Assessment of Salicylic Acid Impacts on Physiological and Biochemical Characteristics under Water Deficit Stress on Pea (*Pisum sativum* L. var. Kashi Nandni)

ABSTRACT

Aims: A study was conducted to investigate the effect of salicylic acid (SA) on garden pea grown under water deficit stress condition to discover the efficacy of foliar application of SA on physiological and biochemical characteristics of garden pea (*Pisum sativum* L.) cv. Kashi Nandni.

Study design: With three replications, the experiment was performed in a split plot design.

Place and Duration of Study: Experiment was carried out in 2019-20 at Vegetable Research Centre, JNKVV, Jabalpur (M.P.), India.

Methodology: Main plots were assigned to irrigated and water deficit stress conditions, and subplots were associated to five dosages of SA (0, 0.25, 0.50, 0.75, and 1 mM). During flowering stage, relative water content (RWC) of leaves, light transmission ratio (LTR), energy interception (EI) were recorded and total soluble solids (TSS) was detected at the time of harvest.

Results: Finding of the research revealed that water deficit stress condition reduces the growth potential of plant via effecting the physiological and biochemical process of plant. The best drought protection was observed when the plant sprayed with 0.50 mM SA in RWC, light transmission %, energy interception and TSS content of garden pea. The analysis of variance shows that the effect of salicylic acid on all characteristics was significant.

Conclusion: According to the findings of this study, water deficit stress causes severe physiological and biochemical malfunctions in plants, resulting in a significant reduction in plant performance. Exogenous application of SA and its derivatives against abiotic stress has the potential to be very useful in agriculture and horticulture.

Keywords: Salicylic acid, water deficit stress, pea, physiological, biochemical

INTRODUCTION

Amongst pulse crops, Pea (*Pisum sativum* L.) is a prominent pulse crop common name as well '*Matar'*. It belongs to the family Fabaceae. It is the important food legume of the world. Pea is native of Ethiopia and is widely grown in temperate countries. India is a densely populated country. The population being increased without check and it is the main handicap in our progress, with the results of that food shortage, malnutrition and poverty occurs. To get over from these problems adoption of intensive cultivation of vegetable crops should be the priority. Several sorts of vegetables are grown in India. Out of them vegetable pea is one of the foremost important leguminous vegetables, having much more protein than other vegetables. Pea is extremely nutritive, containing high percentage of digestible protein together with carbohydrates.

"As the intensity of agriculture and the demand for farmable land increases, abiotic stresses are becoming more frequent. Drought is one of the most important limiting factors of crop production all over the world as compared to other abiotic stress. It inhibits the process of photosynthesis by checking growth and induces stomatal closure" (Nemeth *et al*., 2002). Drought, as an abiotic stress, diminishes food production worldwide. Drought stress during the flowering and pod-filling period has negative effects on morphological, physiological and biochemical activities and ultimately leads to low yield (Coste *et al*., 2001). Numerous plants have optimised their abiotic stress tolerance through physiological, morphological, biochemical, and microbiological changes (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). Plants cope with water deficit stress by generating signalling molecules which thus activate a diverse range of signal transduction pathways.

"Salicylic acid, a stress-related phenolic phytohormone, acts as a signal for induction of specific plant responses to biotic and abiotic stresses" (Lu, 2009). SA is an endogenous signalling molecule that is involved in a wide range of physiological processes, such as growth regulation, photosynthesis, stomatal actions, nutrient absorption, and mechanisms of abiotic stress tolerance (Hayat *et al*., 2010). "Exogenous administration of SA improves plant resistance to water deficit stress and promotes economic yield of the crops *via* triggering morphological response" (Soni *et al*., 2022). "SA is known to play an important role in modulating redox balance through membranes, thus counteracting the negative effects of reactive oxygen intermediates induced by oxidative stress" (Yang *et al*., 2004) by "promoting the production of antioxidant enzymes such as superoxide dismutase" (Singh and Usha 2003). It has been reported that exogenous application of SA is shown to be an effective approach for alleviating the negative impacts of water deficit stress or alleviating drought stress in pea plants (Soni *et al*., 2021). The objectives of the present work were to standardize the optimum concentration of salicylic acid, under different water stress condition.

MATERIALS AND METHODS

The studies were executed at Vegetable Research Centre, Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India during the year 2019-20 with ten treatments. The soil of the experimental field was medium black with good drainage, uniform texture and medium NPK status. The experiment was performed in split plot design with three replications. Water stress factors, such as water deficit stress and irrigated conditions, were allocated to the main plot, while five doses of SA (0.00 mM, 0.25 mM, 0.50 mM, 0.75 mM, and 1.00 mM) were assigned to the subplot. SA was initially dissolved in 100% ethanol, and then water was added dropwise. (ethanol/water, 1/1000, v/v) (Stevens *et al*., 2006). To enhance the crop produce recommended package of practices was followed.

