SYNOPSIS

Dissertation Report

(AGR 596)

"Putrescine and *Glomus* Mycorrhiza Mitigate Salinity Induced Stress Responses in Sorghum (SSV-74)"

Submitted To

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UNDER GUIDANCE OF

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CERTIFICATE

This is to certified that the synopsis entitled **"Putrescine and *Glomus* Mycorrhiza Mitigate Salinity Induced Stress Responses in Sorghum (SSV-74)"** submitted in partial fulfillment of requirements for degree of Master of Science (M.Sc.) in Agronomy by **Mandala Harshavardhan** to Department of Agronomy School of Agriculture, Lovely Professional University, has been formulated and finalized by the student himself on the subject.

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DECLARATION

I hereby declare that the project work entitled **"Putrescine and *Glomus* Mycorrhiza Mitigate Salinity Induced Stress Responses in Sorghum (SSV-74)** is an authentic record of my work carried at **Lovely Professional University** as requirements of project work for the award of degree of Master of Science in Agronomy, under the guidance of **Dr. Prasann kumar**, Assistant Professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.

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INTRODUCTION

Salinity is the saltiness or amount of salt dissolved in a body of water (see also soil salinity). This is usually measured in *g*salt/*kg*sea water (note that this is technically dimensionless). Salinity is an important factor in determining many aspects of the chemistry of natural waters and of biological processes within it, and is a thermodynamic state variable that, along with temperature and pressure, governs physical characteristics like the density and heat capacity of the water. Usually, salinity is measured in units of electrical conductivity (EC_e) of a standard soil paste extract taken from the root zone of the plant. Electrical conductivities are measured on the filtered water extracts from these samples in units of deciSemiens per meter (dS m^{-1}). The soils with ECe<4 dS m^{-1} are considered as non-saline; those with ECe=4 to 16 dS m^{-1} are considered as in saline-phase and those with ECe>16 dS m^{-1} are considered as saline (www.cahe.nmsu.edu). Sorghum (*Sorghum vulgare* L.) belongs to grass family Graminae. It is a short-day C4 plant. The optimum photoperiod which will induce the flower formation is between 10 and 11 hours. Sorghum is one of the main stale foods for the world's poorest and most food-unsecured people across the semi-arid tropics. Globally, sorghum is cultivated on 41 million hectares to produce 64.20 million tonnes, with productivity having around 1.60 tonnes per hectare (DSR annual report, 2010-11, Directorate of Sorghum Research, Hyderabad). India contributes about 16% of the world's sorghum production (DSR annual report, 2010-11, Directorate of Sorghum Research, Hyderabad). It is the fifth most important cereal crop in the country. In India, this crop was one of the major staple cereals during 1950's and occupied an area of more than 18 million hectares but has come down to 7.69 million hectare (DSR annual report, 2010-11, Directorate of Sorghum Research, Hyderabad). Heavy metal contamination is of special concern due to widespread reports emanating both from India and abroad various disease and disorders observed both in human and livestock due to metal toxicity. Sorghum crop responses to salinity depends on several factors such as soil, environment and genetic and their interrelationships. Plants grown in high saline soils experience low water potential and mineral toxicity due to high Na^+ concentration in external solution of plant cells. Plants challenged by this water potential develop a large soil-to-leaf gradient of potentials and are unable to meet transpiration demand, and ultimately may wilt and desiccate. Water stress is therefore considered a component of salinity injury. Na^+ toxicity results in disturbances in the mineral nutrition of the plant. The cell metabolism is seriously affected when cytoplasm Na^+ concentration reaches 50 to 100 Mm . However, under natural conditions, such high salinity level is not experienced by plants at once during the crop growth period. Instead, salinity level increases progressively due to soil drying, which provides ample time for osmotic adjustment of plants to regulate their water uptake. In controlled conditions also, plants adjust osmotically in response to salinity stress, and therefore may overcome the osmotic effect of salt within a few days and symptoms of wilting disappear .The water stress and mineral toxicity induced by soil salinity reduces photosynthesis per unit leaf area indirectly through stomatal closure, and to a smaller extent through direct interference with the photosynthetic apparatus . At whole plant level, soil salinity stress results in delayed flowering, reduced plant height and grain and fodder yields in sorghum, although the degree of these responses varies with the genetic background of the lines . However, grain yield may not decrease until a 'threshold' salinity level is reached. The comprehensive survey of responses of different crop species to salinity stress indicated that 'threshold' level of grain

