DOI: 10.1515/cerce-2016-0009
Available online: www.uaiasi.ro/CERCET_AGROMOLD/
Print ISSN 0379-5837; Electronic ISSN 2067-1865

Cercetări Agronomice în Moldova
Vol. XLIX , No. 1 (165) / 2016: 99-109

EFFECT OF GIBBERELLIC AND SALICYLIC ACIDS PRE-SOAKING ON SEED GERMINATION ATTRIBUTES OF CUCUMBER (*CUCUMIS SATIVUS* L.) UNDER INDUCED SALT STRESS

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Received January 16, 2016. Accepted: March 15, 2016. Published online: April 08, 2016

ABSTRACT. Saline stress is one of the most deleterious abiotic stress determining a considerable reduction in agricultural production. Seed germination is the primitive plant growth stage and considered as vulnerable to saline stress. However, the exogenous application of natural plant growth regulators has been reported as one of the mitigation strategies. A Petri dish experiment under controlled conditions was conducted at King Saud University. The aim was to quantify the negative impact of induced saline stress (NaCl) on seed germination attributes and role of gibberellic acid (GA3) and salicylic acid (SA) to reduce the inhibitory effect of saline stress on cucumber (*Cucumis sativus* L.) seeds. The treatments consist of two sets as: seed pre-soaking solution and NaCl stress. Five pre-soaking solutions were prepared as: H_2O (control), GA3 (100 ppm), GA3 (200 ppm), SA (0.5 ppm) and SA (1.0 ppm). While saline stress was imposed by NaCl at three levels such as: no stress (distilled water, control), mild stress (NaCl, 50 mM) and higher stress (NaCl, 100 mM). Results depicted that NaCl induced stress has significantly affected the all studied germinations attributes. The maximum NaCl stress (100 mM) stood highest in inhibiting seed germination percentage, seedling length, and seedling fresh and dry weights, followed by mild stress and control, respectively. Seed pre-soaking treatments were recorded non-significant for seedling length, fresh and dry weights while significant for germination percentage and number of seeds germinated over period of time. The interaction between seed pre-soaking treatments and induced saline stress was recorded significant. Overall, GA3 at the rate of 100 ppm solution performed a contributory role to mitigate the negative effect of saline stress.

Key words: Seed pre-soaking; NaCl stress; Inhibition; Germination percentage.

INTRODUCTION

Salinity is one of the non-living environmental stresses that affects the

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agricultural production, especially in arid and semiarid regions (Grewal, 2010). It has been estimated that about 50% of the arable land will be affected by salt stress up to 2050 (Munns and Tester, 2008). Seed germination is one of the most salt-sensitive plant growth stages and is severely inhibited under increasing saline stress (Bouda and Haddioui 2011). A number of reports concluded that seed germination of both halophytes (Li *et al.,* 2005; Abdel-Hamid, 2014) and glycophytes (Zhu, 2003) inhibited by salinity, especially when salt concentration was high (Hajiboland *et al*., 2009; Dadkhah 2010; Saadat *et al*., 2012). Salinity reduces germination and its speed, wet and dry weights of root and shoot, and root and shoot lengths in cucumber - *Cucumis sativus* (Baghbani *et al*., 2013). The germination rate, germination energy and enzymatic activity of cucumber seed were inhibited remarkably under NaCl stress (Chang *et al.,* 2010; Zhang and Shang 2010). Seed germination of cucumber, eggplant and sugar beet was only inhibited by higher NaCl concentration (Wang *et al*., 2004; Saadat *et al*., 2012). The results of Yang *et al.* (2004) showed that germination rate of the cucumber seeds treated with 50 mmol/L NaCl was not significantly comparable to control, however, those seeds that were treated with NaCl higher concentrations (≥ 75 mmol/L) showed significantly lower germination rate. Additionally, the fresh weight of shoots and roots of seedling gradually decreased with increasing concentration of NaCl. Abbas *et al.* (2013) mentioned that NaCl levels; 30, 60, 90, 120, and 150 mM led decreased germination percentage, mean germination time, shoot and root lengths and dry weights. Habibi and Abdoli (2013) study on *Lepidium sativum* showed that plumule and radicle lengths were significantly decreased by increased salinity. Many researchers have tried to interpret the mechanism of salt stress and seed germination. The inhibition of seed germination by salinity is relate to the osmotic effect or specific ionic toxicity. Naseri *et al.* (2012) reported that salinity due to the toxic effects of specific ions and a high concentration of salts reduces the water potential, which prevents the absorption of water by seeds. Higher saline stress gives rise to ionic and osmotic effects combined with oxidative damage, as an important factor in determining the sensitivity of plants to various abiotic stresses.

