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Growth, Na^+/K^+ Partitioning and Yield of Chickpea Plants Alleviated From Salt Stress by Magnetopriming

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A B S T R A C T

Chickpea seeds of two varieties magnetoprimed with 100 mT 1h static magnetic field were subjected to salinity stress (80 mM NaCl) from germination to maturity. Physiological traits such as total root length, surface area, volume of the roots, plant height, leaf area and dry weight were significantly improved in plants from magnetoprimed seeds of both the varieties. Total chlorophyll content enhanced significantly under priming in leaves of Pusa 1053 compared to Pusa 256 under all treatments. Under salinity, the Na^+/K^+ ratio was less in plants from primed seeds compared to unprimed seeds irrespective of the plant parts indicating the regulation and partitioning of Na^+ at cellular level which helps in imparting tolerance to chickpea genotypes under stress. We hypothesise that Ca^{2+} mediated signaling that results due to magnetic field exposure (ion cyclotron theory) and salinity stress may signal the activation of SOS pathway that regulates ion homeostasis in plants. Our results also evinced that magnetopriming of dry seeds can be effectively used as a pre-sowing treatment for mitigating adverse effects of salinity in chickpea genotypes.

Introduction

Chickpea (*Cicer arietinum* L.) is a major food legume and an important source of protein for vegetarian population in many countries. Cultivars grown in India are either *microcarpa* also called 'desi' or *macrocarpa* also called 'kabuli' types (Siddique *et al.,* 2002). Chickpea is conventionally cultivated in marginal areas and saline soils (Rao *et al.,* 2002) and like many leguminous crops, is highly sensitive to salinity (Ashraf and Waheed, 1993). It cannot tolerate salinity levels higher than 6 dS/m (Dua, 1992). Salinity inhibits growth of plants by creating an unfavourable osmotic potential in the soil that prevents water uptake

(*Welbaum et al.,* 1990) and by the toxic effects of Na^+ and Cl^- ions on the plants (*Khajesh-Hosseini et al.,* 2003). NaCl toxicity is, in turn, known to disturb nutrient absorption by plants as K^+ absorption is inhibited and Na^+ enhanced (Greenway and Munns, 1980).

Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain using these approaches is limited. The cost associated with implementation of large engineering schemes for reclamation, drainage and irrigation are high and often temporary solution to the problem is achieved. The other approach of exploiting the genetic variation in the germplasm for obtaining salt tolerant traits that can be introgressed in the desired genotype has also met with limited success due to lack of efficient selection criteria, time and labour consuming procedures (Shannon and Grieve, 1999).

Rapid seed germination or early vigour of crop is an adaptive strategy for alleviation from salinity stress under field conditions as seedling establishment can be ensured (Sharma and Sen, 1989). Seed priming is a pre sowing seed enhancement method that is applied to seeds which are sown under different stress conditions to improve the rate and synchronization of seed germination.

Magnetopriming (exposure to static magnetic field) is a non-invasive physical stimulant used for improving vigour and emergence in seeds under non stressed and stressed condition. Numerous reports are available on the stimulating effects of magnetopriming on plant growth and yield under non-stressed condition (Phirke *et al.,* 1996; Harichand *et al.,* 2002; Radhakrishnan and Ranjitha Kumari, 2012). These were supported by response of magnetic field exposure on enhanced level of assimilatory pigments, chlorophyll and average nucleic acids in plants (Racuciu *et al.,* 2006). Under stress condition, magnetically treated plants alleviated inhibitory effects of heat and drought stress (Ruzic and Jerman, 2002) and increased saline-alkali tolerance (Xi *et al.,* 1994).

Electromagnetic treatments led to a significant increase in leaf area, leaf dry weight, mean fruit weight, fruit yield per plant of tomato in normal (De Souza *et al.,* 2006) and late season (De Souza *et al.,* 2005). Exposure to static magnetic field helps maize seedlings to withstand moisture stress conditions by improving the water relations in the seeds (Anand *et al.,* 2012). In the present study, the effect of magnetopriming of chickpea seeds on growth and ionic partitioning under salinity stress was evaluated.

