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Salicylic acid pre-treatment effects on *Beta vulgaris* L. multigerm germination and germination indices

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Key words: Germination percentage, Height reduction, Relative injury rate, Salicylic acid, Seed vigor.

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Abstract

The response of *Beta vulgaris* L. seeds to pre-treatment with salicylic acid (SA) was investigated at the early seedling stage and focused on germination indices to evaluate seed quality at a particular treatment. To achieve this goal, beet seeds were treated with various concentrations of salicylic acid (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6, 6.0 and 6.4 mM) along with control. The experiment was conducted with a dark red variety of beet seeds. Seeds exhibited more tolerance up to 2.0 mM SA treatment. High concentrations (>2.0 mM) of SA delayed the mean germination time of seeds and increased relative injury rate and seedling height reduction. However, germination percentage, germination index, seed vigor, water uptake percentage reduced significantly. Total inhibition of seed germination was found at 5.2 to 6.4 mM SA treatment. Rudimentary seedling growth was observed from 3.6 mM SA to further treatments. From morphological results, this study proposes that up to 2.0 mM of salicylic acid treatment may be helpful in seed germination and seedling growth of the beet. Also, this study might be helpful for further research on the influence of salicylic acid on the growth and development of beets.

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Introduction

In the agricultural production of a vegetable crop, the rapid and uniform germination at sowing is crucial for improving crop yield and quality; it may also affect seedling survival rates and vegetative growth. In the case of beet, most cultivars are multigerm, and they are a tightly compressed collection of one to several fruits, each containing one seed with an embryo. A table beet seed (fruit) is called a seed ball. Generally, each seed ball contains two embryos; however, certain propagules may contain three or four embryos. Because of some significant seed factors that negatively influence beet germination, even in optimum climatic circumstances, nonsynchronous germination occurs in table beet seed batches. Firstly, the seed ball can be surrounded by a mucilaginous layer, mucilage acting as a physical barrier to oxygen diffusion and drastically reducing seed respiration rates. Phenolic chemical inhibitors in the perianth and pericarp tissue in the fruit hinder germination. These factors can lead to dormancy which can be resulted in low and non-uniform germination. In agriculture, seed germination is an important factor that significantly impacts plant growth and yield. Various growth hormones, such as GA, ABA, and ethylene, have been extensively investigated in seed germination for their roles and genetic pathways (Ariizumi, 2007; Robert, 2008; Seo, 2009). Although its specific role and the underlying molecular mechanisms involved in germination under stressful conditions have not been fully understood, SA does have a role in germination (Borsani, 2001; Rajjou, 2006; Alonso, 2009). Therefore, understanding the effect of various concentrations of salicylic acid on seed dormancy and germination is of great significance for improving crop yield and quality.

Salicylic acid is an important plant hormone that naturally occurs in plants in very low amounts. It regulates many aspects of plant growth and development and resistance to various abiotic/biotic stresses. Exogenous salicylic acid has been shown to affect biotic stress resistance and tolerance to a variety of abiotic stresses (heat, UV radiation, drought, osmotic stress, chilling, heavy metals, and salinity), as well as several aspects of plant growth and development, including seed germination, flowering, vegetative growth, fruit yield, senescence, thermogenesis, stomatal closure, and root growth (Malamy, 1992; Hayat, 2010; Rivas-San Vicente, 2011; Miura, 2014 and Khan, 2015). Attempts to discover salicylic acid effector proteins have revealed that SA binds to and changes the activity of several plant proteins, contrasting with the previously thought that hormones work through one or a few receptors (Dempsey, 2017). According to recent research, salicylic acid regulates diverse aspects of plant responses by extensive signaling cross-talk with other growth hormones in abiotic stresses (Achard 2006; Vlot 2009 and Wolters 2009). In view of this background, the various concentration of salicylic acid was applied to study the response on beet germination and germination indices.

Material and methods

Plant materials

The seed of a multigerm variety of dark red beetroot (Kalash Seeds Pvt. Ltd, Jalna), was selected to evaluate the effect of SA on germination and indices. Healthy seeds were surface sterilized with 70% ethanol solution. Seeds were washed five times in a 5% sodium hypochlorite solution, washed thoroughly five times with distilled water, and air-dried with the help of tissue paper.

Salicylic acid treatment

To investigate the influences of various concentrations of salicylic acid on beet seed germination, seeds were Soaked in various concentrations of Salicylic acid (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6, 6.0 and 6.4 mM along with control) for 24 hours at room temperature (27 ± 0.6 °C). The 50 uniformly sized sterilized multigerm were spread and allowed to grow on germination paper in a 150 mm diameter sterilized Petri plate at room temperature (27 ± 0.6 °C). Each germination paper was moistened with respective concentrations of salicylic acid solutions. All the experiments were done with replicates.

Observation and data collection

The number of germinated seeds was observed and counted daily up to 10 days from treatment. Seed with 2 mm radicle emergence from the seed coat, considered as germinated. Water uptake, germination percentage, germination index, relative injury rate, seed vigor, mean germination time, stress tolerance, fresh weight, dry weight was among the parameters investigated in the experiment. Seedlings were selected at random for each salicylic acid treatment to measure morphological characteristics such as seedling height, shoot length, and root length. Multigerm seeds must be counted frequently since the growing seedlings will detach from the cluster, making it difficult to determine the source. Each cluster with only one normal seedling is only counted once.

