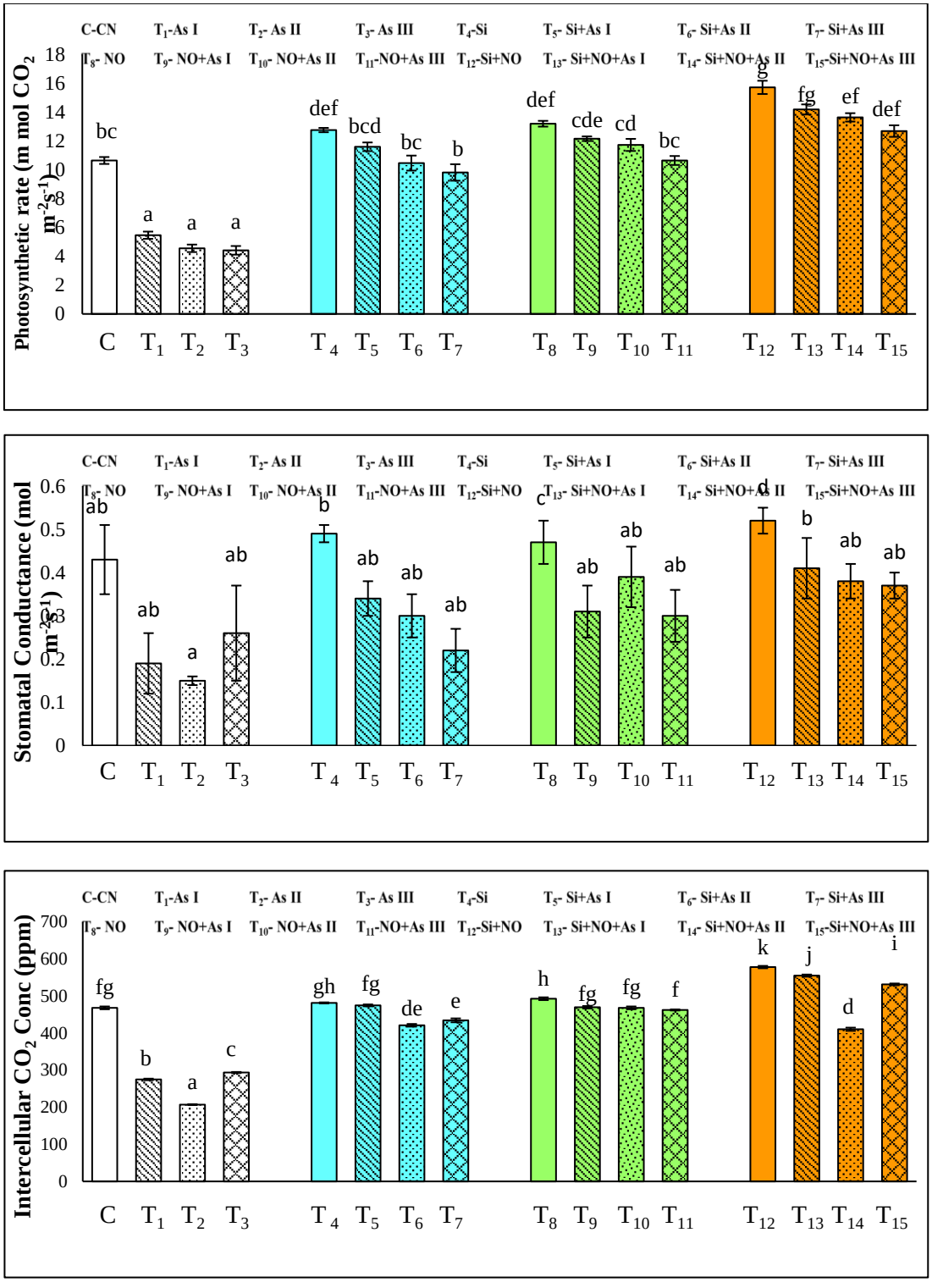

Fig. 6.22 Effect of Si and NO on photosynthetic rate, stomatal conductance and intercellular CO_2 concentration in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

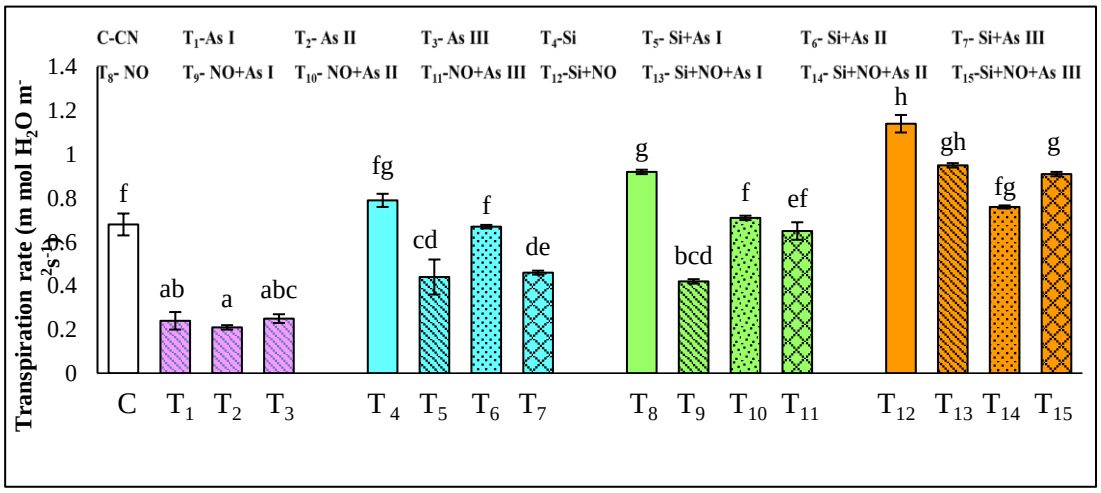

Fig. 6.23 Effect of Si and NO on transpiration rate in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Both Si and NO without As stress had resulted in improved flavonoids in *R. sativus* plants than all other treatments (Fig. 6.24; Table 6.19). A maximum decrease in the flavonoids i.e. 5.41 mg g^{-1} FW was found at T_3. Decrease in flavonoids was found as the As level increased in As alone treated plants. Individual application of Si under stressed conditions increased the flavonoid content from 5.61 to 8.06 mg g^{-1} FW in T_5 plants. In NO plants against As stress, minimum flavonoid content (7.78 mg g^{-1} FW) was noticed in T_{11}. Moreover, the combination of Si and NO greatly mitigated the As toxicity in radish plants with maximum content of 9.41 mg g^{-1} FW at T_{14} treatment. T_5 exhibited 43.67% increase in contrast to T_1 whereas T_9 showed 48.84% elevation than T_1. An augmentation of 18.66% was noticed in T_{14} than T_{10}. T_{14} treatment showed 24.30% elevation as compared to control plants.

Exposure of *R. sativus* plants to the As caused a severe decrease in the phenolic content with a maximum reduction of 4.60 mg g^{-1} FW at T_1 concentration (Fig. 6.24; Table 6.19). T_7 showed 8.31 mg g^{-1} FW phenolic content, whereas T_{11} displayed 7.12 mg g^{-1} FW phenolic content. The maximum phenolic amount of 8.92 mg g^{-1} FW was found at T_{14} treatment, as compared to all other treatments used in the study under stress conditions. T_6 exhibited 46.54% increase in contrast to T_2 whereas T_{10} showed 66.46% elevation than T_2. An augmentation of 16.80% was noticed in T_{13} than T_5. T_{14} treatment showed 8.38% elevation as compared to control plants.

Table 6.19 Effect of Si and NO on metabolites of 30 days old plants of *R. sativus* under As stress

Treatment	Anthocyanin content (mg g^{-1} FW)	Flavonoid content (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)
C (Control)	$8.29^{efg} \pm 0.27$	$7.57^{b} \pm 0.21$	$8.23^{cde} \pm 0.18$
T_1 (AsI)	$5.67^{ab} \pm 0.20$	$5.61^{a} \pm 0.24$	$5.07^{a} \pm 0.19$
T_2 (AsII)	$5.61^{ab} \pm 0.21$	$5.53^{a} \pm 0.20$	$4.92^{a} \pm 0.18$
T_3 (AsIII)	$4.85^{a} \pm 0.19$	$5.41^{a} \pm 0.16$	$4.60^{a} \pm 0.17$
T_4 (Si)	$8.01^{de} \pm 0.16$	$8.86^{bcde} \pm 0.40$	$8.62^{def} \pm 0.15$
T_5 (Si+AsI)	$7.06^{cd} \pm 0.12$	$8.06^{bc} \pm 0.34$	$7.50^{bc} \pm 0.20$
T_6 (Si+AsII)	$6.56^{bc} \pm 0.13$	$8.37^{bcd} \pm 0.32$	$7.21^{b} \pm 0.10$
T_7 (Si+AsIII)	$6.86^{c} \pm 0.16$	$8.04^{bc} \pm 0.17$	$8.31^{cde} \pm 0.17$
T_8 (NO)	$9.14^{fgh} \pm 0.07$	$9.80^{ef} \pm 0.31$	$9.26^{f} \pm 0.13$
T_9 (NO+AsI)	$8.46^{efg} \pm 0.13$	$8.35^{bcd} \pm 0.30$	$7.81^{bcd} \pm 0.22$
T_{10} (NO+AsII)	$8.04^{def} \pm 0.12$	$7.93^{bc} \pm 0.19$	$8.19^{cde} \pm 0.17$
T_{11} (NO+AsIII)	$7.65^{cde} \pm 0.22$	$7.78^{bc} \pm 0.17$	$7.12^{b} \pm 0.19$
T_{12} (Si+NO)	$9.99^{h} \pm 0.41$	$10.79^{f} \pm 0.12$	$10.35^{g} \pm 0.21$
T_{13} (Si+NO+AsI)	$9.32^{gh} \pm 0.20$	$9.04^{cde} \pm 0.16$	$8.76^{ef} \pm 0.15$
T_{14} (Si+NO+AsII)	$8.47^{efg} \pm 0.22$	$9.41^{de} \pm 0.19$	$8.92^{ef} \pm 0.12$
T_{15} (Si+NO+AsIII)	$8.14^{def} \pm 0.28$	$8.87^{bcde} \pm 0.23$	$8.24^{cde} \pm 0.18$

6.1.2.4 Oxidative stress markers

Elevation in the MDA level was observed under As toxicity in radish plants with the lowest and highest MDA content of 9.22 and 9.83 µmol g^{-1} FW at T_2 and T_3 concentrations, respectively (Fig. 6.25; Table 6.20). Addition of Si, NO and Si + NO at As III concentration i.e., T_7, T_{11} and T_{15} showed MDA contents of 5.2, 5.44 and 4.69 µmol g^{-1} FW, respectively. T_5 exhibited 36.38% reduction in contrast to T_1 whereas T_9 showed 37.67% decline than T_1. A decline of 13.78% was noticed in T_{15} than T_{11}. T_{15} treatment showed 35.31% reduction as compared to control plants.

Similar findings were observed in the case of H_2O_2 level. H_2O_2 level was noted to be increased with the increase in the As concentration (Fig. 6.25; Table 6.20). The highest MDA level (12.33 µmol g^{-1} FW) was found at T_3 concentration. Moreover, H_2O_2 levels were lessened as the As level elevated in the case of individual and Si + NO treatments. Minimum H_2O_2 content (4.44 µmol g^{-1} FW) was observed in the T_{15}

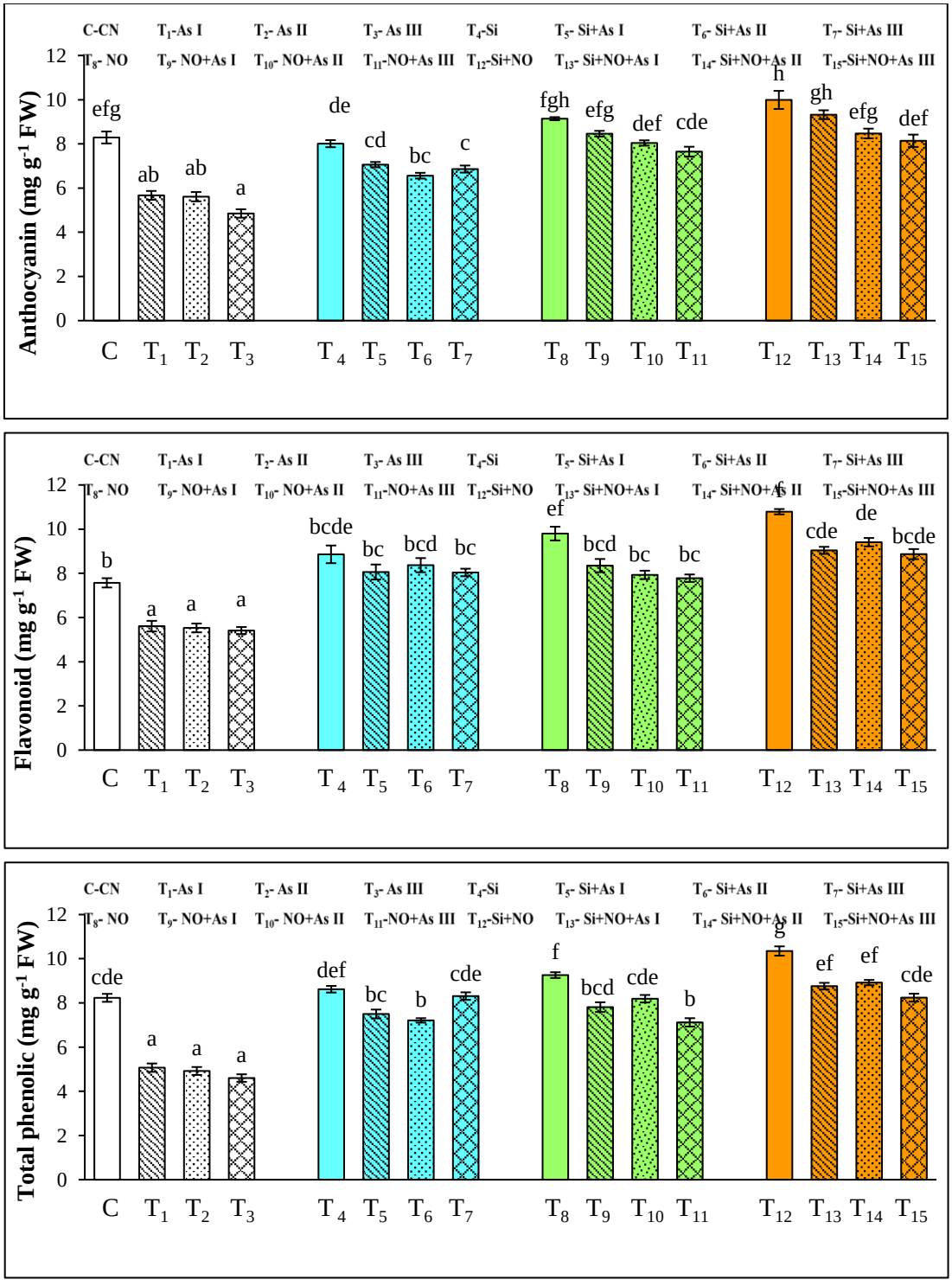

Fig. 6.24 Effect of Si and NO on anthocyanin, flavonoid and phenolic content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.20 Effect of Si and NO on oxidative stress markers of 30 days old plants of *R. sativus* under As stress

Treatment	MDA content (µmol g^{-1} FW)	H_2O_2 content (µmol g^{-1} FW)
C (Control)	$7.25^{c} \pm 0.19$	$7.87^{e} \pm 0.17$
T_1 (AsI)	$9.29^{d} \pm 0.17$	$11.51^{f} \pm 0.24$
T_2 (AsII)	$9.22^{d} \pm 0.37$	$12.22^{f} \pm 0.25$
T_3 (AsIII)	$9.83^{d} \pm 0.27$	$12.33^{f} \pm 0.38$
T_4 (Si)	$6.2^{abc} \pm 0.24$	$7.72^{e} \pm 0.27$
T_5 (Si+AsI)	$5.91^{abc} \pm 0.77$	$7.41^{de} \pm 0.24$
T_6 (Si+AsII)	$5.68^{abc} \pm 0.31$	$7.52^{de} \pm 0.36$
T_7 (Si+AsIII)	$5.2^{ab} \pm 0.38$	$6.96^{cde} \pm 0.26$
T_8 (NO)	$6.7^{bc} \pm 0.23$	$7.88^{e} \pm 0.2$
T_9 (NO+AsI)	$5.79^{abc} \pm 0.26$	$6.92^{cde} \pm 0.19$
T_{10} (NO+AsII)	$6.13^{abc} \pm 0.21$	$6.42^{bcde} \pm 0.29$
T_{11} (NO+AsIII)	$5.44^{ab} \pm 0.19$	$6.14^{bcd} \pm 0.25$
T_{12} (Si+NO)	$5.57^{abc} \pm 0.22$	$5.82^{abc} \pm 0.19$
T_{13} (Si+NO+AsI)	$5.64^{abc} \pm 0.26$	$5.48^{abc} \pm 0.42$
T_{14} (Si+NO+AsII)	$5.32^{ab} \pm 0.2$	$5.25^{ab} \pm 0.43$
T_{15} (Si+NO+AsIII)	$4.69^{a} \pm 0.27$	$4.44^{a} \pm 0.15$

plants. T_5 exhibited 35.62% reduction in contrast to T_1 whereas T_9 showed 39.87% decline than T_1. A decline of 27.68% was noticed in T_{15} than T_{11}. T_{15} showed 43.58% decline than control plants.

6.1.2.5 Arsenic metalloid uptake

Roots exhibited greater metalloid uptake than leaves in 30-days old plants (Fig. 6.26; Table 6.21). Metalloid content of 1.95 and 1.39 mg g^{-1} DW was noticed in roots and leaves, respectively in As alone treated plants. Foliar application of Si showed 1.57 and 1.11 mg g^{-1} DW metal content whereas pre-treatment with NO showed 1.42 and 0.876 mg g^{-1} DW in roots and leaves, respectively under As stress. Si + NO treatment exhibited 1.05 and 0.583 mg g^{-1} DW As contents in roots and leaves, respectively.

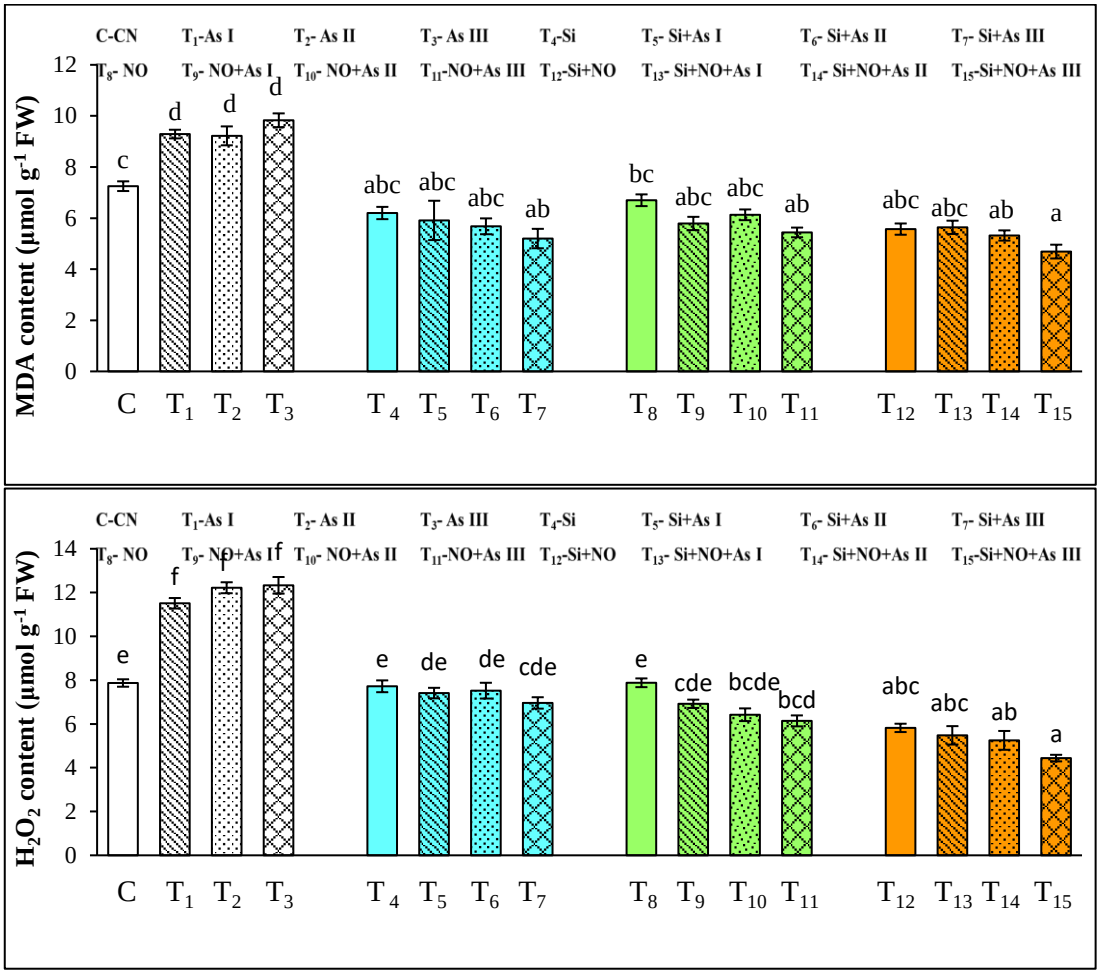

Fig. 6.25 Effect of Si and NO on MDA and H_2O_2 content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6. 21 Effect of Si and NO on metalloid uptake in root and leaves of 30-days old plants of *R. sativus* under As stress

Treatments	**Root (mg g^{-1} DW)**	**Leaves (mg g^{-1} DW)**
C (Control)	ND	ND
T_3 (AsIII)	1.95^{b}±0.222	1.39^{b}±0.154
T_7 (Si+AsIII)	1.57^{ab}±0.119	1.11^{ab}±0.091
T_{11} (NO+AsIII)	1.42^{ab}±0.117	0.876^{a}±0.062
T_{15} (Si+NO+AsIII)	1.05^{a}±0.098	0.583^{a}±0.204

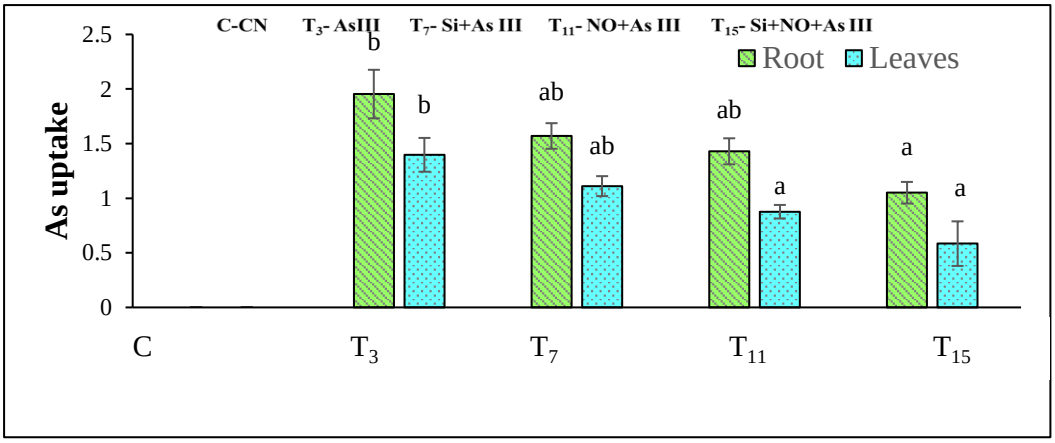

Fig. 6.26: Effect of Si and NO on As uptake in root and leaves in 30 days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean (SEM). Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.2.6 Osmolytes

Proline content was reduced in radish plants when they were exposed to As stress (Fig. 6.27; Table 6.22). Proline contents of 3.3, 3.19 and 2.26 µ mol g^{-1} FW were found at T_1, T_2 and T_3 concentrations, respectively. The control plants showed 4.16 µ mol g^{-1} FW proline content. Si applied control plants (T_4) exhibited 4.52 µ mol g^{-1} FW proline which was 8% more than the control genotypes. Only a 17% diminution was noticed in proline content in Si applied radish plants under As I stress (T_5) when contrasted with Si control plants (T_4). Proline content was increased from 2.26 to 3.06 µ mol g^{-1} FW in T_7 plants. Pre-treatment with NO also improved the proline content under stressed conditions with maximum proline content of 3.81 µ mol g^{-1} FW at T_9. Application of Si and NO further elevated the content of proline under stressed conditions with the greatest content of 4.79 µ mol g^{-1} FW at T_{13}. T_5 exhibited 12.72% increase in contrast to T_1 whereas T_9 showed 15.45% elevation than T_1. An augmentation of 28.76% was noticed in T_{13} than T_5.

Glycine betaine content was inhibited by As stress in radish plants (Fig. 6.27; Table 6.22). T_3 plants exhibited 4.17 µ mol g^{-1} FW glycine betaine content which was a 46% reduction. Si and NO application significantly mitigated the As-induced noxiousness in radish plants by increasing the glycine betaine content. The highest glycine betaine contents in individual application of Si and NO were 7.04 and 8.29 µ mol g^{-1} FW at T_5 and T_9 respectively. The combination of Si and NO control plants (T_{12}) showed 10.11 µ mol g^{-1} FW glycine betaine content. Under stressed conditions, the greatest glycine betaine content of 8.6 µ mol g^{-1} FW was found in T_{13} radish plants when applied with

Si and NO in combination. T_5 exhibited 27.07% increase in contrast to T_1 whereas T_9 showed 49.63% elevation than T_1. An augmentation of 3.73% was noticed in T_{13} than T_9. T_{13} plants showed 10.68% increase than control plants.

