

## Morphological Responses of Pea (*Pisum sativum* L. Var. Kashi Nandni) to Exogenous Application of Salicylic Acid under Water Deficit Stress condition

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**ABSTRACT:** Sustainable agriculture is greatly threatened by the abiotic stress such as drought. The experiment was conducted with a split plot arrangement and replicated thrice. Water stress condition signifies irrigated and water deficit stress conditions. Water stress conditions were assigned to main plots and five doses of SA (0, 0.25, 0.50, 0.75 and 1 mM) were designed to sub plots. Results state that the foliar application of SA (especially 0.5 mM) was effectively improved all morphological parameters under both irrigated and water deficit stress conditions except Leaf area index in which superior result was obtained with 0.75 mM SA. Analysis of variance showed that the effect of salicylic acid on all characteristic was significant except on no. of leaves. Interaction effect was also found significant in the case of total dry weight and LAI. Water deficit stress is one of the crucial factors which create challenges to plant growth and development by altering in its physiological pathways. SA is the modern tool to conquer this emerging issue by creating tolerance mechanism in crop species. The objective of this study was to determine the impact of SA in ameliorating the ill effect of water deficit stress condition in pea.

**Keywords:** Salicylic acid, Pea, Water deficit Stress, Morphological, Growth analytical

### INTRODUCTION

Pea (*Pisum sativum* L.) also known as garden pea, field pea, spring pea, English pea, common pea and green pea belongs to family leguminaceae and is a popular legume vegetable crop for local consumption and exportation. Pea considered as the richest source of protein. This plant is diploid in nature (2n=14) (Hancock, 2004). The primary center of origin for pea is Ethiopia, with a secondary center of diversity is in the Near East (Vavilov, 1949). In general, pea is the source of high yield and maximum profits, especially in export when cultivated for green pods. Therefore, it gains more importance among other legumes. Optimum stage for harvesting is tender pod, over maturity is not considered for consumption purpose (Pavek, 2012).

Soil moisture is a key indicator of crop production and productivity, particularly in arid and semi-arid areas where plants are mostly subjected to water stress situations (Panozzo and Eagles 1999). Drought stress injury could be triggered by a mixture of physiological factors (Jiang and Huang 2001). Drought stress, on the other hand, causes a variety of morphological, physiological, biochemical, and molecular responses in

plants to cope up against water stress. In order to activate defence mechanisms, plants must perceive the stress condition and signal (Park *et al.*, 2007). Multiple biomolecules, such as SA, have been proposed as signal transducers or messengers (Misra and Saxena 2009).

SA is a phytohormone that influences the growth and development of plants (Horvath *et al.*, 2007).

Exogenous administration of SA improves plant resistance to biotic and abiotic stress via multiple mechanisms (Stevens *et al.*, 2006; Horvath *et al.*, 2007; Shi and Zhu 2008; Hayat *et al.*, 2010). However, the effectiveness of SA in stress alleviation completely rely on the type of crop, variety and experiment conditions such as the doses of the SA applied, type of stress and the level and durability of stress (Hayat *et al.*, 2010). Several studies shows that exogenously applied SA revealed remarkable enhancement in the morphological, physiological and growth analytical parameters under water deficit stress (Prabha and Negi 2014). Therefore SA could be used as a potential growth regulator, for improving drought tolerance under abiotic stress conditions (Soni *et al.*, 2021). In actuality, the objective of this study was to see if exogenously administered SA

could mitigate the negative effects of water deficit stress on plant growth and its function.

## MATERIAL AND METHOD

This experiment was conducted during the year of 2018-19 at Horticulture complex, Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). With three replications the experiment was set up in a split plot design. Water stress condition *i.e.* water deficit stress condition and irrigated condition was allotted in main plot and five doses of SA (0.00 mM, 0.25 mM, 0.50 mM, 0.75 mM and 1.00 mM) was allotted as subplot. SA was initially dissolved in absolute ethanol and then added drop wise to the water (ethanol/water, 1/1000, v/v) (Stevens *et al.*, 2006). Seeds were treated with *Trichoderma* and then were sown by hand on 1st week of November 2019. Here water stress condition *i.e.* water deficit stress and irrigated (no water stress) condition. Experimental field were irrigated immediately just after the sowing, and subsequent irrigations were given to the irrigated plots. In the case of irrigated condition frequent irrigation has given within 10 days interval. Moreover, in water deficit stress condition only single irrigation immediately after sowing was given, thereafter no subsequent irrigation was provided. Flowering and pod formation periods are the critical stages in pea and in water deficit stress condition during this critical period stress has been created with the help of tensiometer. Water stress during flowering and subsequent pod-filling stage severely limits yield. Five plants have been taken from each plot for sampling. Plant height were measured as the height of the main stem from the ground level to the apical bud (leaf apex) was measured with the meter scale at 40, 60 and at final harvest. The number of primary branches per plant was counted on the 5 tagged plants in each plot at final harvest, and then mean was determined for each treatment. Total number of leaves was counted from the tagged plants from each plot at 10 days after 1<sup>st</sup> spray and 10 days after 2<sup>nd</sup> spray. Total leaf weights were calculated by taking whole leaves from the individual plant from each plot at 40, 60 and at harvest. Total leaf weight was

