

Impact of salicylic acid and kinetin on morpho-physiological traits, flowering patterns and seed yield in salvia (*Salvia splendens*)

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Abstract

A field experiment was carried out to study the effect of salicylic acid and kinetin on morpho-physiological, floral dynamics, and seed yield in *Salvia splendens* at Banaras Hindu University Varanasi, Uttar Pradesh, India during 2021-22. A randomized block design (RBD) with three replications was used in the design of the experiment. The treatments consisted of varying concentrations of salicylic acid (10 ppm, 20 ppm, 30 ppm, and 40 ppm) and kinetin (5 ppm, 10 ppm, 15 ppm, and 20 ppm) and a control of water spray only. Vegetative growth, flowering, and seed yield parameters were observed at 60 and 90 days after treatment (DAT). The ornamental value of plants was determined largely by plant growth regulators. Results showed that a 15 ppm kinetin spray enhanced plant height (75.34 cm) and plant spread (48.91 cm) at 90 DAT. Salicylic acid at 20 ppm gave the highest number of flower spikes per plant (93.33), number of flowers per plant (1746.00) and fresh weight of flowers (3.57 g). In addition, seed yield per plant (17.39 g) and test weight (2.94 g) were highest at 20 ppm salicylic acid. The research sought to determine the effects of kinetin and salicylic acid on plant responses and to establish a baseline for future work on the mechanisms of action of plant growth regulators (PGRs) in the growth and development of annual flower crops whose demand and scope are increasing.

Key words: Salicylic acid, kinetin, salvia, morpho-physiological traits, flowering, seed yield

Introduction

Salvia splendens is a captivating tender perennial of the Lamiaceae (mint) family, commonly known as *Salvia* or Scarlet sage. This botanical gem is native to Brazil, where it is grown as an annual, and has a stunning display of bright colors, and a rich heritage of medicinal use. The genus name ‘*Salvia*’ means ‘to save’ or ‘to heal,’ in Latin. During the historical reverence of its purported medicinal properties. Its magnificent presence in the garden is the perfect epithet in itself, and indeed, “*splendens*” is a perfect epithet. Usually 1 to 3 feet in height, 8 to 10 inches wide and forming a lush, compact foliage mound, it is typically a perennial. Its low-maintenance nature thrives in full sun to partial shade and well-drained soil environments and is adaptable to many garden settings. Scarlet sage blooms profusely from summer to frost, covering the garden with its intense red tubular flowers. The cultivars range from red, pink, blue, lavender, and orange to white, providing an endless array of colors to use in garden design. With its long-lasting blooms, many cultivars and adaptability to a wide range of growing conditions, it is a much-loved garden asset for those who want to bring colour and ecological life to their outdoor spaces. In terms of exploration and cultivation, scarlet sage remains captivating and heart-winning, as well as a sterling presence in any garden.

Exogenous applications of growth regulators on annual flowers are effective in improving plant growth and yield production. Among

these regulators, Salicylic acid (SA) is an endogenous growth regulator naturally found in plants in very small quantities. SA is considered a crucial chemical messenger that plays a significant role in helping plants tolerate both biotic (caused by living organisms like pests and pathogens) and abiotic (caused by non-living factors like drought and temperature) stresses (Khan et al., 2015; Koo et al., 2020;). Its importance in plants is multifaceted, as it regulates various physiological processes. SA influences seed germination, enhances fruit yield, aids in glycolysis (the breakdown of glucose for energy), promotes flowering in thermogenic plants (those that generate heat), and assists in ion capture and transport within the plant (Dempsey et al., 2024).

Cytokinins, on the other hand, are a class of plant hormones derived from an adenine nitrogenous base. These substances have a broad range of physiological effects on plants. Schmölling (2020) described how cytokinins promote cell elongation and differentiation, enhance the capacity for cell division, and play a crucial role in the formation and activity of apical meristems (the growth regions at the tips of roots and shoots). They are also involved in mobilizing nutrients throughout the plant, breaking apical dominance (the phenomenon where the main central stem of the plant is dominant over other side stems), promoting seed germination, breaking seed dormancy, inducing parthenocarpy (development of fruit without fertilization), stimulating flowering, and delaying senescence (the aging process in plants).

