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Gibberellic Acid Supplements Mitigate the Sodium Chloride Effects on Onion Seed Germination and Its Physio-Chemical Attributes

(Suplemen Asid Giberelik Mengurangkan Kesan Natrium Klorida pada Percambahan Biji Bawang dan Atribut Fisio-Kimianya)

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ABSTRACT

The mitigative effect of gibberellic acid (GA_3) for salt (NaCl) stresses on seed germination attributes of onion (*Allium cepa* L.) cultivar Nasarpuri was assessed. Seeds were moisturized with NaCl (0-, 100- and 200-mM) and GA_3 (250 ppm) before and after sowing for seed germination in 1st week than in 2nd week, GA_3 sprayed once foliarly and NaCl in rooting region. At the end of 1st week, an increase in seed germination rate was observed in seeds supplemented with GA_3 from control (85.0%) to 97.5% and from NaCl stressed seeds (72.5% and 50.0%) to 85.0% and 62.5%, respectively ($p \leq 0.05$). This reduction in seed germination was caused by salt stresses after 96th hours of sowing, inhibition in GA_3-biosynthesis GA_3 and delay in α-*amylases* activation observed in salt stressed seed cultures. The seedling vigor index (SVI) was observed higher in foliarly GA_3 sprayed cultures of both control as well as saline stressed cultures. The seedlings supplemented with GA_3, decrease in malondialdehyde (MDA), H_2O_2, Na^+/K^+, Na^+ and Cl^- contents, while increases seedling biomass, chlorophyll contents, total proteins, and sugars in NaCl stressed seedlings. Interestingly, GA_3 also increased ($p \leq 0.05$) the osmoprotectants in seedlings including abscisic acid (AsA), carotenoids, phenolics and proline contents to depict in stress alleviation. This study may be concluded by the fact that GA_3 minimizes salinity stresses on seed germination as well as further seedling growth with the increased production of organic osmoprotectants as saline stress neutralizers.

Keywords: *Allium cepa* L.; antioxidative responses; gibberellic acid (GA_3); H_2O_2 contents; saline stresses

ABSTRAK

Kesan mitigasi asid giberelik (GA_3) untuk garam (NaCl) menegaskan sifat percambahan benih bawang (*Allium cepa* L.) kultivar Nasarpuri telah dinilai. Benih telah dilembapkan dengan NaCl (0-, 100- dan 200-mM) dan GA_3 (250 ppm) sebelum dan selepas disemai untuk percambahan benih pada minggu pertama berbanding minggu ke-2, GA_3 disembur sekali pada daun dan NaCl di kawasan pengakaran. Pada akhir minggu pertama, peningkatan kadar percambahan biji benih diperhatikan pada benih yang ditambah dengan GA_3 daripada kawalan (85.0%) kepada 97.5% dan daripada benih bertekanan NaCl (72.5% dan 50.0%) kepada 85.0% dan 62.5% masing-masing (p). ≤ 0.05). Pengurangan dalam percambahan benih ini disebabkan oleh tegasan garam selepas 96 jam penyemaian, perencatan dalam GA_3-biosintesis GA_3 dan kelewatan dalam pengaktifan *α-amilase* yang diperhatikan dalam kultur benih bertekanan garam. Indeks vigor ankak benih (SVI) diperhatikan lebih tinggi dalam kultur semburan GA_3 daun bagi kedua-dua kawalan dan juga kultur bertekanan garam. Anak benih yang ditambah dengan GA_3 mengurangkan kandungan malondialdehid (MDA), H_2O_2, Na+/K+, Na+ dan Cl-, sambil meningkatkan biojisim anak benih, kandungan

klorofil, jumlah protein dan gula pada anak pokok bertekanan NaCl. Menariknya, GA_3 juga meningkatkan ($p \leq 0.05$) osmopelindung dalam anak benih termasuk kandungan asid absisik (AsA), karotenoid, fenol dan prolin untuk menggambarkan pengurangan tekanan. Kajian ini boleh disimpulkan oleh fakta bahawa GA_3 meminimumkan tegasan kemasinan pada percambahan biji benih serta pertumbuhan anak benih selanjutnya dengan peningkatan pengeluaran osmopelindung organik sebagai peneutral tegasan garam.

