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Seed Priming with Gibberellic Acid (GA_3) in Sponge-Gourd Modulated High Salinity Stress

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ABSTRACT

The current study was conducted in completely randomized design to investigate the effect of GA_3 in sponge gourd c.v *Jaipuri* under salinity stress. The treatments were comprised of seed priming in water, GA_3 10^{-4}M, and GA_3 10^{-8}M solution with and without high salinity stress (150mM NaCl). Our results shows that seed priming with GA_3 10^{-4}M, significantly minimized number of days to germination (7±2.00), increased number of leaves plant^{-1} (9.76±1.53), leaf area (13.98±0.02 cm^2), root length (14.07±1.15 cm), shoot length (14.03±1.53 cm), shoot (10.13±0.74 gm) and root fresh weight (0.93±0.05 gm) without salinity stress as compared to control. In salinity stress, seed treatment with GA_3 10^{-4}M also repeated the same trend by preventing the adverse effect of salt stress on sponge gourd and significantly increased shoot length (11.93±4.10cm), root length (8.77±0.35cm), shoot (7.33±1.36g) and root fresh weight (0.53±0.14g), leaf area (10.34±0.96 cm^2), number of leaves plant^{-1} (7.67±1.15) as compared to control. Moreover, this study suggests that seed priming of sponge gourd with GA_3 10^{-4} M is well appropriate for the protection of sponge gourd under 150 mM salinity stress.

Keywords: Sponge gourd, GA_3, Plant growth, Salinity stress.

1. Introduction

Among the abiotic stresses, salinity stress due to salinization of agriculture land has severely restricted the yield and thus creating problems directly related to human survival on planet earth [1]. The problem is mostly persisting in arid and semiarid regions of all major global terrestrial portions like Asia, Australia, Africa and Americas [2]. The salinity stress mainly occurs due to the disassociation of NaCl into Na^+ and Cl^- ions, which creates negative water potential and finally hampers nutrient balance. Subsequently, plant growth and development is arrested by hyperosmotic and hyperionic stresses due to the toxicity and apparently identified by signs and symptoms [1, 2, 3]. Various means are currently under practice to strengthen the plant growth under such circumstances. One of them is pre-sowing seed priming treatment to improve the germination and performance of plant growth under such prevailing conditions [4]. In priming the partial hydration of seed begin the germination process and pass the early two phases of water uptake but are briefly re-dried to prevent the radical emergence [5]. Upon sowing, these invigorated seeds quickly assume the germination process and significantly reduce the time to seedling emergence. Various seed priming techniques have been established and the best low cost methods like hydro-priming, priming with plant growth regulators, osmo-priming, solid matrix-priming, and bio- priming are practiced by framers in fields [5]. Nevertheless, seed priming with plant growth regulators has attained great consideration particularly with gibberellin. Gibberellic acid is an essential tetracyclic diterpenoids plant hormone, which play important role in promoting seed germination, stem elongation, leaf expansion and also take part in flowering and seed developmental process [6].

Sponge gourd is a subtropical plant, which requires warm temperatures and long frost-free growing season when grown in temperate regions. Spong gourd (*Luffa cylindrica*) belongs to family *Cucurbitaceae* and commonly known by different names in different part of the word. Previously, a lot of studies have evaluated the effect of growth regulators such as GA_3 and NAA on flowering and fruiting of sponge gourd. These plant growth regulators effect osmotic uptakes of water which preserve a swelling force against the softening of cell wall [7]. Lee [8] studied the Effects of pre-sowing seed treatments with GA_3 and Acetic Acid (IAA) on flowering and yield components in groundnuts and stated that GABA (GA3 1% & SBA *Brassicasteroids* as STC 0.3%) is a plant growth regulator, which can manipulate a variety of growth and yield in various crops. Therefore, the objective of this study was to determine the optimum concentration of GA_3 for sponge gourd seed priming to improve its growth with and without salinity stress.

