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SALICYLIC ACID APPLICATION IMPROVES GROWTH AND ALLEVIATES THE ADVERSE EFFECTS OF HEAT STRESS IN PEA (*PISUM SATIVUM* L.)

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Abstract

High temperature is a major factor limiting plant growth and productivity. Salicylic acid (SA) is an important signal molecule that mitigates the adverse effects of heat stress on plants A field experiments entitled “salicylic acid application improves growth and alleviates the adverse effect of heat stress in pea (*Pisum sativum* L.)” was carried out at The University of Agriculture Peshawar (Amir Mohammad Khan Campus Mardan) in summer 2017. Analysis showed that highest percentage of seedling emergence (73.68 ± SD %), plant height (71.50 ± SD cm), leaves plant^{-1} (202.31 ± SD), pods plant^{-1} (37.60 ± SD), seeds pod^{-1} (7.08 ± SD), 100 seed weight (34.32 ± SD g), yield (5569.48 ± SD kg ha^{-1}), chlorophyll content (50.13 ± SD SPAD), protein content (20.94 ± SD % FW) except total soluble solids (15.27 ± SD ^{0}Brix) were observed in plants which received having both primed seeds and foliar applied SA at 100 ppm. Findings regarding salicylic acid levels revealed that maximum seedling emergence percentage (73.90 %), plant height (71.80 ± SD cm), leaves plant^{-1} (193.76 ± SD), pods plant^{-1} (38.70 ± SD), seeds pod^{-1} (7.58 ± SD), 100 seed weight (37.78 ± SD g), yield (6268.61 ± SD kg ha^{-1}), chlorophyll content (52.03 ± SD SPAD), protein content (21.52 ± SD % FW) except total soluble solids (14.73 ± SD ^{0}Brix) were recorded in plants sprayed with 100 ppm salicylic acid. Interaction between modes of application and 100 ppm salicylic acid was also found significant in most of the studied parameters. So it is concluded that seed priming and again as a foliar application of salicylic acid at 100 ppm improved the physiology of pea and alleviates the heat toxicity in pea plants.

Key words: Heat stress, Salicylic acid, Modes of application, Pea, Growth, Yield, Quality.

Introduction

Heat stress (HS) is a major abiotic stress which limits the plant growth. When the temperature changes and becomes hot enough then it negatively affects the plants functions and ultimately the growth and development. HS affect the process of photosynthesis therefore plants not taking enough nutrition for their growth and development (Song *et al*., 2014). HS also increases the leaf drops from plants, effect flower formation and the production of seeds. Extreme HS cause the plants to wilt (Xu *et al*., 2021).

Pea (*Pisum sativum* L.) is one of the significant vegetable which is cultivated in many countries of the world as well as in different areas of Pakistan. Pea is an important agricultural crop due to its high nutritional benefits as it contains a good amount of useful amino acids, proteins, carbohydrates and various useful vitamins required for human health. It is also packed with some important minerals required for proper growth like iron, zinc and phosphorus. It is a cheaper source of protein as compared to meat and poultry. It is used for treating zinc deficiency in most developing countries because it is cheap (El-Hak *et al*., 2012, Ibrahim & Ramadan, 2015). Pea is very valued by nutritionists all around the world because of its high protein content and is considered a vital source of protein for much of the human population (Hussein *et al*., 2006). Pea is packed with enhanced levels of the amino acids, lysine and tryptophan, two of the nine so called essential amino acids for humans that must be acquired from the diet. These micronutrients are low in cereal grains as compared to pea seeds. Pea contains 24 and 86 percent of protein and total digestible nutrients. These characters made pea an excellent choice not only for humans but also for livestock feed. Pea results in a greater percentage of ingested protein to be broken down to amino acids and oligopeptides capbable of being absorbed into the bloodstream because it contains 5 to 20 percent less trypsin inhibitors than soybean (Kent & Endres, 2003).

Vegetative and reproductive growth of several crops are inhibited with elevated temperature stress (Peet & Willits, 1998; Hussain *et al*., 2006; Singh *et al*., 2007). Crop yield may decrease up to 17% during the growth season when there is increase of 1ºC in the average temperature (Lobell & Asner, 2003). Foliar application or pre-sowing seed treatment with low concentrations of growth hormones can induce thermotolerance in crops (Wahid *et al*., 2007). Yield of horticultural crops can be increased with use of plant growth regulators (Emongor, 1997).