Germination Indices

Germination percentage (GP)

Germination percentage was calculated from the following equation.

Germination (%) = Number of germinated seeds / Total number of seeds × 100

Germination index (GI)

The germination index for each treatment is determined by the formula proposed by (Li 2008).

$GI = \sum Gt / Dt$

Where, Gt is the number of the germinated seed on day t and Dt is the time corresponding to Gt in days.

Relative injury rate (RIR)

The Relative injury rate for each salicylic acid treatment is determined by the formula proposed by (Li 2008).

RIR= Germination (%) in control - Germination (%) in salt treated seeds /Germination (%) in control

Seed vigor

Seed vigor is defined as the ability of a seed to germinate or Properties of the seed or seeds that determine the activity and performance level during germination and seedling emergence. The method given by Abdul-Baki & Anderson (1973) was used to determine the seed vigor value.

Seed vigor= (Length of hypocotyl + length of radical) × Germination (%)/100

Mean germination time (MGT)

MGT refers to the average time a seed needs to initiate and end the germination process. MGT for each salicylic acid treatment is determined by the formula given by (Ellis & Roberts, 1981).

$MGT = \sum (n \times d) / N$

where n is number of seeds germinated on each day, d = number of days from the beginning of the test, and N is total number of seeds germinated at the termination of the experiment.

Stress tolerance (ST)

Stress/salt tolerance can be estimated using the formula below (Tsegay & Gebreslassie, 2014).

ST= Seedling dry weight of salt treatment/ Seedling dry weight in control x 100

Seedling Height Reduction (SHR)

The following equation was used to determine the seedling height reduction, representing the growth suspension in root length and shoots length as a percentage (Islam & Karim, 2010).

SHR= Seedling height at control - Seedling height at saline condition / Seedling height at control

Fresh weight and dry weight

On the day of harvest, the fresh weight of seedlings for each treatment was recorded. Later, seedlings were dried at 78°C for 48 hours to record dry weight.

Water uptake

The dry weight of the seeds is determined by weighing them before sterilization and soaking. The fresh weight of the seeds was recorded after 24 hours of soaking in various concentrations of salicylic acid. Gairola's (2011) method was used to determine the water uptake/moisture percentage of seeds.

Water uptake/moisture percentage = Fresh weight - Dry weight / Fresh weight × 100

Software SPSS 16.0 was used to analyze data to calculate the mean value, standard deviation, and least significant difference (LSD) in the beet for each treatment and control.

Result and discussion

Water uptake (%)

Water is a critical factor in mobilizing vital nutrients required for the germination process. Salicylic acid treatments and their effects on water uptake are shown in Fig. 1. The significant increase in water uptake percentage, i.e., 38.75 ± 0.70 and 38.06 ± 0.83, was recorded at 0.8 and 1.6 mM SA treatment over the control (36.39 ± 0.36) at $P<0.05$ level. The reduction in water uptake percentage was recorded at all the highest concentrations of salicylic acid treatments ranging from 2.4 to 6.4 mM. The obtained results show that the water uptake percentage decreased as the concentration of salicylic acid increased; it may be due to the hypertonic concentrations of solutions.

Table 1. Study on seedling fresh weight and dry weight in *Beta vulgaris* L. under increasing concentrations of salicylic acid.

Treatments	Seedling fresh weight (g)	Seedling dry weight (g)
Control	2.15	0.90
0.4 mM SA	2.95	0.91
0.8 mM SA	2.91	0.96
1.2 mM SA	2.26	0.94
1.6 mM SA	2.85	0.95
2.0 mM SA	2.93	0.95
2.4 mM SA	1.93	0.90

According to Yousof and El-Saidy (2014), Wheat seeds treated with 100 ppm salicylic acid improved seedlings' physiological and chemical characteristics. It has the highest percentage of water uptake, at 6.9%. At 500 ppm SA treatment, there was also a reduction in water uptake compared to the control.

