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Morphological and physiological features of genotypes of *Zea mays* towards salt tolerance influenced by mycorrhizal fungi

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Abstract

Soil salinity poses a significant constraint on plant growth and productivity of maize. Salinity causes reduction in water content in plant tissues, ultimately reducing the photosynthetic capacity and resulting in decreased productivity. Normally in saline soils, chloride ions (Cl−) are particularly considered toxic to certain crops, but in case of maize, sodium ion (Na+) is the main ion responsible for toxicity due to its competition with K+ for binding sites at the plasma membrane. Mycorrhizoremediation which is an enhanced form of phytoremediation is one of the key players in remediation saline soils. Inoculation of these beneficial arbuscular mycorrhizae fungi can alleviate growth inhibition and the adverse effects of salinity in both halophytes and glycophytes by establishing symbiotic relationships with plants. AM fungi colonize the roots of maize plants, perform a crucial role in nutrient cycles in terrestrial ecosystems and own highly efficient and various mitigation mechanisms. Under saline conditions, AM fungi restrict the absorption and translocation of Na+ to shoot tissues and enhance the uptake of K+ in plants. An experiment aiming to enhance salt tolerance in maize through AMF symbiosis was conducted. In this experiment three AMF were evaluated at two salinity levels 66 mM and 100 mM. analysis of data revealed that AMF Ri collect showed showed highest percentage of root colonization at all salinity levels. While plants inoculated with AMF Ce CdG showed highest shoot and root biomass. Furthermore, plants inoculated with Sc CdG and Ce CdG showed a significant reduction in Na+ accumulation and enhance K+ accumulation in shoot and root tissues as compared to non-mycorrhizal plants.

Keywords: Shoot tissues, AMF symbiosis, salinity levels, metal toxicicty.

1. INTRODUCTION

Maize is a member of Paoaceae family. After wheat and rice, maize is the 3rd most important crop globally. The term “mays” originate from Latin language which means “life benefactor” [1]. Nutritional values of maize grains are very high. So, the major aim of maize crop cultivation is to obtain high production of grains yield. Soil salinization is a significant environmental challenge that disrupts plant growth and metabolism. This issue is exaggerated and impacting over 800 million hectares of land worldwide by unsustainable farming practices and climate change [2]. The problem is particularly acute in areas where annual rainfall is low, but evapotranspiration rates are high, such as arid and semi-arid regions. While halophytes (which are known to be resistant to salinity) can grow well under saline conditions, but most crop species, including maize (*Zea mays* L.), are not much salt tolerant and are sensitive to high salt concentrations, which lead to huge decrease in growth and productivity of maize grown in salt affected soils [3].

Salt toxicity causes reduction in water content in plant tissues, ultimately reducing the photosynthetic capacity and resulting in decreased productivity [4, 5]. Normally in saline soils, chloride ions (Cl−) are particularly considered toxic to certain crops and woody species like citrus, legumes, and grapevines. However, in the case of maize, sodium ion (Na+) is the main ion responsible for toxicity caused by salinity. There are various mechanisms in plants to respond salt stress. Some plants respond to salt stress by limiting the water availability to them, and a specific sodium ion related mechanisms which disrupts biochemical processes and lead to reduced growth rates [6, 7]. When salinity level increase beyond tolerance then excess cytosolic Na+ displaces K+, leading to disrupted stomatal regulation, chloroplast deformation and malfunction, and decreased enzyme activation and protein synthesis. Which lead to more severe plant damages in the later stages [8].

Several studies showed that crops like wheat, rice, and maize when grown under saline conditions exhibit a decrease in K+: Na+ ratios as compared to their non-salinized counterparts [9–11]. Damage to structural components of chloroplasts, including membranes, grana, and thylakoids, along with disrupted metabolism in mesophyll cells occurs under high salinity [12]. Therefore, maintaining the K+: Na+ balance (known as "homeostasis" in many studies) and preventing Na+ over-accumulation in the cytoplasm is very important for plants under salt stress. Vascular bundles like xylem are responsible for the transport of Na+ from roots to shoots, and the shoots are primary site of Na+ toxicity [13]. Thus, strategies that enhance the removal of Na+ from shoots while retaining K+ will improve salt tolerance [14-16].

