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Exogenous salicylic acid stimulates secondary metabolites production in red beet

Sandip Palve*, **Digambar Ahire**

P.G. Department of Botany, New Arts, Commerce and Science College, Ahmednagar, India

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Abstract

In the last few years, increasing interest in the foliar application of salicylic acid (plant hormone) on plants has considerably expanded our knowledge concerning its effects on plants' metabolism in stresses such as freezing, drought, disease, insect attack, and nutrient deficiencies. To investigate the effects of salicylic acid (SA) on secondary metabolites (like betanin and vulgaxanthin, total phenol content, anthocyanin, and alkaloids). in beet (*Beta vulgaris* L.), the plants were sprayed with 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2mM of salicylic acid concentrations along with a control. Dark red variety of beet were used in the experiment. Based on preliminary findings and discussion, foliar application of salicylic acid may enhance betanin, vulgaxanthin, total phenol content at 0.8mM, and anthocyanin and alkaloids at 1.2mM.

***Corresponding Author:** Sandip Palve ✉ sandipalve@gmail.com

Introduction

Application of SA increases the production of primary compounds, modulates the absorption and metabolism of mineral elements, essential oil content, yield, and productivity (Gorni *et al.,* 2020). SA Regulates physiological activities in plants such as stomatal closure, food absorption, chlorophyll production, protein synthesis, ethylene biosynthesis inhibition, transpiration, and photosynthesis (Piatelli *et al.,* 1969, Khan *et al.,* 2003 and Shakirova *et al.,* 2003).). Koo *et al.* (2020) suggest that SA is a crucial plant hormone as a plant defence activator and growth regulator. Growth regulators increased plants' secondary metabolites and salt tolerance (Ghassemi-Golezani *et al.,* 2020). Plant growth regulators can improve physiological efficiency through their effect on photosynthesis, flower and fruit formation, and the plant's overall productivity (Asghari & Aghdam 2010). Foliar application of elements often has a more significant impact on plant traits and yield than soil application (Kazemi 2013). Secondary metabolites are important phytochemicals in terms of both economics and ecology. SA stimulates their production by modulating specific gene regulation, and it also interacts with other elicitors to increase secondary metabolite biosynthesis. Therefore, it is highly recommended for commercial secondary metabolite production (Ali 2020). According to Jumali *et al.* (2011), Several molecular investigations have recently revealed that SA can control many aspects of plant growth, secondary metabolite production, and defence response, boosting plant growth and productivity under adverse conditions. SA supplementation successfully stimulated secondary metabolite production, which might be attributed in part to an increase in photosynthetic pigment levels and ROS scavenging capacity, as well as better plant growth and biomass (Maurya *et al.,* 2019). Therefore, the present investigation was undertaken to study the impact of spraying salicylic acid on secondary metabolites of red beet to improve growth, yield, and nutritional value.

Red beetroot is a naturally occurring root vegetable widely consumed as a supplemental juice, powder, bread, gel, boiling, oven-dried, pickled, pureed, or jams since it is a rich nutritious source. It is rich in phytochemicals and bioactive compounds such as betacyanin and betaxanthins, total phenol content, anthocyanin, and alkaloids; there are also other compounds such as flavonoids, saponins, polyphenols, and inorganic nitrate (NO_3). The health-promoting characteristics of beetroot and its metabolites were supported by available data as prospective therapeutic therapies for a variety of metabolic illnesses such as hypertension, diabetes, insulin resistance, and renal dysfunction. Beetroot is certainly one of the ten plants with the highest antioxidant activity (Baiao *et al.,* 2017). Secondary metabolites from plants are a unique source of medications, food additives, flavours, and industrially essential biochemicals. Plants exposed to stress accumulate a variety of metabolites, including numerous elicitors or signal molecules. Secondary metabolites are important in adjusting plants to their environment and overcoming stress conditions. Environmental factors such as temperature, humidity, light intensity, the supply of water, minerals, and CO_2 can cause stressful environmental conditions that can influence growth, productivity, and secondary metabolite production.

Material and method

Different concentrations of salicylic acid were used to investigate the effects on secondary metabolites such as betacyanin and betaxanthins, total phenol content, anthocyanin, and alkaloids in beetroot. The seed of a Detroit dark red variety was obtained from a Kalash seed company in Jalna, Maharashtra. The pilot experiments were carried out in order to establish the best concentration to use in future investigations.