Kata kunci: *Allium cepa* L.; asid giberelik (GA_3); gerak balas antioksidatif; kandungan H_2O_2, tegasan garam

Introduction

The onion (*Allium cepa* L.) is one of most vital medicinal and 2nd most important key vegetable biennial food crops (Manniche 1989; Marles & Farnsworth 1995). Onion is cultivated in almost 170 countries, while Pakistan has got position among first 10 countries in its high yields production (Azam & Shafique 2017; FAO 2021). Onion production is reduced by ~ 1.1% during fiscal year 2020-21 (Abbas & Waheed 2021). This crop likes to grow happily in sunny sheltered areas, while cold is pre-requisite for seedling and hot dry bulb ripening growth (Sumner 2019). Salinity is a crucial abiotic plant factor either in water-limited or canal water irrigated areas. Especially, canal water encourages accumulation of salts to convert fertile to sterile lands (Hillel, Braimoh & Vlek 2008). Salinity led to reduce 2-folds plant growth with prevention of water availability either to roots or tissues (osmotic stress) and toxic ions accumulation in certain tissues (Lima & Leonardo 2008; Munns, Schachtman & Condon 1995). Vegetables including onion are highly-susceptible to water deficit conditions for their bulb growth (Kadayifci et al. 2005). Meanwhile, it is dire need to get per unit high yields by utilization of suitable agronomic techniques to combat saline conditions.

Soil salinity limits the plant growth and its productivity (Allakhverdiev et al. 2000). High soil salt concentrations in particular Na^+ can alter the soil basic texture, which lead to decrease soil porosity, soil aeration and its water conductance capacity. Presently, no economic agronomic technology is available for high crop productions under soil saline conditions. However, development of abiotic stress tolerant crops has been considered a promising technical approach to satisfy increasing food demand of the countries. The natural stress tolerance can be improved with mutation breeding, genetic modifications, and applications of osmoprotectants and growth regulators as supplements (Machado & Serralheiro 2017; Parida, Das & Mittra 2004). Soil salinity changes the balanced relation of root-shoot hormones like as it reduces cytokinins and gibberellins, while abscisic acid increases (Yurekli et al. 2004). Phyto-hormones counteract deleterious salinity effects on plant development (Javid et al. 2011).

Phytohormones mainly regulate plant growth at all stages of its development even from seed germination than further growth of seedlings. As far as there are mechanistic involvement in seed germination to further plant growth, while their ratios are key in plant development regulations. Meanwhile, saline conditions may rapidly and dramatically change their ratios lead to disturbing physiological processes and general growth reduction (Negrao, Schmöckel & Tester 2017). Further, it is stated that salinity consequences appear to effect on hormonal balance rather ionic toxicity (Lerner & Amzallag 1994). As abscisic acid (ABA) increase in leaves is correlated with closure of stomata under stressed conditions (Takemura et al. 2000). Since it is marked that ABA contents are elevated in salt stressed as well as waterlogged plants (Zhang & Zhang 1994) and saline stressed roots (Moons et al. 1995; Roychoudhury et al. 2009). Yet, occasional role of growth regulators to counteract against saline-inhibitory effects on plant metabolism for growth elevations. In this context, suppression of morpho-biochemical parameters in response to saline stressed conditions may be nullified when seeds are presoaked in gibberellic acid (Chauhan et al. 2019; Vetrano, Moncada & Miceli 2020).

Gibberellic acid (GA_3) is one of the most prodigal plant growth hormones among gibberellins. The GA_3 promotes cell division of plant development processes to increase plant height and number of flowers with uniform and reduced flowering time (Srivastava & Srivastava 2007). Its foliar application also enhances plant growth and pod features (Camara et al. 2018; Rahman et al. 2006). The aim of the present study was to measure the mitigation of gibberellic acid on sodium chloride (NaCl) stress on initial seed-germination, further seedling's growth and different morpho-biochemical aspects of onion (*Allium cepa* L.) cv., Nasarpuri seedlings under

aseptic conditions. Role of gibberellic acid (GA_3) foliar spray on seedlings also evaluated to overcome the salt deterioration. This designed research may prove useful for selection of suitable agronomic techniques to utilize saline soil to produce best onion yields.

Materials and Methods

A local onion (*Allium cepa* L.) cv., Nasarpuri was collected from Nuclear Institute for Agriculture, Tandojam. Its healthy seeds were selected and sterilized under aseptic conditions after washing in tap-H_2O. They were stirred in 70% ethanol (v/v) for 1-min then for 15-min in 20% Robin® bleach [5% NaOCl (sodium hypo-chloride)] on magnetic stirrer. These seeds were washed with sterilized dH_2O 3-times (3× for 5-min) than dried in Laminar Air Flow Cabinet. Sterilized seeds were sown in petri-dishes (50 × 12 mm) lined with bed of W hatman No 1 double layered filter paper. Ten seeds that look good in appearance (health-wise) were placed in petri-dishes, which considered as one replicate and such 4 replicates per treatment arranged.

