

Mitigation of Salinity Stress in Chickpea (*Cicer arietinum* L.) by Arbuscular Mycorrhizal Fungi (*Glomus mosseae*) in the Sundarbans Delta Region of India

Varsha Kundu

University of Calcutta

Moushree Sarkar

University of Calcutta

Sabyasachi Kundagrami

skundagrami@gmail.com

University of Calcutta

Research Article

Keywords: Arbuscular mycorrhizal fungi (AMF), Chickpea, *Glomus mosseae*, Salinity

Posted Date: April 16th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-5515052/v1>

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Additional Declarations: No competing interests reported.

Abstract

Salinity stress poses a significant threat to crop productivity, particularly in coastal regions such as the Sundarbans Delta in India. This study investigates the role of arbuscular mycorrhizal fungi (AMF), specifically *Glomus mosseae*, in mitigating salinity-induced stress in chickpea (*Cicer arietinum* L.). Six high-yielding desi-type chickpea lines, including one tolerant (Digbijoy), three moderately susceptible (IC 268971, Radhey, Mahamaya 1), and two highly susceptible (Virat, DCP 93 – 3), were selected for evaluation under controlled greenhouse and field conditions. Salinity stress was induced at 300 mM NaCl in pot experiments, while field trials were conducted in naturally saline soil. Results indicated that salinity significantly reduced key agro-morphological parameters, including seedling length, nodules plant⁻¹, leaf area index, plant height, branches, pods plant⁻¹, pod length, seeds pod⁻¹, 100-seed weight, and seed yield plant⁻¹. However, AMF inoculation mitigated these adverse effects, improving seedling emergence by 45%, plant height by 52%, pod number by 95%, and seed yield by 48% in field conditions. Additionally, AMF inoculation under salinity increased spore population by 535% and structural colonization (mycelium by 181% and arbuscules by 204%). Biochemical traits such as total phenolic content, relative water content, membrane stability index, and chlorophyll levels improved significantly in AMF-treated plants compared to those under saline conditions alone. The observed improvements ultimately led to a notable enhancement in grain yield, demonstrating the efficacy of *Glomus mosseae* in alleviating the adverse effects of salinity stress in chickpea. These results emphasize the role of arbuscular mycorrhizal fungi (AMF) as a viable, environmentally friendly solution to boost chickpea production under saline conditions, presenting a valuable tool for promoting climate-resilient farming in salt-affected areas.

1 Introduction

Chickpea (*Cicer arietinum* L.) is a vital legume crop widely grown in rotation with cereals, contributing to soil nitrogen enrichment and serving as a disease break in cropping systems. It is primarily cultivated in semi-arid regions, where it serves as a key source of nutrition for humans and as animal feed (Chibbarabada et al., 2017). Despite its agricultural significance, chickpea is highly sensitive to salinity stress. Salinity poses a significant challenge to chickpea production, with global annual yield losses estimated at 8–10% (Eckardt et al., 2022; Khatun et al., 2021; Flowers et al., 2010).

Salinity stress significantly impacts chickpea growth and yield by reducing seed germination, seedling vigor, root and shoot length, and photosynthetic activity (Yadav et al., 2020; Khan et al., 2017). Research by Abd-Alla et al. (2019) highlights that salt concentrations ranging from 25 to 150 mM severely impede chickpea development. Salinity also decreases the number of flowers and pods in chickpea genotypes, leading to considerable yield losses. Since chickpeas are commonly cultivated in irrigated soils, salt accumulation often occurs in the topsoil layers due to water evaporation during dry seasons. This salinity buildup lowers the soil's osmotic potential, resulting in water stress and nutrient imbalances that disrupt metabolism and cause cellular damage (Hasanuzzaman et al., 2024; Hanumantha et al., 2016). Addressing these challenges requires the adoption of effective crop management strategies to enhance chickpea productivity in saline environments.

Ensuring food security in the future will require developing salt-tolerant grain legume germplasms that can sustain high yields in saline soils. Advances in biotechnology, genomics, and traditional breeding (Sharma et al., 2018; Duc et al., 2015) have made it possible to create transgenic legume varieties with enhanced performance in salt-affected environments. However, these approaches are often expensive and time-intensive. As an alternative, the use of biofertilizers presents a more sustainable and environmentally friendly solution (Grover et al., 2021; Dawood et al., 2014; Evelin et al., 2009). Among biofertilizers, arbuscular mycorrhizal fungi (AMF) are particularly noteworthy. These fungi, which represent 5–10% of the global soil microbial biomass, form symbiotic relationships with plants that have co-evolved over time. These symbioses not only enhance nutrient uptake but also act as natural defense mechanisms against various stressors, including salinity (Diagne et al., 2020; Kotula et al., 2019; Bonfante and Genre, 2008). Leveraging salt-tolerant microbial inoculants, such as AMF, offers a promising strategy for improving crop performance under saline conditions. Recent studies indicate that AMF can mitigate the effects of salinity on crops by reducing sodium and chloride toxicity (Chouhan et al., 2023; Anand et al., 2022). This environmentally sustainable approach enhances plant resilience to abiotic stress. AMF symbiosis promotes better nutrient absorption, increases the production of osmoregulatory compounds, improves photosynthetic efficiency, and enhances water-use efficiency. Research by Rewald et al. (2015) and Porcel et al. (2012) suggests that both nutritional and molecular mechanisms play a role in reducing salt stress through AMF. Moreover, AMF can improve soil structure, thereby further supporting plant growth under both normal and saline conditions (Tang et al., 2022; Navarro et al., 2014). Studies, such as those by Rabie (2005), have demonstrated that AMF inoculation can significantly boost plant growth and yields in saline soils by optimizing the plant's nutritional status. Thus, integrating AMF inoculation into agricultural practices could be a cost-effective and sustainable method for enhancing the salt tolerance of crops like chickpea, offering protection against the detrimental impacts of salinity stress.