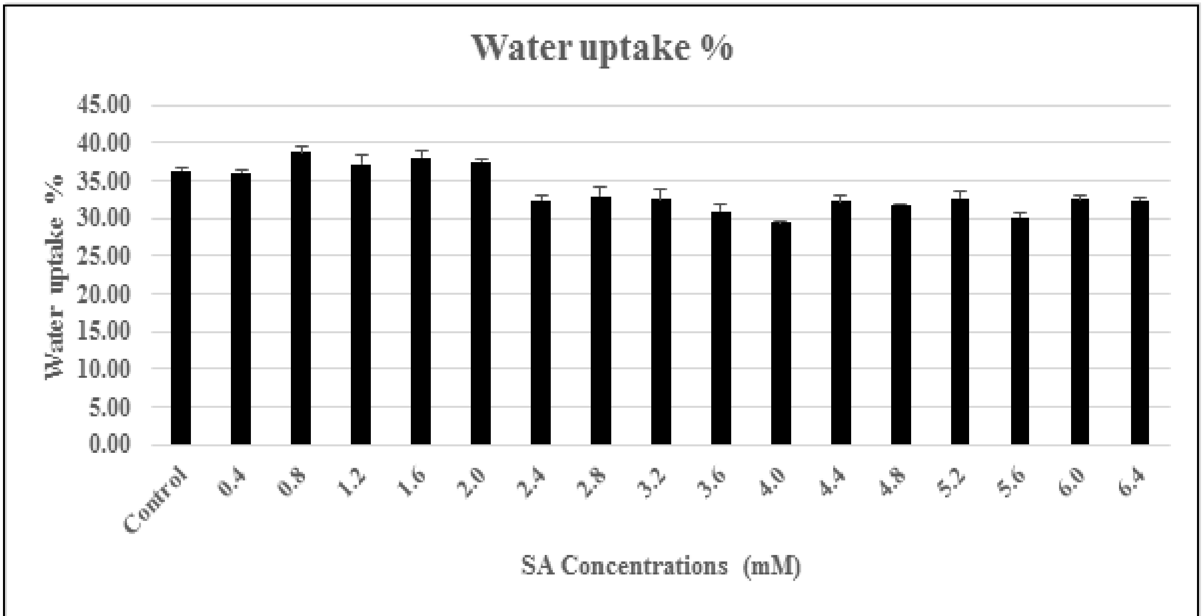

Fig. 1. Study on Water Uptake Under Increasing Concentrations of Salicylic Acid. Values given in mean ± S.D. Bars in each group show significant difference at $p < 0.05$.

This is in agreement with our finding with beet seeds, the effect of salicylic acid is dose-dependent; treatments below 2.4 mM SA showed promising results in beet. Water uptake by seeds is critical for all basic metabolic processes to begin in an early embryo, including respiration and mRNA and protein syntheses. Water uptake also leads to the mobilization of storage compounds like protein and starch, among essential nutrients for a developing embryo. Embryo axial organs cannot absorb the amount of water required for germination in dormant seeds, even if dormant embryos are not experiencing a lack of accessible water. (Obroucheva and Antipova, 2004).

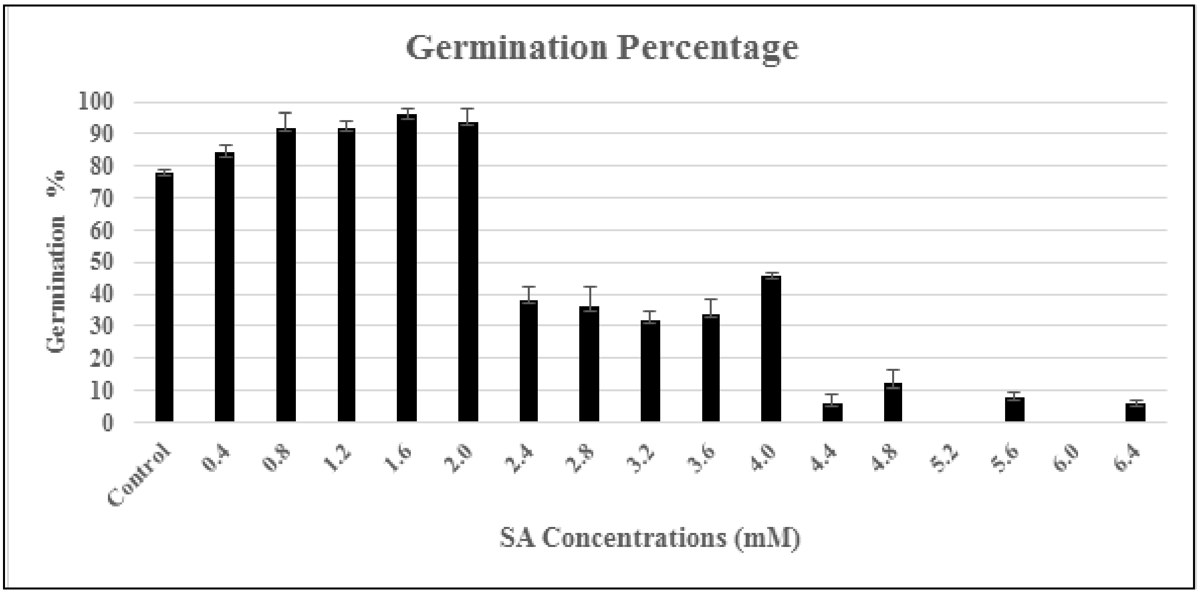

Fig. 2. Study on Germination Percentage Under Increasing Concentrations of Salicylic Acid. Values given in mean ± S.D. Bars in each group show significant difference at $p < 0.05$.

Germination percentage

The percentage of germinated seeds was calculated based on the total number of seeds sown and the total number of seeds that emerged. Salicylic acid treatments and their effects on germination percentage have been shown in Fig. 2. The significant increase in germination percentage i.e., 84 ± 2.6, 92 ± 4.6, 92 ± 2.0, 96 ± 1.7 and 94 ± 3.9 was recorded at 0.4, 0.8, 1.2, 1.6- and 2.0-mM SA treatment over the control (78 ± 1.0) at P<0.05 level. All of the highest concentrations of salicylic acid treatments, ranging from 2.4 to 6.4 mM, resulted in a significant decrease in germination percentage. According to Rajjou (2006), 0.5 mM SA increased germination percentage, i.e., 87%, improved germination vigor under salt stress conditions in Arabidopsis seeds.