Seeds were treated with Trichoderma and then were sown by hand in 2.5 cm depth of soil. At the same time plots were fertilized with NPK. Spacing has been maintained with 30 and 10 cm respectively. 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 day interval. Water deficiency stress conditions treated with a single irrigation immediately after planting. Thereafter no subsequent irrigation was provided. Exogenous application of SA was scheduled at 10 days before flowering and 10 days after flowering. Flowering and pod formation are critical stages in pea, and in water deficit stress conditions, stress was measured using a tensiometer during this critical period. Water stress during flowering and subsequent pod-filling stage severely limits yield. Crop management practices such as hand weeding and application of pendimethalin 0.5 kg ha^{-1} as pre-emergence spray has done as required.

Water stress condition signifies i.e. water deficit stress and irrigated (no water stress) condition. At the flowering stage relative water content of leaves, light transmission (%), energy interception was measured and total soluble solids were measured at the time of harvest. To determine the RWC of leaves; ten sample leaves were obtained from each plant. The fresh weight (FW) of the collected sample leaves was calculated. The identical sample leaves were then determined for saturated weight of leaves (SW). The sample leaves were dipped within the distilled water contained by petri dishes placed in dim light. After four hours, the load of the sample leaves was immediately determined on an electrical balance, and also the results were noted. The sample leaves were then dried in oven to measure the dry weight (DW) of leaves. The labelled paper bags containing the sample leaves were placed within the oven for quarter-hour at 105 °C and later for 72 hours at 70 °C temperature. The relative water content (RWC) of a plant, as described by Slatyer (1967), is a useful indicator of its water balance since it reflects exactly how much water the plant needs to achieve artificial maximum saturation. Relative water content can be calculated by using this formula:

$$RWC = \frac{\text{fresh weight of leaves} - \text{Dry weight of leaves}}{\text{saturated weight of leaves} - \text{Dry weight of leaves}} \times 100$$

“LTR and EI were measured on sunny days between 11:00 and 12:00 hours on the youngest fully expanded leaves of 5 tagged plants per plot by using a portable lux meter. It is the ratio of light intensities at the base of the canopy crown to total incoming radiation and was calculated as per formula given” by Golingai and Mabbayad (1969).

$$\text{LTR (\%)} = \frac{I}{I_0} \times 100 \text{ Where,}$$

I = Light intensity at the base of the plant and I_0 = Total incoming solar radiation

“EI was calculated by recorded data, the total incident light at the canopy crown and transmitted light within the crop were converted into average incident and transmitted energy on the basis of values reported” by Gastra (1963). The efficiency of crop canopy for solar energy interception (EI) can be calculated by using this formula:

$$\text{Energy Interception} = \text{Total Incident Energy} - \text{Transmitted Energy}$$

TSS in the seed was recorded at room temperature using digital refractometer and were expressed in term of ^{0}Brix. Five seeds were taken from each pod from each plot for taking the average value. A small amount of seeds juice was taken in prism of refractometer and the value was recorded. The effect of different treatments was studied and data recorded on physiological and biophysical parameters along with biochemical parameters on five randomly selected plants. The mean data were subjected to statistical analysis following analysis of variance technique (Panse and Sukhatme (1967). The differences between the means of the various treatments and their interactions were compared using the Least Significant Difference (LSD) test at the 5% level of probability. OPSTAT statistical software was used for the statistical analysis, and $p < 0.05$ and $p < 0.01$ were regarded as statistically significant and highly significant, respectively.

RESULTS AND DISCUSSION

Analysis of variance showed that the effect of Salicylic acid on RWC, LTR, EI and TSS content of pea was significant (Table 1). All the parameters of plants treated with SA (0.25 mM, 0.50 mM, 0.75 mM, and 1.00 mM) indicate a substantial increase. RWC was recorded significant with the level of salicylic acid as well as water stress condition, in which irrigated condition showed the maximum RWC with the application of salicylic acid at 0.50 mM and the minimum RWC was recorded in control (0.00 mM) in water deficit stress condition (Table 2). Interaction was found significant in the case of RWC (Table 1). Significant difference in relative water content in leaves was recorded due to different concentration of salicylic acid which is significantly differing from each other in both the water deficit stress as well as irrigated condition (Fig.1 & 2). SA mitigates transpiration rate through stomata closure which lets the plant to accumulate more water in leaves, hence increasing RWC of leaves. Water absorption by the roots and water loss by transpiration are often linked to RWC. Drought stress causes a decrease in RWC in a wide range of plants. According to Nayyar and Gupta (2006), drought stress caused a significant decrease in leaf water potential, relative water content, and transpiration rate, as well as an increase in leaf temperature. The improvement in RWC caused by exogenous SA application could be due to osmotic adjustment caused by the accumulation of compatible solutes such as proline. Because RWC was positively correlated with proline concentration, SA-treated plants showed a slower decrease in RWC during drought stress. Sadeghipour and Aghaei (2012) observed water stress conditions reduced relative water content of common bean but exogenous application of SA (0.50 mM) improved relative water content under both Irrigated and water stress conditions. Hayat *et al*. (2008) reported exogenous application of SA on *Lycopersicum esculentum* L. protected plants against the stress generated by water and significantly improved RWC as compared to control plants. Salicylic acid treated plants exhibited a slower decrease in RWC during drought stress. Razmi *et al*. (2017) explained that water deficit stress decreased relative water content but application of salicylic acid gave better performance to get rid of water deficit stress and increased the RWC.