This study focuses on examining the effects of arbuscular mycorrhizal fungi (AMF) on the growth, physiological, and biochemical traits of chickpea plants under saline stress conditions. Additionally, it aims to develop a practical approach for integrating AMF into chickpea cultivation in salt-affected agricultural regions, addressing a critical challenge in sustainable crop production.

2 Materials and methods

2.1 Plant material

Six high-yielding desi-type chickpea lines having one tolerant (Digbijoy), three moderately susceptible (IC 268971, Radhey, Mahamaya 1) and two highly susceptible (Virat, DCP 93 – 3) [Kundu et al., 2023; Kumar et al., 2020] were picked up for the current study from the germplasm bank at the Department of Genetics and Plant Breeding, University of Calcutta (State-aided University), West Bengal, India. Surface sterilized (1.0% NaOCl) viable seeds of selected germplasms were sown (02.11.2020) in the plastic pots (uniform size, pot diameter 15.24 cm and pot capacity 1 kg dry soil per open pot) containing properly sterilized dry soil collected from university experimental farm. Another field trial was also conducted (06.11.2020) in Farmer's field in Kakdwip, South 24

Parganas, West Bengal, India [GPS coordinates of 21° 52' 59.88" N and 88° 10' 59.88 E] (Fig. 1 and Fig. 2) to show the effect of AMF on the crop in naturally salt affected areas.

2.2 Details of the mycorrhizal inoculum

The culture of *Glomus mosseae* was collected from the Centre for Mycorrhizal Culture Collection (CMCC), a culture repository under The Energy and Resources Institute (TERI), New Delhi for inoculating chickpea plants. The cultures were being maintained and multiplied in sterile pot sand:soil in 1:1 ratio. For pot experiment, the spores of *Glomus mosseae* were added to the sterilized experimental soil as 10 g of trap soil culture (approx. 100 spores g⁻¹ trap soil, M = 80%) per pot. At field condition the AM fungal inoculum was given at the rate of 4690 spores per 50 cm following the method of Ghazi (2002). Non-mycorrhizal soil was used as control for both conditions.

2.3 Salinity treatment

Salt stress was induced by supplementing pot soil with half-strength Hoagland's solution (Hoagland and Arnon, 1950) containing NaCl to achieve a concentration of 300 mM. After seedling emergence, five plants were maintained per pot and watered as required. Salt stress was maintained by irrigating with saline water at 15-day intervals, ensuring the electrical conductivity (EC) of the soil remained at 5.6 mmhos cm⁻¹, following the fertigation technique described by Manasa et al. (2017). Additionally, soil samples collected from the field at the time of sowing indicated an initial salinity level of 7.1 mmhos cm⁻¹, measured using a soil salinity meter.

2.4 Greenhouse and Field Experiment Design

The study was conducted in two phases: a controlled greenhouse experiment followed by a field trial to validate the greenhouse findings through morphological assessments.

2.4.1 Greenhouse Experiment

A total of four treatment groups were established, each consisting of five replicates. Two groups were maintained under non-saline conditions, while the other two were subjected to salinity stress. Among these, one group in each condition was inoculated with AMF. Seeds of selected chickpea germplasms were sown in both inoculated and non-inoculated pots and grown under controlled greenhouse conditions (25°C during the day, 20°C at night, 65% relative humidity, a 16-hour light/8-hour dark photoperiod, and a light intensity of 750 µmol m⁻² s⁻¹).

After sowing, agro-morphological parameters such as days to emergence and shoot-root length were recorded at 10 days after emergence (DAE). At physiological maturity, whole plants were harvested from each treatment/lines/replication in triplicate for the estimation of nodules/plant, plant height, number of branches plant⁻¹, leaf area index, number of pods plant⁻¹, pod length, number of seeds pod⁻¹, 100 seed weight and seed yield plant⁻¹. At physiological maturity, ten plants per treatment were harvested for yield assessment, while dry matter content was determined by oven-drying the plant material at 80°C until a constant weight was achieved.

In addition to agro-morphological traits, biochemical parameters were analyzed only in the greenhouse study to assess the impact of AMF on plant physiology under saline and non-saline conditions. The third top leaf (from the top) of each plant was collected at 75 DAS for biochemical analyses *i.e.*, the estimation of relative water content, membrane stability index, total chlorophyll content, and total phenolic content.

2.4.2 Field Experiment

A field trial was conducted to corroborate the greenhouse findings through morphological characterization. The experiment followed a randomized block design (RBD) with three replications. Two planting beds were prepared: one designated as the Control (C) (non-inoculated) and the other as the Treatment (T) (inoculated). In the T plots, AM fungal inoculum was incorporated into the furrows along with three seeds per treatment and subsequently covered with soil. Irrigation was managed using a drip system to maintain optimal soil moisture levels. To assess the impact of AMF inoculation under saline conditions, only agro-morphological parameters of six chickpea germplasms were recorded in the field study as stated in previous section.

2.5 Determination of arbuscular mycorrhizal colonization

Assessment of AMF colonization in chickpea roots, along with quantification of spore density in the rhizosphere, was conducted using standard methodologies. For root colonization, the fine root samples were carefully washed with ice-cooled water to remove adhering soil, then treated with 10% (w/v) potassium hydroxide (KOH) for clearing, followed by staining with 0.05% (w/v) trypan blue in a lactoglycerol solution, as outlined by Phillips and Hayman (1970). The percentage of root colonization was determined using the gridline intersect method (Giovannetti and Mosse, 1980), by examining stained root segments under a light microscope at 400× magnification for the presence of AM structures such as mycelia, vesicles, and arbuscules. The extent of colonization was calculated using the following formula;

$$\% \text{ Colonization} = \left(\frac{\text{Total number of AM positive segment}}{\text{Total number of segments studied}} \right) \times 100$$

AMF spore density in the rhizosphere was estimated using the wet sieving and decantation method, followed by density separation via sucrose centrifugation, as per the protocol established by Gerdemann and Nicolson (1963). 50 grams of air-dried rhizospheric soil, pre-sieved to remove large particles, was mixed with distilled water, thoroughly stirred, and passed sequentially through sieves with mesh sizes of 250 µm, 100 µm, and 45 µm. The fraction collected on the 45 µm sieve was subjected to centrifugation at 2000 rpm for 5 minutes. The sediment obtained was then mixed with a 50% sucrose solution and centrifuged again at 2000 rpm for 2 minutes. The supernatant, containing AMF spores, was filtered through a 45 µm sieve and washed extensively with distilled water to eliminate any remaining sucrose. Spores were examined and counted using a stereomicroscope at 40× magnification, with

only healthy and intact spores included in the final count. Results were reported as the number of spores per 50 g of soil, representing the AMF spore density within the rhizosphere.

