



Influence of various concentrations of salicylic acid on germination and some morphological characteristics of *Beta vulgaris* L.

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Abstract

Beta vulgaris L., commonly known as beet, is an edible plant of the family Chenopodiaceae. Economically it is the most important crop, as it is the most widely consumed vegetable, processed juice, and food color. The foliar application of several compounds like nutrients or plant hormones may help control growth and development patterns of plant. The current study looked into the effect of the foliar application of salicylic acid on beet growth, like germination percentage, seedling length, plant height, the number of leaves and leaf area. Various concentrations of salicylic acid (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2 mM) were used to evaluate the effect of salicylic acid on beet growth at 30, 60, 90, and 120 DAG (days after germination). Among all these treatments, application of 1.6 and 2.0 mM SA showed increased germination percentage compared to control. The significant increase in height was recorded specifically at 1.2 mM SA over the control at $P < 0.05$ level at 120 days after germination. Significant variation in leaf number was recorded at 1.2 mM SA only. This study concludes that exogenous SA (particularly at 1.2 mM) may help improve plant growth. Diversely, these compounds can have a beneficial or toxic effect depending on their concentrations.

Keywords: *Beta vulgaris* L., foliar spray, salicylic acid, growth parameters

Introduction

Beet is an herbaceous plant native to the Mediterranean, the Atlantic coast of Europe Near East, and India. Beet is grown worldwide; the roots are dark red, white, yellow, swollen, and fleshy. It contains carbohydrates, proteins, and a significant source of vitamins, iron, and other minerals. Beet is mainly used for food, sugar, and food color production. Recent studies have revealed that there has been a rising interest in the biological activity of beetroot and its potential applicability as a health-promoting and disease-preventing functional food. Beetroot is used for food as well as medicinal uses; it acquires anti-inflammatory effects, antioxidant properties. A dark red variety of beetroot is commonly cultivated in Ahmednagar and nearby regions. Ahmednagar is one of the largest districts in the state of Maharashtra, which is predominantly agriculturally based. Out of fourteen tahsils of Ahmednagar district, all remaining thirteen tahsils falls under drought-prone area. The majority of cultivable land is monsoon-dependent, i.e., rainfed^[1]. But due to hot summer and general dryness during the significant part of the year in this area, the plant growth can affect the yield. Therefore, to overcome this problem external application of growth regulators or chemical compounds might be useful. Foliar application of salicylic acid on beet and sunflower plants improved plant growth characteristics, increased their tolerance capacity to abiotic stress, and protected the plant from oxidative stress by increasing antioxidant enzyme activity and increasing the fresh root and shoot mass of the plant^[2, 3, 4, 5].

Salicylic acid naturally occurs in plants in very low amounts and regulates physiological processes such as stomatal closure, organic synthesis, nutrient uptake, chlorophyll synthesis, protein synthesis. It can be produced by either the phenylalanine pathway in the cytosol or the isochorismate

biosynthetic pathway in the chloroplasts. SA has shown to be significant in triggering and regulating a range of biotic and abiotic stress responses. SA is accumulated locally infected tissue and subsequently distributed throughout the plant to cause systemic acquired resistance in non-infected distant regions of the plant as part of the defence mechanisms during plant-microbe interactions. Salicylic acid gets its name from the Latin word Salix, which signifies the willow tree and it has been centuries since the medicinal compound extracted from willow bark was discovered to be beneficial to humans and the plants that produce it. Salicylic acid regulates some aspects of disease resistance and thermogenesis.

Manipulation of salicylic acid levels in plants could be helpful for crop protection^[6]. Foliar application of Salicylic acid to the plants is greater influential in comparison to soil application^[7], and Plant growth regulators can enhance the physiological performance consisting of photosynthetic potential and thereby assist in effective flower formation, fruit and seed development and in the long run, enhance the productivity of the plants^[8]. Foliar application of salicylic acid significantly influenced the top fresh mass and root biomass of some beet varieties, mostly applied after thirty days of planting^[9]. SA is a phytohormone that formed as a benzoic acid derivative via a series of chemical processes. It is involved in several physiological activities like photosynthesis, nutrient absorption, and membrane permeability and assists in the survival of organisms under various biotic and abiotic stress. It also plays a crucial role in systemic acquired resistance^[10]. In view of this background, the present studies were undertaken to evaluate the effect of salicylic acid on the growth parameters of beet.