Table 1. Analysis of variance for the influences of exogenous application of salicylic acid on physiological and biochemical parameters of pea under water deficit stress and irrigated condition

Source of Variation	df	TSS content (^{0}Brix)	RWC (%)	Light transmission ratio (%)	Energy interception (Cal cm^{-2} min^{-1})
Replication	2	0.192	0.802	11.86	0.0045
Water stress condition (W)	1	0.86	409.40**	124.56	0.02
Error(W)	2	0.07	3.97	8.99	0.004
Level of salicylic acid (S)	4	6.64**	140.90**	181.77**	0.02**
Interaction (W X S)	4	0.04	10.27*	20.59	0.001
Error(S)	16	0.11	2.27	13.59	0.004

*** and **: significant at p≤0.05 and p≤0.01, respectively.**

Similar results were observed by Afshari *et al*. (2013). LTR and EI declined in water deficit stress condition as compared to irrigated condition (Table 2). There was significant increase in light transmission (%) and energy interception with SA at 0.50 mM in irrigated and water deficit stress condition as compared to control (Fig 1 & 2). Analysis of variance presents the data that LTR and EI significantly increases in relation to application of salicylic acid (Table 1). Several stresses may directly or indirectly affect the photosynthetic processes, exogenous application of SA has been shown to reduce the deleterious effect of numerous stress conditions, and this protection may be shown in increased photosynthetic capacity in relation to the electron transport chain and PS II quantum yield. (Hovrath *et al*., 2007). Amin *et al*., 2007 also reported that foliar spray of SA caused significant increase in photosynthetic pigments content per leaves of onion.

Effect of salicylic acid in irrigated condition indicated best result followed by water deficit stress condition. Interactions were found non-significant (Table 1). Best result has obtained with the application of 0.50 mM in irrigated condition in all the mentioned characteristics (Fig 2). Analysis of variance showed that the influences of salicylic acid on TSS content were significant (Table 1). Maximum TSS content obtained in irrigated condition with the application of 0.50 mM SA and the minimum TSS was recorded in water deficit stress condition (Table 2).

Best results were obtained in irrigated condition with the application of SA 0.05 mM as compared to water deficit stress condition (Fig 1 and 2). Carbohydrates are considered as the main organic solutes in plants, influenced by drought condition. TSS plays a vital role as compatible solute under drought stress. Siringam *et al*. (2012) reported "in rice that the increase in TSS, decline the water potential, cell turgidity and osmotic adjustment by increasing the storage reserve for the normal functioning of plant cell under water stress condition". The finding are in conformity to that of Al-Desuquy *et al*. (2012), El Tayeb *et al*. (2010) in wheat and Zeid *et al*. (2014) in ajwain (*Trachyspermumammi*). Total soluble solids was analysed and results confirmed that the foliar application of SA with a concentration of 0.50 mM is useful for increasing TSS. In both irrigated and water deficit stress conditions, the treatment 0.50 mM SA followed by 0.75 mM SA delivered the best results and was significantly superior to other treatments. (Fig 1 & 2).

Table 2 .Means of Physiological and biochemical parameters (Relative water content, Light Transmission (%), Energy Interception, TSS content) of pea influenced by salicylic acid in water deficit stress and irrigated condition.

