

Arbuscular Mycorrhiza Fungi and Polyamines in Mitigation of Rhizosphere Salts: With Special Reference to Leaf Pigmentation

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Abstract

The growth of plants is suppressed by salt-stressed soils. Crops grown in salt soil suffer from high osmotic stress and toxicity, malignancies in the soil and reduced crop productivity. Sorghum accumulates solutes and osmotically adjusts in response to developing water stress, apparently more so than maize. This would allow sorghum to maintain stomatal opening and carry on photosynthesis longer as the soil water depletes, and possibly also aid in delaying canopy senescence induced by water stress. The best mitigation effect in T8 treatments, increased chlorophyll by 61.84% at 60DAS, increased by 65.25% in T9 at 60DAS. Compared with T1, the indices of chlorophyll were significantly improved by 58.55 percent when treated with a higher dose of mycorrhiza T5 in 90DAS.

Keywords: Agriculture, Biotic, Crop, Dose, Economy, Forage, Sorghum

INTRODUCTION

Sorghum is moderately tolerant to salinity. As EC increased from 11 to 18 dS m⁻¹, grain yield was reduced from 50 percent to 100 percent [1, 2 and 3]. Much of the temperate effects on sorghum have already been discussed under Growth and Development. Leaf extension closely parallels air temperature to approximately 34 °C [4 and 5]. Pollination and fruit setting may fail when night temperatures fall below 12-15 °C at flowering, and pollens produced below 10°C and above 40 °C are most likely non-viable [6 and 7]. Sorghum grain contains around 1.5 percent nitrogen and 0.25 percent phosphorus. For a high yield of 8 tonnes, the grain alone removes 120 kg of N and 20 kg of P. To achieve this yield, fertilization must account also for the N and P in the stover residue and the efficiency of applied nutrients and native soil supply [8 and 9]. For water-limited situations, fertilization rates would be adjusted downward. In areas prone to terminal drought, care must be taken to avoid too much N supply early in the season because the resultant fast early growth would exhaust water stored in the soil and accentuate the terminal drought damage [10 and 11].

Saline soil is usually well-defined to exceed 4 dS m⁻¹ (approximately 40 mM NaCl) at 25°C and to have exchangeable sodium of 15 percent in the area of the root (ECe) in electrically-driven conductivity (EC) [12 and 13]. In this ECe, the output of most crop plants is reduced but many crops have lower ECes. The global figure is estimated to be high in salinity, at 20% of total crops and 33% in irrigated farmlands [18 and 19]. Besides, salinized areas rise by 10% a year of different geographical areas, counting squat precipitation, high surface development, indigenous rock weathering, saltwater irrigation, and poor culture. More than 50% of the arable land is estimated to be salinized by 2050 [15 and 16]. It has been estimated.

Soil salinity is an enormous problem for irrigated agriculture [18, 19 and 20]. In the warm and dry parts of

the world, soils are often saline with low agricultural potential. In these areas, most plants are irrigated, which leads to secondary salinization of 20 percent of irrigated soil worldwide to exacerbate the problem [21, 22 and 23]. In many countries, the salinization of nearly 1 billion ha worldwide, which represent approximately 7 percent of the continental extent of the world, is recognized as the main threats to the environment or health of humans, approximately ten times the size of a nation like Venezuela or 20 times the size of France [24, 25, 26 and 27]. Approximately 7 million hectares of soil in India has been estimated to cover saline soil. Most of them occur in the soil of Punjab, Haryana, U.P. Bihar and certain parts of Rajasthan. Also, salt-land areas are largely affected by the arid tracts of Gujarat, Rajasthan and semi-Arid tracts of Gujarat, Madhya Pradesh, Maharashtra, Karnataka, and Andhra Pradesh. Salinity affects nearly every aspect of plant development including germination, vegetative growth, and breeding. Soil salinity places ion toxicities and osmotic stress on plants, and thus restricts water intake from the soil. Nutrient deficiency (N, Ca, K, P, Fe, Zn) and oxidative stress on plants. Soil salinity reduces phosphorus plant uptake (P) significantly because Ca ions precipitate phosphate ions. All of these factors harm physiological and biochemical plant growth and development.