Table 6.22 Effect of Si and NO on osmolytes of 30 days old plants of *R. sativus* under As stress

Treatment	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
C (Control)	4.16^{d}±0.54	7.77^{cd}±0.43
T_1 (AsI)	3.3^{b}±0.46	5.54^{ab}±0.18
T_2 (AsII)	3.19^{b}±0.46	4.61^{a}±0.32
T_3 (AsIII)	2.26^{a}±0.26	4.17^{a}±0.25
T_4 (Si)	4.52^{de}±0.61	8.75^{de}±0.42
T_5 (Si+AsI)	3.72^{bc}±0.64	7.04^{bcd}±0.31
T_6 (Si+AsII)	3.54^{bc}±0.61	6.11^{abc}±0.21
T_7 (Si+AsIII)	3.06^{b}±0.48	5.5^{ab}±0.14
T_8 (NO)	4.8^{def}±0.8	9.09^{de}±0.47
T_9 (NO+AsI)	3.81^{bc}±0.61	8.29^{de}±0.48
T_{10} (NO+AsII)	3.52^{bc}±0.6	7.37^{bcd}±0.45
T_{11} (NO+AsIII)	3.44^{b}±0.7	7.07^{bcd}±0.86
T_{12} (Si+NO)	5.51^{g}±0.46	10.11^{e}±0.2
T_{13} (Si+NO+AsI)	4.79^{def}±0.68	8.6^{de}±0.25
T_{14} (Si+NO+AsII)	4.58^{de}±0.8	7.6^{cd}±0.14
T_{15} (Si+NO+AsIII)	4.19^{d}±0.75	7.23^{bcd}±0.42

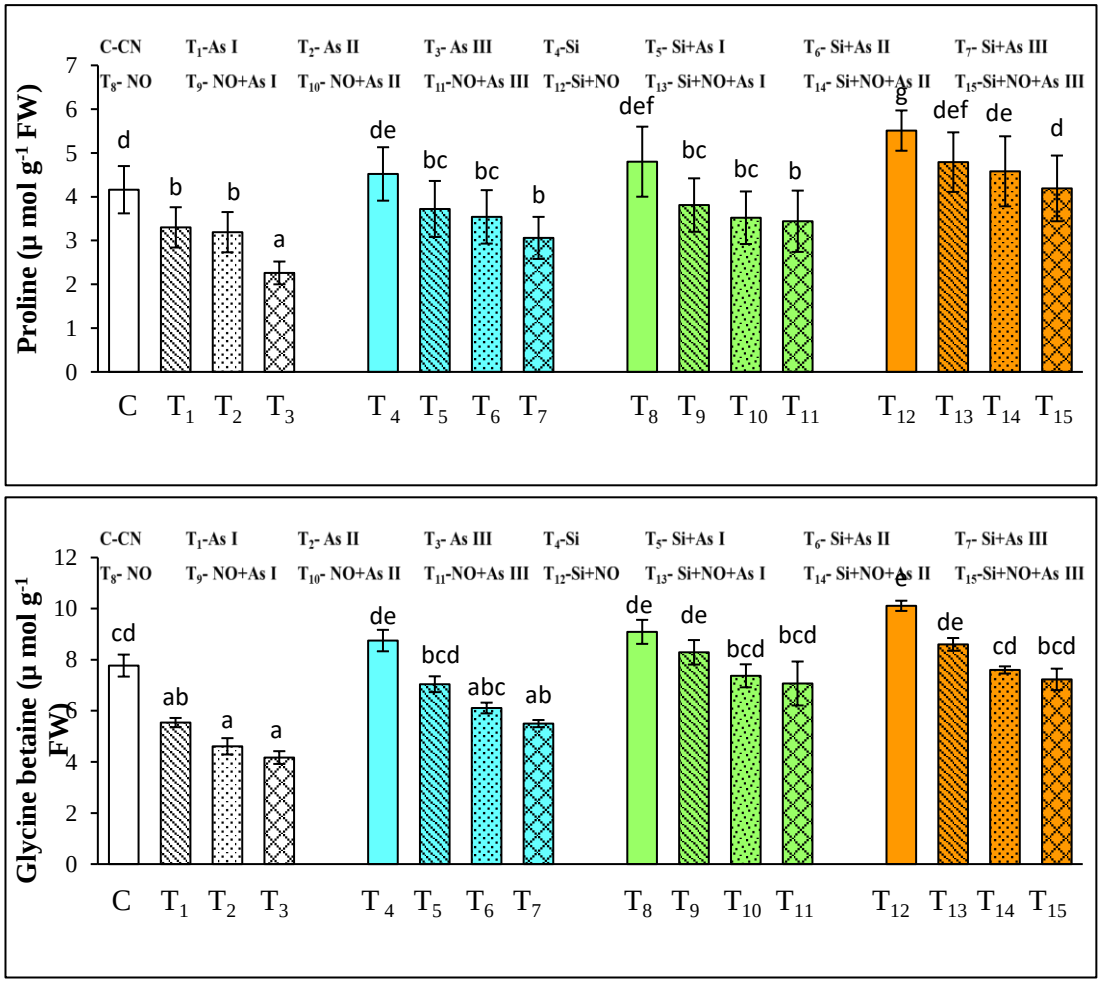

Fig. 6.27 Effect of Si and NO on proline and glycine betaine content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.2.7 Total carbohydrates

Arsenic stress decreased the carbohydrates content with a minimum content of 2.63 mg g^{-1} FW at T_3 stressed plants (Fig. 6.28; Table 6.23). Plants exposed to As I (T_1) showed a 38% reduction when contrasted against control plants. Foliar spray of Si under stressed conditions showed maximum and minimum carbohydrates content in T_6 and T_7 with 5.65 and 4.73 mg g^{-1} FW contents. Pre-treatment with NO in radish plants under stressed conditions exhibited the highest carbohydrate content of 5.81 mg g^{-1} FW in T_{10} plants. Combination of Si and NO showed a minimum carbohydrate content of 6.14 mg g^{-1} FW at T_{14} concentration. T_6 exhibited 57.82% increase in contrast to T_2 whereas T_{10} showed 62.29% elevation than T_2. An augmentation of 42.24% was noticed in T_{13} than T_5. T_{13} plants showed 19.73% increase than control plants.

Table 6.23 Effect of Si and NO on total carbohydrates of 30 days old plants of *R. sativus* under As stress

Treatment	Total carbohydrates (mg g^{-1} FW)
C (Control)	$6.13^{efg}\pm0.25$
T_1 (AsI)	$3.8^{abc}\pm0.22$
T_2 (AsII)	$3.58^{ab}\pm0.3$
T_3 (AsIII)	$2.63^{a}\pm0.29$
T_4 (Si)	$7.48^{h}\pm0.17$
T_5 (Si+AsI)	$5.16^{de}\pm0.2$
T_6 (Si+AsII)	$5.65^{def}\pm0.27$
T_7 (Si+AsIII)	$4.73^{bcd}\pm0.25$
T_8 (NO)	$6.98^{fgh}\pm0.18$
T_9 (NO+AsI)	$5.08^{cde}\pm0.1$
T_{10} (NO+AsII)	$5.81^{def}\pm0.19$
T_{11} (NO+AsIII)	$4.71^{bcd}\pm0.33$
T_{12} (Si+NO)	$8.91^{i}\pm0.35$
T_{13} (Si+NO+AsI)	$7.34^{gh}\pm0.18$
T_{14} (Si+NO+AsII)	$6.14^{efgh}\pm0.4$
T_{15} (Si+NO+AsIII)	$6.78^{fgh}\pm0.16$

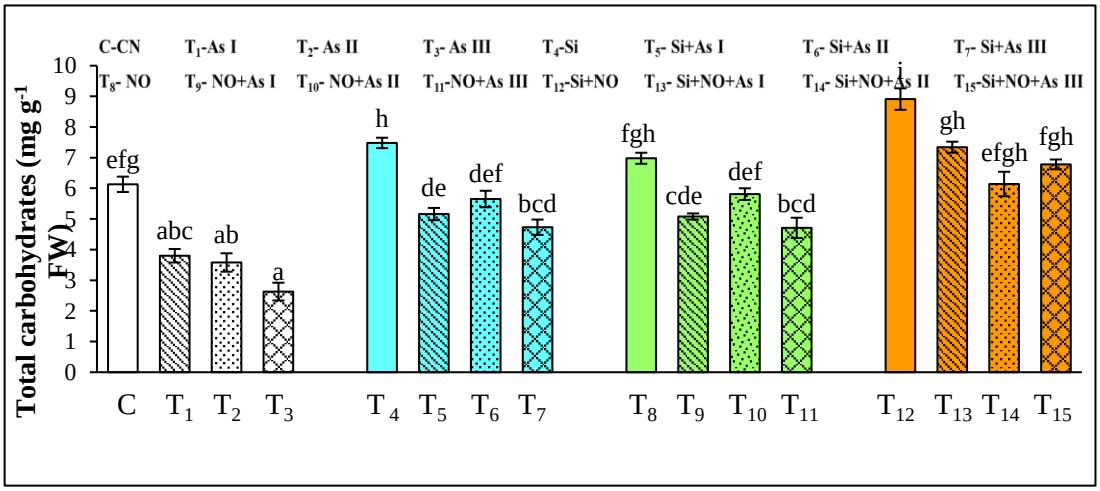

Fig. 6.28 Effect of Si and NO on total carbohydrates in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

6.1.2.8 Protein content and antioxidant defense system

6.1.2.8.1 Protein content and antioxidative enzymes

Protein content was reported to be reduced in radish plants by their exposure to As stress (Fig. 6.29; Table 6.24). Protein contents of 7.22, 7.17 and 6.61 mg g^{-1} FW were

observed at T_1, T_2 and T_3 concentrations, respectively. Silicon control radish (T_4) plants showed 10.23 mg g^{-1} FW content while under As stress, Si exhibited 8.52 mg g^{-1} FW at T_5. Pre-treatment of radish plants with NO under stressed conditions also increased the protein content with the highest protein of 8.89 at T_9. Coupled application of Si and NO exhibited 9.44, 8.5 and 8.75 mg g^{-1} FW protein contents at T_{13}, T_{14} and T_{15} concentrations, respectively. T_6 exhibited 18.68% increase in protein content in contrast to T_2 whereas T_{10} showed 18.41% elevation than T_2. An augmentation of 10.79% was noticed in T_{13} than T_5. T_{13} plants showed 12.91% increase than control plants.

Arsenic exposure resulted a decrease in SOD activity with 6.77, 6.22 and 5.21 UA mg^{-1} protein at T_1, T_2 and T_3 concentrations, respectively (Fig. 6.29; Table 6.24). Silicon control plants (T_4) showed 9.13 UA mg^{-1} protein. T_5 showed a 14% increase in SOD activity when compared to As I stressed plants. Pre-treatment with NO under stressed conditions improved the SOD enzyme activity with the highest activity of 7.32 UA mg^{-1} protein in T_9 plants. Highest SOD activity (9.54 UA mg^{-1} protein) under As toxicity was found in T_{13} treated plants. T_5 exhibited 14.18% increase in contrast to T_1 whereas T_9 showed 8.12% elevation than T_1. An augmentation of 23.41% was noticed in T_{13} than T_5. T_{13} plants showed 2.06% increase than control plants.

Control plants showed 7.56 UA mg^{-1} protein CAT activity, whereas T_1, T_2 and T_3 treated plants showed 6.69, 6.03 and 6.18 UA mg^{-1} protein (Fig. 6.29; Table 6.24). Application of Si under stressed conditions exhibited 7.04, 7.22 and 7.08 UA mg^{-1} protein at T_5, T_6 and T_7 concentrations. Highest CAT activity under As stress was found in T_{11} with 7.74 UA mg^{-1} protein in the case of NO pre-treated plants. Combination of Si and NO showed the highest CAT activity of 9.85 UA mg^{-1} protein at T_{14} level. T_5 exhibited 5.23% increase in contrast to T_1 whereas T_9 showed 14.49% elevation than T_1. An augmentation of 36.42% was noticed in T_{14} than T_6. T_{14} plants showed 30.29% increase than control plants.

Arsenic exposure led to the maximum reduction in the APX enzyme activity with the highest reduction of 14.54 UA mg^{-1} protein at T_3 concentration (Fig. 6.30; Table 6.24). Application of Si and NO alone markedly increased the APX level. The maximum content of 19.79 UA mg^{-1} protein in the case of Si treatment was noticed at T_5 while in NO treated plants, higher APX activity (20.79 UA mg^{-1} protein) was in T_9 plants. Under

As stress, the highest APX enzyme activity of 22.61 UA mg^{-1} protein was noticed at combined treatments of Si + NO at T_{14}. T_5 exhibited 16.54% increase in contrast to T_1 whereas T_9 showed 22.43% elevation than T_1. An augmentation of 12.93% was noticed in T_{14} than T_{10}. T_{14} plants showed 19.75% increase than control plants.

Table 6.24 Effect of Si and NO on protein content and antioxidative enzymes of 30 days old plants of *R. sativus* under As stress

Treatment	Protein content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
C (Control)	8.36^{bcde} ±0.23	7.88^{def}±0.18	7.56^{bcde} ±0.23	18.88^{cdef} ±0.42
T_1 (AsI)	7.22^{abc} ±0.28	6.77^{bcd}±0.25	6.69^{abc} ±0.24	16.98^{abc} ±0.22
T_2 (AsII)	7.17^{ab} ±0.1	6.22^{ab}±0.19	6.03^{a} ±0.33	15.72^{ab} ±0.48
T_3 (AsIII)	6.61^{a} ±0.11	5.21^{a}±0.24	6.18^{ab} ±0.27	14.54^{a} ±0.27
T_4 (Si)	10.23^{fg} ±0.23	9.13^{gh}±0.18	9.15^{fghi} ±0.22	21.31^{fgh} ±0.33
T_5 (Si+AsI)	8.52^{cde} ±0.11	7.73^{cdef}±0.2	7.04^{abcd} ±0.46	19.79^{cdefg} ±0.44
T_6 (Si+AsII)	8.51^{cde} ±0.26	7.34^{bcde}±0.16	7.22^{abcde} ±0.22	18.10^{bcde} ±0.14
T_7 (Si+AsIII)	7.46^{abcd} ±0.24	6.27^{ab}±0.12	7.08^{abcde} ±0.22	17.28^{abcd} ±0.53
T_8 (NO)	10.41^{fg} ±0.19	8.67^{fgh}±0.4	8.51^{efgh} ±0.33	22.79^{hi} ±0.69
T_9 (NO+AsI)	8.89^{e} ±0.28	7.32^{bcde}±0.26	7.66^{cde} ±0.27	20.79^{efgh} ±0.5
T_{10} (NO+AsII)	8.49^{cde} ±0.13	6.61^{bc}±0.36	7.00^{abcd} ±0.22	20.02^{defgh} ±0.47
T_{11} (NO+AsIII)	7.23^{abc} ±0.28	6.14^{ab}±0.12	7.74^{cdef} ±0.26	18.63^{cdef} ±0.42
T_{12} (Si+NO)	10.82^{g}±0.25	11.14^{i}±0.11	10.11^{i} ±0.15	24.60^{i} ±0.54
T_{13} (Si+NO+AsI)	9.44^{ef} ±0.13	9.54^{h}±0.31	9.29^{ghi} ±0.22	20.55^{efgh} ±0.73
T_{14} (Si+NO+AsII)	8.5^{cde} ±0.19	8.89^{fgh}±0.14	9.85^{hi} ±0.18	22.61^{ghi} ±0.74
T_{15} (Si+NO+AsIII)	8.75^{de} ±0.51	8.17^{efg}±0.13	8.18^{defg} ±0.34	19.40^{cdef} ±1.03

Activity of POD enzyme was highly reduced in T_1 concentration with 13.89 UA mg^{-1} protein activity when compared to control plants (Fig. 6.30; Table 6.25). Control plants of Si and NO (T_5 and T_9) showed 20.98 and 21.85 UA mg^{-1} protein POD activities which were greater than the control plants. Under stressed conditions, the highest activities with regard to individual application of Si and NO were shown at T_7 and T_9 concentrations with 19.12 and 19.8 UA mg^{-1} protein POD activities, respectively. Maximum POD activity among all the treatments under As stress was

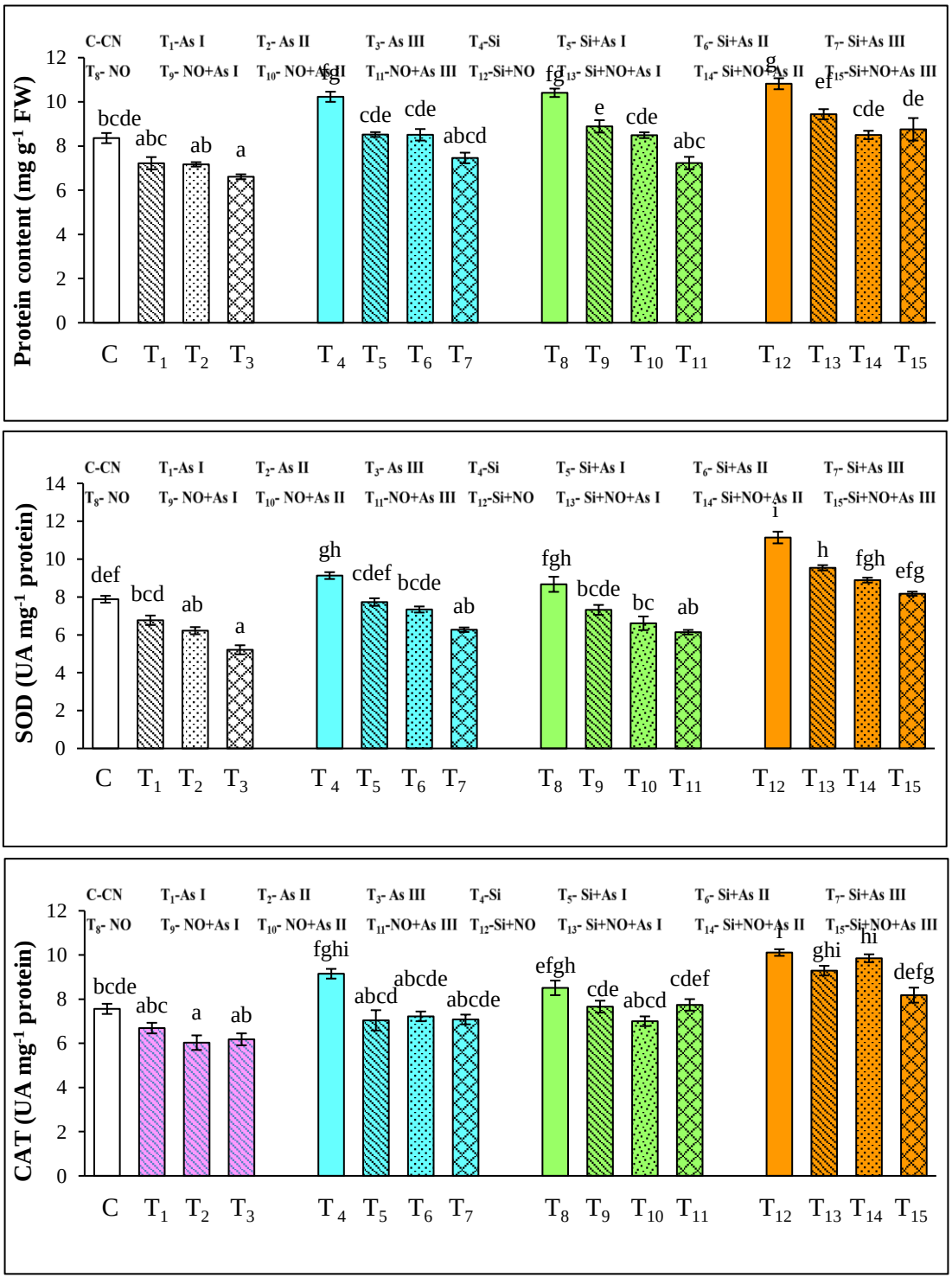

Fig. 6.29 Effect of Si and NO on protein, SOD and CAT enzyme activity in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

noticed at T_{14} in the case of the combination of Si and NO with 19.35 UA mg^{-1} protein. T_7 exhibited 36.76% increase in contrast to T_3 whereas T_{11} showed 35.33% elevation than T_3. An augmentation of 2.54% was noticed in T_{14} than T_{10}.

The functioning of the GR enzyme was negatively affected by As stress with minimum GR activity of 4.05 UA mg^{-1} protein at T_3 concentration (Fig. 6.30; Table 6.25). Enzymatic activity of GR was observed to be diminished as the As level elevated. Foliar spray with Si under As stress increased the GR activity. Activity of GR enzyme was enhanced from 4.05 to 6.24 UA mg^{-1} protein in T_7 plants. Pre-treated NO plants showed the highest and lowest GR activity at T_9 and T_{11} with 7.49 and 5.82 UA mg^{-1} protein, respectively. Coupled application of Si and NO in case of As exposure showed the highest GR activity (8.7 UA mg^{-1} protein) at T_{13} level. T_5 exhibited 31.65% increase in contrast to T_1 whereas T_9 showed 39.47% elevation than T_1. An augmentation of 16.15% was noticed in T_{13} than T_9. T_{13} exhibited 21.84% increase than control plants.

Activity of GPOX enzyme was reduced in As stressed plants with 3.55, 4.2 and 2.79 UA mg^{-1} protein at T_1, T_2 and T_3 concentrations, respectively (Fig. 6.31; Table 6.25). Individually, Si upregulated the GPOX activity than As alone treated plants. Addition of Si under As showed maximum GPOX activity of 6.52 UA mg^{-1} protein at T_5. Application of NO under As stress upsurged the GPOX level with the highest activity of 6.18 UA mg^{-1} protein in T_{10} treated plants. Further, GPOX enzyme, in the case of coupled treatment of Si and NO exhibited maximum and minimum activity of 7.42 and 6.19 UA mg^{-1} protein at T_{14} and T_{15} concentrations, respectively. T_5 exhibited 83.66% increase in contrast to T_1 whereas T_9 showed 60.28% elevation than T_1. An augmentation of 20.06% was noticed in T_{14} than T_{10}. T_{14} exhibited 19.13% increase than control plants.

DHAR enzyme level was lessened in radish plants by their exposure to As stress (Fig. 6.31; Table 6.25). Among As alone treated plants, minimum DHAR activity of 5.45 UA mg^{-1} protein was found in T_3 stressed plants. In the case of Si application under As, the highest activity (9.2 UA mg^{-1} protein) was at T_5. Application of NO also increased the DHAR activity under stressed conditions, among which the lowest activity of 8.05 UA mg^{-1} protein was found at T_{11}. Si + NO further augmented the

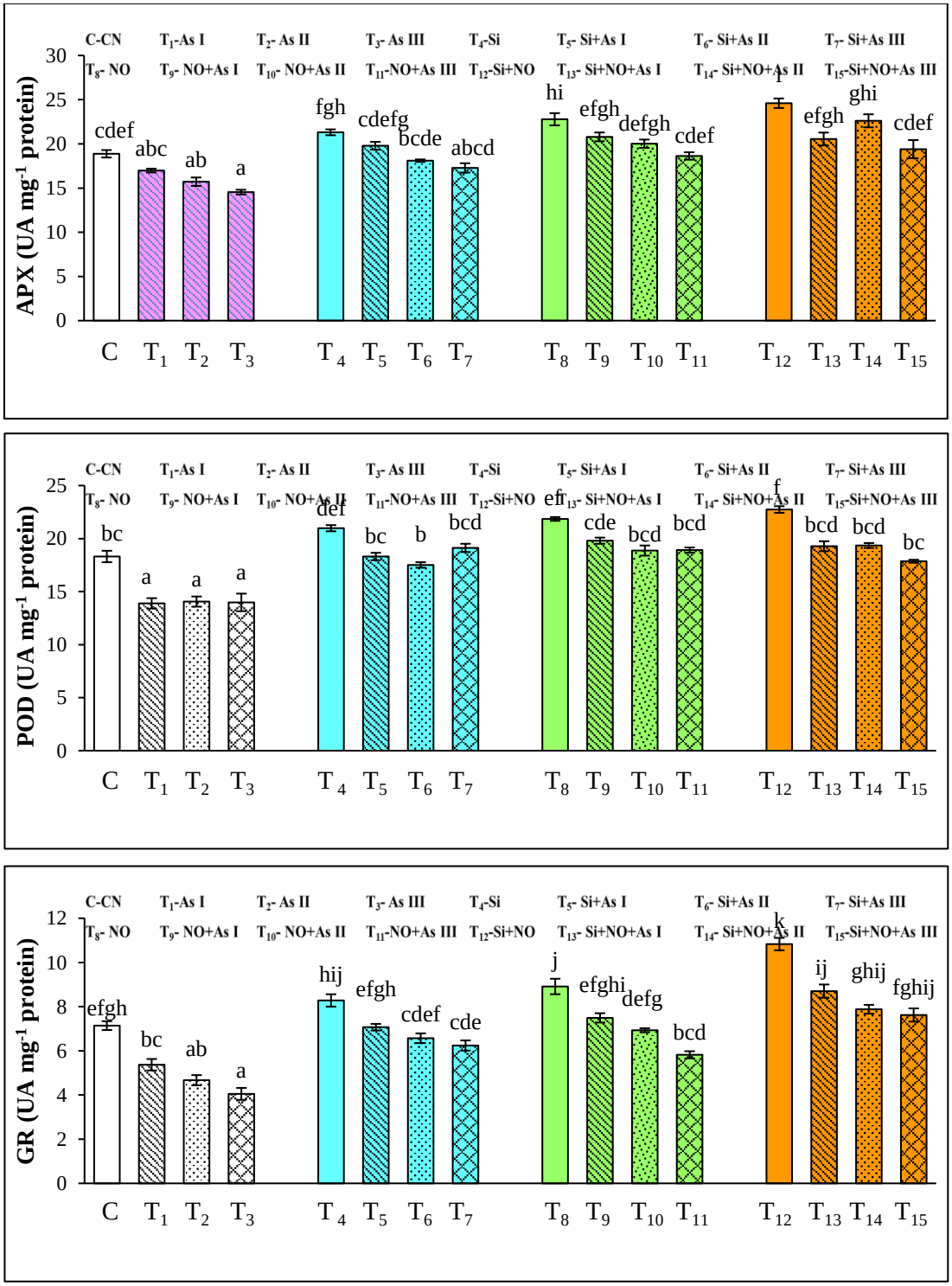

Fig. 6.30 Effect of Si and NO on APX, POD and GR enzyme activity in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.25 Effect of Si and NO on antioxidative enzymes of 30 days old plants of *R. sativus* under As stress

Treatment	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
C (Control)	18.31^{bc} ±0.54	7.14^{efgh}±0.2	6.48^{efg}±0.17	8.97^{cdefg}±0.5
T_1 (AsI)	13.89^{a} ±0.49	5.37^{bc}±0.26	3.55^{bc}±0.2	7.27^{abc}±0.28
T_2 (AsII)	14.06^{a} ±0.48	4.67^{ab}±0.23	4.2^{ab}±0.2	6.69^{ab}±0.21
T_3 (AsIII)	13.98^{a} ±0.83	4.05^{a}±0.27	2.79^{a}±0.11	5.45^{a}±.27
T_4 (Si)	20.98^{def} ±0.29	8.28^{hij}±0.28	7.24^{fgh}±0.29	10.63^{fghi}±0.32
T_5 (Si+AsI)	18.31^{bc} ±0.35	7.07^{efgh}±0.15	6.52^{efg}±0.24	9.2^{cdefg}±0.1
T_6 (Si+AsII)	17.51^{b} ±0.26	6.57^{cdef}±0.22	6.1^{def}±0.24	8.28^{bcde}±0.16
T_7 (Si+AsIII)	19.12^{bcd} ±0.39	6.24^{cde}±0.23	5.08^{cd}±0.29	7.67^{bcd}±0.18
T_8 (NO)	21.85^{ef} ±0.17	8.91^{j}±0.35	7.19^{fg}±0.18	11.39^{hi}±0.69
T_9 (NO+AsI)	19.8^{cde} ±0.29	7.49^{efghi}±0.21	5.69^{de}±0.22	9.57^{defgh}±0.51
T_{10} (NO+AsII)	18.87^{bcd} ±0.48	6.93^{defg}±0.09	6.18^{def}±0.23	8.68^{bcdef}±0.6
T_{11} (NO+AsIII)	18.92^{bcd} ±0.24	5.82^{bcd}±0.16	5.64^{de}±0.25	8.05^{bcde}±0.61
T_{12} (Si+NO)	22.74^{f} ±0.31	10.83^{k}±0.28	8.41^{h}±0.15	12.37^{i}±0.26
T_{13} (Si+NO+AsI)	19.28^{bcd} ±0.47	8.7^{ij}±0.3	6.72^{efg}±0.28	10.91^{ghi}±0.38
T_{14} (Si+NO+AsII)	19.35^{bcd} ±0.23	7.88^{ghij}±0.2	7.42^{gh}±0.16	9.98^{efgh}±0.23
T_{15} (Si+NO+AsIII)	17.86^{bc} ±0.14	7.62^{fghij}±0.3	6.19^{def}±0.24	9.17^{cdefg}±0.19

DHAR activity under stressed conditions with maximum DHAR activity of 10.91 UA mg^{-1} protein at T_{13}. T_5 exhibited 26.54% increase in contrast to T_1 whereas T_9 showed 31.63% elevation than T_1. An augmentation of 18.58% was noticed in T_{13} than T_5.