Plant hormones are active members of the signal cascade involved in the induction of plant stress responses (Pedranzani *et al*., 2003). Concentrated attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators. It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones. However, incorporation of plant growth regulators during pre-soaking, priming and other pre-sowing treatments in many vegetables crops has improved seed performance. Several of studies indicated that GA3 has broad effects on many of the biological processes which occur in plants, such as increased germination percentage in many plants under normal conditions (Nasri *et al.*, 2012; Kandil *et al.*, 2014). The application of GA3 was found to alleviate the adverse effects of saline stress on the seed germination, lengths, fresh and dry weights of the root and shoot, chlorophyll and carotenoid contents of wheat *Triticum aestivum* L. (Turkyilmaz, 2012). Alonso-Ramírez *et al.* (2009) mentioned that exogenous application of GA3 improved tolerance under abiotic stress by induction and increment of the endogenous levels of salicylic acid. Salicylic acid (SA) is one of plant growth regulators, which is a phenolic compound naturally occurring in plants at very low concentrations, but it has significant impact on the various aspects of the plant life (Hayat and Ahmad, 2007; Gharib and Hegazi, 2010). Several application methods, as soaking seeds in SA, adding SA to the hydroponic solution and irrigating or spraying with SA solution have been shown to protect various plant species against abiotic stresses, such as salinity (El Tayeb, 2005; Szepesi *et al.*, 2009). Numerous studies showed that SA improves germination under salt stress conditions, as in pea (McCue *et al.*, 2000) and tomato seeds (Szepesi *et al.*, 2005). Zhang and Shang (2010) reported that cucumber seed treated with 0.1 mmol L^{-1} SA increases germination rate and enzymes activity under NaCl stress.

Cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae family. The fruits of this plant are an important part of traditional Mediterranean diet since antiquity, its pulp and peel extracts contain a high concentration of lactic acid (~7–8% w/w), which can explain the traditional use of cucumber in skin treatment (Sotiroudis *et al.*, 2010). Cucumber fruit contains calcium (20 mg/100 g), iron (0.7 mg/100 g), thiamin (0.3 mg/100 g), niacin (0.2 mg/100 g) and riboflavin (0.01 mg/100 g), and some natural antioxidants, which prevent cardiovascular diseases and cancer (Trichopoulou *et al.*, 2000). Cucumber plants from the greenhouse are important in arid and semi-arid, irrigated using saline groundwater, and are grown for local consumption. From the foregoing, it is important to intensify research on the effects of saline stress on cucumber seed germination and strategies to mitigate these effects in order to establish healthy seedling and continuous productivity. Therefore, the aim of the present study was to determine the extent of cucumber seeds exposed to saline stress and the effect of pre-soaking with different levels of SA and GA3 on seed germination attributes.