Apart from these intrinsic protective systems of plants, soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) can help plants to mitigate salinity stress by forming associations. Inoculation of these beneficial fungi can alleviate growth inhibition and the adverse effects of salinity in plants by establishing symbiotic relationships [17]. Mycorrhizoremediation which is an enhanced form of phytoremediation is one of the key players in remediation of soils [18]. AMF, which colonize the roots of maize plants, perform a crucial role in nutrient cycles and own highly efficient and various mitigation mechanisms. Previous studies have showed that under saline conditions, AM fungi restrict the absorption and translocation of Na+ to shoot tissues and enhance the uptake of K+ in plants [19].

Plants which form mycorrhizal symbiotic associations with fungi typically exhibit lower Na+ concentrations accumulation in shoots and higher K+: Na+ ratios as compared to non-mycorrhizal plants under saline conditions [20, 21]. Which reveals that AM fungi can aid plants in alleviating NaCl-induced ionic imbalance and preserving the structural and functional disturbance of cells and/or organelles [22].

2. MATERIALS AND METHODS

2.1 Experimental design

Thes research followed a CRD factorial design with four AMF inoculations: (1) non-mycorrhizal, (2) AM fungus *Rhizophagus intraradices* (Ri collect), (3) AM fungal strain *Septoglomus constrictum* isolated from CdG (Sc CdG), and (4) AM fungal strain *Claroideoglomus etunicatum* isolated from CdG (Ce CdG). Three levels of salinity (controlled, 66 mM and 100 mM) were used for each AM fungi.

2.2 Soil and biological materials

Loamy soil was sieved and mixed with quartz sand. Then this mixture was sterilized for three consecutive days by steaming at 100 °C for 1 hour. The pH of original soil was 8.2, organic matter 1.5%, and nutrient concentrations per kg of soil were nitrogen 1.9 g, phosphorus 1 g, and potassium 6.9 g. while 0.5 dS/m was the EC of the soil. Three seeds were cultivated in each pot and at seedling stage were thinned to single seedling per pot.

2.3 Inoculation treatments

The AM species were isolated from areas which were severely affected by salinity and desertification. Then in an open-pot culture of *Zea mays* L., mycorrhizal inoculum was produced consisting of soil, spores, mycelia, and infected root fragments.

2.4 Symbiotic development

After clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactic acid (v/v), Phillips and Hayman [23] method was used to visually observe the percentage of infection in mycorrhizal maize roots. Then extent of mycorrhizal colonization was calculated by gridline intersect method [24].

2.5 Determination of mineral nutrients

Acid digestion g of grounded leaf and root dry material was done for the extraction of K+ and Na+. Then samples after mixing with 4 mL of HNO3 and 1 mL of H2O2, were heated for 20 minutes at 220 °C, and then for at least 4 hours, these samples were cooled at room temperature. Subsequently, the samples were diluted with water and analyzed using an ICP plasma analyzer. These extractions were replicated on three different plants from each treatment to ensure accuracy.

Through the process of aqueous extraction from 0.2 g of dry plant material Cl+ were extracted using 10 mL of deionized water. Then after shaking 2 hours, extract was filtered through Whatman filter paper. Quantification was done on three plants from each treatment.

2.6 Statistical analysis

This experiment was conducted according to CRD factorial design and both factors were replicated three times to ensure accuracy. Statistical analysis of data

was done using the Statistix 8.1 software. Initially, a factorial ANOVA was performed, followed by LSD means comparison test. Then the percentage contribution of AM fungi to mitigate various levels of salt stress was calculated.

3. RESULTS AND DISCUSSION

3.1 Least Significance Difference Test (LSD)

3.1.1 Symbiotic Development

AM root colonization percentage was generally enhanced with increasing salinity levels in all cases, (Table-1). Ri collected inoculated plants showed the highest AM root colonization rates (up to 83%), within each salinity level. Similarly, Sc CdG and Ce CdG inoculated plants also showed high root colonization levels as shown in Table-1 [25].

