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Comparative Effect of NaCl and PEG on Physiological and Biochemical Attributes during Vegetative Stage of Tomato

Sandeep Kumar[1,2], Teg Bahadur Singh[1,3], Rajneesh K. Agnihotri*[4] and Purti Chaturvedi[5]

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A B S T R A C T

Abiotic stresses are major constraints for global crop production. A major problem of arid and semi-arid regions is environmental stresses like metal, salinity and drought. Among various abiotic stresses, salinity and drought have become a severe threat to ensure food security by affecting about one-third of the irrigated land on earth. Limited water and hot dry climates frequently cause salinity problem that limit or prevent higher crop production. Drought induced by polyethylene glycol (PEG-6000) and salinity induced by sodium chloride (NaCl) were compared to see their effects on growth parameters such as root and shoot length, leaf area, root development and biochemical parameters like chlorophyll, proline and carbohydrate (soluble sugar, reducing sugar and starch) contents of tomato in pot experiment. Three different treatments of PEG (-0.40, -0.50 and -0.75MPa) and NaCl (0.1, 0.2 and 0.3M) were given to tomato plant. Results of this experiment showed significant reduction in all physiological and biochemical parameters (except proline and reducing sugar) with increasing concentration of PEG and NaCl. Proline content was found maximum by treatment of NaCl as compared to PEG but reducing sugar slightly increased by PEG treatment. NaCl treatment was observed to induce high salinity stress. The application of calcium nitrate (5 mM, used as nitrogen source) mitigated the adverse effects of PEG and NaCl. The present experiment suggested that application of calcium nitrate can enhance the growth of tomato plant under salinity and drought stresses.

***Key words*:** Tomato, Salinity, Leaf area, Drought, Proline, Chlorophyll, Carbohydrate

Tomato (*Solanum lycopersicum* L) is one of the most consumed vegetable that cultivated worldwide. It is the most common vegetable subsequently potato, lettuce and onion. It is important for iron absorption, vitamin A, C and it is also maintain healthy bones and teeth. Tomato contains a wide array of beneficial nutrients and antioxidants, including α-lipoic acid, lycopene, choline, folic acid, β-carotene and lutein [1]. Stress is a response against any factor of the environmental which affect plants biological activities and may lead to damage or injury. More than US$12 Billion of crop productivity reduced annual losses due to soil salinity [2]. Salinity is a worldwide problem of the soil, especially semi-arid and arid region, more than 50% crop productive reduced due to salinity [3]. Around 930 million ha or 7% of the world's lands affected mainly by salinity stress [4]. Under drought stress condition, Plants accumulate various beneficial chemical compounds such as proline act as osmolytes or osmoprotectant [5], which perform an important role in defense against abiotic or salinity stress, on the other hands if higher proline accumulation plants could be abiotic stress tolerance [6]. Similarly, salinity adversely affects germination, physiology and productivity of the plant by causing imbalance, osmotic potential and uptake nutrient and water. Proline acts as an organic nitrogen reserve during detoxification of biotic or abiotic stress as well as membrane stabilization and osmotic adjustment [7]. Plant scientists should be developed the salt-tolerant crops through genetic approaches because population growth and salinization increased simultaneously [8]. Photosynthetic activity, negative effect of ions i.e., Na^+ and Cl^- in metabolic pathways and decrease turgescence pressure are three main reasons which is affected due to salinity [9-10]. Chlorophyll contents play important role in photosynthetic and contributed to plant growth and development, which is highly sensitive to biotic and abiotic stress [11-12.

Nitrogen supply in the form of calcium nitrate highly reduced the harmful effects of drought (caused by PEG) by

* Rajneesh K. Agnihotri
✉ rk_agnihotri@rediffmail.com

[1-5] Department of Botany, School of Life Sciences, Khandari Campus, Dr. B. R. Ambedkar University, Agra - 282 005, Uttar Pradesh, India

[2] Department of Botany, Chaudhary Charan Singh University, Meerut - 250 004, Uttar Pradesh, India

[3] Department of Botany, Faculty of Sciences, Dayalbagh Educational Institute, Dayalbagh Agra - 282 005, U.P.

enhancing of antioxidant defenses, nitrogen assimilation and provides drought tolerance [13]. Calcium play important role in the reduction of salinity effect. It has been also reported that calcium has the capability to reduce sodium toxicity [14]. The combined application of NaCl and $CaCl_2$ were reported to increased antioxidant activity in salinity stress [15].

