Effects of sodium nitroprusside and potassium silicate on the growth and flowering of *Gazania rigens* (L.)

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Abstract

Gazania, belonging to the family Asteraceae, is widely grown in gardens and other environments. The purpose of this study was to evaluated the effects of sodium nitroprusside and potassium silicate treatments on the growth and flowering of gazania. For this purpose, a completely randomized design was used with three levels of sodium nitroprusside (SNP) (25, 50, and 100 µM/l) and three levels of potassium silicate (PS) (25, 50, and 100 mg/l) and control. Traits under investigation included fresh and dry weight of shoot and root, number of flowers, root volume, longest root length, plant height, cell membrane stability index, petiole carotenoid, leaf chlorophyll, protein, superoxide dismutase (SOD) and peroxidase (POD) enzyme activity, and flower longevity. It was observed that shoot fresh/dry weight, flower number, root length, plant height, carotenoid, and leaf chlorophyll increased under 100 mg/l PS treatment. The results suggest that the application of 50 µM/l SNP has favorable effects on root fresh/dry weights, root volume, cell membrane stability index, protein, SOD and POD activity. Over the growth stage, 50 mg/l PS was found to be the best treatment to maintain flower longevity with 7.2 days.

Keywords: sodium nitroprusside, potassium silicate, Gazania, flower longevity, carotenoid

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Introduction

A species of flowering plant belonging to the family Asteraceae is *Gazania rigens* (syn. G. splendens). It can grow both annually in cold climates and as a perennial in temperate regions. Due to its floral and therapeutic properties, this plant has attracted a lot of attention from domestic farmers. The plant is extremely important economically for its medicinal properties (Youssef Moustafa et al., 2007).

Salinity is a major problem affecting crop production all over the world. In fact, 20% of cultivated land in the world and 33% of irrigated land are salt-affected and degraded (Machado and Serralheiro, 2017). In arid and semi-arid regions, several interrelated factors could restrain sustainable agricultural development, including a lack of fresh irrigation water, soil salinity, and an increase in evapotranspiration (Qadir et al., 2008). The salt content in the soil adversely impacts soil productivity which limits the growth and ruined plant performance (Pessarakli and Szabolcs, 2010). According to Machado and Serralheiro (2017), increment levels of soil salinity are attributed to the irrigation of crops with saline water.

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Foliar spraying on plants to promote growth and lessen the negative effects of salt stress is a useful advancement towards a replacement technique in agriculture (Gomaa et al., 2021). Along with serving as a source of potassium (K) and more soluble silicon (Si) than other fertilizers, potassium silicate ($K_2 SiO_3$) is used in plants (Rodrigues et al., 2009). Silicate can develop root structure, plant improvement, leaf firmness, photosynthesis, and water contents. It also can be regarded as one of the most remarkable factors in plant productivity, principally in reducing the adverse effects of salt and oxidative stress (Liang et al., 2003). Potassium (K) is a primary macronutrient for overall plant growth, yield potential, product quality, and stress resistance of plants (Hasanuzzaman et al., 2018). It can be applied to decrease the adverse impact of salt tension in plants. Potassium silicate (PS) could develop yield-linked properties and quality and element (N, P, K) uptake (Gomaa et al. 2021). Hafez et al., (2021) indicated that the use of $K_2 SiO_3$ under the saline water irrigation treatment had the capability to reduce the levels of exchangeable sodium percentage (ESP) in the soil.

Nitric oxide (NO) is an endogenous signaling molecule that plays an important role in plant ontogenesis and responses to different stresses (Doncheva et al., 2009). Utilizing exogenous chemical donors such as sodium nitroprusside (SNP) is one of the most popular ways to examine the effects of nitric oxide on plants (Denton et al., 2012). Nitric oxide also impacts lignification and sodium nitroprusside was shown to ameliorate the vase life of different flowers, including *Rosa sp.*, *Gladiolus communis*, and *Dianthus caryophyllus* by decreasing ethylene synthesis and senescence-related genes and by increasing antioxidant activity (Naing et al., 2017). Also, it has beneficial effect in terms of recovery from salinity, drought etc. Accordingly, the exogenous application of NO may result in an enhanced crop yield under adverse conditions owing to its role in regulating mechanisms related to increased tolerance to abiotic stress (Nabi et al., 2019). Mirakhorli et al (2021) observed that NO fertilization may operate particular signaling cascades, therewith boosting the growth, efficiency, and immunity system of plants. Besides, they indicated that the foliar application technique was more useful than the soil solution.