MATERIALS AND METHODS

The experiment was carried out at Science College, King Saud University, Saudi Arabia, in April 2015. Healthy seeds of cucumber were selected then sterilized with sodium hypochlorite solution 5% (v/v) for five minutes and washed with sterilized distilled water. Germination experiment was carried out using 30 seeds distributed on Petri dishes for five replications. Each experimental dish was moistened by 5 ml of distilled water (control) or with same quantity of the respective test solution. All solutions were freshly prepared as stock solutions in distilled water, for each experiment, and diluted to the appropriate concentrations before further use. The treatments consist of two sets as seed pre-soaking solution and NaCl stress. Five pre-soaking solutions were prepared as: H_2O (control), GA3 (100 ppm), GA3 (200 ppm), SA (0.5 ppm) and SA (1.0 ppm). While saline stress was imposed by NaCl using three concentrations, such as: distilled water (control), NaCl (50 mM) and NaCl (100 mM). Cucumber seeds were pre-soaked in mentioned solutions for 12 hours. The pre-soaked seeds were then placed in 9 cm Petri dishes containing double layer of Whatman No.1 filter paper. Experiment was designed in randomized complete block design (RCBD) in combination of NaCl stress treatments. All Petri dishes were then transferred to a controlled plant growth chamber under 16 hours' photoperiod, 24±2°C temperature system and 32±5% relative humidity (RH). Distilled water or test solutions were added to each Petri dish, during the experiment as required. The number of germinated seeds were recorded daily, starting from second day over the period of experiment. Seeds that had their radicle length of at least 2 mm were counted as germinated. The germination percentage, hypocotyl (seedling) length, seedling fresh and dry weight were measured at the end of experiments (8^{th} day). Germination percentage was calculated according to the given formula (Bybordi, 2010).

$$GP\ (\%) = n \div N \times 100 \quad (1),$$

where n is the number of germinated seed on the eighth day and N is the number of all seeds.

Hypocotyl length was measured using laboratory ruler. Fresh weight was determined using electric balance while to determine the seedling dry weight, seedlings were dried at 70°C to a constant weight. Data collected was subjected to statistical analysis using software, STATISTICAL ANALYSIS SYSTEM (SAS), version 9.0 (Cary, NC, USA) (SAS, 2001). Means were compared using least significant difference (LSD) at 95 percent confidence interval.

RESULTS AND DISCUSSION

Germination percentage

The germination percentage of cucumber seeds was significantly ($p \leq 0.01$) depressed by increase in NaCl stress (*Table 1*). Control (NaCl0) resulted in maximum germination percentage (92.00%), followed by NaCl50 (85.86%) and NaCl100 (73.34%), respectively (*Table 2*). The germination percentage of cucumber seeds was also recorded highly significant for pre-soaking treatment (*Table 1*). The pre-soaking treatment as GA3100 resulted in highest germination percentage (87.77%). However, GA3 200 also produced statistically overlapping results with it. The minimum germination percentage (77.55%) was observed in SA 1.0 (*Table 2*). Saline stress into pre-soaking treatment interaction also resulted in significant differences. In general, the combinations of lower NaCl stresses (NaCl0, NaCl50) with H_2O, GA3 100 and GA3 200 were recorded highest in germination percentage. Contradictorily, interaction among higher NaCl stresses and salicylic acid resulted lower germination percentage. The lowest germination (63.33%) was recorded in NaCl100 x H_2O (*Table 3*). Overall, GA3 and SA pre-soaking treatments had improved the germination percentage, as compared to control (H_2O), even under saline stress conditions. The findings of current experiment were supported by similar studies, which proved the role of GA3 in plant biological processes during seed germination. GA3 increases germination percentage under salt stress by enhancing the mobilization of starch reserves and increasing amylase activity in cotyledons, which ultimately leads to better seedling's growth. This assertion was supported by Jamil and Rha (2007), Atia *et al.* (2009) and Kandil *et al.* (2014). The positive contribution of GA3 in controlling the inhibitory effects of NaCl stress on germination percentage was reported in *Beta vulgaris* L. (Kandil *et al.,* 2014), chickpea *Cicer arietinum* L. (Lgbal *et al.,* 2001) and wheat *Triticum durum* (Asahina *et al.,* 2002). The increase in germination percentage under saline stress, due to SA in present experiment was in line to the findings of Bahrani and Pourreza (2012), who reported that lower doses of SA promoted the seed germination in cucumber while extremely higher concentrations inhibited the germination percentage. Contradictorily, Guan and Scandalios (1995), for maize (*Zea mays*), and Aldesuquy (1999), for lupine - *Lupinus termis*, have reported that SA resulted in lower germination percentage. However, they did not specify the tolerability range of the mentioned crops.