The lowest MDHAR activity of 6.74 UA mg^{-1} protein was in T_3 plants (Fig. 6.31; Table 6.26). Application of Si (T_4) showed 13.39 UA mg^{-1} protein whereas Si supplementation under stressed conditions exhibited 10.25, 9.02 and 8.58 UA mg^{-1} protein activity at T_5, T_6 and T_7 concentrations, respectively. Only a 25% decline in its amount was noticed in T_{11} than their respective control genotypes (T_8). Only 21, 26 and 30% reductions were found in T_{13}, T_{14} and T_{15} stressed plants when applied with a combination of Si and NO than T_{12} plants. T_5 exhibited 25.76% increase in contrast to T_1 whereas T_9 showed 33.61% elevation than T_1. An augmentation of 15.31% was noticed in T_{13} than T_5. T_{13} showed 4.14% elevation than control plants.

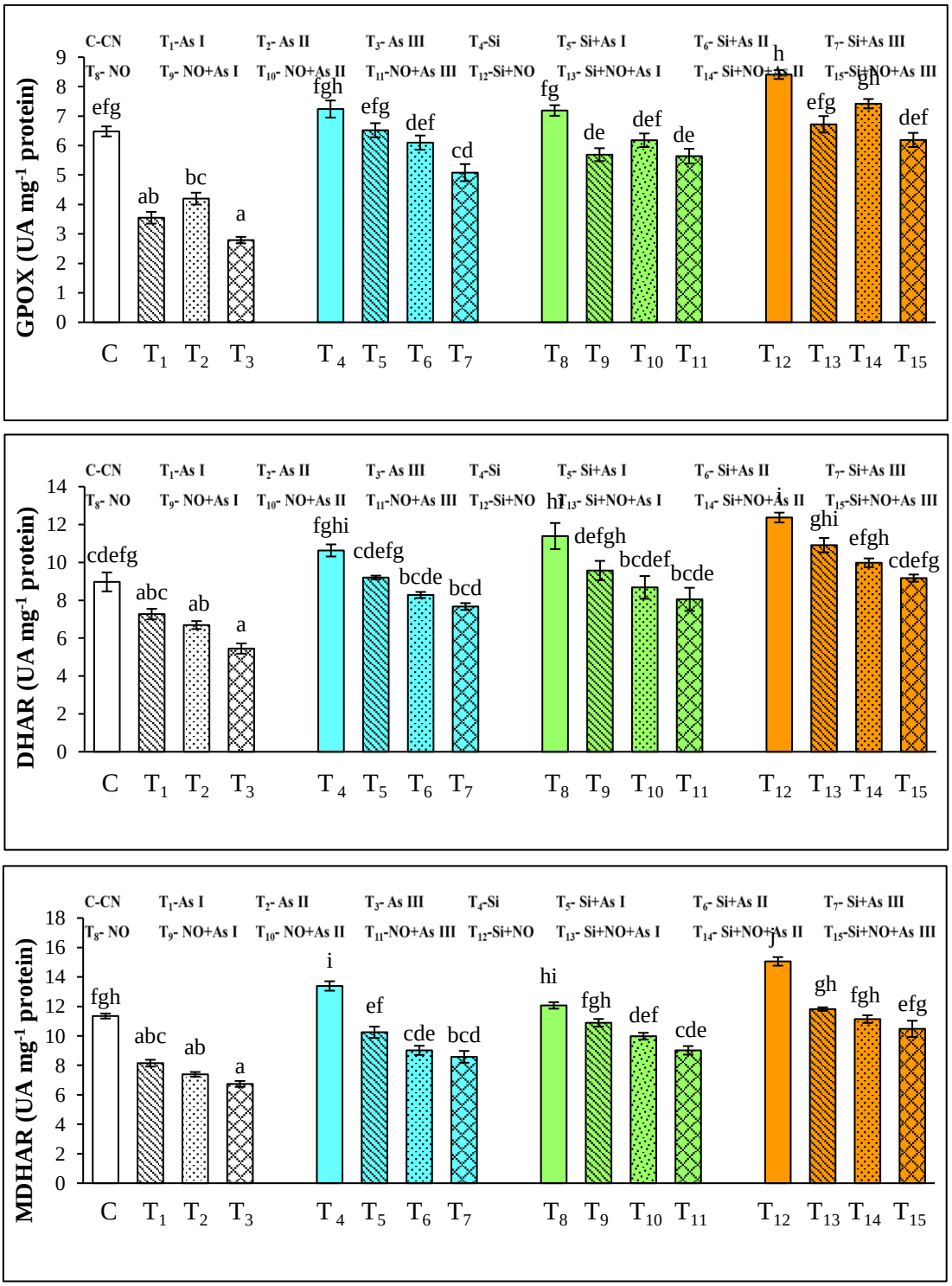

Fig. 6.31 Effect of Si and NO on GPOX, DHAR and MDHAR enzyme activity in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

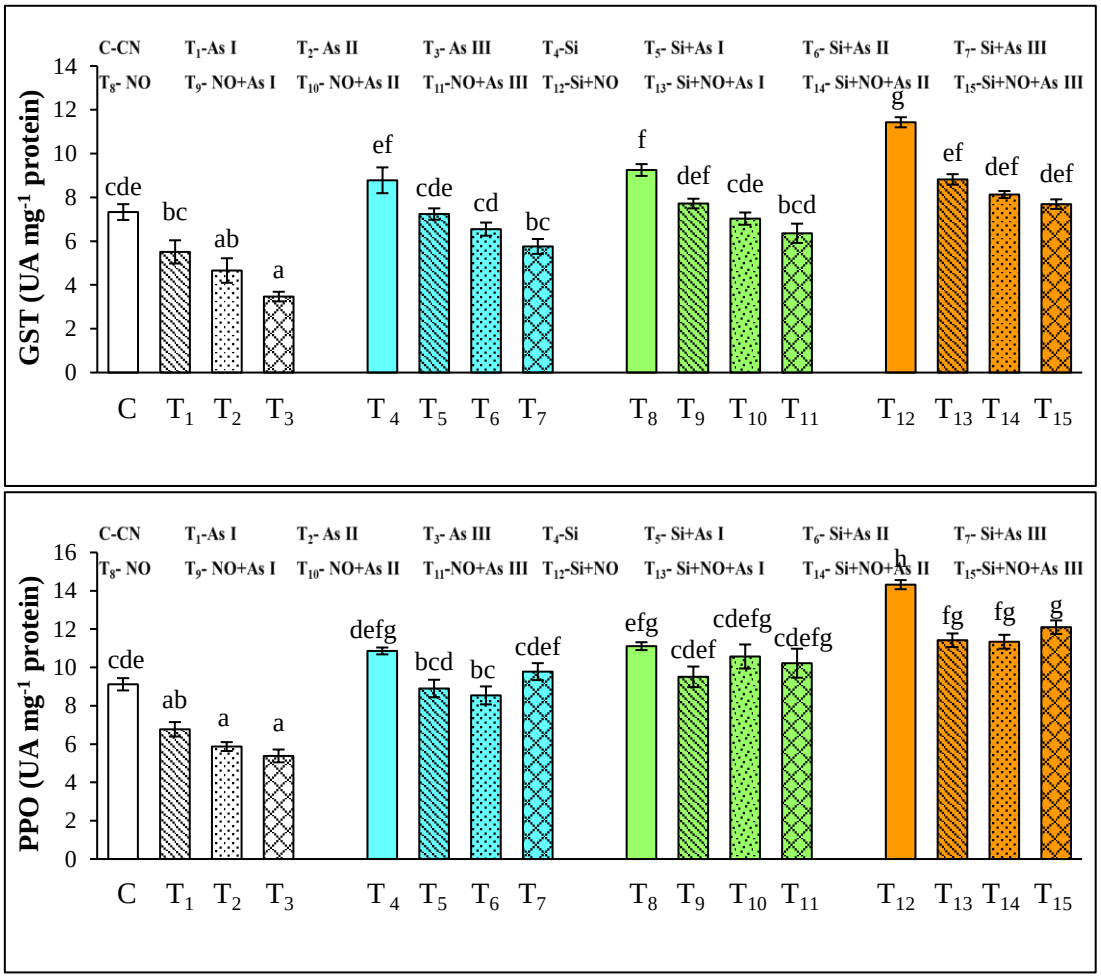

Fig. 6.32 Effect of Si and NO on GST and PPO enzyme activity in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Arsenic stress diminished the GST activity in 30-days old radish plants with a minimum activity of 3.47 UA mg^{-1} protein in T_3 stressed plants (Fig. 6.32; Table 6.26). Individual treatment of Si and NO under stressed conditions resulted in maximum GST activity under As I stress with 7.24 and 7.72 UA mg^{-1} protein GST activities at T_5 and T_9, respectively. Combined Si and NO control plants (T_{12}) exhibited 11.43 UA mg^{-1} protein activity while in T_{13}, T_{14} and T_{15}, combination of Si and NO showed 8.82, 8.13 and 7.69 UA mg^{-1} protein activities, respectively. T_5 exhibited 31.39% increase in contrast to T_1 whereas T_9 showed 40.10% elevation than T_1. An augmentation of 14.24% was noticed in T_{13} than T_9. T_{13} showed 20.32% elevation than control plants.

Minimum PPO activity was noticed in As III treated plants with 5.38 UA mg^{-1} protein (Fig. 6.32; Table 6.26). However, treatment of Si under As stress caused maximum

PPO activity of 9.78 UA mg^{-1} protein at T_3 concentration. Whereas with regard to NO-treated plants under stressed conditions, maximum PPO activity of 10.57 UA mg^{-1} protein was observed at T_{10}. Highest and lowest PPO activities in the case of Si and NO combination were found at T_{15} and T_{14} with 12.1 and 11.34 UA mg^{-1} protein activities, respectively. T_7 exhibited 81.78% increase in contrast to T_3. An augmentation of 18.39% was noticed in T_{15} than T_{11}. T_{13} showed 25.21% elevation than control plants.

Table 6.26 Effect of Si and NO on antioxidative enzymes of 30 days old plants of *R. sativus* under As stress

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
C (Control)	$11.35^{fgh}\pm0.17$	$7.33^{cde}\pm0.36$	$9.12^{cde}\pm0.32$
T_1 (AsI)	$8.15^{abc}\pm0.23$	$5.51^{bc}\pm0.53$	$6.77^{ab}\pm0.37$
T_2 (AsII)	$7.39^{ab}\pm0.16$	$4.66^{ab}\pm0.56$	$5.87^{a}\pm0.23$
T_3 (AsIII)	$6.74^{a}\pm0.2$	$3.47^{a}\pm0.22$	$5.38^{a}\pm0.33$
T_4 (Si)	$13.39^{i}\pm0.32$	$8.78^{ef}\pm0.59$	$10.86^{defg}\pm0.17$
T_5 (Si+AsI)	$10.25^{ef}\pm0.38$	$7.24^{cde}\pm0.26$	$8.9^{bcd}\pm0.45$
T_6 (Si+AsII)	$9.02^{cde}\pm0.31$	$6.55^{cd}\pm0.3$	$8.54^{bc}\pm0.46$
T_7 (Si+AsIII)	$8.58^{bcd}\pm0.4$	$5.76^{bc}\pm0.34$	$9.78^{cdef}\pm0.44$
T_8 (NO)	$12.07^{hi}\pm0.22$	$9.25^{f}\pm0.27$	$11.11^{efg}\pm0.20$
T_9 (NO+AsI)	$10.89^{fgh}\pm0.26$	$7.72^{def}\pm0.22$	$9.51^{cdef}\pm0.53$
T_{10} (NO+AsII)	$9.98^{def}\pm0.23$	$7.03^{cde}\pm0.28$	$10.57^{cdefg}\pm0.62$
T_{11} (NO+AsIII)	$9.02^{cde}\pm0.29$	$6.36^{bcd}\pm0.44$	$10.22^{cdefg}\pm0.75$
T_{12} (Si+NO)	$15.06^{j}\pm0.29$	$11.43^{g}\pm0.23$	$14.32^{h}\pm0.23$
T_{13} (Si+NO+AsI)	$11.82^{gh}\pm0.12$	$8.82^{ef}\pm0.24$	$11.42^{fg}\pm0.35$
T_{14} (Si+NO+AsII)	$11.14^{fgh}\pm0.26$	$8.13^{def}\pm0.16$	$11.34^{fg}\pm0.36$
T_{15} (Si+NO+AsIII)	$10.48^{efg}\pm0.55$	$7.69^{def}\pm0.22$	$12.1^{g}\pm0.35$

6.1.2.8.2 Non-enzymatic antioxidants

The levels of ascorbic acid were lowered in the case of As treated plants (Fig. 6.33; Table 6.27). The lowest ascorbic acid content (4.04 µg g^{-1} FW) was found at T_2 concentration. Maximum ascorbic content i.e., 7.20 µg g^{-1} FW was noted at Si + NO treated plants i.e., T_{12}. At T_{13}, T_{14} and T_{15} in the case of Si and NO combination plants, ascorbic acid contents of 6.43, 6.72 and 6.31 µg g^{-1} FW, respectively were observed. T_6 exhibited 11.13% increase in contrast to T_2 whereas T_{10} showed 25% elevation than T_2. An augmentation of 33.06% was noticed in T_{14} than T_{10}. T_{14} showed 12.56% elevation than control plants.

Glutathione content was found to be diminished as As level elevated (Fig. 6.33; Table 6.27). Minimum glutathione content i.e., 5.02 µg g^{-1} FW was at T_3 concentration which

was a 39% decline than control plants. Si and NO (control) genotypes (T_4 and T_8) exhibited an elevation of 5% and 1%, respectively than control plants. Alone treatment of Si showed 7.48, 7.71 and 8.09 µg g^{-1} FW at T_5, T_6 and T_7 concentrations, respectively. Combined supplementation with Si and NO under As stress increased the glutathione content to 8.99, 8.83 and 8.58 µg g^{-1} FW in T_{13}, T_{14} and T_{15} stressed plants, respectively. T_6 exhibited 52.07% increase in contrast to T_2 whereas T_{10} showed 50.09% elevation than T_2. An augmentation of 16.03% was noticed in T_{14} than T_{10}. T_{13} showed 9.50% elevation than control plants.

Table 6.27 Effect of Si and NO on non-enzymatic antioxidants of 30 days old plants of *R. sativus* under As stress

Treatment	**Ascorbic acid (µg g^{-1} FW)**	**Glutathione (µg g^{-1} FW)**	**Tocopherol content (µg g^{-1} FW)**
C (Control)	5.97^{def} ±0.19	8.21^{bcdef} ±0.25	6.56^{fgh}±0.29
T_1 (AsI)	4.58^{abc} ±0.17	5.34^{a} ±0.24	3.07^{a}±0.15
T_2 (AsII)	4.04^{a} ±0.20	5.07^{a} ±0.21	4.34^{abc}±0.32
T_3 (AsIII)	4.18^{ab} ±0.10	5.02^{a} ±0.25	4.05^{ab}±0.34
T_4 (Si)	5.08^{bcd} ±0.27	8.60^{def} ±0.20	7.66^{hi}±0.27
T_5 (Si+AsI)	4.55^{abc} ±0.14	7.48^{bcd} ±0.20	5.75^{defg}±0.17
T_6 (Si+AsII)	4.49^{ab} ±0.23	7.71^{cde} ±0.16	6.4^{efgh}±0.13
T_7 (Si+AsIII)	5.06^{bcd} ±0.15	8.09^{b} ±0.18	5.12^{bcde}±0.2
T_8 (NO)	6.55^{fg} ±0.16	8.26^{cdef} ±0.14	6.93^{ghi}±0.18
T_9 (NO+AsI)	5.49^{cde} ±0.16	7.50^{bcd} ±0.21	5.08^{bcd}±0.16
T_{10} (NO+AsII)	5.05^{bcd} ±0.10	7.61^{bcd} ±0.18	5.6^{cdef}±0.31
T_{11} (NO+AsIII)	4.74^{abc} ±0.16	7.42^{bc} ±0.16	4.3^{abc}±0.35
T_{12} (Si+NO)	7.20^{g} ±0.11	10.14^{g} ±0.14	7.92^{i}±0.18
T_{13} (Si+NO+AsI)	6.43^{fg} ±0.14	8.99^{fg} ±0.27	6.11^{defg}±0.17
T_{14} (Si+NO+AsII)	6.72^{fg} ±0.23	8.83^{ef} ±0.25	5.8^{defg}±0.22
T_{15} (Si+NO+AsIII)	6.31^{efg} ±0.20	8.58^{def} ±0.30	6.59^{fgh}±0.29

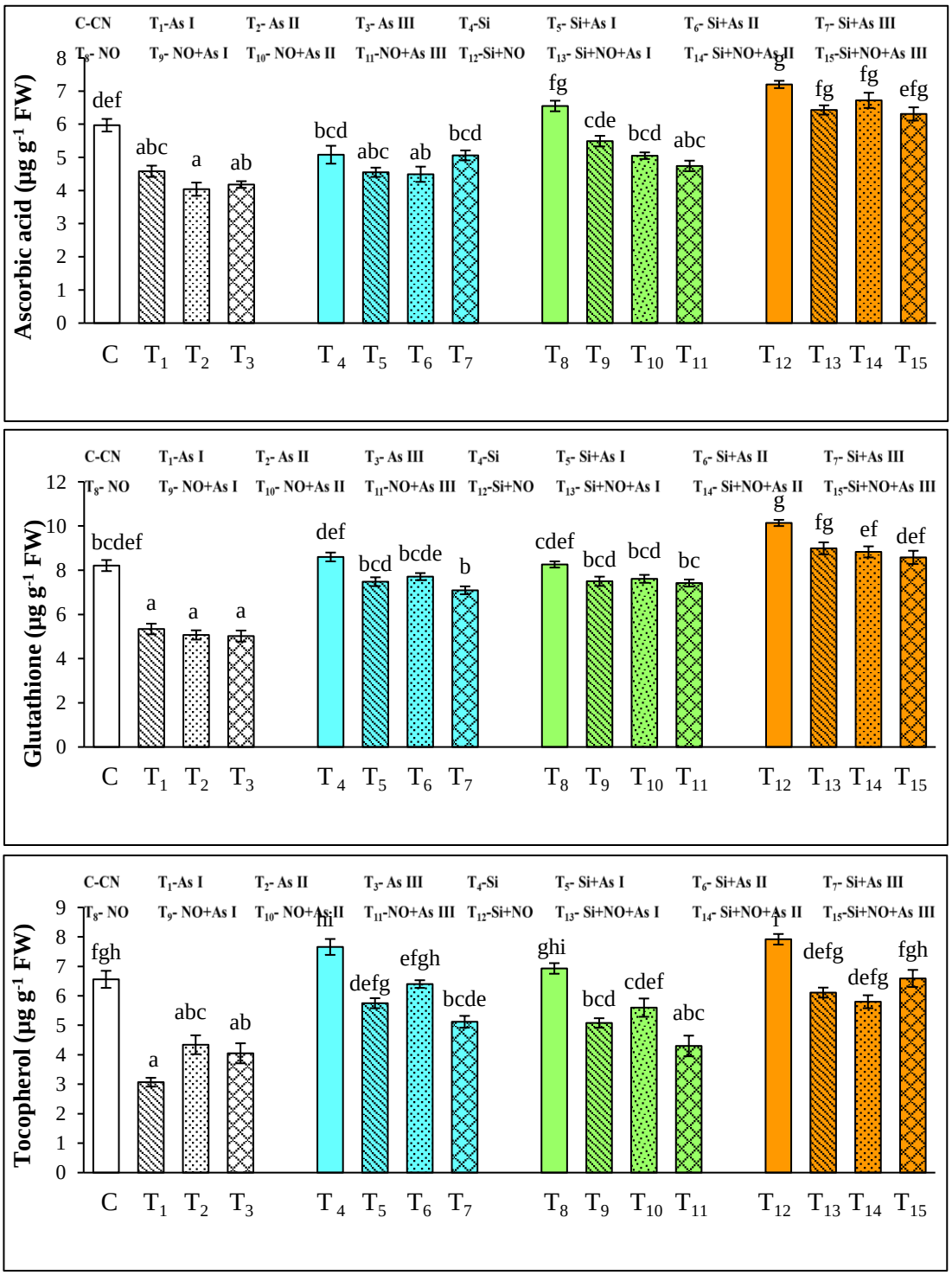

Fig. 6.33 Effect of Si and NO on ascorbic acid, glutathione and tocopherol content in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Tocopherol content was negatively associated with As treatment as As metalloid decreased the tocopherol content (Fig. 6.33; Table 6.27). T_1 treatment showed 3.07 µg g^{-1} FW amount which was the minimum among all three As concentrations used in the study. In individual application of Si and NO under As stress, highest tocopherol content was noticed at T_6 and T_{10} with 6.4 and 5.6 µg g^{-1} FW, respectively. The highest tocopherol content of 6.59 µg g^{-1} FW was found in T_{15} plants. T_6 exhibited 47.46% increase in contrast to T_2 whereas T_{10} showed 29.03% elevation than T_2. An augmentation of 3.57% was noticed in T_{14} than T_{10}.

6.1.2.9 Gene expression

Lowest expression of SOD gene was observed in control plants with 0.95 fold change (Fig. 6.34; Table 6.28). Under As III stress, 1.94 fold change in gene expression was notice. Individual treatment with Si and NO under As stress showed 3.5 and 5.6 fold change. However, co-application of Si + NO further induced the gene expression of SOD gene with 7.12 fold change under stressed conditions.

Similarly, expression of CAT gene was also influenced with minimum expression in control plants with 1.22 fold change (Fig. 6.34; Table 6.28). A fold change of 1.57 was found in As III stressed plants. Application of Si and NO alone under stressed conditions increased the expression of CAT gene with 5.67 and 5.41 fold change, respectively. Application of Si and NO in coupled manner resulted in 8.62 fold change in CAT expression under As III which was highest among all the samples.

Table 6.28 Effect of Si and NO on relative gene expression of SOD and CAT in *R. sativus* plants under As stress

Treatment	**Relative gene expression**	
	SOD	**CAT**
C (Control)	0.95^{a}±0.1	1.22^{a}±0.22
T_3 (AsIII)	1.94^{ab}±0.47	1.57^{a}±0.39
T_7 (Si+AsIII)	3.5^{b}±0.53	5.67^{b}±0.49
T_{11} (NO+AsIII)	5.6^{c}±0.25	5.41^{b}±0.22
T_{15} (Si+NO+AsIII)	7.12^{c}±0.48	8.62^{c}±0.3

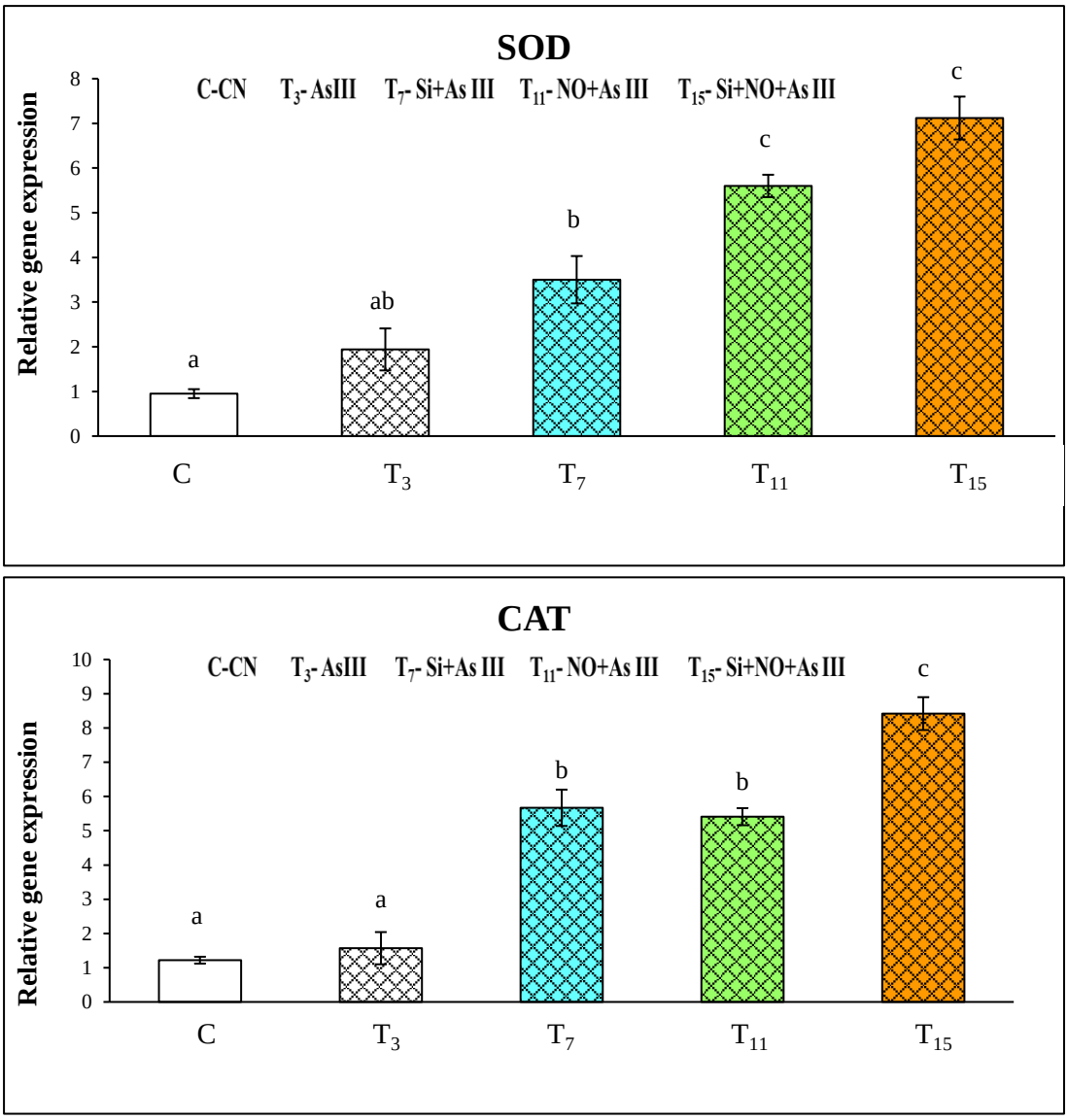

Fig. 6.34 Effect of Si and NO on relative gene expression of SOD and CAT genes in 30-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

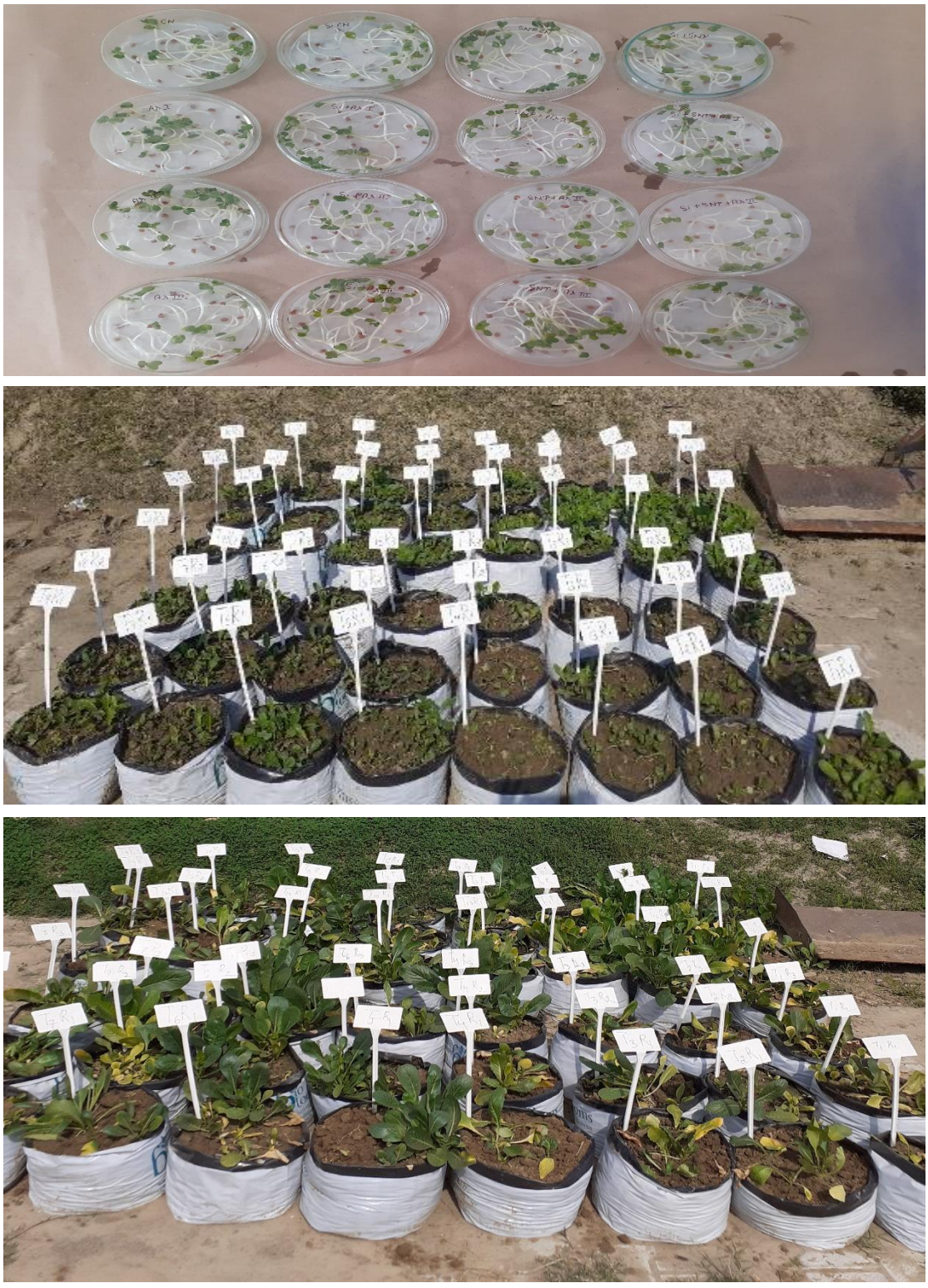

Fig. 6.35 Individual and combined effect of Si, NO and As in 7 days seedlings and 30 and 60 days old plants of *R. sativus*.