Cytokinins, also play a role in the development and protection of cellular structures they play a key role in the maintenance of the integrity of cellular system (Cortleven, 2023). The induction and activation of protein synthesis needed to form the photosynthetic system, which is essential for the plant's energy production and overall health is regulated by cytokinins (Kieber and Schaller, 2018).

Although the roles of salicylic acid (SA) and cytokinins in enhancing plant growth and stress tolerance have been well documented, there is a gap in knowledge of how these compounds affect the growth, flowering and seed yield of annual ornamental plants such as *S. splendens*. SA has been shown to play a role in stress tolerance, physiological regulation and flowering induction, while cytokinins have been implicated in cell division, nutrient mobilization and delayed senescence, however, the combined application of SA and cytokinins, and their detailed effects on the morpho-physiological traits of *S. splendens* have been largely unexplored. As annual flowering crops are increasingly demanded, and growth regulators could be used to optimize the cultivation of *S. splendens*, this study fills this gap by evaluating the effects of varying concentrations of SA and kinetin on *S. splendens*.

Materials and methods

The experimental investigation was conducted at the research farm of the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India, spanning the duration of 2021 to 2022. The seeds of salvia (*S. splendens* L.) procured from the Horticulture unit at BHU, Varanasi. Nursery establishment commenced in 3.6 x 1 m² bed in Nov 2021. Seeds were sown in rows, covered with a fine layer of farm yard manure (FYM), and gently irrigated for a germination period of 10 days. The climate of Varanasi is humid subtropical with large variations between summer and winter temperatures. During the experiment, the maximum temperature ranged 18-42°C and minimum temperature 6-23°C. Fog is common in winter and summer areas accompanied by hot dry winds called loo. The total annual rainfall during the year was 664 to 1044 mm, mainly distributed for six to eight months extending from June to October with the peak period of rainfall from July to August.

Management practices were diligently implemented, including regular irrigation to sustain optimal moisture levels and manual weeding to ensure a weed-free nursery bed. The nursery bed was shielded with a polyethylene sheet to safeguard the emerging seedlings from avian predators and chilling winds. After 25 days, robust seedlings, bearing 3-4 leaves, were deemed ready for transplantation. Field preparation was completed by 29 Nov, 2021, followed by transplanting at a spacing of 60 cm between rows and 50 cm between seedlings. Transplantation was executed in the evening to mitigate potential sun exposure. Irrigation protocols involved initial daily watering utilizing a watering can, followed by weekly flooding to meet the moisture requirements of the growing plants.

The treatments for the experiment included salicylic acid at concentrations of 10 ppm, 20 ppm, 30 ppm, and 40 ppm, and kinetin at concentrations of 5 ppm, 10 ppm, and 15 ppm, alongside a control group treated with water spray. The treatment with salicylic acid at 10 ppm was labeled as T₁, while the other salicylic acid treatments were T₂ (20 ppm), T₃ (30 ppm), and T₄

(40 ppm). The kinetin treatments were T₅ (5 ppm), T₆ (10 ppm), T₇ (15 ppm) and T₈ (20 ppm). The control group was labeled as T₉. These treatments were administered post-transplantation, and the experiment was conducted under natural open-field conditions.

The experimental design employed a Randomized Block Design (RBD) with three replications. Data on growth, flowering, and seed yield were recorded throughout the study and subjected to statistical analysis using analysis of variance (ANOVA) techniques as per the methodology prescribed by Koladiya *et al.* (2012).

Results and discussion

Plant height: At 60 DAT, the highest plant height (33.37 cm) was observed in treatment T₈ followed by T₇ (30.93 cm) and T₆ (29.53 cm). At 90 DAT, maximum plant height (75.34 cm) was recorded in treatment T₇ followed by T₂ (72.98 cm) whereas, minimum plant height (66.19 cm) was found in control (Table 1). This could be related to kinetin's potential to boost protein synthesis, cell division and growth. Similar, results were observed by Youssef *et al.* (2014) in *Hippeastrum vittatum*.

Number of primary and secondary branches per plant: Data presented in Table 1 revealed significant differences in different concentrations of treatment on the number of primary and secondary branches per plant. The highest number of primary branches per plant (14.66) was observed in treatment T₆ which was at par with T₂ (13.91) and T₄ (13.33). At 90 DAT, the maximum number of primary branches per plant (18.91) was observed in treatment T₇ which was at par with all the treatments except T₈ and T₉ whereas, the minimum number of primary branches per plant (15.41) was observed in T₉ (control). Plants treated with T₇ observed the highest number of secondary branches per plant (20.62) followed by T₈ (17.91). However, the minimum number of secondary branches per plant (11.58) was recorded in control. The role of kinetin in promoting the lateral branches that counteract the action of apical dominance which resulted in the promotion of horizontal growth. Results were consistent with the findings of Singh *et al.* (2018) in marigolds and Abou-El-Ghait *et al.* (2018) in chrysanthemum.