2. Material and Methods

Experimental site and weather conditions

A pot experiment was conducted at the Research Greenhouse of University of Haripur, Haripur, during spring 2013. The climatic conditions of this locality are considered favorable for different vegetables growth in spring season with low incidence of pest and pathogen thus yielding good quality and healthy crop. The average mean minimum temperature 15.2°C and maximum up to 22.3°C was recorded in spring season. The relative humidity remained in range from 53.9 to 97.9% during experiment.

Treatment structure and seed priming with GA_3

The experiment was consist of seed priming in two different GA_3 concentrations with and without salinity stress (NaCl=150mM). Thus total six treatments replicated three times were used in our experiment. Before sowing, seed of sponge gourd C.V '*Jaipuri*' were primed for 24hours in their respective one liter of GA_3 (Sigma-Aldrich, USA) solutions (10^{-4} M and 10^{-8} M) and for control purpose in double distilled water at room temperature in dark condition. During seed priming, fresh air was continuously bubbled in GA_3 solutions and double distilled water with the help of aeration pump. After priming, seeds were three times surfaced washed with autoclave double distilled water and dried with sterilized tissue towels. The seeds were further forced air dried with electric fan until it attained the original weight.

Following are the treatments structure

T1 Control (seed priming in DDW).

T2 GA_3 10^{-4} M.

T3 GA_3 10^{-8} M.

T4 Control (seed priming in DDW) + 150mM NaCl.

T5 GA_3 10^{-4} M+ 150mM NaCl.

T6 GA_3 10^{-8} M+ 150mM NaCl.

Experimental design, growth media and soil characteristics

The experiment was laid out in completely randomized design (CRD) having three replications. The gray black clay loam soil was used for experimental purpose and composite soil samples were collected before experiment for chemical analysis such as pH, electrical conductivity and TDS. The pots were filled in equal ratio 1:1:1 of gray black clay loam soil, compost and farm yard manure and supplemented with recommended basal dose of NPK before planting. The five treated seeds were placed in each earthen pot and cover up with lose soil immediately.

Cultural practices and salinity stress

During growth period cultural practices were uniformly applied to all treatments. Plants were regularly irrigated with double distilled water as per their requirements. The sponge gourd seeds were left to grow for twenty five days for obtaining well establish seedlings plants. Equal amount of DDW, 150 Mm NaCl in strength was applied to designated pots for induction of salinity stress. Before salinity stress induction, designated pots were irrigated with normal DDW to avoid leaching and retain the total amount of NaCl in root zone.

Plants harvesting and different growth parameters

After two weeks of salinity stress, representative seven plants were randomly selected in each treatment for harvesting and observations on different growth parameters. The performance of plant growth under different treatments was judged by parameters like number of days taken to germination, plant height (cm), number of leaves plant^{-1}, leaf area (cm^2), root length (cm), shoot fresh weight (gm) and root fresh weight (gm). Days taken to germination were counted from the day first of sowing till full emergence of all seeds. Shoot length of seven plants was measured from the base of stem up to top of shoot with the help of steel ruler marked in cm. Root length of the same plants was also measured from base up to the root tip. Numbers of leaves were counted on randomly selected seven plants from all replications in each treatment. Representative 15 leaves from all replication in each treatment were carefully detached and measured by leaf area measurement machine (LI-3100C, USA) in cm^2. After harvesting the plants were immediately cut into shoot and root portion and then shoot fresh weight and root fresh weight were measured with digital balance.

Statistical Analysis

The data were statistically analyzed using analysis of variance techniques appropriate for completely randomized design. Mean values were compared using DMRT and considered significant at 0.05 level of probability.

3. Results and Discussion

Soil characteristics

The chemical analysis of soil used as a plant growth media in earthen pots revealed that it has almost neutral pH 7.44±0.39, electrical conductivity 197±6.59 µScm^{-1} and the total dissolved solids (TDS) 117±4.83 mgL^{-1}.

Seed germination

The data on number of days taken to germination are presented in Figure 1. Seed priming with GA_3 10^{-4} M has significantly enhanced germination and reduced the number of days (7±2.00) taken to germination which was followed by seed priming in GA_3 10^{-8}M (10±1.00 days). Contrarily, results shows that maximum number of days (15±1.53) taken to germination was observed in control seeds primed with DDW.