Material and Methodology

The present investigation aims to study the effects of different salicylic acid treatments on beet. The seed of a

variety of dark red beetroot selected for the present study was obtained from Kalash Seeds Pvt. Ltd, Jalna, Maharashtra. The Heavy-duty plastic pots (34 cm diameter top, 30 cm depth.) were filled with 14 kg of sundried garden soil and a 3:1 ratio of well-rotten compost. The pilot experiments have been carried out to determine the optimum concentration of SA for further studies. Various concentrations of salicylic acid like 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2 mM were sprayed manually using a 1000 mL hand sprayer twice a day for three days every week. All the experiments were done with replicates.

The percentage of germination is calculated from the treated and untreated seedlings. Initially the seeds were soaked in above mentioned concentrations of salicylic acid at room temperature for 24hrs. The germination percentage and seedling development was studied during its early stage, which is up to 10 days. The number of seeds showing emergence of the radical was counted from the seeds kept in Petri plates lined on moist germination paper and expressed as percentage. The software SPSS 16.0 was used to calculate the mean value, standard deviation and least significant difference (LSD) in the beet for each treatment and control.

Results and Discussion

Effect of salicylic acid on germination percentage and seedling length

In the present study, the increased levels of salicylic acid show the effects on plants germination have been depicted Fig.1. (a). Among all these salicylic acid treatments, application of 1.6 and 2.0 mM SA showed increased

germination percentage i.e., 73 and 75% over control (68%). While lower germination percentage, i.e., 56%, was recorded at a higher level of concentrations of salicylic acid 3.2 mM SA. Pre-treatment with 1.0 to 2.0 mM salicylic acid is one of the most efficient strategies for promoting germination and growth of sugar beet seedlings [11]. The physiological priming of beet seeds alters seed germination and vigor potential, and the response varies according to the cultivar and type of conditioning applied. Physiological measurements combined with global expression profiling were used to evaluate the effect of salicylic acid (SA) on the elicitation of defense mechanisms in *Arabidopsis thaliana* seeds and seedlings. However, germination decreased and seedling growth reduced compared to control by increasing salicylic acid up to 3.0 mM in the seed of *Vicia faba* (L.) reported by Soliman [12]. Results were recorded that pre-treatment of beet seeds with salicylic acid increased the seedling length of beet seeds compared with higher concentrations and control (Fig.1.b). The significant increase in seedling length i.e., 3.5 ± 0.6 , 4.1 ± 0.5 and 4.1 ± 0.6 cm was recorded at 0.4, 0.8 and 1.2 mM SA treatment over the control (2.8 ± 0.4) at $P < 0.05$ level. The minimum seedling length, i.e., 1.9 ± 0.5 , 1.2 ± 0.3 , and 0.9 ± 0.1 cm, was recorded at the highest concentrations of 2.4, 2.8, and 3.2 mM SA treatment. The obtained results shows that, as the concentration of salicylic acid increased, seedling length decreased. The findings of this study clearly show that salicylic acid has a variable effect on seed germination and seedling growth in beet seed, the effect of SA depends upon its concentration.