The seeds were treated with different treatments as shown in Table 1. Seeds were moisturized with deionized distilled water (ddH_2O) and considered as control, while others moisturized with 250 ppm gibberellic acid (GA_3), with and without salt (NaCl) stresses of 0 mM (control), 100 mM NaCl and 200 mM NaCl. According to Table 1, this treatment was applied at seed germination stage (a.), while at seedling growth stage salt stress of NaCl applied on same as above in rooting region, while GA_3 foliarly sprayed at plumules or shoots. The concentrations of treatments applied at both growth stages were the same. These above treated cultures were incubated in dark for over-night at 25±2 °C then for next 13-days under 16/8-hours day and night conditions (@ 27 µmol m^{-2} s^{-1}) for 14-days.

At the end of first week, seed germination (SG) rate was determined with this formula; SG (%): (No of seeds germinated/Total number of seeds) × 100, while seedling vigor index (SVI): Mean germination % age × Mean seedling length (Mahender, Anandan & Pradhan 2015). After 2-weeks of sowing, different growth-related parameters were measured in the treated and non-treated seed-cultures like as fresh weight (FW) of shoots and roots (g) and their lengths were also measured. Dry weights (DW) of shoots and roots (g) were taken after drying these stuffs at 80 °C for 48 h. Their relative water contents (RWC) were calculated with formula; *RWC (%): [(FW - DW)/TW]x100* (Catsky 1974; Turner 1981).

The mineral nutrients including Na^+, K^+ and Cl^- contents were measured in dried leaves of seedling. Wet digestion procedure was followed by adding 0.1 g sample in 5 mL digestion solution (HNO_3:H_2O with 5:1, v/v). Filtrate of above clarified digestate sample was subjected to mass-spectrometry (ICP-MS, Finnigan Element XR, Germany) to measure Na^+ and K^+ ionic contents via previously reported methods (Colomer-Winter et al. 2018), while Cl^- also estimated on potentiometer (Chapman & Pratt 1961).

TABLE 1. Composition of treatments, schedule, and growth stages for applications

#s	Treatments	Composition	Treatments applications
01.	T_0	ddH_2O (control)	
02.	T_1	250 ppm GA_3	Seed germination stage (1st-week). Seeds were moisturized with GA_3 and NaCl
03.	T_2	100 mM NaCl	
04.	T_3	100 mM NaCl + 250 ppm GA_3	Foliar spray on 7th day of seedling (2nd-week): NaCl sprayed at root region and GA3 foliarly sprayed on plumules of shoots
05.	T_4	200 mM NaCl	
06.	T_5	200 mM NaCl + 250 ppm GA_3	

ddH_2O: Deionized distilled water; GA_3: Gibberellic acid; NaCl: Sodium chloride

Chlorophyll and carotenoids were analyzed in fresh tissues following Arnon (1949) and Wellburn (1994) methods, while total proline by Bates, Waldren and Teare (1973). Similarly, total proteins and carbohydrates were determined with folin-phenolic reagents method of Lowry and Rosebrough (1951) and Waterborg (2009), and Dubois et al. (1956), respectively. Reducing sugar contents measured with Miller's method (1959). Briefly 2 mL sample mixed with 2 mL DNS (dinitro-salicylic acid) then heated at 100 °C for 5 min. Its OD540 was read when cooled down to room temperature.

The hydrogen peroxide (H_2O_2) contents were determined by formation of the complex of H_2O_2 with titanium tetrachloride as by Brennan and Frenkel (1977). For lipid peroxidation analysis, thiobarbituric acid (TBA) reaction method was employed for MDA (malondialdehyde) contents analysis (Heath & Packer 1968; Lutts et al. 1996). Ascorbic acid was determined by homogenization of 0.20 g fresh leaves in TCA (Tricholoroacetic acid, 2 mL; 10 % (w/v)). Its supernatant was reduced into dehydroascorbate (DHA) with DTT (dithiothreitol) and $FeCl_3$. The OD_{525} of above mixture was read (Law, Charles & Halliwell 1983), while DHA contents are calculated by difference of ASC and AsA (ascorbic acid). Total phenolics were determined by folin-ciocalteu's reagent method (Ti et al. 2014) with spectrophotometer.