recorded separately with the help of weighing balance. Dry weight was recorded after the weight of leaves became stable in 7-10 days. LAI was measured with the help of leaf area meter at 40, 60 and at final harvest. LAI is the leaf area (X) or assimilatory surface area over a certain ground area (Y) and is calculated by the formula given as under Watson (1952):

$$LAI = \frac{X}{Y}$$

X = Leaf Area

Y = Ground Area

Leaf Area was calculated using Leaf Area Meter. Following the analysis of variance technique (Panse and Sukhatme 1967) the averaged data were analysed statistically. Least Significant Difference (LSD) test at 5% level of probability was applied to compare differences among treatments` means and their interactions. Statistical analysis was performed using OPSTAT statistical software;  $p < 0.05$  and  $p < 0.01$  were considered statistically significant and highly significant, respectively.

## RESULT AND DISCUSSION

Morphological parameters like plant height, number of branches per plant, total number of leaves, leaf fresh weight and total dry weight were investigated. In the present study there is a significant difference in all the parameters after the application of SA. Results are non-significant in the case of number of leaves either in water stress condition or level of SA (Table 3). Interaction effect between water stress condition and salicylic acid was also found significant in total dry weight of leaf at 60 DAS and harvest along with Leaf area index at harvest (Table 3).

At 60 DAS and at harvest there was significant increase in plant height after the application of salicylic acid as compared to control condition in water deficit stress condition (Table 1). While at 40 DAS result was non-significant, highly significant result obtained with an application of SA at the concentration of 0.50 mM followed by 0.75 mM SA in both irrigated as well as water deficit stress condition.

**Table 1: Means of plant height (cm), number of branches and no. of leaves of pea as influenced by salicylic acid in water deficit stress and irrigated condition at different interval.**

SA concentration	Plant height (cm)						Number of branches		Number of leaves			
	40 DAS		60 DAS		At harvest		At harvest		10 days after 1 <sup>st</sup> spray		10 days after 2 <sup>nd</sup> spray	
	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated
0.00 mM	28.48	33.46	38.80 <sup>c</sup>	40.20 <sup>d</sup>	45.47 <sup>d</sup>	48.68 <sup>c</sup>	1.26 <sup>c</sup>	1.38 <sup>c</sup>	54.20	59.73	74.93	79.73
0.25 mM	34.70	36.16	41.00 <sup>bc</sup>	46.20 <sup>bc</sup>	49.73 <sup>bc</sup>	53.93 <sup>ab</sup>	1.73 <sup>bc</sup>	1.80 <sup>bc</sup>	56.53	61.66	75.13	82.60
0.50 mM	36.40	39.60	50.00 <sup>a</sup>	55.40 <sup>a</sup>	55.66 <sup>a</sup>	58.13 <sup>a</sup>	2.26 <sup>a</sup>	2.40 <sup>a</sup>	63.53	64.86	79.33	87.60
0.75 mM	35.93	37.86	46.13 <sup>ab</sup>	49.40 <sup>b</sup>	52.21 <sup>b</sup>	54.78 <sup>ab</sup>	1.77 <sup>b</sup>	1.90 <sup>b</sup>	62.93	62.13	77.53	85.13
1.00 mM	34.25	34.20	41.66 <sup>bc</sup>	43.27 <sup>cd</sup>	48.17 <sup>cd</sup>	50.53 <sup>bc</sup>	1.32 <sup>bc</sup>	1.41 <sup>c</sup>	57.33	53.93	75.00	68.60

Different letters in each column indicate significant difference at  $p < 0.05$ . Any two means not sharing a common letter differ significantly from each other at 5% probability.

**Table 2: Means of leaf fresh weight (g), leaf dry weight (g) and leaf Area Index of pea as influenced by salicylic acid in water deficit stress and irrigated condition at 40, 60, and at harvest.**