Materials and Methods

Seed material and salinity treatment

Chickpea seeds of kabuli (Pusa 1053) and desi (Pusa 256) were exposed to static magnetic field of 100 mT for 1 h as described in our previous study (Thomas *et al.,* 2013). Seeds had 7.6% moisture and 98 % germination at the beginning of the experiment. Seeds were sown in pots filled with sandy loam soil (sand, silt and clay as 62.5, 25.8 and 11.7% respectively; pH, 7.9; electrical conductivity = 1.26 dSm^{-1}).

Salinity was artificially created in the pots by maintaining the salinity level from germination to reproductive stage following the procedure of Vadez *et al.,* (2007). The saline treatment was applied as 80 mM solution of NaCl in sufficient volume to wet the soil to field capacity. The pH and electrical conductivity for saline soil was 8.1 and 6.3 dSm^{-1} respectively. The saline treatment was applied at sowing and thereafter, the pots were watered with tap water and maintained close to field capacity (by gravimetric determination). In both non saline and saline pots, six chickpea seeds were planted and later

thinned to four plants pot^{-1}. There were three replicate pots for each cultivar, magnetic treatment and salinity level. In each experiment the design was completely randomized block design with two factors (magnetic treatment and salinity). Two experiments were planted side by side: one for the evaluation of biomass and the other for seed yield/pot.

Root and shoot growth parameters

The root samples were washed gently with a stream of water for complete separation of roots from the soil. Scanning and image analysis for root characteristics (total root length, surface area and volume) was carried out using Root Scanner (LA 1600). Plant height was measured with the meter scale from the soil line to the shoot tip. Leaves were separated from the stem of the plants and their total area was measured using leaf area meter (LICOR-100 automatic leaf area meter, Lincoln, USA). These leaves were then dried along with stem part in a hot air oven at 80^{o}C till constant weight of shoot was obtained.

Net rate of photosynthesis and chlorophyll content

Net rate of photosynthesis was measured at 70 DAS on the second mature leaf from the top with LI-6400 system (LICOR, USA) by giving constant light of 1000 µmol m^{-2}s^{-1}. Air temperature during measurement was 26^{o}C and ambient CO_2 was 380 ppm. Net rate of photosynthesis was expressed as µmol CO_2 m^{-2}s^{-1}. Chlorophyll was determined by the non-maceration technique given by Hiscox and Israelstam (1979). Fresh leaf samples (100 mg) from different treatments were extracted with 10 ml DMSO (Dimethyl Sulphoxide) in a capped vial kept in an oven at 65^{o}C for about 3 hours and the absorbance read at 645 and 663 nm in a UV visible spectrophotometer.

Yield components

Five replicates per treatment were harvested at physiological maturity and biomass, number of pods, seed number and seed weight per plant were recorded.

Na^+ and K^+ content in the roots, stem and leaves of the plants

Sodium and potassium content was measured in dried samples of root, stem and leaves of the plant after harvest following the procedure of Miller (1998). One gram of dried plant sample (root, stem and leaves) was digested with 10 ml of triacid mixture HNO_3: H_2SO_4: $HClO_4$; (9: 2: 1) and digested on a hot plate at 60^{o}C for half an hour. The temperature was subsequently increased to 120°C and 250°C and mixture heated for another half an hour at each temperature till the solution became clear. After cooling at room temperature the volume of the solution was made to 50 ml and filtered before taking the reading with a Flame Photometer.

Statistical analysis

The experimental design was completely randomized block design. Values for growth and yield were means of 5 replicates per treatment, and the values of chlorophyll content, photosynthesis rate and sodium and potassium content were means of 3 replicates. The growth and yield parameters were subjected to analysis of variance, and least significant difference between means performed at probability level of $P< 0.01$ Data for other parameters were expressed as mean values + SEM.

Results and Discussion

Root growth characteristics

A noticeable increase in root growth in terms of total root length, root surface area and root

volume was observed in magnetoprimed seeds under saline and non-saline conditions (Fig. 1). Root surface area and volume increased by two fold in roots from magnetoprimed Pusa 1053 under non-saline and saline conditions. On the other hand, magnetopriming resulted in greater increase in these root parameters (3.6 fold in root volume and 2.5 fold in surface area respectively) in Pusa 256 under non saline compared to saline conditions (Fig. 1).