Salicylic acid (SA) has been reported to increase tolerance to several abiotic stresses including heat stress. In micro plants of potato, thermotolerance was induced with the use of salicylic acid (Gutierrez *et al*., 1998). Senaratna *et al.* (2003) noted that when seeds were imbibed in aqueous solutions of salicylic acid (0.1-0.5 mM) resulted in 100% plant survival percentage and tolerance against several stresses The present research work was planned to use salicylic acid for induction of heat tolerance in pea plants under high summer temperature. This research is regionally important as pea constitutes an important but underutilized horticultural crop in the Peshawar region of Pakistan due to the high temperatures experienced in this area during periods when a pea could be cultivated.

Materials and Methods

The experiment entitled “salicylic acid application improves growth and alleviates the adverse effect of heat stress in pea (*Pisum sativum* L.)” was conducted at Amir Mohammad Khan (AMK) Campus Mardan, The University of Agriculture Peshawar, during 2017.

Experimental design: The study was laid out using a Randomized Complete Block Design (RCBD) with split plot arrangement having three replications. In this experiment have two factors such as modes of application and salicylic acid levels were tested. Modes of application were kept in main plots and salicylic acid levels in sub plots. Modes of application consisted of seed treatment, seed plus foliar treatment and foliar treatment while the salicylic acid levels consisted of the following concentrations 0, 50, 100 and 150 ppm. For the seed treatment the pea seeds were soaked in solutions of salicylic acid (50, 100 and 150 ppm) dissolved in water. Soaking time was 6 hours. While for the foliar treatment, each solution of salicylic acid was sprayed after 25 days of sowing the pea seed. The plants of the control group were sprayed 25 days after sowing with plain water. All foliar applications were made early in the morning (From 9:00 AM to 10:00 AM) for better absorption and long lasting effect. A hand operated sprayer was used to spray distil water for control plants or the salicylic acid solution uniformly on each experimental unit.

Experimental site: The study site is located at an altitude of 335 m above sea level. Mardan climate is subtropical with hot summer and cool winter getting <300 mm annual rainfall. The maximum temperature in summer reaches 48°C, while the minimum temperature in the winter season falls to 2°C. Data on rainfall and temperature were recorded at the research station situated near the Amir Mohammad Khan Campus Mardan and presented in the (Table 1).

Agronomic treatments: Distance between rows and plants were 50 cm and 10 cm, respectively. Climax variety of pea was used in this research. Normally in Pakistan pea are sown in the month of mid-October to mid-November. In this experiment pea seeds were sown in summer on 1st April, 2017. A total of twelve treatments were replicated three times. The soil was ploughed 1 month before planting and applied with well-rotten farm yard manure at 25 t ha^{-1}. Basal dose of phosphorus and potassium at 50 kg•ha^{-1} was applied at the time of sowing, while nitrogen was applied at 25 kg ha^{-1} in the form of urea (CH_4N_2O) having two split doses (half at the time of sowing and the other half one month after sowing. Weed emergence was controlled manually. All cultural practices were performed upon on the requirements of the crop.

Weather data of Mardan (Experimental site): (Fig. 1) shows maximum and minimum temperature of the experimental site during the time period of the whole experiment.

Treatments: Factor A was modes of application having three levels M_1 = Seed Treatment (ST), M_2 = Seed + Foliar Treatment (SFT) and M_3 = Foliar Treatment (FT) which were applied to the main plots, while factor B was Salicylic acid levels (ppm) having four levels S_1 = 0, S_2 = 50, S_3 = 100 and S_4 = 150 which were applied to the subplots.

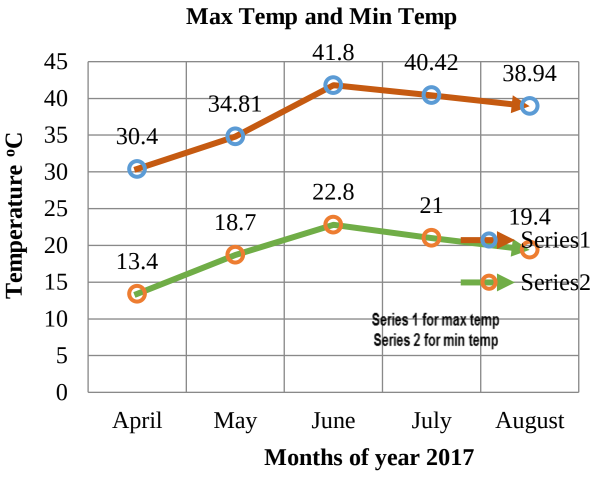

Fig. 1. Mean monthly maximum and minimum temperature of experimental cite Mardan during growing season 2017.