Plant spread (cm): Treatment T₇ recorded the highest plant spread (22.89 cm), which was significantly at par with T₁ (21.79 cm), T₂ (22.74 cm), T₅ (22.15 cm) and T₈ (21.64 cm) whereas, minimum plant spread (19.12 cm) was observed in T₉ (control). However, at 90 DAT, treatment T₇ observed the highest plant spread (48.91 cm) followed by T₈ (46.55 cm) whereas, T₉ (control) obtained the smallest plant spread (Table 1). The role of growth regulator kinetin which apart from vertical growth also increases the number of branches resulting in the promotion of horizontal growth. This finding was also supported by Sahib and Abbas (2019) in carnation (cv. Corolla); Kumar *et al.* (2017) in marigold.

Internodal length: Maximum length of internode was obtained in treatment T₇ (8.14 cm) followed by T₂ (7.46 cm) and T₁ (7.31 cm). However, minimum internodal length (5.60 cm) was noticed in T₉. The administration of kinetin at high concentrations enhanced plant height, hence this outcome might be related to plant height. Plant height was also highest at this treatment and internode distance was greatest at this kinetin levels.

Number of leaves per plant: Maximum number of leaves per plant (192.85) was obtained in treatment T₇ followed by T₁ (183.96) and T₄ (181.99). However, minimum number of leaves

Table 1 Effect of salicylic acid and kinetin on vegetative parameters of salvia

Tr. No.	PH (60 DAT)	PH (90 DAT)	NPBP (60 DAT)	NPBP (90 DAT)	NSBP (90 DAT)	PS (60 DAT)	PS (90 DAT)	IL	NLP	FWL	DWL	LA	LAI
T1	27.67	67.11	12.66	17.41	13.66	21.79	42.75	7.31	183.96	3.67	0.64	84.11	0.52
T2	28.44	72.98	13.91	18.83	16.45	22.74	44.12	7.46	180.66	3.47	0.82	96.30	0.58
T3	26.92	67.90	12.41	17.08	15.99	19.90	40.75	6.63	180.83	4.16	0.82	87.15	0.53
T4	27.33	70.20	13.33	18.58	16.08	19.39	38.77	7.03	181.99	4.33	0.88	100.15	0.61
T5	28.80	71.39	12.12	17.91	11.79	22.15	43.08	6.50	160.58	3.65	0.72	99.97	0.54
T6	29.53	71.50	14.66	17.74	15.99	20.82	42.94	6.12	177.15	4.32	0.89	107.52	0.64
T7	30.93	75.34	11.74	18.91	20.62	22.89	48.91	8.14	192.85	4.41	1.12	131.80	0.85
T8	33.37	66.53	11.08	16.24	17.91	21.64	46.55	6.18	169.83	5.01	1.19	77.52	0.44
T9	22.46	66.19	9.99	15.41	11.58	19.12	35.29	5.60	164.38	4.49	0.93	66.80	0.37
C.D. (5%)	1.83	1.45	1.89	1.95	1.63	1.63	1.26	0.49	6.43	0.40	0.14	1.70	0.02

PH: Plant height (cm); NPBP: No. of primary branches plant⁻¹; NSBP: No. of secondary branches plant⁻¹; PS: Plant spread (cm); IL: Internodal length (cm); NLP: No. of leaves plant⁻¹; FWL: Fresh weight of leaves (g); DWL: Dry weight of leaves (g); LA: Leaf area (cm²); LAI: Leaf area index; T1: Salicylic acid (10 ppm); T2: Salicylic acid (20 ppm); T3: Salicylic acid (30 ppm); T4: Salicylic acid (40 ppm); T5: Kinetin (5 ppm); T6: Kinetin (10 ppm); T7: Kinetin (15 ppm); T8: Kinetin (20 ppm); T9: Control

per plant (160.58) was noticed in treatment T5 followed by T9 (164.38). This was mostly owing to kinetin's role in cell division, expansion and cell number stimulation. Similar conclusions were drawn by Basit *et al.* (2018) in marigold and Gad *et al.* (2016) in ixora.