3.1.2 Plant biomass production

Shoot biomass production was negatively affected across all treatments with increasing salt application as shown in Table-1, at 100 mM salinity level the most significant decrease was observed. While in case of root biomass, only in non-mycorrhizal plants with increase in salinity root biomass production was decreased. From analyzing Table- 1 it is evident that Sc CdG and Ri collect did not enhance shoot biomass and root biomass, while shoot biomass was enhanced in plants inoculated with Ce CdG as compared to non-mycorrhizal plants [26, 27].

Table-1: LSD mean values ± Standard Error for AM Root Colonization Percentage, Shoot Dry Weight and Root Dry Weight

Salt Leval	Arbuscular Mycorrhizar	AM root colonization	Shoot Dry Weight	Root Dry Weight
0 mM	NM	00.00 ± 00 ^{I}	4.30 ± 0.102 ^{D}	2.17 ± 0.103 ^{A}
0 mM	Ri Collect	64. 78 ±0.453 ^{D}	3.73 ± 0.119 ^{FG}	1.70 ± 0.038 ^{C}
0 mM	Sc CdG	37.713 ± 0.405 ^{H}	3.93 ± 0.045 ^{E}	1.45 ± 0.031 ^{DE}
0 mM	Ce CdG	56.55 ± 0.357 ^{F}	5.12 ± 0.058 ^{B}	2.28 ± 0.052 ^{A}
66 mM	NM	00.00 ± 00 ^{I}	3.83 ± 0.045 ^{EF}	1.58 ± 0.047 ^{CD}
66 mM	Ri Collect	83.027 ± 0.537 ^{A}	3.59 ± 0.043 ^{GH}	1.46 ± 0.035 ^{DE}
66 mM	Sc CdG	56.257 ± 0.398 ^{F}	3.67 ± 0.050 ^{FG}	1.38 ± 0.024 ^{E}
66 mM	Ce CdG	61.33 ± 0.339 ^{E}	5.31 ± 0.043 ^{A}	2.21 ± 0.062 ^{A}
100 mM	NM	00.00 ± 00 ^{I}	3.70 ± 0.018 ^{FG}	1.57 ± 0.05 ^{CD}
100 mM	Ri Collect	80.68 ± 0.571 ^{B}	3.41 ± 0.032 ^{HI}	1.65 ± 0.043 ^{C}
100 mM	Sc CdG	51.54 ± 0.686 ^{G}	3.39 ± 0.027 ^{I}	1.48 ± 0.035 ^{DE}
100 mM	Ce CdG	72.99 ± 0.514 ^{C}	4.85 ± 0.042 ^{C}	1.92 ± 0.037 ^{B}

3.1.3 Accumulation of Potassium ions in Shoots and Roots

K+ accumulation in root tissues across all treatments were reduced with increasing salinity in the growing medium while plants inoculated with Ri collect, at 0 mM and 100 mM NaCl, maintained similar K+ levels at both as shown in Table-2. Conversely, with higher salinity levels K+ accumulation in shoot tissues increased. At 100 mM NaCl, plants inoculated with Sc CdG showed higher accumulation of K+. All mycorrhizal treatments resulted in greater K+ accumulation in roots compared to non-mycorrhizal plants as shown in Table- 2. AM as well as non-AM showed not much significant differences in root K+ accumulation at these salt levels. While in case of shoots, NM and plants inoculated with Ri collect consistently showed lower K+ accumulation at all salinity levels, than plants inoculated with other two AM fungal strains (Table-2) [27].

3.1.4 Accumulation of Sodium ions in Shoots and Roots

When salinity was applied to growth medium, Na+ accumulation in both shoot and root tissues of maize were significantly enhanced. In root tissues, within each salt level, at 0 mM NaCl, it was observed that, two AMF enhanced Na+ accumulation as compared to NM plants. However, at 66 mM salinity and 100 mM salinity, accumulation of Na+ in roots didn't show prominent variances among AM treatments. At 100 mM salinity, significantly lower Na+ levels were found plants inoculated with Ce CdG. While in case of shoot tissues at 0mM NaCl, for all AM treatments Na+ levels were very low as shown in Table- 2. At 66 mM and 100 mM salinity, Na+ accumulation was increased for all treatments. Mycorrhizal plants showed the lowest Na+ accumulation especially those inoculated with Ce CdG, while NM plants exhibited the highest Na+ accumulation in shoots (Table-2) [28].