For determination abscisic acid (ABA), fresh leaf was extracted with acetonitrile (with 12 % 2,6-di-tertiary-butyl-p-cresol) and cleaned with chloroform and HPLC preparative. After dryness, 500 µL methanol was added, while its 6 µL was injected for analysis onto HPLC by following Majcherczyk, Rakoczy and Huttermann (1986) method. The gibberellic acid (GA_3) was extracted from 3 g fresh tissues in 25 mL methanol and partitioned with a series of different solvents (n-hexane, n-butanol, ethyl acetate polyvinyl polypyrolidone). Mixture was dried and mixed in 100 µL methanol, while its 6 µL injected onto analytical HPLC as performed by Lin and Stafford (1987). For salt, tolerance index (TI) and sensitivity index for seedling growth were determined following Mbarki et al. (2020) with formulas like as TI (%) = (TDW Salt/TDW control) × 100, where TDW is total DW and SI (%) reduction= ((salt treatment-control)/ control) × 100) for seedling growth.

The data of 4 replicates per treatment was subjected to Statistix version 8.1 (Analytical Software, Miller Landing Rd, Tallahassee) for computation of analysis of variance (ANOVA) at 5% difference level. The means values of treatment groups were compared with least significant difference (LSD) test as suggested by Calinski (1981) and Steel and Torrie (1980).

Results and Discussion

Seed germination has been a key factor in yield production of crop plants. In this research, potential attributes of seed germination were assessed in onion (*Allium cepa* L.) cultivar Nasarpuri. Seeds were moisturized with different levels of salt (control (dH_2O) 0 mM-, 100 mM-, and 200 mM-NaCl) stresses along-with to check the mitigative effects of gibberellic acid (250 ppm GA_3) against saline toxic conditions. Generally, salinity changes the soil osmolarity, which hinders imbibition of seeds for water thus impeding or inhibition of seed-germination occurs. Results in Table 2 shows the decrease in seed germination, GA_3 contents and *α-amylases* activities with increase in NaCl levels of stresses, while GA_3 increased each in both saline stressed conditions including control (non-saline, seed moisture with dH_2O) seed sown cultures (01-week culture) significantly. This impede of seed germination by salt stress is established when salinity levels exceed, from plant salt-tolerance limits (Belaqziz, Romane & Abbad 2009). The GA_3 can break these saline induced limits in seeds with alleviation of NaCl stress and early activation of *α-amylases* lead to increase in germination rate (Kaur, Gupta & Kaur 1998; Liu et al. 2018). Overall seedling vigor index (%) was decreased by NaCl and increased with GA_3 foliarly spray (Kandil et al. 2014; Salih et al. 2022).

Responses of seedlings under salt stressed conditions result into complex interaction among different morphophysiological and biochemical processes (Nawaz et al. 2010). Overall, ANOVA in Table 3 showed highly significant NaCl inhibitory effects on seedling's growth (root and shoot length, plant biomass and relative water contents (RWC), while these attributes increased when germinated seedlings foliarly sprayed with GA_3 either treated or untreated with salinity stresses. Foliar GA_3 spray showed significant growth enhancing impacts on plant shoot height, its biomass and relative water contents in both shoots and roots (Aglaia et al. 2011; Ali et al. 2015), while salinity reduces growth-parameters than control seedlings (Nasri et al. 2017). Similar results due to salinity decline in growth as compared to control have been observed (Table 3), which might be resulted by removal of potassium ions (K^+) via roots due to sodium (Na^+) and chloride (Cl^-) ions accumulation (Table 5). Meanwhile, they generate physiological discrepancy as

TABLE 2. Mitigative effects of gibberellic acid (GA_3) on seed-germination related parameters of onion (*Allium cepa* L.) cv., Nasarpuri seeds germinated under different salinity (0 mM-, 100 mM, 200 mM-NaCl) stresses

#s	Treatments	Rate of SG (%)	GA_3 (ηg g^{-1} FW)	α-Amy (U. $Seed^{-1}$)	SVI (%)
01.	T_0	^{b}85.00±2.887	^{b}0.419±0.003	^{b}22.07±0.069	^{b}36.61±0.544
02.	T_1	^{a}97.50±2.500	^{a}0.461±0.002	^{a}23.98±0.201	^{bc}36.07±0.666
03.	T_2	^{c}72.50±2.500	^{c}0.353±0.005	^{d}15.33±0.064	^{ab}39.63±1.153
04.	T_3	^{b}85.00±2.887	^{c}0.399±0.004	^{c}17.65±0.250	^{a}41.64±1.961
05.	T_4	^{c}50.00±4.082	^{c}0.347±0.004	^{f}12.05±0.064	^{c}31.22±2.928
06.	T_5	^{d}62.50±2.500	^{d}0.383±0.004	^{e}14.56±0.184	^{ab}37.45±1.282
i.	*F-significance*	34.40***	121.0***	849.0***	4.680***
ii.	*LSD±SEC*	8.754±4.167	0.012±5.4E-03	0.469±0.223	4.872±2.319
iii.	*G-Mean±SE*	75.42±2.946	0.397±0.024	17.61±0.877	8.817±0.619