Since importance of gibberellin has been known for its active role in seed germination and at least it perform two mechanisms during this process [9]. One mechanism is to weaken tissue surrounding the embryo by loosening cell wall and endosperm degradation. In second identified mechanism, gibberellin strengthens and enhances the growth of embryo. That is why the enhancement of germination process has decreased the number of days taken to germination in seed primed with high concentration of GA_3 10^{-4} M [9]. There are very few reports on seed priming in vegetable crops and mostly experiments were performed on agronomic crops. Our results are in agreement with those of Sedghi et al. [10], who studied the effect of seed priming with plant growth regulators naphthalene acetic acid (NAA), Gibberellic acid (GA_3) and dry seed as a control treatment on medicinal pumpkin. There results showed that seed priming with GA_3 has significantly higher germination percentage and emergence rate then the rest of treatment. Here, it can be deduced from the report of Sedghi et al. [10], that highest emergence rate (75.2) per day in seed primed with GA_3 will have reduced the number of days taken to germination. Our results are further confirmed by those of Andreoli and Khan [11], who reported that the germination time of pepper and tomato seed primed in GA solution for different periods reduced the average germination time by 2-3 days.

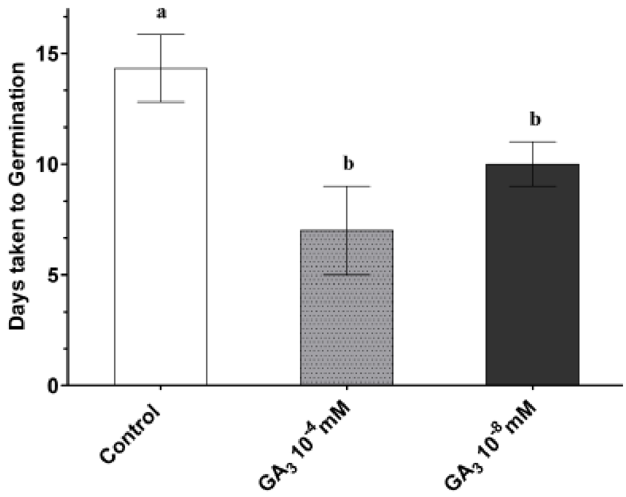

Figure1. Effect of seed priming with GA_3 and water in sponge gourd on number of days taken to germination. Each bar represent mean ± SE. Bars with same letter are not significantly different at $P< 0.05$ as analyzed by DMRT.

Shoot lengths

Seed priming with different concentration of GA_3 for 24hours has significantly affected sponge gourd shoot length with and without salinity stress as shown in Figure 2. The results indicate that seed priming with GA_3 10^{-4} M has significantly increased shoot length both in normal (14.03±1.53 cm) and salinity stress condition (11.93±4.10 cm). However, there was no significant difference in shoot length of seeds primed with GA_3 10^{-8}M and water in salinity stress free conditions. Salinity stress has significantly reduced shoot length (7.1±0.83 cm) in control followed by seed primed with GA_3 10^{-8}M. Early and fast germination results in better stand establishment of crop. Bejandi et al.[12] studied the effect of soybean seed priming in water, auxin and gibberellin with three different rates of sulfur on growth parameters under field saline condition (3.61 ds m^{-1}). Our results are in agreement with Bejandi et al. [12], as they obtained 18% increase in soybean shoot length of primed seed after 100 days of sowing. However, seed priming of soybean in gibberellin also increased chlorophyll and relative water contents as compared with other treatments. Results of significant increase in sponge gourd shoot length primed with GA_3 10^{-4} M with and without salt stress are also in line with those of Pill and Kilian (2000) [13]. As they reported that seed priming of Parsley with 1 mM GA_3 at 30°C produced 25% longer hypocotyl length and 65% heavier than control treatment and attributed this to the growth stimulation of hypocotyls, leaf petioles and laminas.