Shoot growth characteristics

Plants from magnetoprimed seeds showed a significant increase in height in both the genotypes under non saline and saline soils. Genotypic variation was observed under saline conditions with 97 and 65 % enhancement in Pusa 256 and Pusa 1053 respectively (Table 1). Leaf area per plant also increased when the seeds were magnetoprimed before sowing. The difference was more marked in Pusa 1053 under salinity with the magnitude of increase being up to 89% compared to 27% in Pusa 256 (Table 1). Shoot dry weight enhanced significantly in magnetoprimed seeds of both the varieties under non saline conditions. Salinity affected shoot growth adversely in both genotypes but the advantage of magnetopriming was more evident in Pusa 256 as shoot dry weight increased to 6.18 g/plant in comparison to 3.6 g/plant in unprimed seeds (Table 1).

Net rate of photosynthesis and chlorophyll content

Magnetopriming increased net photosynthesis rate in both the genotypes under non saline and saline conditions. Salinity resulted in 62 and 66% reduction in net photosynthetic rate in plants from unprimed seeds of Pusa 256 and Pusa 1053 respectively. Magnetopriming compensated this decline by significantly increasing net photosynthetic rate by 26 and 15% in Pusa 256 and Pusa 1053 respectively over unprimed seeds (Fig. 2a). Leaves of plants from magnetoprimed seeds showed 50 and 43% increase in total chlorophyll content under non-saline and 50 and 12% under saline conditions in Pusa 1053 and Pusa 256 respectively (Fig. 2b).

Yield components

The biomass of magnetoprimed seeds increased under both the growing conditions. Under salinity, the difference in dry weight between plants from primed and unprimed seeds was more marked in Pusa 256 (75%) than in Pusa 1053 (58%) (Table 2). Under non- saline conditions, both the genotypes recorded similar increase in biomass in primed seeds in comparison to unprimed seeds.

The number of pods/plant also increased in plants from magnetoprimed seeds under saline and non- saline conditions. Under non saline conditions there was no genotypic variation as magnetoprimed seeds of both the varieties showed 23% increase in pod number whereas under saline conditions an improvement of 34 % was seen in Pusa 1053 and 41% in Pusa 256 compared to their respective unprimed seeds (Table 2). Magnetopriming also resulted in increase in the number of seeds/plant under both the growing conditions with marked improvement in Pusa 1053. Seed weight per plant significantly enhanced in plants from magnetoprimed seeds with 24 and 15% in Pusa 1053 and Pusa 256 respectively under normal conditions. Under saline conditions, Pusa 1053 showed a 32% increase in yield with no significant variation in Pusa 256.

Sodium and potassium content in roots, stems and leaves of plants

Sodium and potassium concentration was measured in roots, stems and leaves of the plants of both the varieties grown under salinity.

Fig.1 Effect of magnetopriming on a) Total root length b) Root surface area and c) Root volume at 70 DAS in chickpea genotypes Pusa 1053 and Pusa 256 under non-saline and saline conditions. Values are means of 3 measurements. Bars represent ± SE (n= 3). UPC= unprimed control; MPC = magnetoprimed control; UPS = unprimed saline; MPS = magnetoprimed saline