SG: Seed germination; Amy: Amylases (U. $Seed^{-1}$, after 96-hrs); SVI: Seedling vigor index; SEC: Standard error for comparison; G: Grand. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent different among the treated groups by LSD (Least Significant Difference) test and * & *** for p-values are significant and highly significantly respectively affected with treatments at $p \leq 0.05$

potassium ions are important for protein biosynthesis and metabolism (Ibrahim et al. 2018). Exogenous application of GA_3 enhances growth parameters of seedlings grown under salinity stress because it might be partially diminishing the saline toxic effects by increasing antioxidative contents and accumulation of osmolytes (Chauhan et al. 2019; Gharib et al. 2018).

Root-shoot lengths and their biomass including RWC increases with foliar application phytohormones positively and significantly (Table 3), while decreased when salinity level increased and could be proportional to increase in Na^+ as shown in Table 5 (Ghodrat & Rousta 2012; Ibrahim et al. 2021). Growth of seedlings also depends on chlorophyll biosynthesis, in our results chlorophyll (*Chl* a, *Chl* b) contents, *Chl* a-*Chl* b ratios and malondialdehyde (MDA) reduced significantly with increasing toxic salt-concentration due to decrease in assimilation of photosynthetic apparatus. Foliar GA_3 spray has positive effects that's-why all chlorophyll (*Chl* aa, b and total chlorophyll) contents observed increased in control as well as among both NaCl stressed levels. Meanwhile, carotenoids and *Chl* ab/carotenoids showed reversed results in comparison to chlorophyll contents (Table 4). This mitigating effects of GA_3 caused the decrease in adverse damaging effects of salinity in case chlorophyll contents. Reduction in chlorophyll contents under stressed conditions is related to the sensitivity of chlorophyll biosynthesis to accumulated Na^+ and Cl^- ions due to inhibition of 5-amino-levulinic acid biosynthesis (Wu et al. 2018). Decrease in chlorophyll contents ($p \leq 0.05$) under oxidative stresses of NaCl applications lead to reactive oxygen species (ROS) production initiates the chloroplast breakdown due to disruption of enzymes involved in chlorophyll biosynthesis (Ahmad et al. 2021), while reversed phenomena is observable with foliarly phytohormonal applications (Ahanger et al. 2019).

TABLE 3. Mitigative effects of gibberellic acid (GA_3) on seedling growth of onion (*Allium cepa* L.) cv., Nasarpuri seeds germinated under different salinity (0 mM-, 100 mM, 200 mM-NaCl) stresses

#s	Treatments	SL (cm)	RL (cm)	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)	SRWC (%)	RRWC (%)
01.	T_0	^{b}23.21± 0.603	^{b}14.46± 0.187	^{b}7.318± 0.112	^{b}1.070± 0.027	^{b}2.479± 0.0210	^{b}0.534± 0.004	^{d}66.09± 0.771	^{abc}50.07± 0.918
02.	T_1	^{a}27.03± 0.353	^{a}17.53± 0.107	^{a}8.141± 0.105	^{a}1.330± 0.009	^{a}2.658± 0.046	^{a}0.617± 0.006	^{d}67.31± 0.925	^{a}53.60± 0.559
03.	T_2	^{d}18.29± 0.174	^{c}12.90± 0.113	^{d}6.103± 0.126	^{c}0.899± 0.003	^{d}1.611± 0.010	^{c}0.486± 0.009	^{c}73.55± 0.692	^{bc}45.98± 0.834
04.	T_3	^{c}20.46± 0.373	^{c}13.46± 0.081	^{c}7.000± 0.064	^{b}1.075± 0.018	^{c}1.811± 0.011	^{bc}0.508± 0.006	^{c}74.12± 0.373	^{a}52.68± 1.003
05.	T_4	^{e}16.08± 0.247	^{d}11.58± 0.751	^{f}4.848± 0.063	^{d}0.636± 0.016	^{f}0.836± 0.012	^{c}0.303± 0.008	^{a}82.76± 0.337	^{ab}52.20± 2.418
06.	T_5	^{e}16.69± 0.319	^{d}11.16± 0.067	^{e}5.188± 0.044	^{d}0.641± 0.019	^{e}1.083± 0.012	^{d}0.348± 0.023	^{b}79.12± 0.399	^{c}45.54± 4.363
i.	*F-significance*	124.0***	50.30***	199.0***	248.0***	1046***	108.0***	108.0***	2.640**
ii.	*LSD±SEC*	1.126± 0.536	0.967± 0.460	0.270± 0.128	0.051± 0.024	0.067± 0.032	0.034± 0.016	1.857± 0.884	6.388± 3.041
iii.	*G-Mean±SE*	20.30± 0.815	13.51± 0.455	6.433± 0.246	0.942± 0.052	1.746± 0.139	0.466± 0.023	73.83± 1.255	50.01± 1.023