Bearing in mind above facts, this study was performed to evaluate the effect of salinity (NaCl) and drought stress (PEG) on growth and biochemical parameters of tomato as well as the role of calcium nitrate in mitigating the response induced by NaCl and PEG in tomato. It was used with the purpose of finding a cure for salinity and drought stress that can be efficient and cost effective.

MATERIALS AND METHODS

Experimental design

To determine the effect of salinity (NaCl) and water stress (PEG) on vegetative growth of tomato (*Solanum lycopersicum* L) var. NBH-333 F1 hybrid was used for this purpose. This experiment was conducted in greenhouse of the Department of Botany, School of Life Sciences Khandari Campus, Dr. B. R. Ambedkar University, Agra at average temperature 30±7ºC and humidity between 85-90% during the month of June- September. In this study the chemicals used were obtained from Qualigen and Merck companies, India. Distilled water was used for preparation of all the solutions.

Pot experiment

Earthen pots were filled with 5 kg sieved soil and Farmyard Manure (3:1 of soil and FYM) and 10 surface sterilized seeds were sown in each pot and maintained eight plants per pot. Pots were organized in a completely randomized design and after one week of seed germination when secondary leaf emerged out from the seedling then treatments of PEG (-0.40, -0.50 and -0.75MPa) and NaCl (0.1, 0.2 and 0.3M) in each pot were given twice in a week followed by irrigation with distilled water. Nitrogen source in the form of calcium nitrate (5 mM) was also supplied twice in a week. This experiment was performed in a greenhouse. Plants were harvested after one month.

Growth parameters

For the measurement of root and shoot length of plants were taken in three replicate from pot. Root and shoot length were measured with the help of scale. Leaf area was determined by using standard graph papers methods. Secondary and tertiary roots were counted manually.

Biochemical parameters

Proline estimation

Bates *et al.* [16] method was followed for quantification of proline content in leaves. 200 mg fresh leaves were homogenized with 10 ml of 3% sulphosalicylic acid. Mixture was centrifuged and supernatant separate out. 2 ml supernatant, 2 ml glacial acetic acid and 2 ml freshly prepared ninhydrin solution (1.25g ninhydrin was dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) were added. The mixture was boiled for 1 hour in water bath and then cooled at ice bath for terminate the reaction then added 4 ml toluene. All components were mixed well and allowed to stand for sometimes till it become clear then separate the toluene (upper layer) and the absorbance was measured at 520 nm against toluene is blank. Standard curve was prepared by using pure proline. To calculate the amount of proline content the following formula was used:

$$\text{Amount of proline } (\mu g/g) = \frac{A\ 520}{2} \times \frac{\text{vol. of toluene}}{115.5} \times \frac{\text{weight of sample}}{5}$$

Carbohydrates

Dried powder (50 mg) was extracted with 80% ethanol at room temperature for 1 hour and centrifuged at 10,000 rpm at 25ºC for 30 minutes. The pellet was re-extracted with 80% ethanol and centrifuged at 10,000 rpm for 10 minutes at 25ºC. Both the supernatant were pooled and the volume was reduced to 25 ml in vacuo (oven), which as used for the analysis of total soluble and reducing sugars. Pellets were used for estimation of starch [17], total soluble sugar [18] and reducing sugar [19].

Chlorophyll estimation

For estimation of chlorophyll in leaves, Brougham [20] method was adopted. 1 g sample of green leaf was weigh and ground in chilled mortar and pestle. Chlorophyll content was extracted with 80% chilled acetone (20 ml distilled water + 80 ml acetone) by repeated homogenization. Supernatant was filtered and make up to 100 ml with 80% acetone.