6.1.3 60-days old plants

6.1.3.1 Plant growth

The minimum root length of 5.5 cm was found at T_3 concentration (Fig. 6.36; Table 6.29). Root length was observed to be increased from 6.96 to 8.96 cm in T_5 plants. Whereas in the case of NO-treated plants under unstressed conditions (T_8), a root length of 10.4 cm was observed. The results also exhibited that Si and NO suggestively alleviated the As triggered noxiousness by increasing the root length in radish plants. Among the NO pre-treated seedlings under As stress, the highest root length of 9.7 cm was observed in T_9. Further, Si + NO treatment also increased the root length in response to As stress with highest root length of 11.33 cm at T_{13}. It was increased by 28.73% in T_5 than T_1. An increase of 39.36% was found in T_9 than T_1. T_{13} treatment exhibited 16.80% increase than T_9. T_{13} showed 29.78% elevation than control plants.

Fresh weight was significantly affected under metal stress with maximum reduction in fresh weight (1.86 g) in T_3 plants (Fig. 6.36; Table 6.29). T_5 plants increased the fresh weight to 5.21 g. In NO pre-treated plants, the highest and lowest fresh weights i.e., 4.77 and 3.78 g were noticed at T_9 and T_{11}, respectively. Combination of Si and NO was confirmed to be more significant in improving fresh weights as compared to their individual treatments in response to As stress. The greatest increase in fresh weight in the case of combined application was reported at T_{13} with 5.66 g fresh weight. It was increased by 9.22% in T_5 than T_9. T_{13} treatment exhibited 18.65% increase than T_9. T_{13} showed 27.76% elevation than control plants.

T_1, T_2 and T_3 plants showed 1.33, 0.95 and 0.79 g dry weights (Fig. 6.36; Table 6.29). An increase in dry weights was found in As-treated plants when applied with Si. Application of Si and NO in individual manner under As stress showed maximum 2.69 and 2.55 g dry weights at T_5 and T_9, respectively. Better results were exhibited by Si + NO in the case of dry weights when compared with their individual treatments. The highest dry weight of 3.66 g was noticed in at T_{13}. It was increased by 5.49% in T_5 than T_9. A 36.05% increase was noticed in T_{13} than T_5. T_{15} treatment exhibited 34.10% increase than T_9.

Arsenic exposure to radish plants resulted in decreased relative water content with the lowest relative water content of 61.45% at at T_3 (Fig. 6.37; Table 6.29). Foliar

application of Si under unstressed conditions (T_4) showed 84.03% relative water content. T_5, T_6 and T_7 showed 82, 75.48 and 73.57% relative water content. Pre-treatment with NO under stressed conditions showed maximum relative water content of 80.37% at T_9 followed by 79.57% at T_{11}. The relative water content of 83.15% was noticed at T_{13} when applied with Si and NO together. It was increased by 21.40% in T_5 than T_1. An increase of 18.99% was found in T_9 than T_1. T_{13} treatment exhibited 3.45% increase than T_9.

Table 6.29 Effect of Si and NO on morphological parameters and relative water content of 60 days old plants of *R. sativus* under As stress

Treatment	Root length (cm)	Fresh weight (g)	Dry weight (g)	Relative water content (%)
C (Control)	$8.73^{de}\pm0.14$	$4.43^{cde}\pm0.18$	$1.73^{bcd}\pm0.1$	$78.46^{bcd}\pm2.55$
T_1 (AsI)	$6.96^{bc}\pm0.17$	$2.84^{ab}\pm0.14$	$1.33^{abc}\pm0.07$	$67.54^{ab}\pm1.79$
T_2 (AsII)	$6.06^{ab}\pm0.2$	$2.25^{a}\pm0.17$	$0.95^{ab}\pm0.17$	$76.99^{bcd}\pm4.02$
T_3 (AsIII)	$5.5^{a}\pm0.26$	$1.86^{a}\pm0.06$	$0.79^{a}\pm0.18$	$61.45^{a}\pm2.58$
T_4 (Si)	$10.06^{fgh}\pm0.26$	$5.95^{gh}\pm0.37$	$3.42^{fgh}\pm0.24$	$84.03^{cd}\pm2.6$
T_5 (Si+AsI)	$8.96^{def}\pm0.29$	$5.21^{efgh}\pm0.22$	$2.69^{ef}\pm0.14$	$82^{cd}\pm4.18$
T_6 (Si+AsII)	$8.13^{cd}\pm0.23$	$4.42^{cde}\pm0.16$	$2.33^{de}\pm0.18$	$75.48^{bcd}\pm4.65$
T_7 (Si+AsIII)	$7.9^{cd}\pm0.23$	$3.91^{cd}\pm0.19$	$1.74^{bcd}\pm0.23$	$73.57^{abc}\pm4.60$
T_8 (NO)	$10.4^{gh}\pm.32$	$5.6^{fgh}\pm0.14$	$3.6^{gh}\pm0.18$	$86.31^{cd}\pm1.49$
T_9 (NO+AsI)	$9.7^{efg}\pm0.26$	$4.77^{cdef}\pm0.18$	$2.55^{def}\pm0.17$	$80.37^{bcd}\pm0.92$
T_{10} (NO+AsII)	$8.43^{de}\pm0.27$	$4.3^{cde}\pm0.1$	$2.16^{cde}\pm0.28$	$76.6^{bcd}\pm3.49$
T_{11} (NO+AsIII)	$7.86^{cd}\pm0.31$	$3.78^{bc}\pm0.08$	$1.42^{abc}\pm0.13$	$79.57^{bcd}\pm1.67$
T_{12} (Si+NO)	$12^{i}\pm0.17$	$6.18^{h}\pm0.26$	$3.98^{h}\pm0.11$	$88.16^{d}\pm1.1$
T_{13} (Si+NO+AsI)	$11.33^{hi}\pm0.23$	$5.66^{fgh}\pm0.34$	$3.66^{gh}\pm0.12$	$83.15^{cd}\pm0.93$
T_{14} (Si+NO+AsII)	$10.3^{gh}\pm0.28$	$4.95^{defg}\pm0.15$	$2.99^{efg}\pm0.08$	$81.5^{cd}\pm1.28$
T_{15} (Si+NO+AsIII)	$9.7^{efg}\pm0.17$	$3.99^{cd}\pm0.17$	$2.32^{de}\pm0.13$	$79.54^{bcd}\pm0.87$

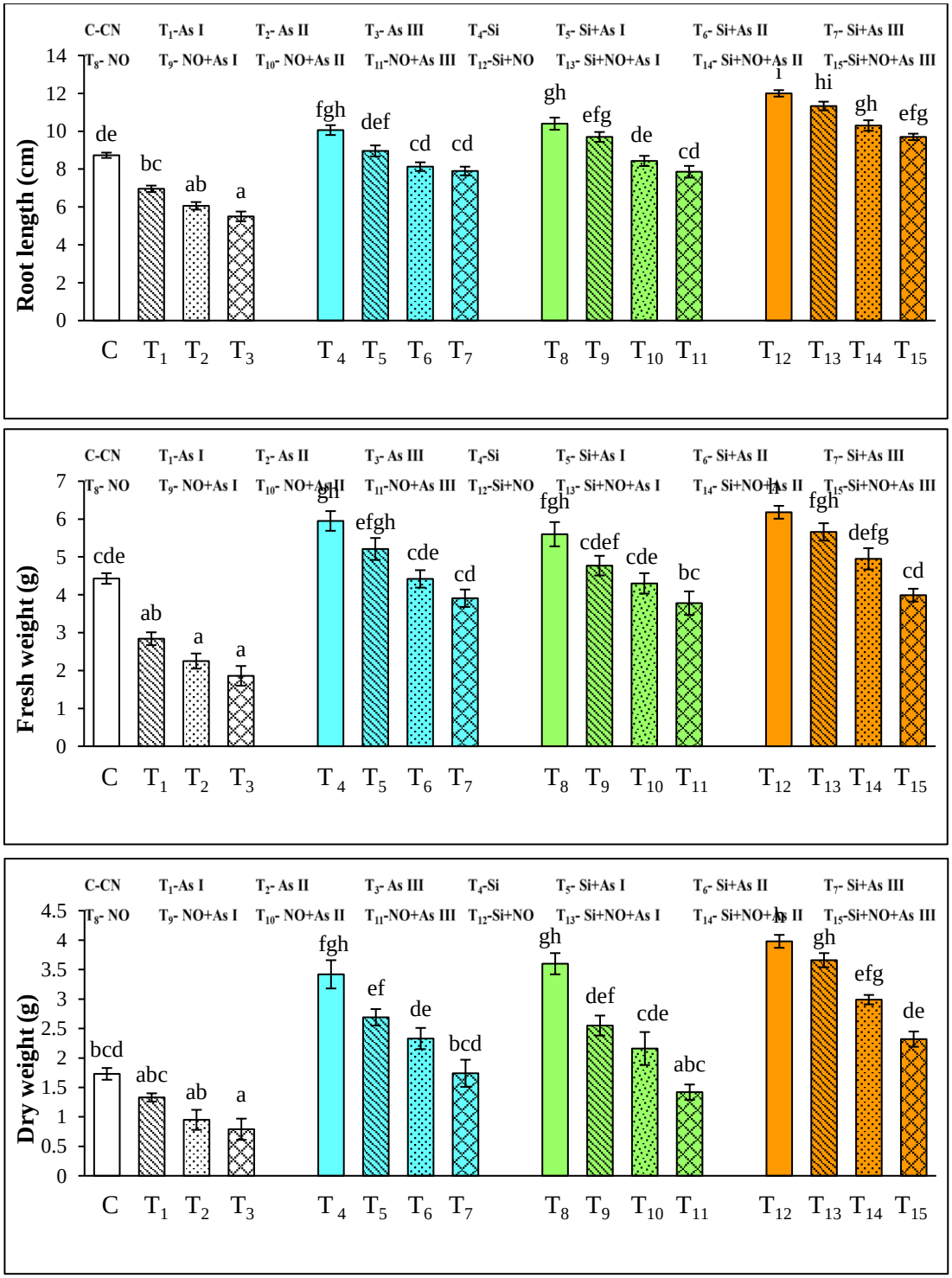

Fig. 6.36 Effect of Si and NO on root length, fresh and dry weight in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

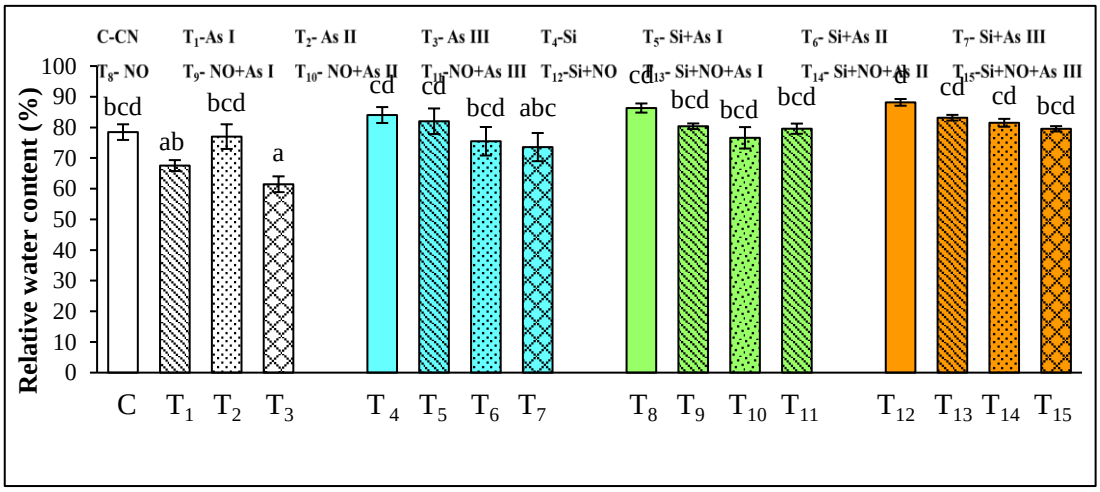

Fig. 6.37 Effect of Si and NO on relative water content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

2.1.3.2 Photosynthetic activity

2.1.3.2.1 Photosynthetic pigments

Photosynthetic pigments were noticed to be lessened by As stress (Fig. 6.38; Table 6.30). Almost 46% reduction was found at T_3 concentration in case of total chlorophyll content, when compared with control plants i.e., from 0.976 to 0.653 mg g^{-1} FW. Individual level application of Si and NO upregulated its content under As toxicity, among which the highest contents i.e., 1.126 and 1.111 mg g^{-1} FW were detected at T_5 and T_9, respectively. Synergistic application of Si and NO showed better results in improving total chlorophyll contents under stress conditions, as compared to their individual treatments. The highest 1.321 mg g^{-1} FW amount under stressed conditions was noticed in the plants having T_{13} treatment. It was increased by 31.54% in T_5 than T_1. An increase of 29.78% was found in T_9 than T_1. T_{13} treatment exhibited 17.31% increase than T_9. T_{13} showed 35.34% elevation than control plants. Similar findings were observed in the case of chl a and chl b in radish plants. The lowest content of chl a (0.445 mg g^{-1} FW) and chl b (0.315 mg g^{-1} FW) were found at T_3. A significant alleviation of the As-induced malicious effects was observed by Si treatment via an enhancement in the amount of pigments. Combination of Si and NO further increased the chl a and b contents under stress conditions with the highest 0.879 and 0.754 mg g^{-1} FW contents, respectively at T_{13} concentration.

Table 6.30 Effect of Si and NO on photosynthetic pigments of 60 days old plants of *R. sativus* under As stress

Treatment	Total chlorophyll (mg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)
C (Control)	0.976^{cd}±0.26	0.73^{bcde}±0.09	0.654^{cde}±0.04
T_1 (AsI)	0.856^{b}±0.18	0.588^{abc}±0.06	0.523^{bc}±0.04
T_2 (AsII)	0.769^{a}±0.15	0.512^{ab}±0.04	0.449^{ab}±0.05
T_3 (AsIII)	0.653^{a}±0.05	0.445^{a}±0.07	0.315^{a}±0.04
T_4 (Si)	1.456^{hi}±0.12	0.866^{ij}±0.05	0.767^{hij}±0.05
T_5 (Si+AsI)	1.126^{fgh}±0.06	0.765^{fgh}±0.05	0.646^{fgh}±0.06
T_6 (Si+AsII)	0.983^{cde}±0.06	0.698^{def}±0.08	0.574^{def}±0.1
T_7 (Si+AsIII)	0.776^{bc}±0.06	0.569^{cdef}±0.07	0.463^{cd}±0.07
T_8 (NO)	1.35^{hi}±0.06	0.832^{hi}±0.03	0.732^{ij}±0.03
T_9 (NO+AsI)	1.111^{efgh}±0.09	0.735^{fgh}±0.09	0.632^{fghi}±0.04
T_{10} (NO+AsII)	0.968^{cdef}±0.08	0.684^{efg}±0.14	0.535^{def}±0.03
T_{11} (NO+AsIII)	0.846^{cd}±0.1	0.595^{bcd}±0.06	0.428^{cd}±0.05
T_{12} (Si+NO)	1.673^{i}±0.05	0.965^{j}±0.04	0.856^{j}±0.05
T_{13} (Si+NO+AsI)	1.321^{ghi}±0.03	0.879^{ghi}±0.23	0.754^{ghi}±0.04
T_{14} (Si+NO+AsII)	1.132^{defg}±0.07	0.768^{fgh}±0.09	0.684^{efg}±0.02
T_{15} (Si+NO+AsIII)	0.964^{cdef}±0.08	0.684^{fgh}±0.06	0.553^{de}±0.08

It was found that As stress decreased the carotenoids amount (Fig. 6.39; Table 6.31). A 41% reduction was noticed in T_3 than control ones. Carotenoid content was upsurged from 0.678 to 0.845 mg g^{-1} FW in T_5 plants, in contrast to T_1 plants. NO against As stress also increased the carotenoid content with the highest content (0.787 mg g^{-1} FW) at T_9. Si + NO further boosted their content in order to mitigate the As-induced toxic effects. The highest carotenoid content of 0.912 mg g^{-1} FW was noticed at T_{13} concentration while a minimum of 0.786 mg g^{-1} FW was at T_{15}. It was increased by 24.63% in T_5 than T_1. An increase of 16.07% was found in T_9 than T_1. T_{13} treatment exhibited 15.88% increase than T_9. T_{13} showed 4.22% elevation than control plants.

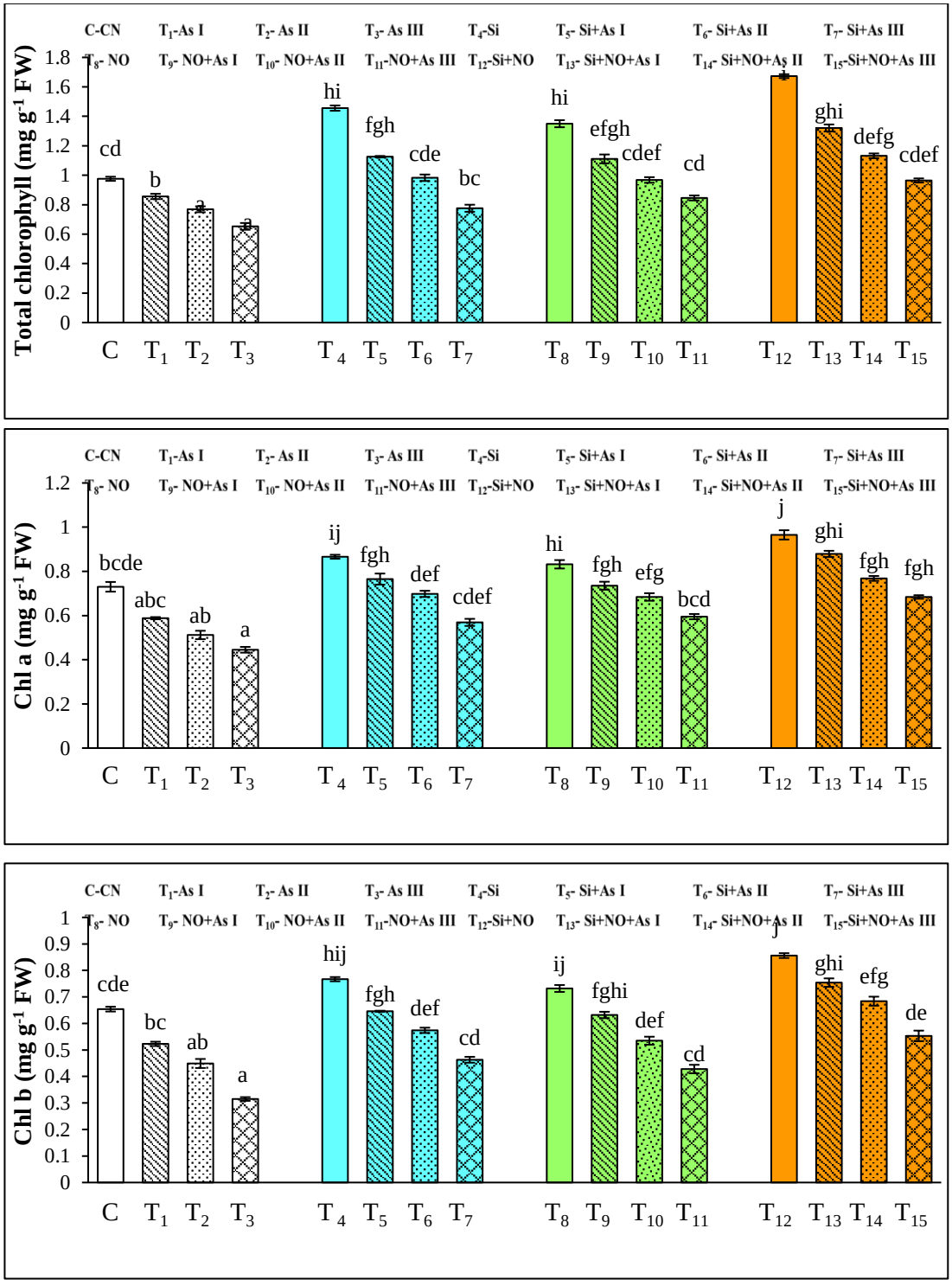

Fig. 6.38 Effect of Si and NO on total chlorophyll, chl a and chl b in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Xanthophyll amount was also noticed to be reduced in 60-days old radish plants by their exposure to As (Fig. 6.39; Table 6.31). The minimum xanthophyll content of 6.71 mg g^{-1} FW was in T_3. With the increase in the As concentration, xanthophyll content was found to be decreased in the case of As, Si, NO and Si + NO treatments. Individually applied Si and NO increased the xanthophyll content under stressed conditions. The highest xanthophyll level of 10.45 and 10.83 mg g^{-1} FW in the case of individual application of Si and NO, respectively were noticed at T_5 and T_9 concentration. The coupled application of Si and NO showed 11.58. 11.05 and 10.47 mg g^{-1} FW xanthophyll contents at T_{13}, T_{14} and T_{15}, respectively. It was increased by 20.11% in T_5 than T_1. An increase of 24.48% was found in T_9 than T_1. T_{13} treatment exhibited 6.92% increase than T_9. T_{13} showed 2.38% elevation than control plants.

Table 6.31 Effect of Si and NO on photosynthetic pigments of 60 days old plants of *R. sativus* under As stress

Treatment	**Carotenoid (mg g^{-1} FW)**	**Xanthophyll (mg g^{-1} FW)**
C (Control)	0.875^{de}±0.26	11.31^{fgh}±0.29
T_1 (AsI)	0.678^{b}±0.07	8.7^{bcd}±0.23
T_2 (AsII)	0.634^{ab}±0.09	7.66^{ab}±0.21
T_3 (AsIII)	0.512^{a}±0.05	6.71^{a}±0.25
T_4 (Si)	0.978^{hi}±0.07	12.53^{ghi}±0.13
T_5 (Si+AsI)	0.845^{ef}±0.08	10.45^{def}±0.19
T_6 (Si+AsII)	0.803^{de}±0.04	9.17^{bcde}±0.24
T_7 (Si+AsIII)	0.747^{cd}±0.08	8.28^{abc}±0.43
T_8 (NO)	0.943^{gh}±0.05	12.88^{hi}±0.33
T_9 (NO+AsI)	0.787^{de}±0.056	10.83^{efg}±0.30
T_{10} (NO+AsII)	0.726^{cd}±0.07	10.33^{cdef}±0.53
T_{11} (NO+AsIII)	0.677^{c}±0.072	9.06^{bcde}±0.49
T_{12} (Si+NO)	1.1^{i}±0.05	13.56^{i}±0.22
T_{13} (Si+NO+AsI)	0.912^{fgh}±0.14	11.58^{fgh}±0.65
T_{14} (Si+NO+AsII)	0.827^{efg}±0.06	11.05^{fgh}±0.49
T_{15} (Si+NO+AsIII)	0.786^{de}±0.06	10.47^{def}±0.43

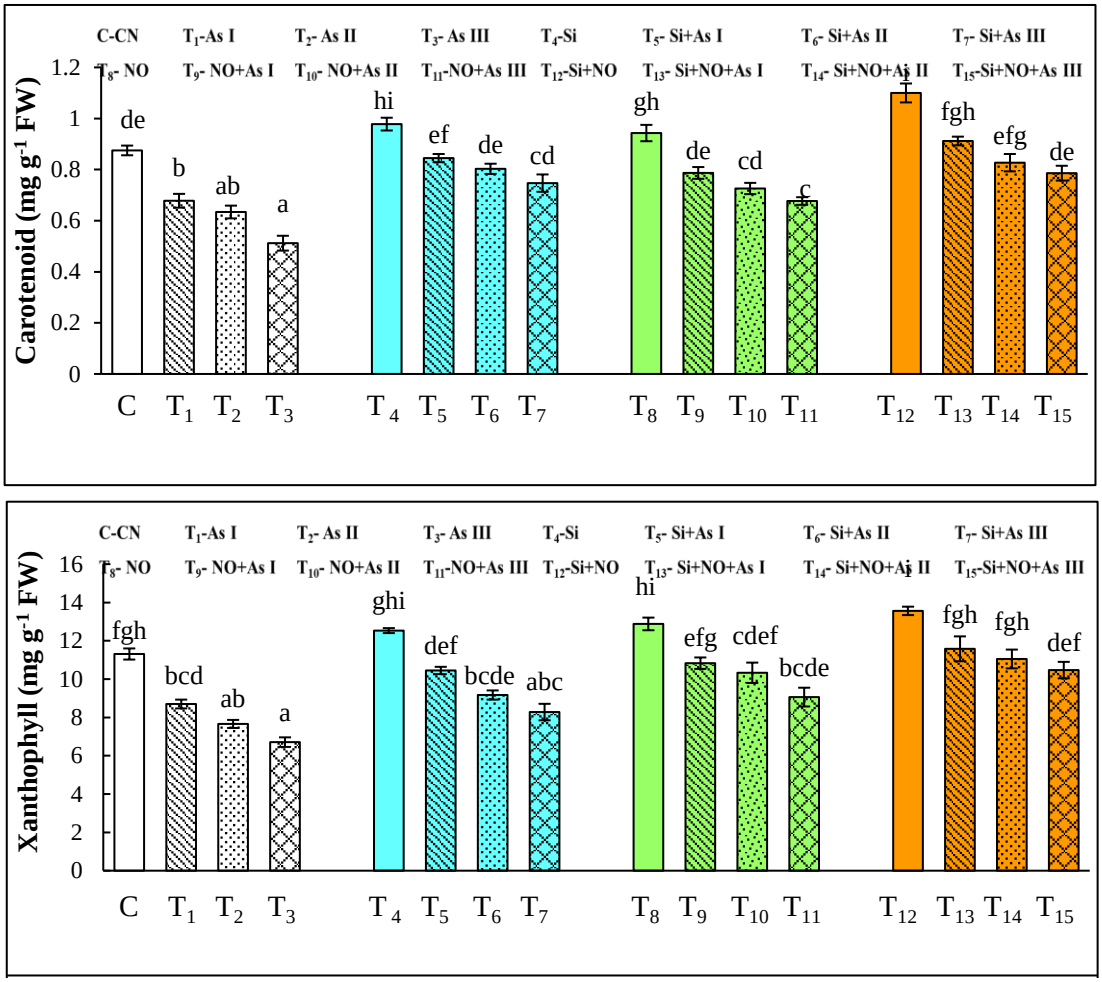

Fig. 6.39 Effect of Si and NO on carotenoid and xanthophyll content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.3.2.2 Gas exchange parameters

The photosynthetic rate was noticed to be reduced under As toxicity with the lowest 7.17 m mol CO_2 m^{-2} S^{-1} at T_2 (Fig. 6.40; Table 6.32). Si raised its rate from 9.14 to 13.47 m mol CO_2 m^{-2} S^{-1} at T_5 concentration. Pre-treatment with NO also improved the photosynthetic rate under As stress with the highest of 14.32 m mol CO_2 m^{-2} S^{-1} at T_{10}. Application of Si exhibited a higher photosynthetic rate than NO application. Si + NO further increased the photosynthetic rate against As toxicity with the maximum photosynthetic rate of 17.25 m mol CO_2 m^{-2} S^{-1} at T_{13}. It was increased by 47.37% in T_5 than T_1. An increase of 56.23% was found in T_9 than T_1. T_{13} treatment exhibited 20.79% increase than T_9. T_{13} showed 9.52% elevation than control plants.