2.6 Determination of relative water content (RWC)

Top fully expanded leaves were separated and were weighted to record the fresh weight, the leaf samples were then put in double distilled water for 4 hours, removed, cleaned with tissue paper and weighted to record turgid weight. The same leaf samples were put into the oven at 65° C for 48 hours to record the dry weight. Relative water content (RWC) of leaf was estimated using the following formula of Smart and Bingham (1974);

$$RWC = \left(\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \right) \times 100$$

2.7 Determination of membrane stability index (MSI)

For estimation of MSI, 100 mg of fresh leaf samples were taken in test tubes containing 10 mL double distilled water in two sets. One set was kept in water bath for 30 mins at 40° C and the electrical conductivity (EC) was recorded as C₁. Another set was kept in water bath at boiling temperature (100° C) and EC was recorded as C₂. MSI was calculated using the following formula of Sairam and Srivastava (2002);

$$MSI = [1 - (C_1 / C_2)] \times 100$$

2.8 Total chlorophyll content

Total chlorophyll content *i.e.* total photosynthetic pigments were estimated at vegetative, flowering, and pod filling stage of the crop. The data was collected and average values were calculated for all these stages. Fresh shoot tissues were finely chopped and placed in test tubes containing 10 ml of dimethyl sulfoxide (DMSO). The tubes were covered with aluminum foil and incubated in an oven at 65°C for 4 hours. Following incubation, the absorbance of the extracted chlorophyll solution was measured at wavelengths of 663 nm and 645 nm. Chlorophyll content was determined following the method described by Arnon (1949). Total chlorophyll (mg/g fresh weight) was calculated by summing chlorophyll a and chlorophyll b, using the following equations:

$$\text{Chlorophyll a (mg/g fresh weight)} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \left(\frac{V}{1000} \times W \right)$$

$$\text{Chlorophyll b (mg/g fresh weight)} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \left(\frac{V}{1000} \times W \right)$$

Here A represents the absorbance at the specified wavelengths, V is the volume of the extract in ml, and W is the weight of the fresh leaf sample in g.

2.9 Determination of total phenolic content

Total phenolic content was determined by the method of Bray and Thorpe (1954). For phenol determination, 100 mg fresh leaf sample was homogenized with 10 ml of 70% acetone and suspension was centrifuged at 6000x g for 10 minutes. To 1 ml supernatant, 1 ml of Folin-Ciocalteu reagent (1 N) and 2 ml of Na₂CO₃ (20% w/v) were added and final volume was made up to 10 ml with distilled water. The mixture was heated in a boiling water bath for one minute and cooled to room temperature. O.D. of solution was measured at 650 nm wavelength on UV-Vis spectrophotometer.

2.10 Statistical analysis

The recorded data were analysed by analysis of variance technique using statistical software SPSS-21.

3 Results and discussion

3.1 Influence of salt stress on agro-morphological parameters of chickpea in presence and absence of AMF

The study on effect of salinity stress on growth parameters in both inoculated and non-inoculated chickpea reported that salt stress significantly reduced the morphological as well as yield parameters in all the tested lines. Salinity stress caused significant reduction in days to emergence, seedling length, leaf area, nodules plant⁻¹, plant height, branches plant⁻¹, pods plant⁻¹, pod length, seeds pod⁻¹, 100 seed weight and seed yield plant⁻¹ by 200, 76, 13, 75, 36, 22, 39, 12, 32, 21 and 39% respectively relative to control (Table 1, Fig. 3). Such reduction under salinity may be due to osmotic stress and increased rate of ion transfer (Farooq et al., 2017). However, application of AMF mitigates the salinity induced reduction in all these parameters in all the lines.

Table 1

Influence of salt stress (300 mM NaCl) on agro-morphological parameters of chickpea in presence and absence of AMF in