3.1.5 Accumulation of Chloride ions in Shoots and Roots

With increase in salinity levels in the growth medium, Cl- accumulation was increased in both root and shoot tissues as shown in Table-3. Analysis of Table-3 data within each salt level revealed that the accumulation of Cl- in roots didn't show prominent differences. However, in case of shoots, plants inoculated with the Sc CdG and Ce CdG at 0 mM NaCl, accumulated more Cl- than NM. Conversely, no prominent variances were observed among fungal treatments at higher salt levels. While NM plants

consistently showed that when salinity level was enhanced, the accumulation of Cl- was also enhanced. Ce CdG inoculated maize plants exhibited the minimum Cl- accumulation at both saline levels.

Table-2: LSD mean values ± Standard Error for AM Potassium Content in Shoot, Potassium Content in Root and Sodium Content in Shoot

Salt Leval	Arbuscular Mycorrhizar	Potassium Content in Shoot	Potassium Content in Root	Sodium Content in Shoot
0 mM	NM	17.69 ± 0.291 ^{G}	11.66 ± 0.278 ^{C}	0.11 ± 0.012 ^{F}
0 mM	Ri Collect	18.65 ± 0.257 ^{G}	12.90 ± 0.412 ^{B}	0.22 ± 0.018 ^{F}
0 mM	Sc CdG	27.10 ± 0.439 ^{D}	14.95 ± 0.401 ^{A}	0.31 ± 0.015 ^{F}
0 mM	Ce CdG	27.46 ± 0.594 ^{D}	13.22 ± 0.371 ^{B}	0.43 ± 0.019 ^{F}
66 mM	NM	22.29 ± 0.440 ^{EF}	7.62 ± 0.233 ^{I}	12.54 ± 0.251 ^{B}
66 mM	Ri Collect	22.74 ± 0.474 ^{EF}	8.48 ± 0.318 ^{H}	7.06 ± 0.198 ^{D}
66 mM	Sc CdG	34.58 ± 0.475 ^{B}	9.06 ± 0.290 ^{FG}	6.76 ± 0.057 ^{D}
66 mM	Ce CdG	33.10 ± 0.954 ^{C}	9.60 ± 0.368 ^{E}	4.51 ± 0.042 ^{E}
100 mM	NM	21.62 ± 0.286 ^{F}	6.86 ± 0.146 ^{J}	18.70 ± 0.443 ^{A}
100 mM	Ri Collect	23.29 ± 0.296 ^{E}	10.60 ± 0.368 ^{D}	11.48 ± 0.245 ^{C}
100 mM	Sc CdG	38.25 ± 0.461 ^{A}	8.96 ± 0.010 ^{G}	12.88 ± 0.452 ^{B}
100 mM	Ce CdG	32.46 ± 0.436 ^{C}	9.47 ± 0.247 ^{EF}	7.19 ± 0.117 ^{D}

Table-3: LSD mean values ± Standard Error for Sodium Content in Root, Chloride Content in Shoot and Chloride Content in Root