Table 1 - Analysis of variance (ANOVA) summary of various germination parameters of cucumber seeds exposed to different NaCl stress levels and pre-soaking treatments

Sources of variations	Seedling dry weight (mg)	Seedling fresh weight (mg)	Germination percentage (%)	Seedling length (mm)
NaCl stress (S)	4058.04**	44772.2**	2260.09**	67651.4**
Pre-soaking (PS)	34.74^{NS}	191.3	259.87**	50.7 ^{NS}
S x PS	18.61**	166.4*	118.83**	1427.6**
CV	28.95	10.79	4.82	9.46

Seedling length

Saline stress has significantly ($p \leq 0.01$) hampered the seedling length of cucumber (*Table 1*). Control (NaCl0) resulted in maximum (126.85 mm), which was significantly higher than other two stress levels, and was followed by NaCl50

(112.23 mm). However, the maximum stress (NaCl100) has seriously reduced the seedling length (30.33 mm) much lower as compared to others. Pre-soaking treatments have not produced any significant differences ($p \leq 0.05$) for cucumber seedling length (*Tabs. 1 and 2*). On the other hand, NaCl stress and pre-soaking interaction was recorded significant ($p \leq 0.01$). NaCl0 x GA3 100 (145.73 mm) found highest in seedling height followed by NaCl0 x H_2O (136.47 mm), which was also statistically at par. However, the lowest seedling heights were observed in higher NaCl stress (NaCl100) and pre-soaking combinations (*Table 3*). The inhibitory effect of saline stress on seedling length is also reported by Baghbani *et al.* (2013), who tested five genotypes of cucumber. Habibi and Abdoli (2013) reported the same finding for *Lepidium sativum*, while Bahrani and Pourreza (2012) for wheat. Contradictory to our findings, Turkyilmaz (2012) has claimed increase in seedling length of wheat when GA3 and SA were applied.

Table 2 - Individual effect of various NaCl stress levels and pre-soaking treatments on germination and seedling parameters of cucumber

Treatments	Seedling dry weight (mg)	Seedling fresh weight (mg)	Germination percentage (%)	Seedling length (mm)
NaCl0	35.64 A	114.68 A	92.00 A	126.85 A
NaCl50	35.40 A	109.48 A	85.86 B	112.23 B
NaCl100	13.27 B	38.92 B	73.34 C	30.33 C
LSD	**4.59**	**5.36**	**2.28**	**4.81**
H_2O	27.26	84.80	81.77 C	87.46
GA3 100	30.08	92.20	87.77 A	91.84
GA3 200	28.93	90.86	86.91 AB	90.11
SA 0.5	26.22	84.66	84.66 BC	91.17
SA 1.0	27.42	85.93	77.55 D	88.42
LSD	**NS**	**NS**	**2.95**	**NS**

Seedling fresh weight

Seedling fresh weight of the cucumber was recorded highly significantly ($p \leq 0.01$), differing under various NaCl stress levels (*Table 1*). Although the maximum seedling fresh weight (114.68 mg) was recorded for control treatment (NaCl0), it was statistically at par to NaCl50 (109.48 mg). The highest saline stress (NaCl100) has hampered the seedling fresh weight to much higher extents, valued only 39.92 mg (*Table 2*). Pre-soaking treatments have not resulted significant differences among mean seedling fresh weights of cucumber (*Table 1*). However, the interaction among various NaCl stress levels and pre-soaking solutions was recorded significant ($p < 0.05$) for seedling fresh weight of cucumber. Saline stress control (NaCl0) in combinations of H_2O, GA3 100 and GA3 200 was recorded highest in production of fresh weight as: 117.20 mg, 125.40 mg and 120.40 mg, respectively. Likewise, germination percentage and seedling height, highest concentration of NaCl (NaCl100) was noted significantly decreasing the fresh weight of cucumber, regardless of the pre-soaking treatments (*Table 3*). The similar findings were also reported by Turkyilmaz (2012) for wheat. Yang *et al.* (2004) claimed that the NaCl stress beyond 50 mM concentration can seriously decrease the seedling fresh weight. Turkyilmaz (2012) reported that there is no direct effect of SA application on seedling fresh weight of wheat, however, GA3 can produce positive contribution in seedling fresh weight.