		Days to emergence				Seedling length (7 DAE)				Number of nodules plant ⁻¹				Leaf Area index				Plant h
		T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁
T	Digbijoy	2.0	2.0	4.0	3.0	125.0	138.2	70.0	125.7	10.0	10.0	6.0	10.0	3.6	3.6	3.5	55.7	60.2
MS	IC 268971	2.0	2.0	6.0	3.0	132.6	142.3	29.0	124.7	10.0	11.0	2.0	10.0	3.3	3.5	2.9	57.1	60.4
	Radhey	2.0	2.0	6.0	3.0	155.7	166.9	22.0	147.9	9.0	9.0	2.0	9.0	3.2	3.2	2.8	59.5	65.8
	Mahamaya ₁	2.0	2.0	5.0	4.0	151.2	161.3	39.0	140.8	9.0	10.0	2.0	11.0	3.3	3.4	2.7	52.0	57.7
HS	Virat	2.0	2.0	7.0	3.0	125.0	144.6	20.0	125.4	10.0	8.0	1.0	9.0	2.3	2.6	1.9	54.6	59.9
	DCP 93 – 3	2.0	2.0	8.0	4.0	160.5	179.1	27.0	155.7	9.0	9.0	1.0	9.0	3.5	3.6	2.8	59.1	63.4
Mean		2.0	2.0	6.0	3.3	141.7	155.4	34.5	136.7	9.5	9.5	2.3	9.7	3.2	3.3	2.8	56.3	61.2
CD		0	0	0.5	0.8	6.5	4.9	7.8	6.3	0.2	0.4	0.2	0.2	0.2	0.3	0.2	1.0	1.2
		Number of pods plant ⁻¹				Pod length (mm)				Number of seeds pod ⁻¹				100 seed weight (g)				Seed y
		T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄	T ₁
T	Digbijoy	79.5	82.6	68.5	70.6	1.8	1.8	1.7	1.8	2.1	2.2	2.0	2.1	14.2	14.2	13.9	14.0	18.6
MS	IC 268971	75.6	80.2	50.3	70.2	1.7	1.7	1.5	1.7	2.3	2.3	1.6	2.0	13.6	13.6	11.0	13.2	16.6
	Radhey	82.1	86.3	49.6	71.6	1.7	1.7	1.5	1.7	2.1	2.3	1.5	1.9	10.3	10.3	9.0	10.0	20.2
	Mahamaya ₁	80.2	85.9	52.0	76.5	1.6	1.5	1.5	1.6	2.1	2.4	1.7	2.0	13.5	13.2	11.2	13.0	19.1
HS	Virat	79.3	84.7	38.6	65.2	1.7	1.7	1.4	1.6	2.2	2.6	1.0	1.6	14.2	14.1	9.1	13.9	18.0
	DCP 93 – 3	78.1	82.0	30.2	69.1	1.6	1.5	1.3	1.5	2.1	2.2	1.2	1.7	14.3	14.3	9.6	13.8	19.1
Mean		79.1	83.7	48.2	70.6	1.7	1.7	1.5	1.7	2.2	2.3	1.5	1.9	13.4	13.3	10.6	12.9	18.6
CD		1.2	1.4	1.6	1.2	0.6	0.5	0.5	0.6	0.5	0.4	0.6	0.4	1.0	1.2	1.9	1.3	1.0
T1 = Control; T2 = Control + AMF; T3 = Saline; T4 = Saline + AMF																		

Field study under saline location confirms the results (Table 2) obtained from the green-house data. Similar trend of growth pattern was reported under saline location. Here, in saline soil (control) average days to emergence was 6 days whereas in AMF inoculated field (treatment) the days to emergence was 3.3 days, which was 45% improvement over control. Inoculation with AMF also significantly increase the value of other tested parameters namely seedling length, nodules plant⁻¹, leaf area index, plant height, number of branches plant⁻¹, number of pods plant⁻¹, pod length, seeds pod⁻¹, 100 seed weight and seed yield plant⁻¹ by 296, 322, 7, 52, 66, 95, 42, 36, 32 and 48% respectively over control. Moreover, highly susceptible lines also showed remarkable yield improvement over control in both greenhouse and field condition (about 46–69% for Virat and 100% for DCP 93 – 3) in presence of Gm under salt stress. AMF inoculation has been widely documented to trigger diverse beneficial responses in host plants, encompassing morphological, physiological, and biochemical enhancements (Abd Allah et al., 2019; Begum et al., 2019; Hashem et al., 2016). These effects are largely attributed to AMF-mediated improvements in soil solution chemistry, which enhance nutrient availability-particularly phosphorus-and facilitate efficient nutrient uptake (Rao and Chaitanya, 2016; Ahanger et al., 2014; Ahmad et al., 2014). Such symbiotic interactions enable plants to better tolerate osmotic stress, maintain cellular homeostasis, and sustain growth under salinity. From an agricultural perspective, AMF application offers a viable strategy to improve crop performance in salt-affected soils, thereby enhancing food security in vulnerable regions. Environmentally, AMF contributes to soil health restoration, reducing the need for synthetic fertilizers and promoting microbial biodiversity (Singh et al., 2024). Economically, the increased yield and reduced input costs associated with AMF use present a cost-effective, sustainable alternative for farmers. Overall, these findings highlight AMF as a promising bioresource for improving chickpea productivity under salinity stress, aligning with the goals of climate-resilient and sustainable agriculture (Kotula et al., 2019; Chouhan et al., 2023).

Table 2

Field study: Influence of salt stress at a farmer's field at Kakdwip, West Bengal on agro-morphological parameters of chickpea in presence and absence of AMF

		Days to emergence		Seedling length (7 DAE)		Number of nodules plant ⁻¹		Leaf Area index		Plant height (cm)		Number of branches plant ⁻¹	
		Saline	S + AMF	Saline	S + AMF	Saline	S + AMF	Saline	S + AMF	Saline	S + AMF	Saline	S + AMF
T	Digbijoy	4	3	70	125.7	6	10	3.5	3.6	58.6	61.9	5.0	5.5
MS	IC 268971	6	3	29	124.7	2	10	2.9	2.9	40.2	59.6	3.6	6.5
	Radhey	6	3	22	147.9	2	9	2.8	3.2	39.2	52.9	3.9	6.2
	Mahamaya ₁	5	4	39	140.8	2	11	2.7	3	38.6	51.8	4.0	5.9
HS	Virat	7	3	20	125.4	1	9	1.9	2.1	25.4	58.2	2.4	5.9
	DCP 93 – 3	8	4	27	155.7	1	9	2.8	3	22.9	56.8	2.6	5.0
Mean		6	3.3	34.5	136.7	2.3	9.7	2.8	3	37.5	56.9	3.5	5.8
CD		0.5	0.8	7.8	6.3	0.2	0.2	0.2	0.3	1.6	1.0	1.7	1.2
		Number of pods plant ⁻¹		Pod length (mm)		Number of seeds pod ⁻¹		100 seed weight (g)		Seed yield plant ⁻¹ (g)			
		Saline	S + AMF	Saline	S + AMF	Saline	S + AMF	Saline	S + AMF	Saline	S + AMF		
T	Digbijoy	68.2	70.6	1.5	1.8	1.9	2.1	13.0	13.9	16.8	18.5		
MS	IC 268971	30.6	70.2	1.3	1.7	1.5	2.0	9.9	13.6	10.2	15.9		
	Radhey	31.2	71.6	1.3	1.7	1.5	1.9	9.8	10.1	10.9	18.4		
	Mahamaya ₁	30.9	76.5	1.3	1.6	1.4	2.1	9.6	12.3	11.0	15.2		
HS	Virat	22.5	55.2	1.0	1.6	0.9	1.7	7.2	12.4	7.1	12.0		
	DCP 93 – 3	22.8	59.1	1.0	1.5	1.0	1.7	7.6	12.9	6.3	12.6		
Mean		34.4	67.2	1.2	1.7	1.4	1.9	9.5	12.5	10.4	15.4		
CD		1.9	1.6	0.5	0.8	0.5	0.8	0.6	1.0	1.9	2.1		