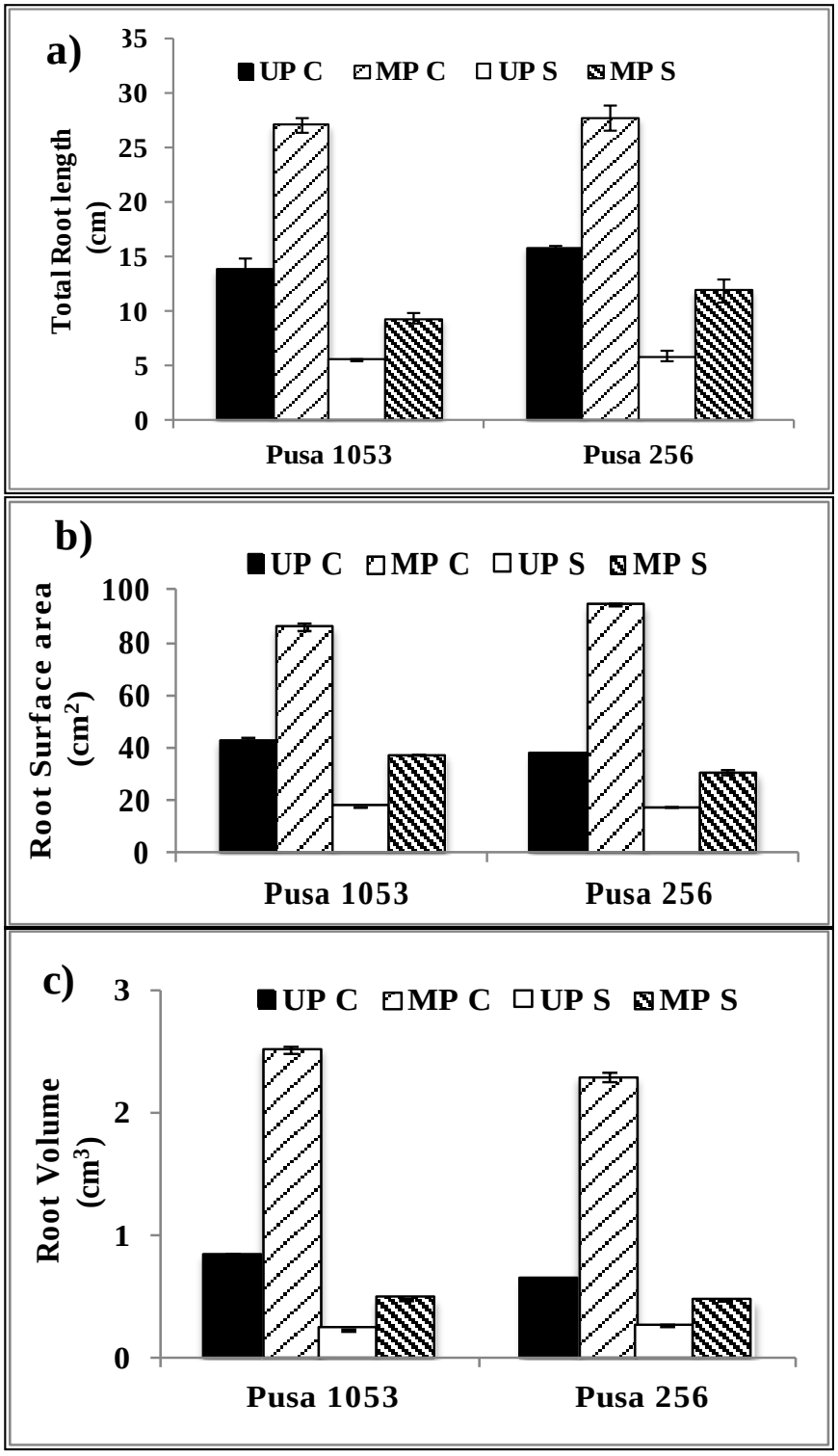

Fig.2 Effects of magnetopriming on a) photosynthesis b) total chlorophyll content of 70 days old magnetoprimed and unprimed chickpea genotypes Pusa 1053 and Pusa 256 under non-saline and saline conditions. Values are means of 3 measurements. Bars represent ± SE (n= 3). UPC= unprimed control; MPC = magnetoprimed control; UPS = unprimed saline; MPS = magnetoprimed saline