Salt Leval	Arbuscular Mycorrhizar	Sodium Content in Root	Chloride Content in Shoot	Chloride Content in Root
0 mM	NM	3.35 ± 0.060 ^{H}	21.05 ± 0.567 ^{H}	28.99 ± 0.520 ^{G}
0 mM	Ri Collect	3.81 ± 0.085 ^{H}	26.97 ± 0.705 ^{G}	32.28 ± 0.743 ^{F}
0 mM	Sc CdG	8.75 ± 0.284 ^{G}	32.20 ± 0.578 ^{F}	33.26 ± 0.629 ^{F}
0 mM	Ce CdG	10.63 ± 0.408 ^{F}	25.38 ± 0.412 ^{G}	32.18 ± 0.393 ^{F}
66 mM	NM	26.24 ± 0.448 ^{B}	42.66 ± 0.719 ^{C}	55.85 ± 0.765 ^{C}
66 mM	Ri Collect	23.88 ± 0.424 ^{C}	36.97 ± 0.845 ^{E}	58.84 ± 0.637 ^{B}
66 mM	Sc CdG	20.79 ± 0.469 ^{D}	39.57 ± 0.801 ^{D}	54.27 ± 0.358 ^{D}
66 mM	Ce CdG	19.10 ± 0.456 ^{E}	32.44 ± 0.736 ^{F}	56.71 ± 1.165 ^{C}
100 mM	NM	30.26 ± 0.461 ^{A}	50.04 ± 0.879 ^{A}	56.59 ± 0.736 ^{C}
100 mM	Ri Collect	25.87 ± 0.655 ^{B}	42.34 ± 0.589 ^{C}	60.24 ± 1.031 ^{A}
100 mM	Sc CdG	30.11 ± 0.644 ^{A}	46.01 ± 0.618 ^{B}	53.06 ± 0.927 ^{E}
100 mM	Ce CdG	23.22 ± 0.388 ^{C}	36.60 ± 0.764 ^{E}	52.76 ± 0.546 ^{E}

3.2 Percentage effect of Arbuscular Mycorrhizae Fungi on Salinity

3.2.1 Shoot Biomass and Root Biomass

Figure-1 showed that various AM fungi showed significant impact on shoot biomass and root biomass. Arbuscular Mycorrhizae Ce CdG causes significant increase in both shoot biomass and root biomass irrespective of salt stress. It causes 23% increase in shoot biomass at 66 mM salt level and 13% increase at 100 mM salt level. It also helped maize plants to maintain roots network with increase in salinity. So Arbuscular Mycorrhizae Ce CdG can be considered as a salt resistant fungus, and it can be useful to develop symbiosis with crops to grow in saline conditions.

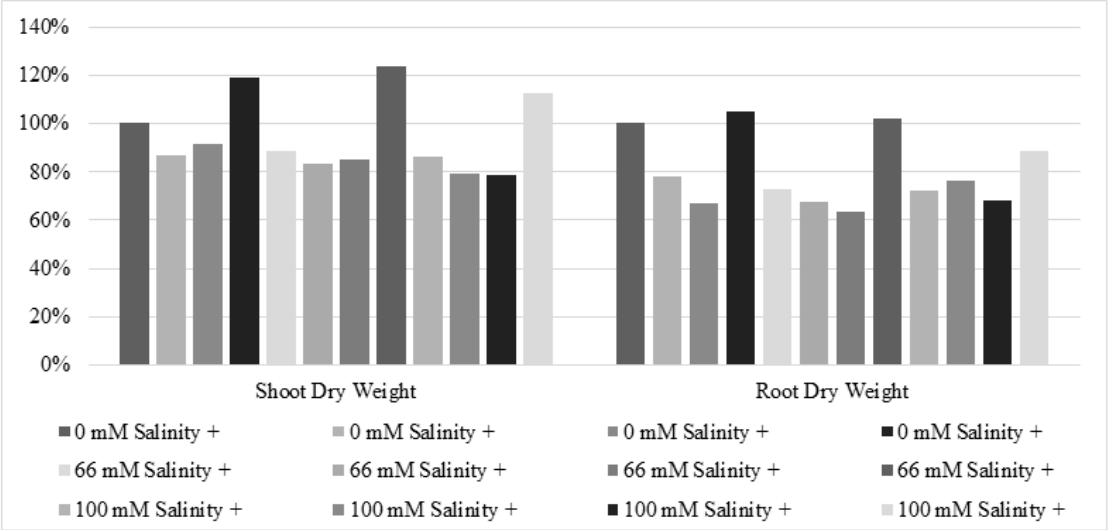

Fig-1: Graphical Representation of Percentage effect of Arbuscular Mycorrhizae Fungi on Salinity for Dry Shoot Weight and Dry Root Weight

3.2.2 Potassium Content

Plants grown under saline conditions generally accumulate more Na+ in tissues. AM fungi restricts the absorption and translocation of Na+ to shoot tissues and enhances the uptake of K+ in plants. Figure- 2 showed that various AM fungi showed significant impact on potassium level in shoot and root. Arbuscular Mycorrhizae Sc CdG and Ce CdG caused significant increase in accumulation of K+ in shoots and roots. At 100 mM salinity level, Sc CdG and Ce CdG caused 116% and 83% more K+ accumulation in shoot respectively. While in roots at 100 mM salinity level, none of AM species accumulate more K+ as compared to their values at 0 mM salinity level.

