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Research Article

Salicylic acid can enhance quality characteristics, growth and productivity of strawberry (*Fragaria* × *ananassa* CV Camarosa) under drought stress condition

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Abstract

Abiotic stress from drought inhibits plant growth and decreases yields. Salicylic acid (SA) is classified as a compound that effectively reduces crop susceptibility and environmental stress by mitigating the harmful effects of many stressors. Numerous levels of SA protect several plant species from environmental stresses by initiating different processes involved in the stress tolerance mechanism. Fruit productivity and quality under dry stress conditions were examined by evaluating the effects of the application mode and various concentrations of SA. The split factorial experiment based on a completely randomized block design with four replicates in the Jiroft University Research Greenhouse was carried out. In this study, drought stress is the main factor at three levels (control, moderate and severe stress). Factorial treatment included interaction at two levels of (SA application method as foliar spray and soil drench) and (SA concentration include control, 1 and 1.5 mM SA). The results showed that relative water content (RWC), electrolyte leakage, titratable acidity (TA), average fruit weight, fruit yield, and the number of fruits per plant were affected by the interaction of drought stress, SA concentration and consumption method. Strawberry fruit production per plant was highest with 315.20 g from non-stress treatment with 1.5 mMof SA. Under severe drought stress, the maximum fruit production per plant of 201.80 g with 1.5 mM of SA was obtained, which increased the strawberry fruit productivity with an ascending concentration of SA. In strawberry plants, drought stress manifests in elevated levels of antioxidant enzyme activities, including catalase (CAT) and peroxidase (POD) in the leaves. Interestingly, moderate and high drought stress led to improvements in certain fruit quality indices such as vitamin C content, compared to those not subjected to stress. Another significant finding was that the application of SA increased the activities of CAT and POD enzymes, while also elevating the concentrations of chlorophyll and proline considerably. Finally, it is recommended to use 1.5 mM of SA as a foliar spray under drought stress conditions on greenhouse strawberries.

Keywords: Electrolyte leakage, Foliar spray, Fruit weight, Relative water content

Introduction

Strawberry is considered one of the most important fruits of the Rosaceae family. Strawberry fruits contain important minerals, fibres, vitamins (in particular ascorbic acid) and antioxidant compounds such as pigments (anthocyanin), phenolic compounds and carotenoids. (Maksimovic *et al*., 2015; Darwish *et al*., 2021). Strawberry (*Fragaria* × *ananassa* CV Camarosa) is an octaploid plant (2n = 8x = 56) (Edger *et al*., 2019), which is an important small fruit in the world. Strawberries have good taste and appearance and are nutrient-rich (Li *et al*., 2021). On the other hand, the short production period of this product has made it possible for its fans to produce it out of season, and it is available fresh all year round (Morgan, 2003). The total world production of strawberries was 8,861,381 tonnes, which was harvested from 384,668 ha. In 2020 more than 8,861,381 tons of strawberries were produced worldwide (FAO, 2020). China ranks first with a production of 3,221,557 tons, followed by the USA (1,055,963 tons), Egypt (597,029 tons), Mexico270 (557,514 tons), Turkiye (546,525 tons) (FAO Stat

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2020). The south of the Kerman region in Iran, with particular climatic conditions, is one of the most important areas for the production of strawberries in autumn and winter. According to the latest statistics published by the Ministry of Agricultural Jihad in Iran, of the 351 hectares of cultivated area and 15,760 tons of greenhouse strawberry products produced in Iran in 2021, 234 hectares (67%) and 6,407 tons (41%) belong to the Jiroft region (Anonymous, 2020). Plants are constantly affected by adverse environmental factors while growing. Water stress is one of the limiting factors that strongly affects the growth and development of the plant and causes severe damage to its growth and yield. (Ghadirnezhad Shiade *et al*., 2022; El-Mogy *et al*., 2022). Drought is a global phenomenon that endangers the successful production of agricultural products every year with varying intensities (Nassiri Mahallati *et al*., 2022). Extreme drought stress will lead to nutritional imbalances and impair the metabolic function of cells, resulting in plant mortality (Kardoni *et al*., 2019: Li *et al*., 2022). Drought stress has been reported to affect the growth and development of strawberries (Merlaen *et al*., 2019). Therefore, enhancing the tolerance to drought stress and improving the yield and quality of strawberries are one of the main challenges (Li *et al*., 2022). One of the compounds that effectively create tolerance and resilience to drought stress in plants is the quasi-hormonal salicylic acid (SA) compound (Fathi *et al*., 2022). SA is a phenolic compound produced by root cells and plays an important role in plant growth and development (Kaya *et al*., 2020; Maleki and Fathi, 2019). SA (2-hydroxybenzoic acid) is an endogenous growth regulator that affects the physiological and biochemical functions of plants. As a natural regulatory molecule, it is crucial in establishing and signaling defense responses against various biotic and abiotic stresses (Ghadirnezhad Shiade *et al*., 2022). Applying SA in response to drought stress has altered some physiological responses in plants (Ghaderi *et al*., 2015). The mechanism of action of SA against drought stress is related to its role in regulating antioxidant enzymes and its combination with active oxygen species in plants (Fathi *et al*., 2022). It has been reported that the use of SA under stress conditions alters the amount of plant hormones and activates plant protection mechanisms against stress (Fahad *et al*., 2015). Researchers have claimed that SA significantly increases drought tolerance and can increase and stabilize crop production under stress conditions (Sharma *et al*., 2017; Sahraei *et al*., 2018). The objective of this study was to study the application of salicylic acid (method of use and dose) as an increase in the resistance of strawberries to drought stress conditions.

Materials and methods

Study location: The present study was conducted at the Jiroft University Research Greenhouse, situated at coordinates 28.540945" N 57.863858" E, during the year 2018. The region where the greenhouse is located experiences a humid subtropical climate, classified as a semi-arid area characterized by hot and lengthy summers, followed by mild and brief winters. The annual precipitation in this region is approximately 120 mM, with the majority of rainfall occurring between the months of January and April. For the experimental setup, hot distilled water was utilized to prepare the desired concentration of the SA (salicylic acid) solution. The SA treatments were systematically arranged in the subplots, and a total of six applications were administered at 10-day intervals, comMencing from 25 days after transplanting.