SL: Shoot length; RL: Root length; SFW: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; SRWC: Shoot relative water contents; RRWC: Root relative water contents; SEC: Standard error for comparison; G: Grand. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent different among the treated groups by LSD (Least Significant Difference) test and * & *** for p-values are significant and highly significantly respectively affected with treatments at $p \leq 0.05$

In Table 5, the results showed an increase in ionic contents in seedlings when stressed with NaCl. Salt stress has augmented the accumulation of Na^+ and Cl^- with the decrease in K^+ that were observed maximum at 200 mM NaCl stressed seedlings. At germination stage, screening of salt stress tolerance in seedlings we calculated sensitivity index (SI) by using seed germination rate under all stressed conditions. Table 5 shows significant impact of salt stress on SI and our seedling growth showed slight good tolerance to salt stress. Meanwhile, maintenance of shoot growth toward salt dilution or salt exclusion uptake, accumulation of limited Na^+ lead to more vigorous growth rate could be achieved by GA_3 applications (Hamayun et al. 2010). For salt tolerance index (TI), our one-way ANOVA shows significant adjustments in seedling growth under NaCl stressed and foliarly GA_3 sprayed conditions. GA_3 showed that high variation in fresh and dry matters of seedlings ($p \leq 0.05$). Salt stresses induces Na^+ 'inclusion mechanism', while applications of growth regulators and accumulation of secondary metabolites induce 'exclusion mechanisms' for toxic ions accumulation (Tuna et al. 2008).

TABLE 4. Mitigative effects of gibberellic acid (GA_3) on pigmentation and carotenoids of onion (*Allium cepa* L.) cv., Nasarpuri seeds germinated under different salinity (0 mM-, 100 mM-, 200 mM-NaCl) stresses

#s	Treatments	*Chl* a (mg g^{-1})	*Chl* b (mg g^{-1})	*Chl* ab (mg g^{-1})	*Chl* a/ *Chl* b	*Carot* (mg g^{-1})	*Chl* ab/ Carot	*MDA* (µmol g^{-1})
01.	T_0	^{b}4.241±	^{b}3.664±	^{b}7.905±	^{b}1.158±	^{bc}1.182±	^{b}6.691±	^{c}162.4±
		0.019	0.015	0.009	0.010	0.002	0.007	3.230
02.	T_1	^{a}4.442±	^{a}3.760±	^{a}8.202±	^{a}1.182±	^{c}1.158±	^{a}7.086±	^{d}154.9±
		0.016	0.011	0.027	0.002	0.010	0.036	2.979
03.	T_2	^{d}3.328±	^{d}3.338±	^{d}6.666±	^{d}0.997±	^{b}1.231±	^{c}5.416±	^{b}173.7±
		0.012	0.008	0.020	0.001	0.001	0.019	2.016
04.	T_3	^{c}3.660±	^{c}3.551±	^{c}7.211±	^{c}1.031±	^{d}0.997±	^{a}7.234±	^{c}164.5±
		0.006	0.007	0.012	0.001	0.001	0.006	1.796
05.	T_4	^{f}2.954±	^{f}3.156±	^{f}6.110±	^{f}0.936±	^{a}1.388±	^{e}4.405±	^{a}187.2±
		0.015	0.013	0.004	0.008	0.024	0.076	2.196
06.	T_5	^{c}3.094±	^{c}3.220±	^{c}6.314±	^{c}0.961±	^{b}1.236±	^{d}5.122±	^{b}173.8±
		0.015	0.009	0.020	0.005	0.038	0.144	1.968
i.	*F-significance*	1774***	524.0***	2454***	333.0***	46.20***	288.0***	21.70***
ii.	*LSD±SEC*	0.043±	0.032±	0.051±	0.017±	0.056±	0.204±	7.204±
		0.021	0.015	0.024	7.96E-03	0.027	0.097	3.429
iii.	*G-Mean±SE*	3.620±	3.449±	7.068±	1.044±	1.198±	5.992±	169.4±
		0.117	0.047	0.163	0.020	0.025	0.224	2.323