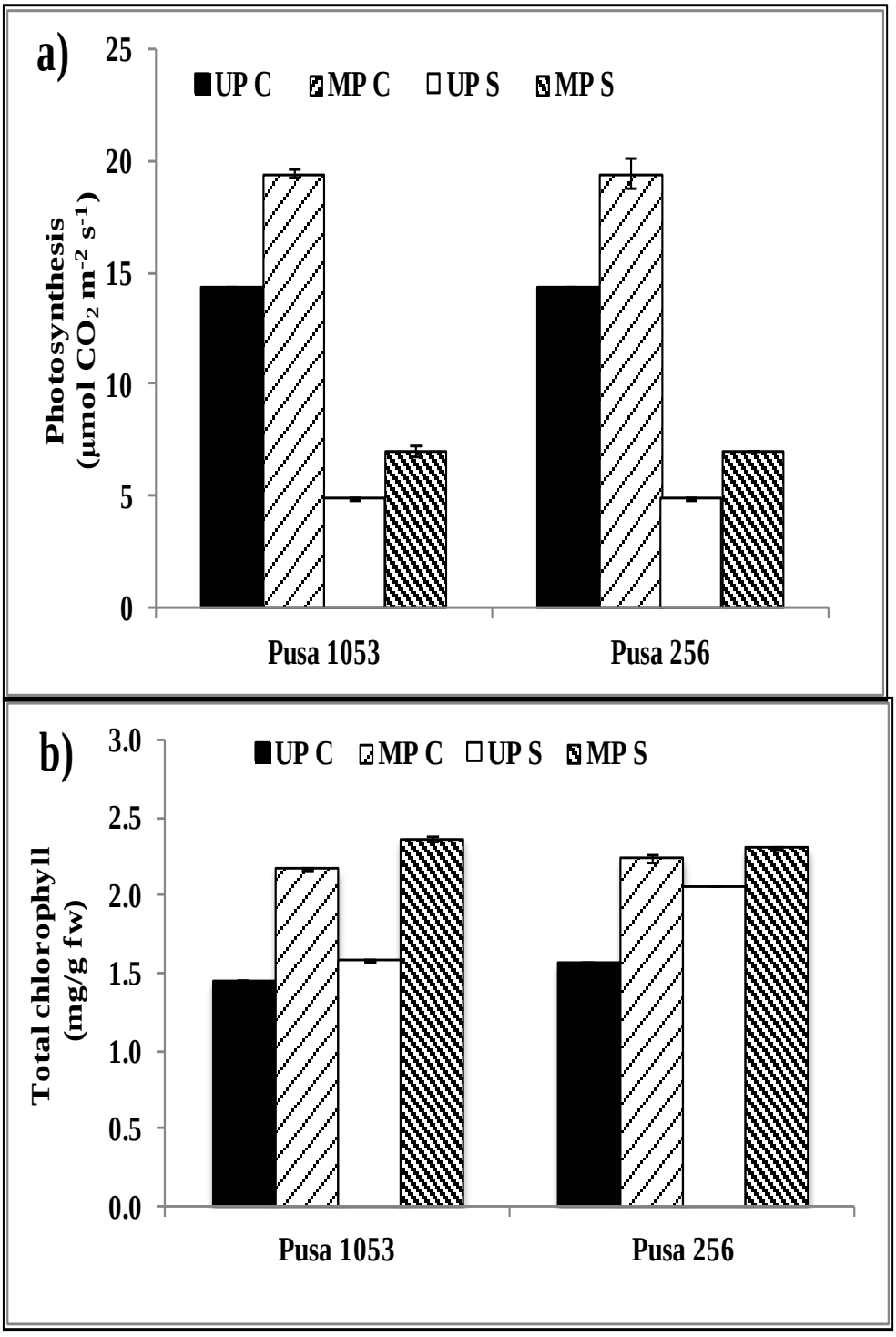

Fig.3 Effects of magnetopriming on a) sodium content b) potassium content and c) Na^+/K^+ ratio in root, stem and leaves of magnetoprimed and unprimed chickpea genotypes Pusa 1053 and Pusa 256 under salinity. Values are means of 3 measurements. Bars represent ± SE (n= 3). UPS = unprimed saline; MPS = magnetoprimed saline