Following Arnon's [21] technique, the amount of chlorophyll a and b was determined by measuring the absorbance in a double beam UV spectrophotometer (Systronics 128) at 663, 645 and 470nm.

The chlorophyll and carotenoids contents were estimated using the following standard formula as given below:

$$\text{Chl 'a' (mg/g F.W)} = \frac{12.7(A\ 663) - 2.69(A\ 645) \times V}{1000 \times W}$$

$$\text{Chl 'b' (mg/g F.W)} = \frac{22.9(A\ 645) - 4.68(A\ 663) \times V}{1000 \times W}$$

$$\text{Carotenoids } (\mu g/mg\ F.W) = \frac{[1000A470 - 1.28(\text{Chl a}) - 56.7(\text{Chl b})]}{256 * 0.906}$$

Where,

A = Absorbance of chlorophyll extract on specific induced wavelength.

V = Final volume of extract in a mixture of 80% acetone.

FW = Fresh weight of tissue (mg.)

Statistical analysis

All experiments were performed in triplicates. The obtained data were analyzed by analysis of variance (ANOVA) using SPSS software (Version 17). The mean values were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05\%$.

RESULTS AND DISCUSSION

Growth parameters

The effect of salinity and drought stress on the vegetative growth of tomato in presence and absence of nitrogen illustrated in (Fig 1-2). It showed that both salinity and drought reduce the vegetative growth in absence of nitrogen. While in presence of nitrogen the vegetative growth

of tomato seedlings increased even under the salt and drought stress.

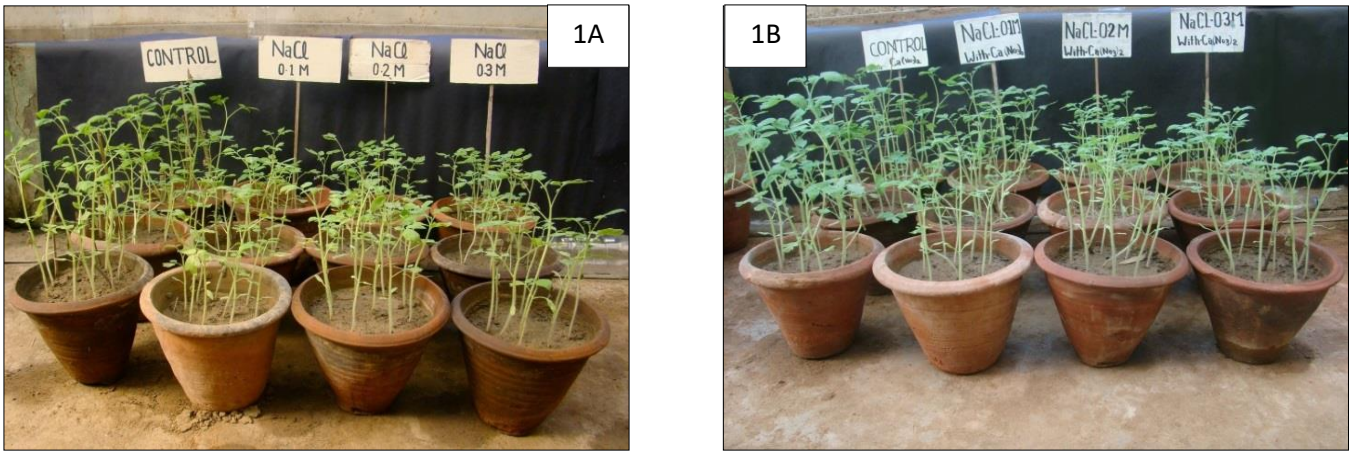

Fig 1 Effect of NaCl without nitrogen (1A) or with nitrogen (1B) on vegetative growth of tomato in pot experiments