Chl: Chlorophyll; Carot: Carotenoids; MDA: Malondialdehyde; SEC: Standard error for comparison; G: Grand. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent different among the treated groups by LSD (Least Significant Difference) test and * & *** for p-values are significant and highly significantly respectively affected with treatments at $p \leq 0.05$

With the increase in Na^+ and Cl^- ions in shoots caused to decline in chlorophyll (*Chl* b, *Chl* a and *Chl* ab) contents, total proteins and total sugars as shown in Table 6, while increase in reducing sugars, ascorbic acid (AsA), hydrogen peroxides (H_2O_2), MDA, phenolics and proline contents was observed in saline stressed seedling shoots (Tables 4 & 6). These contents further increased with foliar GA_3 spray in both (100 mM-, 200 mM-) NaCl stressed seedling cultures while increase was observed higher in 100 mM-NaCl stressed seedlings (Table 6). Simultaneous increase in H_2O_2 and MDA contents in seedling means ROS generation and cellular damages has been initiated due to NaCl stresses. Meanwhile, increase in phenolics, AsA and proline are preventors for ROS formation and cell injury to save photosynthetic or other cellular components to continue their growth. The presence of higher Na^+ and Cl^- ions disturb the activities of those enzymes which are involved in chlorophyll biosynthesis (Ahanger et al. 2019). Under these circumstances, osmolyte proline has antioxidant properties which aids signal transformation metabolism (Dar et al. 2021). In our research, proline value was higher in NaCl as well as NaCl with foliar GA_3 applied seedlings than control. It was measured that there is directly proportional relation among the proline level of salt stress same as studied previously (Nounjan & Theerakulpisut 2012). Under high NaCl stresses, MDA and H_2O_2 concentrations observed higher which are symptoms of injury. For the purpose of preventing compartmental injury, biosynthesis of phenolics and others also considered as secondary metabolites including free proline are observed higher in stressed conditions. Both MDA and H_2O_2 decreased with foliarly GA_3 spray, that means GA_3 involved in initiation of osmoprotectants biosynthesis for saving the injured tissues.

TABLE 5. Mitigative effects of gibberellic acid (GA_3) on mineral ionic contents of onion (*Allium cepa* L.) cv., Nasarpuri seeds germinated under different salinity (0 mM-, 100 mM-, 200 mM-NaCl) stresses

#s	Treatments	Na^+ (mg g^{-1})	K^+ (mg g^{-1})	Cl^- (mg g^{-1})	Na^+/K^+	Na^+/Cl^-	K^+/Cl^-	STI (%)	SI (%)
01.	T_0	^{c}5.575± 0.149	^{b}9.925± 0.170	^{c}6.300± 0.158	^{c}0.562± 0.010	^{bc}0.887± 0.034	^{b}1.578± 0.049	^{b}100.0± 0.000	^{b}147.9± 2.098
02.	T_1	^{f}5.000± 0.0913	^{a}10.60± 0.187	^{f}5.575± 0.118	^{c}0.472± 0.013	^{b}0.897± 0.014	^{a}1.904± 0.057	^{a}107.2± 0.960	^{a}165.8± 4.560
03.	T_2	^{c}9.600± 0.178	^{d}6.800± 0.108	^{b}13.10± 0.178	^{c}1.412± 0.008	^{d}0.734± 0.021	^{d}0.520± 0.013	^{d}65.02± 0.875	^{d}61.12± 1.043
04.	T_3	^{d}8.150± 0.104	^{c}7.450± 0.065	^{d}9.725± 0.1377	^{d}1.094± 0.018	^{bc}0.838± 0.007	^{c}0.767± 0.016	^{c}73.06± 0.678	^{c}81.08± 1.135
05.	T_4	13.35± ^{a}0.119	^{f}5.375± 0.193	^{a}16.08± 0.138	^{a}2.493± 0.087	^{c}0.831± 0.014	^{c}0.334± 0.012	^{f}33.73± 0.768	^{f}-16.45± 1.221
06.	T_5	^{b}11.23± 0.165	^{c}6.325± 0.110	^{c}10.88± 0.149	^{b}1.775± 0.011	^{a}1.033± 0.025	^{d}0.582± 0.016	^{c}43.69± 0.523	^{c}8.275± 1.213
i.	*F-significance*	550.0***	200.0***	735.0***	420.0***	21.80***	378.0***	1714***	1046***
ii.	*LSD±SEC*	0.411± 0.195	0.436± 0.208	0.439± 0.209	0.111± 0.052	0.063± 0.030	0.098± 0.046	2.111± 1.005	6.702± 3.190
iii.	*G-Mean±SE*	8.817± 0.619	7.746± 0.398	10.28± 0.764	1.301± 0.146	0.870± 0.020	0.948± 0.122	70.45± 5.605	74.62± 13.91

Na^+: Sodium ion; K^+: Potassium ion; Cl^-: Chloride ion; STI: Salinity tolerance index; SI: Sensitivity index; SEC: Standard error for comparison; G: Grand. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent different among the treated groups by LSD (Least Significant Difference) test and * & *** for p-values are significant and highly significantly respectively affected with treatments at $p \leq 0.05$.

