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Research Article

Enhancing Seed Germination and Yield Performance of Onion (*Allium cepa* L.) under Saline Stress using Plant Growth Regulators and Ascorbic Acid

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ABSTRACT

The study aimed to examine the protective role of plant growth regulators and ascorbic acid against NaCl-induced oxidative damage in onion, to enhance its salinity tolerance mechanism. Onion seeds were subjected to salt stress (10 dS/m) and primed with 30 ppm gibberellic acid (GA_3), 1-naphthylacetic acid (NAA), salicylic acid (SA), and ascorbic acid (AsA). Additionally, onion plants were sprayed with 100 ppm of these chemicals. The application of 30 ppm GA_3, AsA, and SA significantly enhanced seed germination percentage, vigor, energy of germination, and shoot-root length, while reducing mean germination time. In contrast, NAA negatively affected germination by lowering energy of germination and shoot-root length. The highest germination percentage (87.92%) was recorded with SA, followed by GA_3 at 69.58%, while the lowest (49.17%) was observed in the control and NAA treatments. Foliar application of 100 ppm SA resulted in the highest bulb yield (15.04 t/ha) under saline stress, along with improvements in yield-contributing parameters. This yield was statistically similar to those obtained from GA_3 and AsA treatments. In contrast, NAA treatment showed no significant difference from the control. The study highlights SA as a potential PGR for improving onion productivity, offering a valuable approach for mitigating salt stress in onion cultivation.

Introduction

Onion (*Allium cepa* L.) is a popular spice and a commercial vegetable crop worldwide (Teshika et al., 2019). Conventional cultivation methods with poor yield rates reduce the total onion production (Aboukhadrah et al., 2017). The yield can be increased by using suitable varieties and balanced nutrition-based agronomic practices (Garg et al., 2018). The alluvial regions of Bangladesh have a great opportunity for onion cultivation if farmers can reduce the saline effect of these areas. Salt stress hampers germination of seeds, reduces plant growth, inhibits plant development, and induces immature senescence (Das and Biswas, 2022; Sabagh et al., 2021). It can cause a state of seed dormancy by germination inhibition (Benadjaoud et al., 2022) and dying of seed before germination (Ibrahim, 2016). It also induces ion-toxicity and osmotic stress, which can affect water absorption and physiological functions of seed (Balasubramaniam et al., 2023; Hasegawa et. al., 2000). Plant growth regulators (PGRs) and ascorbic acid (Vitamin C) can influence the physiologic reactions (Noein and Soleymani, 2022; Sravani et al., 2020), including accelerated germination by stimulating metabolism in seed (Quamruzzaman et al., 2021; Khan et al., 2020; Moori and Eisvand, 2017). Therefore, this study applied four chemicals- gibberellic acid (GA_3), 1-naphthylacetic acid (NAA), salicylic acid (SA), and ascorbic acid (AsA) – at low concentration (30 ppm during seed germination test and 100 ppm on yield test) to explore their regulatory effect. GA_3 has important role on seed germination by activating enzymes (Sakhabutdinova et al., 2003; Davière and Achard, 2013), and regulating lipid peroxidation (Siddiqui et al., 2020). NAA, a well-known auxin, can significantly increase the vegetative growth of onion (Singh et al., 2019). When soil salinity

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increases, NAA can enhance seed peroxidase activity, catalase activity, and cell membrane integrity (Yuan et al., 2011; Ahmad, 2016; Li et al., 2019). SA, a phenolic compound, contributes to salinity tolerance by osmotic potential, enzyme activation, and ion balance (Ashraf and Ozturk, 2008; Singh and Gautam, 2013; Soliman et al., 2016). AsA is an essential antioxidant in plants (Hossain et al., 2017). AsA, a key antioxidant, reduces the negative impact of soil salinity on plants (Chen et al., 2021; Afzal et al., 2005), and can stimulate antioxidant enzyme activity (Dolatabadian et al., 2008). Due to the increase in food demand in developing countries and the need to improve crop productivity in saline-prone areas, there is a growing interest in develop unconventional methods and explore new sources of bioactive compounds that can enhance onion production. Therefore, the objective was to evaluate the protective roles of plant growth regulators and ascorbic acid in mitigating oxidative damage and improving seed germination of onion under saline stress.