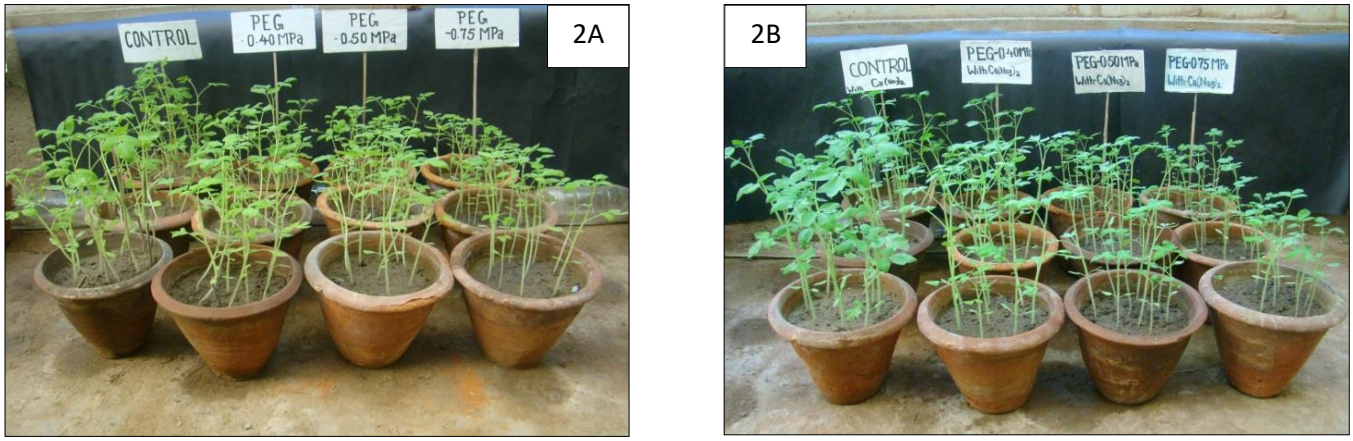

Fig 2 Effect of PEG without nitrogen (2A) or with nitrogen (2B) on vegetative growth of tomato in pot experiments

Root and shoot length

The increasing concentration of salinity highly decreased the root and shoot length, leaf area and intermodal length [22]. The maximum reduction in root length was observed by treatment of 0.3M NaCl and -0.75MPa and decreased root length up to 69.18% and 127.48% respectively, in comparison to control. Shoot length also decreased under salinity stress and drought stress in comparison to control (Table 1). The application of calcium nitrate reduced the salinity effect on root and shoot length. Similarly, the study of [23] showed that salinity effect can be reduced by the application of calcium nitrate. PEG (Polyethylene glycol) was observed more phytotoxic as compared to NaCl.

Table 1 Effect of NaCl and PEG shoot and root length (cm) in tomato grown without or with nitrogen

Osmotic stress	Conc.	Shoot length (cm)		Root length (cm)	
		0 mM N	5 mM N	0 mM N	5 mM N
Control	0.0	33.93±1.01^{a}	37.96±1.09^{a}	10.76±1.12^{a}	12.10±1.10^{a}
	0.1	26.46±1.24^{b}	30.16±1.54^{b}	8.53±0.69^{ab}	9.30±0.87^{ab}
NaCl (M)	0.2	23.56±0.48^{b}	26.26±0.73^{cd}	7.46±1.06^{bc}	8.83±1.62^{ab}
	0.3	20.46±0.69^{c}	23.93±1.02^{d}	6.36±0.50^{bc}	7.70±1.15^{b}
PEG (MPa)	-0.40	24.76±0.98^{b}	27.66±1.30^{bc}	7.80±1.17^{b}	9.13±1.24^{ab}
	-0.50	20.06±0.89^{cd}	24.93±0.95^{cd}	6.23±0.69^{bc}	7.50±1.38^{b}
	-0.75	17.30±1.30^{d}	19.30±1.11^{e}	4.73±0.69^{d}	5.83±0.61^{b}

Data are the mean values of three replicates. Means±SE sharing the same letter do not differ significantly (p≤0.05) as determined by Duncan's multiple range test. Error bars (┬) show SE