The lowest stomatal conductance of 0.44 m mol H_2O m^{-2} s^{-1} was found at T_3 (Fig. 6.40;

Table 6.32). Individual supplication Si and NO in radish plants caused elevation in the stomatal conductance under As stress. T_4 and T_8 exhibited greater stomatal conductance of 1.54 and 1.8 m mol H_2O m^{-2} s^{-1}, respectively, than control genotypes. Under As stress, Si and NO alone showed maximum stomatal conductance of 1.21 and 1.48 m mol H_2O m^{-2} s^{-1} at T_6 and T_{11}, respectively. Combined application of Si with NO exhibited highest and lowest stomatal conductance of 1.73 and 1.35 m mol H_2O m^{-2} s^{-1} in T_{13} and T_{15} plants, respectively. It was increased by 45.20% in T_5 than T_1. An increase of 86.30% was found in T_9 than T_1. T_{13} treatment exhibited 27.20% increase than T_9. T_{13} showed 13.81% elevation than control plants.

Intercellular CO_2 concentration was noticed to be reduced in 60-days old radish plants by their exposure to As stress (Fig. 6.40; Table 6.32). Control plants reported 533.66 ppm intercellular CO_2 concentration. T_1, T_2 and T_3 plants showed 375.33, 332 and 358.66 ppm intercellular CO_2 concentrations, respectively. Individual application of Si and NO showed the highest intercellular CO_2 concentration of 519 and 490.66 ppm, respectively at T_5 and T_9. T_{12} exhibited 655.33 ppm intercellular CO_2 concentration which was the highest among all treatments used in this study. Under As stress, a maximum intercellular CO_2 concentration of 548.33 ppm was found in T_{13} plants. It was increased by 38.27% in T_5 than T_1. An increase of 30.72% was found in T_9 than T_1. T_{13} treatment exhibited 2.74% increase than T_5.

Control plants showed 1.35 m mol H_2O m^{-2} S^{-1} transpiration rate whereas T_1, T_2 and T_3 plants exhibited 0.89, 0.71 and 0.67 m mol H_2O m^{-2} S^{-1} rates, respectively (Fig. 6.41; Table 6.32). Application of Si under stressed conditions showed a maximum transpiration rate of 1.16 m mol H_2O m^{-2} S^{-1} at T_5, whereas NO also showed the highest transpiration rate of 1.39 m mol H_2O m^{-2} S^{-1} at T_{11} concentration. Combined treatment of Si with NO displayed the highest and lowest transpiration rate at T_{13} and T_{14} with 1.6 and 1.44 m mol H_2O m^{-2} S^{-1} transpiration rates, respectively. It was increased by 30.33% in T_5 than T_1. An increase of 46.06% was found in T_9 than T_1. T_{13} treatment exhibited 37.93% increase than T_9. T_{13} showed 18.51% elevation than control plants.

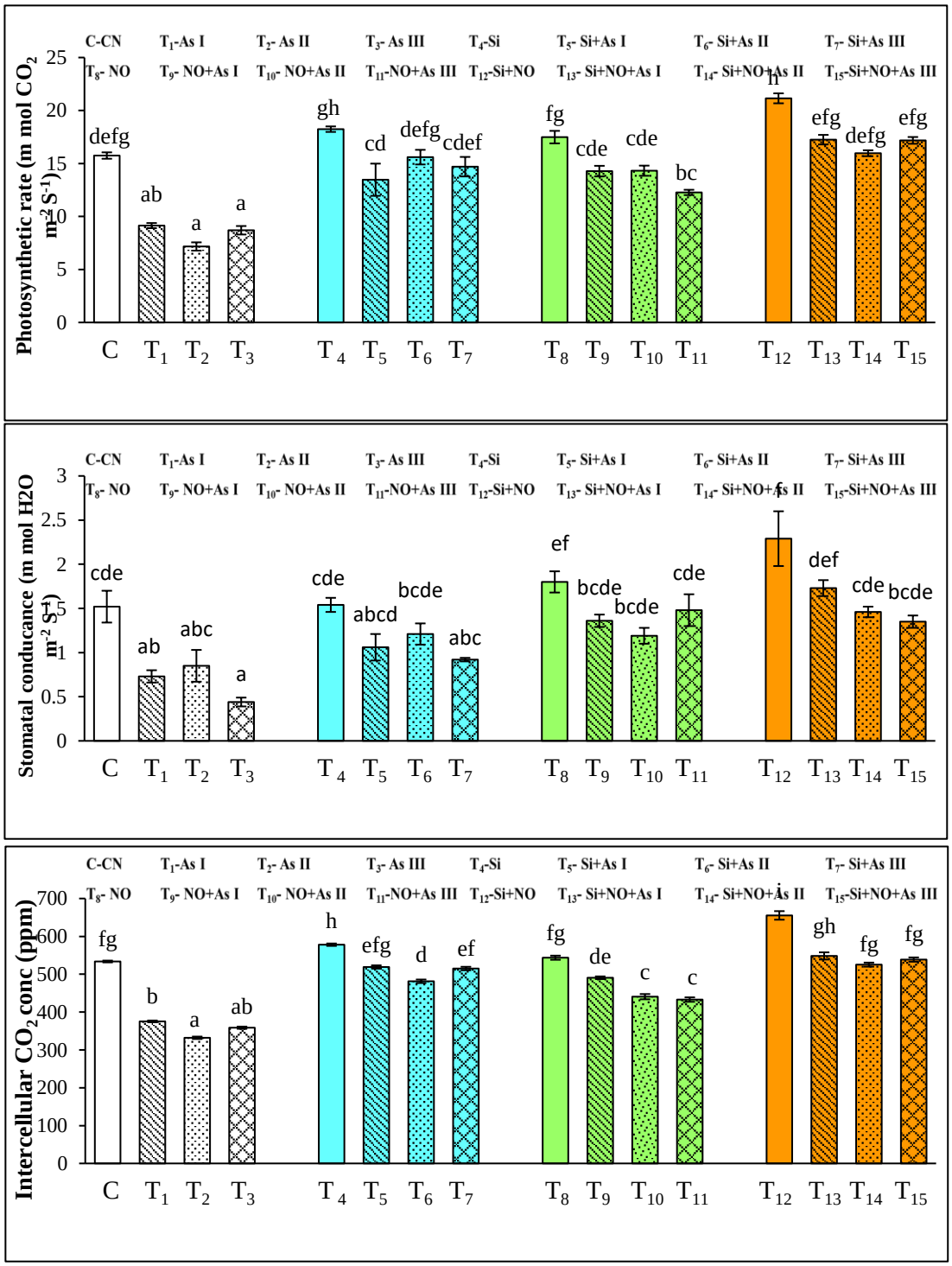

Fig. 6.40 Effect of Si and NO on photosynthetic rate, stomatal conductance and intercellular CO_2 concentration in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Table 6.32 Effect of Si and NO on gas exchange parameters of 60 days old plants of *R. sativus* under As 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})
C (Control)	15.75^{defg}±0.3	1.52^{cde}±0.18	533.66^{fg}±2.4	1.35^{cd}±0.07
T_1 (AsI)	9.14^{ab}±0.24	0.73^{ab}±0.07	375.33^{b}±2.33	0.89^{ab}±0.32
T_2 (AsII)	7.17^{a}±0.39	0.85^{abc}±0.18	332^{a}±3.21	0.71^{a}±0.02
T_3 (AsIII)	8.7^{a}±0.4	0.44^{a}±0.05	358.66^{ab}±2.9	0.67^{a}±0.01
T_4 (Si)	18.24^{gh}±0.26	1.54^{cde}±0.08	578^{h}±3.21	1.62^{ef}±0.27
T_5 (Si+AsI)	13.47^{cd}±1.52	1.06^{abcd}±0.15	519^{efg}±4.35	1.16^{bc}±0.12
T_6 (Si+AsII)	15.6^{defg}±0.69	1.21^{bcde}±0.12	481.33^{d}±4.63	1.03^{b}±0.12
T_7 (Si+AsIII)	14.7^{cdef}±0.92	0.92^{abc}±0.02	515^{ef}±4.61	0.99^{bc}±0.15
T_8 (NO)	17.49^{fg}±0.59	1.8^{ef}±0.12	543.66^{fg}±5.45	1.8^{f}±0.26
T_9 (NO+AsI)	14.28^{cde}±0.5	1.36^{bcde}±0.07	490.66^{de}±3.52	1.3^{cd}±0.04
T_{10} (NO+AsII)	14.32^{cde}±0.48	1.19^{bcde}±0.09	440.66^{c}±6.96	1.11^{bc}±0.09
T_{11} (NO+AsIII)	12.27^{bc}±0.25	1.48^{cde}±0.18	433^{c}±5.85	1.39^{cd}±0.32
T_{12} (Si+NO)	21.15^{h}±0.47	2.29^{f}±0.31	655.33^{i}±11.31	2.17^{g}±0.03
T_{13} (Si+NO+AsI)	17.25^{efg}±0.45	1.73^{def}±0.09	548.33^{gh}±9.49	1.6^{de}±0.07
T_{14} (Si+NO+AsII)	15.97^{defg}±0.28	1.46^{cde}±0.06	525.33^{fg}±5.36	1.44^{cd}±0.04
T_{15} (Si+NO+AsIII)	17.18^{efg}±0.32	1.35^{bcde}±0.07	538.66^{fg}±5.680	1.49^{de}±0.36

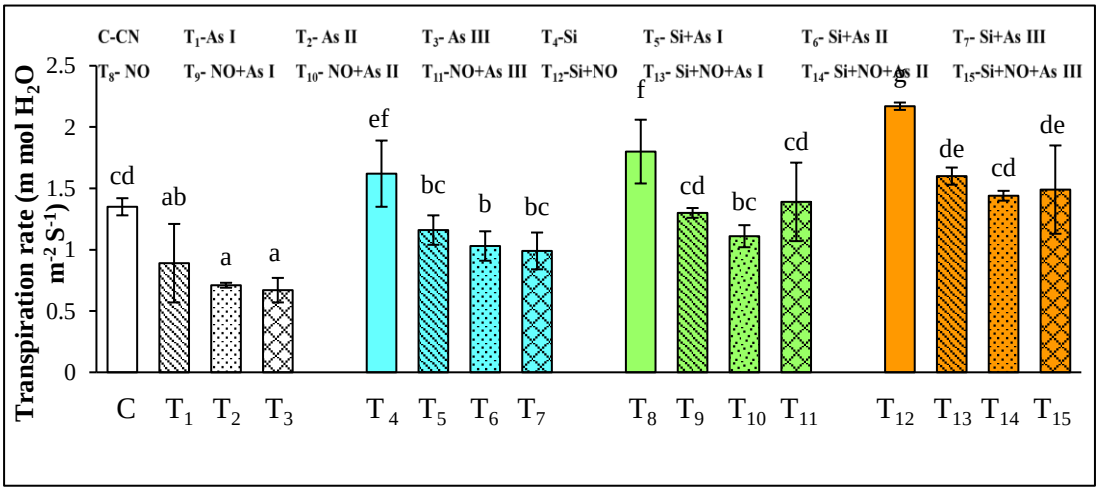

Fig. 6.41 Effect of Si and NO on transpiration rate in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.3.3 Metabolites

Anthocyanin content drastically diminished in As stressed plants (Fig. 6.42; Table 6.33). The lowest anthocyanin content of 6.3 mg g^{-1} FW was noticed in T_3. Under

stressed conditions, Si improved their amount with 10.15, 9.41 and 8.7 mg g^{-1} FW content at T_5, T_6 and T_7, respectively. Among NO-pre-treated plants under stressed conditions, maximum anthocyanin content (10.92 mg g^{-1} FW) was at T_9. Si + NO control plants (T_{12}) exhibited an anthocyanin content of 13.18 mg g^{-1} FW which was the highest among all 16 treatments used in this study. Under As stress, the maximum content (11.7 mg g^{-1} FW) was in T_{13} plants. It was increased by 24.38% in T_5 than T_1. An increase of 33.82% was found in T_9 than T_1. T_{13} treatment exhibited 7.14% increase than T_9. T_{13} showed 15.38% elevation than control plants.

Table 6.33 Effect of Si and NO on metabolites of 60 days old plants of *R. sativus* under As stress

Treatment	Anthocyanin (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)
C (Control)	10.14^{cde}±0.08	8.71^{efgh}±0.32	11.21^{ef}±0.22
T_1 (AsI)	8.16^{abc}±0.26	6.82^{bc}±0.24	9.76^{bcd}±0.15
T_2 (AsII)	6.98^{ab}±0.30	6.16^{ab}±0.16	8.89^{b}±0.17
T_3 (AsIII)	6.30^{a}±0.50	5.49^{a}±0.15	7.37^{a}±0.28
T_4 (Si)	11.49^{ghi}±0.23	9.65^{hi}±0.18	12.12^{fg}±0.26
T_5 (Si+AsI)	10.15^{efgh}±0.21	8.16^{def}±0.21	10.97^{def}±0.27
T_6 (Si+AsII)	9.41^{defg}±0.32	7.51^{cd}±0.22	10.2^{bcde}±0.31
T_7 (Si+AsIII)	8.70^{def}±0.23	6.7^{bc}±0.25	9.33^{bc}±0.32
T_8 (NO)	12.16^{fgh}±0.29	9.37^{gh}±0.18	12.71^{gh}±0.21
T_9 (NO+AsI)	10.92^{defgh}±0.33	8.75^{fgh}±0.21	11.04^{def}±0.34
T_{10} (NO+AsII)	10.33^{cde}±0.53	8.28^{defg}±0.18	10.37^{cde}±0.38
T_{11} (NO+AsIII)	9.67^{bcd}±0.37	7.62^{cde}±0.27	9.33^{bc}±0.3
T_{12} (Si+NO)	13.18^{i}±0.32	10.52±0.29	13.71^{h}±0.26
T_{13} (Si+NO+AsI)	11.70^{hi}±0.33	9.24^{fgh}±0.08	12.07^{fg}±0.13
T_{14} (Si+NO+AsII)	10.76^{efgh}±0.31	8.97^{fgh}±0.09	11.16^{ef}±0.13
T_{15} (Si+NO+AsIII)	9.86^{efgh}±0.73	8.35^{defg}±0.13	9.99^{bcde}±0.16

Flavonoid content was negatively associated with As treatment as As stress decreased the its content (Fig. 6.42; Table 6.33). T_3 showed 5.49 mg g^{-1} FW flavonoid content which was the minimum among all three As concentrations used in the study. Si and NO alone and together under stress increased the flavonoid content. Maximum flavonoid content was noticed at T_5 and T_9 concentration with 8.16 and 8.75 mg g^{-1} FW, respectively when applied alone. The uppermost flavonoid amount of 9.24 mg g^{-1} FW was found at T_{13} treatment, as compared to all other treatments used in the study under

stress conditions. It was increased by 19.64% in T_5 than T_1. An increase of 28.29% was found in T_9 than T_1. T_{13} treatment exhibited 5.6% increase than T_9.

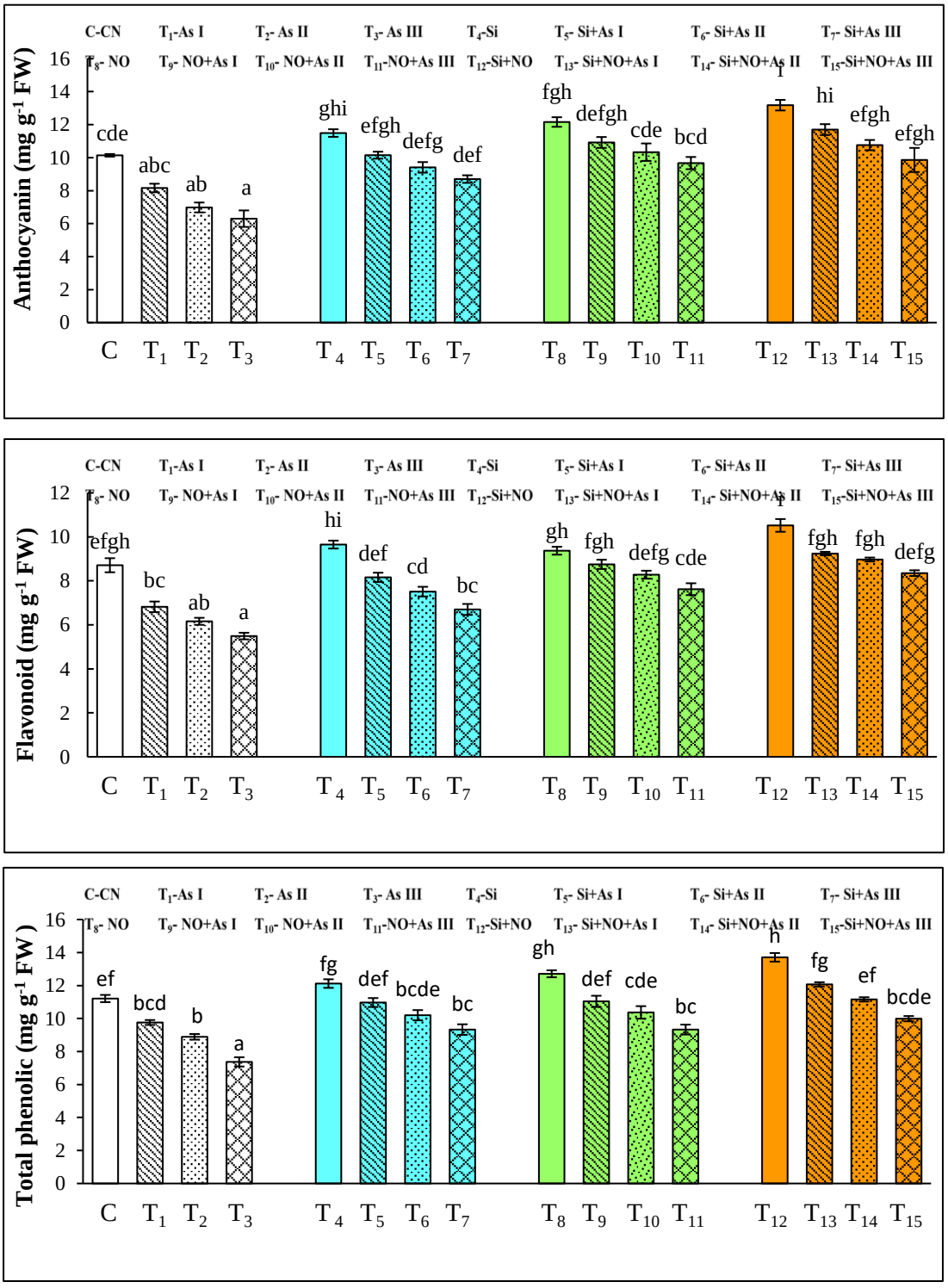

Fig. 6.42 Effect of Si and NO on anthocyanin, flavonoid and phenolic content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Control plants showed 11.21 mg g^{-1} FW phenolics which was further decreased in As treated plants (Fig. 6.42; Table 6.33). Minimum phenolic content of 7.37 mg g^{-1} FW was noticed in T_3 plants. Si and NO alone and together significantly boosted the As-mediated decline in phenolic content. Phenolic contents shown by Si and NO treated plants at T_7 and T_{11} were 9.33 mg g^{-1} FW. Further, Si + NO in the case of T_{13}, T_{14} and T_{15} showed 12.07, 11.16 and 9.99 mg g^{-1} FW phenolic contents, respectively. It was increased by 12.39% in T_5 than T_1. An increase of 13.11% was found in T_9 than T_1. T_{13} treatment exhibited 9.32% increase than T_9. T_{13} showed 7.67% elevation than control plants.

6.1.3.4 Oxidative stress markers

An increase of 14.75 µmol g^{-1} in MDA content was noticed in T_3 when compared with control plants (Fig. 6.43; Table 6.34). However, treatment of Si and NO under As stress reduced the level of MDA. With regard to the individual application of Si and NO, the highest decline in MDA content under As stress was observed at T_5 and T_9 with 8.1 and 8.33 µmol g^{-1} FW contents. MDA content was diminished by 6.72 µmol g^{-1} FW in T_{13} treatment. It was reduced by 34.14% in T_5 than T_1. A decline of 32.27% was found in T_9 than T_1. T_{13} treatment exhibited 19.32% decrease than T_9.

Table 6.34 Effect of Si and NO on oxidative stress markers of 60 days old plants of *R. sativus* under As stress

Treatment	MDA (µmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)
C (Control)	9.35^{defg}±0.19	9.89^{f}±0.24
T_1 (AsI)	12.3^{h}±0.23	13.68^{g}±0.22
T_2 (AsII)	13.45^{hi}±0.38	14.79^{gh}±0.37
T_3 (AsIII)	14.75^{i}±0.36	15.73^{h}±0.34
T_4 (Si)	7.52^{bc}±0.2	6.12^{ab}±0.17
T_5 (Si+AsI)	8.1^{cd}±0.17	7.01^{bcd}±0.12
T_6 (Si+AsII)	8.9^{def}±0.31	7.79^{cde}±0.24
T_7 (Si+AsIII)	9.54^{efg}±0.37	8.43^{def}±0.32
T_8 (NO)	7.15^{bc}±0.23	6.6^{abc}±0.31
T_9 (NO+AsI)	8.33^{cde}±0.17	7.37^{bcd}±0.25
T_{10} (NO+AsII)	10.52^{g}±0.2	8.34^{de}±0.34
T_{11} (NO+AsIII)	9.69^{fg}±0.16	8.98^{ef}±0.19
T_{12} (Si+NO)	5.56^{a}±0.23	5.41^{a}±0.38
T_{13} (Si+NO+AsI)	6.72^{ab}±0.2	6.08^{ab}±0.46
T_{14} (Si+NO+AsII)	7.26^{bc}±0.1	6.26^{abc}±0.25
T_{15} (Si+NO+AsIII)	8.11^{cd}±0.15	7.17^{bcd}±0.17

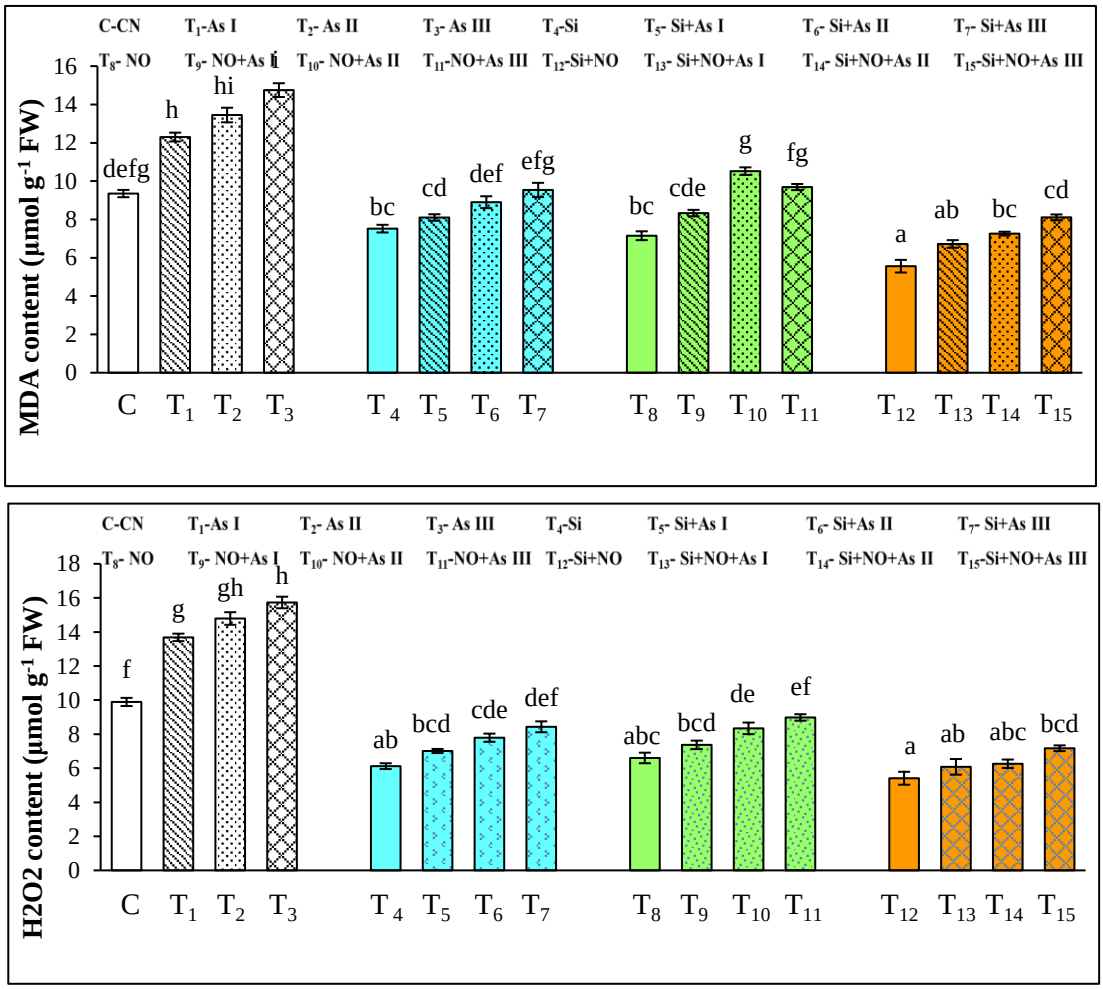

Fig. 6.43 Effect of Si and NO on MDA and H_2O_2 content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

The level of H_2O_2 was significantly greater in radish seedlings upon their exposure to As stress (Fig. 6.43; Table 6.34). Level of H_2O_2 was elevated to 13.68, 14.79 and 15.73 µmol g^{-1} FW in T_1, T_2 and T_3 plants. Whereas, the treatment with Si, NO and Si + NO against As stress depressed the H_2O_2 level. The maximum decrease in the H_2O_2 level (6.08 µmol g^{-1} FW) was at T_{13}. It was reduced by 48.75% in T_5 than T_1. A decline of 46.12% was found in T_9 than T_1. T_{13} treatment exhibited 17.50% decrease than T_9. T_{13} showed 38.52% reduction than control plants.