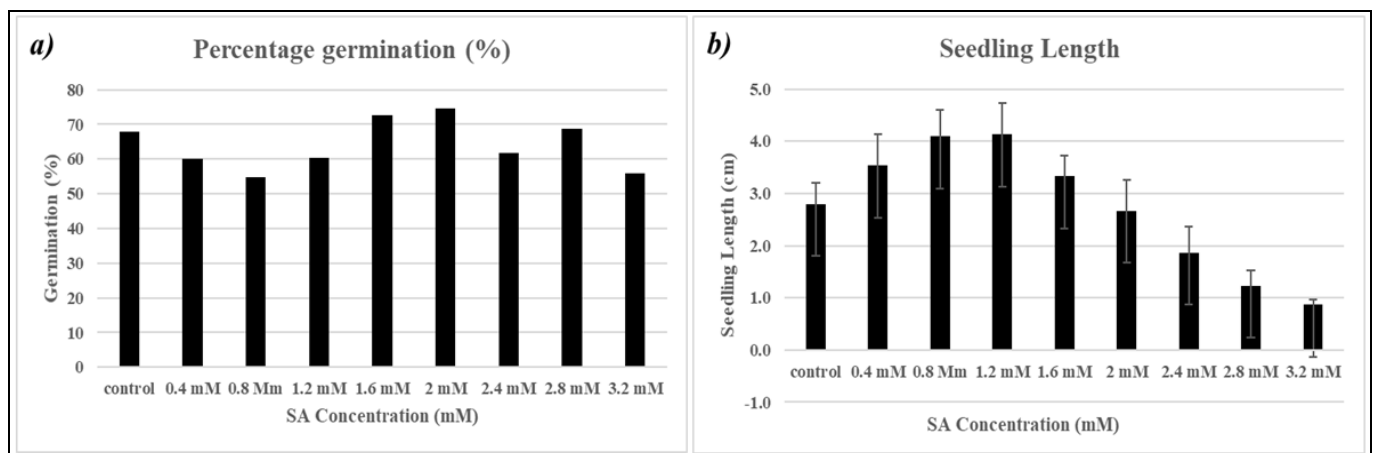


Fig 1: Study on a) germination percentage and b) seedling growth in *Beta vulgaris* L. Under Increasing Concentrations of Salicylic Acid. Values given in mean \pm S.D. Bars in each group show significant difference at $p < 0.05$

Effect of salicylic acid on height

For each treatment of salicylic acid, the plant height was measured at 30, 60, 90, and 120 days after germination, from ground level to the tip of the leaf (Figure 2.). The plant height was measured in centimeters using a meter scale. The significant increase in height (26.5 ± 0.72 cm) was recorded specifically at 1.2 mM SA treatment, over the control (24.38 ± 0.54 cm) at $P < 0.05$ level at 120 days after germination (Fig.3.d).

Results in this study agree with several reports related to applications of SA increased plant growth and those parameters that were reduced by drought, salinity, and water

stress [13, 14, 15]. Salicylic acid is a phenolic endogenous plant growth regulator that is important in regulating plant growth, development, and responses to environmental stresses. Exogenous application of S.A. significantly increased nutrient uptake and leaf concentrations of K, Ca, Zn and Fe, as it significantly reduced leaf Na concentration under salinity [16]. Application of SA increased the height more than the control in *Panicum miliaceum* L. [17]. In contrast, at 90 DAG, plant height, i.e., 19.64 ± 1.13 cm, decreased significantly at 3.2 mM SA treatment over the control (23.62 ± 0.67 cm) at $P < 0.05$ level (Fig.3. c.).

