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Effect of AM fungi during salt stress on biochemical content in Ginger (*Zingiber officinale* Rosc.)

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ABSTRACT

Ginger (*Zingiber officinale* Rosc.) is a highly-grown spice crop; its aromatic rhizomes are commercially important due to its high importance in the diet as a spice and some medicinal values. Irrigation methods in India increase salt content in the soil. Arbuscular Mycorrhizal (AM) fungi assist plants under salt stress. However, the vital role of mycorrhizal fungi in ginger salt tolerance has not been evaluated yet and needs to emphasize on its evaluation. The present investigation was conducted to assess the efficacy of AM fungi on ginger plants grown under different salt concentrations. In the current investigation level of Chlorophyll, nucleic acids like DNA and RNA, Proteins, Proline, reducing sugars, and total soluble carbohydrates contents have been evaluated to estimate the Growth and biochemical parameters. The study revealed that AM fungi significantly contributed to the salt stress tolerance of Ginger plants. Statistical analysis found an enormously significant correlation between growth parameters and salt tolerance. Pearson correlation coefficient has been used as testimony, resulting in a positive correlation of the use of AM fungi on ginger plant's Growth and biochemical contents.

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1 Introduction

Ginger (*Zingiber officinale* Rosc.), a perennial herbaceous crop from the family Zingiberaceae, originated in South East Asia. It is being cultivated as a spice and condiment which enhance the flavour of the food (Park and Pezzuto 2002). Ginger is mainly grown in the countries like India, China, Nigeria, Australia, Jamaica etc. Among the ginger-cultivating countries, China and India are reported as leading ginger producers in the world (Blumenthal et al. 2000). Ginger rhizomes are used in the treatment of various ailments such as headache, cold, nausea and emesis (Blumenthal et al. 2000). The antioxidant (Nile and Park 2015), and anticancer activities of the ginger rhizome have also been well reported (Citronberg et al. 2013).

Now in these days, soil salinity is a significant issue, and according to Ruiz-Lozano et al. (2001), saline soils occupy near about 7 % surface of the land, and this percentage continuously increases and lead to almost half of the cultivable land loss till 2050 (Wang et al. 2003). Around 15 million hectares of cultivable fields worldwide and approximately 7.7 billion hectares of total land are likely unnatural due to excessive salt (Sheng et al. 2008). Yamato et al. (2008) recorded the presence of AM Fungi in saline environments and reported that the association of AM fungi with plant enhance the Growth and biomass of the host plant. Generally, salt concentration decreases plant growth and yield, specifically in dry regions. Rabie (2005) also testified that AM fungi augment the survival capacity of plants during salinity stress and improve the nutrient absorption and water uptake capacity. Most recent studies noted that AM-colonized plants are more vigorous than non-colonized plants underneath salinity (Al-Karaki 2000). Previous research on the efficacy of AM during salinity stress showed that symbiosis of AM fungi positively affected nutrients uptake and signifies that the effect of salinity stress is controlled by AM fungi (Dastogeer et al. 2020; Liu et al. 2023; Aziz et al. 2023). AM fungi effectively boost the availability of different macro and micro-nutrients, which improves photosynthetic efficiency and plant biomass (Chen et al. 2017; Mitra et al. 2020).

The effects of salt stress on the photosynthetic compound like Chlorophyll in Ginger have not been evaluated yet. Similarly, reports on the salt stress effect on Ginger's nucleic acid content are also unavailable. Ginger is an essential component of the human diet, and its assessment concerning nutritional value is highly needed. Protein, reducing sugars, and total soluble carbohydrates are essential content as far as diet is concerned, so the effect of salt stress on the analysis of these contents is also very important. During the stress conditions, proline acts as an osmolyte, and its content can help to conclude the intensity of salt stress on Ginger (Singh et al. 2017; El Moukhtari et al. 2020; Spormann et al. 2023). The present investigation is primarily being undertaken for two aspects; one is to assess biochemical content in the Ginger rhizome during different concentrations of salt stress, and the other is to evaluate the influence of AM Fungi during salt stress with the help of measuring Chlorophyll and Proline content. The nucleic acid content in Ginger has not been evaluated yet; this is the first research attempt in this area. These studies would help know Ginger's food value in diet and AM fungi's value when plants are under salt stress.

2 Materials and methods

2.1 Set up of pot for experiment

The pot matrix to carry out this work was prepared by Soil, FYM and coarse sand in a ratio of 3:1:1, respectively. This mixture was autoclaved at 15 lbs for about 60 min. It was then added to selected pots, per Doganlar et al. (2010).