The world's major cereal after rice, wheat, maize, and barley is sorghum [*Sorghum bicolor* (L.) Moench]. It is India's third-largest basic food grain after rice and wheat in the semiarid Tropics (SAT) [1, 2 and 3] for millions of poor and most food-insecure individuals. Where in India it is commonly called *jwaarie*, *jowar*, *jola*, or *jondhalaa*, sorghum is one of the staple sources of nutrition. An Indian bread called *bhakri*, *jowar roti*, or *jolada rotti*, is prepared from this grain [4, 5, and 6]. While the rainy-season sorghum grain is used mostly for animal/poultry feed, post-rainy season produce is used for human

consumption. In SAT India, sorghum is truly a dual-purpose crop; both grain and stover are highly valued outputs. In large parts of SAT India, sorghum stover represents up to 50% of the total value of the crop, especially in drought years [7, 8, 9 and 10]. Sorghum also offers great potential to supplement the fodder requirement of the growing dairy industry in India because of its wide adaptation, rapid growth and high green-fodder yields as well as good quality [11, 12, and 13]. The leading producers of sorghum bicolor in 2011 were Nigeria (12.6%), India (11.2%), Mexico (11.2%), and the United States (10.0%). Sorghum grows in a wide range of temperatures, high altitudes, and toxic soils. It has four features that make it one of the most drought-resistant crops i.e. very large root-to-leaf surface area ratio, at the time of drought it will roll its leaves to lessen water loss by transpiration, in drought conditions, it will go into dormancy rather than dying and leaves are protected by a waxy cuticle [14, 15, 16 and 17]. In India, rainy season (*Kharif*) sorghum is sown between the second week of June and the first week of July, with rains of the southwest monsoon. However, sorghums are prone to fungal attacks leading to grain mold if late-season rains occur during grain maturity. Post-rainy season (*Rabi*) sorghum is sown generally from the last week of September to the second week of October and is generally exposed to low winter temperatures at sowing resulting in low germination and poor stand establishment. Late sown *Rabi* season crops are exposed to terminal drought when grown on black soils (Vertisols) with stored soil moisture and are prone to disease such as charcoal rot [18, 19, 20 and 21]. Sweet sorghum is special-purpose sorghum with a sugar-rich stalk, almost like sugarcane. Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability [22, 23, 24 and 25].

As a C₄ crop, sorghum does not tolerate cool temperature regimes. For seed germination, the minimum temperature is about 8°C, and optimum temperature, 21–35°C [26, 27, 28, 29 and 30]. Under field conditions, minimum soil temperature in the range of 15–18°C is required for 80 percent emergence in 10-12 days. Normally in the field emergence takes 5–10 days. Panicle initiation takes place after approximately one-third of the growth cycle after the last leaf has initiated and about one-third of the total leaf area has developed. Rapid leaf development and stem elongation follow panicle initiation. The rapid growth of the panicle starts after all but the last two or three leaves emerge. By the time the flag leaf is visible, all but the final 3 to 4 leaves are fully expanded and light interception is approaching its maximum; a few lower leaves may begin to senesce if nitrogen is not plentiful or the crop is planted very densely [31 and 32]. Sorghum is considered to be drought resistant, especially in comparison to maize. A part of the perceived resistance may be because sorghum cultivars grown in water-limited areas are the short-season type, thus their water requirement is less than that of maize, a crop generally with a longer life cycle [27, 28 29 and 30]. If water stress is severe enough at flowering to cause head blast (death of a portion or whole head) tillers

may emerge from nodes high on the stem to form branch heads to produce grain and compensate for at least part of the loss, provided that harvest can be delayed [31 and 32]. Sorghum accumulates solutes and osmotically adjusts in response to developing water stress, apparently more so than maize [2, 3, 6, 7 and 9]. This would allow sorghum to maintain stomatal opening and carry on photosynthesis longer as the soil water depletes, and possibly also aid in delaying canopy senescence induced by water stress. In addition to stomatal closure, sorghum leaves roll noticeably under water stress, reducing the effective transpiration surface. The rolling is attributed to turgor changes in the rows of motor cells along the midrib and veins on the upper surface of the leaf. Motor cells are also present in maize leaves, but maize leaves roll only minimally under water stress. Leaf growth by expansion is highly sensitive to water stress in sorghum [11, 12 13 and 18 and 19].

MATERIALS AND METHODS

This was the pot for the experiment with a 30 cm diameter and a 25 cm height and ten kg of soil each along with a small hole underneath it. Under the work plan, targeted pots with Endomycorrhiza have been inoculated. Salinity stresses created by the exogenous application of NaCl at the concentration of 8dsm⁻¹ per 10kg of soil. Putrescine was applied at the rate of 1.5 and 3.0 mM through the foliar spray at the fifteen days of interval. The various measurements were taken at three stages such as 30, 60, and 90DAS (Table 1 and 2).