Fresh and dry weight of leaves (g): Highest fresh weight of leaves (5.01 g) was observed in treatment T8 whereas, lowest fresh weight of leaves (3.47 g) was observed in treatment T2. Highest dry weight of leaves (1.19 g) was observed in treatment T8 which was statistically at par with T7 (1.12 g) whereas, minimum dry weight of leaves (0.64 g) was found in T1 (salicylic acid 10 ppm). The result depicted in Table 1 showed the significant differences in fresh and dry weight of leaf due to the effect of salicylic acid and kinetin. This was due to cytokinin's role in encouraging xylem differentiation and vascular strand formation, which resulted in increased water and nutrient absorption from the soil, resulting in increased fresh weight and dry weight of leaves. Similar results were reported by Abou- El-Ghait *et al.* (2018) in chrysanthemum and Ramy *et al.* (2019) in Gaillardia.

Leaf area (cm²): Table 1 shows that the T7 recorded the largest leaf area (131.80 cm²) followed by T6 (107.52 cm²) and T4 (100.15 cm²) whereas, the smallest leaf area (37.63 cm²) was found in T9 (control). This was because of an increase in leaf size due to cell division and expansion. Comparable conclusions were reported by Farjadi-Shakiba *et al.* (2012) in Persian cyclamen and Abbasi *et al.* (2015) in zinnia.

Leaf area index (LAI): Maximum leaf area index (0.85) was found in treatment T7 followed by T6 (0.64) and T4 (0.61). However, minimum leaf area index (0.37) was noticed in T9. As the number of leaves increased in this treatment due to enhanced cell division, expansion, and stimulation of cell numbers, the leaf area index also increased. Similar findings were observed by Abbaszadeh *et al.* (2019) in *Echinacea purpurea*.

Days to bud initiation: Salicylic acid 30 ppm had an early bud initiation (51.58 DAT) followed by T6 (53.32 DAT). T4 showed the longest bud initiation time (Fig. 1). This is because the stimulating effect of salicylic acid accelerates the production of secondary metabolites, with its molecular mechanisms, similar to those of phenolic compounds, playing a role in bud-stimulating behavior. Similar conclusions were drawn by Vijayakumar *et al.* (2017) in China aster and Zeb *et al.* (2017) in zinnia.

Days to flower anthesis: Treatment T3 observed the earliest flower anthesis. T1 obtained the maximum number of days for

flower anthesis (Fig. 1). Salicylic acid acts as a blooming time manager and interacts with both photoperiod-dependent and self-governing pathways to induce the opening of flowers and involved in physiological activities, causing early flowering and floral bud sprouts. The results are supported by findings of Vijayakumar *et al.* (2017) in China aster and Pawar *et al.* (2018) in gladiolus.

Days to withering of flower: Fig. 1 revealed that the maximum days taken for flower withering (92.24 DAT) was observed in treatment T4 (salicylic acid 40 ppm) which was at par with T2 (91.83 DAT) and T1 (90.94 DAT). The earliest flower withering (85.49 DAT) was observed in treatment T9 followed by T3 (85.99 DAT). Salicylic acid increases the blooming period of plants and alters the flowering anatomy by reducing electrolytic leakage which causes a delay in flower withering. Comparable findings were reported by Ramy *et al.* (2019) in Gaillardia.

Spike length (cm): Data in Fig. 1 enumerate that spike length ranged from 24.32 cm to 32.86 cm, which varied significantly. The longest spike length (32.86 cm) was observed in treatment T7 which was significantly comparable to all treatments except T8 and T9. T9 showed the shortest spike length (24.32 cm). This result may be correlated to plant height because kinetin application at high concentrations increased plant height. The treatment resulted in the largest plant height and the longest spike length. The results are found to be corroborated with Kumar *et al.* (2014) in gladiolus cv. Jessica and Pawar *et al.* (2018) in gladiolus.

Rachis length (cm): Treatment T6 showed the highest rachis length (25.57 cm) which was significantly at par with T7 (Fig. 1). However, the lowest rachis length (15.27 cm) was observed in treatment T9 (control). This may be because, at this level of kinetin concentration, the plant height and spike length increased, resulting in the maximum rachis length. A similar finding was reported by Pawar *et al.* (2018) in gladiolus.