Materials and Methods

Planting materials and crop management

The germination test of Onion (*Allium cepa* L.) cultivar 'Taherpuri' (a local variety) was conducted in the laboratory, and a field trial was carried out under environmental conditions favorable for onion cultivation. 25% compost fertilizer was mixed with the soil during field preparation. Weeding was performed as necessary to control the unwanted weeds. Treatment solutions were applied to the foliage starting at 30 DAS and repeated at 15-day intervals. Saline irrigation water (10 dS/m) was applied every 10 days to impose salt stress throughout the crop growth period for all treatments. In addition, to maintain consistent soil moisture, regular irrigation with non-saline water was applied at 3–5 day intervals.

Treatments for seed germination test

The plant growth promoting chemicals- GA_3 (T_1), NAA (T_2), SA (T_3) and AsA (T_4) were used at concentrations of 30 ppm for seed germination test. Distilled water was used as control (T_0) treatment. A 10 dS/m saline solution was prepared using distilled water through measuring by an electrical conductivity (EC) meter. Each chemical was first weighed, and then dissolved in sterile distilled water and agitated at 180 rpm for 1 hour to prepare the 30 ppm treatment solutions. Petri dishes with a tissue were sterilized under UV light for an hour, after which 100 seeds were spread on each dish. 10 dS/m saline solution was applied to moisten the tissue and induce saline stress during seed germination. Treatment solution were applied from the second day until the seventh day after seed placement. Following treatment, the petridishes were incubated in an incubation chamber at 25 °C for 7 days.

Treatments for yield performance test

The plant growth promoting chemicals- GA_3 (T_1), NAA (T_2), SA (T_3) and AsA (T_4) were applied on foliar region at 100 ppm concentrations for yield performance test. Distilled water was used as control (T_0) treatment for foliar application. Cultural operations along with treatments application were carried out uniformly throughout the experimental period.

Germination Percentage (GP)

Seed germination was calculated through following equation mentioned by Ellis and Roberts (1981):

$$GP = \frac{n}{N} \times 100$$

where: n = number of germinated seeds and N = total number of seed tested.

Mean Germination Time (MGT)

Early Seedling's mean germination time was calculated according to the formula:

$$MGT = \frac{\sum(n \times d)}{\sum n}$$

where: n = germinated seed numbers on days (d) and d = number of days counted (Ellis and Roberts, 1981).

Germination Index (GI)

The following formula mentioned by Benech Arnold et al. (1991) determines the germination, which is a measurement of percentage and speed of germination:

$$GI = (7 \times n1) + (6 \times n2) + \ldots + (1 \times n7)$$

where: n1, n2…. n7 = germinated seed number on the first, second and subsequent days until the 7^{th} day.

Seedling Vigor Index (SVI)

According to Abdul-Baki and Anderson, (1970), following equation was used to determine the SVI:

SVI = (Average shoot length + Average root length) × Germination percentage

Energy of Germination (EG)

Energy of germination was recorded on the fourth day after sowing relative to the number of seeds tested according to Ruan et al. (2002).

$$EG = \frac{\text{Germinated seed no. after seven day}}{\text{Germination day no.}} \times 100$$

Yield

The mean plant height, leaf and bulb diameter were measured and expressed in centimeter. Weight of each bulb was calculated in kilograms considering the planting spacing of 10 cm (plant-to-plant) × 30 cm (row-to-row). The mean values were recorded considering 100 plants per 3 m^2 plot.

$$\text{Bulb yield per ha} = \frac{\text{Yield per plot(kg)}}{\text{Area (m2)} \times 1000} \times 10000$$

Statistical analysis

All data obtained were analyzed following the procedure outlined by Gomez and Gomez (1984). Data were organized in a completely randomized design (CRD), and analyses of variance (ANOVA) were obtained using Minitab Statistical Software 21 (Minitab Inc., State College, PA, USA), and Graphs were designed using Microsoft Excel. The significance of difference between the pairs of means was separated by least significant difference (LSD test at 5% levels of probability.