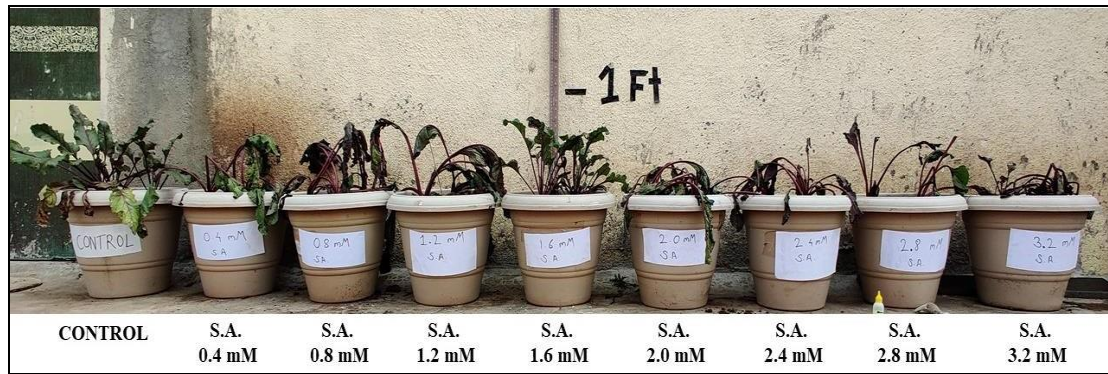


Fig 2: Progressive height in beta vulgaris l. at 120 days under application of salicylic acid.

At 120 days after germination, the SA treatment of 2.0, 2.4, 2.8 and 3.2 mM showed a significant decrease in height i.e., 23.0, 23.4, 23.0 and 20.0 cm respectively over the control (24.38 cm) at $P < 0.05$ level (Fig.3. d.). A significant decrease in height was recorded from 2.0 mM SA and all the higher levels. Some plants died at a higher level of SA treatment after 60 days.

On the other hand, in plant biology, the role of salicylic acid in plant growth and development is still a contentious issue. Salicylic acid dose-dependently plays a vital role in plant

growth under normal and saline conditions. SA reduced the plant height and thickened the stem. The foliar application of two levels of Salicylic acid is combined with two levels of nutrient solution strength on sweet basil [18]. In our study, while low concentrations of exogenous salicylic acid show a positive effect on height, while higher concentrations have the opposite effect on plant height, these parameters were significantly reduced by 2.0 to 3.2 mM SA treatment compared to control plants.