Since source and concentration have an impact on the rate at which this element polymerizes in solution, they both affect the optimum foliar uptake of K. and the plant reaction. In fact, there are concerns about the best K source and dosage for foliar spraying in garden and bedding plants. Previous studies on container-grown floriculture crops have either focused on a small number of species or have been conducted in hydroponics with purified water. However, there has only been limited research on plants grown in soil media. The use of Si, K, and NO in commercial gazania culture has not been tested, and as a result, there has been a very slow pace of research. Therefore, the aim of this study was to investigate if foliar application of potassium silicate and sodium nitroprusside will change the enzyme activity or affect the morphological and physiological characteristics of gazania.

Materials and Methods

Plant material and treatment

This study was performed in a commercial greenhouse under 22-23 ℃, relative humidity of 50-60%, and light intensity of 60-70 µM/m^2/sec. Seeds with suitable germination qualities were supplied from the seed bank of the Institute of Medicinal Plants. Six plants were kept in each pot after thinning out from each cultivation of twenty seeds. During the growth season, other activities were regularly carried out. The experiment was arranged in a completely randomized design with 6 treatments and 3 replications (CRD). The treatments consisted of SNP at three levels of 25, 50, and 100 Mm, PS at three concentrations of 25, 50, and 100 mg/l and control (plants sprayed with water). For foliar application, treatments were applied after emerging flower bud, as a fine mist on the upper surface of the leaves followed by four times spraying during growth season with 3-day intervals (Dallagnol et al., 2012).

Evaluation of traits was performed about 10 days after the last foliar application. Samples were placed in nylon bags and sent to the lab. Evaluated characteristics included plant height, number of flowers, fresh and dry weight of shoots and roots, cell membrane stability index, petiole carotenoid content, total leaf chlorophyll, protein content, SOD and POD activity, and flower longevity.

Quality attributes

Fresh weight of fresh plant was recorded, using a digital scale with an accuracy of 0.01. Weight loss was estimated in each replication and was noted initially and after 15, 30, and 45 days during storage. The longest root length and plant height were measured with a ruler. Samples of both roots and shoots were weighed by a digital scale with an accuracy of 0.01 after being dried for 72 hours around 60 ℃ (Hosseinzadeh Rostam Kalaei et al., 2022). The root volume of each plant was recorded by a calibrated stone after washing the roots. Also, the number of flowers in each pot was counted. Flower longevity on the plant from the time of flower opening and the appearance of color to wilting, paleness, and shedding of flowers was calculated and expressed as the day (Ezhilmathi, 2007).

Nitro blue Tetrazolium (NBT) approach was used to test the SOD activity. Liquid nitrogen was used to grind the 200 mg samples, which were then homogenized using phosphate buffer (pH 7.0) and 0.5 mM EDTA. It was then centrifuged in a rotor for 15 minutes at 4 ℃ at 18,000 rpm. To evaluate the SOD activity, 1500 ml of phosphate buffer (50 mM), 300 ml of sodium carbonate (50 mM), 300 ml of methionine (12 mM), 300 ml of nitro blue tetrazolium chloride (75 mM), 300 l of riboflavin (1 mM), and 300 l of the enzyme extract were utilized. The sample's absorbance at 560 nm was measured using a spectrophotometer. The decrease of NBT is 50% inhibited by one unit of SOD enzyme activity (Giannopolitis and Ries, 1997). POD was assayed following the procedure outlined by Polle et al. (1994). The carotenoid content was obtained using fruit ground with 5% of 80% acetone. The extract was then poured into a falcon and refrigerated for 4 hours at 4 ℃. The absorption was noted by the spectrophotometer at 480 and 510 nm (Mostofi and Najafi, 2005).

The amount of protein was determined by Bradford (1976) method by extracting a protein extract of 0.05 g from plant dry matter and adding 4 ml of tris hydrochloric acid buffer to it. The samples were mixed with a vortex shaker for 20 min. After that, they received a 30 min centrifugation at a speed of 5000 rpm and the upper phase containing the total protein was separated. Then, 5 ml of Bradford solution was added to 0.1 ml of protein extract from each sample and then vortexed for 20 min and the adsorption was recorded at 595 nm.

The chlorophyll content was estimated according method of Soroori and Danaee (2023). Total chlorophyll content was measured by the spectrophotometer at wavelengths of 663 and 645 nm, and was expressed as mg g^{-1} FW leaves.