SA	RWC (%)	LTR (%)	EI	TSS (^{0}Brix)

concentration					(Cal cm^{-2} min^{-1})			
	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated	Water deficit stress	Irrigated
0.00 mM	45.21^{c}	50.65^{d}	64.47^{c}	66.59^{b}	0.27^{c}	0.33^{b}	9.31^{d}	9.55^{d}
0.25 mM	49.35^{b}	54.98^{c}	69.98^{abc}	71.18^{b}	0.32^{bc}	0.37^{ab}	10.88^{bc}	11.44^{b}
0.50 mM	54.54^{a}	65.97^{a}	75.85^{a}	84.29^{a}	0.41^{a}	0.47^{a}	12.11^{a}	12.44^{a}
0.75 mM	50.35^{b}	59.01^{b}	70.81^{ab}	78.56^{a}	0.36^{ab}	0.45^{a}	11.18^{b}	11.64^{b}
1.00 mM	46.64^{c}	52.42^{cd}	69.34^{bc}	70.21^{b}	0.31^{bc}	0.32^{b}	10.55^{c}	10.66^{c}

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

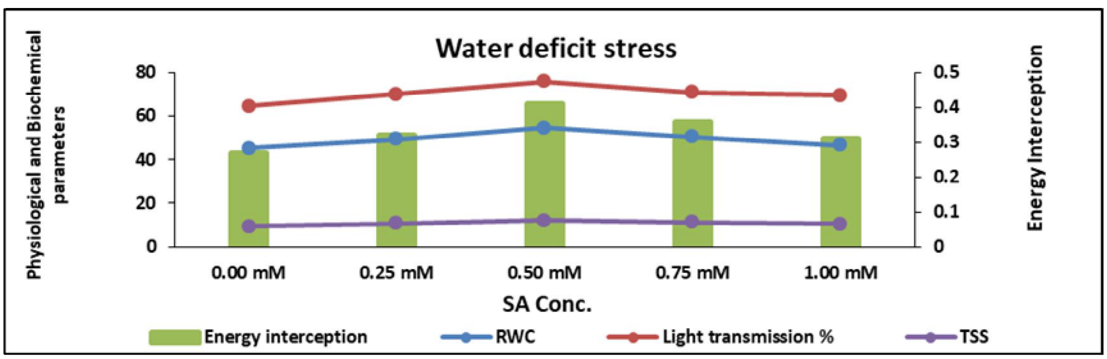

Fig. 1.The mean values of physiological and biochemical parameters of pea under water deficit stress condition

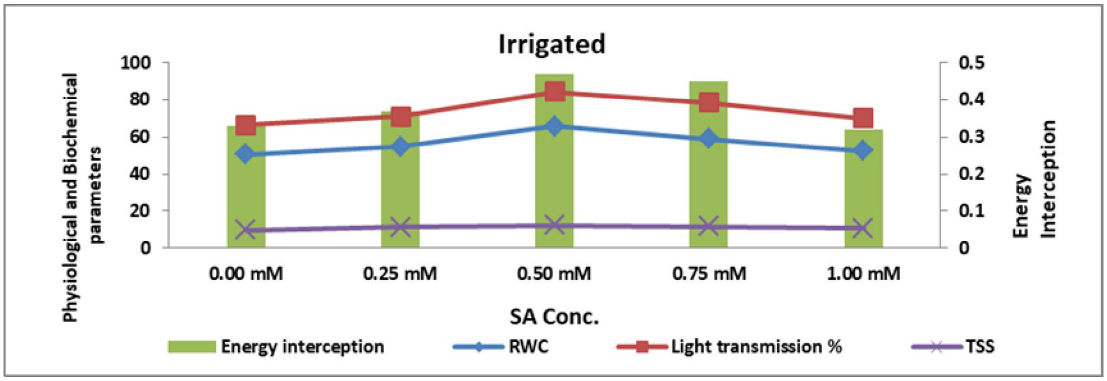

Fig. 2.The mean values of physiological and biochemical parameters of pea under Irrigated condition

CONCLUSION

The physiological and biochemical aspects of pea are adversely affected by environmental stress, such as water deficit stress. Plants by themselves would not be able to mitigate the negative effects in this situation, so the pea plant requires exogenous support to cope with drought stress. This is made possible by the foliar application of SA to carry out various processes that improve the physiological and biochemical phenomenon of plant body. Plant performance under water scarcity stress situations causes a drop in yield attributes, however exogenous administration of SA not only reduces the negative effects of stress while facilitating high crop yields. We found that water stress reduced RWC, LTR, EI and TSS 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. The present investigation suggests that application of SA may help in ameliorate the adverse effects of drought in pea. Salicylic acid is currently receiving special consideration as it is a crucial signal molecule for the development of various plant stress resistance mechanisms. Although salicylic acid has primarily been discussed in relation to biotic and abiotic stresses, numerous studies have demonstrated that salicylates play a significant role in modulating how plants react to a variety of abiotic stresses, including UV light, drought, salinity, chilling stress, and heat shock. It has become evident that SA can be used as an appropriate alternative to PGR chemicals for mitigate water deficit stress of agro-horticultural crops to assure high economic return and food safety concern.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscripts.

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