TABLE 6. Mitigative effects of gibberellic acid (GA_3) on physio-chemical contents of onion (*Allium cepa* L.) cv., Nasarpuri seeds germinated under different salinity (0 mM-, 100 mM-, 200 mM-NaCl) stresses

#s	Treatments	Proteins (mg mL^{-1})	Sugars (mg mL^{-1})	R. sugars (mg mL^{-1})	AsA (µmol g^{-1})	H_2O_2 (µmol g^{-1})	Proline (mg mL^{-1})	Phenolics (mg g^{-1})
01.	T_0	^{b}8.090± 0.025	^{a}84.54± 1.056	^{f}31.79± 0.062	^{f}0.475± 0.004	^{c}0.138± 0.003	^{f}4.439± 0.020	^{f}0.919± 0.006
02.	T_1	^{a}8.242± 0.033	^{a}85.95± 0.531	^{e}34.25± 0.074	^{e}0.503± 0.011	^{e}0.129± 0.004	^{e}5.034± 0.027	^{e}0.967± 0.009
03.	T_2	^{d}6.980± 0.071	^{c}72.54± 0.468	^{d}36.74± 0.139	^{d}0.636± 0.010	^{b}0.163± 0.004	^{d}6.544± 0.016	^{d}0.998± 0.008
04.	T_3	^{c}7.715± 0.023	^{b}74.605± 0.500	^{e}41.88± 0.099	^{e}0.678± 0.005	^{e}0.140± 0.004	^{c}7.405± 0.010	^{c}1.182± 0.004
05.	T_4	^{f}4.974± 0.054	^{e}64.72± 0.348	^{b}47.13± 0.171	^{b}0.707± 0.006	^{a}0.182± 0.004	^{b}8.759± 0.022	^{b}1.260± 0.005
06.	T_5	^{e}5.354± 0.059	^{d}67.42± 0.267	^{a}52.66± 0.208	^{a}0.746± 0.004	^{a}0.178± 0.005	^{a}9.191± 0.015	^{a}1.330± 0.010
i.	*F-significance*	881.0***	222.0***	3510***	233.0***	28.20***	10336***	525.0***
ii.	*LSD±SEC*	0.142± 0.067	1.740± 0.828	0.403± 0.192	0.022± 0.010	0.013± 5.99E-03	0.056± 0.027	0.022± 0.011
iii.	*G-Mean±SE*	6.892± 0.270	74.960± 1.673	40.741± 1.530	0.624± 0.021	0.155± 0.005	6.895± 0.367	1.109± 0.033

R: Reducing; AsA: Ascorbic acid; H_2O_2: Hydrogen peroxide; SEC: Standard error for comparison; G: Grand. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent different among the treated groups by LSD (Least Significant Difference) test and * & *** for p-values are significant and highly significantly respectively affected with treatments at $p \leq 0.05$

Conclusions

Seed germination is key to final yields-production of crops. Both germination and early stages of seedling growth are considered as sensitive phases for abiotic stresses, especially for saline conditions. This study showed a highly significant salt (NaCl) effect on reduction in rate of seed germination and further growth of seedlings, while gibberellic acid (GA_3) has alleviated its effects. When seeds are exposed to GA_3, it induces early increased *α-amylases* activities as well as increase in these both significantly increases seed germination rates in saline stressed seed-cultures. The same pattern has also observed, increase in seedling vigor index (SVI) with foliarly spray of GA_3. The GA_3 spray decreases malondialdehyde (MDA), H_2O_2, Na^+/K^+, Na^+ and Cl^- contents in NaCl stressed seedlings. Interestingly, GA_3 further increases ($p \leq 0.05$) osmoprotectants including abscisic acid (AsA), proline, total sugars, carotenoids, phenolics and proline contents under saline stressed conditions, which means that they depict in stress alleviation. This study may be concluded by the fact that application of GA_3 minimizes salinity stresses on germination of seeds and seedling growth with the production of organic osmoprotectants as stress neutralizers.

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