Experimental setup and plant resources: The experiment had three factors: Drought stress at three levels (no stress, 100% field capacity; moderate stress, 75% FC; severe stress, 50% FC); salicylic acid application method at two levels (foliar spraying and soil drench); and salicylic acid concentration at three levels (control, 1 and 1.5 mM SA). The transplants of the strawberry cultivar “Camarosa” were planted on September 28 in individual pots (30 cm diameter × 30 cm height). Camarosa’ Strawberry is widely cultivated in Iran, especially in the south of Kerman (Jiroft). The pots were filled with sand, perlite and a little peat. Before planting, the excess leaves and roots of the seedlings were trimmed and disinfected. All flowers were removed at transplanting and at the first flowering stage. Salicylic acid was applied once every 10 days, both to the soil and as a foliar spray, with the specified concentrations.

Trait assessment, plant growth indicators: The strawberry plant was well maintained until its fruit developed a vivid and juicy display. When these fruits reached a specific color and texture indicative of their optimal quality, the harvest was initiated. The fruits were then carefully transported from the harvest location to the laboratory for further analysis and research. After one hundred days of transplanting, an evaluation was conducted to determine the traits of strawberry plants in each experimental pot. Several traits were examined, including the number of fruits per pot, plant productivity, single fruit weight, number of fruits, fruit weight average, and quality characteristics of the strawberry fruit. The evaluation aimed to investigate the potential differences in these traits among the plants and experimental treatments. Precise measurements were taken to ensure all results were collected accurately for further analysis. To determine the vitamin C content in a fruit, a mixture of 10 cc of fruit juice, 2 cc of solution containing starch (1 gram of starch dissolved in 100 cc of distilled water), and 20 cc of distilled water was prepared. The resulting solution was then titrated using potassium iodide in a burette tube until a clear color change was observed. The number obtained from the titration process was then recorded and multiplied by a factor of 17.6 to obtain the total vitamin C content. To determine the fruit acidity level, strawberry juice was mixed with phenolphthalein

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and 1% alcohol in a ratio of 5 cc and 5 drops, respectively. The resulting solution was titrated until the color turned pale, and the measurements were noted down. To assess the water status of a given plant, an empirical procedure was performed. A sample of five fully-grown leaves was taken from each plant, and their fresh weights were recorded individually (FW). The leaves were then immersed in distilled water and left for five hours to achieve turgidity, after which their weight was measured and recorded as the turgid weight (TW). Subsequently, these leaves were placed in an oven and dried for 24 hours at a temperature of 70°C, and their dry weight was measured and recorded as well (DW). With these values obtained, the relative water content (RWC) was determined by performing a calculation prescribed by Baris and Weatherley, (1962).

$$RWC\% = \frac{Wf-Wd}{Wt-Wd} \times 100$$

DW: Leaf dry weight, TW: Leaf turgescence weight, FW: Fresh weight of leaves

To measure the Electrolyte leakage of the leaves, parts from the veins of the leaf edge with a weight of 0.1 g were obtained and placed in a closed container in 15 ml of water for 24 hours. The primary ion leakage (EC1) was then read by an EC meter. The closed containers were then placed in a freezer for 24 hours and then placed at normal room temperature for 24 hours. The secondary ion leakage (EC2) was then measured. These two readings were used to calculate the ion leakage. Additionally, parts of the leaf veins, with a weight of 0, were used in the procedure. A total of one gram of the material was obtained and deposited into a sealed container that contained fifteen milliliters of water. This mixture was left for 24 hours. After that, the primary ion diffusion from the material was measured by an EC meter. Then, the sealed container was placed in a freezer for 24 hours and then returned to normal room temperature for 24 hours. After that, the secondary ion leaching from the material was read and recorded. The total ion leakage was then calculated according to established methodology (Sairam and Srivastava, 2001).

$$EL\% = \frac{EC_1}{EC_2} \times 100$$

EL: Electrolytic leakage – EC_1 and EC_2 electrolytic conductivity respectively before and after boiling bath.

Titrarable acidity (TA): To determine the titratable acidity (TA) of the juice, a measure of its acidity, a process was carried out involving the combination of 10 ml of undiluted juice with distilled water in a 1:2 volume ratio. This mixture was then subjected to titration with a 0.1 normal solution of sodium hydroxide (NaOH), until it attained a pH value of 8.2. The data obtained from this exercise was then analyzed using a specified formula to yield the desired results:

$$\%TA = Z = \frac{V*N*Meq}{Y} \times 100$$

Where Z: Percentage of acidity, V: Volume of sodium hydroxide used (ml), N: Sodium hydroxide normality, and Y: Volume of bulk (ml). SSC: TA ratio was calculated by dividing SSC by TA (Ting and Russeff, 1981).

Proline content and antioxidant enzymes activity: The free proline content was measured as described previously by Bates *et al*. (1973). Briefly, 0.2 g of leaf samples were extracted in sulfosalicylic acid (3% 10 ML). Then, the samples were filtered using filter paper (Whatman one). Next, 2 ML of filtrate solution was added to ninhydrin and 100% glacial acetic acid (2 ML). The samples were heated in a water bath at 100 °C for an hour. The process was stopped by immersing the samples in ice water for 15-20 min, and 4 mL of toluene was added and shaken in a test tube for 15-20 s. The samples were left standing until the separation of the toluene phase from the sample solution phase. The toluene phase was measured using a spectrophotometer, with the 520 nm absorbance and proline levels expressed in umol. g^{-1}. Peroxidase (POD) (EC 1.11.1.7) was assessed according to the method of Scebba *et al*. (2001). One unit of POD enzyme activity was considered as an increase of 0.01 per minute in the absorbance at 470 nm.

Statistical analysis: The analysis of the data was done using SAS software. The comparison of data averages was done by the least significant difference (LSD) test at a probability level of five percent. The analysis results are graphically represented by Excel software.