Results

Seed germination

The result of seed germination test is presented in Table 1. Seeds began to germinate one day after incubation. The highest germination percentage (87.92%) was observed in treatment T_4 (SA) after 7 days, which was statistically similar to T_1 (GA_3) (69.58%). The lowest germination percentage (49.17%) was recorded in both T_0 (control) and T_2 (NAA). Treatments had a significant effect on mean germination time (MGT). The highest MGT (4.94 days) was recorded in T_0 (control), statistically similar to T_2 (NAA) (4.54 days). The lowest MGT was observed in T_1 (GA_3) (3.98 days), which was statistically similar to T_2 (NAA) (4.54 days), T_3 (AsA) (4.22 days) and T_4 (SA) (4.24 days). The germination index was also significantly influenced by treatments. The result showed that, among all treatments the highest germination index (266) was recorded in seeds treated with SA, which was statistically similar to GA_3 (224). The lowest index (121.67) was recorded in T_0 (control), statistically similar to T_2 (NAA) (136). The highest seedling vigor index (SVI) (519.54) was found in the SA treatment, statistically similar to GA_3 (492.11) and AsA (384.54). The lowest SVI (139) was recorded in T_2 (NAA), which was statistically lower than the control (255.08), suggesting that NAA may have a negative effect on seedling vigor under saline condition. Energy of germination (EG) also showed significant variation ($p < 0.001$) among treatments. The highest EG (747.62) was recorded in SA treatment (T_4), which was significantly superior to all other treatments. GA_3 (580.95), AsA (514.29) showed statistically similar EG values, while the control (252.38) had lowest EG, statistically similar to NAA (304.76). This result demonstrates that NAA at 30 ppm had no significant effect on onion seed germination under salt stress, whereas SA at the same concentration significantly improved germination performance.

Table 1. Influence of GA_3, SA, NAA and AsA at 30 ppm on energy of germination of onion seeds under salinity stress.

Treatments	GP	MGT	GI	SVI	EG
T_0	49.17 ± 4.58^{c}	4.94 ± 0.19^{a}	121.67 ± 7.85^{c}	255.08 ± 14.74^{bc}	252.38 ± 54.92^{c}
T_1	69.58 ± 1.10^{ab}	3.98 ± 0.03^{b}	224 ± 4.51^{ab}	492.11 ± 35.31^{a}	580.95 ± 4.76^{b}
T_2	49.17 ± 1.10^{c}	4.54 ± 0.14^{ab}	136 ± 6.43^{c}	139 ± 24.02^{c}	304.76 ± 31.23^{c}
T_3	67.92 ± 7.98^{bc}	4.24 ± 0.20^{b}	202.33 ± 15.96^{b}	384.54 ± 57.39^{ab}	514.29 ± 8.25^{b}
T_4	87.92 ± 1.10^{a}	4.22 ± 0.04^{b}	266 ± 4^{a}	519.54 ± 10.50^{a}	747.62 ± 28.97^{a}
CV(%)	11.25	5.48	10.4	15.96	11.31
$LSD^{(5\%)}$	13.246	0.437	35.959	103.98	98.844
Level of significance	**	**	**	**	**

(T_0 – Distilled water; T_1 - 30 ppm GA_3; T_2 - 30 ppm NAA; T_3 - 30 ppm AsA; T_4 - 30 ppm SA).
NS = non-significant
* statistically significant at 5% level of significance
** statistically significant at 1% level of significance

The data on fresh weight, dry weight, radicle and plumule length of germinated seedlings are presented in Table 2. The highest seedling fresh weight (26.93 g) was observed in the SA treatment, which was statistically similar to GA_3 (26.07 g) and AsA (24.52 g). Seed priming with 30 ppm SA produced the highest fresh weight, while the NAA treatment had a negative impact on seedling fresh weight. However, NAA positively affected seedling dry weight for the physically unchanged material content influenced by NAA, which can be compared with the result of Singh et al. (1995). The greatest length of plumule (4.65 cm) and radical length (2.43 cm) were recorded in the GA_3 treatment under saline stress, indicating a significant effect. In contrast, NAA treatment negatively affected both plumule and radicle growth compared to the control. A similar pattern was reported in pumpkin by Rafique et al. (2011), where seeds treated with SA and AsA under salt stress improved seedling development.

Table 2. Influence of GA_3, SA, NAA and AsA at 30 ppm on germinated seedlings of onion under salinity stress.

Treatment	Seedling Fresh Weight	Seedling Dry Weight	Plumule Length	Radicle Length
T_0	$16.08±0.99^{b}$	$3.08±0.13^{a}$	$3.87±0.05^{a}$	$1.36±0.16^{ab}$
T_1	$26.07±0.39^{a}$	$2.85±0.06^{a}$	$4.65±0.11^{b}$	$2.43±0.56^{a}$
T_2	$9.68±0.82^{c}$	$3.17±0.04^{a}$	$1.94±0.38^{a}$	$0.87±0.17^{b}$
T_3	$24.52±1.68^{a}$	$3.09±0.11^{a}$	$4.00±0.02^{a}$	$1.62±0.16^{ab}$
T_4	$26.93±0.67^{a}$	$3.00±0.05^{a}$	$4.54±0.15^{a}$	$1.37±0.09^{ab}$
CV (%)	8.46	4.89	8.63	32.9
$LSD_{0.05}$	3.179	0.271	0.596	0.902
Level of significance	**	NS	**	*