The foliar treatment includes a series of increasing concentrations of salicylic acid 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2mM along with control was used for the experiment. All of the experiments were carried out with replicates. The influence of different salicylic acid levels on some morphological characteristics were evaluated after 30 days interval (Palve *et al.,* 2021), while secondary metabolites were estimated such as betalain (betanin and vulgaxanthin), total phenol content, anthocyanin and alkaloids.

Betalain

For the determination of the betalain content, water extraction was prepared by following the method reported by Cai *et al.* (1998b) with slight modifications. A fresh beetroot sample weighed exactly 50g broken down using a blender with 150ml of distilled water. After adding diatomaceous earth to the suspension, the mixture was filtered through Whatman no. 1 paper. With the help of distilled water, the filtrate was washed until the liquid passing through the filter was clear. The water extracts were firstly purified to remove soil and small particles by centrifugation at 10,000× g for 15 min at room temperature. Analysis of the extracts prepared from plant material of different SA treatments was performed using a spectrophotometer (Shimadzu UV-1900). Phosphate buffer (0.05 M, pH 6.5) was used as blank and also used to dilute the sample with too high concentrations. Three wavelengths, i.e., 538nm for Betanin, 476nm for vulgaxanthin I, and 600nm for the presence of impurities, measured the absorption of betalain.

The absorption of betanin constituent ($A_{Betanin}$) calculated by following formula.

$$A_{Betanin} = 1.095 \times (A_{538} - A_{600})$$

Equation for impurity absorbance

$$A_{Impurities} = A_{538} - A_{Betanin}$$

For the determination of vulgaxanthin absorption ($A_{Vulgaxanthin}$ I)

$$A_{Vulgaxanthin\ I} = A_{476} - A_{Impurities} - A_{Betanin} / \text{ratio } A_{538}/A_{476}$$

Anthocyanin content

As described by Mancinelli *et al.,* The fresh sample of beet root was homogenized and filtrated in methanol containing 1% (v/v) HCl. A spectrophotometer was used to read the filtration at 530 and 657nm.

Determination of alkaloids

Method by Adham *et al.,* used for determination of alkaloids, The percentage of alkaloid was calculated as follows:

Percentage of total alkaloid (%) = Weight of residue / Weight of sample taken ×100

Total phenolic content (TPC)

Folin–Ciocalteau method was used to determine TPC by Singleton & Rossi (1965). Extract of beetroot (100 µL) mixed with 0.5ml of Folin–Ciocalteau reagent and 1mL of 20% (w/v) sodium carbonate. After incubating samples for 2hrs in the dark, the absorbance was measured at 760nm using a UV-visible spectrophotometer. The calibration curve deduced the TPC mg of gallic acid equivalent (GAE) per kg of fresh weight based on the measured absorbance.

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

Betalains

Betalain is a relatively new term for a group of water-soluble plant colorants that includes betacyanins, which have a red-violet color, and yellow betaxanthins. Nitrogen-containing water-soluble pigments called betalains are widely used as a natural food colorant. Under the application of the salicylic acid, the maximum content of betanin (1869mg kg^{-1} F.W.) was recorded at 0.8mM SA over the control (1648mg kg^{-1} F.W.), while, amount of betanin decreased significantly from 2.0mM SA, same trend was observed in case of vulgaxanthin (Fig. 1.). SA concentration (400µM) elicited an increase in betacyanin synthesis in *A. tenella* (Rodrigues-Brandão *et al.,* 2014). According to Boonsnongcheep *et al.,* 2010 and Korsangruang *et al.,* 2010 secondary metabolism of plants can be affected by salicylic acid. It has been extensively researched regarding signalling mechanisms and pest and disease responses (Fujita *et al.,* 2006). Signal molecules are involved in signal transduction systems, which trigger biosynthetic reactions catalysing the formation of defence compounds such as phenols, terpenoids, alkaloids, or pathogenesis-related proteins (Chaman *et al.,* 2003; Wen *et al.,* 2005).

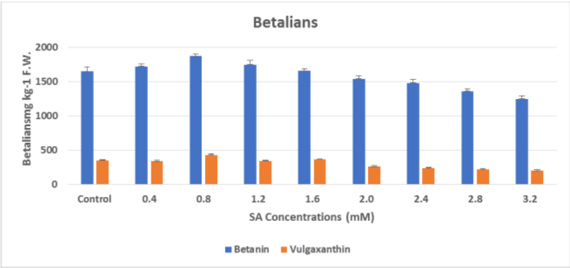

Fig. 1. Study on Betanin and Vulgaxanthin Under Increasing Concentrations of Salicylic Acid.