Results and discussion

Relative water content (RWC): The findings obtained from the variance analysis indicate that the Relative Water Content (RWC) was impacted by varying degrees of drought stress, with a 5% probability. Salicylic acid also had an effect on RWC, with a significance level of 5%. Moreover, a combination of drought stress and salicylic acid yielded an interaction effect with a probability level of 1%. The way the treatment was administered also had an effect, with a 5% probability linked to the method of consumption. Similarly, the interaction effect of drought stress and method of consumption showed a 1% probability level. Additionally, when salicylic acid was coupled with a particular method of application, it exhibited a significant impact, with a 5% probability level. Further, an intricate interplay of three factors, drought stress, salicylic acid, and method of application, revealed an interaction effect with a probability level of 1% (Table 1). According to the findings in table 2, the application method of salicylic acid (SA) played a significant role in the RWC of the plant. The highest RWC percentage, 99.65%, was observed with the Soil drench, under non-stress conditions (control), and a SA concentration of 1 mM. On the other hand, the lowest RWC, at 34.4%, was observed with the same application method, but under severe stress treatment and a SA concentration of 0 mM. From these results, it can be deduced that applying salicylic acid at a concentration of 1 mM in non-stress conditions using the Soil drench method can maintain the relative water content of the plant at its highest

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Table 1. Analysis of variance (mean square) of quality traits of strawberries

S.O.V	DF	mean square			
		RWC	Electrolyte leakage	amount of acidity (TA)	Average fruit weight
Repeat	2	10.71 ^{ns}	5.62 ^{ns}	1.78 ^{ns}	2.78 ^{ns}
drought stress	2	90.21*	1239.79**	8.04 ^{ns}	91.76**
Error a	4	9.07	5.73	20.27	4.28
SA	2	65.58*	63.92**	4.91 ^{ns}	9.85*
Drought stress × SA	4	256.87**	31.14**	35.76**	31.67**
application method	1	87.37*	411.85**	159.20**	0.97 ^{ns}
Drought stress × application method	2	148.60**	324.35**	7.70 ^{ns}	49.12**
SA pplication method	2	80.20*	158.22**	97.04**	26.76**
Drought stress × SA × application method	4	256.30**	141.26**	38.62**	10.04*
Error b	30	17.79	6.13	8.03	90.82
C.V	-	5.04	25.79	16.45	10.28

**** Means significant at 1% probability levels,* Means significant at 5% probability levels, n.s No means**

Table 2. The mean comparison of quality characteristics of strawberries grown under drought, Salicylic acid concentration, and application method

Treatments	RWC (%)	electrolyte leakage	Titratable acidity amount (TA)	Number of fruits	fruit weight average (g)
Foliar spraying× (0)SA×severe stress	75.24 ^{ef}	23.20 ^{a}	12.60 ^{cd}	12.33 ^{de}	12.82 ^{gh}
Add to soil× (0)SA× severe stress	80.19 $^{c-f}$	23.86 ^{a}	13.93 ^{cd}	13.33 $^{c-e}$	13.71 $^{f-h}$
Foliar spraying× (1)SA× severe stress	75.66 ^{def}	17.11 ^{b}	20.71 ^{a}	16 $^{b-d}$	11.63 ^{h}
Add to soil× (1)SA× severe stress	92.81 ^{b}	5.26 ^{c}	22.20 ^{a}	11.33 ^{e}	20.72 $^{a-c}$
Foliar spraying× (1.5)SA× severe stress	85.94 $^{b-e}$	18.08 ^{ab}	15.50 ^{c}	16.33 ^{bc}	16.86 $^{d-f}$
Add to soil× (1.5)SA× severe stress	82.20 $^{b-e}$	19.01 ^{ab}	12.96 ^{cd}	13.33 $^{c-e}$	14.46 $^{e-h}$
Foliar spraying× (0)SA× moderate stress	85.31^{b-e}	6.26 ^{c}	15.40 ^{c}	6 ^{f}	14.90 $^{e-g}$
Add to soil× (0)SA× moderate stress	85.69 $^{b-e}$	8.17 ^{c}	17.86 ^{b}	7.33 ^{f}	14.35 $^{e-h}$
Foliar spraying× (1)SA× moderate stress	86.48 $^{b-d}$	10.58 ^{bc}	14.90 ^{c}	11.33 ^{e}	18.40 $^{b-d}$
Add to soil× (1)SA× moderate stress	70.83 ^{f}	4.16 ^{c}	21.501 ^{a}	12 ^{e}	14.98 $^{e-g}$
Foliar spraying× (1.5)SA×moderate stress	83.20 $^{b-e}$	17.88 ^{ab}	17.90 ^{b}	12 ^{e}	17.6 ^{de}
Add to soil× (1.5)SA× moderate stress	86.04 $^{b-e}$	18.95 ^{ab}	16.96 ^{bc}	5 ^{f}	14.82^{e-h}
Foliar spraying× (0)SA× non stress	88.60 ^{bc}	5.36 ^{c}	20.32 ^{ab}	23.33 ^{a}	22.09 a
Add to soil× (0)SA× non stress	83.33 $^{b-e}$	5.22 ^{c}	21.86 ^{a}	18.21^{ab}	22.34 ^{a}
Foliar spraying× (1)SA× non stress	84.42 $^{b-e}$	4.34 ^{c}	21.60 ^{a}	17.33 ^{b}	19.55 $^{a-d}$
Add to soil× (1)SA× non stress	99.65 ^{a}	6.37 ^{c}	20.53 ^{ab}	12.67 $^{c-e}$	19.35 $^{a-d}$
Foliar spraying× (1.5)SA× non stress	77.54 $^{c-f}$	5.96 ^{ab}	14.90 ^{c}	16.33 ^{bc}	19.61 $^{a-d}$
Add to soil× (1.5)SA× non stress	78.84 $^{c-f}$	6.85 ^{ab}	17.43 ^{b}	19 ^{b}	21.64 ^{ab}

**** Means significant at 1% probability levels,* Means significant at 5% probability levels, n.s No means**

value. These findings emphasize the importance of choosing the appropriate application method and concentration of salicylic acid when treating plants. In a study by Ibrahim *et al*. (2021), it was reported that water stress had a significant effect on various parameters in the growth of strawberry plants. The experiment yielded significant results regarding the transpiration rate, net photosynthesis, stomatal conductance, leaf relative water content, number of flowers and fruits, proline content, as well as the length, diameter, weight, and total soluble solids of Earlibrite, California, and Sweet Charlie strawberry cultivars. These findings could have potential implications for the cultivation of strawberry plants under different conditions.