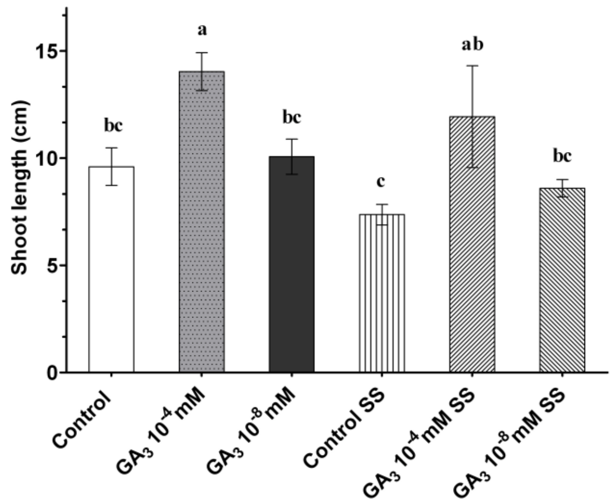

Figure 2. Effect of seed priming with GA_3 in sponge gourd on plant height with and without salinity stress. Each bar represent mean ± SE. Bars with different letter(s) are significantly different at $P< 0.05$ as analyzed by DMRT

Number of leaves

Only seed priming with GA_3 10^{-4} M has significantly increased number of leaves (9.76±1.53) in sponge gourd plants without salinity stress (Figure 3). This was followed by seed priming in GA_3 10^{-4}M with salinity stress and GA_3 10^{-8}M without salinity stress, respectively. No significant difference was observed for the minimum number of leaves plant^{-1} in control without salinity stress and control and seed priming in GA_3 10^{-8}M with salinity stress.

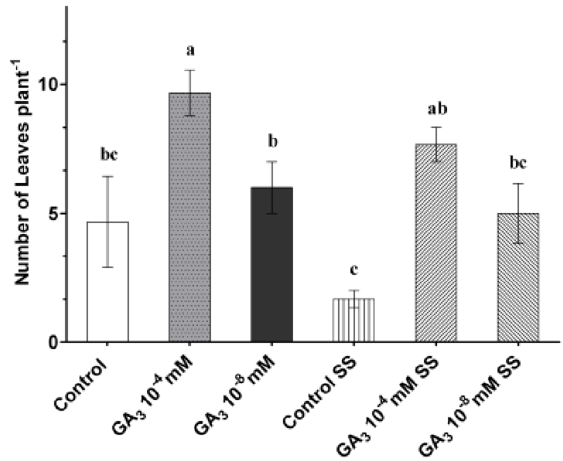

Figure 3. Effect of seed priming with GA_3 in sponge gourd on number of leaves plant^{-1} with and without salinity stress. Each bar represent mean ± SE. Bars with different letter(s) are significantly different at $P< 0.05$ as analyzed by DMRT.

Leaf Area (cm^2 plant^{-1})

Seed priming with GA_3 10^{-4} M has also significantly increased leaf area (13.98±0.02 cm^2) in sponge gourd without salinity stress and followed by the same treatment in salinity stress (Figure 4) as compared to other treatments. No significant difference was observed for the leaf area in control without salinity stress and control and seed priming in GA_3 10^{-8}M with salinity stress.

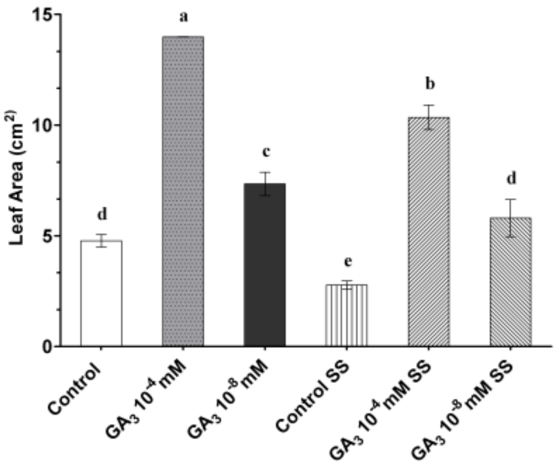

Figure 4. Effect of seed priming with GA_3 in sponge gourd on leaf area with and without salinity stress. Each bar represent mean ± SE. Bars with different letter are significantly different at $P < 0.05$ as analyzed by DMRT.