Combined analysis of variance (ANOVA) study showed a varying treatment effect on the observed parameters (Table 3). Highly significant interaction effect of germplasm x treatment was observed on days to emergence, number of pods plant⁻¹, pod length and seed yield plant⁻¹. Meanwhile, the single factor treatment significantly/ highly significantly affected all observed parameters except number of branches plant⁻¹ and 100 seed weight. Significant germplasm x treatment interaction effect suggested that the chickpea germplasms were not consistent in their ranks in the observed parameters, where the rank changed following the change of the treatment (inoculated and non-inoculated) (Mau et al., 2017).

Table 3

ANOVA showing F-values and level of significance for agro-morphological parameters of six germplasms of chickpea in presence and absence of AMF under saline location

F-value (calculated)							
SOV	df	Days to emergence	Seedling length (7 DAE)	Number of nodules plant ⁻¹	Leaf area index	Plant height (cm)	Number of Branches plant ⁻¹
Germplasms (G)	5	11.75***	23.26***	6.25**	0.35	10.43**	0.82
Treatment (T)	1	76.21**	16.45*	4.29*	1.20**	7.21*	3.70
G x T	1	12.16***	1.14*	0.89	0.72	1.39	2.29*
SOV	df	Number of pods plant ⁻¹	Pod length (mm)	Number of seeds pod ⁻¹	100 seed Weight (g)	Seed yield plant ⁻¹ (g)	
Germplasms (G)	5	0.61**	13.14**	49.21*	2.46	12.52*	
Treatment (T)	1	29.51***	7.25*	21.42**	7.16	16.76*	
G x T	1	7.20***	3.49***	9.13*	3.59	5.66***	
Level of Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; where, df = degrees of freedom, DAE: days after emergence							

3.2 Influence of salinity on mycorrhizal root colonization

Salinity not only negatively impacts the host plant but also adversely affects AMF. Control plants, which were not pre-inoculated with AMF, were colonized by AMF in non-sterile field soil, though the level of colonization was significantly lower compared to seedlings that were pre-inoculated. With increasing salinity, spore density and root colonization rates declined. Salinity stress led to a marked reduction in mycorrhizal colonization, spore numbers, mycelium, and arbuscules, consequently decreasing the intensity of colonization in the uninoculated plots. Specifically, the spore population decreased by 39%, while the numbers of mycelium and arbuscules were reduced by 45% and 25%, respectively, compared to non-saline conditions (Table 4). However, AMF inoculation under salt stress resulted in a significant increase in total spore population (535%) and structural colonization, with mycelium and arbuscule numbers increasing by 181% and 204%, respectively. These results are consistent with the findings of Abeer et al. (2016).

Table 4

Influence of salinity (300 mM NaCl) on mycorrhizal root colonization in chickpea

Non saline			Saline			Saline + AMF		
		Total spore/50 g soil	Total colonization %		Total spore/50 g soil	Total colonization %		Total spore/50 g soil
			Mycelium (M)	Arbuscules (A)		Mycelium (M)	Arbuscules (A)	
T	Digbijoy	100	40	10	59	24	8	319
MS	IC 268971	98	38	12	62	29	10	410
	Radhey	102	42	11	56	22	11	408
	Mahamaya 1	96	40	10	58	28	9	412
HS	Virat	100	39	13	65	19	5	400
	DCP 93 – 3	110	46	12	69	11	8	395
Mean		101.0	40.8	11.3	61.50	22.17	8.50	390.6
SEM ±		2.6	6.3	2.9	6.29	1.40	0.91	3.61
								0.72
								1.14

3.3 Influence of salt stress on bio-chemical parameters of chickpea in presence and absence of AMF in green house condition

Salinity stress is a major abiotic factor limiting chickpea productivity, particularly in vulnerable coastal ecosystems. Our study demonstrates that inoculation with *Glomus mosseae* significantly mitigates the adverse effects of salinity on key physiological and biochemical parameters in chickpea, enhancing stress tolerance under both greenhouse and field conditions.

Results depicting the impact of AMF and salinity individually as well as combined on physiological and biochemical parameters were presented in Table 5 and Fig. 4. Total chlorophyll, relative water content and membrane stability index were reduced by 33, 43 and 36% due to salinity stress, however amelioration