Electrolyte leakage: The amount of electrolyte leakage experienced in a plant's system can be indicative of its ability to withstand harsh environmental conditions. Among these conditions, drought stress and the application of salicylic acid in varying methods were found to have a significant impact on electrolyte leakage. This effect was observed at a noteworthy level of 1%, as stated in Table 1. A review of the mean interaction impact of drought stress, salicylic acid, and the approach of administering exhibited that the absence of salicylic acid during drought stress situations produced a noteworthy amplification in electrolyte leakage. Conversely, salicylic acid utilization with a concentration of 1.5 mM throughout all stress degrees equally induced an upsurge in electrolyte leakage. In alternative treatments, the extent of electrolyte leakage was limited, and there was no visible discrepancy among them. When faced with the burden of stress, studies have shown that the employment of salicylic acid with a concentration level of 1 mM can lead to a reduction in electrolyte leakage in soil. Additionally, research conducted by Karlidag *et al*. (2011) discovered that the application of SA had a positive impact on the total soluble solids (TSS) content of strawberry fruits when compared to the control, during both tested growing seasons. According to a recent study conducted

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Table 3. Analysis of variance (mean square) of growth traits and yield of strawberries

Sources of changes	DF	M.S		
		Number of fruits in plant	Average fruit weight	Plant yield
Repeat	2	3.24 ^{ns}	2.78 ^{ns}	124.37 ^{ns}
Drought stress	2	245.13**	91.76**	483.31**
Error a	4	6.68	4.28	174.46
Salicylic acid (SA)	2	57.79**	9.85*	305.83 ^{ns}
Drought stress×SA	4	25.99**	31.67**	173.77**
method of Application	1	101.40**	0.97 ^{ns}	259.22**
Drought stress × method of application	2	11.24*	49.12**	714.27*
Method of application ×SA	2	20.13**	26.76**	337.38 ^{ns}
Method of application ×SA× drought stress	4	41.04**	10.04*	285.72 ^{ns}
Error b	30	3.49	90.82	166.61
Coefficient of Variation (CV)	-	11.97	10.28	18.20

**** Means significant at 1% probability levels,* Means significant at 5% probability levels, n.s No means**

by El-Beltagi in 2022, the application of TR through the leaves of plants resulted in notable enhancements in the function of stomata, photosynthesis, and the efficient usage of water. In addition, the study findings indicated that the transpiration rate of plants decreased significantly as compared to a control group. Moreover, foliar application with high TR levels, specifically at a rate of 1 ppm, when combined with well-watered irrigation at 100% WHC, presented even further improvements, displaying higher rates of photosynthesis in both seasons than low-level TR and control plants.

Titrable acidity (TA): According to table 2, it was found that the use of SA for soil application had a significant impact on the level of TA (total acidity) present in strawberry fruits. At a 1 mM SA treatment during severe stress, the highest TA content recorded was 22.20. Remarkably, this result highlights the efficacy of SA application in increasing the acidity levels of strawberries during times of stress. In contrast, the non-stress level treatment with 0 mM (control) produced the least amount of TA at 4.31. These findings suggest that the use of SA for soil application can greatly benefit strawberry fruit quality, particularly during periods of heightened stress. Climate change has brought about numerous ecological impacts, such as drought and extreme heat. One of the repercussions of this shift is the damage that is caused to the cells of plants due to increased drought stress. Under these conditions, there is an increase in the leakage of electrolyte and a subsequent destruction of the cell wall. The cytoplasmic membrane of plants becomes highly vulnerable, and as a result, the contents of the cells tend to leak out. However, researchers have found that applying salicylic acid can alleviate these issues to a certain extent. This treatment encourages the production of polyamines such as putrescine, spermidine and spermine which play a critical role in preserving the integrity of the membrane when the plant is subjected to dry conditions. This finding has been documented in studies such as that conducted by Nemeth *et al*. (2002). Upon analysis of table 2, it can be deduced that in the absence of drought stress, both foliar and soil applications not incorporating salicylic acid or incorporating it with a concentration of 1 mM retained TA at the maximum value. It is interesting to note that the utilization of salicylic acid with a concentration of 1 mM resulted in an elevation of TA in moderate and severe stress conditions, as indicated in table 2. The results of the conducted experiment revealed that the application of SA had a significant impact on the Triacontanol (TA) content of fruits when compared to untreated fruits. Specifically, when applying SA at concentrations of 1 and 1.5 mM, the TA content was found to be considerably higher. These findings align with a previous study conducted by Lolaei *et al*. (2012), where it was concluded that the pre-harvest application of SA led to an improvement in the TA content of strawberry fruits as compared to the control group (Shafiee *et al*., 2010). Karlidag *et al.* (2009b) have conducted studies on strawberries that showed no significant change in total acidity (TA) after salicylic acid (SA) foliar application. Similarly, Lu *et al*. (2011) reported similar observations in experiments conducted on pineapple fruit. In our own experiment, we also did not observe any changes in TA after SA foliar application during the different storage periods, as compared to the control group. In accordance with the findings of both Karlidag *et al*. (2009 a) and Asghar, (2006), a notable improvement in desired traits within strawberry fruits could be achieved through the application of salicylic acid at concentrations of either 1 mM or 2 mM via foliar means. Specifically, this treatment was found to heighten the total soluble solids and TA properties of said fruits to a meaningful degree. It has been stated that SA holds a significant role in the vast network of signal transduction. Furthermore, its involvement varies greatly depending on the specific system in question. Recent studies have also shown that by increasing the biosynthesis of pigments in photosynthesis, SA can effectively stimulate the growth of strawberry plants. This, in turn, leads to an enhanced yield of strawberries, ultimately increasing their overall value and nutritional benefits (Bano and Qureshi, 2017).