In *Vicia faba* (L.), a 0.25 mM salicylic acid concentration significantly increased germination percentage under salt stress (Anaya, 2018). The study by Bahrani & Pourreza (2012) agrees with the SA improving germination percentage. However, more than 3 mg/Litre application reduced germination percentage in wheat under salt conditions. Sakhabutdinova (2003) found that in SA-treated wheat seedlings, high ABA levels were retained, resulting in antistress responses. The maintenance of proline accumulation SA is thought to stimulate cell division in the apical meristem of seedling roots in increased plant development and protective and growth promotion abilities. As well as being treated with SA, plant hormones are maintained, especially auxin and cytokinin, which reduce stress-induced inhibition of wheat growth (Yousof and El-Saidy, 2014). Salicylic acid pre-treatment on beet seeds altered seed germination and vigor potential and the response can be varied according to the crops and method of application. Pre-treating beet seeds with lower than 2.0 mM SA is one of the most efficient methods for promoting germination and growth.

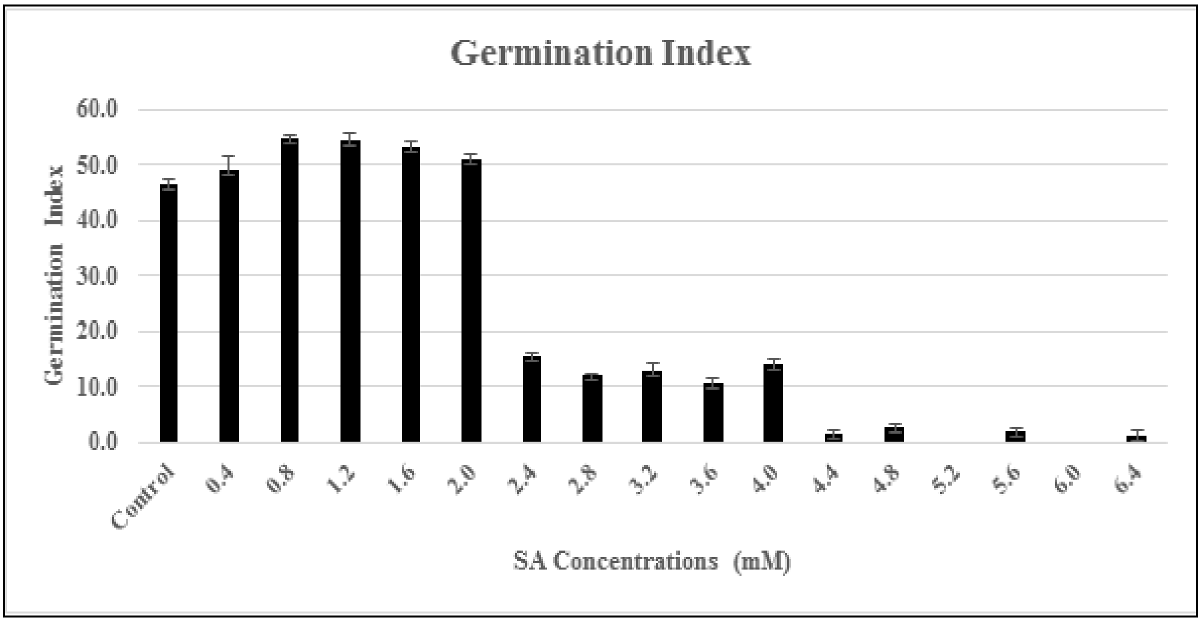

Fig. 3. Study on Germination Index Under Increasing Concentrations of Salicylic Acid. Values given in mean ± S.D. Bars in each group show significant difference at $p < 0.05$

Germination index (GI)

In this study, the speed of germination and germination (%) was measured in the form of germination index. Red beet had different responses in germination index at different levels of SA. Germination index was initially made for all salicylic acid treatments with the control have been represented in Fig. 3. At the $P<0.05$ level, the 0.4, 0.8, 1.2, 1.6 and 2.0 mM SA treatments resulted in a significant increase in germination index (49.2 ± 2.4, 55.0 ± 0.3, 54.4 ± 1.5, 53.4 ± 1.0 and 51.2 ± 0.8) over the control (46.6 ± 0.7).