Values given in mean ± S.D. *Bars in each group show significant difference at p < 0.05*

Anthocyanin

Anthocyanins are the most common and widely used natural pigments in the red-purple color spectrum. The effects of increasing salicylic acid levels on anthocyanin were recorded in Fig. 2. There was no significant increase or decrease in anthocyanin content noted at all salicylic acid treatments over the control (0.62 ± 0.02µg/100g F.W.) at P<0.05 level. lowest anthocyanin content i.e., 0.56 ± 0.01µg/100g F.W. recorded at 3.2mM SA. On the contrary, Ginger plants treated with 10^{-5} M of SA showed the highest levels of anthocyanin i.e., 0.442mg g^{-1} DW (Ghasemzadeh *et al.,* 2012). Treatment with salicylic acid significantly increases the production of procyanidin and anthocyanin in grape cells (Obinata *et al.,* 2003). SA-induced increases in phenylalanine ammonia-lyase and chalcone-flavanone isomerase enzyme activities are linked to the production of these phenolics. But in case of beet there was no significant effect was noticed with anthocyanin.

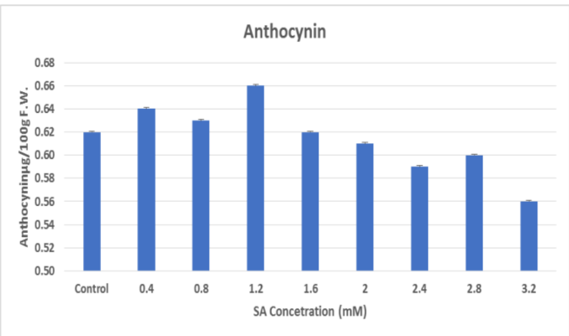

Fig. 2. Study on Anthocyanin Under Increasing Concentrations of Salicylic Acid.

Values given in mean ± S.D. *Bars in each group show significant difference at p < 0.05*

Alkaloids

Alkaloids have pharmacological effects and are used in the pharmaceutical industry. They have a bitter taste that prevents insects from feeding on the plant's leaves (Odoh *et al.,* 2012). The effects of increasing salicylic acid levels on alkaloids have been shown in Fig., 3. The significant increase in alkaloid content i.e., 2.14 ± 0.03 g/100g D.W. was recorded at 1.2mM SA treatment over the control (2.03 ± 0.08) at P<0.05 level. Highest concentrations from (2.4mM) of salicylic acid treatments induced stress on plants which affected alkaloid content. According to Idrees *et al.* (2013), foliar spray of $10^{(-5)}$ M SA significantly increased the content of anticancer alkaloids vincristine (22.2%) and vinblastine (50.0%) in *Catharanthus roseus* (L.) treated with 150mg kg $^{(-1)}$ Ni. *Stemona* sp. plantlet elicited with 100 M salicylic acid for one week produced 1.69 fold more 1′,2′-didehydrostemo-foline and 1.61 fold more stemofoline than the control (Chaichana and Dheeranupattana 2012). SA is one of the numerous phenolic compounds that contain an aromatic ring with a hydroxyl group or its derivative found in plants. It has previously been used as a potent enhancer of some secondary metabolites, for example, exogenous application of SA improves alkaloids production (Idrees *et al.,* 2011 and Pitta–Alvarez, 2000).

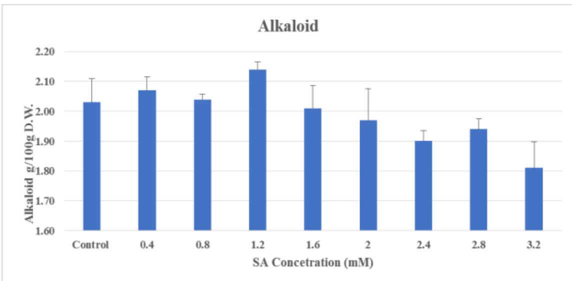

Fig. 3. Study on Alkaloids Under Increasing Concentrations of Salicylic Acid.