Leaf area

Reduction in plant growth due to salinity is commonly expressed by a reduced leaf area and stunted shoots [24]. In this experiment leaf area was reduced 69.85 % under 0.3M NaCl. NaCl was proved more toxic at high concentration. Leaf area was also reduced up to 107.18 % under -0.75 MPa. With the application of nitrogen leaf area was reduced 55.04 % under 0.3M of NaCl while decreased up to 63.39 % by PEG treatment at -0.75MPa as compare to control (Fig 3A). Plant growth reduces under salinity is a matter of controversy. It has been related to salt-induced disturbance of water balance and, in the extreme, to a loss of leaf turgor, which can decrease leaf

expansion and photosynthetic leaf area [25-26]. Salinity and water deficiency lead to decrease plants metabolic activities and finally decrease plant growth. Reduction of plant water uptake under saline conditions could be related to reductions in morphological and physiological parameters like leaf area, stomatal density, and stomatal closure [27].

Table 2 Effect of NaCl and PEG on secondary and tertiary root development in tomato grown without or with nitrogen

Osmotic stress	Conc.	No. of secondary roots		No. of tertiary roots	
		0 mM N	5 mM N	0 mM N	5 mM N
Control	0.0	25.44±1.79^{a}	27.22±0.78^{a}	26.77±2.88^{a}	35.88±3.03^{a}
	0.1	22.22±2.53^{ab}	24.11±2.51^{ab}	14.33±2.44^{b}	27.44±2.35^{b}
NaCl (M)	0.2	18.55±2.39^{ab}	23.77±2.88^{ab}	11.88±2.43^{b}	25.88±2.16^{b}
	0.3	17.77±1.85^{b}	21.33±1.73^{ab}	10.76±2.24^{bc}	17.88±1.39^{c}
PEG (MPa)	-0.40	20.88±2.04^{ab}	22.44±3.04^{ab}	9.22±1.36^{bc}	14.66±277^{cd}
	-0.50	18.33±2.22^{b}	21.33±1.73^{ab}	8.33±1.82^{bc}	12.11±1.42^{cd}
	-0.75	16.77±1.92^{b}	19.11±2.35^{b}	4.77±1.54^{c}	8.66±1.94^{d}

Data are the mean values of three replicates. Means±SE sharing the same letter do not differ significantly (p≤0.05) as determined by Duncan's multiple range test. Error bars (┬) show SE

Root development

Secondary root number highly decreased under drought condition as compared to control and NaCl. PEG was slightly more toxic than NaCl while tertiary roots numbers were highly reduced compared to control and NaCl (Table 2). Salinity and drought stress induced by NaCl and PEG, respectively on secondary and tertiary root of tomato and increased with the application of nitrogen in the form of calcium nitrate. Data of **(**Table 2) also represent the effect of nitrogen in combating the stress induced by NaCl and PEG on secondary and tertiary root of tomato. [28] reported that among *Hordeum spp*., the growth of *Hordeum vulgare* was more adversely affected by salinity compared to wild species. In another study growth of barley seedlings was inhibited at 150 mM NaCl [29]. In other studies, it was reported that the growth of aerial organ was inhibited under salt stress by the decrease of root development [30-31].

Biochemical Parameters

Proline content

In the present investigation, it was observed that proline content in leaf increased with increase in concentration of NaCl and PEG. Increased level of proline in PEG induced water stressed plants may be an adaptation to overcome the stress conditions. Proline accumulates under stress condition contribute to osmotic adjustment and thereby helps the plant to tolerate stress [32-33]. Proline content was highly increased under salinity stress (NaCl) as compared to drought (PEG) and control. The application of calcium nitrate, over all proline content was decreased (Fig 3B). NaCl treatments were more toxic than PEG treatment. Similarly, proline content in leaves increased significantly with an increase in PEG and NaCl concentration [34]. Proline content increased significantly in the leaves of all the genotypes of chilli as the salt concentration increased [7]. [35] reported 10 fold increase in proline accumulation under PEG induced water stress condition in tomato plants. The accumulation of proline under drought stress condition is well established in other plants like in Ragi [36], sesame [37] and bhindi [38].