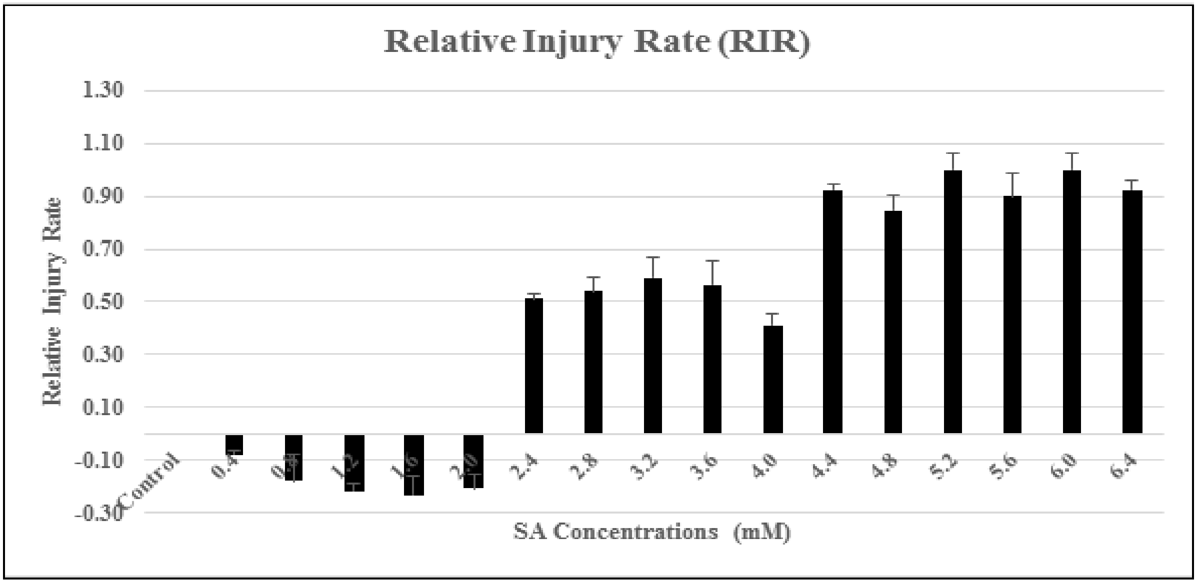

Fig. 4. Study on Relative Injury Rate Under Increasing Concentrations of Salicylic Acid. Values given in mean ± S.D. Bars in each group show significant difference at $p < 0.05$.

All of the salicylic acid treatments with the highest doses, ranging from 2.4 to 6.4 mM, resulted in a significant reduction in germination index. 0.25 mM SA improved germination index in *Vicia faba* L. under salt stress attained the 1.1 and 1 seed/day (Anaya, 2018). Wheat seeds treated with 100 ppm salicylic acid improved germination index, i.e., 67.5; on the other hand, at a higher level of concentration (500 ppm SA), there was also a significant reduction in germination index, i.e., 49.5 compared to the control (According to Yousof and El-Saidy 2014). As the concentration of SA increased, the emerging index decreased. Increased SA levels resulted in increased ABA production, preventing seed germination (Wu *et al.*, 1998). Germination index was significantly improved up to 2.0 mM SA; above that, a significant decrease was recorded at higher concentrations in beet seeds.

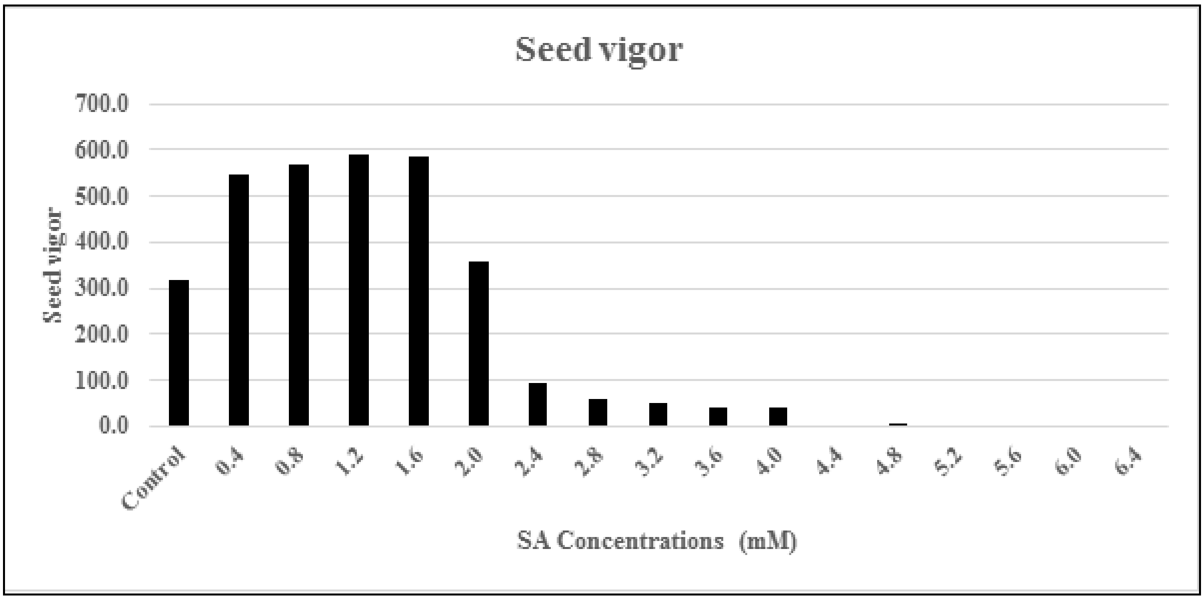

Fig. 5. Study on Seed Vigor Under Increasing Concentrations of Salicylic Acid. Values given in mean.

Relative injury rate (RIR)

Salicylic acid treatment and its effects on relative injury rate are shown in Fig. 4. The significantly reduced relative injury rate was recorded at 0.8, 1.2, 1.6 and 2.0 mM salicylic acid treatments. All of the salicylic acid treatments above 2.4 mM show a significantly increased relative injury rate.