(T_0 – Distilled water; T_1 - 30 ppm GA_3; T_2 - 30 ppm NAA; T_3 - 30 ppm AsA; T_4 - 30 ppm SA).
NS = non-significant
* statistically significant at 5% level of significance
** statistically significant at 1% level of significance

Shoot-Root length (cm)

The treatments had a significant influence on young seedlings' shoot-root length (Figure 1). The highest shoot-root length ratio, indicating balanced shoot and root growth, was observed in the GA_3 treatment. SA treatment stimulates shoot growth more than root growth. AsA also showed a similar trend under saline condition. Conversely, NAA treatment negatively impacted the shoot-root ratio, likely due to toxic effects under salt stress.

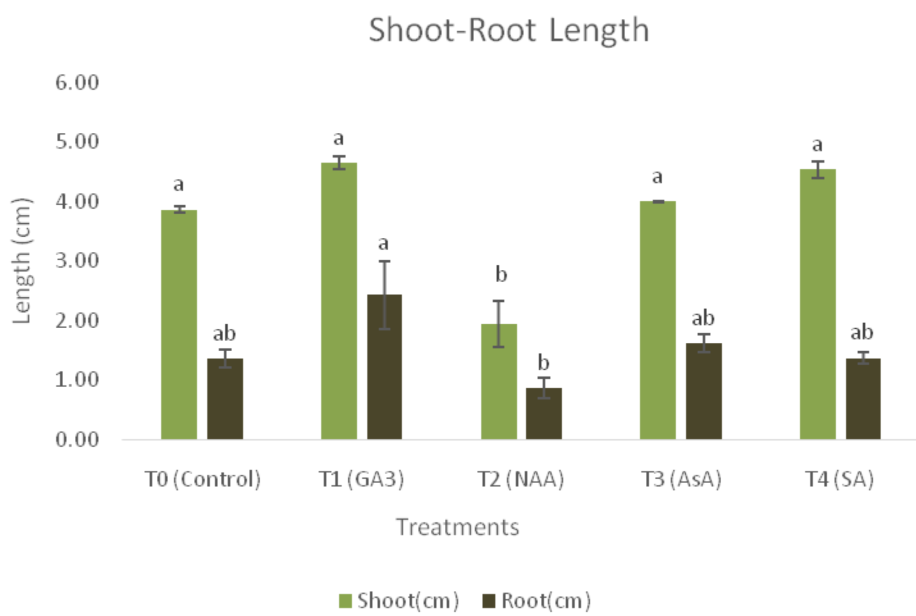

Figure 1. Influence of GA_3, SA, NAA and AsA at 30 ppm on shoot-root length of young onion seedlings under salinity stress. The different values indicate the shoot and root length of young seedlings after seven days.
(T_0 – Distilled water; T_1 - 30 ppm GA_3; T_2 - 30 ppm NAA; T_3 - 30 ppm AsA; T_4 - 30 ppm SA).

Yield attributing parameters

The result of yield contributing parameters of onion are presented in Table 3. Plant height was non-significantly (P>0.005) influenced by treatments. However, the highest plant height (17.87 cm) was observed in plants treated with 100 ppm GA_3, which was 1.1 cm, 0.5 cm, and 1.6 cm higher than those treated with 100 ppm NAA, AsA and SA, respectively. Leaf diameter was significantly (P<0.05) affected by treatments under 10 dS/m salinity. The highest diameter (0.79 cm) was recorded in plants treated with 100 ppm GA_3, which was 0.9 cm, 1.0 cm, and 0.5 cm higher than those treated with 100 ppm NAA, AsA and SA, respectively. However, these values were statistically close to one another. Bulb diameter showed significant variation among treatments. The highest bulb diameter (3.14 cm) was observed in plants treated with 100 ppm SA, which was statistically similar to the GA_3, AsA treatments. Similarly, the highest bulb weight (45.13 g per plant) was recorded in the 100 ppm SA treatment, which was also statistically similar to GA_3 and AsA treatments. No significant difference were found between the T_2 (NAA) and T_0 (control) treatments in terms of bulb weight.

Table 3. Influence of GA_3, SA, NAA and AsA regulators at 100 ppm concentration on the total yield attributing parameter of onion under salt stress.