of 15, 49 and 7% over saline condition due to inoculation of *Glomus mossae* was observed in the tested chickpea lines. The significant alteration in pigment content under salinity stress may be attributed to the increased activity of chlorophyll-degrading enzymes and the inhibition of chlorophyllase by the accumulated ions (Guo et al., 2014). In saline soils, plants experience physiological drought because Na^+ and Cl^- ions bind to water, making it less available for plant mobilization (Klinsukon et al., 2021; Füzy et al., 2008), which leads to reduced plant growth and yield. Several studies have shown that plants inoculated with AMF maintain better water conductance in their roots, while also enhancing stomatal conductance and transpiration compared to uninoculated plants (Kumar et al., 2020; Kotula et al., 2019; Colla et al., 2008). Furthermore, AMF inoculation supports the host plant in nutrient acquisition, which ultimately improves the photosynthetic rate and water osmotic homeostasis (Porrás-Soriano et al., 2009; Sheng et al., 2008). Specifically, AMF boosts photosynthetic efficiency by enhancing Rubisco activity, improving electron transport rates, facilitating ATP synthesis, and rising the ATP-to-ADP ratio in leaf tissues. These effects are facilitated by higher nitrogen (N) and phosphorus (P) levels in the leaves (Bechtaoui et al., 2021; Kaschuk et al., 2009). Membrane stability, an indicator of cell membrane integrity under stress, was significantly compromised by salinity, as reflected in the decreased MSI. This reduction is primarily due to the overproduction of reactive oxygen species (ROS), which cause lipid peroxidation and cellular damage (Abbaspour et al., 2021). AMF inoculation likely curtailed ROS diffusion by enhancing the plant's antioxidant defense and scavenging capacity, thus protecting membrane integrity and reducing oxidative stress. This protective role aligns with recent findings in AMF-associated legumes under saline conditions (Tang et al., 2022; Kotula et al., 2019). Salinity significantly increased the amount of total phenolic content in all the lines but AM inoculation under salinity increased the amount of phenolic over respective controls in all the tested lines (Table 5 and Fig. 4). Present study revealed that salinity stress caused 19% increase in total phenolic content but when the salt-treated plants inoculated with AMF (salinity + AMF) recorded further increase of total phenolic content by 37% over the plant under salt stress without AMF. Phenolics are secondary metabolites with strong antioxidant properties, and their accumulation under stress conditions is a well-documented adaptive response to mitigate oxidative damage (Jerbi et al., 2022; Wada et al., 2014). The enhanced synthesis of phenolics in AMF-inoculated plants likely results from upregulation of key biosynthetic enzymes such as phenylalanine ammonia-lyase (PAL), contributing to improved stress resilience. Similar trends have been reported in other legumes and cereals under AMF and salinity interaction (Mitra et al., 2021; Tomar and Agarwal, 2013).

Collectively, these findings elucidate the multifaceted role of *G. mosseae* in enhancing chickpea salinity tolerance through physiological stabilization, improved water and nutrient relations, membrane protection, and increased antioxidant capacity. This study not only confirms previous observations but extends current understanding by demonstrating the effectiveness of AMF in the unique agro-climatic conditions of the Sundarbans Delta. The integration of AMF into chickpea cultivation systems offers a promising, sustainable strategy to enhance crop productivity in saline-prone regions, with broader implications for climate-resilient agriculture.

Table 5
Influence of salt stress (300 mM NaCl) on Relative water contents (RWC), Membrane Stability Index (MSI), Chlorophyll content and total phenolic content of chickpea in presence and absence of AMF in green house condition

		RWC				MSI			
		Control	Control + AMF	Saline	Saline + AMF	Control	Control + AMF	Saline	Saline + AMF
T	Digbijoy	43.0	45.2	32.3	43.5	70.2	71.5	65.8	67.0
MS	IC 268971	42.3	44.5	23.5	40.2	49.6	51.6	35.9	40.2
	Radhey	41.5	42.6	25.6	40.9	50.3	53.9	32.5	35.0
	Mahamaya 1	42.9	43.9	25.2	39.8	49.5	50.9	34.3	36.5
HS	Virat	43.5	44.5	19.5	26.9	51.6	53.8	19.6	21.2
	DCP 93 – 3	44.6	45.9	20.0	26.4	50.9	52.7	18.2	20.2
Mean		42.97	44.43	24.35	36.28	53.68	55.73	34.38	36.68
CD		1.02	0.98	1.00	0.89	0.79	0.96	0.84	1.03
		Chlorophyll content				Total phenolic content (mg/g)			
		Control	Control + AMF	Saline	Saline + AMF	Control	Control + AMF	Saline	Saline + AMF
T	Digbijoy	28.62	28.93	27.24	28.51	11.12	15.12	15.20	16.30
MS	IC 268971	26.69	26.79	20.36	22.35	12.19	18.01	13.13	19.19
	Radhey	27.58	27.69	20.55	22.49	11.23	18.11	14.22	18.21
	Mahamaya 1	26.33	26.86	21.32	22.00	11.14	19.12	12.02	19.11
HS	Virat	26.50	26.97	10.12	15.69	11.20	18.00	13.19	19.09
	DCP 93 – 3	26.96	27.00	9.55	14.87	11.19	14.12	13.09	19.14
Mean		27.11	27.37	18.19	20.99	11.35	17.08	13.48	18.51
CD		0.96	1.02	0.99	0.89	1.06	0.98	0.97	1.06

4 Conclusion

This study provides compelling evidence that arbuscular mycorrhizal fungi (AMF), specifically *Glomus mosseae*, play a pivotal role in enhancing the growth, yield, and physiological resilience of chickpea (*Cicer arietinum* L.) under salinity stress. Salinity-induced reductions in critical agro-morphological and biochemical parameters were significantly ameliorated by AMF inoculation in both greenhouse and field conditions. Notably, AMF symbiosis markedly improved seedling emergence, plant height, nodulation, pod formation, seed weight, and overall yield, even in highly susceptible chickpea genotypes. These improvements are attributed to AMF-mediated mechanisms including improved water-use efficiency, stabilization of membrane integrity, increased chlorophyll biosynthesis, and elevated production of phenolic compounds which contribute to osmotic adjustment and antioxidative defense. Thus, AMF inoculation proved effective in both controlled and field conditions, improving soil health and crop performance in saline environments, making it a valuable biofertilizer for sustainable agriculture. As soil salinization worsens, integrating AMF into chickpea cultivation offers a cost-effective, eco-friendly solution to boost productivity. Future research should explore large-scale trials, molecular insights, and tailored AMF consortia to reduce reliance on chemical inputs and support climate-resilient farming.

Declarations

Acknowledgements

All the authors are grateful to Vice-Chancellor, University of Calcutta for providing the infrastructural facilities to carry out this work.