For determination of electrolytic conductivity, the samples were placed at a water bath in 30 ℃ for 60 min; then, the EC_1 level was recorded by an EC meter. The falcons were then transferred to an autoclave at 120 ℃ for 20 min at 1.2 atm. After cooling, the EC_2 was recorded. Finally, cell membrane stability index was expressed as percentage (Singh et al., 2008).

Statistical Analysis

SPSS software was used for the statistical analysis of the data. Data values were compared using LSD test at 1% and 5% probability levels.

Results

PS and SNP affected shoot fresh and dry weights, root fresh weight, cell membrane stability index, plant height, carotenoid, anthocyanin content, chlorophyll, SOD, POD, and flower longevity ($P \geq 0.05$). In this experiment, protein, root dry weight, and root length of plants sprayed with PS and SNP were significantly ($P \geq 0.01$) different from control (Table 1).

According to Table 2, traits increased in all treatments during growth but declining trend in control treatment was more than others. Exposure to 100 ppm SP resulted in the shoot fresh weight and shoot dry weight increasing to 33.16 g and 6.61 g, respectively. PS at concentration of 50 ppm was more effective than other sources, enhancing fresh and dry weights of roots by 6.18 g and 1.16 g, respectively. PS applications increased the amount of root volume

Table 1
Analysis of variance of application of sodium nitroprusside coupled with potassium silicate on the growth and flowering traits of *Gazania rigens* (L.)

S.O. V	DF	Mean Square													
		Shoot Fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Flower number	Root volume	Root length	Plant height	Cell stability	Carotenoid	SOD	POD	Protein	Shelf life
Treatment	6	144.021**	11.153**	3.764**	1.674*	13.804**	36.027*	17.139**	42.301**	239.257**	0.055**	74.701*	687.69**	2.574*	12.68**
error	-	0.079	0.22	0.019	0.007	0.024	0.065	0.075	0.079	0.294	0.003	0.017	0.405	0.012	0.023
CV	-	9.47	11.39	10.77	11.36	10.98	9.41	10.96	10.73	11.25	10.99	11.18	10.21	10.58	11.29

ns, *, and ** indicate non-significant and significant at P≤0.05 and P≤0.01, respectively.

Table 2
Effect of application of sodium nitroprusside coupled with potassium silicate on the growth and flowering traits of *Gazania rigens* (L.)

No.	Treatment	Shoot fresh weigh t (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Flower number
1	Control	21.52 ± 0.09f	3.36 ± 0.07f	4.26 ± 0.04e	1.16 ± 0.02f	4.5 ± 0.11f
2	SNP 25 µM	26.14 ± 0.07d	5.28 ± 0.05d	4.89 ± 0.04cd	1.62 ± 0.05d	6.1 ± 0.05d
3	SNP 50 µM	31.79 ± 0.15b	6.26 ± 0.08b	5.42 ± 0.05b	1.85 ± 0.03c	7.9 ± 0.03a
4	SNP 100 µM	24.81 ± 0.07e	4.43 ± 0.06e	4.67 ± 0.05d	1.40 ± 0.05e	5.1 ± 0.15e
5	PS 25 ppm	27.55 ± 0.06cd	5.62 ± 0.06c	4.96 ± 0.05c	1.43 ± 0.01e	6.1 ± 0.11d
6	PS 50 ppm	28.63 ± 0.05c	5.74 ± 0.11c	6.18 ±0.03a	2.42 ± 0.02a	6.5 ± 0.08c
7	PS 100 ppm	33.16 ± 0.12a	6.61 ± 0.06a	5.65 ± 0.05b	2.04 ± 0.04b	7.6 ± 0.07b
No.	**Treatment**	**Root volume (cm3)**	**Root length (cm)**	**Cell membrane st ability (%)**	**Carotenoid (mg/g FW)**	**Chlorophyll (mg/g FW)**
1	Control	19.54 ± 0.11f	17.18 ± 0.11f	66.87 ± 0.09g	0.661 ± 0.01f	7.235 ± 0.03f
2	SNP 25 µM	22.71 ± 0.05d	19.04 ± 0.05de	73.56 ± 0.07e	0.735 ± 0.01d	7.982 ± 0.02d
3	SNP 50 µM	23.86 ± 0.03c	20.35 ± 0.03c	77.64 ± 0.05c	0.811 ± 0.01bc	8.959 ± 0.01b
4	SNP 100 µM	21.98 ± 0.15e	18.96 ± 0.15e	71.25 ± 0.07f	0.709 ± 0.01e	7.262 ± 0.08e
5	PS 25 ppm %	23.05 ± 0.11cd	19.61 ± 0.11d	74.91 ± 0.06d	0.807 ± 0.02c	8.262 ± 0.04cd
6	PS 50 ppm	25.83 ± 0.08a	20.87 ± 0.08b	82.68 ± 0.05a	0.827 ± 0.01b	8.612 ± 0.07c
7	PS 100 ppm	24.47 ± 0.17b	21.23 ± 0.17a	78.35 ± 0.07b	0.894 ± 0.01a	9.543 ± 0.05a
No.	**Treatment**	**Protein (µg/g FW)**	**SOD (Unit enzyme/g F W)**	**POD (Unit enzyme/g F W)**	**Plant height (cm)**	**Flower longevity (day)**
1	Control	2.39 ± 0.04e	39.12 ± 0.40f	106.89 ± 0.4e	18.75 ± 0.11g	3.8 ± 0.04g
2	SNP 25 µM	3.38 ± 0.04c	45.83 ± 0.10c	119.81 ± 0.3c	21.27 ± 0.05d	5.4 ± 0.04e
3	SNP 50 µM	3.79 ± 0.05ab	47.25 ± 0.40a	131.39 ± 0.4a	22.67 ± 0.03c	6.3 ± 0.05c
4	SNP 100 µM	2.93 ± 0.01d	42.81 ± 0.10e	117.72 ± 0.6d	19.34 ± 0.15f	4.9 ± 0.05f
5	PS 25 ppm %	3.54 ± 0.05bc	43.49 ± 0.20d	121.55 ±0.5bc	20.69 ± 0.11e	5.9 ± 0.05d
6	PS 50 ppm	3.89 ± 0.03a	46.76 ± 0.20b	132.57± 0.4a	23.83 ± 0.08b	7.2 ± 0.03a
7	PS 100 ppm	3.66 ± 0.01ab	46.08 ± 0.30bc	124.61 ± 0.2b	24.41 ± 0.017a	6.9 ± 0.05b