Leaf Fresh weight						Total Dry weight						Leaf Area Index						
SA concentration	40 DAS		60 DAS		At harvest		40 DAS		60 DAS		At harvest		40 DAS		60 DAS		At harvest	
	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated
0.00 mM	3.68	5.45	5.09 <sup>c</sup>	6.21 <sup>b</sup>	8.87 <sup>c</sup>	11.13 <sup>b</sup>	0.84	1.03	1.12 <sup>d</sup>	1.07 <sup>d</sup>	1.20 <sup>c</sup>	1.37 <sup>d</sup>	0.76	1.26	0.77 <sup>b</sup>	0.84 <sup>c</sup>	1.15 <sup>c</sup>	1.44 <sup>c</sup>
0.25 mM	4.10	5.66	6.19 <sup>b</sup>	6.96 <sup>ab</sup>	9.55 <sup>c</sup>	12.26 <sup>ab</sup>	1.05	1.03	1.26 <sup>c</sup>	1.19 <sup>c</sup>	1.49 <sup>b</sup>	1.76 <sup>b</sup>	0.82	1.07	1.23 <sup>ab</sup>	1.40 <sup>b</sup>	1.42 <sup>d</sup>	1.55 <sup>d</sup>
0.50 mM	4.87	5.92	6.99 <sup>a</sup>	8.30 <sup>a</sup>	11.08 <sup>a</sup>	12.92 <sup>a</sup>	1.31	1.46	1.44 <sup>a</sup>	1.74 <sup>a</sup>	1.70 <sup>a</sup>	1.86 <sup>a</sup>	0.97	1.64	1.50 <sup>a</sup>	1.16 <sup>c</sup>	1.99 <sup>b</sup>	1.99 <sup>b</sup>
0.75 mM	4.78	5.55	6.26 <sup>b</sup>	5.33 <sup>ab</sup>	10.62 <sup>ab</sup>	11.62 <sup>b</sup>	1.23	1.36	1.36 <sup>b</sup>	1.63 <sup>b</sup>	1.64 <sup>a</sup>	1.83 <sup>a</sup>	1.53	1.13	1.75 <sup>a</sup>	1.85 <sup>a</sup>	2.15 <sup>a</sup>	2.26 <sup>a</sup>
1.00 mM	4.67	5.41	5.94 <sup>b</sup>	6.58 <sup>ab</sup>	9.66 <sup>bc</sup>	11.20 <sup>b</sup>	0.70	1.17	1.24 <sup>c</sup>	1.33 <sup>c</sup>	1.54 <sup>b</sup>	1.51 <sup>c</sup>	1.02	1.08	0.88 <sup>b</sup>	0.96 <sup>d</sup>	1.62 <sup>c</sup>	1.63 <sup>c</sup>

Different letters in each column indicate significant difference at  $p < 0.05$ . Any two means not sharing a common letter differ significantly from each other at 5% probability.

**Table 3: Analysis of variance for the influences of exogenous application of salicylic acid on morphological parameters and growth analytical parameters of pea under water deficit stress and irrigated condition.**

Source of Variation	df	Mean sum of squares														
		Plant height at 40 DAS	Plant height at 60 DAS	Plant height at harvest	Total No. of branches per plant	No. of leaves at 10 days after 1 <sup>st</sup> spray	No. of leaves at 10 days after 2 <sup>nd</sup> spray	Leaf fresh weight At 40 DAS	Leaf fresh weight At 60 DAS	Leaf fresh weight At harvest	Total dry weight at 40 DAS	Total dry weight at 60 DAS	Total dry weight at harvest	LAI at 40 DAS	LAI at 60 DAS	LAI at harvest
Replication	2	69.4	16.48	2.75	0.0085	38.68	286.78	2.07	0.0035	0.052	0.031	0.002	0.0045	0.004	0.029	0.0015
Water stress condition (W)	1	39.91	85.45	65.78	0.08	18.26	141.64	10.46*	1.87**	33.61**	0.25	0.09*	0.17*	0.35*	0.002	0.08**
Error(W)	2	45.03	22.53	18.50	0.09	34.12	64.04	0.17	0.01	0.040	0.03	0.002	0.006	0.01	0.07	0.00
Level of salicylic acid (S)	4	44.31	157.99**	85.70**	1.00**	78.96	118.58	0.58	2.45**	2.73**	0.26	0.22**	0.23**	0.19	0.92**	0.82**
Interaction (W X S)	4	5.38	5.42	0.88	0.001	22.03	56.78	0.32	0.05	0.08	0.04	0.05**	0.01**	0.25	0.06	0.02**
Error(S)	16	51.84	7.46	4.17	0.06	41.14	80.21	0.54	0.04	0.08	0.10	0.001	0.001	0.11	0.14	0.002

\* and \*\*: significant at  $p < 0.05$  and  $p < 0.01$ , respectively.

The maximum number of branches plant<sup>-1</sup> was recorded with a spray of salicylic acid at 0.50 mM followed by 0.75mM SA in irrigated along with water deficit stress condition (Table 1). All the concentrations of the SA were significant in relation with each other. Number of leaves was observed non-significant as depicted in (Table 1). It shows that foliar application of salicylic acid with different concentration in pea plants had no effect on number of leaves (Table 1).