2.2 Ginger rhizomes cultivations

Fresh Ginger rhizomes of Satara variety procured from Satara, Maharashtra state, India. Rhizomes having buds were cut in pieces of 25-30g and treated for half an hour with 0.1% of the laboratory grade $HgCl_2$; this was followed by the washing of rhizomes with sterile water. Later, a soil-AM combination was prepared using 0.2 kg of autoclaved soil mixture and an assortment of five selected AM species, i.e. *Acaulospora appendiculata*, *A. gerdemanni*, *Glomus convolutum*, *G. fasciculatum* and *Scutellospora calospora.*

2.3 Salt stress treatment

The surface sterilized rhizomes were grown in each pot. All pots were kept inside the net house in a completely randomized block design with a sufficient water supply generally for 30 days at every five days interval. These rhizomes germinated within 2 to 3 weeks. The salinity treatment started one month later, growing it. Sodium chloride (NaCl) was used to give salt stress treatment. Four different concentrations, i.e., 25 mM, 50 mM, 75 mM and 100 mM of sodium chloride, were made using distilled water as per Dhanapackiam and Iliyas (2010) and have been used for treatment. For every concentration, three replicates were made along with control pots to evaluate the effect of salt concentration on the various growth parameters of Ginger under AM-supplemented soil. Consequent usages of different salt concentrations were administered with a gap of 5 days for the next three months. Every time 500 ml of NaCl of various intensities is introduced at each pot. Initially, salinity was escaped to escape plants from stress jolt. Ninety days after salinity treatment, plants were used for testing biochemical contents.

2.4 Isolation and quantification of Chlorophyll

Chlorophyll pigments were isolated from leaves of salinity-stressed Ginger treated with AM fungi (experimental) and without AM fungi (control). The estimation of pigments was carried out

using Arnon's (1949) method. Leaves are used from both mycorrhizal and control plants. The absorbance of the blank and leaf extract was recorded at 645 nm and 663 nm with a UV-visible spectrophotometer.

2.5 Isolation and Estimation of DNA

DNA isolation was carried out using ginger rhizomes by Dellaporta et al. (1983) method and estimated by Burton's (1956) method. Absorbance for the developed blue solution and blank was noted at 600 nm. DNA content in plant samples was calculated by using a standard graph.

2.6 Isolation and Estimation of RNA

RNA isolation is performed as per Brawerman's (1974) method and estimated by Bial's (1902) method. Absorbance noted with 660 nm range versus blank. The standard graph was used to estimate the RNA amount in plant samples.

2.7 Estimation of Protein

Protein estimation was performed using the method of Lowry et al. (1951). The reaction was carried out to quantify protein as per Lowry's method. At the end of the reactions, the mixture's changed colour was measured at 660 nm. The protein content in the sample was estimated by comparing it with a standard graph.

2.8 Estimation of Proline

Proline estimation was done with the Bates et al. (1973) method. Salt and water-stressed plant parts like rhizomes and leaves were used to estimate proline. For this, 0.5 g. samples were used for the estimation of proline. The absorbance of the mixture was noted at 520 nm. Standard proline was used to plot standard graphs and calculate proline content in plant samples.

2.9 Estimation of reducing sugars

Dinitrosalicylic acid (DNSA) reagent was used to estimate reducing sugars, as Miller (1959) described. At the end of the reaction, the mixture was cooled. D-glucose was used to plot a standard graph and calculate different amounts of reducing sugars in plant samples.

2.10 Estimation of total soluble carbohydrates

Hedge et al. (1962) method was used to estimate total soluble carbohydrates. 1 g of rhizomes was used for estimation purposes. An absorbance read at 630 nm, and standard graph was used to estimate total soluble carbohydrates in plant samples.

2.11 Statistical Analysis

Data obtained were analyzed with MS Excel 2016. The variance between the control and experimental plants was calculated using a t-test. A comparison of the data was made using Pearson's correlation coefficient.