Pedicle length (cm): The length of pedicle varied between treatments, ranging from 6.49 cm to 9.87 cm. Treatment T5 (kinetin 5 ppm) observed the longest pedicle length (9.87 cm) which was significantly at par with T1 (9.58 cm), T2 (8.99 cm), T4 (8.66 cm) and T8 (9.66 cm) whereas, the smallest length of the pedicle (6.49 cm) was found in treatment T6 (Table 2). This could be owing to the kinetin effect, which speeds up pedicle meristematic cell division. A similar conclusion was drawn by Zeb *et al.* (2017) in zinnia cultivars, El Bably *et al.* (2017) in *Clivia miniata* L.

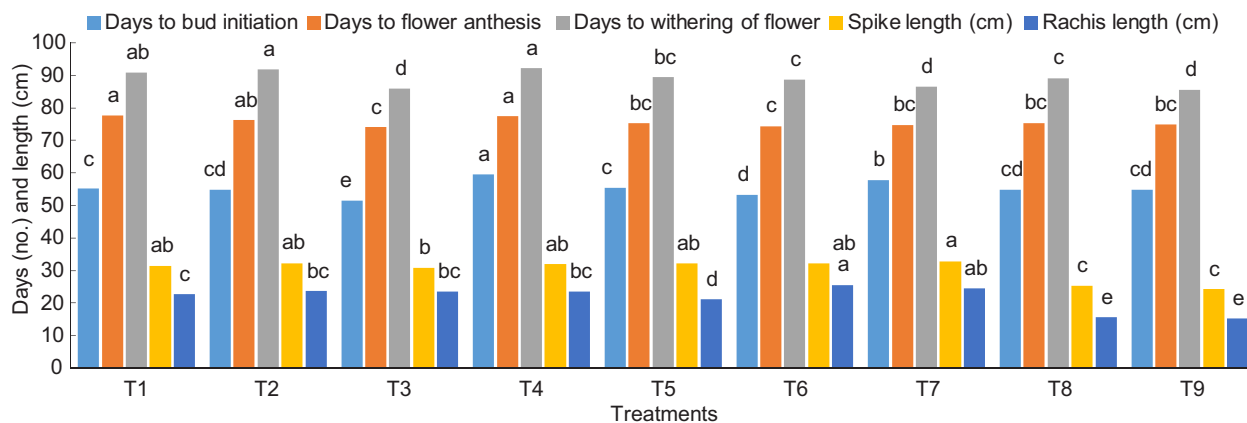


Fig. 1. Effect of salicylic acid and kinetin on flowering parameters of salvia. T1: Salicylic acid (10 ppm); T2: Salicylic acid (20 ppm); T3: Salicylic acid (30 ppm); T4: Salicylic acid (40 ppm); T5: Kinetin (5 ppm); T6: Kinetin (10 ppm); T7: Kinetin (15 ppm); T8: Kinetin (20 ppm); T9: Control

Number of spikes per plant: An analysis of data in Table 2 indicated that the impact of salicylic acid and kinetin on the number of spikes per plant differed significantly. The maximum number of spikes per plant was recorded in treatment T7 (19.40), which was significantly at par with all treatments except T3, T5 and T9. The minimum number of spikes per plant (16.34) was noticed T3. This would suggest that kinetin plays a part in lowering the inhibitors while maintaining a steady level of promoter content to bring on the highest amount of spike/plant. Similar results were reported by Pawar *et al.* (2018) in gladiolus, Singh *et al.* (2009) in tuberose and Kumar *et al.* (2014) in gladiolus cv. Jessica.

Number of flowers per spike: Data in Table 2 demonstrates that the mean performance of number of flowers per spike varied significantly from 66.12 to 93.33. The highest number of flowers per spike (93.33) was noticed in treatment T2 which was at par with T7 (92.03). T9 (control) showed the lowest number of flowers per spike (66.12). SA is a new-generation hormone that promotes and boosts blooming in plants inducing and boosting thermogenesis in the staminate part of the flower up to 14°C. Similar findings were reported by Wu and Chang (2012) in *Phalaenopsis* orchids and Kumar *et al.* (2014) in gladiolus cv. Jessica.