Observation Recorded

The observations were recorded three stages such as 30, 60, and 90 DAS. The recorded observations of biochemical parameters and the standard procedure adopted during the study are given below:

Chlorophyll content (mg g⁻¹ fresh weight)

The chlorophyll content in the leaf of sorghum was estimated by the method of Arnon DI. (1949). Chlorophyll is extracted in 80% acetone and the absorbance is measured at 645 nm and 663 nm. The amount of chlorophyll is calculated using the absorbance coefficient.

Reagent

Acetone (80%, pre-chilled)

Procedure

Chlorophyll was extracted from 100 mg of the sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 10 min. The extraction was repeated until the residue became colourless. The volume in the flask was made up to 100 ml with 80% acetone. The absorbance of the extract was read using a spectrophotometer (Spectramax M2) at 645 and 663nm against 80% acetone blank. The amount of the chlorophyll content was calculated by using the formula as given below.

Chlorophyll 'a' (mg/g Fresh Weight) = 12.7(A663)-

$$2.69(A645) \times \frac{V}{1000 \times W}$$

Chlorophyll 'b' (mg/g Fresh Weight) = 22.9(A645)-

$$4.68(A663) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g Fresh Weight)} = 20.2(A645) + 8.02(A663) \times \frac{V}{1000 \times W}$$

where, V= final volume of the extract, W= fresh weight of the leaves, A=Absorbance at the specific wavelength, The value is expressed as the mg/g fresh weight

Table 1. Name of the Treatments and symbol used respectively

Treatments	Symbol Used For Respective Treatments
T0	Control
T1	NaCl (8 dsm ⁻¹)
T2	Endomycorrhizal fungi
T3	Putrescine (1.50 mM)
T4	Putrescine (3.0 mM)
T5	NaCl (8 dsm ⁻¹) + Endomycorrhizal fungi(150 spores per pot)
T6	NaCl (8 dsm ⁻¹) + Putrescine (1.5 mM)
T7	NaCl (8 dsm ⁻¹) + Putrescine (3.0 mM)
T8	NaCl (8 dsm ⁻¹)+Putrescine (1.50 mM) + Endomycorrhizal fungi (AMF)(150 spores per pot);
T9	NaCl(8dsm ⁻¹) + Putrescine(3.00mM) + Endomycorrhizal fungi (AMF)(150 spores per pot)

Table 2. Layout Details

Sl. No	Particulars	Action
1.	Layout	CRD
2.	Treatments	10
3.	Replications	3
4.	Total number of pots	30
5.	Soil per pot	15kg
6.	Genotype	SSV74

RESULTS AND DISCUSSION

Chlorophyll a

In a sorghum variant SSV74 under the stress of salinity, the effect of putrescine and mycorrhiza and the combination of these on chlorophyll was studied. The data was registered at the time of sowing (DAS) 30, 60 and 90 days (Table 3 & Fig. a). It is obvious that the mean salinity stress (T1) compared to control (T0), was reduced significantly by 32.0% by 26.83 percent and 27.69% at intervals 30, 60 and 90 DAS. The mitigation effect was demonstrated by exogenous application of endomycorrhiza in the earth (T2) by increasing the amount of chlorophyll by 42.94 percent, 4.14 and 4.14 percent compared with T1 by 30, 60 and 90 DAS. T3 compared to T1 was significantly higher in chlorophyll by 20.01%, 16.90%, and 15.53% in proposed DAS, when compared. In comparison to T1 exogenous use of putrescine (T4), chlorophyll has increased by 24.94%, 13.23%, 13.98%, and the proposed DAS, which has an attenuating effect. The average chlorophyll-a in the treatment of a higher dose of mycorrhiza has been significantly improved compared to T1, by 44.66 percent, 59.39%, and 41.75%. Similarly, chlorophyll increased substantially by 16.07%, 15.99% and 21.97% at proposed DAS, when comparing T6 with T1. Compared to T1, average chlorophyll a was increased by 33.14%, 19.08%, and 20.19% when treated with a high dosage of putrescine (T7). The combination of

putrescine and mycorrhiza has shown the best mitigation effect in T8, with chlorophyll increasing by 52.15%, 61.84%, and 44.51% for T1 in the proposed DAS. When treatment T9 was compared with treatment T1 then significant chlorophyll a was increased by 54.47%, 54.60%, and 50.20%, respectively. Based on the above results obtained, it was found that the combined application of mycorrhiza and putrescine showed the best mitigation effect in crops concerning salinity stress.