6.1.3.5 Arsenic metalloid uptake

Roots of radish showed higher As content than shoots in 60-days old plants. In As III stressed plants, 2.89 and 1.57 mg g^{-1} DW contents were found in roots and leaves of radish, respectively (Fig. 6.44; Table 6.35). Further, Si and NO treatments reduced the As content. In roots, As contents of 2.02, 1.88 and 1.46 mg g^{-1} DW were noticed in Si, NO and Si + NO treatments under As III stress, respectively. While in leaves, Si and NO individually and Si + NO together treated plants showed 1.1, 0.88 and 0.72 mg g^{-1} DW under As III stress, respectively.

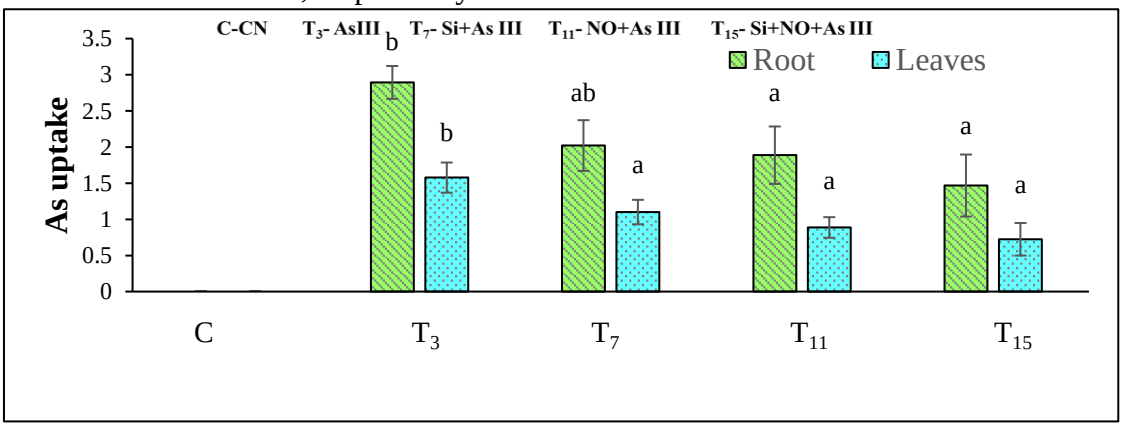

Fig. 6.44: Effect of Si and NO on As uptake in root and leaves in 60 days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean (SEM). Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.35 Effect of Si and NO on As metalloid uptake in 60 days old plants of *R. sativus* under As stress

Treatments	**Root (mg g^{-1} DW)**	**Leaves (mg g^{-1} DW)**
C (Control)	ND	ND
T_3 (AsIII)	2.89^{b}±0.22	1.57^{b}±0.20
T_7 (Si+AsIII)	2.02^{ab}±0.35	1.1^{a}±0.16
T_{11} (NO+AsIII)	1.88^{a}±0.39	0.88^{a}±0.14
T_{15} (Si+NO+AsIII)	1.46^{a}±0.42	0.72^{a}±0.22

6.1.3.6 Osmolytes

Proline content was significantly inhibited by As in radish plants. At T_1, T_2 and T_3, proline contents were 4.62, 4.09 and 3.54 µ mol g^{-1} FW, respectively (Fig. 6.45; Table 6.36). Individual application of Si raised the proline level under stress conditions. Proline content was increased from 0.7 to 6.15 µ mol g^{-1} FW in T_5 plants. Proline amount was raised by pre-treatment with NO under As stress with maximum content of 7.06 µ mol g^{-1} FW at T_9. Under As stress, maximum proline level of 8.38 µ mol g^{-1} FW was detected in T_{13}. It was increased by 33.11% in T_5 than T_1. An increase of

52.81% was found in T_9 than T_1. T_{13} treatment exhibited 44.98% increase than T_9. T_{13} showed 4.14% elevation than control plants.

A decline of 9.5, 8.71 and 7.35 µ mol g^{-1} FW in glycine betaine content were observed in T_1, T_2 and T_3 plants, respectively (Fig. 6.45; Table 6.36). However, individual as well as in combination, Si and NO brought forth a significant alleviation of the As noxiousness in radish by enhancing the glycine betaine content. Glycine betaine contents were 13.65, 11.9 and 11.14 µ mol g^{-1} FW at T_5, T_6 and T_7, respectively upon the application of Si. Plants pre-treated with NO showed glycine betaine contents of 14.79, 13.05 and 11.98 µ mol g^{-1} FW under T_9, T_{10} and T_{11}, respectively. Furthermore, Si and NO treated plants under unstressed conditions (T_{12}) showed the highest quantities of glycine betaine (18 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 As level escalated in case of coupled application of Si and NO with highest activity (15.05 µ mol g^{-1} FW) at T_{13}. It was increased by 43.68% in T_5 than T_1. An increase of 55.68% was found in T_9 than T_1. T_{13} treatment exhibited 1.75% increase than T_9. T_{13} showed 18.03% elevation than control plants.

Table 6.36 Effect of Si and NO on osmolytes of 60 days old plants of *R. sativus* under As stress

Treatment	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
C (Control)	5.78^{bcd}±0.24	12.75^{de}±0.41
T_1 (AsI)	4.62^{abc}±0.19	9.5^{b}±0.21
T_2 (AsII)	4.09^{ab}±0.35	8.71^{ab}±0.25
T_3 (AsIII)	3.54^{a}±0.19	7.35^{a}±0.26
T_4 (Si)	7.43^{def}±0.29	15.09^{gh}±0.23
T_5 (Si+AsI)	6.15^{bcd}±0.21	13.65^{efg}±0.19
T_6 (Si+AsII)	5.58^{abcd}±0.31	11.9^{cd}±0.32
T_7 (Si+AsIII)	4.84^{abc}±0.38	11.14^{c}±0.41
T_8 (NO)	8.47^{fg}±0.4	16.35^{h}±0.17
T_9 (NO+AsI)	7.06^{def}±0.36	14.79^{fg}±0.21
T_{10} (NO+AsII)	6.3^{cde}±0.49	13.05^{de}±0.25
T_{11} (NO+AsIII)	5.32^{abcd}±0.51	11.98^{cd}±0.39
T_{12} (Si+NO)	10.17^{g}±0.19	18^{i}±0.22
T_{13} (Si+NO+AsI)	8.38^{efg}±0.31	15.05^{gh}±0.21
T_{14} (Si+NO+AsII)	7.41^{def}±0.71	13.56^{ef}±0.17
T_{15} (Si+NO+AsIII)	6.86^{cdef}±0.74	12.27^{cde}±0.26

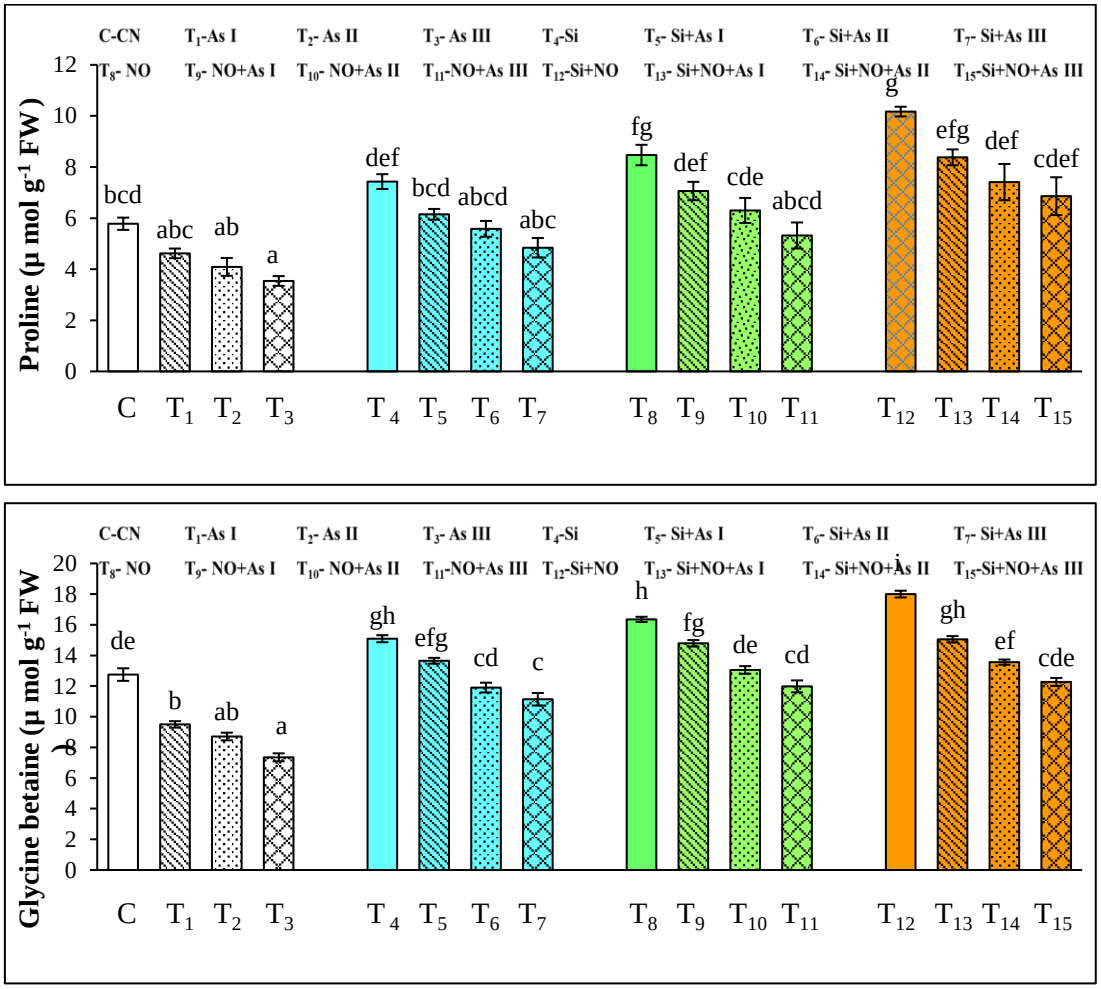

Fig. 6.45 Effect of Si and NO on proline and glycine betaine content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.3.7 Total carbohydrates

Total carbohydrate content was decreased by As stress in radish plants with the lowest content (5.43 mg g^{-1} FW) at T_3 (Fig. 6.46; Table 6.37). T_4 exhibited 11.68 mg g^{-1} FW carbohydrate amount. It was observed to be decreased from 9.5 mg g^{-1} FW at T_5 to 7.46 mg g^{-1} FW at T_7 concentration. In T_{11} applied plants, minimum total carbohydrate content (7.75 mg g^{-1} FW) was noticed in case of NO alone applied genotypes. Combined application of Si and NO under stressed conditions further increased the total carbohydrates content with maximum content of 10.57 mg g^{-1} FW at T_{13}. It was increased by 25.66% in T_5 than T_1. An increase of 24.07% was found in T_9 than T_1. T_{13} treatment exhibited 20.80% increase than T_9.

Table 6.37 Effect of Si and NO on total carbohydrates of 60 days old plants of *R. sativus* under As stress

Treatment	Total carbohydrates (mg g^{-1} FW)
C (Control)	8.75^{cd}±0.22
T_1 (AsI)	7.56^{bc}±0.25
T_2 (AsII)	6.39^{ab}±0.23
T_3 (AsIII)	5.43^{a}±0.23
T_4 (Si)	11.68^{f}±0.19
T_5 (Si+AsI)	9.5^{de}±0.22
T_6 (Si+AsII)	8.38^{cd}±0.23
T_7 (Si+AsIII)	7.46^{bc}±0.27
T_8 (NO)	11.56^{f}±0.21
T_9 (NO+AsI)	9.38^{de}±0.3
T_{10} (NO+AsII)	8.31^{cd}±0.41
T_{11} (NO+AsIII)	7.75^{bc}±0.26
T_{12} (Si+NO)	14.6^{g}±0.32
T_{13} (Si+NO+AsI)	10.57^{ef}±0.24
T_{14} (Si+NO+AsII)	9.64^{de}±0.37
T_{15} (Si+NO+AsIII)	8.24^{cd}±0.26

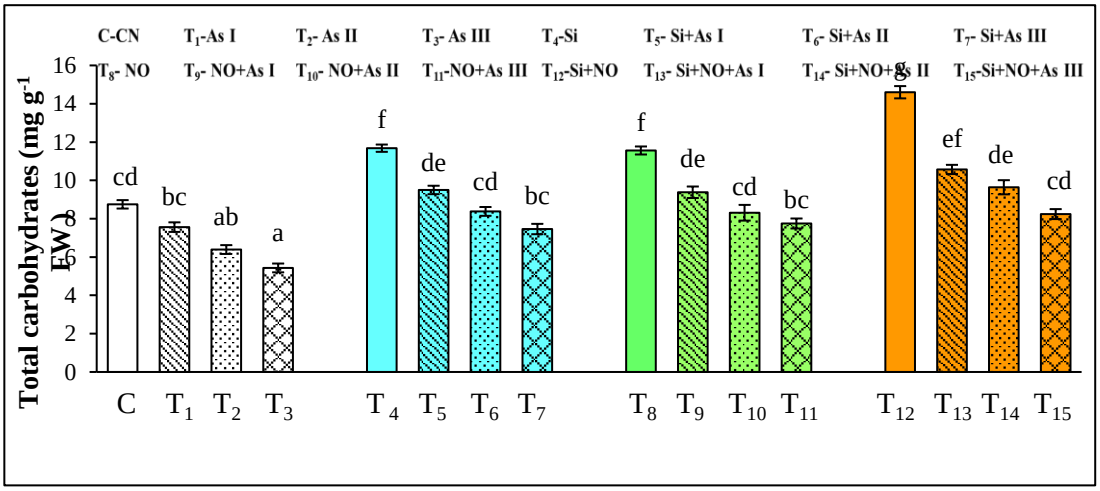

Fig. 6.46 Effect of Si and NO on total carbohydrates in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.3.8 Protein content and antioxidant defense system

6.1.3.8.1 Protein content and antioxidative enzymes

Control plants showed 10.49 mg g^{-1} FW amount (Fig. 6.47; Table 6.38) while T_1 stressed plants showed 8.02 mg g^{-1} FW. A 38% decline was found in T_3 treatment when compared with control plants. In the case of Si-applied plants under stressed conditions, the maximum protein content of 9.07 mg g^{-1} FW was in T_5 plants. Nitric oxide pretreatment also improved the protein content under As stress with the highest of 9.38

mg g^{-1} FW at T_9. Among all 16 treatments, Si + NO control plants (T_{12}) showed the highest protein content of 11.89 mg g^{-1} FW while under stressed conditions, the highest protein content of 10.07 mg g^{-1} FW was in T_{13} treated plants. It was increased by 13.09% in T_5 than T_1. An increase of 16.95% was found in T_9 than T_1. T_{13} treatment exhibited 7.35% increase than T_9. T_{13} showed 16.32% elevation than control plants.

SOD activity was found to be decreased under As stress with the lowest SOD activity of 6.23 UA mg^{-1} protein in T_1 plants (Fig. 6.47; Table 6.38). A 35% decrease in SOD was detected in T_3 than control genotypes. An 18% increase in the SOD level was found in T_4 than control genotypes. Under As stress, Si application increased the SOD activity from 6.23 cm to 11.76 UA mg^{-1} protein at T_5. NO-pre-treated plants also increased the SOD activity under As stress with the highest of 11.93 UA mg^{-1} protein at T_9. Si + NO further improved the SOD level from 6.53 to 11.8 UA mg^{-1} protein at T_{14}. It was increased by 88.76% in T_5 than T_1. An increase of 91.49% was found in T_9 than T_1. T_{13} treatment exhibited 1.44% increase than T_5.

As stress declined the CAT activity with the lowest (6.13 UA mg^{-1} protein) at T_3 concentration (Fig. 6.47; Table 6.38). Individual Si and NO control plants i.e., T_4 and T_8 showed an 11 and 19% increase in CAT activity than control plants. Si addition under As stress displayed highest activity of 9.96 UA mg^{-1} protein in T_5 plants. Similarly, NO pre-treatment to radish plants also showed the highest CAT activity of 10.44 UA mg^{-1} protein at T_9. Coupled application of Si and NO under As toxicity exhibited higher and lower activities of 11.93 and 10.43 UA mg^{-1} protein in T_{13} and T_{15} plants, respectively. It was increased by 20.58% in T_5 than T_1. An increase of 26.39% was found in T_9 than T_1. T_{13} treatment exhibited 14.27% increase than T_9.

The enzymatic activity of APX was noticed to be declined under As stress in radish plants (Fig. 6.48; Table 6.38). T_1 treated plants showed a decline in APX activity (20.26 UA mg^{-1} protein) than the control plants. Maximum APX activity (23.11 UA mg^{-1} protein) in Si treated plants was shown by T_5 plants under As toxicity. APX activity was enhanced from 20.26 to 24.20 UA mg^{-1} protein at T_9 when pre-treated with NO, in contrast to T_1 plants. Si + NO further upregulated the APX activity under stressed conditions with the highest of 25.97 UA mg^{-1} protein activity T_{13}. It was increased by 14.06% in T_5 than T_1. An increase of 29.91% was found in T_9 than T_1. T_{13} treatment exhibited 7.31% increase than T_9.

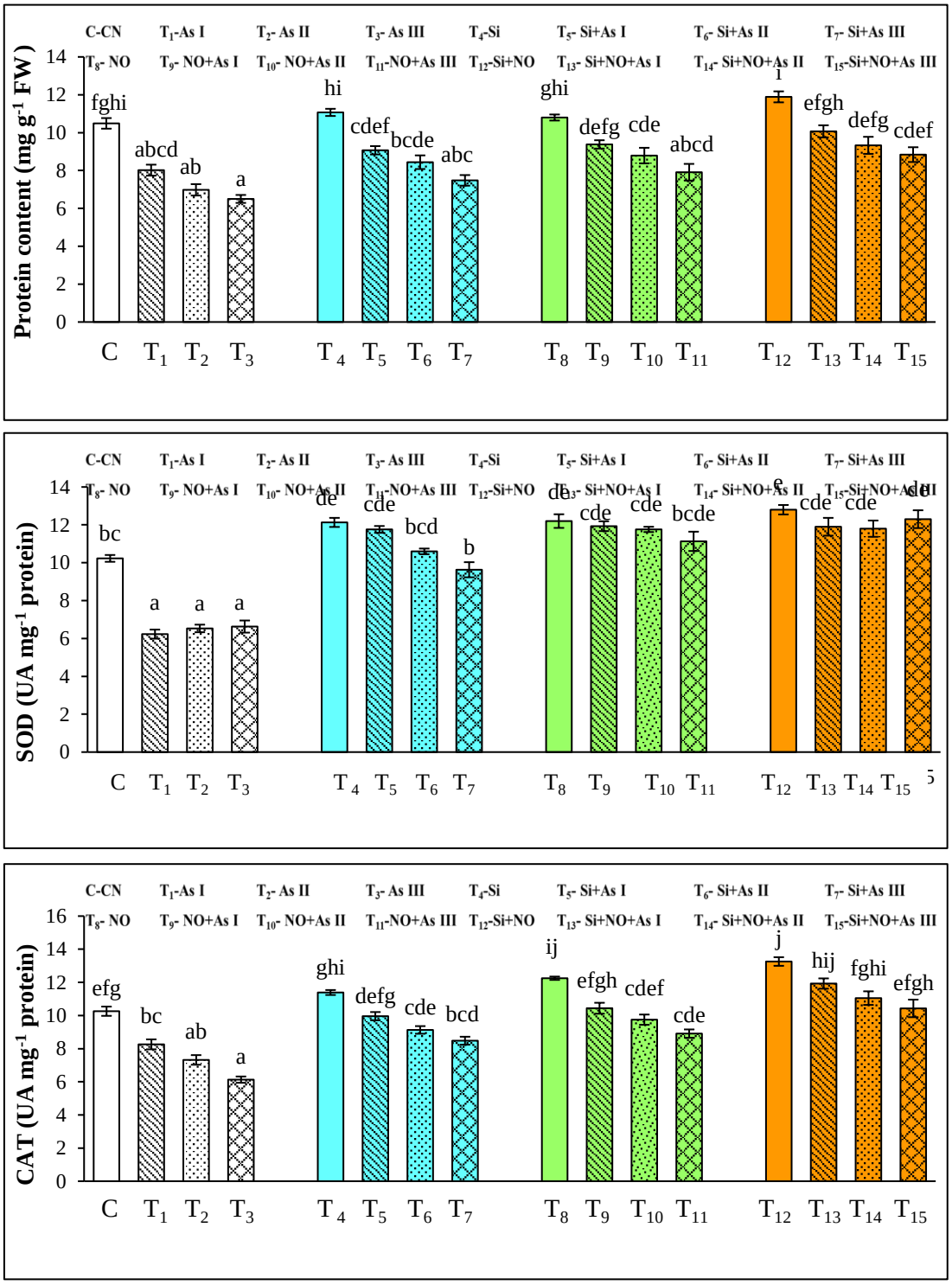

Fig. 6.47 Effect of Si and NO on protein, SOD and CAT enzyme activity in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

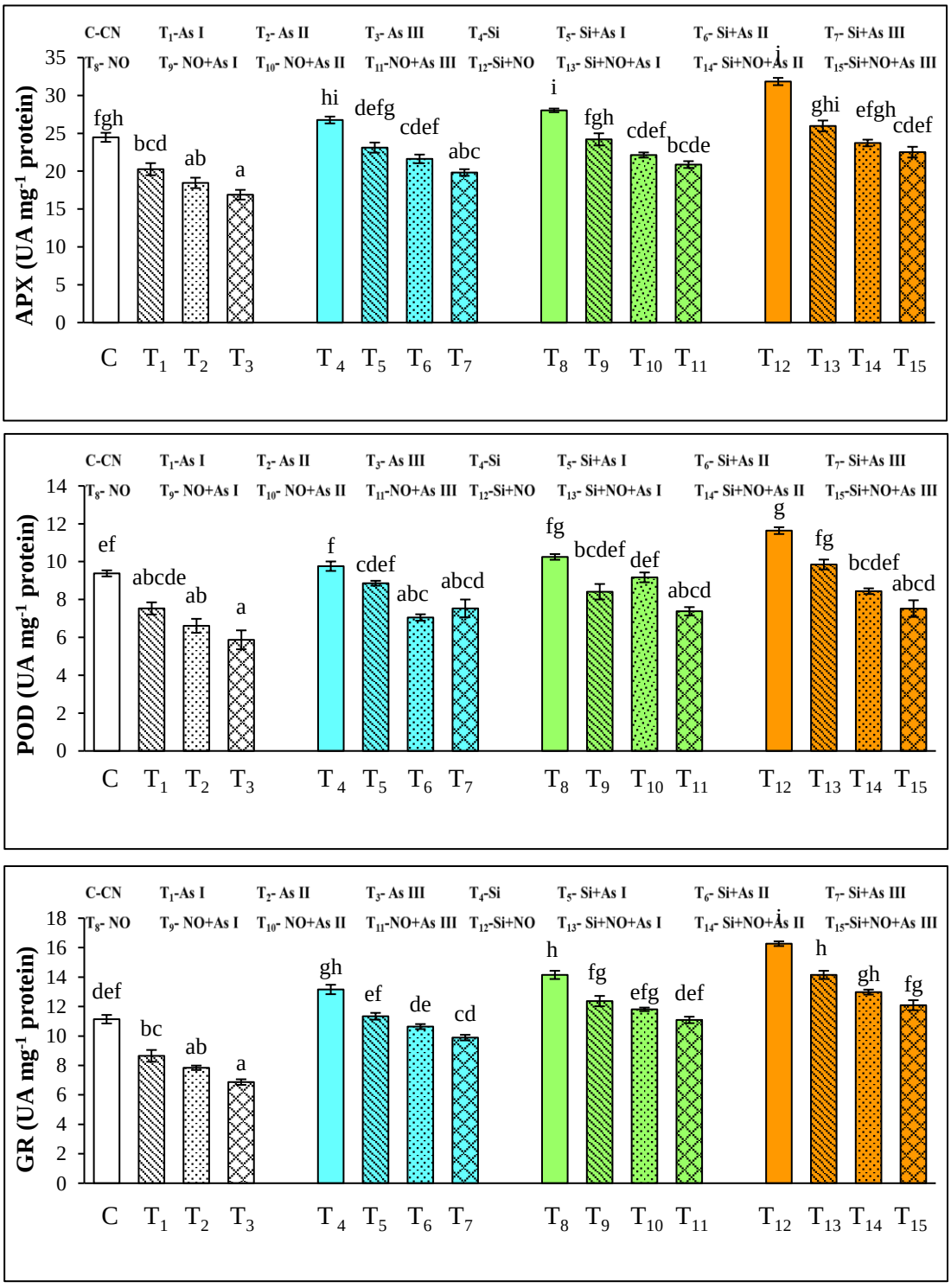

Fig. 6.48 Effect of Si and NO on APX, POD and GR enzyme activity in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

Table 6.38 Effect of Si and NO on protein content and antioxidative enzymes of 60 days old plants of *R. sativus* under As stress

Treatments	Protein content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
C (Control)	10.49^{fghi}±0.28	10.23^{bc} ±0.18	10.26^{efg}±0.28	24.47^{fgh}±0.60
T_1 (AsI)	8.02^{abcd}±0.29	6.23^{a} ±0.24	8.26^{bc}±0.3	20.26^{bcd}±0.80
T_2 (AsII)	6.98^{ab}±0.3	6.53^{a} ±0.20	7.32^{ab}±0.29	18.45^{ab}±0.69
T_3 (AsIII)	6.5^{a}±0.21	6.63^{a} ±0.32	6.13^{a}±0.19	16.89^{a}±0.64
T_4 (Si)	11.07^{hi}±0.19	12.13^{de} ±0.24	11.39^{ghi}±0.15	26.76^{hi}±0.45
T_5 (Si+AsI)	9.07^{cdef}±0.22	11.76^{cde} ±0.18	9.96^{defg}±0.25	23.11^{defg}±0.67
T_6 (Si+AsII)	8.44^{bcde}±0.36	10.6^{bcd} ±0.15	9.13^{cde}±0.23	21.62^{cdef}±0.57
T_7 (Si+AsIII)	7.48^{abc}±0.28	9.63^{b} ±0.40	8.48^{bcd}±0.24	19.82^{abc}±0.44
T_8 (NO)	10.8^{ghi}±0.16	12.2^{de} ±0.36	12.25^{ij}±0.1	28.03^{i}±0.24
T_9 (NO+AsI)	9.38^{defg}±0.22	11.93^{cde} ±0.26	10.44^{efgh}±0.33	24.20^{fgh}±0.80
T_{10} (NO+AsII)	8.79^{cde}±0.41	11.76^{cde} ±0.14	9.75^{cdef}±0.31	22.13^{cdef}±0.34
T_{11} (NO+AsIII)	7.91^{abcd}±0.44	11.13^{bcde} ±0.51	8.91^{cde}±0.25	20.87^{bcde}±0.45
T_{12} (Si+NO)	11.89^{i}±0.29	12.8^{e} ±0.25	13.26^{j}±0.26	31.84^{j}±0.48
T_{13} (Si+NO+AsI)	10.07^{efgh}±0.32	11.9^{cde} ±0.47	11.93^{hij}±0.31	25.97^{ghi}±0.72
T_{14} (Si+NO+AsII)	9.33^{defg}±0.45	11.8^{cde} ±0.43	11.05^{fghi}±0.41	23.72^{efgh}±0.44
T_{15} (Si+NO+AsIII)	8.84^{cdef}±0.39	12.3^{de} ±0.47	10.43^{efgh}±0.53	22.51^{cdef}±0.71

Performance of POD was lessened with the increase in the As concentration (Fig. 6.48; Table 6.39). A maximum decline in POD activity (5.87 UA mg^{-1} protein) was observed at T_3. Activity of POD was raised from 7.53 to 8.86 UA mg^{-1} protein in T_5, in contrast to T_1 plants. Pre-treatment with NO against As stress also improved the POD activity with the highest activity (8.41 UA mg^{-1} protein) at T_9. Si + NO boosted the POD level to diminish the As-induced toxic effects. Maximum POD activity i.e. 9.85 UA mg^{-1} was noticed at T_{13} while minimum POD activity i.e. 5.87 UA mg^{-1} protein was at T_{15}. It was increased by 17.66% in T_5 than T_1. An increase of 11.68% was found in T_9 than T_1. T_{13} treatment exhibited 17.12% increase than T_9. T_{13} showed 5.01% elevation than control plants.