sorghum is 6.8 dS m^{-1} and that grain yield starts declining at 6.8 dS m^{-1} and the reduction are up to 25% at 7 dS m^{-1} and 50% at 10 dS m^{-1}. A polyamine is an organic compound having two or more primary amino groups-NH2. They are also known as a group of natural compounds with an aliphatic nitrogen structure present in almost all living organisms. It plays an important role in many physiological processes such as cell growth and development. Polyamines which are commonly found are putrescine. Spermidine, spermine etc. the diamine putrescine, the triamine spermidine and the tetramine spermine are ubiquitously found in plant cells while other polyamines are of more limited occurrence. In animals, their levels are maintained from both the diet and denovo synthesis. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase. Polyamine can also be synthesized from the aminoacid arginine and methionine. The first step in the pathway is the production of ornithine from arginine by the mitochondrial enzyme arginase. Ornithine is then decarboxylated by ornithine decarboxylase to produce putrescine. Polyamine is found in high concentration in the mammalian brain. The polyamines declines with the ages in the organism. Mycorrhiza is a fungus having a symbiotic or mutualistic relationship with the rhizosphere or roots of the plants. They share a mutualistic relationship in which the mycorrhiza forms a network of filaments that associates with the plant roots which helps them in uptaking the mineral nutrients or water while the mycorrhiza are benefitted by getting access to carbohydrates such as glucose and sucrose. The carbohydrates are translocated from their source to root tissues and onto the plant fungal partners. Some plant roots may be unable to uptake nutrients that are chemically or physically immobilized. For example, phosphate ions and micronutrients such as ions. Polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes (Farooq *et al*., 2009). They are the recent additions to the class of plant growth regulators, and also considered as a secondary messenger in signaling pathways (Kusano *et al*., 2008). Polyamines are involved in abiotic stress tolerance in plants (Nayyer *et al*., 2005). Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss, and stimulation of protein, nucleic acid and protective alkaloids (Sharma, 1999). Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions (Roberts *et al*., 1986). Polyamines also protect membranes from oxidative damages as they as free radical scavengers (Besford *et al*., 1993).

HYPOTHESIS

For the present study, I select sorghum plant, because

[A] The research of the effect of the salinity on plant mainly focuses on food crops such as rice, wheat and maize, but less on sorghum plants;

[B] Sorghum which is often using as feed sources and quality assurance particular in concern with salinity is not known that's why evidence of accects of salinity in sorghum is still a point of research.

[C] Level of sensitivity of sorghum for salinity is not known; by conducting this type of experiment, I will be able to detect level of sensitivity which will be beneficial for plant breeders for development of salinity tolerant sorghum.

OBJECTIVES

The objectives of my work are to study:

1. The effect of salinity stress on various morphological, biochemical and yield attributes in sorghum.

2. The effect of polyamines (putrescine) and mycorrhiza in ameliorating salinity induced stress in sorghum.

3. The scavenging capacity of sorghum plant for salinity present in the soil.

REVIEW OF LITERATURE

Soil salinity among others is an important abiotic constraint for crop productivity in semi-arid tropics (SAT) of the world, where sorghum is cultivated in vast areas for food/feed/fodder uses. Saline soils are those soils with higher levels of soluble salts, such as sulfates (SO_4), carbonates (CO_3) and chlorides (Cl). These soils often exhibit a whitish surface crust when dry (www.cahe.nmsu.edu). The increased demand for sorghum, especially for feed/fodder uses driven by enhanced demand for milk and milk products in SAT regions imposes extension of sorghum cultivation in saline soils.

Keeping in view the objectives of the study,an attempt was made in this chapter to review the available literature which are directly or indirectly related to the study and are presented under the following sub-headings.

Dagar;J.C. (2005) The state-of-the-art of salinity management in India and the latest developments in salinity research have been discussed in this paper. Special issues related to databases to document the extent of land degradation due to salinity, vegetation of saline habitats, salinity development and management, poor quality water management, agroforestry and arable crops suited to saline environment have been included. This paper deals with the main thrust areas of research with achievements and research gaps, some broad issues to be tackled, the challenges ahead and the issues, which will need our research in next 2-3 decades.