Table 3. Chlorophyll a of Sorghum during Rabi

Treatments	Chlorophyll a (30 DAS)	Chlorophyll a (60 DAS)	Chlorophyll a (90 DAS)
T0	4.008 ^c ±0.292	5.022 ^c ±0.037	4.866 ^c ±0.037
T1	2.722 ^d ±0.011	3.675 ^e ±0.019	3.519 ^e ±0.019
T2	4.771 ^b ±0.019	3.827 ^e ±0.118	3.671 ^e ±0.118
T3	3.403 ^{de} ±0.152	4.322 ^d ±0.128	4.166 ^d ±0.128
T4	3.627 ^d ±0.013	4.247 ^d ±0.256	4.091 ^d ±0.256
T5	4.919 ^b ±0.013	6.197 ^b ±0.084	6.041 ^b ±0.084
T6	3.243 ^e ±0.057	4.666 ^{cd} ±0.031	4.510 ^{cd} ±0.031
T7	4.072 ^c ±0.047	4.565 ^d ±0.144	4.409 ^d ±0.144
T8	5.689 ^a ±0.015	6.498 ^b ±0.155	6.342 ^b ±0.155
T9	5.979 ^a ±0.016	7.224 ^a ±0.205	7.068 ^a ±0.205

Table 4. Chlorophyll b of Sorghum during Rabi

Treatments	Chlorophyll b (30 DAS)	Chlorophyll b (60 DAS)	Chlorophyll b (90 DAS)
T0	0.342 ^{bcd} ±0.150	3.350 ^a ±0.105	3.140 ^a ±0.105
T1	0.881 ^{abcd} ±0.118	2.060 ^c ±0.123	1.850 ^c ±0.123
T2	0.129 ^e ±0.063	1.238 ^c ±0.067	1.028 ^c ±0.067
T3	0.497 ^{abcd} ±0.031	1.313 ^e ±0.047	1.103 ^c ±0.047
T4	0.174 ^{cd} ±0.165	1.722 ^d ±0.074	1.512 ^d ±0.074
T5	1.069 ^{ab} ±0.101	1.764 ^d ±0.105	1.554 ^d ±0.105
T6	0.989 ^{abc} ±0.692	0.303 ^g ±0.027	0.093 ^g ±0.027
T7	0.743 ^{abcd} ±0.246	0.709 ^f ±0.056	0.499 ^f ±0.056
T8	0.487 ^{abcd} ±0.038	2.717 ^b ±0.025	2.507 ^b ±0.025
T9	0.342 ^{bcd} ±0.150	3.404 ^a ±0.052	3.194 ^a ±0.052

Chlorophyll b

The impact and combinations of putrescine and mycorrhizae on chlorophyll b have been studied in the salinity stress of sorghum SSV74. Thirty, 60 and 90 days of sowing (DAS) data was collected (Table 4 & Fig. b). The average chlorophyll b was significantly decreased at intervals 30 60 and 90 DAS by 61.23%, 38.0% and 41.08% when exposed to the stress of salinity (T1) when compared with control (T0). The mitigation effect has been shown by exogenous application to soil of endomycorrhiza (T2), which increased chlorophyll b by 45.35%, 39.88% and 44.41% in comparative terms to T1 in 30-, 60- and 90 DAS. When compared with T1, T3 increased substantially by 43.63%, 36.24%, and 40.36% at proposed DAS when compared with T1. Compared with T1, the exogenous use of putrescine (T4) demonstrated a decreasing effect by 40.28%, 16.39% and 18.25% on the proposed DAS by increasing chlorophyll-b. The average of chlorophyll b in treatments with higher doses of mycorrhiza (T5) has been significantly increased compared with T1 by 17.58%, 14.38%, and 16.01%. In comparison with T1, T6 also significantly increased chlorophyll b with 10.89%, 18.29%, and 19.97% with the

proposed DAS when compared with T1. The average chlorophyll b has been significantly increased with the higher dose of putrescine (T7) compared with T1 by 15.66%, 15.58%, and 17.02%. The combination of putrescine with mycorrhiza showed the best effect of mitigation in T8 by an increase of 44.68%, 31.87% and 35.49% in T1 in proposed DAS treatment. In comparison

with T1, T9 was then significantly increased in chlorophyll b by 44.98%, by 65.25% and by 72.66%, respectively. Based on the above results obtained, it was found that the combined application of mycorrhiza and putrescine showed the best mitigation effect in crops concerning salinity stress.