Values given in mean ± S.D. *Bars in each group show significant difference at p < 0.05*

Total phenolic content (TPC)

The phenolics are widely distributed among bioactive compounds and have the ability to scavenge free radicals, superoxide, and hydroxyl radicals by a single-electron transfer (Deng *et al.,* 1997). The observations for the increasing levels of salicylic acid treatment and their effects on total phenolic content were initially made for all salicylic acid treatments with the control have been depicted in Fig., 4. The significant increase in TPC i.e., 1453 ± 29.6 and 1357 ± 32.5mg kg^{-1} F.W. was recorded at 0.8 and 1.2mM SA treatment over the control (1281 ± 17.9) at $P<0.05$ level. Highest concentrations of salicylic acid treatments, ranging from 2.0, 2.4, 2.8 and 3.2mM, resulted in a significantly reduced TPC i.e., 1197 ± 38.0, 1150 ± 27.9, 1102 ± 47.4 and 1056 ± 54.1mg kg^{-1} F.W. 400 µM SA concentration elicited an increase in total phenol synthesis in *A. tenella* (Rodrigues-Brandão *et al.,* 2014). SA stimulates phenylalanine ammonia-lyase activity in sweet cherry fruit, producing the main phenolic compounds and the synthesis of new polyphenolic substances (Yao & Tian 2005).

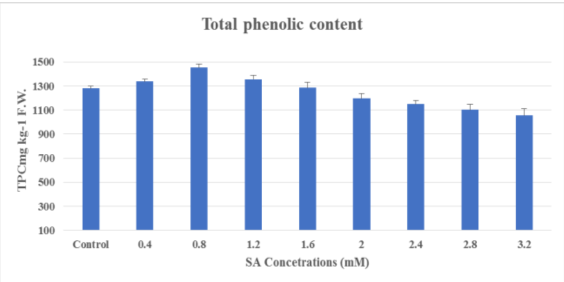

Fig. 4. Study on Total phenolic content (TPC) Under Increasing Concentrations of Salicylic Acid.

Values given in mean ± S.D. *Bars in each group show significant difference at* $p < 0.05$

Conclusion

The foliar application of salicylic acid effectively alters the content of secondary metabolites such as betacyanin and betaxanthins, total phenols and alkaloids in beetroot. Positive or negative effects of SA is crop species and concentrations dependent. In present study SA shows promising results in beet up to 1.2mM concentration. Based on the initial findings and discussion, it is possible to conclude that foliar application of salicylic acid may enhance betanin, vulgaxanthin, total phenol content at 0.8mM, and alkaloids at 1.2mM. However, the treatment >2.0mM SA showed a decreasing trend in secondary metabolites at higher levels of concentrations. There was no significant increase or decrease in anthocyanin content noted at all salicylic acid treatments compared to control. This means that foliar application of salicylic acid of *Beta vulgaris* L. with this low concentration of salicylic acid will enhance the secondary metabolites. This might have a good impact on crop management and crop economy. Based on the results of this study, SA treatment of dark red beet with low concentrations such as 0.8 and 1.2mM is recommended.

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References

Adham AN. 2015. Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in Mentha piperita, Mentha longifolia and Osimum basilicum. Journal of Pharmacognosy and Phytochemistry **3(6),** 130-139.

Ali B. 2020. Salicylic acid: An efficient elicitor of secondary metabolite production in plants. Biocatalysis and Agricultural Biotechnology, 101884.

Asghari M, Aghdam MS. 2010. Impact of salicylic acid on post-harvest physiology of horticultural crops. Trends in Food Science & Technology **21(10),** 502-509.

Baião DDS, da Silva DV, Del Aguila EM, Paschoalin VMF. 2017. Nutritional, bioactive and physicochemical characteristics of different beetroot formulations. Food additives **6(6)**.

Boonsnongcheep P, Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathanturarug S. 2010. Growth and isoflavonoid accumulation of Pueraria candollei var. candollei and P. candollei var. mirifica cell suspension cultures. Plant Cell, Tissue and Organ Culture (PCTOC) **101(2),** 119-126.

Chaichana N, Dheeranupattana S. 2012. Effects of methyl jasmonate and salicylic acid on alkaloid production from *in vitro* culture of Stemona sp. International Journal of Bioscience, Biochemistry and Bioinformatics **2(3),** 146.

Chaman ME, Copaja SV, Argandoña VH. 2003. Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. Journal of Agricultural and Food Chemistry **51(8),** 2227-2231.

Deng W, Fang X, Wu J. 1997. Flavonoids function as antioxidants: by scavenging reactive oxygen species or by chelating iron? Radiation Physics and Chemistry **50(3),** 271-276.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Current opinion in plant biology **9(4),** 436-442.