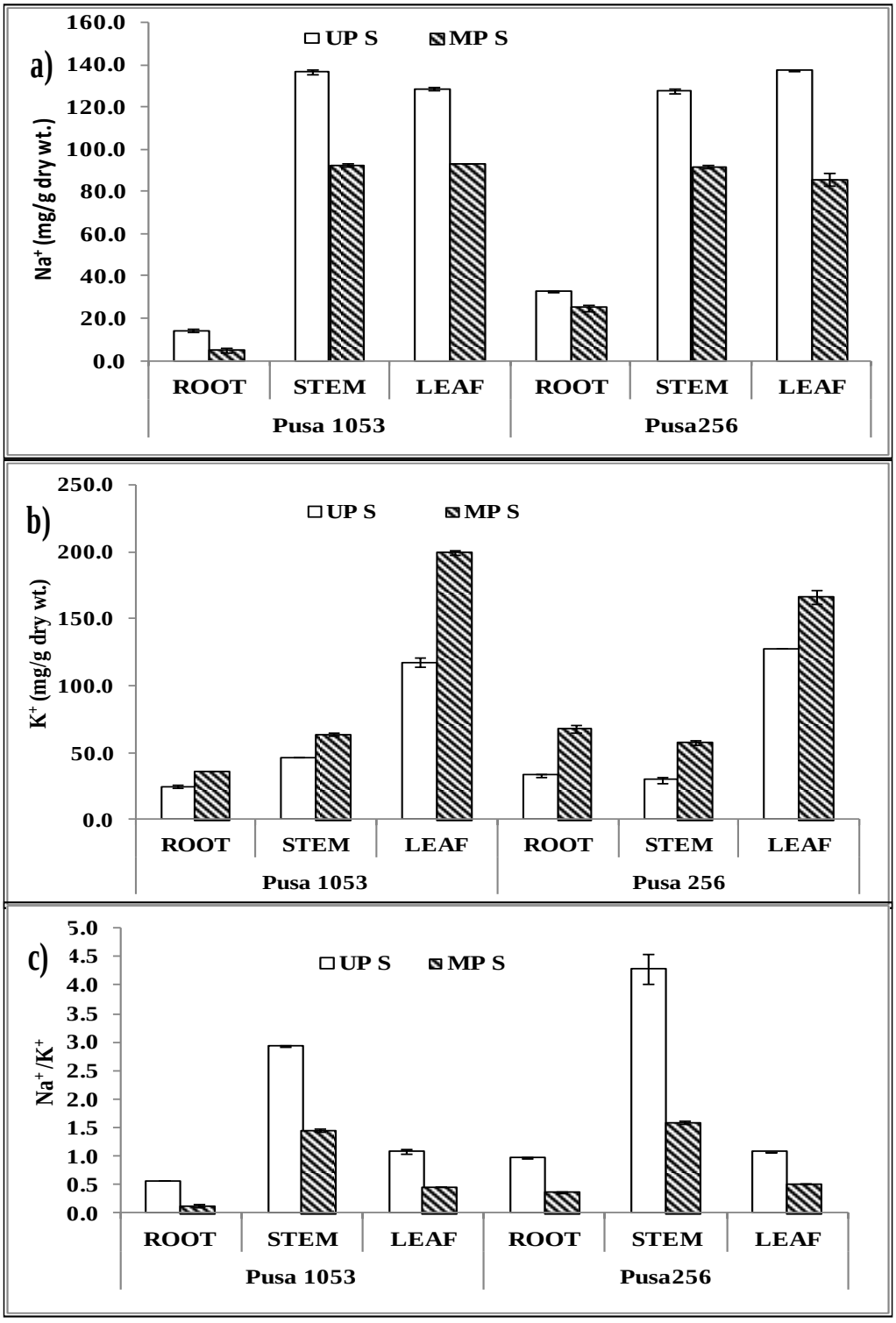

Table.1 Effect of magnetopriming on plant height, leaf area/plant and shoot dry weight/plant at 70 DAS of chickpea genotypes Pusa 1053 and Pusa 256 under non-saline and saline conditions. Values are means ± S.E. (n=5). UP= Unprimed, MP = Magnetoprimed. LSD (P < 0.01) Plant height = 1.68; Leaf area/ plant = 72.39; shoot dry weight/ plant = 1.34

	Pusa 1053				**Pusa 256**			
	CONTROL		SALINE		CONTROL		SALINE	
	UP	MP	UP	MP	UP	MP	UP	MP
Plant height (cm)	29.34±0.33	34±0.35	9.53±0.35	15.74±0.72	25.19±0.45	29.27±0.24	5.04±0.47	9.91±0.19
Leaf area/plant (cm^2)	264.22±6.90	331.85±5.71	69.91±5.66	132.21±1.60	197.17±1.26	312.67±2.91	75.15±1.02	96.59±1.48
Shoot dry weight/plant (g)	7.19±0.17	10.37±0.17	1.95±0.05	2.53±0.09	7.64±0.17	9.47±0.07	3.60±0.11	6.18±0.13