Carbohydrate content

Sugars act as antioxidant and it performs as true ROS scavenger under salt stress by increase the sugars level that interact with cell membranes [39]. Accumulation of osmo-protective sugars contribute to maintain the ion partition and homeostasis in the plant cell and helps in proper cell functions and play important role in abiotic stress tolerance as trehalose most promising osmo-protective sugar [40]. Starch content decrease under salinity stress because of break down and converted into monosaccharides and transfer to cytosol for synthesis of other sugars [40]. Reduction in starch content was observed as compared to control under NaCl as well as PEG. With the application of calcium nitrate, over all starch content increase **(**Fig 3C). Thus, nitrogen source was protective for starch content in test plant even if it could not eliminate the effect of stress completely. Reducing sugars similarly increased under salinity and drought stress. Reducing sugar slightly decreased with the application of calcium nitrate with NaCl and PEG **(**Fig 3D). Synthesis of sugar takes place through carbon assimilation that plays diverse roles under salinity stress tolerance [40]. Soluble sugars reduced under salinity as compared to control, but high reduction was observed under PEG treatments and proved more harmful. However, the application of nitrogen enhanced the soluble sugar content (Fig 3E). It had been proved that salinity and drought stress cause a significant decrease in net CO_2 assimilation rate in plants [9].

Pigment content

Chlorophylls

Salinity extremely affects photosynthetic processes due to decreasing chlorophyll content and commonly shows adverse effects on membrane stability [41]. Salinity decreased chlorophyll (a, b) and carotenoid contents in green gram seedlings [42]. Decreasing chlorophyll contents leads to reduction of excited electrons during photosynthetic mechanism through the formation of ROS [43].

Chlorophyll 'a' was decreased more by treatment of NaCl in comparison to PEG **(**Fig 3F). With the application of nitrogen in the form calcium nitrate Chl 'a' increase. Chlorophyll 'b' highly affected under drought condition as compared to salinity. Chlorophyll 'b' was increased with the application of nitrogen **(**Fig 3G). Carotenoids content also decreased in similar way. However, with the application of calcium nitrate carotenoids content also increased (Fig 3H).

Our study showed that NaCl treatment caused reduction in the overall growth of all tomato plants as compared to

control plants. Other workers have also showed a significant decrease in photosynthesis in plants exposed to salinity [44-46]. [47] observed decrease in total chlorophyll content in tomato with the increasing the level of salinity [48-50].

Chlorophyll content was also affected during the present investigation showed that long progressive stress along with other environmental factor may affect photosynthetic ability of the plant system. In our present report it was observed that Chl. 'a' more sensitive under salinity and Chl. 'b' under drought stress. PEG induced water stress causes decrease in total chlorophyll content in rice leaves [51].