Seedling dry weight

Likewise, seedling fresh weight and dry weight of cucumber seedlings was also significantly ($p \leq 0.01$) reduced by saline stress (*Table 1*). The control (NaCl0) and mild saline stress (NaCl50) had not produced any significant differences and were resulted in 35.64 mg and 35.40 mg of dry weight, respectively. Contradictorily, maximum level of NaCl stress (NaCl100) was recorded significantly lower (13.27), as compared to other two treatments (*Table 2*). Following the same fashion, pre-soaking treatments of various hormonal concentrations had not produced any significant differences ($p \leq 0.05$) for cucumber seedling dry weight (*Tabs. 1 and 2*).

Table 3 - Interactive effect of NaCl and pre-soaking treatments on various germination and seedling parameters of cucumber

Treatment combinations	Seedling dry weight (mg)	Seedling fresh weight (mg)	Germination percentage (%)	Seedling length (mm)
NaCl0 x H_2O	35.13 A	117.20 ABC	96.66 A	136.47 AB
NaCl0 x GA3 100	39.40 A	125.40 A	96.66 A	145.73 A
NaCl0 x GA3 200	38.60 A	120.40 AB	96.00 A	134.40 B
NaCl0 x SA 0.5	31.86 A	105.00 D	90.66 B	110.07 C
NaCl0 x SA 1.0	33.20 A	105.40 CD	80.00 D	107.60 CD
NaCl50 x H_2O	35.40 A	102.80 D	85.32 C	97.60 D
NaCl50 x GA3 100	36.86 A	111.20 BCD	87.99 BC	99.07 D
NaCl50 x GA3 200	33.53 A	111.80 BCD	88.66 BC	103.20 CD
NaCl50 x SA 0.5	34.46 A	109.60 BCD	87.33 BC	132.73 B
NaCl50 x SA 1.0	34.93 A	112.00 BCD	80.00 D	128.53 B
NaCl100 x H_2O	11.26 B	34.40 E	63.33 F	28.34 E
NaCl100 x GA3 100	14.00 B	40.00 E	78.66 D	30.73 E
NaCl100 x GA3 200	14.66 B	40.40 E	76.06 DE	32.73 E
NaCl100 x SA 0.5	12.33 B	39.40 E	75.99 DE	30.73 E
NaCl100 x SA 1.0	14.13 B	40.40 E	72.66 E	29.13 E
LSD	**10.26**	**11.99**	**5.11**	**10.76**

The interaction among saline stress and pre-soaking treatments stood highly significant ($p \leq 0.01$) for seedling dry weight of cucumber (*Table 1*). Regardless of pre-soaking treatments, control (NaCl0) and mild saline stress (NaCl50) have not produced any significant differences. However, higher saline stress (NaCl100) in combinations of pre-soaking treatments were recorded significantly lower (*Table 3*). Baghbani *et al.* (2013) reported similar findings, when five genotypes of cucumber were test for saline stress response, a significant decrease in seedling dry weight was observed. Turkyilmaz (2012) also reported the significant reduction in seedling dry weight of *Triticum aestivum* L. under saline stress.