Number of fruits: As shown in table 2, the analysis

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of the mean interaction effect of drought stress, salicylic acid, and application method revealed that foliar spraying of salicylic acid at concentrations of 0, 1, and 1.5 mM in non-stress conditions resulted in the highest fruit productivity per plant, at 23.33. During severe stress periods where salicylic acid was not consumed, a significantly lower fruit yield was observed per plant when compared to those that consumed 0 mM salicylic acid (as indicated by table 2). This observation suggests that salicylic acid plays a crucial role in inducing flowering and increasing fruit production. This is attributed to the presence of a free hydroxyl group on the benzoic acid ring, as proposed by Raskin, (1992). A study by Raskin (1992) revealed that salicylic acid holds the ability to enhance the process of flowering and facilitate flower opening in orchids. In a recent study by Hasan *et al*. (2021), it was found that subjecting strawberry cv. Chandler to an SA concentration of 4 mM resulted in an improvement in plant height, leaf count per plant, as well as the average weight of fruits produced. Furthermore, the postharvest properties of the fruits, such as the total soluble solids and total sugar contents, were also positively affected. Drought stress poses a significant threat to the growth and productivity of strawberry plants, as it adversely affects the nutrient-absorption process. This, in turn, leads to reduced flowering and ultimately results in a decrease in the average fruit weight. These findings have been documented by Klamkowski and Treder (2008). Additionally, a comparison of different conditions revealed that the highest yield was observed under non-stress conditions combined with the application of salicylic acid. The interaction effect of drought stress and salicylic acid played a pivotal role in determining the overall yield of the plant. Based on the findings, it was determined that a concentration of 5 mM of salicylic acid weighing 315.20 g had been utilized during the experimental treatment. However, despite this, a noticeable discrepancy existed between this approach and the implementation of salicylic acid under the given stress level. More specifically, it was determined that the consumption of 1 mM of salicylic acid was not feasible at this stage of stress. Nevertheless, the results indicated that during moderate stress conditions, the usage of 1 mM of salicylic acid resulted in a similar performance to that achieved with the control in the absence of stress, as depicted in figure 1. In conditions of severe stress, the yield saw a notable decrease. However, the implementation of salicylic acid in a quantity of 1.5 mM has successfully mitigated the effects of drought stress by decreasing its intensity.

Effect of drought stress and SA on fruit weight average: Upon analysis of the average interaction effect between three factors, namely drought stress, salicylic acid, and method of application, on the average fruit weight, it was deduced that regardless of the level and method of application of salicylic acid, the highest fruit weight was observed in the absence of drought stress. Interestingly, it was observed that salicylic acid did not produce the highest fruit weight when consumed under non-stress conditions. The recorded fruit weight was found to be 22.34 g. Table 2 demonstrates that the weight of fruits attained the minimum level when subjected to severe stress conditions, and the presence of varying amounts of salicylic acid. When studying the interplay of drought stress, salicylic acid, and method of application, it was determined that the highest average fruit weight was observed in non-stressful (control) conditions, regardless of the quantity or mode of applying salicylic acid. The application of salicylic acid can have significant effects on fruit growth, particularly when it comes to managing stress conditions. According to the data presented in table 2, using a concentration of 1 mM or 1.5 mM of salicylic acid in non-stressful conditions through either soil or foliar application can lead to an improvement in average fruit weight. However, when there is severe stress, there seems to be a negative impact on fruit weight, as evidenced by the lowest average fruit weight observed under such conditions. Interestingly, the use of salicylic acid at the same concentrations in severe stress conditions also resulted in a decrease in average fruit weight, as outlined in table 2. Crop scientists warn that the effects of drought stress on strawberry plants can have severe ramifications on agricultural productivity if not managed properly (Senaratna *et al*., 2000). In recent research, salicylic acid has been shown to have the ability to enhance the resilience of tomatoes and beans to a variety of environmental stressors, including heat, cold, and drought. The findings of Adak *et al*. (2018) shed light on a different plant, the strawberry, and investigated how different irrigation systems impact four distinct cultivars - Camarosa, Albion, Amiga, and Rubygem. The irrigation systems consisted of control (30% water), and water restriction (15% drainage). The study revealed that fruit weight dropped by 59.72%, and yield per area plunged by 63% in underwater stress conditions. Additionally, they also evaluated the quality and biochemical aspects of the strawberries. According to the report, when plants were exposed to water stress conditions, 62% of them showed signs of being overwhelmed. It was found that the severe drought stress, which was calculated at 40% of the water holding capacity (WHC), had a significant effect on reducing the average fruit fresh weight, the number of fruits produced per plant, the total yield that each plant yielded, as well as the early yield and total yield, when compared to plants that were receiving the recommended level of irrigation, which stood at 100% of the WHC (El-Beltagi *et al*., 2022).

Fruits yield: According to the findings illustrated in figure 1, it was observed that the most productive fruit yield, measuring at 315.2 g and 298.1 g, was obtained under the application of 1 and 1.5 mM SA treatment, as well as in the non-stress control group. On the other hand, the lowest fruit yield of merely 104.8 g and 117.9 g was recorded for crops that were exposed to harsh/moderate stress levels in the control experiment.