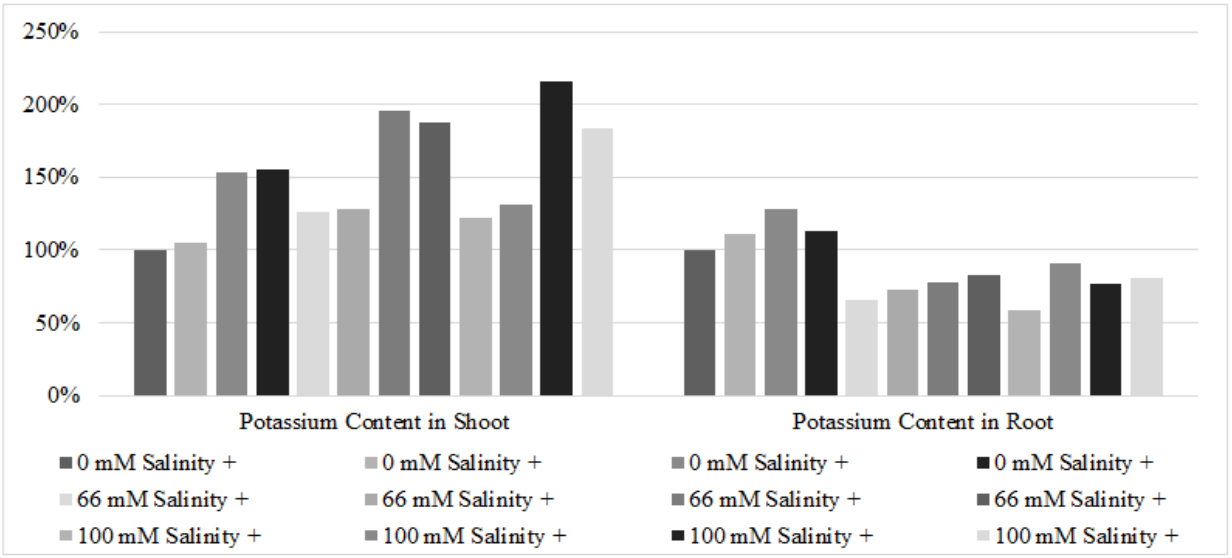

Fig-2: Graphical Representation of Percentage effect of Arbuscular Mycorrhizae Fungi on Salinity for Potassium Content in Shoot and Root

3.2.3 Sodium Content

Normally in saline soils, chloride ions (Cl−) are particularly considered toxic to certain crops and woody species like citrus, legumes, and grapevines. However, in the case of maize, sodium ion (Na+) is the main ion responsible for toxicity caused by salinity. Figure- 3 showed that none of AM fungi showed significant impact Na+ accumulation as compared to their controlled values. But at 66 mM salinity and 100 mM salinity, Arbuscular Mycorrhizae Ce CdG causes significant reduction in Na+ accumulation in both shoots and roots as compared to non-mycorrhizal plants.