Treatment	Plant height (cm)	Leaf diameter (cm)	Bulb Diameter (cm)	Bulb weight (g)	Yield (t/ha)
T_0	12.92±0.4^a	0.46±0.04^b	1.94±0.04^b	18.55±2.93^b	6.18±0.98^b
T_1	17.87±0.57^a	0.79±0.04^a	3.08±0.21^a	31.31±3.52^{ab}	10.44±1.17^{ab}
T_2	16.71±2.03^a	0.7±0.11^{ab}	2.44±0.07^b	28.82±3.21^b	9.61±1.07^b
T_3	17.3±0.1^a	0.69±0.06^{ab}	3.06±0.1^a	30.35±3.21ab	10.12±1.07^{ab}
T_4	16.24±0.86^a	0.74±0.04^{ab}	3.14±0.15^a	45.13±3.75^a	15.04±1.25^a
CV	11.06	16.91	8.25	18.74	18.74
LSD	3.262	0.207	0.411	10.51	3.503
Level of significance	NS	*	**	**	**

(T_0 – Distilled water; T_1 - 100 ppm GA_3; T_2 - 100 ppm NAA; T_3 - 100 ppm AsA; T_4 - 100 ppm SA)
NS = non-significant
* statistically significant at 5% level of significance
** statistically significant at 1% level of significance

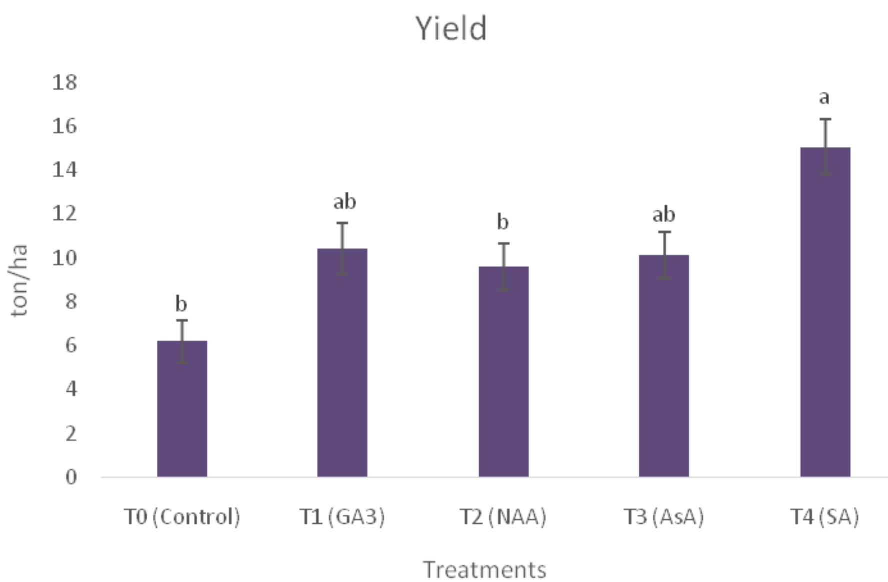

Figure 2. Influence of GA_3, SA, NAA and AsA at 100 ppm on bulb yield of onion under salt stress. (T_0 – Distilled water; T_1 - 100 ppm GA_3; T_2 - 100 ppm NAA; T_3 - 100 ppm AsA; T_4 - 100 ppm SA).

Yield was significantly (P<0.001) influenced by all the treatments except NAA (Table 3). The highest bulb yield (15.04 t/ha) was recorded in plants treated with 100 ppm SA under salt stress, which was statistically similar to the yields from GA_3 and AsA treatments. There were no significant difference between NAA treatment and the control (Figure 2.). Overall, the results indicated that vegetative growth was more enhanced by GA_3, while SA treatment contributed most effectively to bulb bulb production by mitigating the adverse effects of soil salinity.