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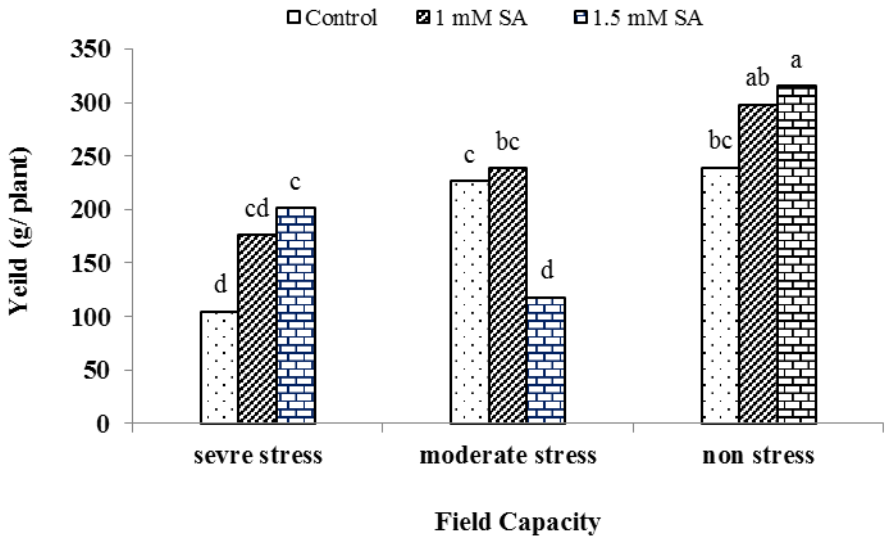


Figure 1. The interaction effect of drought stress and salicylic acid on strawberry plant productivity. Means with at least one shared letter based on the least significant difference test have no significant difference.

These results align with a prior investigation that gauged the impact of drought stress on strawberry yield, which also emerged with similar results as those outlined above (El-Beltagi *et al*., 2022). The findings of the research revealed that the foliar application of salicylic acid (SA) at a concentration of 1.5 mM had the most significant impact on crop yield improvement. Furthermore, an empirical investigation of the correlation between drought stress and application methods indicated that foliar spraying with SA was markedly more effective in mitigating the effects of stress, resulting in higher yields in extreme stress conditions. A figurative representation of the data further underscored the promising results obtained from using SA as a foliar spray in stress management, which could have far-reaching positive implications for crop production. Salicylic acid is a beneficial substance when it comes to increasing fruit yield, both for a single plant and for an entire field. According to recent research by Tawseef *et al*. (2017), they found that the best outcome was achieved by using a 2 mM concentration of salicylic acid, leading to significantly higher yields compared to the control group. In fact, the highest fruit yield of 161.85 g per plant and, 11989 kg per hectare was obtained only with the application of 2 mM salicylic acid. Another study by Adak *et al*. (2018) may have similar findings on this subject, but further research is yet to be conducted in this area. A study was conducted to investigate the characteristics of different strawberry cultivars, namely Camarosa, Albion, Amiga, and Rubygem. The study aimed to assess the influence of distinct irrigation regimes, consisting of control (30%) and water stress conditions (15% drainage), on the yield, quality, and biochemical aspects of these cultivars. The findings of the research indicated that the fruit weight of the strawberries decreased significantly by 59.72% and the yield per unit area dropped by 63.62% underwater stress conditions in comparison to the control group. These results suggest that drought stress can have a substantial impact on crop productivity, including the growth and development of strawberries. It has been noted by Serrano *et al*. (1992) that in order to attain desirable economic gains from the cultivation of strawberries, it is paramount that the crop receives sufficient irrigation.

Table 2 revealed a significant impact of drought stress and mode of application on the yield of strawberry fruits, with a p value of less than 0.01. Amongst all the treatments, it was observed that the non-stress or control treatment had the highest fruit yield, measuring 300.07 g as seen in figure 2. In contrast, the yield appeared to diminish under severe drought stress conditions, ranging from 150 g per plant when grown in soil under stress to 238.3 g under foliar spraying under severe drought stress (Figure 2). In the study conducted, it was found that the plant's fruit yield was notably low when subjected to severe stress under the control treatment, with only 104.8 g produced. However, the experiment also demonstrated that the application of foliar SA had a significant impact on increasing the yield of strawberries. In particular, administering 1 and 1.5 mM of SA showed the highest increase in fruit production (Figure 2). The findings of this research were discussed in a review article by Karlidag *et al*. (2009a). It was posited that when strawberry plants were treated with SA on their branches and leaves, the resulting yield increased. These findings were supported by observed results. Furthermore, Senaratna *et al*. (2000) demonstrated that tomato and bean plants exposed to conditions of heat, cold, and drought stress showed improved resistance when treated with salicylic acid. In fact, it was found that when wheat plants were subjected to drought stress and also received a foliar application of SA, their growth was significantly enhanced (Shakirova *et al*., 2003; Iqbal and Ashraf, 2006). Over the years, there

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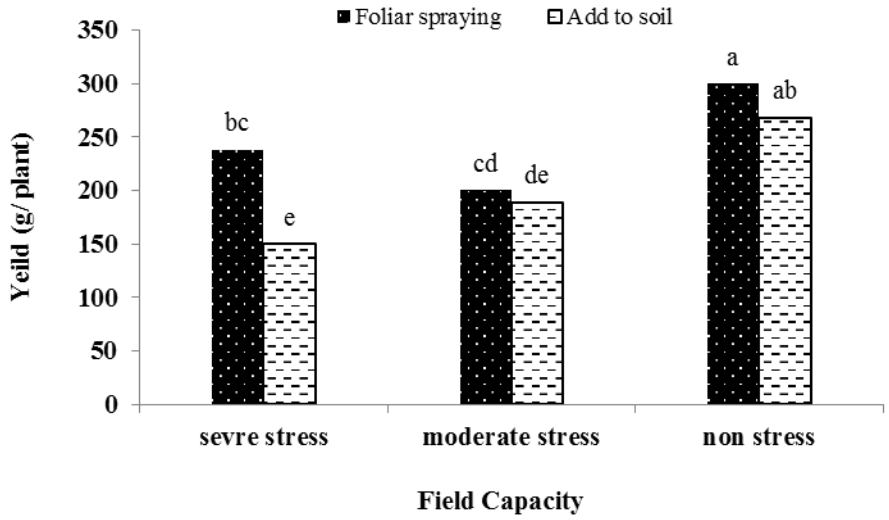


Figure 2. The interaction effect of drought stress and method of application on strawberry plant yield. Means with at least one comMon letter based on the least significant difference test have no significant difference.

have been several studies conducted to explore the effects of foliar application of salicylic acid on yield and its components in crops like maize and wheat. These studies have shown that this method can considerably amplify the yield and its components in these plants. For instance, research conducted by Shehata *et al*. (2001) and Abdel-Wahed *et al*. (2006) on corn showed that foliar application of salicylic acid had a significant impact on yield. Similar results were observed in maize and wheat plants through experiments conducted by Shakirova *et al*. (2003) and Iqbal and Ashraf, (2006). Thus, it can be inferred that the foliar application of salicylic acid has proven to be an effective agricultural practice to enhance crop yield.