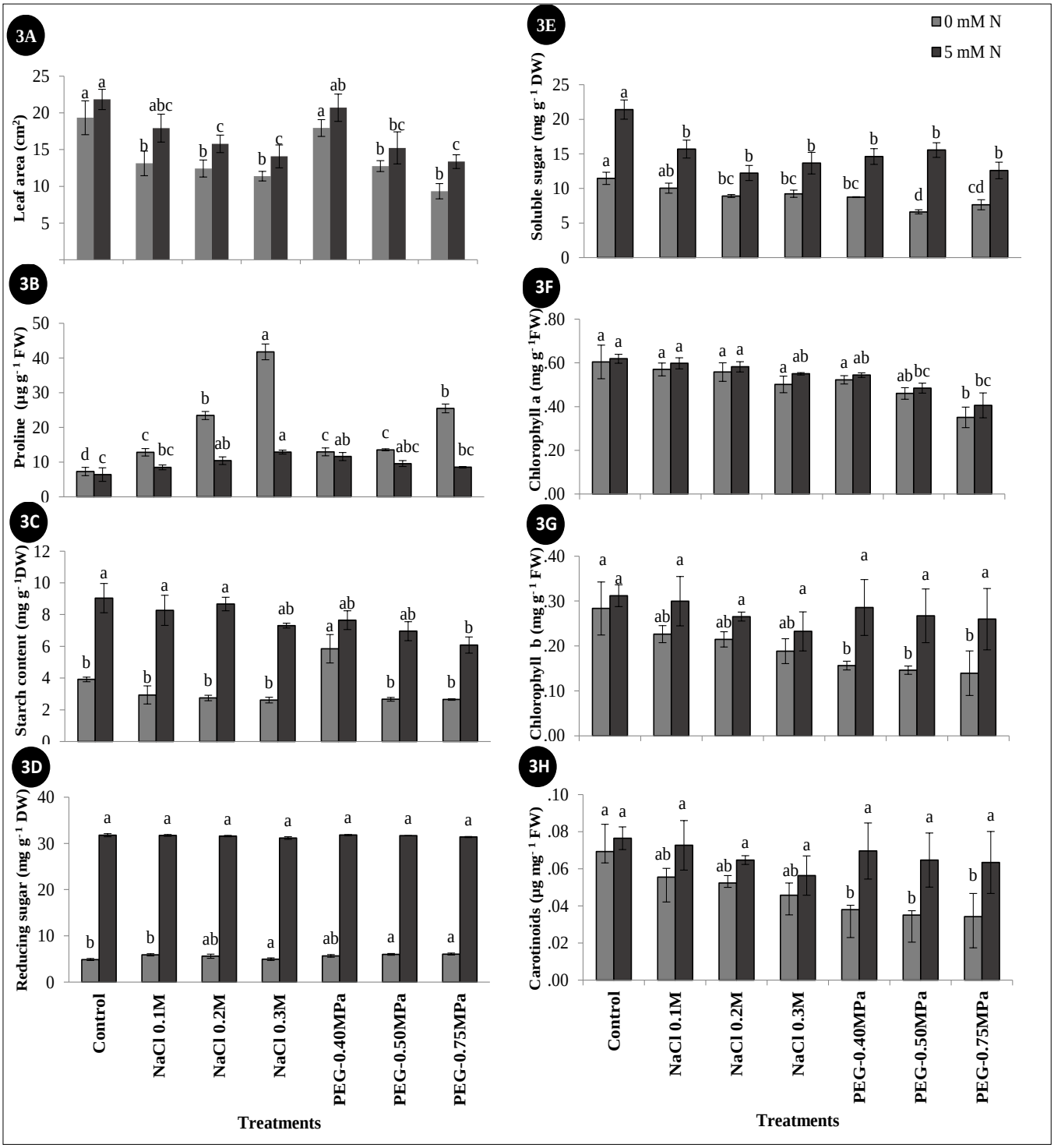

Fig 3 Effect of NaCl and PEG on leaf area (3A), proline (3B), starch (3C), reducing sugar (3D), soluble sugar (3E), chlorophyll a (3F), chlorophyll b (3G), carotenoids (3H) in tomato grown without or with nitrogen. Bars sharing the same letter (s) do not differ significantly ($p \leq 0.05$) as determined by Duncan's multiple range test. Error bars (┬) show SE

CONCLUSION

This study indicates that salinity and drought stress lead to a significant decrease in the growth parameter like root and soot length, leaf area and root development. Biochemical parameters like chlorophyll, soluble sugar and starch decreased in response to increasing concentration of NaCl and PEG, while increase in proline content and slightly reducing

sugar was observed with these treatments. This experiment also confirmed that the salinity and drought stress can be reduced by the application of calcium nitrate and helpful where this problem occurred. Tomato was found quite sensitive to salinity and drought stress. Salinity is a worldwide problem so further investigations are needed to improve the understanding the effect of salinity and drought stress during vegetative growth of tomato. Tomato is relatively sensitive to salinity like other vegetable crops. It is an important crop that gives vegetable yield during dry spells when other vegetables will have wilted and dried up. Further studies and determination about growth, physiological and biochemical parameters might lead to development of drought stress tolerant varieties and cultivars of tomato on saline soils, particularly Agra region.

Conflict of interest

Authors declare that there is no conflict of interest.

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