Incorporating plant growth regulators during presoaking, priming, and other treatments in many vegetables has improved seed performance (Halmer 2004). Reduction in relative injury was observed at 1.5 mM salicylic acid treatment in chickpea genotypes in drought stress (Patel and Hemantaranjan, 2013). Heat stress and relative injury may be alleviated by a treatment containing 200 mM SA and 10 mM $CaCl_2$, which could influence breeding programs to improve poinsettia cultivars (Lin *et al.*, 2019). According to Li (2017), under chilling stress, SA and H_2O_2 synergistically stimulated hormone metabolism and signal transduction, along with increased energy supply and antioxidant enzyme activities, all of which were linked to reduced chilling injury and improved chilling tolerance in maize seed.

Seed vigor

The performance of seeds planted to regenerate the crop is directly affected by seed viability and vigor. The observations for the various concentrations of salicylic acid treatment and their effects on Seed vigor have been depicted in Fig. 5.

In all salicylic acid treatments with different concentrations from 0.4 - 6.4 mM along with control, the general trend was associated with a decrease in seed vigor index. 0.4, 0.8, 1.2, and 1.6 mM SA presented the best seed performance observed to have the highest seed vigor values, i.e., 546.0, 570.4, 588.8, and 585.6 compared to other treatments and control (319.8). Because the high concentrations impeded root and shoot growth, no results were obtained at 4.4, 5.2, 5.6, 6.0, and 6.4 mM SA. Two enzymes (Malate synthase and isocitrate lyase) activities are good indicators of seedling emergence potential and seed vigor in the sugar beet (de Los Reyes *et al.*, 2003). There are some contradictory data on the effect of salicylic acid on seed germination and seedling establishment, implying that these enzymes might inhibit seed vigor (e.g., 3 to 5 mM in *Zea mays*; Guan and Scandalios, 1995) or promote in wheat and pea (Shakirova *et al.*, 2003; McCue *et al.*, 2000). Our finding suggests that the exogenous application of salicylic acid can exhibit both the effects dosage dependently, i.e., inhibition or promoting germination. Lower concentrations of salicylic acid below 2.0 mM can promote germination, and concentration above in can suppress the germination in the dark red variety of beet.

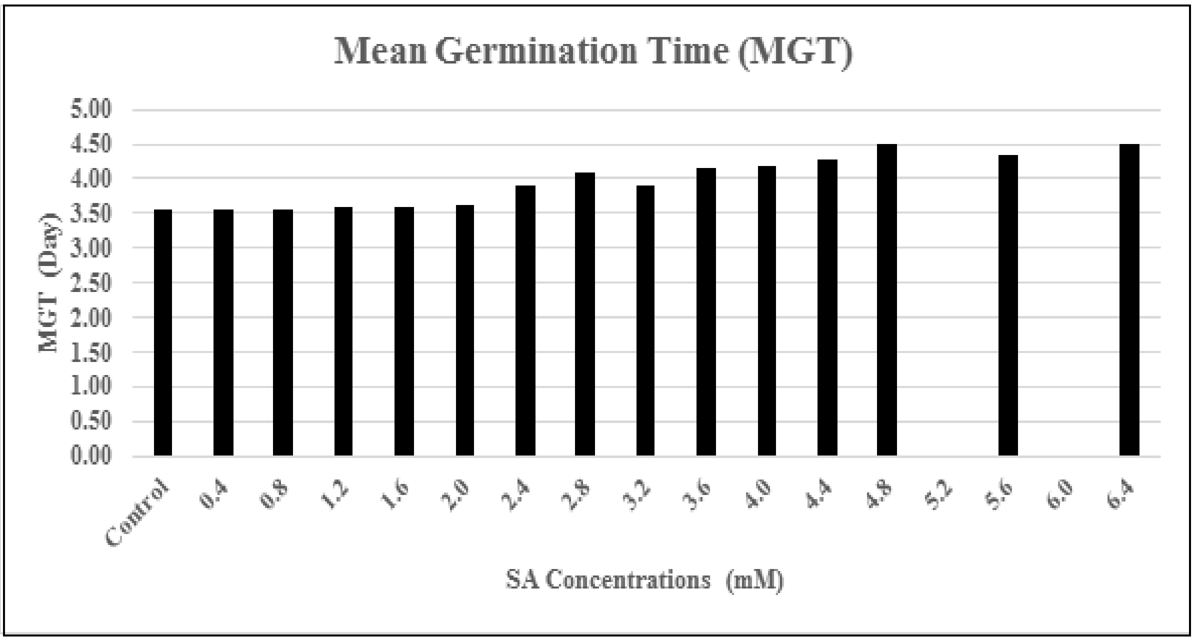

Fig. 6. Study on Mean Germination Time Under Increasing Concentrations of Salicylic Acid. Values given in mean.