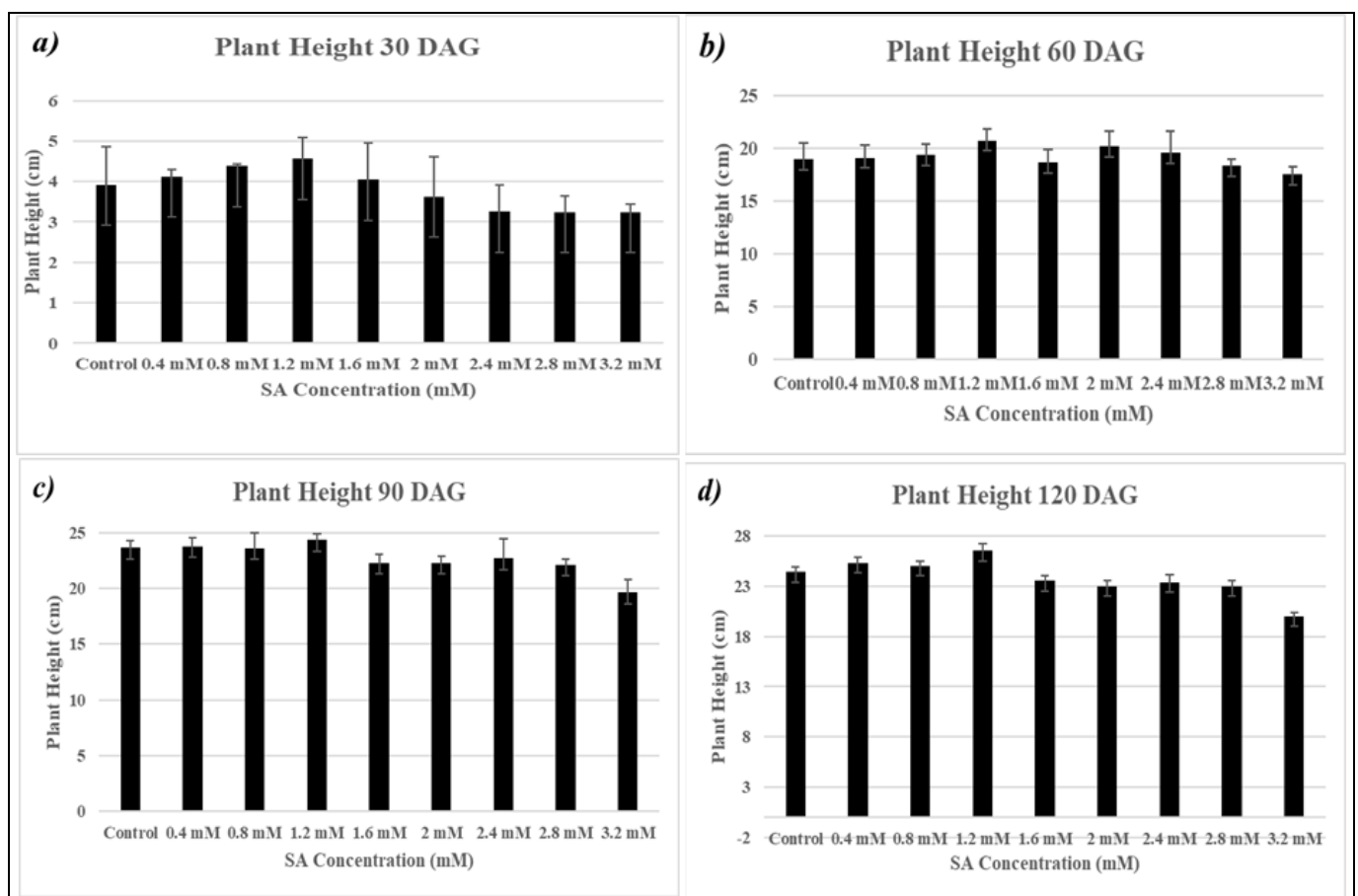


Fig 3: Study on mean progressive height (a,b,c,d) in beta vulgaris l. under increasing concentrations of salicylic acid. Values given in mean \pm s.d. bars in each group show significant difference at $p < 0.05$

Effect of salicylic acid on leaf number and senescent leaves

Growth observations for the leaf number and senescent leaves were initially made for all salicylic acid treatments with control, regularly at 30, 60, 90, and 120 days after germination. The observations of the increasing levels of

foliar salicylic acid with their effects on the leaf number and number of senescent leaves of plants have been depicted in Figure 4. From the results, it is clear that under foliar application of salicylic acid treatment of 1.2 mM at 30,60 and 90 DAG (days after germination), the significant increase ($P < 0.05$ level) in leaf number, i.e., 7 ± 1.0 , 10 ± 1.7

and 14 ± 1.0 was recorded over the control, i.e., 4 ± 0.1 , 7 ± 1.7 and 10 ± 1.0 respectively (Fig.4. a,b,c.). The same trend was observed at 120 days after germination at $P < 0.05$ level, a significant increase in leaf number (13 ± 1.1 & 14 ± 1.1)

recorded at 0.4 and 1.2 mM SA over the control, i.e., 10 ± 0.6 (Fig.4. d.). No significant difference in leaf number of plants has been observed up to 120 days for all treatments, except at 90 DAG;