Data are means ± standard error (n=3); the means followed by the same letter are not different according to the LSD test. Potassium Silicate: PS; Sodium nitroprusside (SNP)

(25.83 cm^3) was observed under 50 ppm PS treatment. Root lengths of the plants treated with 50 ppm PS were observed to reach up to 21.23 cm.

Discussion

Potassium silicate reduces water consumption, improves drought tolerance, and enhances the productivity under deficit irrigation (Ma et al., 2004). In our study, PS treatment reduced foliar transpiration and increased shoot and root fresh weight. This is because of the regulation of a silica gel layer that attaches cellulose to epidermal cells, decreasing water loss, and subsequently the increment activity of aquaporin, a protein linked to the gained water transportation in plants (Chen, 2016). The advantages of Si in raising the performance of water utilization in a K-deprived plant are significance. This is because climate change has induced prolonged droughts in crops, restricting water availability and harming the physiological aspects of the crop (Habermann 2021). A same effect of enhanced dry matter in K-deficient plants treated with silicon through nutrient solution was reported in some species including *Glycine max* and *Sorghum bicolor* (Ma et al., 2004; Miao et al., 2021). The incremented dry weight effects on *Bracteantha bracteatum*, *Lobelia* spp., and *Verbena officinalis* with silicon nutrient are considerable and require further research (Neil et al., 2010). Potassium has promoted root length, vegetative growth, and osmoregulation (Hasanuzzaman et al., 2018).

The plants treated with 100 ppm PS showed maximum height (24.41 cm) compared to control. Substrate-incorporated PS supplement led to maximum stem height of *Zinnia elegans* although weekly application of high concentration of PS delayed anthesis and decreased shoot length in zinnia and *Helianthus annuus* and was coupled with disordered flowers (Neil et al., 2010). These results agreed with Danaee and Abdossi (2020) who indicated that different levels of silicon and nano-silicon improved the plan growth.

Significant difference was found in cell membrane index for 50 ppm PS which was still higher after growth period. This is in line with Miao et al. (2010) studying *Glycine max* plants that took silicon through nutrient supplement. The efficacy of silicon in decreasing electrolyte leakage is because of its ability to induce greater plasma membrane protection.