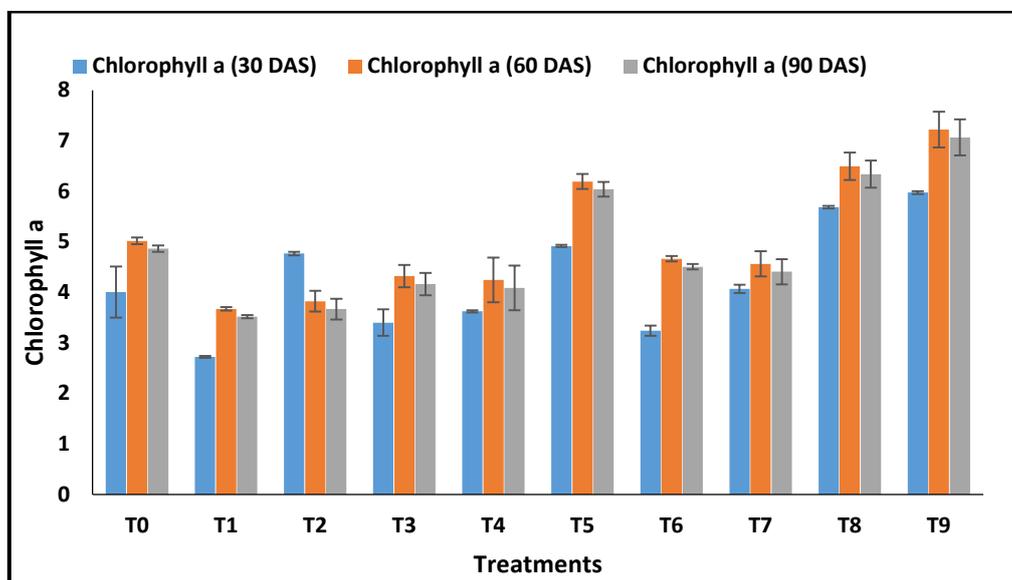


Figure a. Chlorophyll a of Sorghum during Rabi

where T0-Control; T1- NaCl (8 dsm^{-1}); T2-Endomycorrhizal fungi (AMF); T3-Putrescine (1.50 mM); T4-Putrescine (3.0 mM); T5- NaCl (8 dsm^{-1}) + Putrescine 1.5 mM; T6- NaCl (8 dsm^{-1}) + Putrescine (3.00mM); T7- NaCl (8 dsm^{-1}) + Endomycorrhizal fungi (AMF); T8- NaCl (8 dsm^{-1}) + Putrescine (1.50 mM) + Endomycorrhizal fungi (AMF); T9- NaCl (8 dsm^{-1}) + Putrescine (3.00 mM) + Endomycorrhizal fungi (AMF)