Reddy;B.V.S *et al.* (2007) The increased demand for sorghum (one of the important food/fodder/feed crops in the semi-arid tropics) driven by enhanced demand for milk and milk products imposes extension of sorghum cultivation in saline soils, which severely limits crop productivity. The development and adoption of cultivars tolerant to salinity is the cost-effective approach to enhance sorghum productivity in saline soils. However, attempts to breed sorghum for salinity tolerance are limited owing to the complexity in screening for and inheritance of salinity tolerance. In this article we have reviewed and discussed the screening methods and selection criteria used to breed sorghum for salinity tolerance.

Kafi;M *et al.* (2011) Salinity stress during early growth and panicle differentiation declined the plant height and tiller number. The highest biological yield was obtained from the control treatment, but it was the lowest when plants were salinized throughout the growing season. When plants were stress-free at 2-3 early stages and then subjected to salt stress, reductions in total dry matter were remarkably less than those experienced when salinity was imposed in later growth stages, especially if salinity occurred at a late individual stage. Continuation of salt stress from emergence to both blooming and soft dough stages led to remarkably adverse effects on grain yield. The effect of salinity appears to be most effective on

yield components that are growing or developing at the time the salt stress is imposed. The critical period of salinity stress for biological yield was more distinct than that of the grain yield.

Gupta.B and Bingru.H (2014) Salinity is a major abiotic stress limiting growth and productivity of plants in many areas of the world due to increasing use of poor quality of water for irrigation and soil salinization. Recent research has identified various adaptive responses to salinity stress at molecular, cellular, metabolic, and physiological levels, although mechanisms underlying salinity tolerance are far from being completely understood.

METHODOLOGY

The pot experiment will be conducted in the poly house of the School of Agriculture, Lovely Professional University, Punjab, India with one genotype of sorghum SSV 74. Sorghum genotype will be taken from Directorate of Sorghum Research, Hyderabad. Pot size for experiment will be diameter: 30 cm and height 25 cm. One best concentration after initial screening within the range of 1-100 ppm of sodium chloride will be finally selected. There is one concentration of sodium chloride (after screening), will be applied in soil for creating stress in sorghum plant. Putrescine will be applied at the rate of 1.50 mM and 3.0 mM through foliar application. Seed treatment with mycorrhiza (*Glomus* spp.) will be done. The various measurements will be made at three stages such as 30 DAS, 60 DAS, 90 DAS and 120 DAS.

EXPERIMENTAL DETAILS

1. Genotype: SSV-74

2. No. of treatment

a. Control

b. NaCl concentration 1

c. Endomycorrhizal fungi (AMF), *Glomus* species

d. Putrescine 1(1.50 mM)

e. Putrescine 2 (3.0 mM)

f. NaCl concentration 1 + Putrescine 1

g. NaCl 1 + Putrescine 2

h. NaCl 1 + Endomycorrhizal fungi (AMF), *Glomus* species

i. NaCl 1 + Putrescine 1(1.50 mM) + Endomycorrhizal fungi (AMF), Glomus species

j. NaCl 1 + Putrescine 2 (3.00 mM) + Endomycorrhizal fungi (AMF), *Glomus* species

3. Replication: Three

4. Treatment: 10

5. Total number of pots: 10 x 3= 30

6. Design: CRD

OBSERVATIONS TO BE RECORDED

The various measurements will be made at three stages such as 30 DAS, 60 DAS and 90 DAS.

1. Morpho-physiological parameters

a. Germination percentage

b. Plant Height (cm)

c. Leaf area (cm x cm)

d. Leaf number

e. Days to 50% anthesis

f. Days to maturity

g. Internodal length (cm)

h. Stem girth (cm)

i. Panicle length

2. Biochemical parameters

a. Chlorophyll content (Arnon, 1949)

b. Total soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

c. Total soluble protein (Bradford method, 1976)

d. Lipid peroxidation (Malondialdehyde-MDA) (Hodges *et al*., 1991)

e. Catalase (EC. 1.11.1.6) (Teranish *et al*., 1974)

f. Membrane stability (Sullivan method, 1972)

g. Phenylalanine ammonia lyase (EC. 4.3.1.5) (Brueske, 1980)

h. Superoxide dismutase (SOD) (EC. 1.15.1.1) (Dhindsa *et al*., 1981)

i. Transportable ratio (Using Atomic absorption spectrophotometer in different parts of plant including developing seeds).