SOD activity was affected by 50 µM SNP since this treatment resulted in 46.76 Unit enzyme/g FW during growth time. Moreover, 50 ppm PS with 132.57 Unit enzyme/g FW reinforced POD activity. Ahmad et al. (2016) showed that potassium improved ionic equivalency and antioxidant enzyme activity. PS has a positive impact on plant growth, productivity, and performance (Hafez et al., 2021). Consequently, increasing the functioning of enzyme-mediated antioxidants along with salt content tension sustains plasma-membrane activity, e.g., through regulating the penetrance, which is associated with maximum root activities, improving the root's ability to accumulate vital nutrients (Ahmad et al., 2019). NO, a form of reactive nitrogen species (RNS), can limit the negative effects of reactive oxygen species (ROS) by acting as a network splitter and by increasing the gene synthesis of antioxidant enzymes (Nadeem et al., 2019). The research strongly supports the idea that NO, which is a medium for enhancing plant resistance to abiotic stress, is linked to a significant ROS detoxification by defense mechanisms. NO plays a role in expanding the antioxidant chain in plants. Additionally, NO has been shown to possess antioxidant properties, playing a role in the reactive oxygen species detoxifying process and therefore helping to prevent lipid peroxidation and oxidative damage to proteins (Chen et al., 2018). In spite of the fact that the role of NO is relatively well-understood in drought conditions, salinity, and heavy metal contamination (Khalil, 2019), its collaboration in herbicide-induced phytotoxicity, such as glyphosate, remains inexpertly investigated.

Carotenoid content was significantly greater in 100 ppm PS than the other treatments (0.894 mg/g FW). Chlorophyll level of gazania leaves significantly increased to 9.543 mg/g FW under 50 ppm PS treatment. Treatment of 50 ppm PS with 3.89 µg/mg FW, demonstrated maximum protein content. Silicon increases the plant prospective by decreasing sodium ion uptake and enhancing potassium ion absorption in the leaves (Yaghubi et al., 2016). Potassium has been presented to boost physiological activities including chlorophyll pigments, stomata movement, and water condition (Hasanuzzaman et al., 2018). Si reduces oxidative damage to cells and enhance a certain amount of chlorophyll, and free of enzymes antioxidants component like carotenoids. These silicon properties boost levels of activity in photosynthesis (Chen, 2016), decrement transpiration and raising water usage performance, and consequently potassium-deficient plants produce less dry matter (Ma, 2004; Mia et al., 2010; Chen, 2016; dos Santos Sarah, 2021). Barros et al. (2018) found the same result in *Phaseolus vulgaris* L. after foliar applying of Si. Accumulation of silicon in hydroponically-grown bean crops sprayed with silicon barricaded pigment destruction. This shows the impact of silicon on leaf epidermis, which defends the photosynthetic tissues. Souza Junior et al. (2021) evaluated the principal efficacy of controlling silicate sources similar to the one used in the present study compared to the PS, and reported enhancement in accumulation of silicon, the percentage of chlorophyll, photosystem II performance, and therefore the dry matter efficiency of *Gossypium herbaceum* L. This development in pigments boosted by silicon improves the rate of photosynthesis via expanding electrons transportation and activating the genes connected with photosynthesis as well as Rubisco enzyme (Maghsoudi et al., 2016). Carotenoid acts as a non-enzymatic antioxidant that eliminates singlet oxygen (O^{-2}), a primarily harmful ROS, which causes lipid peroxidation, destruction of electrolytes inside of cells, and resistance of the lipid bilayer membrane. Additionally, since silicon functions as an attachment in light absorption for photosynthesis and protects the amount of chlorophyll from photooxidation in the reaction focus, the rise in carotenoids affected by its treatment is crucial for the stabilization in the photosynthetic system (Lichtenthaler, 1987). Improved enzymatic activity in plants treated with different levels of sodium nitroprusside as found in the present study agrees with the findings of Danaee and Abdossi (2020).

The maximum flower number was observed under 50 µM SNP treatment. Also, 50 ppm PS with 7.2 days resulted in the flowers with the longest life while control treatment with 3.8 days lasted the for the shortest time. SNP is one of the most extensively studied NO donors. In the present study, we found that SNP enhanced number of flowers. Our results are in line with Pagnussat et al. (2004), who found that NO is involved in the auxin response throughout the adventitious rooting procedure in *Cucumis sativus* via activating protein kinase cascades in response to certain mitogens. The favorable morphological effects of silicon are similar to the findings reported by other researchers e.g., Kamenidou et al. (2008, 2009), who reported that silicon content and form have a major impact on plants' morphology.

Conclusion

In sum, foliar spraying of PS enhanced the total chlorophyll and the amount of antioxidant carotenoid compounds while decreasing the amount of electrolyte leakage, all of which favored the rate of photosynthesis. Therefore, form and concentration of applied silicon significantly impacts on flower longevity life. On the other hand, SNP increased flower number, and gazania plants can all benefit from PS and SNP supplementation.

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