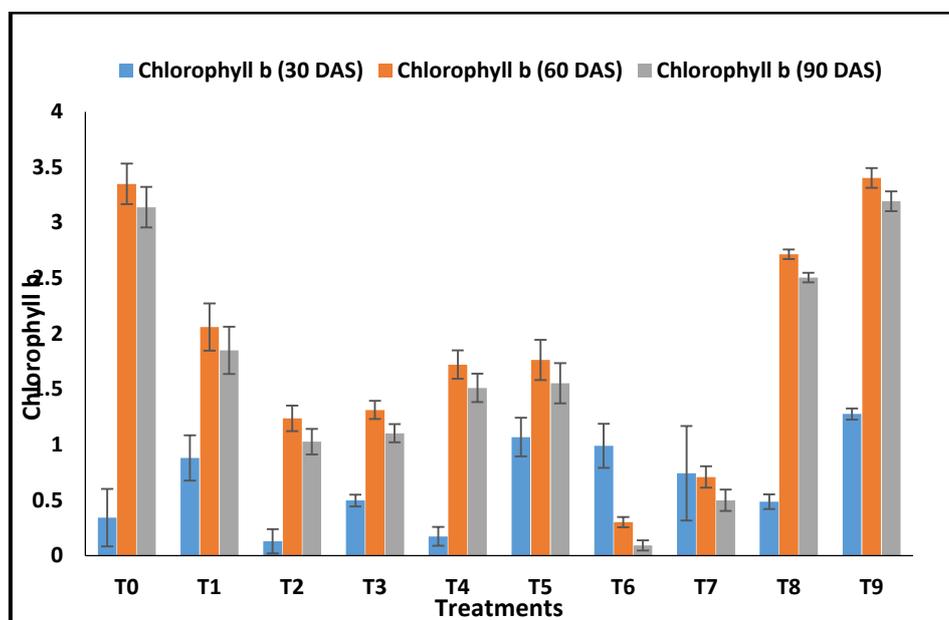


Figure b. Chlorophyll b of Sorghum during Rabi

where T0-Control; T1- NaCl (8 dsm^{-1}); T2-Endomycorrhizal fungi (AMF); T3-Putrescine (1.50 mM); T4-Putrescine (3.0 mM); T5- NaCl (8 dsm^{-1}) + Putrescine 1.5 mM; T6- NaCl (8 dsm^{-1}) + Putrescine (3.00mM); T7- NaCl (8 dsm^{-1}) + Endomycorrhizal fungi (AMF); T8- NaCl (8 dsm^{-1}) + Putrescine (1.50 mM) + Endomycorrhizal fungi (AMF); T9- NaCl (8 dsm^{-1}) + Putrescine (3.00 mM) + Endomycorrhizal fungi (AMF)

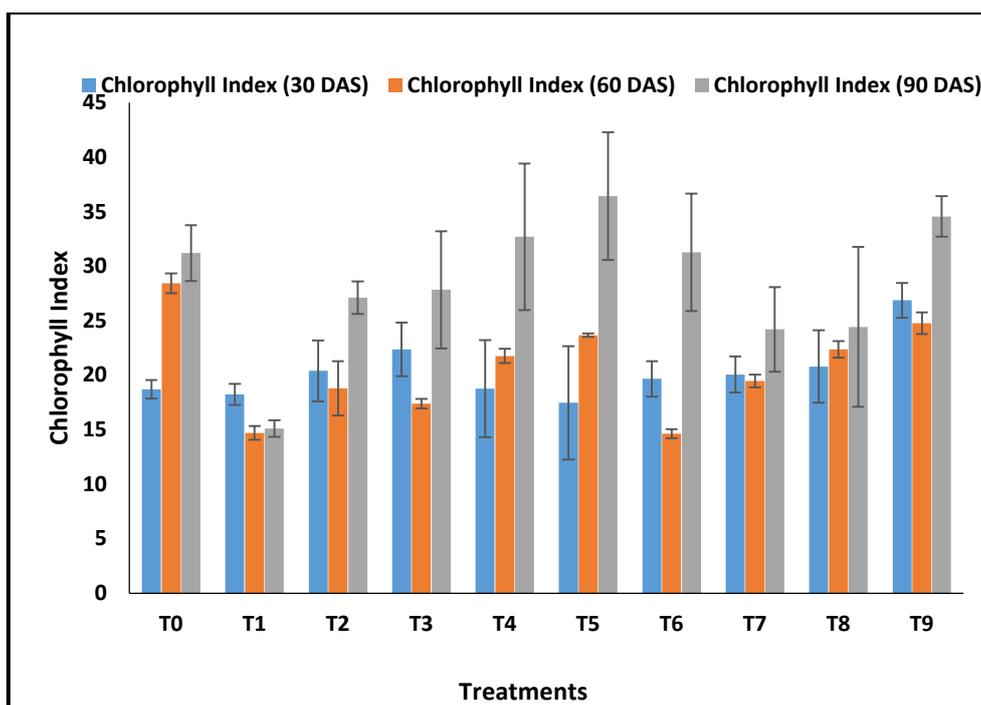


Figure c. Chlorophyll Index of Sorghum during Rabi

where T0-Control; T1- NaCl (8 dsm^{-1}); T2-Endomycorrhizal fungi (AMF); T3-Putrescine (1.50 mM); T4-Putrescine (3.0 mM); T5-NaCl (8 dsm^{-1}) + Putrescine 1.5 mM; T6- NaCl (8 dsm^{-1}) + Putrescine (3.00mM); T7- NaCl (8 dsm^{-1}) + Endomycorrhizal fungi (AMF); T8- NaCl (8 dsm^{-1}) + Putrescine (1.50 mM) + Endomycorrhizal fungi (AMF); T9- NaCl (8 dsm^{-1}) + Putrescine (3.00 mM) + Endomycorrhizal fungi (AMF)