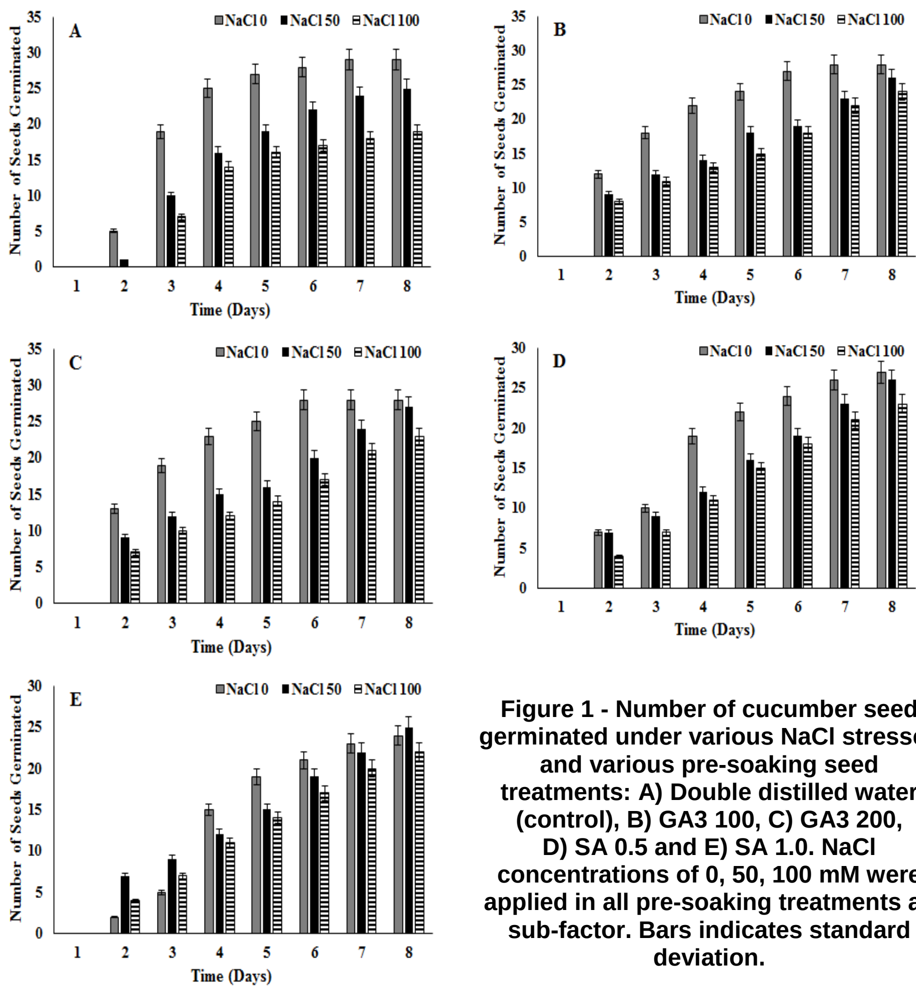

Figure 1 - Number of cucumber seed germinated under various NaCl stresses and various pre-soaking seed treatments: A) Double distilled water (control), B) GA3 100, C) GA3 200, D) SA 0.5 and E) SA 1.0. NaCl concentrations of 0, 50, 100 mM were applied in all pre-soaking treatments as sub-factor. Bars indicates standard deviation.

Number of seed germinated

Number of germinated seeds of cucumber were counted on daily basis continually for eight days, starting from 24 hours after sowing. Saline stress has significantly reduced the number of seeds germinated in H_2O pre-soaked seeds. Control saline stress (NaCl0) produced consistently higher number of germinated seeds, followed by NaCl50. However, the highest NaCl stress 100 mM has seriously suppressed the number of seed germination. A similar general trend was also observed for GA3 100, GA3 200, SA 0.5 and SA 1.0 pre-soaking treatments. However, seed pre-soaking treatments, except H_2O, have decreased the relative impact of salinity on seed germination, when compared for NaCl50 and NaCl100 stress levels. GA3 200 and SA 1.0 performed relatively effective role in reducing the deleterious impact of saline stress on seed germination in cucumber (*Fig. 1*). The current findings are primary in their category and have not been reported previously.

CONCLUSION

It was concluded that saline stress can seriously hamper cucumber seed germination attributes. However, seed pre-soaking with gibberellic acid (GA3) and salicylic acid (SA) can contribute in mitigation of deleterious effects of saline stress and can improve seed germination percentage and number. GA3 at the rate of 100 ppm stood positively highest and may be further explored for general application to improve germination characteristics of cucumber under saline stress.

Acknowledgements. This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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