Treatment of plants with Si + NO had caused in highest improvement in the GR activity as compared to all other treatments (Fig. 6.48; Table 6.39). Maximum decline in the

GR activity (6.87 UA mg^{-1} protein) was observed at T_3. Application of Si alone under stressed conditions increased the GR activity of 11.34 UA mg^{-1} protein in T_5 plants. In plants pre-treated with NO against As stress, the lowest GR activity (11.09 UA mg^{-1} protein) was observed in T_{11} plants. Furthermore, the combination of NO, Si and As greatly mitigated the As toxicity in plants with the highest GR activity of 14.15 UA mg^{-1} protein at T_{13}. It was increased by 31.09% in T_5 than T_1. An increase of 43% was found in T_9 than T_1. T_{13} treatment exhibited 24.77% increase than T_5. T_{13} showed 27.01% elevation than control plants.

Functioning of GPOX enzyme was observed to be diminished under As exposure in 60-days old radish plants (Fig. 6.49; Table 6.39). Minimum GPOX activity of 5.23 UA mg^{-1} protein was noticed at T_3 concentration. A 19, 26 and 41% reduction were observed at T_1, T_2 and T_3 concentrations, respectively than control ones. In the case of Si application under As stress, only a reduction of 15, 27 and 35% were noticed at T_5, T_6 and T_7 concentrations. T_5 and T_9 showed the highest GPOX activity of 9.15 and 8.17 UA mg^{-1} protein, respectively. T_{12} showed 11.8 UA mg^{-1} protein GPOX activity. The highest GPOX level of 10.26 UA mg^{-1} protein under stressed conditions was at T_1, T_2 and T_{13} when Si and NO were applied together. It was increased by 28.69% in T_5 than T_1. An increase of 14.90% was found in T_9 than T_1. T_{13} treatment exhibited 25.58% increase than T_9. T_{13} showed 15.67% elevation than control plants.

Arsenic stress led to a maximum decrease in the DHAR activity with the highest reduction of 7.11 UA mg^{-1} protein at T_3 (Fig. 6.49; Table 6.39). Si and NO under As stress upsurged the DHAR activity with the highest GST activity of 12.56 and 12.1 UA mg^{-1} protein at the combination of T_5 and T_{10}, respectively. Under stressed conditions, the highest DHAR activity of 14.19 UA mg^{-1} protein was detected in a combined treatment of Si and NO at T_{14}. It was increased by 24.60% in T_5 than T_1. An increase of 18.35% was found in T_9 than T_1. T_{14} treatment exhibited 16.21% increase than T_9.

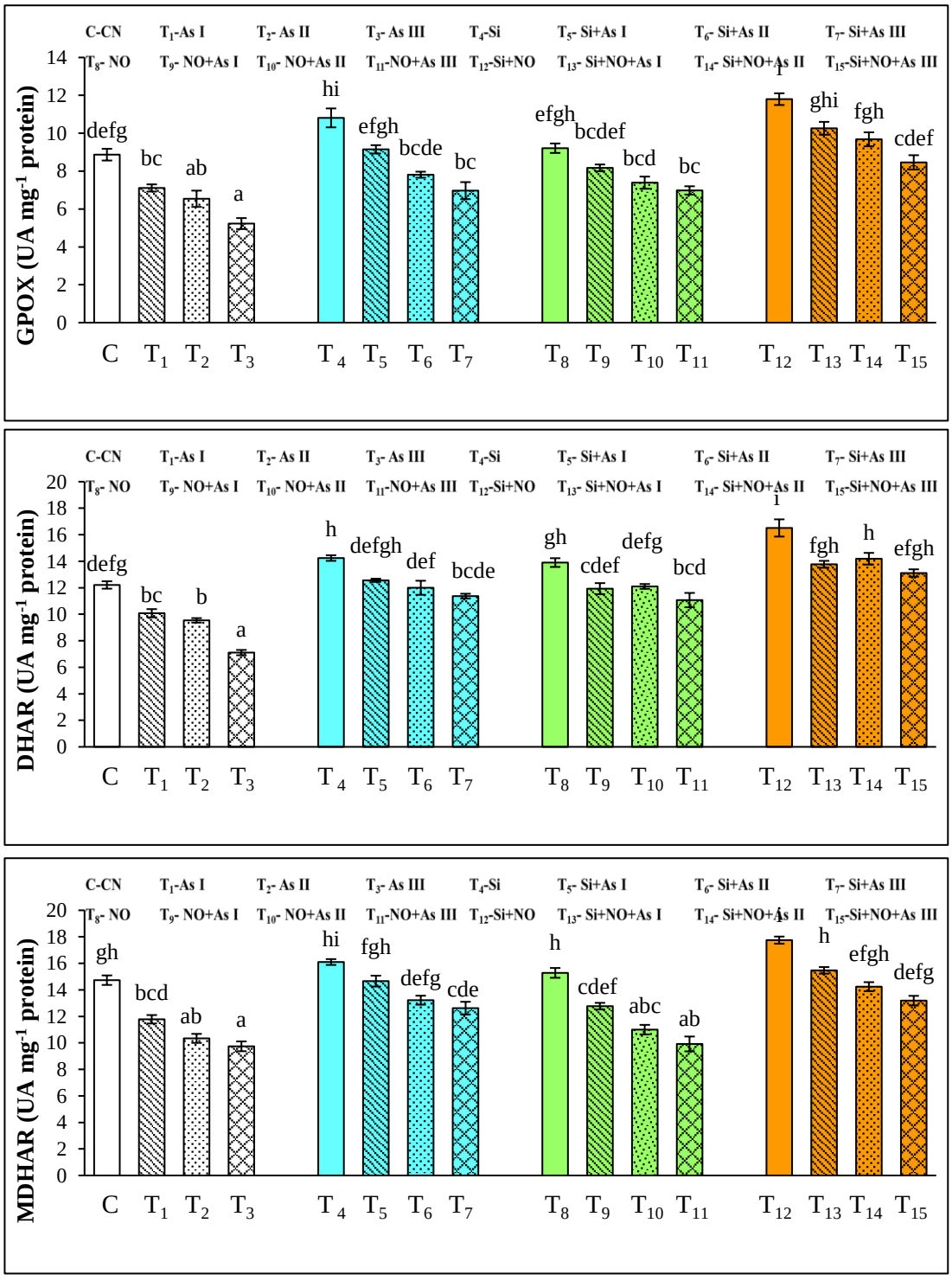

Fig. 6.49 Effect of Si and NO on GPOX, DHAR and MDHAR enzyme activity in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at $P< 0.05$.

Table 6.39 Effect of Si and NO on antioxidative enzymes of 60 days old plants of *R. sativus* under As stress

Treatment	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
C (Control)	9.38^{ef}±0.16	11.14^{def}±0.29	8.87^{defg}±0.31	12.21^{defg}±0.28
T_1 (AsI)	7.53^{abcde}±0.32	8.65^{bc}±0.4	7.11^{bc}±0.19	10.08^{bc}±0.31
T_2 (AsII)	6.61^{ab}±0.37	7.84^{ab}±0.15	6.54^{ab}±0.43	9.54^{b}±0.17
T_3 (AsIII)	5.87^{a}±0.5	6.87^{a}±0.19	5.23^{a}±0.29	7.11^{a}±0.2
T_4 (Si)	9.76^{f}±0.25	13.16^{gh}±0.32	10.81^{hi}±0.5	14.24^{h}±0.21
T_5 (Si+AsI)	8.86^{cdef}±0.13	11.34^{ef}±0.23	9.15^{efgh}±0.22	12.56^{defgh}±0.12
T_6 (Si+AsII)	7.05^{abc}±0.17	10.64^{de}±0.17	7.81^{bcde}±0.16	12^{def}±0.53
T_7 (Si+AsIII)	7.53^{abcd}±0.47	9.89^{cd}±0.19	6.97^{bc}±0.29	11.37^{bcde}±0.18
T_8 (NO)	10.25^{fg}±0.15	14.15^{h}±0.28	9.21^{efgh}±0.25	13.90^{gh}±0.33
T_9 (NO+AsI)	8.41^{bcdef}±0.41	12.37^{fg}±0.35	8.17^{bcdef}±0.18	11.93^{cdef}±0.42
T_{10} (NO+AsII)	9.17^{def}±0.26	11.81^{efg}±0.12	7.39^{bcd}±0.32	12.10^{defg}±0.18
T_{11} (NO+AsIII)	$7.^{38abcd}$±0.22	11.09^{def}±0.22	6.98^{bc}±0.22	11.07^{bcd}±0.54
T_{12} (Si+NO)	11.64^{g}±0.18	16.27^{i}±0.16	11.8^{i}±0.31	16.51^{i}±0.65
T_{13} (Si+NO+AsI)	9.85^{fg}±0.26	14.15^{h}±0.28	10.26^{ghi}±0.34	13.77^{fgh}±0.26
T_{14} (Si+NO+AsII)	8.44^{bcdef}±0.15	12.98^{gh}±0.17	9.68^{fgh}±0.37	14.19^{h}±0.44
T_{15} (Si+NO+AsIII)	7.52^{abcd}±0.44	12.09^{fg}±0.34	8.46^{cdef}±0.38	13.11^{efgh}±0.28

T_1 plants showed a decline in MDHAR activity (9.74 UA mg^{-1} protein), as compared to control plants (Fig. 6.49; Table 6.40). Foliar application of Si under stressed conditions alleviated the As-induced toxicity by improving the MDHAR activity. Maximum MDHAR activity (14.66 UA mg^{-1} protein) in the case of individual Si was shown by T_5 plants. Enzymatic activity of MDHAR was enhanced from 11.78 to 12.78 UA mg^{-1} protein at T_9 when pre-treated with NO, in contrast to T_1 plants. Si + NO further augmented the MDHAR activity under stressed conditions with a maximum of 15.47 UA mg^{-1} protein activity at T_{13}. It was increased by 24.44% in T_5 than T_1. An increase of 8.40% was found in T_9 than T_1. T_{13} treatment exhibited 21.14% increase than T_9. T_{13} showed 5.02% elevation than control plants.

Arsenic stress decreased the GST activity in 60-days old radish plants (Fig. 6.50; Table 6.40). Minimum GST activity (5.51 UA mg^{-1} protein) was noticed at T_3 concentration. T_1 plants showed 7.08 UA mg^{-1} protein GST activity. With regard to the Si application

under As stress, only a reduction of 24, 33 and 41% were noticed at T_1, T_2 and T_3 concentrations, respectively. Si and NO under stressed conditions showed the highest and lowest GST activity of 8.89 and 9.92 UA mg^{-1} protein at T_5 and T_9, respectively. Among individual applications of Si and NO, NO exhibited better results in improving GST activity under stressed conditions, as compared to Si application. T_{12} showed 13.04 UA mg^{-1} protein GST activity which was the highest among all 16 treatments. Highest and lowest GST activity i.e., 11.07 and 9.44 UA mg^{-1} protein under stressed conditions was at T_{13} and T_{15} when Si and NO were applied together. It was increased by 25.56% in T_5 than T_1. An increase of 40.11% was found in T_9 than T_1. T_{13} treatment exhibited 11.59% increase than T_9. T_{13} showed 21.38% elevation than control plants.

Activity of PPO enzymes was reduced in As stressed radish plants (Fig. 6.50; Table 6.40). Minimum PPO activity of 9.25 UA mg^{-1} protein was observed at T_1. Further, T_5 and T_9 caused the highest PPO activity of 13.25 and 13.88 UA mg^{-1} protein. T_{14} caused the highest PPO activity of UA mg^{-1} protein. It was increased by 33.83% in T_5 than T_1. An increase of 18.48% was found in T_9 than T_1. T_{13} treatment exhibited 18.32% increase than T_9. T_{13} showed 0.87% elevation than control plants.

Table 6.40 Effect of Si and NO on antioxidative enzymes of 60 days old plants of *R. sativus* under As stress

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
C (Control)	$14.73^{gh}\pm0.36$	$9.12^{cde}\pm0.18$	$13.76^{fgh}\pm0.25$
T_1 (AsI)	$11.78^{bcd}\pm0.32$	$7.08^{ab}\pm0.21$	$9.9^{ab}\pm0.24$
T_2 (AsII)	$10.34^{ab}\pm0.34$	$6.31^{ab}\pm0.38$	$10.43^{abc}\pm0.31$
T_3 (AsIII)	$9.74^{a}\pm0.37$	$5.51^{a}\pm0.32$	$9.25^{a}\pm0.52$
T_4 (Si)	$16.1^{hi}\pm0.22$	$11.83^{hi}\pm0.29$	$14.83^{h}\pm0.36$
T_5 (Si+AsI)	$14.66^{fgh}\pm0.41$	$8.89^{cde}\pm0.32$	$13.25^{efgh}\pm0.22$
T_6 (Si+AsII)	$13.22^{defg}\pm0.34$	$7.84^{bcd}\pm0.34$	$11.80^{cde}\pm0.23$
T_7 (Si+AsIII)	$12.62^{cde}\pm0.48$	$6.89^{ab}\pm0.37$	$12.44^{def}\pm0.19$
T_8 (NO)	$15.28^{h}\pm0.37$	$11.32^{gh}\pm0.1$	$14.40^{gh}\pm0.25$
T_9 (NO+AsI)	$12.77^{cdef}\pm0.25$	$9.92^{efg}\pm0.27$	$11.73^{cde}\pm0.45$
T_{10} (NO+AsII)	$11^{abc}\pm0.36$	$9.31^{cde}\pm0.3$	$12.97^{efg}\pm0.38$
T_{11} (NO+AsIII)	$9.92^{ab}\pm0.56$	$7.76^{bc}\pm0.36$	$11.09^{bcd}\pm0.28$
T_{12} (Si+NO)	$17.75^{i}\pm0.27$	$13.04^{i}\pm0.24$	$16.73^{i}\pm0.3$
T_{13} (Si+NO+AsI)	$15.47^{h}\pm0.25$	$11.07^{fgh}\pm0.3$	$13.88^{fgh}\pm0.19$
T_{14} (Si+NO+AsII)	$14.24^{efgh}\pm0.34$	$10.43^{efgh}\pm0.42$	$13.41^{efgh}\pm0.46$
T_{15} (Si+NO+AsIII)	$13.19^{defg}\pm0.36$	$9.44^{def}\pm0.36$	$12.74^{defg}\pm0.19$

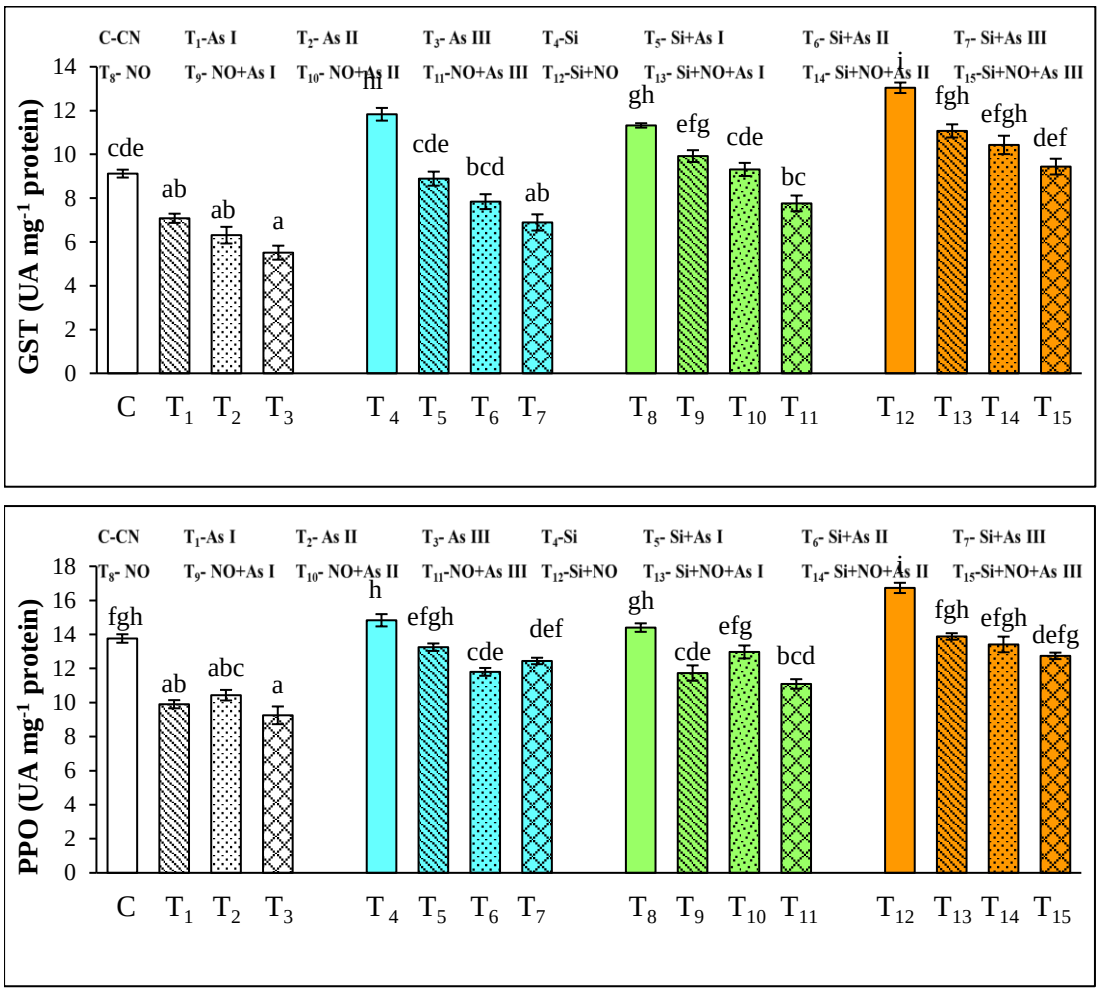

Fig. 6.50 Effect of Si and NO on GST and PPO enzyme activity in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.1.3.8.2 Non-enzymatic antioxidants

Content of ascorbic acid was greatly diminished in radish plants under As stress (Fig. 6.51; Table 6.41). The lowest amount of 7.97 µg g^{-1} FW was detected in T_3 plants. Control plants showed 11.93 µg g^{-1} FW ascorbic acid content. T_4 and T_8 plants exhibited 14.36 and 15.1 µg g^{-1} FW ascorbic acid content, respectively. Application of Si under stressed conditions showed maximum ascorbic acid content (12.28 µg g^{-1} FW) at T_5. Pre-treatment with NO also improved the levels of ascorbic acid under As stress with a maximum of 13.16 µg g^{-1} FW at T_9. In the coupled application of Si and NO under As toxicity, its amount was increased from 10.08 to 14.75 µg g^{-1} FW at T_{13}, in contrast to T_1 treated plants. It was increased by 21.82% in T_5 than T_1. An increase of 30.55% was found in T_9 than T_1. T_{13} treatment exhibited 12.08% increase than T_9. T_{13} showed 23.63% elevation than control plants.

Glutathione content was reduced from 11.26 to 9.51 µg g^{-1} FW when concentration

reduced from T_1 to T_3 concentration (Fig. 6.51; Table 6.41). Glutathione content was found to be increased from 9.51 to 12.05 µg g^{-1} FW in T_7 concentration, in contrast to T_3 plants. NO pre-treated plants under As stress showed maximum glutathione content of 14.24 µg g^{-1} FW at T_9. Si + NO further upregulated the glutathione content under stressed conditions. The Si + NO treated plants showed 16.36, 15.12 and 14.49 µg g^{-1} FW amount at T_{13}, T_{14} and T_{15}, respectively. It was increased by 24.42% in T_5 than T_1. An increase of 26.46% was found in T_9 than T_1. T_{13} treatment exhibited 14.88% increase than T_9. T_{13} showed 17.19% elevation than control plants.

Tocopherol level of control plants was 13.85 µg g^{-1} FW which got reduced in the As stressed genotypes (Fig. 6.51; Table 6.41). The lowest tocopherol content of 7.08 µg g^{-1} FW was detected at T_3. Individual treatment of Si and NO under As stress exhibited the highest tocopherol content of 12.98 and 11.5 µg g^{-1} FW in T_5 and T_9, respectively. T_{13} showed maximum tocopherol content of 14.79 µg g^{-1} FW. It was increased by 38.23% in T_5 than T_1. An increase of 22.47% was found in T_9 than T_1. T_{13} treatment exhibited 28.60% increase than T_9. T_{13} showed 6.78% elevation than control plants.

Table 6.41 Effect of Si and NO on non-enzymatic antioxidants of 60 days old plants of *R. sativus* under As stress

Treatment	Ascorbic acid (µg g^{-1} FW)	Glutathione (µg g^{-1} FW)	Tocopherol content (µg g^{-1} FW)
C (Control)	11.93^{cde}±0.22	13.96^{def}±0.36	13.85^{gh}±0.37
T_1 (AsI)	10.08^{bc}±0.27	11.26^{abc}±0.29	9.39^{bc}±0.15
T_2 (AsII)	9.16^{ab}±0.48	10.4^{ab}±0.33	$8.^{31ab}$±0.32
T_3 (AsIII)	7.97^{a}±0.57	9.51^{a}±0.63	7.08^{a}±0.16
T_4 (Si)	14.36^{fg}±0.36	16.28^{gh}±0.26	15.14^{hi}±0.2
T_5 (Si+AsI)	12.28^{de}±0.39	14.01^{ef}±0.23	12.98^{efg}±0.32
T_6 (Si+AsII)	10.98^{bcd}±0.38	12.89^{cde}±0.3	11.36^{cdef}±0.22
T_7 (Si+AsIII)	9.34^{ab}±0.39	12.05^{bcd}±0.21	9.73^{bcd}±0.77
T_8 (NO)	15.1^{gh}±0.2	17.25^{h}±0.35	14.11^{gh}±0.35
T_9 (NO+AsI)	13.16^{ef}±0.25	14.24^{ef}±0.53	11.5^{def}±0.52
T_{10} (NO+AsII)	11.64^{cde}±0.27	13.18^{cde}±0.2	10.32^{bcd}±0.51
T_{11} (NO+AsIII)	10.74^{bcd}±0.32	12.64^{cde}±0.36	8.85^{ab}±0.26
T_{12} (Si+NO)	16.95^{h}±0.2	19.86^{i}±0.39	17.13^{i}±0.21
T_{13} (Si+NO+AsI)	14.75^{fg}±0.22	16.36^{gh}±0.46	14.79^{gh}±0.61
T_{14} (Si+NO+AsII)	13.01^{ef}±0.39	15.12^{fg}±0.13	13.15^{fgh}±0.22
T_{15} (Si+NO+AsIII)	12.37^{de}±0.52	14.49^{efg}±0.45	11.11^{cde}±0.32

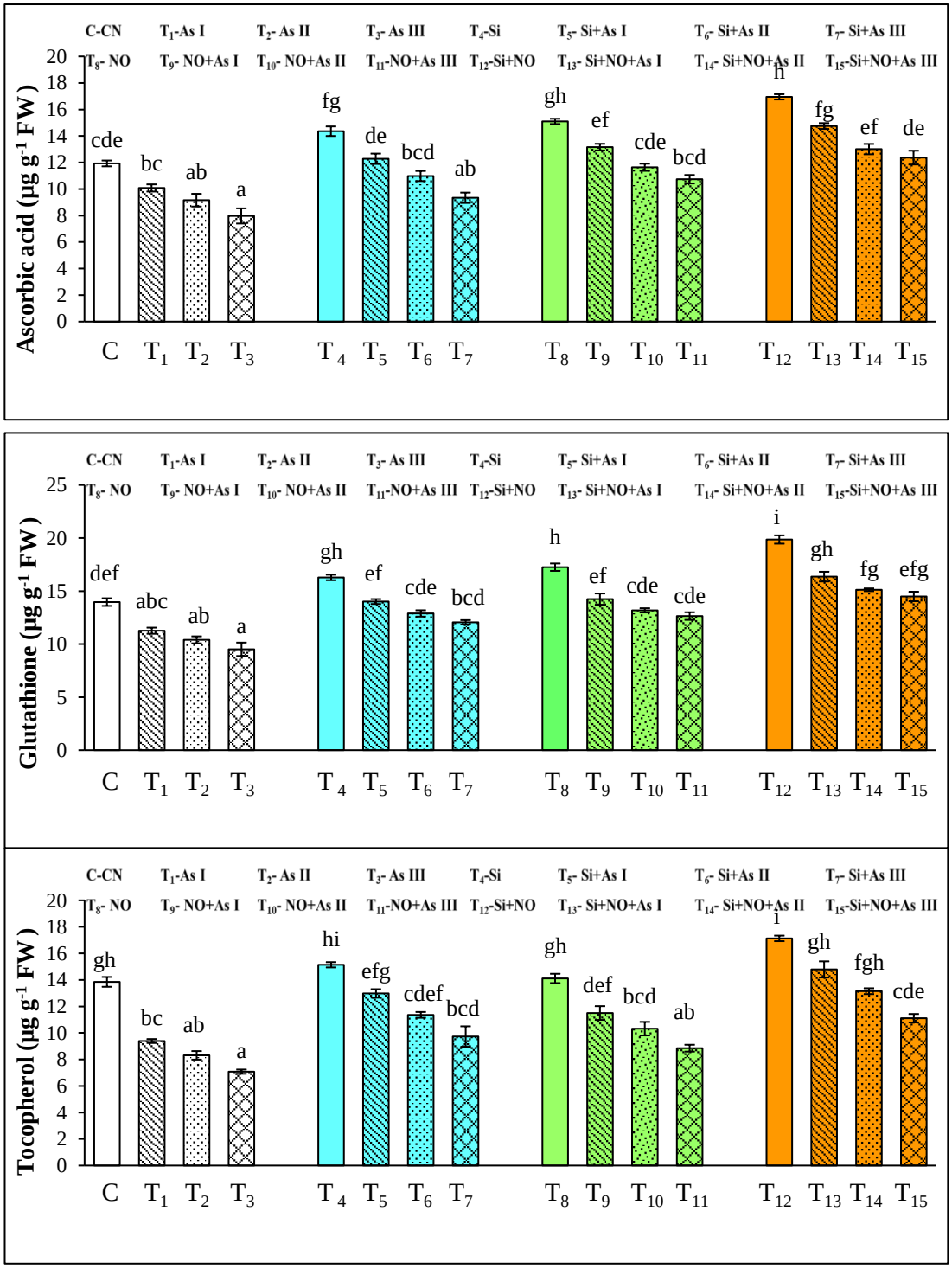

Fig. 6.51 Effect of Si and NO on ascorbic acid, glutathione and tocopherol content in 60-days old plants of *R. sativus* under As stress. Each value denotes the mean of three replicates for every treatment level along with standard error of mean. Means within a column followed by the dissimilar letter are significantly different at P< 0.05.