These results were in agreement with those obtained by El-Saadony *et al.* (2017) who sprayed pea plants with salicylic acid at 100 ppm and recorded maximum plant height. Hegazi and El-Shraiy (2007) showed similar results, demonstrating that foliar treatment of salicylic acid at 10<sup>-2</sup> M has a beneficial influence on vegetative growth parameters such as plant height, which is enhanced by SA application as compared to control. This result is also similar to Prabha and Negi (2014) who presented that salicylic acid was responsible for the significant increase in the morphological characters like plant height (cm), number of branches per plant in capsicum. On the other hand the concentration of 0.50 mM, was shown to be more efficient than the other dosages of SA used. Hesami *et al.* (2012) also studied that plant height of coriander crop can be improved by suitable soil water content and SA application in low concentrations. The reduction in plant height and number of branches as a result of water deficit stress condition could be due to decrease in cell expansion, more leaves senescence and low turgor pressure under dry condition. The stomatal conductance gets reduced under situation of drought stress. As a result, CO<sub>2</sub> fixation drops and photosynthetic rate declines. This reduction in growth parameters may be due to the reduction of water flow from the xylem to the different cells, which regulates cell division, elongation, development as well as the decline in chlorophyll content and lipid peroxidation in the cell membrane. The pivotal role of SA on growth characters may be due to enhanced activity of antioxidant enzyme which protects the plants from the oxidative damage, reducing ROS (reactive oxygen species) levels and lipid peroxidation as well as ion leakage (Nazar *et al.*, 2011). SA at 0.50 mM on pea plants significantly increased leaf fresh weight and total dry weight. As depicted in Table 2 there was significant increase in leaf fresh weight, total dry weight of leaves and leaf area index after the application of salicylic acid at 60 DAS and at harvest in water stress condition. Application of salicylic acid at 0.50 mM followed by 0.75 mM was showed remarkable enhancement in total fresh and dry weight of leaves (Table 2). Leaf expansion is extremely influenced by water status of plants, especially in sensitive plants like pea where water plays a vital role in leaves development of plant (Jelonek *et al.*, 2009). In addition, there was direct correlation between leaf weight and leaf area (Liu and Stutzel 2004) therefore decrease in leaf area was main reason of decline in leaf dry weight and fresh weight. Mardani *et al.* (2012) showed that foliar application of SA enhanced leaf fresh and dry weight under high level of water deficit stress.

Significant increase in leaf area index was observed after the application of salicylic acid at 0.75mM both irrigated and water deficit stress condition (Table 2). In, irrigated condition plant shows more LAI compared with water deficit stress condition and is associated with the lowest rate of reduction in stomatal conductance in irrigated condition. Increased cell osmotic pressure due to accumulation of soluble sugars and other osmotically active substances such as proline possibly explain why SA increases LAI in water-stressed plants. Drought resistance is usually increased by the accumulation of several osmotic molecules, such as soluble carbohydrates and proline, which are required for the osmotic adjustment mechanism (Idrees *et al.*, 2010). Foliar application of SA had significant effect on LAI and the result of the present study is in propinquity with that reported by Afsari *et al.* (2013) in cowpea, Razmi *et al.* (2017) in soybean. Abdelaal (2015) showed that the exogenous application of SA works tremendously on leaf area (cm<sup>2</sup>) per plant of faba bean under drought stress.

## CONCLUSION

Drought stress causes severe physiological and biochemical malfunctions in plants, which leads to a drastic reduction in plant performance. Environmental stress, such as water stress, adversely influences the morphological along with growth analytical parameters of pea. Plants alone would not be able to alleviate the negative consequences of water stress in this circumstance therefore; there is a need of exogenous support to pea plant to cope drought stress which is facilitated by the application of SA to carry out various processes which enhance the physiological and biochemical phenomenon of plant body. Salicylic acid is a phytohormone that helps plants copes with abiotic and biotic stresses. Recent study state that water deficit stress reduced plant height, no. of branches, leaf fresh weight, leaf dry weight and leaf area index of pea. SA application improved all measured traits not only under irrigated but also under water deficit stressed plants. The effect of 0.50 mM SA was more effective and considerable in all the parameters. In the case of Leaf area index 0.75mM SA was effective. The present investigation suggests that application of SA may help in decrease the adverse effects of drought in pea. Also it was observed that exogenous application of SA and its derivatives against abiotic stress could have a substantial practical application in agriculture and horticulture.

## FUTURE SCOPE

1. Salicylic Acid does not only stimulate physio-morphological changes in the plants but also immunize the plant through 'systemic acquired resistance' (SAR) to withstand several disease infection, as a result the overall production cost can be reduced by eliminating fungicide costs. Thus, more intensive study needs to conduct.

2. Salicylic acid is not much expensive chemical but farmers need to be trained for how much concentration along with how to apply this chemical in field.

**Conflict of Interest.** There is no conflict of interest.

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