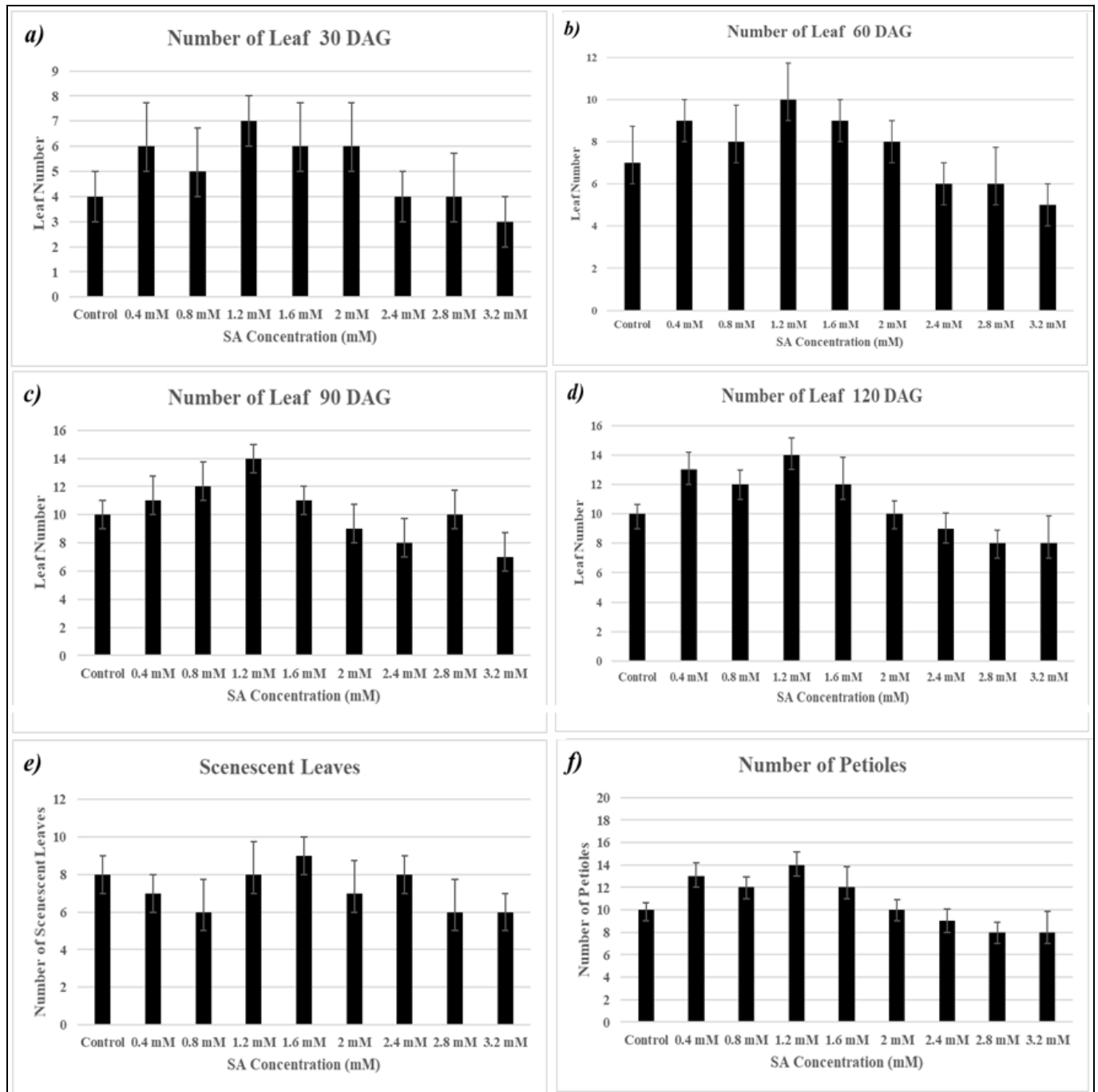


Fig 4: Study on leaf number (a,b,c,d), senescent leaves (e) and number of petioles (f) in *Beta vulgaris* L. under increasing concentrations of salicylic acid. values given in mean \pm s.d. bars in each group show significant difference at $p < 0.05$

the significant decrease in leaf number (7 ± 1.7) was recorded at 3.2 mM SA over the control at $P < 0.05$ level treatment. When compared to control, there is no or minimal influence on the number of senescent leaves. The number of petioles, i.e., 13 ± 1.18 and 14 ± 1.18 , increased significantly at 0.4 and 1.2 mM SA treatments over the control (10 ± 0.65) at the $P < 0.05$ level (Fig.2. e.). On the other hand, Salicylic acid treatment enhanced the number of leaves significantly when administered at low concentrations, according to Hayat [19]. Application of exogenous SA to improve salt tolerance of *Lycopersicon*