Funding

There are no funders to report for this submission.

Author contributions

Varsha Kundu: Conceptualization, Methodology, Validation, Investigation, Data curation, Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing. **Moushree Sarkar:** Conceptualization, Supervision, Data analysis, Software, Validation, Writing – review & editing. **Sabyasachi Kundagrami:** Conceptualization, Supervision, Data curation, Validation, Writing – review & editing. All the authors read and approved the final manuscript.

Data availability

All the data generated or analysed during this study are included in this published article.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Research involving human participants and/or animals

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All the authors gave their consent for publication of the results.

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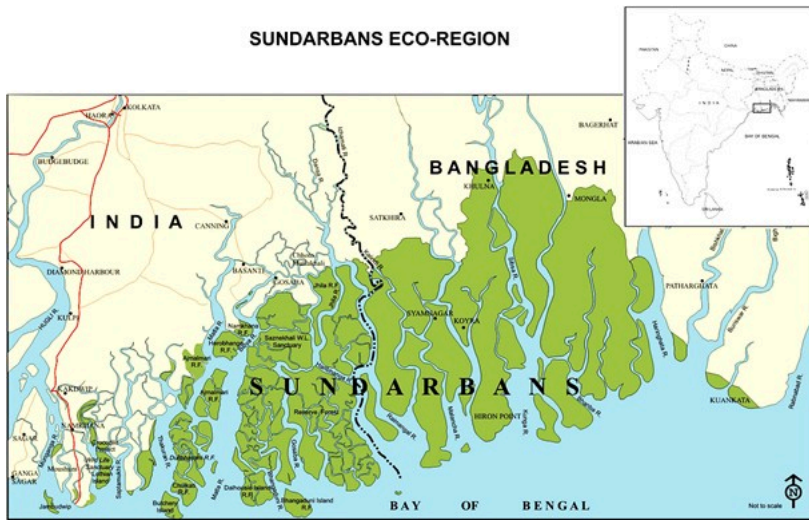


Figure 1

Map of Sundarbans UNESCO World Heritage Site (Source: State of Environment Report-II, West Bengal, 2021)