Root length (cm)

Significant variation was observed in sponge gourd root length primed with different GA concentrations under normal and salinity stress (Figure 5). Sponge gourd seed primed in GA_3 10^{-4} M solution showed significant increase (14.07±1.15 cm) in root length under no salinity stress conditions as compared to the other treatments with or without salinity stress. This was followed by the root length of seed primed in GA_3 10^{-8} M without salinity stress and GA_3 10^{-4} M with salinity stress, respectively. Salinity stress adversely affected the plant growth in control plants and significantly reduced the root length (2.97±0.51 cm) in sponge gourd seed primed with water. Ghodrat and Rousta [14] reported the significant stimulatory effect of seed priming with GA_3 on root length under saline condition. They treated corn seed with three different GA_3 concentrations for 12 hours and salinity stress condition was imposed at different levels of 0, 5, 10, 12 and 15dsm^{-1}. Seed primed with 2.5mgL^{-1} GA_3 and 1.5mgL^{-1} GA_3 increased 36% and 125% root length in salinity stress of 5 and 10dsm^{-1}, respectively. It clearly reveals the growth promotory effect of seed priming with GA_3 and overcome the salinity stress in roots. Our results are also in agreement with Sadeghi et al [15], as they observed longer radicle length of milk thistle treated with gibberellic acid.

Shoot fresh weight

Data regarding shoot fresh weight of sponge gourd plants primed in different GAs concentration with or without salinity stress are depicted in Figure 6. Plants of sponge gourd seed

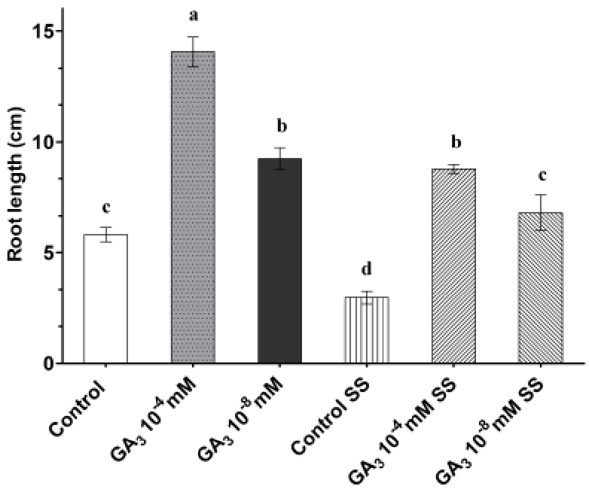

Figure.5 Effect of seed priming with GA_3 in sponge gourd on root length with and without salinity stress. Each bar represent mean ± SE. Bars with different letter are significantly different at *P*< 0.05 as analyzed by DMRT.

primed in GA_3 10^{-4} M without salinity stress yielded significantly highest amount of shoot fresh weight (10.13±0.74gm) as compared to the other treatments and followed by the same treatment under salinity stress. The minimum shoot fresh weight (1.88±0.65g) was recorded in control plants of seeds primed with water under salinity stress. Our results are in conformity with those of Ghodrat and Rousta [14], they recorded significantly higher fresh weight of corn seedlings under three extreme levels (10, 12, 15 dsm^{-1}) of salinity stress for the seeds primed in $1.5mgL^{-1}$ GA_3. Similarly, Pill and Kilian [13], obtained maximum shoot dry weight for the parsley seeds primed in 1 $mMGA_3$ at 30°C.