esculentum Mill. According to the researchers, foliar application of salicylic acid boosted the number of leaves and overall growth of tomato plants [20]. As per the findings, tolerant genotypes maintained low leaf temperature in high-temperature conditions. SA improved their ability to maintain low leaf temperature, which was beneficial to the plant's normal metabolic processes. According to Szepesi [21], The improved CO₂ assimilation photosynthetic rate and increased mineral uptake stressed plants under SA treatment might account for this beneficial effect of SA. Lower SA concentrations increased photosynthetic activity and

increased the number of leaves in basil and marjoram [22, 23]. On the contrary, according to Farahbakhsh [24], Salinity and SA interaction had no significant effect on the number of leaves of plants. The application of salicylic acid had no influence on the number of leaves in *Zea mays* reported by El-Katony [25].

Effect of salicylic acid on leaf area

For each treatment of Salicylic acid, the leaf area was determined by the non-destructive graph paper method at 30, 60, 90, and 120 DAG. From the results, it is clear that under foliar application of salicylic acid at 30 DAG, a significant increase ($P < 0.05$ level) in leaf area, i.e., 224 ± 7 , 230 ± 4.5 , 257 ± 5.2 , 192 ± 8.1 , 193 ± 9 and 204 ± 4.5 cm² over the control (164 ± 6.5 cm²) was recorded at 0.4, 0.8, 1.2, 1.6, 2.8-, and 3.2-Mm SA, followed by a significant decrease in leaf area (152 ± 6.5 cm²) at 2.4 mM SA treatment (Fig.5. a.). However, as the concentration of salicylic acid increased, the significant decrease in leaf area was recorded at 60, 90 days after germination, 1.6 to 3.2 mM SA and at 120 DAG 0.8, 1.6 to 3.2 mM SA over the control at $P < 0.05$ level (Fig.5.b & c).

Foliar application of one millimolar SA significantly increased the coriander leaf area Observed over the control

[26]. The additive effect of 0.1 mM SA on leaf area under water stress was reported in *Coriandrum sativum* L. [27]. The use of salicylic acid encourages cell division and growth [19]. According to Delavari [27], SA enhances the leaf area of sweet basil plants. [14] discovered that SA had an additive effect on leaf area during water stress in maize. Application SA on drought-stressed maize crop Increased leaf area, improved drought tolerance, increased photosynthetic pigments, and RWC, improved leaf gas exchange characteristics, increased antioxidant enzyme activities, reduced MDA, H₂O₂, O₂ contents [28]. However, the significant reduction ($P < 0.05$ level) in leaf area recorded for the salicylic acid treatment of 1.6, 2.0, 2.4, 2.8 and 3.2 mM at 60 ($312, 251, 101, 212$ and 188 cm²), 90 ($272, 211, 149, 149$ and 146 cm²) and 120 ($242, 181, 119, 119, 116$ cm²) days after germination compared to control i.e., 360 ± 7.2 , 320 ± 4.4 and 302 ± 4.4 , respectively (Fig.5. a, b & c). Under water stress, a drop-in leaf turgor and photosynthesis limit cell expansion and development under water stress, resulting in a reduction in leaf area [29]. Under drought stress, the reduction in leaf area could be viewed as a water loss avoidance mechanism [30] to avoid excessive transpiration due to low stomatal density [31].