Over the years, there have been several studies conducted to explore the effects of foliar application of salicylic acid on the yield and its components in crops like maize and wheat (Shakirova *et al*., 2003; Iqbal and Ashraf, 2006) and corn (Shehata *et al.,* 2001). These studies have shown that this method can considerably amplify the yield and its components in these plants. For instance, research conducted by Shehata *et al*. (2001) and Abdel-Wahed *et al*. (2006) on corn showed that foliar application of salicylic acid had a significant impact on yield. Similar results were observed in maize and wheat plants through experiments conducted by Shakirova *et al*. (2003) and Iqbal and Ashraf, (2006). Thus, it can be inferred that the foliar application of salicylic acid has proven to be an effective agricultural practice to enhance crop yield. In the realm of plant growth, drought stress can be a problematic force to contend with. It has been noted by researchers Mozafari *et al*. (2019), Zhang *et al*. (2019), and Mustafa *et al*. (2021) that exposure to drought can result in oxidative damage within plants, which can be assessed through the level of lipid peroxidation observed in various plant species, including wheat, the comMend reed, and strawberry plants. There have been experiments conducted to aid in mitigating these negative effects that drought stress introduces. For instance, the use of salicylic acid has been observed to have a beneficial impact on the growth of strawberry plants. In particular, Jamali and Neville (2011) found that salicylic acid-treated strawberry plants exhibited an 11% increase in chlorophyll content compared to untreated plants. This improvement in overall health was reflected through an increase in fruit yield as well, as evidenced by Raskin, (1992).

According to table 4, it was found that the use of SA for soil application had a significant impact on the level of POD amount. At a 1.5 mM SA treatment during severe stress, the highest POD content recorded was 4.06. However, severe drought treatment significantly increased CAT and POD (Table 4), activities and proline (Figure 3) compared to the non-stress condition. In contrast, foliar applications with SA at rates of 1 and 1.5 ppm increased the activity of CAT, POD, and Proline, both under moderate and severe drought stress conditions (Table 4 and Figure 3). However, severe drought treatment significantly increased CAT and POD (Table 4) activities and proline (Figure 3) compared to the non-stress condition. In contrast, foliar and soil applications with SA at rates of 1 and 1.5 ppm increased the activity of CAT, POD and proline in comparison with SA (0). Results showed that both drought stress levels (severe stress and moderate stress) caused a marked increase in the CAT, POD and proline parameters of strawberry plants compared with the non-stress condition. Under the non-stress condition, SA foliar and soil applications at rates of 1 and 1.5 ppm significantly increased the vitamin C parameter of strawberry plants in comparison with severe stress and moderate stress was happened (Table 4). Foliar application with a high SA rate (1.5 ppm) under non-stress (Control) showed a higher Chlorophyll (SPAD) amount in comparison with the low level and the severe and moderate stress (Table 4). Severe drought stress significantly reduced the Chlorophyll (SPAD) amount

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Table 4. The mean square investigated the quality characteristics of strawberries

Treatments	POD (IU.mg protein^{-1})	CAT (IU.mg protein^{-1})	Chlorophyll (SPAD)	Vitamin C (mg.100 g FW^{-1})
Foliar spraying× (0)SA×severe stress	3.2 ^{d}	2.13 ^{e}	26.33 ^{lm}	25.06 ^{h}
Add to soil× (0)SA× severe stress	3.1 ^{e}	2 ^{f}	26.13 ^{m}	24.83 ^{i}
Foliar spraying× (1)SA× severe stress	2.96 ^{f}	2.23 ^{d}	26.7 ^{k}	27.03 ^{c}
Add to soil× (1)SA× severe stress	3.06 ^{e}	2.1 ^{e}	26.5 ^{kl}	26.36 ^{d}
Foliar spraying× (1.5)SA× severe stress	4.06 ^{a}	2.83 ^{a}	29.9 ^{g}	27.63 ^{a}
Add to soil× (1.5)SA× severe stress	3.86 ^{b}	2.63 ^{b}	29.5 ^{h}	27.46 ^{b}
Foliar spraying× (0)SA× moderate stress	2.2 ^{k}	1.33 ^{g}	28.2 ^{i}	23.23 ^{l}
Add to soil× (0)SA× moderate stress	2 ^{m}	1.2 ^{i}	27.8 ^{j}	23.03 ^{m}
Foliar spraying× (1)SA× moderate stress	2.86 ^{g}	1.36 ^{g}	30.5 ^{f}	25.13 ^{h}
Add to soil× (1)SA× moderate stress	2.7 ^{h}	1.26 ^{h}	29.9 ^{g}	24.73 ^{f}
Foliar spraying× (1.5)SA×moderate stress	3.3 ^{c}	2.33 ^{c}	30.7 ^{ef}	25.43 ^{g}
Add to soil× (1.5)SA× moderate stress	3.1 ^{e}	2.1^{e}	30.56 ^{f}	25.13 ^{h}
Foliar spraying× (0)SA× non stress	2.1 ^{l}	0.83 ^{m}	31.03 ^{d}	22 ^{o}
Add to soil× (0)SA× non stress	2 ^{m}	0.8 ^{m}	30.85 ^{de}	22.26 ^{n}
Foliar spraying× (1)SA× non stress	2.33 ^{j}	1.03 ^{k}	32.46 ^{c}	24.23 ^{j}
Add to soil× (1)SA× non stress	2.2 ^{k}	0.9 ^{l}	32.3 ^{c}	24.03 ^{k}
Foliar spraying× (1.5)SA× non stress	2.4 ^{i}	1.2 i	35.06 ^{a}	26.1^{e}
Add to soil× (1.5)SA× non stress	2.3 ^{j}	1.1 j	34.66 ^{b}	25.73 ^{f}