Discussion

Plant Growth Regulators (PGRs) play a crucial role in the germination, growth, development, and yield of onions. These are chemical substances that influence physiological processes in plants at very low concentrations (Sharma et al., 2016). From the results, it was found that under salt stress onion seed primed with of 30 ppm gibberellic acid (GA_3), ascorbic acid (AsA), and salicylic acid (SA) significantly improved germination percentage, seedling vigor, germination energy, and the shoot-root length, while also reducing the mean germination time. Conversely, 30 ppm naphthalene acetic acid (NAA) had a negative impact, lowering both germination energy and the shoot-root length (Singh et al., 2017). The highest germination percentage (87.92%) was achieved with SA (T_4), followed by GA_3 (T_1) at 69.58%, whereas the lowest percentages were observed in the control (T_0) and NAA (T_2) treatments, each around 49.17%. Root-shoot length, and fresh and dry weight of young seedlings were significantly increased by 30 ppm AsA and SA treatments under both normal and saline conditions, similar observations were reported for pumpkin seeds treated with SA and AsA under saline conditions (Rafique et al., 2011). Qamar et al. (2022) reported that seed priming with AsA and SA promoted heat-stress tolerance and enhanced growth and productivity of Indian squash. Afzal et al. (2005) also found that germination percentage was significantly increased by AsA and SA seed treatments, although mean germination time was unaffected. Similar results heve been reported in tomato, where SA and AsA treatments improved seedling vigor compared to untreated seeds (Mirabi and Hasanabadi, 2012). This indicates that seed priming with PGRs significantly enhanced the germination index under salt stress (Li et al., 2019). Foliar application of 100 ppm SA produced the highest bulb yield (15.04 t/ha) (Ghani et. al., 2021; Kka, 2021), with comparable yields from GA_3 (10.44 t/ha) and AsA (10.12 t/ha) treatments (Mahmoud et al., 2024), while the NAA (9.61 t/ha) treatment did not show any significant difference from the control (6.18 t/ha). Based on statistical analysis, two distinct treatment groups were identified: T_2 (NAA) and T_0 (control) with lower performance, and T_1 (GA_3), T_3 (AsA), and T_4 (SA) with significantly better performance. SA and GA_3 demonstrated positive effects on plant growth, bulb diameter, and yield under saline conditions (Ghani et. al., 2021). These findings align with previous research suggesting that specific concentrations of PGRs can improve plant resilience and productivity under stress conditions (Atif et al., 2020; Zahra et al., 2011; Hye et al., 2002), while excessively high concentrations may reduce yield (Ünlükara et al., 2007; Hakim et al., 2014). Ascorbic acid also had a positive effect in reducing salt-induced damage (Khan and Ashraf, 2008). Subimal Mondal and Shukla (2005) reported that GA_3 was most effective for producing larger bulbs and improving the overall yield quality of onion. Deb et al. (2024) examined the effect of GA_3 and boron on summer tomato and found that GA_3 alone improved growth and yield more than boron including the highest yield and plant performance. Kale et al. (2024) reported that PGRs had significant impact on vegetative growth, yield, and quality of onion, with the highest bulb yield achieved through a 0.6% foliar spray, which was statistically at par with the 1.4% soil drenching treatment when combined with the recommended dose of fertilizers. Singh and Usha (2003), and Czerpak et al. (2002) found that an exogenous infusion of SA improved the photosynthetic rate by the enhancement of chlorophyll a, b, and carotenoids. In addition to controlling physiological and biochemical functions in plants, salicylic acid has the potential to be a growth regulator that might enhance plant development in saline condition. AsA also had a positive effect in reducing salt-induced damage. This might be because of the increased activity of reactive oxygen species (ROS) brought on by salinity. GA_3 enhanced plant development through improved photosynthetic capability led to the accumulation of the most photosynthates, which also redistributed to the storage organ (Sarkar et al., 2018). Therefore, GA_3, SA, and AsA at appropriate concentrations, along with effective application methods such as seed priming and foliar spray, can significantly enhance onion germination, growth, and yield under both normal and stress conditions.

Conclusion

The study demonstrated that the onion seed germination and yield under salt stress can be effectively improved through the application of plant growth regulators (PGRs) or ascorbic acid (ASA) at a concentration of 30 ppm for seed priming and 100 ppm for foliar spraying. Among the treatments, salicylic acid (SA) resulted in significantly higher germination rates and the greatest bulb yield. Although gibberellic acid (T_1) and ascorbic acid (T_3) showed statistically similar but slightly lower performance compared to SA, they still provided notable improvements over the control. Conversely, naphthalene acetic acid (NAA, T_2) had a detrimental effect on early growth parameters such as germination energy, seed vigor index, and shoot-root development, indicating its unsuitability under saline conditions at the tested concentrations. While higher concentrations of certain PGRs may promote yield, they may also hinder seed germination, as observed with NAA. In conclusion, the results suggest that salicylic acid (SA) is the most effective treatment for enhancing both seed germination and bulb yield of onion under salt stress, making it a promising and practical option for onion cultivation in saline-prone areas.

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