3 Results and Discussion

3.1 Estimation of Chlorophyll

Chlorophyll was extracted using ginger leaves as per Arnon's (1949) method. Among the tested salt concentrations, the highest concentration for all types of Chlorophyll (Chlorophyll a, chlorophyll b and total chlorophylls) was reported at a salt concentration of 25 mM, while the lowest was at 100 mM salt concentration. Further, all chlorophyll concentrations for 25 mM saline treatment are at par with the control. Chlorophyll content decreased as the concentration of saline solution increased (Table 1). Further, the concentration of all types of Chlorophyll in AM-treated plants was higher than in non-mycorrhizal plants, and this might be due to the higher photosynthetic efficacy of AM-treated plants. Results of the study show that the chlorophyll contents were reduced distinctly with elevated salinity. Further t-test for all chlorophyll contents in all experimental plants was significant at $P < 0.05$ level. Pearson's correlation test showed a negative correlation between chlorophyll content and to increase in salt concentration Table 1.

Salt stress significantly affects genetic potential, leading to several growth limitations (Gama et al. 2007). The amount of Chlorophyll lessens due to either slow formation or quick collapse, showing

Table 1 Effect of salt concentration and mycorrhiza treatment on chlorophyll content of Ginger

Salt Concentration	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophylls (mg/g)	
	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)
25 mM	1.312 ± 0.002	1.466 ± 0.02***	1.354 ± 0.003	1.474 ± 0.09*	2.666 ± 0.09	2.940 ± 0.003**
50 mM	1.107 ± 0.002	1.251 ± 0.01***	1.217 ± 0.001	1.430 ± 0.001***	2.324 ± 0.01	2.680 ± 0.002***
75 mM	0.970 ± 0.003	1.132 ± 0.002***	1.068 ± 0.003	1.327 ± 0.001***	2.038 ± 0.002	2.459 ± 0.001***
100 mM	0.634 ± 0.004	0.743 ± 0.001***	0.846 ± 0.002	0.906 ± 0.001***	1.480 ± 0.002	1.710 ± 0.002***

Results are given as mean ± SD, based on the measurement of individual samples; t-tests with significant differences (P< 0.05) between means are indicated by different * signs (*significant at <0.05 level, ** significant at <0.001 level and *** significant at <0.0001 level).

that there was a mechanism of photoprotection through the reducing absorbance of light, which has resulted in decreasing chlorophyll contents (Elsheery and Cao 2008). Smirnoff (1996) opined that salt stress might initiate the symptoms of oxidative stress, which has resulted in the inhibition of chlorophyll synthesis. Inoculation of plants with AM fungi activates different enzymatic activities, including Phosphoenolpyruvate carboxylase (PEPC) and rubisco, which enhances the rate of photosynthesis (Kaddes et al. 2019). These findings are contested with the various other plants such as maize (Rahmaty and Khara 2011), *Ocimum* (Heidari 2012), Barley (Meddich et al. 2021) and grapes (Ye et al. 2022). These researchers noted a decrease in chlorophyll content with increased salt concentration.

3.2 Estimation of DNA and RNA

Contradictory to chlorophyll content, the lowest DNA was isolated from the 25 mM salt concentration controlled plants, while it was highest in the 100 mM salt concentration. Similarly, the experimental plants' lowest DNA concentration was isolated from the 25mM and the highest at 100 mM saline concentrations. The DNA in non-treated plants was higher than in experimental ginger plants (Table 2). Similar trends have been recorded for the RNA content, and the lowest and highest salt concentrations were recorded at 25mM and 100mM for both control and experimental plants, respectively. Furthermore, DNA content was higher as compared to RNA in both sets. DNA and RNA were elevated as the concentration of salt increased in both experimental and controlled plants. The DNA and RNA within the AM-treated plants were noted to be lower than non-treated plants in all concentrations of salt. Further, RNA among control plants was reported to be higher than experimental ginger plants. Statistically, the t-test for both nucleic acid contents showed substantial differences at $P< 0.05$. Pearson's correlation test showed a positive correlation between DNA and RNA content with an elevation in salt concentration (Table 2).

The salinity stress might have resulted in a decrease in cell size. The smaller size of the cell increases its number per gram of tissue. Thus, the amount of high DNA ultimately results in the elevation of RNA content per gram. In experimental plants, AM made nutrients available to roots and mobilized soil nutrients, specifically phosphate and abetted roots, to absorb other soil nutrients. This occasioned in the healthy and vigorous Growth of experimental plants. Due to robust Growth, the number of cells per gram of plant tissue decreased. The decreased number of cells affected nucleic acid content in plant cells. Thus, a smaller number of cells per gram of plant tissue resulted in less DNA and RNA in experimental plants. Similar outcomes were documented by Gomathi and Vasantha (2006) in sugarcane. Studies on nucleic acid contents in rhizomatous crops concerning salt stress have not been done, and it is the first report on the effect of salt stress on nucleic acid contents in Ginger.