Table.2 Effect of magnetopriming on biomass/plant, number of pods/plant, seed number/ plant and seed weight per plant at harvest of chickpea genotypes Pusa 1053 and Pusa 256 under non-saline and saline conditions. Values are means ± S.E. (n = 5). UP= Unprimed, MP = Magnetoprimed. LSD (P < 0.01) Biomass/plant = 7.68; No. of pods/plant = 5.13; Seed no. /plant = 3.23; Seed weight/ plant = 2.18

	Pusa 1053				**Pusa 256**			
	CONTROL		SALINE		CONTROL		SALINE	
	UP	MP	UP	MP	UP	MP	UP	MP
Biomass/ plant (g)	28.64 ± 0.65	44.23 ± 0.79	13.56 ± 0.65	21.45 ± 0.71	23.67 ± 0.93	35.65 ± 0.65	12.25 ± 0.67	21.43 ± 0.56
No. of pods/plant	16.2±0.58	20±0.89	9.42±0.75	12.62±0.61	20±1.30	24.6±0.75	11.2±0.73	15.82±0.37
Seed no./plant	32.4±1.17	40±1.79	18.83±1.49	25.23±1.21	40±2.61	49.2±1.49	22.4±1.47	23.64±0.75
Seed weight (g/plant)	6.40±0.71	7.97±0.73	3.64±0. 51	4.82±0. 45	6.00±0. 36	6.90±0. 80	3.30±0. 37	3.55±0.62

In general, sodium content was low and potassium more in roots, stem and leaves from magnetoprimed plants in comparison to unprimed plants. Roots of Pusa 1053 showed upto 66% less sodium content than roots from unprimed treatment. Magnitude of variation in potassium content was more in roots and stem of Pusa 256 in comparison to leaves (102, 93 and 30% for roots, stem and leaves respectively). On the contrary Pusa 1053 showed 70% more K^+ content in the leaves from magnetoprimed plants compared to unprimed (Fig. 3b).

In saline condition, the Na^+/K^+ ratio was more in plants from unprimed seeds compared to primed seeds irrespective of the organs and the genotypes. Roots of both the genotypes under salinity showed a marked differentiation in the Na^+/K^+ ratio when unprimed and primed seeds were compared (76 and 62% for Pusa 1053 and Pusa 256 respectively) (Fig. 3c).

The presence of high concentration of salts in the soil lowers soil water potential, thus reducing the ability of plants to take up water. This osmotic or water deficit effect of salinity may be reduced to some extent by change in rooting pattern that facilitates better uptake of water. In our study, a significant increase in root growth parameters, promoted by magnetopriming indicated the alterations in root architecture that may help in adaptation to salinity stress. It has been observed that maize seedlings adapt to low water potential by making the walls in the apical part of the root more extensible (*Wu and Cosgrove*, 2000). This was accomplished in part by increase in expansin activity and in part by other more complex changes in the cell wall. It is possible that plants from magnetoprimed seeds have greater modulation of both the activity of wall enzymes and physical properties of the wall matrix that help in better root length and root surface area. *Rajendra et al.,* (2005) correlated the increase of root growth to increase in mitotic index as well as ^{3}H-thymidine incorporation into DNA in seeds of *Vicia faba* exposed to 100 µT power frequency magnetic fields.

Plants from magnetoprimed seeds showed a significant increase in rate of photosynthesis and chlorophyll content over the plants from unprimed seeds under normal and saline conditions. It has been reported that magnetoprimed seeds had a long lasting stimulatory effect on plants as higher performance index for photosynthesis contributed to higher efficiency of light harvesting that consequently increased biomass in plants from treated plants (*Shine et al.,* 2012). Also, exposure to magnetic field increased photochemical activities in a unit of chlorophyll molecule resulting in increase of green pigment of wheat and bean (*Lebedev et al.,* 1977). Our studies corroborate with these findings as an increase in net photosynthetic rate could explain increased growth in terms of increase in height, leaf area and biomass of the plants from magnetoprimed seeds of both the genotypes under non saline and saline soils.