3. Anatomical features

Transverse section of root to study mycorrhizal colonization (Microscopic conventional method)

4. Yield and quality attributes

1. Number of panicle
2. Number of grains per panicle
3. Test weight
4. Seed soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)
5. Seed total protein content (Bradford method, 1976)

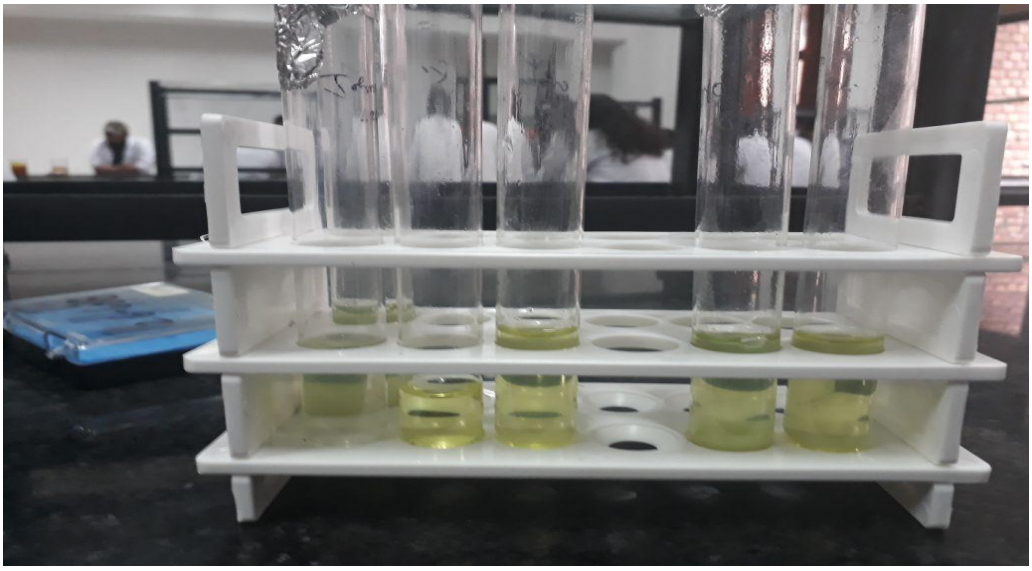
6.5 Screening test

Biochemical observation

a. Estimated the Total Soluble sugar (Anthrone method)(in Seeds)

S.No.	Blank reading	0.000
1	R1	0.824
2	R2	0.801
3	R3	0.863

Figure 1: Total Soluble Sugar



(Source: Photograph by M. Harsha vardhan,2018,unpublished)

b. Estimated the Total Soluble Protein (Bradford method)

S.No.	Blank reading	0.000
1.	R1	0.022
2.	R2	0.024
3.	R3	0.028

c. Estimated the Total Lipid (Sadashivam and Manickyam)

Weight of Petri plate(X1) -7.56g

Weight along with sample(X2) -12.01g

X2-X1=12.01-7.56=4.5g of fatty acids per 1g of Sorghum seed powder

Since Sorghum is a both Kharif and *Rabi* season crop during this semester I have done a screening test for determination of lethal concentration of Salinity in laboratory by using Sodium chloride(NaCl) in small cups with different concentrations from 10 ppm to 100 ppm.

d. Seed germination

Number of germinated seeds

S.No.	NaCl(ppm)	DAS-4	DAS-5	DAS-6	DAS-7
1.	**Control**	3	5	5	5
2.	**10**	1	2	3	3
3.	**20**	1	3	3	5
4.	**30**	1	1	3	4
5.	**40**	2	2	3	4
6.	**50**	1	1	3	5
7.	**60**	1	3	3	3
8.	**70**	1	1	2	5
9.	**80**	1	2	4	5
10.	**90**	0	2	3	5
11.	**100**	0	1	3	4

e. Morphological characteristics

Sl. No.	Concentration (ppm)	Plant height(cm)	Leaves number
1.	**Control**	14	3
2.	**10**	2.1	3
3.	**20**	26	3
4.	**30**	22.3	3
5.	**40**	21.5	3
6.	**50**	18	3
7.	**60**	22	3
8.	**70**	25.1	3
9.	**80**	27.3	3
10	**90**	22	3
11.	**100**	23.6	3