3.3 Estimation of Proteins

Protein content was elevated with the intensity of salt in both sets of experiments. The lowest and highest protein content was reported at 25 mM and 100 mM salinity in both sets of experiments. Further, protein content in treated plants was recorded as lower than those of the non-treated plants for all the salinity concentrations. Additionally, plants with higher salinity have higher protein requirements (Table 2). Plants accumulate and use stored proteins to survive under stress conditions to protect cells from various stresses (Wang et al. 2003). Further, it was also reported that plants accumulated proteins during salt stress conditions which can be utilized in future in physiological processes. These results agree with the findings of Doganlar et al. (2010) in tomato cultivars. Sibole et al. (2003) also noted the salt stress among clover (*Medicago citrana* L.) and augmented the soluble proteins in seedlings compared to control plants. Similarly, Chao et al. (1999) noted an increase in tomato protein content in response to salinity stress. A similar type of protein content improvement was reported in *Asparagus* (Matsubara et al. 2014), clover plants (Xie et al. 2020) and Onion plants (Metwally et al. 2021). Saboor et al. (2021) noted that AM fungi assist the maize plant during salt stresses.

Table 2 Effect of salt concentration and mycorrhiza on DNA, RNA and Protein content of Ginger

Salt Concentration	DNA (µg/g)		RNA (µg/g)		Proteins (mg/g)	
	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)
25 mM	361.68 ± 0.24	341.24 ± 0.32***	320.61 ± 0.45	312.20 ± 0.42***	3.196 ± 0.01	3.032 ± 0.04**
50 mM	381.30 ± 0.11	372.83 ± 0.24***	350.15 ± 0.28	341.94 ± 0.20***	3.304 ± 0.05	3.076 ± 0.01**
75 mM	436.44 ± 0.09	418.99 ± 0.11***	388.30 ± 0.11	379.07 ± 0.11***	3.576 ± 0.01	3.365 ± 0.01***
100 mM	496.26 ± 0.05	483.48 ± 0.09***	422.15 ± 0.15	413.74 ± 0.07***	3.676 ± 0.01	3.445 ± 0.01***

Results are given as mean ± SD, based on measurement on individual samples; Significant differences (P< 0.05) between means are indicated by Asterix (*significant at <0.05 level, ** significant at <0.001 level)

Table 3 Effect of salt concentration and mycorrhiza on Proline content in leaves and rhizome of Ginger

Salt Concentration	Proline leaves		Proline Rhizome	
	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)
25 mM	0.65 ± 0.04	0.54 ± 0.03**	0.38 ± 0.03	0.32 ± 0.04^{ns}
50 mM	0.80 ± 0.05	0.69 ± 0.02**	0.67 ± 0.04	0.58 ± 0.05*
75 mM	1.63 ± 0.07	1.47 ± 0.05*	1.26 ± 0.05	1.19 ± 0.04^{ns}
100 mM	2.32 ± 0.05	2.25 ± 0.06^{ns}	2.19 ± 0.04	2.12 ± 0.04^{ns}

Results are given as mean ± SD, based on the measurement of individual samples. Significant differences (p< 0.05) between means are indicated by different letters (^{ns} not significant at >0.05 level *significant at <0.05 level, ** significant at <0.001 level)

3.4 Estimation of Proline

Proline content was also elevated with the upliftment in the salt concentration in both sets of experiments and the lowest proline content was noted at 25 mM of salinity, while the highest was noted at 100 mM salinity. Like protein, the proline content in AM-treated plants was recorded as lower than those of the non-treated plants among all the salt concentrations (Table 3). Further t-tests for leaf proline contents showed significant differences at P<0.05 levels for 25 mM, 50 mM and 75 mM salt concentration in leaves and 50 mM salinity in the rhizome. Pearson's correlation test showed a positive correlation of proline content with respect to increased salt concentration in both leaves and rhizome (Table 3).

The buildup of proline has a vital role in osmotic balance and salt defence mechanism and generally accumulates in plants facing salinity stress. A defence response of proline in maintaining the osmotic pressure in a cell was observed by de Lacerda et al. (2003). Osmoregulation under water scarcity and salinity is essential for protein stabilization and preventing enzymes from heat denaturation. Lower osmotic potential might cause proline accumulation within plant tissues (Buhl and Stewart 1983). AMF improves the plant defence mechanism and produces low-molecular-weight compounds like proline (Bhosale and Shinde 2011; Vergara et al. 2018; Pirzadah et al. 2019). Among halophytes and glycophytes accumulation of proline is done as a non-toxic and protective osmolyte. Apart from osmolyte, proline also contributes to scavenging reactive oxygen species (ROS) and stabilizes subcellular structures (Fougere et al. 1991; Torabi and Halim 2010; Bhosale and Shinde 2011). Liu et al. (2022) have reported that AM fungi have a significant role in the secretion of osmolyte-like proline in Rice plants.