Yield increase in magnetoprimed plants was a consequence of increase in biomass that led to increase in the number of pods and seeds in Pusa 1053. Recently, *Turner et al.,* 2013 associated salinity tolerance of chickpea genotypes with higher shoot biomass and consequently increased pod and seed number. *Singh et al.,* (2004) also reported a positive correlation of yield with biomass and chlorophyll content in chickpea. The improvement induced by the magnetic treatment was consistent with the results of other studies (*Phirke et al.,* 1996; *Amaya et al.,* 1996) where root, stem and plant fresh weight enhanced in plants from primed seeds. Seed priming of chickpea with water and mannitol has been reported to improve

seedling growth under water deficit stress (*Kaur et al.,* 2002). *Musa et al.,* (1999) reported that over-night priming of chickpea seeds gave better crop production in Bangladesh. Priming of seeds with water promoted seedling vigour, yield and crop establishment of chickpea, maize and rice in India (*Harris et al.,* 1999).

Na^+/K^+ ratio is widely used as a selection criterion for screening of genotypes under salinity. Roots of both varieties showed lesser sodium content than stem and leaves irrespective of priming treatment in the saline conditions. This indicates that sodium exclusion occurs at the level of roots in both the genotypes under saline conditions. Also, sodium content decreased in all plant parts from magnetoprimed seeds of both the varieties compared to unprimed counterparts. The concentration at which NaCl accumulates in the stem depends on the salt concentration in the soil solution, percentage of salt taken up by the roots and percentage of water retained in the leaves. Magnetoprimed seeds showed a selective uptake, as K^+ concentration was higher compared to Na^+ in roots, stem and leaves. This is due to well established antagonistic effect on uptake of these two elements (*Qadar* 1988). The lower Na^+/K^+ ratio in plants from magnetoprimed seeds was advantageous for imparting tolerance to chickpea genotypes. The low Na^+/K^+ in roots and leaves indicate the regulation and partitioning of Na^+ at cellular level. Roots of both the genotypes showed a marked differentiation in the Na^+/K^+ ratio between unprimed and primed seeds (76 and 62% for Pusa 1053 and Pusa 256 respectively). Leaves from magnetoprimed plants of both the genotypes have 50% less Na^+/K^+ suggesting their protection from sodium build up in leaves. It is observed that salt tolerant genotypes have lower rate of Na^+ loading and better capacity to sequester it as it enters the leaf (*Davenport et al.,* 2005).

Experimental evidence exists to suggest that exposure to salt stress generates the increase in Ca^{2+} in cytoplasm of root cells that acts as a second messenger for adaptive signaling (*Tracy et al.,* 2008). This in turn leads to Ca^{2+}-dependent increased activity of SOS1, a plasma membrane Na^+-H^+ antiporter that facilitates adaptation through sodium efflux (*Chung et al.,* 2008). Similarly, the ion-cyclotron resonance hypothesis that explains the magnetic field induced stimulation of growth in plants postulates that the ion cyclotron resonance may interfere with the Ca^{2+} ion sequestering, thereby, enabling the rise in free Ca^{2+} concentration in the system. We speculate that the increased Ca^{2+} concentration as a result of magnetic field exposure and salinity may signal the Ca^{2+} mediated activation of SOS pathway that regulates ion homeostasis in plants. It will be of interest to determine the expression pattern of *SOS* genes to establish their role in Na^+/K^+ partitioning in plant organs from magnetoprimed seeds. The increased uptake of Ca^{2+} ions in rice seedlings grown from seeds exposed to pulsed magnetic field have also been found to be responsible for better growth of leaf, meristematic tissues in stems and roots (Saktheeswari and Subrahmanyam 1989). The increase in leaf area and shoot dry weight in plants from magnetoprimed seeds grown under saline conditions may have resulted from modulation of Ca^{2+} levels. Our study elucidates that magnetopriming of chickpea seeds with static magnetic field of 100 mT for 1h has the capacity to increase the growth parameters in roots, rate of photosynthesis and chlorophyll content that help the plants to adapt to salinity stress. Lower Na^+/K^+ ratio in different plant parts helps in imparting tolerance to magnetoprimed plants of chickpea under salinity stress. Integrated at whole plant stress response level, all these responses lead to increase in yield in magnetoprimed seeds under non saline and saline conditions.

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