Mean germination time (MGT)

The average time for a seed to commence and complete the germination process is referred to as mean germination time. The observations for the increasing levels of salicylic acid treatment and their effects on mean germination time that were initially made for all salicylic acid treatments with the control have been depicted in Fig. 6. Seeds treated with 0.4, 0.8, 1.2, 1.6, and 2.0 mM SA showed a faster germination rate (near about 3.6 days) and maximum seedling length compared to remaining treatments (4.8 and 6.4 mM), which showed a delay in germination time (> 4.5 days) and showed radicle emergence only. According to Li (2017), Under chilling stress, SA + H_2O_2 shortened seed germination time and enhanced seed vigor and seedling growth compared to untreated maize seed. In sesame, 0.9 % solutions of salicylic acid showed a lower mean germination time compared to untreated seeds (Ahmad *et al.*, 2019). Application of Salicylic acid improved mean germination time and other characteristics; It might be due to salicylic acids' role in increasing oxygen, nutrient uptake, and α-amylase activity (Alamri *et al.*, 2018). According to Liu *et al.* (2018), reduced α-amylase activity inhibits seed germination.

Stress tolerance (ST)

In the present study, the stress tolerance was recorded up to 2.8 mM salicylic acid treatments (Fig., 7). The highest tolerance, i.e., 107.0 %, was recorded at 0.8 mM salicylic acid treatment compared to control (100.0 %). The 1.0 mmol/L SA treatment to the salty (NaCl) medium increased the germination rate significantly at all salt concentrations, i.e., 100, 200, 300, and 400 mmol/L (Jini and Joseph 2017). By increasing enzymatic antioxidants, exogenously administered chemicals can drastically increase plant growth and development and promote plant stress/salt tolerance (Gossett *et al.*, 1994). SA reduced the adverse effects of salt on *D. superbus* development and adaption, which was attributable to well-developed chloroplasts and high antioxidant enzyme activity. SA treatment promotes radicle cell proliferation by causing meristem cells to divide and expand (Boukra *et al.*, 2013). The interplay between salinity and SA may stimulate salt-resistance genes and affect germination by boosting physiological activity and mobilization of the reserved material required for growth (Szalai *et al.*, 2005).

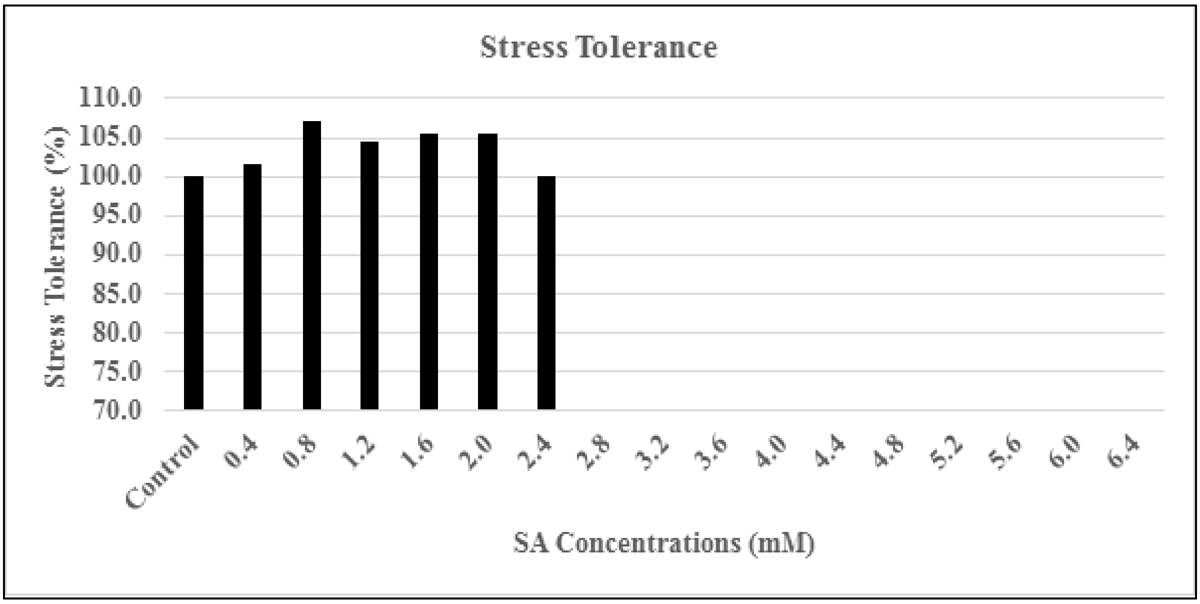

Fig. 7. Study on Stress Tolerance Under Increasing Concentrations of Salicylic Acid. Values given in mean.

Seedling Height Reduction (SHR)

The decrease in seedling height reduction percentage i.e., -37, -34, -37 and -33 % was recorded at 0.4, 0.8, 1.2 and 1.6 mM SA treatment over the control (0.0%). All of the highest concentrations of salicylic acid treatments, ranging from 2.4 to 6.4 mM, showed increased seedling height reduction (Fig. 8). Complete reduction in seedling height recorded at 4.8 mM SA. Because the high concentrations inhibited root and shoot growth, no results were obtained at 4.4, 5.2, 5.6, 6.0, and 6.4 mM SA. Jam *et al.* (2012) reported that at 2500 µM SA concentration increases 42 % shoot length compared to the control in safflower seeds. In sesame, *0.6 %, 0.8%, and 0.9% of SA treatment recorded taller seedlings, i.e., 8.4 cm (Ahmad et al. 2019). The* positive effects of exogenous SA on seedling growth in some plants are not understood perfectly. According to Shakirova *et al.* (2003), SA possibly regulates elongation and cell division along with auxin. Auxin oxidation is prevented by salicylic acid (Fariduddin *et al.*, 2003). Salicylic acid's low concentration and low pH could activate the proton pump in the membrane, increasing nutrient absorption and inducing osmotic pressure (McCue *et al.*, 2000). It affects the rate of biosynthesis, and absorption considerably rises. Lower pH causes cell walls to weaken as assimilation progresses, allowing cell elongation to occur, resulting in increased seedling length. Pre-treatment increased amylase activity, turning seed reserves into simple compounds, resulting in higher seedling growth Kaur *et al.* (2002).