Ghasemzadeh A, Jaafar HZ, Karimi E. 2012. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber officinale Roscoe*) varieties. International journal of molecular sciences **13(11),** 14828–14844. https:// doi.org /10.3390/ijms131114828

Ghassemi-Golezani K, Hassanzadeh N, Shakiba MR, Esmaeilpour B. 2020. Exogenous salicylic acid and 24-epi-brassinolide improve antioxidant capacity and secondary metabolites of Brassica nigra. Biocatalysis and Agricultural Biotechnology **26,** 101636.

Gorni PH, Pacheco AC, Moro AL, Silva JFA, Moreli RR, de Miranda GR, ... da Silva RMG. 2020. Salicylic acid foliar application increases biomass, nutrient assimilation, primary metabolites and essential oil content in *Achillea millefolium* L. Scientia Horticulturae **270,** 109436.

Idrees M, Naeem M, Aftab T, Khan MMA. 2011. Salicylic acid mitigates salinity stress by improving antioxidant defence system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. Acta Physiologiae Plantarum **33(3),** 987-999.

Idrees M, Naeem M, Aftab T, Khan MMA. 2013. Salicylic acid restrains nickel toxicity, improves antioxidant defence system and enhances the production of anticancer alkaloids in *Catharanthus roseus* (L.). Journal of hazardous materials **252,** 367-374.

Jumali SS, Said IM, Ismail I, Zainal Z. 2011. Genes induced by high concentration of salicylic acid in'*Mitragyna speciosa*'. Australian Journal of Crop Science **5(3),** 296-303.

Kazemi M. 2013. Foliar application of salicylic acid and calcium on yield, yield component and chemical properties of strawberry. Bulletin of Environment, Pharmacology and Life Sciences **2(11),** 19-23.

Khan W, Prithiviraj B, Smith DL. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of plant physiology **160(5),** 485-492.

Koo YM, Heo AY, Choi HW. 2020. Salicylic Acid as a Safe Plant Protector and Growth Regulator. The plant pathology journal **36(1),** 1–10.

Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathanturarug S. 2010. Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in *Pueraria candollei* var. candollei and P. candollei var. mirifica cell suspension cultures. Plant Cell, Tissue and Organ Culture (PCTOC) **103(3),** 333-342.

Mancinelli AL, Yang CP, Rabino I, Kuzmanoff KM. 1976. Photocontrol of Anthocyanin Synthesis: V. Further Evidence against the Involvement of Photosynthesis in High Irradiance Reaction Anthocyanin Synthesis of Young Seedlings. Plant physiology **58(2),** 214–217.

Maurya B, Rai KK, Pandey N, Sharma L, Goswami NK, Rai SP. 2019. Influence of salicylic acid elicitation on secondary metabolites and biomass production in in-vitro cultured *Withania coagulans* (L.) Dunal. Plant Archives **19(1),** 1045-1308.

Obinata N, Yamakawa T, Takamiya M, Tanaka N, Ishimaru K, Kodama T. 2003. Effects of salicylic acid on the production of procyanidin and anthocyanin in cultured grape cells. Plant Biotechnology **20(2),** 105-111.

Odoh UE, Ezugwu CO, Okoro EC. 2012. Quantitative phytochemical, proximate/nutritive composition analysis of *Beta Vulgaris* Linnaeus (Chenopodiceae). Planta Medica **78(11),** PI116.

Palve SB, Ahire DD, Arsule CS. 2021. Influence of various concentrations of salicylic acid on germination and some morphological characteristics of *Beta vulgaris* L. International Journal of Botany Studies **6(5),** 1387-1393.

Piattelli M, De Nicola MG, Castrogiovanni V. 1969. Photocontrol of amaranthin synthesis in Amaranthus tricolor. Phytochemistry ***8*(4),** 731-736.

Pitta–Alvarez SI, Spollansky TC, Giulietti AM. 2000. The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. Enzyme and Microbial Technology **26(2-4),** 252-258.

Rodrigues-Brandão I, Kleinowski AM, Einhardt AM, Lima MC, Amarante LD, Peters JA, Braga EJB. 2014. Salicylic acid on antioxidant activity and betacyan in production from leaves of *Alternanthera tenella*. Ciência Rural **44,** 1893-1898.

Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant science **164(3),** 317-322.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture **16(3),** 144-158.

Wen PF, Chen JY, Kong WF, Pan QH, Wan SB, Huang WD. 2005. Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. Plant Science **169(5),** 928-934.

Yao H, Tian S. 2005. Effects of pre-and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biology and Technology **35(3),** 253-262.