Table 5. Chlorophyll Index of Sorghum during Rabi

Treatments	Chlorophyll Index (30 DAS)	Chlorophyll Index (60 DAS)	Chlorophyll Index (90 DAS)
T0	18.700 ^h ±0.493	28.433 ^a ±0.521	31.200 ^{ab} ±1.484
T1	18.233 ^b ±0.555	14.700 ^e ±0.361	15.100 ^c ±0.436
T2	20.400 ^b ±1.617	18.800 ^{ef} ±1.442	27.100 ^b ±0.862
T3	22.367 ^{ab} ±1.424	17.400 ^f ±0.252	27.833 ^{ab} ±3.105
T4	18.767 ^b ±2.571	21.767 ^d ±0.376	32.700 ^{ab} ±3.884
T5	17.467 ^b ±3.005	23.667 ^{bc} ±0.088	36.433 ^a ±6.267
T6	19.667 ^b ±0.940	14.633 ^e ±0.240	31.267 ^{ab} ±3.113
T7	20.067 ^b ±0.953	19.467 ^e ±0.338	24.200 ^{bc} ±2.248
T8	20.800 ^b ±1.914	22.367 ^{cd} ±0.437	24.433 ^{bc} ±4.236
T9	26.867 ^a ±0.921	24.767 ^{ab} ±0.578	34.567 ^{ab} ±1.068

Chlorophyll Index

The effect and combination of putrescine and mycorrhiza on the chlorophyll index were investigated in the salinity stress of the SSV74 sorghum species. Thirty, 60, and 90 days after the sowing (DAS) data were collected (Table 5 and Fig. c). It can be seen that when exposed to salinity stress (T1) about control (T0) in intervals 30, 60 and 90 DAS, the average chlorophyll index was significantly lowered by 2.49%, 48.30%, and 51.60%. Exogenous application of endomycorrhiza in soil (T2) showed a mitigation effect by increasing 10.62%, 21.80%, and 44.28% compared to 30.60 and 90 DAS for the chlorophyll index. Compared to T1, T3 was significantly increased with the chlorophyll index by 18.48%, 15.51%

and 45.74% with the proposed DAS when compared to T1. In contrast to T1, an increase of the chlorophyll index by 2.84 percent, 32.46 percent and 53.82 percent on the proposed DAS was the effect of an exogenous application of putrescine (T4). When treated with a higher dose of mycorrhiza (T5), the mean index of chlorophyll was significantly increased compared to T1 by 4.38%, 37.88%, and 58.55%. Likewise, when T6 was compared to T1, a significant increase in the chlorophyll index at the proposed DAS increased with 7.28%, 0.4%, and 51.77%. When treated with a high dose of putrescine (T7), the average chlorophyll index was improved significantly compared to T1 by 9.13 percent by 24.4 and 37.0 percent. In T8 treatment, the combination of putrescine and mycorrhiza showed the best mitigation effect with an increase in chlorophyll index of 12.34%, 34.27% and 38.19% concerning T1 at the proposed DAS. When treatment T9 was compared with treatment T1 then a significant chlorophyll index was increased by 32.13%, 40.64%, and 56.31%, respectively. Based on the above results obtained, it was found that the combined application of mycorrhiza and putrescine showed the best mitigation effect in crops concerning salinity stress.

CONCLUSION

Ion toxicity and osmotic imbalances were observed to cause salinity stress and cause plant oxidative stress. Arbuscular mycorrhizae (AM) is considered as bio-enhancers of saline soil capable of developing salinity tolerances on cultivated plants. Sorghum compatibility with polyamines and mycorrhizas to reduce stress on

salinity. The best mitigation effect in T8 treatments, increased chlorophyll by 61.84% at 60DAS, increased by 65.25% in T9 at 60DAS. Compared with T1, the indices of chlorophyll were significantly improved by 58.55 percent when treated with a higher dose of mycorrhiza T5 in 90DAS.

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Author Contributions

The study was designed by P.K. and M.H, the morphological protocolizations were established, experiments were carried out and the data analysed and interpreted were collected. The paper has been written by P.K. and M.H.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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