Figure2: Morphological characters

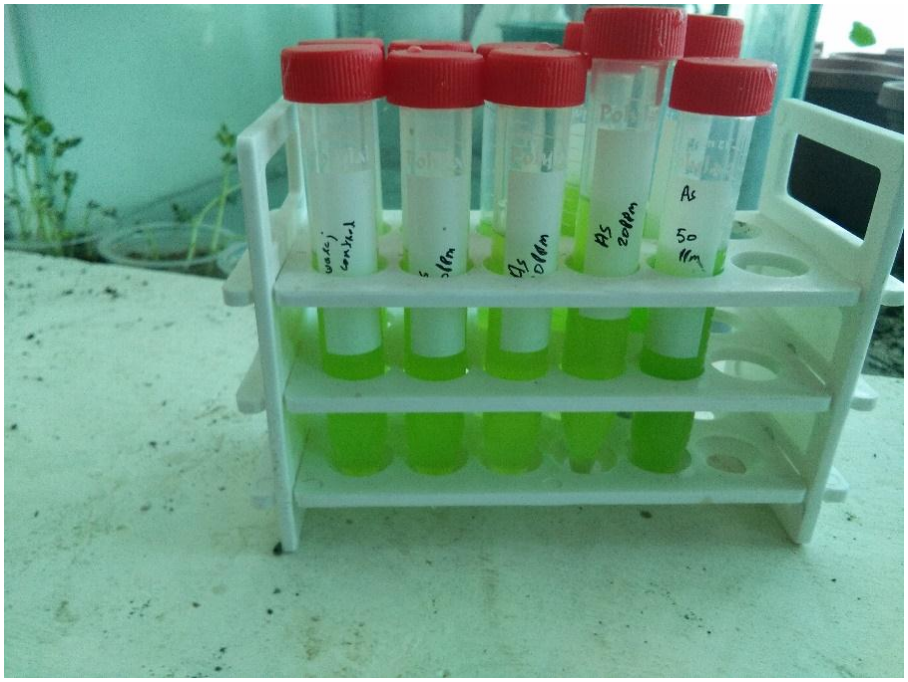
(Source: Photograph by M. Harsha vardhan, 2018, unpublished)

f. Estimated the chlorophyll content by Arnon DI method.

Chlorophyll content (mg/g fresh weight):

TREATMENTS (NaCl)(ppm)	OPTICAL DENSITY					
	663nm	645nm	410nm	480nm	510nm	590nm
Control	0.636	0.643	0.638	0.386	0.071	0.081
10	0.423	0.487	0.456	0.439	0.478	0.431
20	0.659	0.657	0.791	0.451	0.084	0.099
30	0.610	0.581	0.882	0.387	0.060	0.089
40	0.684	0.654	0.755	0.426	0.070	0.096
50	0.689	0.653	0.823	0.437	0.069	0.093
60	0.587	0.564	0.765	0.385	0.075	0.078
70	0.583	0.563	0.684	0.356	0.046	0.078
80	0.695	0.722	0.903	0.439	0.074	0.106
90	0.704	0.695	0.859	0.461	0.088	0.100
100	0.868	0.864	1.026	0.562	0.093	0.129
Reference	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3: Estimation of Chlorophyll



(Source: Photograph by M. Harsha vardhan, 2018, unpublished)

PLAN OF WORK

1st Year

(a) Morpho-physiological parameters

(b) Determination of biochemical parameters

(c) Anatomical features

(d) Yield and quality attributes

2nd Year

The experiment conducted in 1st year will be repeated during the IInd year.

CONCLUSION

Higher the concentration of the Salinity more is the deteriortation effect it has shown. At100ppm concentration of NaCl the plant showed less germination as well as the chlorophyll content measured were seen to be low in 90ppm and 100ppm compared to other NaCl concentration to other NaCl concentration. So the experiment will be conducted using 100ppm in pot cultivation.

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