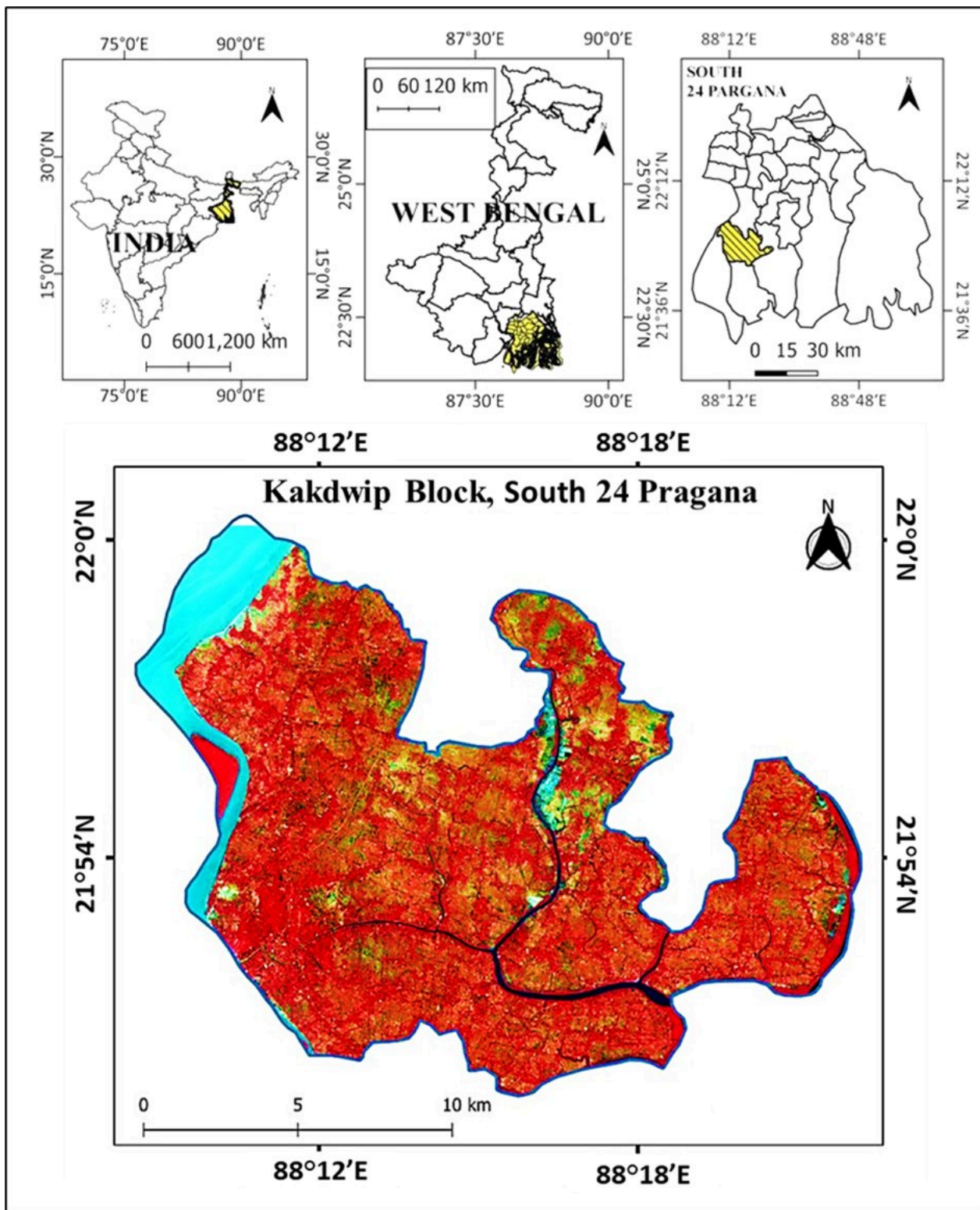


Figure 2

Location map of the study area

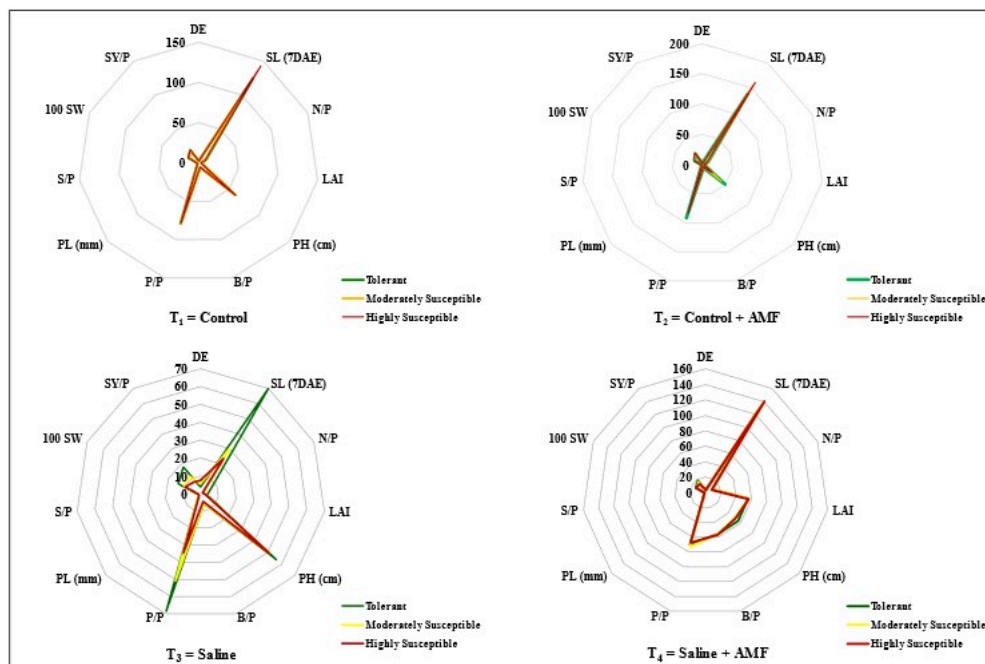


Figure 3

Representation of agro-morphological parameters of chickpea in control (T₁), control + AMF (T₂) saline (T₃) and Saline + AMF (T₄) treated plants under greenhouse condition. Radial graphs represent results relative to the higher value (indicated as 100%) for each parameter; DE = Days to emergence, SL (7DAE) = Seedling length (7DAE), N/P = Number of nodules plant⁻¹, LAI = Leaf Area Index, PH = Plant height (cm), B/P = Number of branches plant⁻¹, P/P = Number of pods plant⁻¹, PL = Pod length (mm), S/P = Number of seeds pod⁻¹, 100 SW = 100 seed weight, SY/P = Seed yield plant⁻¹ (g).

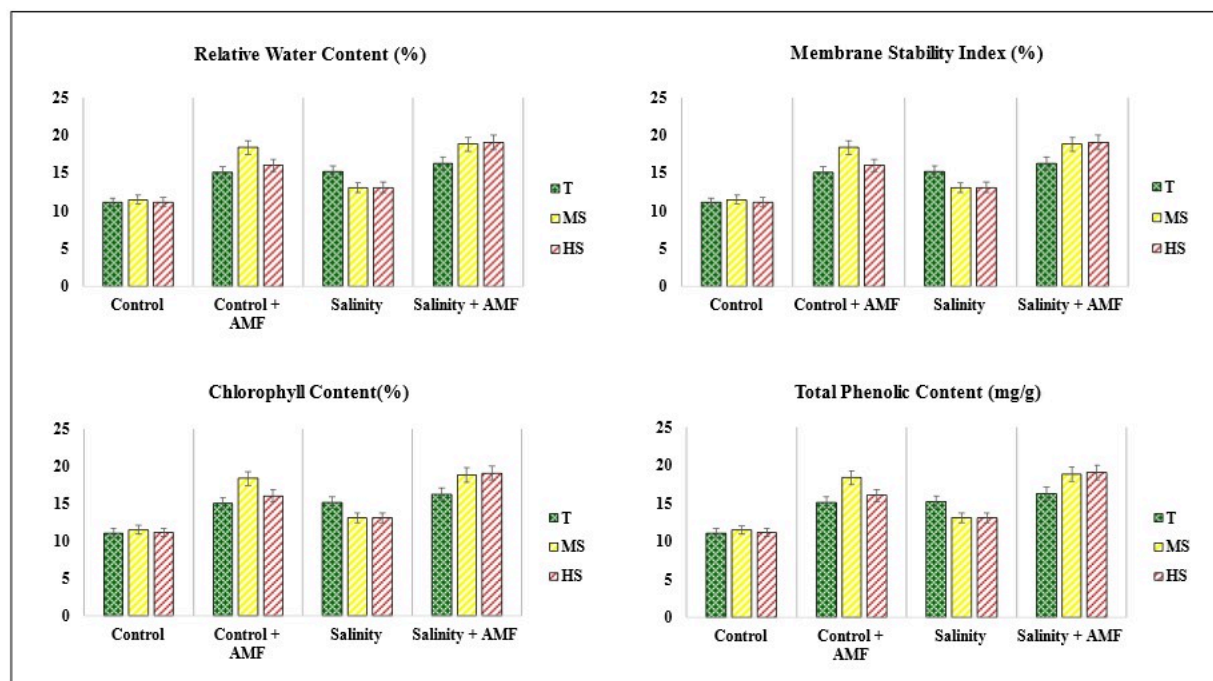


Figure 4

Influence of salt stress (300 mM NaCl) on relative water contents (RWC), membrane stability index (MSI), total chlorophyll content and total phenolic content of chickpea in presence and absence of AMF in green house condition [T: Tolerant, MS: Moderately susceptible, HS: Highly susceptible]