Number of flowers per plant: The maximum number of flowers per plant was observed in treatment T2 salicylic acid 20 ppm (1746.00) which was at par with T7 (Table 2). The fewest number of flowers per plant (1175.00) was observed in treatment T9 (control). SA improves mRNA and protein transcription and translation, which aids in the development of new isozymes and increases the number of flower buds, which is an exogenous flowering regulator. This observation was supported by Zeb *et al.*

(2017) in zinnia and Basit *et al.* (2018) in marigold.

Fresh weight and dry weight of flowers (g): Salicylic acid 20 ppm recorded the highest fresh weight of flowers (3.57 g) which was at par with T4 (3.52 g) and T8 (3.29 g). In contrast, T9 showed the lowest fresh weight of flowers (2.34 g). This could be owing to kinetin's role in delaying senescence and causing solute transfer from older areas into the treated zone. Similar findings were suggested by Farjadi-Shakiba *et al.* (2012) in Persian cyclamen, Ghorbani *et al.* (2013) in violet flower, Zeb *et al.* (2017) in zinnia and Ramy *et al.* (2019) in gaillardia. Table 2 depicts the effect of salicylic acid and kinetin on dry weight of flower (g). It was found that there was no significant difference among the treatments with respect to dry weight of flowers. Similar observations were reported by Abou-El-Ghait *et al.* (2018) in chrysanthemum and Ramy *et al.* (2019) in gaillardia.

Number of pods per plant: A higher number of pods per plant (1657.16) were found in treatment T4 whereas, T9 showed the lowest number of pods per plant (Table 2). This may be due to the role of salicylic acid in improving mRNA and protein transcription and translation, which aids in the development of new isozymes and increases the number of pods/plants. Similar findings were reported by Maddah *et al.* (2007) in chickpea.

Seed yield/plant and 1000 seed weight: The highest seed yield per plant (17.39 g) was found in treatment T2 followed by T4 and T6 (Table 2). However, the lowest seed yield per plant (9.63 g) was observed in T5. The highest test weight (2.94 g) was observed in treatment T2 which was at par with T1 (2.91 g) whereas, T9 showed the lowest test weight (2.41 g). This could be due to salicylic acid ability to boost CO₂ assimilation and as

Table 2. Effect of salicylic acid and kinetin on flowers and seed yield in salvia

Treatment	Pedicle length (cm)	No. of spike/plant	No. of flowers/spike	No. of flowers/plant	Fresh weight of flowers (g)	Dry weight of flowers (g)	No. of pod/plant	Seed yield/plant (g)	1000 seed weight (g)
T1	9.58	18.91	69.16	1,281.62	2.77	0.35	1,176.50	9.66	2.91
T2	8.99	19.37	93.33	1,746.00	3.57	0.38	1,535.75	17.39	2.94
T3	8.32	16.34	74.83	1,223.75	2.42	0.32	1,139.75	11.84	2.59
T4	8.66	19.30	78.47	1,542.25	3.52	0.38	1,657.16	13.78	2.50
T5	9.87	16.98	79.22	1,342.86	2.65	0.33	1,303.36	9.63	2.79
T6	6.49	18.85	84.37	1,590.41	3.17	0.37	1,554.08	12.01	2.79
T7	7.66	19.40	92.03	1,741.33	3.03	0.36	1,492.00	11.64	2.74
T8	9.66	18.70	79.91	1,582.25	3.29	0.36	1,542.50	11.14	2.81
T9	8.24	16.94	66.12	1,175.00	2.34	0.29	1,138.25	9.75	2.41
LSD (5%)	1.53	1.64	1.30	134.08	0.32	NS	127.70	1.85	0.10

a result, photosynthetic rate, as well as an increase in mineral intake, resulting in the highest seed output per plant. Similar results were observed by Kumar *et al.* (2014) in China aster and Khattak *et al.* (2021) in sunflower.

This study on *S. splendens* revealed that flower spike count, total flower production, seed yield per plant, and test weight of seeds (1000 seeds) were found to be enhanced by salicylic acid at 20 ppm. It stimulated early bud initiation and flower anthesis at 30 ppm, and delayed flower withering and increased pod production at 40 ppm. The practical applications of these results are for both horticulturists and researchers. To

maximize flower and seed yield, ornamental plant growers and seed producers should use salicylic acid at 20 ppm. These insights can provide a basis for the optimization of growth conditions and yield and quality in *S. splendens* cultivation.

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