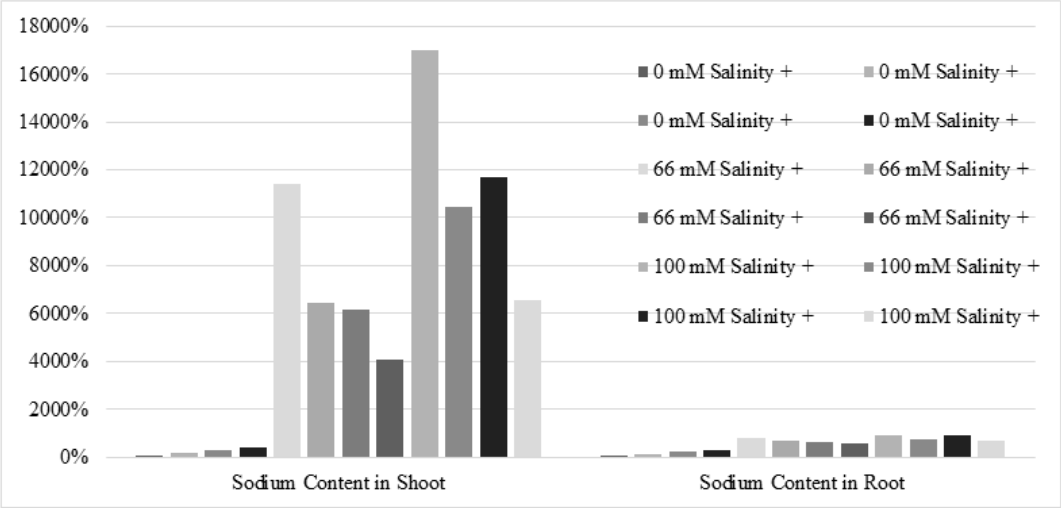

Fig-3: Graphical Representation of Percentage effect of Arbuscular Mycorrhizae Fungi on Salinity for Sodium Content in Shoot and Root

3.2.4 Chloride Content

In the case of maize, chloride ions (Cl−) accumulation is not considered much toxic. Figure- 4 showed that Cl- accumulation was increased with increase in salinity levels. As compared to non-mycorrhizal plants, arbuscular mycorrhizae Sc CdG and Ce CdG causes significant decrease in Cl- accumulation at 66 mM and 100 mM salinity levels in both shoots and roots.

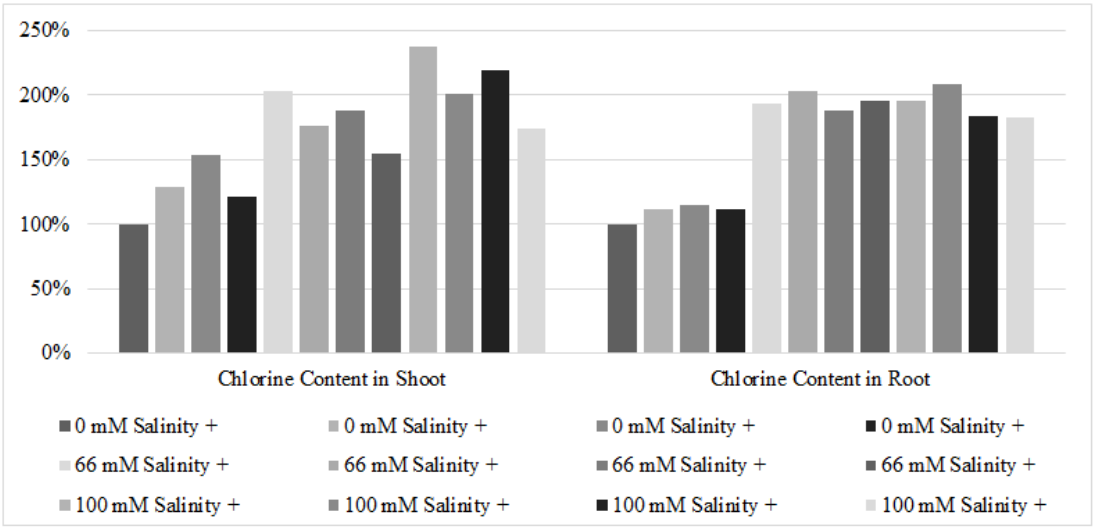

Fig-4: Graphical Representation of Percentage effect of Arbuscular Mycorrhizae Fungi on Salinity for Chloride Content in Shoot and Root

CONCLUSION

From this study it can be concluded that the AMF species enhance the tolerance of maize to salt stress. Our findings highlight that AMF has great importance for the maintenance of ionic balances for salt tolerance in maize plants. In this experiment Ce CdG and Se CdG were the most effective AM fungi. These species exhibited better ability to alleviate the inhibitory effects of salt stress. Understanding of the molecular mechanisms of salt tolerance can be acquired through characterizing the ion transporters of these salt-tolerant fungi. For sustainable agricultural practices, these results offer significant potential for implementing in salinized soils to improve crop performance and yield.

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