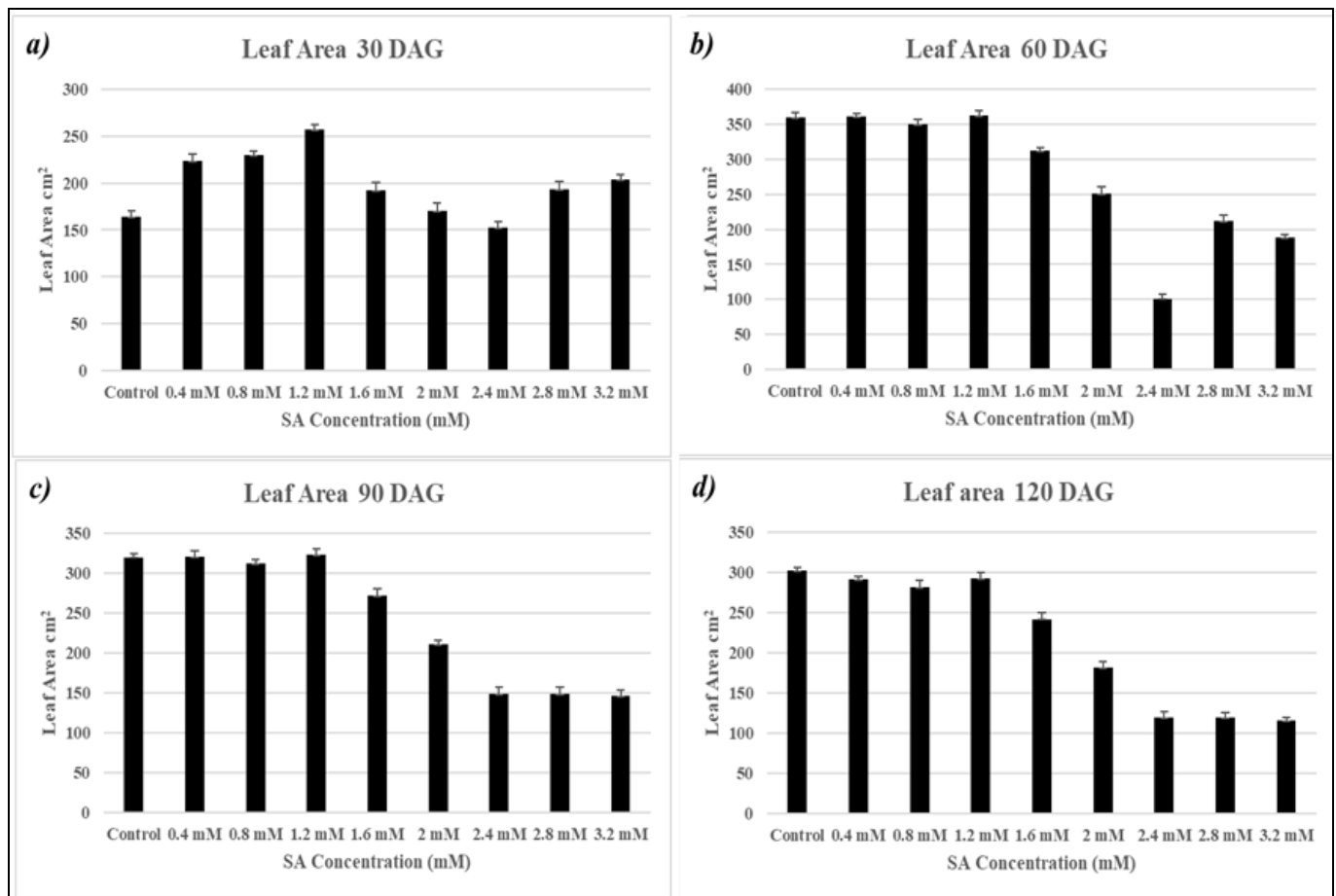


Fig 5: Study on leaf area (a, b, c, d) in beta vulgaris l. under increasing concentrations of salicylic acid. values given in mean \pm s.d. bars in each group show significant difference at $p < 0.05$

Conclusion

In this study, the effect of pre-soaking beet seeds in salicylic acid was influential; the different application forms like 1.6 and 2.0 mM SA significantly increased germination percentage. In the case of foliar application, results suggest that the application of 1.2 mM SA significantly influenced

the top fresh mass of beet in terms of plant height, leaf number, and leaf area. On the other hand, these compounds can have a beneficial or toxic effect depending on their concentrations. Higher concentrations of salicylic acid were stressful to beet foliage; leaf area decreased significantly. Overall, lower salicylic acid treatment was the most

effective, followed by reduced frequency of applications to alleviate the injurious effects in beet.

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