Fresh weight and dry weight

The increase in Fresh seedling weight, i.e., 2.95, 2.91, 2.26, 2.85, and 2.93 g, was recorded at 0.4, 0.8, 1.2, 1.6, and 2.0 mM SA treatment over the control (2.15 g) (Table 1.). All of the highest concentrations of salicylic acid treatments, ranging from 2.8 to 6.4 mM, did not show a sufficient amount of germinated seeds to calculate fresh weight compared to control. There was no significant difference in dry weight of seedlings among all the treatments of salicylic acid with control.

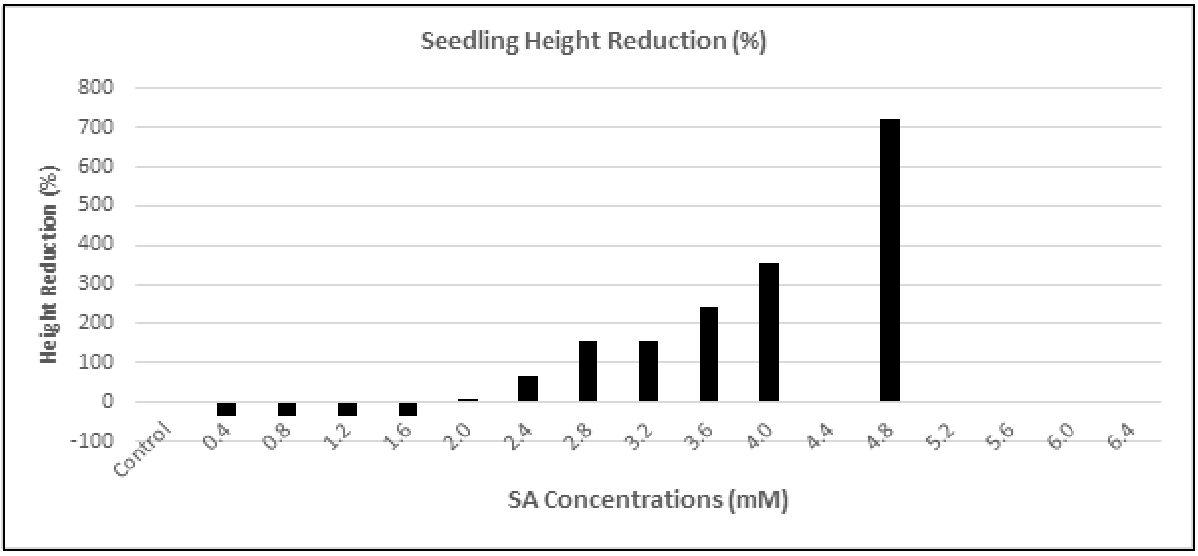

Fig. 8. Study on Seedling Height Reduction (%) Under Increasing Concentrations of Salicylic Acid. Values given in mean.

In order to accumulate dry weight, they must be grown for much longer than ten days. *0.9%* and 0.7% SA treatment recorded increased shoot weight (0.67 mg plant^{-1}), similarly 0.9% SA showed increased root weight i.e., 0.38 mg plant^{-1} (Ahmad et al., 2019). Baninasab (2010) reported, SA treatment significantly increased root shoot fresh/dry weight under drought-stressed cucumber. Under saline conditions, El-Tayeb (2005) discovered that pre-treatment of SA improved the fresh and dry weights, water, photosynthetic pigments, insoluble saccharides, phosphorus content, and peroxidase activity dry weight of barley seedlings.

Conclusion

The pre-treatment of salicylic acid on beet seeds significantly alters the Water uptake, Germination percentage, Germination index, Relative injury rate, Seed vigor, Mean germination time, Stress tolerance, Seedling Height Reduction, and Fresh weight. The positive or negative effect of salicylic acid varies according to the crop plants and concentrations.

The result of our finding suggests that salicylic acid shows promising results in beet seed dose-dependently. Pre-treating beet seeds with < 2.0 mM SA is one of the most efficient methods for promoting germination and growth. However, the treatment >2.0 mM SA showed a significant decrease/inhibition in germination traits at higher levels of concentrations. This means that seed pre-treatment of *Beta vulgaris L.* with this low concentration of salicylic acid will speed up the mean germination time and enhance the establishment of seedlings. This has a good impact on crop management during the planting and germination stages. Based on the results of this study, seed pre-treatment of *dark red beet* with low concentrations below 2.0 mM is recommended.

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