**** Means significant at 1% probability levels,* Means significant at 5% probability levels, n.s No means**

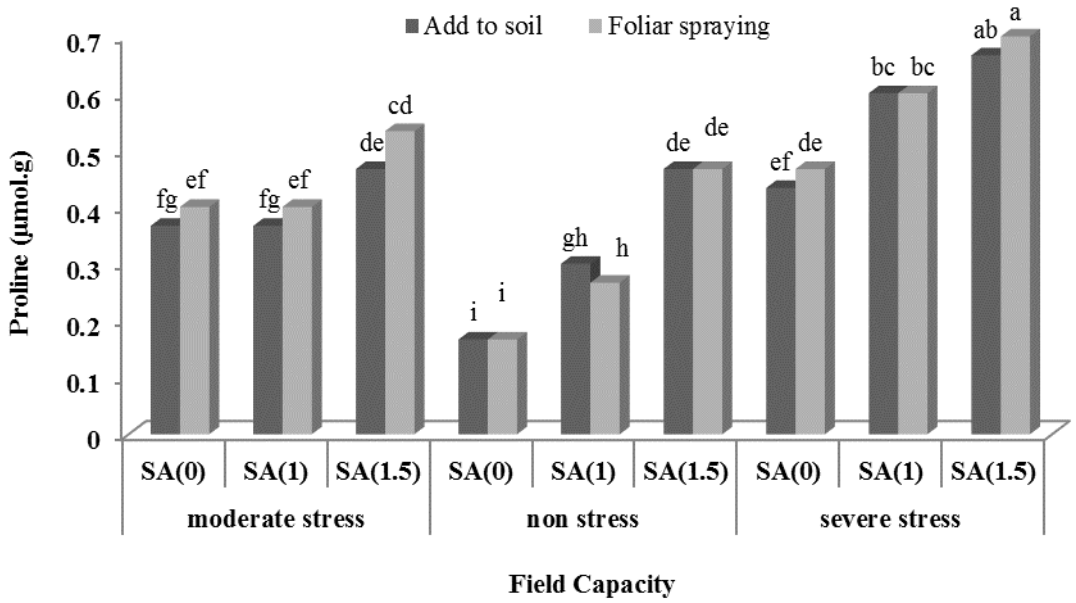


Figure 3. The interaction effect of drought stress and method of application on strawberry proline amount. Means with at least one comMon letter based on the least significant difference test have no significant difference.

(Table 4). According to findings of study Meighani and Roozkhosh (2023) in pomegranate (*Punica granatum*) indicate that the application of melatonin resulted in a substantial reduction in weight loss, the suppression of polyphenol oxidase and peroxidase activities, and a delay in the loss of total anthocyanin and phenolic content, These effects ultimately contributed to an increase in total antioxidant activity (Meighani and Roozkhosh, 2023).

In our results, chlorophyll content decreased due to drought stress, which is in harmony with a previous study (Ghaderi and Siosemardeh, 2011). However, the proline amount was higher in severe stress and moderate stress than in the non-stress condition (Figure 3). Foliar and soil application with SA improved under severe stress and moderate stress (Figure 3). While significant decreases were observed in the proline amount in treatment non-stress and SA (0) compared to severe stress and moderate stress (Figure 3). The improvement in plant growth could be due to the role of TR in enhancing the photosynthesis process by enhancing chlorophyll synthesis and increasing the number and size of chloroplasts (Alharbi *et al*., 2021; Borowski *et al*., 2000). This result is in agreement with previous works on some crops such as mung bean (Rady *et al*., 2020), sunflower (Aziz *et al*., 2013) and *Foeniculum*

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vulgare mill. (Ghanbari-Odivi *et al*., 2013). Results indicated that combining carboxymethyl cellulose (CMC) and putrescine (PUT) could effectively preserve the integrity of strawberries during cold storage (Meighani and Roozkhosh, 2024).

Conclusion

It has been observed that the utilization of salicylic acid has led to an enhancement in the plant's water status, membrane durability, acidity, CAT, POD enzymes, proline content, vitamin C and overall average yield of strawberry fruits. Drought stress increased the activities of CAT and POD enzymes, as well as proline content. Furthermore, the detrimental impacts of drought stress on the plant have been alleviated to a great extent, owing to the application of salicylic acid. In the study, it was revealed that various levels of stress did have an impact on the plant yield as well as the number of fruits that grew on each plant. Additionally, the weight of each fruit was also found to be influenced by these stress levels. As the amount of drought stress increased, there was a noticeable decrease observed in both the yield and its different components. Furthermore, the research also demonstrated that the water relative content level, rate of electrolyte leakage, fruit acidity, and average fruit weight were all significantly affected by a combination of stress factors. These included the amount of drought stress experienced, the concentration of salicylic acid present, and the method of consumption used. The use of SA was effective in minimizing the damage to the strawberry plant. It has been found through experimental research that the optimal yield of strawberry fruit is achieved when treated with 1.5 mM of salicylic acid in non-stress conditions. This treatment produced an impressive 315.20 g of fruit per plant. However, under more challenging conditions of severe drought stress, the same treatment with salicylic acid produced a slightly lower yield of 201.80 g per plant. Interestingly, this result was still better than other treatments that did not involve the use of salicylic acid. Further research showed that by increasing the concentration of salicylic acid, the yield of strawberry fruit also increased. Overall, it was concluded that foliar application of salicylic acid produced superior results, with a yield of 246 g per plant in comparison to other treatments. The study found that the application of salicylic acid through foliar spraying with a concentration level of 1.5 mM can mitigate the negative effects of drought stress and consequently increase crop yield. Therefore, it is suggested that SA at this particular concentration level could be an effective measure to boost strawberry production in drought conditions. The results indicate that the soil application of SA, when compared to the foliar spraying method, is less effective in enhancing yield. These findings imply that employing foliar spraying techniques using salicylic acid could be a promising strategy to optimize strawberry growth and production while improving resilience to unfavorable environmental factors such as drought stress. The empirical data derived from the conducted experiment indicates that when exposed to drought stress conditions in greenhouse cultivation, strawberry yield may be enhanced as a consequence of incorporating SA at a concentration level of 1.5 mM.

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