6.2 Discussion

6.2.1 Plant growth

In our study, As stress caused a decrease in the germination percentage of radish at various As concentrations. However, exogenous application of Si and NO significantly induced seed germination in radish. Similar results were noticed by Kopyra and Gwozdz (2003) in lupin, Hu et al. (2007) in wheat and Habib et al. (2010) in rice by the application of NO under stressed conditions. This NO-mediated increase in seed germination might be associated with the regulation of hormones production by NO. These hormones are crucial in stimulating seed germination (Beligni & Lamattina, 2000; Parani et al., 2004). It was shown by several studies that the process of seed germination is positively regulated by NO in various plant species under stressed environments (Singh et al., 2008, 2009; Xiong et al., 2010). Seed germination was increased by NO under stressed conditions as it improves the water absorption, H_2O level and activities of α-amylase and protease enzymes as reported by Hu et al. (2007) that NO stimulated the α-amylase activity under Cu-stress. Hence, the stimulated activity of α-amylase and protease might be the reason for the NO mediated elevation in germination percentage of *R. sativus* under As stress. Reactive oxygen species scavenging and protection of biomolecules, like nucleic acids and protein might be another reason for NO mediated acceleration of seed germination.

In our research, it has been detected that growth and the biomass were reduced in *R. sativus* under As stress. Degradation of chlorophyll pigments might be the reason for the poor growth of stem against As toxicity. According to Gupta et al. (2018), Jin et al. (2010) and Shri et al. (2009) studies in *Allium cepa,* fescue leaves and *Oryza sativa* respectively, As showed a similar dose-dependent decline in the plant growth and biomass. The impaired growth of radish might be attributed to the fact that the integrity of the plasma membrane gets disturbed by As exposure which ultimately results in declined relative water content and the suppression of various biochemical aspects (Meharg & Hartley-Whitaker, 2002; Panda et al., 2010).

However, application of Si and NO upgraded these growth parameters under As pollution. Our results are similar to Ahmad et al. (2021) in which they found an upsurge in length and biomass by the Si + NO treatment under As stress in *B. juncea* plants.

Silicon and NO decrease the metal uptake and improves the accumulation of essential nutrients and photosynthetic pigments which eventually upsurge the growth and biomass against metal stresses as indicated by Amooaghaie and Enteshari (2017), Amooaghaie et al. (2018) and Verma et al. (2013) in NO treated genotypes and by Ashfaque et al. (2017) and Wu et al. (2018) in Si applied genotypes. Silicon gets accumulated in leaf bundle cells which subsequently alter the structure of chloroplasts. This results in the increased potential of plants to use light and ultimately raises the plant biomass. Silicon-triggered improvement in the PS II efficiency is also a reason for the enhancement in the plant biomass (Adrees et al., 2015; Torabi et al., 2013). Plant height and biomass were found to be increased in Si applied *Spinacia oleracea* against As toxicity (Saleem et al., 2022). Silicon provides mechanical support and improves plant growth under stressed conditions which is the reason for Si-mediated mitigation of As stress even at high As concentrations (Singh et al., 2019). Nitric oxide-facilitated increase in growth parameters is similar to the findings of Souri et al. (2020) in *Isatis cappadocica*, Singh et al. (2016) in *Oryza sativa*, Singh et al. (2009) in *Oryza sativa* and Ghorbani et al. (2021a) in *Solanum lycopersicum* under As stress. Pigment synthesis and stomatal functioning were improved in NO treated *Vicia faba* plants when exposed to As via increasing the relative water content (Mohamed et al., 2016).

6.2.2 Photosynthetic system

6.2.2.1 Photosynthetic pigments

Process of photosynthesis is performed by chlorophylls because they convert light into chemical energy which is then converted to ATP (Zhou et al., 2018). Rate of photosynthesis get directly influenced if there is any variation in the pigments content (Zhang et al., 2011). Our study exhibited that As treatment reduced the level of pigments. A decline in the synthesis of porphobilinogen (PBG), an intermediate molecule for the formation of chlorophyll is responsible for the reduction in these pigment levels (Cenkci et al., 2010). Our results are similar to Choudhury et al. (2011) who also found a decrease in photosynthetic pigments in *Oryza sativa* exposed to As stress. A diminution in the functioning of photosynthesis-related enzymes could be the reason for the diminution of these parameters (Islam et al., 2013). Photosystem II efficiency was reduced by As which ultimately diminish the content of photosynthetic pigments (Baker, 2008). In crops such as bean, oat and red clover, a significant

reduction in carotenoid content was noticed in As polluted soil (Mascher et al., 2002). Arsenic induced damage to the thylakoid membrane cause the decline in carotenoids (Bhat et al., 2021). Carotenoid level was lowered as described by Zhou et al. (2018) in *Acorus calamus* under Sb stress and suggested that the lack of electron transfer under stressed conditions was the reason for reduction in pigment level.

In our study, Si and NO improved the various photosynthetic parameters under As stress. Similar reports of elevation in photosynthetic pigments were noticed by Wu et al. (2018) on cabbage by the leaf application of Si and by Khator et al. (2021) on *Brassica juncea* by the application of NO under Cd stress. Carotenoids can protect chlorophyll from photooxidation by regulating the ROS level in the chloroplast (Amooaghaie et al., 2018). Similarly, improvements in photosynthetic pigments were observed by Paula et al. (2015) in Zn stressed *Zea mays* by Si application and by Nabaei and Amooaghaie (2020) in Cd-stressed *Catharanthus roseous* plants by NO treatment. Similar to our results, the combined treatment of Si and NO significantly improved the pigments level, as compared to their individual application by diminishing the ROS burst in As-exposed mustard (Ahmad et al., 2021). Synthesis of chlorophyll pigments and diminution in As accumulation in plant parts are the two foremost mechanisms for the increase in pigments level by Si under As stress (Kashif et al., 2021). Das and Biswas (2022) also reported an escalation in the levels of chlorophyll, carotenoid, and xanthophyll by the individual and joint supplementation of Si and selenium under As stress in rice. It has also been shown that the photosynthetic functioning of wheat seedlings was amended by Si under As (Sil et al., 2019). NO-mediated upsurge in the contents of photosynthetic pigments under As in *Vicia faba* and *Nasturtium officinale* respectively (Ahmad et al., 2020; Namdjoyan & Kermanian, 2013), Cu in *Oryza sativa* (Mostofa et al., 2014) and Cd stress in perennial grass (Wang et al., 2013b) has been reported. De novo formation of chlorophylls and related protein compounds by NO was another reason for this NO mediated upsurge in pigments level (Ahmad et al., 2016).

6.2.2.2 Gaseous exchange characteristics

It has been shown by several studies that various environmental stresses alter the gas exchange characteristics in plants (Ahmad et al., 2011; Asgher et al., 2014; Iqbal et al., 2015). In our study, As metalloid reduced all the gaseous exchange parameters. Anjum et al. (2017) also testified a similar deterioration in gas exchange parameters in *Zea*

mays under Cd and As stress. Cadmium mediated decline in the stomatal conductance was a result of reduced functions of stomata which ultimately resulted in decreased photosynthesis (Khan et al., 2016; Per et al., 2016). Similar mechanism could be the reason for As mediated decrease in stomatal conductance. Bean genotypes exposed to Zn stress showed reduced stomatal functions which were the major factor for the diminution in photosynthetic activity. Moreover, the carboxylase functioning of Rubisco was affected due to inhibition of CO_2 flow which is attributed to the Cd-induced decrease in intercellular spaces of leaf mesophyll (Vassilev et al., 2011).

Our study showed an increase in the gas exchange attributes by Si and NO treatment under As stress in radish. The net photosynthetic rate was reported to be increased whereas stomatal limitations were decreased by Si in *Oryza sativa* under As pollution (Hu et al., 2013). Silicon applied rice plants showed significant improvement in the gas exchange measurements (Sanglard et al., 2014). These parameters were enhanced by Si in hydroponically grown cotton, rice and cucumber against Cd contamination (Farooq et al., 2013; Feng et al., 2010; Nwugo & Huerta, 2008). Results similar to our results were noticed by Fatma et al. (2016) in mustard when treated with NO. Farnese et al. (2017) also found upsurge in internal CO_2 concentration by the exogenous application of NO in As polluted *Pistia stratiotes*. Ahmad et al. (2018) also displayed NO induced increase in the level of gas exchange attributes.

6.2.3 Metabolites

Under stressful environments, phenolic compounds, produced by shikimic acid or phenylpropanoid pathways, exhibit antioxidant properties (Ren & Sun, 2014). Present study showed that contents of metabolites (flavonoid, anthocyanin and phenolics), were noticed to be decreased under all three concentrations of As. However, Si and NO escalated the metabolite contents under stressed conditions. González-Moscoso et al. (2019) reported similar findings of an elevation in the flavonoids and phenolic content in Si NPs treated tomato fruits under As stress. Flavonoids regulate ROS homeostasis under stress conditions due to their antioxidant potential. An upsurge in the concentrations of phenols was noticed by Vega et al. (2019) in the shoots and the roots of barley by Si application under Al toxicity. NO-mediated increase in the phenolic and anthocyanin content was reported in coriander seedlings under As toxicity in combination with triacontanol (Asadi Karam et al., 2017). Si and NO-mediated increase

in the phenolic compounds in radish under As stress might be due to the fact that they prevent reactions to $O_2^{\cdot -}$ formation to decrease the ROS synthesis.

6.2.4 Oxidative stress

Higher As content cause overaccumulation of ROS. Biomolecules get damaged through As-induced ROS burst (Shaibur et al., 2008, Singh et al., 2009). This ROS burst alters the membrane integrity via increasing the MDA and H_2O_2 levels in *Raphanus sativus*. Present research work showed elevation in the MDA and H_2O_2 levels in As alone genotypes. Treatment with Si and NO diminished the level of MDA and H_2O_2 under As toxicity. Similar outcomes were described by Praveen et al. (2019) and Pandey et al. (2016) in *B. juncea* and by Boorboori et al. (2020) and Tripathi et al. (2013) in *Oryza sativa* by Si under stressed conditions. Nitric oxide also declined MDA and H_2O_2 contents as shown by Jin et al. (2010) in tall fescue, Kaya et al. (2019) in *Capsicum annuum*, Andrade et al. (2016) in *Eichhornia crassipes*, Singh et al. (2009) in *Oryza sativa* and by Singh et al. (2013) in *Luffa acutangular*. There are two possible mechanisms for the NO-mediated decline in oxidative stress and damage under As stress a) NO prevents oxidative damage by changing highly toxic products into less toxic products by directly scavenging ROS and b) by stimulating cellular defense system of plants by NO through acting as a signaling moiety (Gill et al., 2013; Xiong et al., 2010).

6.2.5 Metalloid uptake

Arsenic concentration was higher in As treated *Raphanus sativus* in the present study, whereas Si and NO declined its content in roots, shoot and leaves of *R. sativus* grown under stressed conditions. It was found from the results that more As content get accumulated in roots of *Raphanus sativus*, as compared to shoot or leaves. Ahmad et al. (2021) also reported declined level of As in individual and combination of Si and NO treated plants and also found less As content in shoot compared to root in *Brassica juncea*. Silicon application also showed lower metal contents in shoot in comparison to roots in field-grown cereals (Sohail et al., 2020). Usman et al. (2019) testified that reduced metal contents in shoot tissues were shown by most of the plants as compared to the root tissues. Casparian strips get thickened by the application of Si which subsequently results in lowering metal transport in plants. This further causes Si and lignin deposition in the dermal region. Silicon-induced decrease in As content might be

due to the fact that it reduces the content of free As ions by limiting apoplasmic transport of heavy metals. On the other hand, it also shows elevation in essential ions passage in the various plant tissues (Saleem et al., 2022). Silicon reduces metal absorption by roots via stimulating the formation of root exudates in order to chelate metals (Etesami & Jeong, 2018; Kidd et al., 2001).

Praveen and Gupta (2018) noticed that NO treatment under As stress in rice diminished the As concentration in root and shoot with lower As content in the shoot as compared to root tissues. Ahmad et al. (2020b) also showed similar results in *Vicia faba*. A decline in the As uptake and/or translocation to the shoot might be the cause for the minor levels of As in *R. sativus* shoot treated with NO, as compared to roots. Phytochelatins synthesis and ABC transporters were activated by NO (Grün et al., 2006), which further cause sequestration of As^{3+}-PC complex inside the vacuoles (Song et al., 2010). Another reason for lower content of As in shoot of *Raphanus sativus* by pre-treatment with NO under As stress may be that the majority of the As that collected in the root was gathered in the root vacuole as As^{3+}-PC complex due to which transportation of As to shoot get reduced.

6.2.6 Osmolytes

The osmotic potential of plants is reduced by various environmental stresses, which in turn augment electrolyte leakage, lessens turgor and hinder growth. Plants produce osmolytes to cope with these stresses (Arif et al., 2021). Osmolytes were found to avert the noxious impacts of metals by amending the chelation of metal ions (Anjum et al., 2015). In response to serious As toxicity, proper hydration level within the cells is maintained by osmolytes (Ahmad et al., 2018; Kumar et al., 2019). Osmolytes were noticed to be declined in the present study under As stress. However, Si and NO treatment escalated their content in *Raphanus sativus* under As stress conditions. Fatemi et al. (2020) in *C. sativum*, Howladar et al. (2018) in *Triticum aestivum* and Yazdani et al. (2021) in *Glycyrrhiza glabra* under Pb, Cd and Al stress respectively, reported the enhanced osmolytes content in exogenous applied Si plants. Accumulation of compatible osmolytes was noticed to be improved by NO supplementation in various plant plants. This increase in the osmolytes level can maintain redox homeostasis and enzyme functioning through ROS scavenging and can protect key cellular processes to maintain cellular osmolarity (Ahanger & Ahmad, 2019). The application of NO

increased the functioning of pyrroline-5-carboxylate synthetase (P5CS1) to improve the proline accumulation in soybean plants against As toxicity (Chandrakar & Keshavkant, 2019). Nitric oxide-mediated enhancement in the proline level is most likely due to upsurge in *P5CS1* activity, which further prevents As (Chandrakar et al., 2017; Rajeb et al., 2014). The plant's antioxidant and glyoxalase systems were reported to be regulated by osmolytes to enhance stress endurance (Hasanuzzaman et al., 2014). Carboxylase functioning of the Rubisco was protected by proline (Sivakumar et al., 2000) to prevent photoinhibition. According to Khan et al. (2014), escalation in glycine betaine level led to enhanced photosynthesis under salinity stress. Choudhury et al. (2011) and Siddiqui et al. (2015a) observed improved proline accumulation in *Oryza sativa* and *Withania somnifera*, respectively under As stress. Modulations in the biosynthesis pathways cause marked variations in the osmolytes production (Ahmad et al., 2013). Silicon and NO-mediated accumulation of osmolytes under stressed conditions may have contributed to withstand As induced noxious impacts.

6.2.7 Carbohydrates

The starch builds up in chloroplasts and is distributed to other components to supply energy in the cells and needs C fixation during photosynthesis for its production. Metal stress can considerably influence carbon metabolism which ultimately reduces the nutritional value of plants (Wahid et al., 2007). Energy for cellular metabolism is provided in large part by the carbohydrates created during photosynthesis and also helps to protect cellular components (Muller et al., 2011). In our study, total carbohydrate content was noticed to be diminished under As stress in radish. But application of Si and NO opposed the As induced effects by increasing the total carbohydrate content. Carbohydrates accumulation in plants regulates the oxidative pentose phosphate pathway by performing ROS scavenging via acting as a free radical scavenger (Ende and Peshev, 2013; Hu et al., 2012; Van den Ende & Valluru, 2009). Carbohydrates content was enhanced by NO application in As exposed *Pistia stratiotes* (Farnese et al., 2017), *Satureja Hortensis* (Azizi et al., 2021) and *Vigna radiata* (Khan et al., 2020a) under Cd stress. Nitric oxide-mediated escalation in the carbohydrate content in radish plants might be attributed to an increase in net photosynthetic rate. Organic molecules needed during stress environments are provided and synthesized by carbohydrates and also are the major source of energy (Siddiqui et al., 2019, 2020).

6.2.8 Antioxidant defense system

6.2.8.1 Antioxidative enzymes

Arsenic stress cause oxidative stress by damaging the ETC in cellular organelles (Nahar et al., 2022). Enzymes such as SOD, CAT, GR, APX, GPX, MDHAR, DHAR, GST and POD perform ROS scavenging (Das & Roychoudhury, 2014). Each antioxidative enzyme performs a unique function to balance ROS level which includes i) removal of $O_2^{\bullet-}$ radicals by SOD; ii) CAT mediated transformation of H_2O_2 into H_2O and O_2; iii) role of POD in H_2O_2 scavenging in the extra-cellular space; iv) GST performs glutathione conjugation to electrophilic or hydrophobic compounds; v) APX scavenge H_2O_2 to H_2O and vi) DHAR and MDHAR induced maintenance of ascorbate pool; and (Rajput et al., 2021). Growth of plants gets hampered under As stress by directly binding to the thiol groups of antioxidant enzymes (Sharma, 2012). Our results showed diminished levels of SOD, CAT, GR, POD APX, GPOX, MDHAR, DHAR, GST and PPO enzymes under As stress. Similarly, decrease in activities of GR and GPOX enzymes were described by Bianucci et al. (2017).

In this particular study, Si and NO treatment escalated the functioning of various antioxidative enzymes. Similar results were noticed in *B. juncea* (Pandey et al., 2016) under As stress, mustard (Abd_Allah et al., 2019) under Ni stress, *Oryza sativa* (Geng et al., 2018) under As stress, *Triticum aestivum* (Xuebin et al., 2020) under Cd stress and in *Oryza sativa* (Hasanuzzaman et al., 2019) under Ni toxicity when supplied with Si. Supplementation with SNP resulted in increased functioning of APX, GR, DHAR, and POX enzymes in *Phaseolus vulgaris* (Talukdar, 2013a). A similar SNP-mediated increase in CAT, POX and APX enzymes was indicated by Farnese et al. (2013) in lettuce that were exposed to As. These outcomes suggest that NO acts as a signaling moiety by augmenting the functioning of antioxidative enzymes. The up-regulation of antioxidant enzyme coding genes may be responsible for the stimulated action of defense system by NO as seen in our study (Ahmad et al., 2016). Ahmad et al. (2016) suggested that plant metabolism may have been protected by an increase in the functioning of GR and APX enzymes by the application of NO via escalating the enzymes associated with the ascorbate-glutathione pathway and GSH/GSSH ratio. Production of highly toxic $OH^{\cdot}$ radicals are prevented by CAT whereas the formation of superoxide radicals is reduced through increase in the GR activity (Ahmad et al.,

2010). Functioning of SOD and POD enzymes was enhanced in NO supplemented tomato plants which ultimately mitigated the boron-induced oxidative stress (Kaya & Ashraf, 2015). CAT enzyme involves in the neutralization of H_2O_2 in the cytoplasm or ascorbate-glutathione cycle in the chloroplasts while $O_2^{-\cdot}$ radicals are detoxified by the SOD enzyme.

Talukdar (2013b), Yadav and Srivastava (2015) and Siddiqui et al. (2015b) reported a similar increase in the antioxidant functioning in fenugreek, *Zea mays* and *Ocimum tenuiflorum*, respectively under As stress. Up-regulation in the expression of different SOD and APX isozymes exhibiting coordinated functioning with CAT is responsible for protection against As-induced oxidative damage (Talukdar, 2013b). Inhibition of toxic $OH^{\cdot}$ radical production and protection of structure and functioning of enzymes resulted in enhanced cellular functioning which might be attributed to the augmented functioning of APX, DHAR, MDHAR and GR enzymes (Mittler, 2017). Ahmad et al. (2018, 2020b) indicated that NO upsurged the functioning of these enzymes under Cd and As toxicity in tomato and faba bean, respectively. Metal detoxification is facilitated by redox status adjustment and antioxidative machinery (Zeng et al., 2011). Level of ROS in plants were reduced in the present study due to Si and NO-mediated improvement in the antioxidative system upon exposure to As toxicity. Mitigation of As stress via Si and NO application may be due to the following mechanisms a) decline in the metal accumulation in the root by influencing root cell wall organization b) reducing the oxidative damage by ROS scavenging and c) maintaining the functioning of stress-associated genes. Arsenic phytotoxicity can be mitigated by stimulation of antioxidative enzymes and the glyoxalase system (Ghorbani et al., 2021b). Therefore, our findings confirm that Si and NO application effectively strengthened the immune response by boosting the activity of antioxidative enzymes against As phytotoxicity.

6.2.8.2 Non-enzymatic antioxidants

Non-enzymatic antioxidants like glutathione show a significant effect in improving plant tolerance and metal detoxification of heavy metals. Membrane structure within the cells is stabilized by glutathione and ascorbic acid as both of these exhibit reductant properties. Non-enzymatic antioxidants directly scavenge $OH^{\cdot}$ radicals (Foyer & Noctor, 2011). In our research, Si, NO and Si + NO treatments alone and together under As stress upsurged the amount of these antioxidants. Similarly, Si showed improvement

in the contents of antioxidants under As stress as reported by Kaya and Ashraf (2022) in tomato, Das et al. (2018) in rice, González-Moscoso et al. (2019) in tomato and Sil and Biswas (2020) in wheat. Similarly, Singh et al. (2021b) in soybean, Asadi Karam et al. (2017) in coriander and Ahmad et al. (2020b) in *Vicia faba* also reported NO-mediated upsurge in the levels of antioxidants under As pollution.

6.2.9 Gene expression

Our study showed that expression of SOD and CAT genes was stimulated under stressed environments. Treatment with Si and NO further improved their expression. Khandekar and Leisner (2011) displayed that in *Arabidopsis thaliana*, Si elevated the SOD gene level under Cu stress. Si mediated improved tolerance of rice seedlings to Zn toxicity might be due to the enhanced expression of photosynthetic related genes (Song et al., 2014). Similarly, application of Si regulated the expression of polyamine associated genes to alleviate the Cd stress in *T. aestivum* (Howladar et al., 2018). Similarly, antioxidative genes were stimulated by NO in Ni stressed rice (Rizwan et al., 2018). Exogenous application of NO mitigated the As toxicity in *B. juncea* plants by regulating the expression of nitrogen and PIN genes (Praveen et al., 2019). Moreover, Si and NO also improved the expression of the SOD gene under Cu stress in *Salvia officinalis* (Pirooz et al., 2021). Nitric oxide-mediated metal detoxification might be due to the upregulation of antioxidant enzymes to facilitate the gene expression. Moreover, Si and NO-mediated increase in the expression of SOD and CAT genes of *R. sativus* is also significant for reducing lipid peroxidation levels to improve membrane integrity by increasing H_2O_2 scavenging. The findings of the present research suggest that Si-facilitated upsurge in antioxidative enzymes might be modulated through NO-dependent escalation in performance of antioxidant enzymes and gene expression.

Chapter 7 Summary and Conclusions

There is a need of reducing As toxicity in radish by the use of effective and eco-friendly substances. Therefore, this research work was conducted to evaluate the influence of Si and NO in ameliorating As toxicity in *Raphanus sativus*. In the present study, length, biomass and vigor index were diminished under As stress. However, Si and NO treatment improved these attributes in seedlings and plants under As stress. Relative water content was lowered under As stress but increased by Si and NO application. Contents of photosynthetic pigments i.e. chlorophyll, carotenoid and xanthophylls were noticed to be declined under As toxicity. The highest reduction in photosynthetic pigments was at 0.7 mM concentration. Further, Si and NO application led to increased contents of these photosynthetic pigments. As stress also caused a reduction in the gas exchange attributes. But, the application of Si and NO enhanced these gas exchange attributes.

Contents of metabolites (anthocyanin, flavonoids, and phenolics) were recorded to be decreased in As exposed seedlings and plants of *R. sativus*. However, Si and NO led to an upsurge in the contents of anthocyanin, flavonoids and phenolics. Supplementation with Si and NO also showed better results in increasing metabolite content as compared to their individual applications. Arsenic stress elevated the level of MDA and H_2O_2. Maximum elevation in the MDA and H_2O_2 were at 0.7 mM concentration. However, Si and NO reduced their level to diminish oxidative stress. High membrane and nuclear damages were seen in As treated seedlings, while treatment with Si and NO resulted in reduction of membrane and nuclear damages.

High As content was noticed in As-treated seedlings and plants. Roots showed higher As contents as compared to shoot tissues. However, As content was declined in Si and NO-treated seedlings and plants. Contents of osmolytes were diminished in As stressed plants. The highest reduction in the osmolytes contents was at 0.7 mM concentration. Silicon and NO treatment increased their contents under stressed conditions. Contents of total carbohydrates and proteins were noticed to be declined under As stress. However, supplementation of Si and NO led to elevation in total carbohydrates and proteins amount in seedlings and plants of *R. sativus*. Activities of antioxidative enzymes were decreased in As-treated seedlings and plants. However, application of Si and NO under stressed conditions increased their activities. It was observed that contents of non-enzymatic antioxidants were reduced under As toxicity. Whereas,

application with Si and NO increased their contents in seedlings and plants under stressed conditions. Gene expression of SOD and CAT genes were upregulated under As stress. However, application of mitigants in individual plus combined manner further increased their expressions under stressed conditions.

Overall, it was concluded from the present study that use of Si and NO is an advantageous strategy to alleviate the As-induced toxic effects in *Raphanus sativus* by improving their morphological, physicochemical and molecular aspects.

Chapter 8
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Appendices

List of Publications

1. **Bhardwaj, S.,** Verma, T., Kaur, J., Singh, A.D., Bhardwaj, R., Sharma, N.R., Ansari, S., Raza, A., Vara Prasad, P.V., Thakur, U., & Kapoor, D. (2024). Silicon and nitric oxide modulate growth attributes, antioxidant defense system and osmolytes accumulation in radish (*Raphanus sativus* L.) under arsenic toxicity. *Plant stress, 12, 100473.*
2. **Bhardwaj, S.,** Verma, T., Raza, A., & Kapoor, D. (2023). 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, 92*(3), 763-782.

List of Conferences Attended

1. Oral presentation on the title "Alleviation of Arsenic stress in radish plants by the application of Silicon and Nitric oxide" 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 "Silicon and nitric oxide mediated mechanism of arsenic alleviation in radish seedlings" at "**International Conference on Sustainability: Life on Earth 2021"**, 17-18 December 2021, organized by Lovely Professional University, Phagwara, Punjab
3. Poster presentation on the title "Role of Indole-3-Acetic Acid in the Alleviation of Lead Induced Phytotoxicity in *Silybum marianum* (L.)" at e- International Conference on **Plant Biodiversity and Environment (ICPBEC-2021)** held at Hansraj Mahila Maha Vidyalaya, Jalandhar in collaboration with Punjab Biodiversity Board (20-21 May, 2021).
4. Poster presentation entitled "Alleviation of heavy metal induced toxicity in *Psoralea corylifolia* by exogenous application of PGR" at National conference on **"Microbial Bioprospecting: Present and future scope**" held on 6^{th}-7^{th} March, 2020 organized by Association of Microbiologist of India- LPU unit, at Lovely Professional University, Punjab.