3.5 Reducing sugars

A significant improvement in the concentration of reducing sugars was recorded with the progress of salt concentration, and the lowest amount of reducing sugars was recorded at 25 mM salinity. In comparison, the highest was recorded at 100 mM salinity in both sets of experiments (Table 4).

Level of reducing sugars within the AM treated Ginger was lower than the non-treated Ginger at various salt concentrations. During salt stress along with other essential solutes, sugars accumulate for adjustments in plants (Baki et al. 2000). Excessive amounts of sugars in the cytoplasm block the expression of Rubisco (Sewada et al. 1992). Therefore, the rate of glucose synthesis and alternations in metabolic processes might contribute to salt sensitivity in plants. The results recorded in the present investigation match with the results obtained by Kerepesi and Galiba(2000) in wheat plants, Amirjani (2011) in rice plants and Meng et al. (2021) in trifoliate orange. The t-test for reducing sugars content showed a significant difference at P<0.05 (Table 4).

3.6 Total soluble carbohydrates

The increased salinity at both experimental sets elevated the total soluble carbohydrate content. The lowest amount of total soluble carbohydrates was noted in 25 mM salinity, whereas the highest

Table 4 Effect of salt concentration and mycorrhiza on reducing sugars and total soluble carbohydrates content of Ginger

Salt Concentration	Reducing Sugars		Total soluble carbohydrates	
	Control (Only Salt)	Experimental (Salt + AM)	Control (Only Salt)	Experimental (Salt + AM)
25 mM	75.81 ± 0.07	63.09 ± 0.07***	203.01 ± 0.08	223.32 ± 0.04***
50 mM	91.42 ± 0.07	74.76 ± 0.06***	262.66 ± 0.09	281.77 ± 0.06***
75 mM	176.42 ± 0.07	130.94 ± 0.09***	356.36 ± 0.08	393.33 ± 0.04***
100 mM	200.71 ± 0.02	187.51 ± 0.05***	452.66 ± 0.09	528.77 ± 0.07***

Results are given as mean ± SD, based on the measurement of individual samples; Significant differences (p< 0.05) between means are indicated by different letters (*** significant at <0.0001 level)

amount of carbohydrate was noted at 100 mM salinity of AM-treated and non-treated sets of treatments. Further, a remarkable increase in the total soluble carbohydrates was recorded in the AM-treated plant as compared to the non-treated plants (Table 4). Preferential partitioning of carbohydrates is generally facilitated by salinity (Schellenbaum et al. 1998). Plants, facing sodium salt stress, always store more starch (Rathert, 1984). Total soluble carbohydrates act as a solute which is synthesized and accumulated in the cytosol under salinity stress. Due to the association of AM, all treated plants absorbed more nutrients, resulting in enhanced total soluble carbohydrate contents among treated plants as there was an increase in salt stress; the amount of carbohydrates was alleviated because plants store more carbohydrates due to stress for utilization in coming adverse conditions. Similar results were reported by Munns and Weir (1981) in wheat, where the amount of total soluble carbohydrates elevated with stress. Increases in total soluble carbohydrates have also been recorded in various crops such as *Sesbania grandiflora* (Dhanapackiam and Iliyas 2010), Onion (Metwally 2020), Wheat (Gupta et al. 2021) and *Cicer arietinum* plants (Garg and Cheema 2021).

Conclusions

The study results show that applying Arbuscular Mycorrhiza helps overcome the adverse effect of salinity stress and enhances photosynthesis efficiency and nutrient storage. Further, the impact of salinity stress was also recorded on the nucleic acid, protein and proline contents and increasing the salinity level enhances the nucleic acid, protein and proline content. The AM-treated plants showed fewer amounts of nutrient, protein and proline content. Reducing sugars and total soluble carbohydrate content due to the treatment of AM fungi indicates an accumulation of sugars for osmotic adjustments in plants during salt stress.

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Conflicts of interest and financial disclosures

As the authors, I, Dr Kishor S. Bhosale, declare that no financial, academic, commercial or political interest could have influenced the findings noted in this article.

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