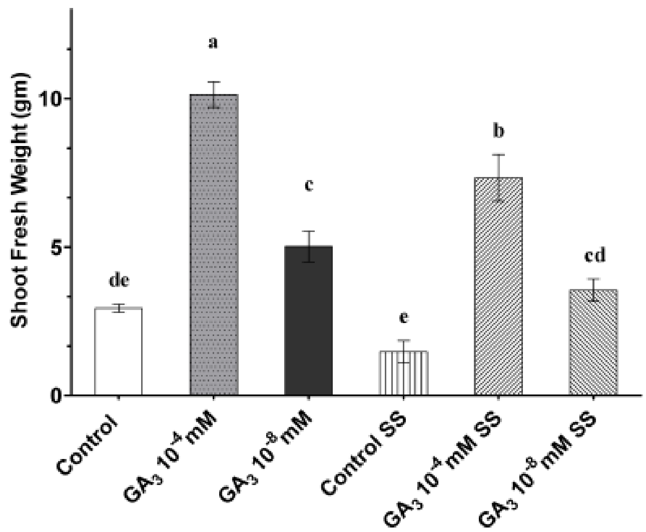

Figure 6. Effect of seed priming with GA_3 in sponge gourd on shoot fresh weight with and without salinity stress. Each bar represent mean ± SE. Bars with different letter(s) are significantly different at *P*< 0.05 as analyzed by DMRT.

Root fresh weight

Data on fresh root weight was taken to observe the growth promotory effect of seed priming in GA_3 with or without salinity stress (Figure 7). The results indicate that the maximum fresh root weight (0.93±0.05gm) was attained by plants treated with GA_3 10^{-4} M without salinity stress as compared to other treatments with or without salinity stress. Moreover, there was no significant difference in root fresh weight of sponge gourd seed primed in GA_3 10^{-4} M under salinity stress and GA_3 10^{-8} M without salinity stress at 95% confidence interval. Likewise the other plant growth parameters salinity stress has also affected the root fresh weight of control plants primed in water and significantly reduced (0.15±0.02gm) as compared to the other treatments. Our results are in line with Sheykhbaglou et al. [16]. They observed that sorghum seed priming with gibberellin produced highest seedling (including both radical and plumule) dry weight under higher level of drought stress and it is known fact that the deleterious consequences of drought stress are similar to that of salinity.

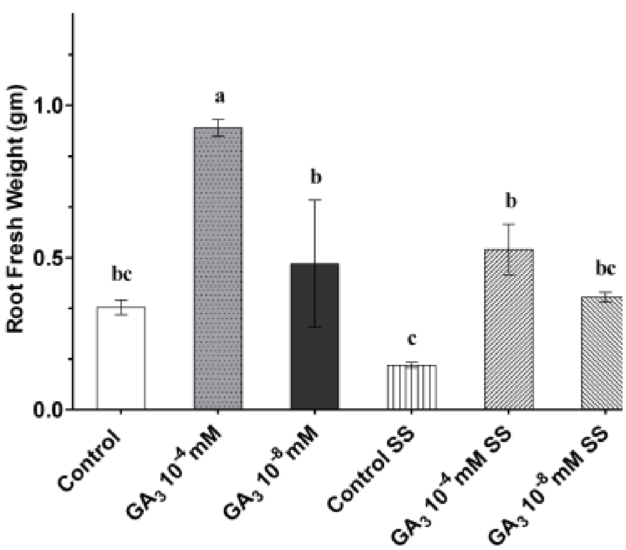

Figure 7. Effect of seed priming with GA_3 in sponge gourd on fresh root weight with and without salinity stress. Each bar represent mean ± SE. Bars with different letter(s) are significantly different at *P*< 0.05 as analyzed by DMRT.

Conclusion

The aforementioned description clearly indicates the importance of GA_3 for sponge gourd under salinity stress. Our results show that overall seed priming in GA_3 enhanced seed germination and prevented the adverse effects of salt stress in sponge gourd. However, seed priming with GA_3 10^{-4} M has significantly minimized days to germination, increased plant height, number of leaves per plant, leaf area, root length, root fresh weight and shoot fresh weight with and without salinity stress. Hence, this study suggests that seed priming of sponge gourd with GA_3 10^{-4} M is well appropriate for the protection of sponge gourd under 150 mM salinity stress. Furthermore, the outcome of this study will add clear dimensions of seed priming with GA_3 for its protective role and better growth in sponge gourd under salinity stress.

Acknowledgement

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