Salicylic acid solution preparation

Molecular weight: 138.121g/mol

For 50 ppm of salicylic acid, 50 mg salicylic acid powder was dissolved in 1000 ml of distil water. Similarly, for 100 ppm and 150 ppm of salicylic acid solutions, 100 mg and 150 mg salicylic acid powder was dissolved in 1000 ml of distil water respectively.

Parameter studied

Data was recorded on;

Measurement of pea growth and yield

Seedling emergence percentage: Seedling emergence percentage was calculated after the germination of the seeds with the below procedure seedling emergence.

$$\% = \frac{\text{Number of seedling emerged}}{\text{Number of seed sown}} \times 100$$

Plant height (cm): It was measured using measuring tape. Five plants height was from ground level to the plant part furthest from the ground in each treatment measured which were selected randomly, and then the average height was computed from these five measurements.

Number of leaves, seeds and pods per plant: Five plants leaves were counted in each treatment which were selected randomly and then their average was computed.

Fifty pods were selected in each treatment randomly and the seeds they contained were counted and then average seeds per pod were computed.

It was counted in five plants selected randomly in each treatment and then average was computed.

100 seed weight (g): Fifty randomly selected pods were selected three time and the number of seeds counted and weighed. This number was then divided by the total number of seeds and multiplied by 100 to produce the 100 seed weight. This number was then averaged among the three different estimates to determine the 100 seed weight.

Yield (kg ha^{-1}): Seed yield•plot^{-1} was measured and the seed yield•hectare^{-1} was calculated using the following method:

$$\text{Yield per hectare} = \frac{\text{Plot yield (kg)}}{\text{Plot area (m}^2\text{)}} \times 1000\ (\text{m}^2)$$

Measurement of pea qualitative parameters

Chlorophyll content (SPAD): C*hlorophyll* meter was used to calculate chlorophyll content for 5 randomly selected leaves in each treatment and then the average chlorophyll content was determined.

Protein content (%): Protein content in pea fruit samples were determined by adding 20% (W/V) tricholoroacetic acid and then were dissolved in 1% (w/v) sodium hydroxide. Method of Lowry *et al*., (1951) were adopted for protein determination.

Total soluble solids (°brix): The Total Soluble Solids (TSS) in pea seeds were calculated by placing a drop of extruded "juice" from a seed on the prism of a refractometer and recording the observations through the eyepiece of the refractometer reporting the units in "°Brix". One-degree Brix is the sugar content of an aqueous solution equivalent to 1 gram of sucrose in 100 grams of solution. It can also provide an estimate of TSS.

Statistical Analysis

The data recorded were analyzed statistically to find difference between treatments and their interactions with Statistical software STATISTIX 8.1 for calculating analysis of variance and least significance difference (Steel and Torrie, 1980). The statistical software 8.1 (GenStat, 2005) was used.

Results

Seedling emergence (%): According to (Table 2) seedling emergence percentages was affected by different modes of application and SA levels significantly. Their interaction was also found to be significant (Fig. 2A). Regarding modes of application, maximum seedling emergence percentage (73.68 ± SD %) was recorded for seed plus foliar SA treatment, while minimum seedling emergence percentage (51.82 ± SD %) was recorded for foliar treatment. Regarding SA levels, maximum seedling emergence percentage (73.90 ± SD %) was found in plants treated with 100 ppm foliar spray, while minimum seed emergence percentage (55.44 ± SD %) was observed in control plants (sprayed with only water). The interaction between modes of application and SA levels showed that maximum seedling emergence percentage (84.60 ± SD %) was found in plants which received 100 ppm salicylic acid in seed plus foliar treatment, while minimum seedling emergence percentage (54.66 ± SD %) was noted in control plants.

Table 1. Weather data of Mardan (Experimental site) Pakistan during the growing season of 2017. Temperature values are the monthly average plus or minus the standard deviation. Precipitation is the cumulative monthly precipitation.

Year	Month	Temperature (°C)		Cumulative monthly precipitation (mm)
		Mean monthly maximum	Mean monthly minimum	
2017	April	30.40 ± S.D	13.40 ± S.D	9.40 ± S.D
	May	34.81 ± S.D	18.70 ± S.D	4.50 ± S.D
	June	41.80 ± S.D	22.80 ± S.D	25.60 ± S.D
	July	40.42 ± S.D	21.00 ± S.D	15.62 ± S.D
	August	38.94 ± S.D	19.40 ± S.D	22.40 ± S.D
	Average	**37.67 ± S.D**	**21.06 ± S.D**	**Total 77.52 ± S.D**

Table 2. Changes in morphological attributes of peas as affected by different modes of application and salicylic acid levels.

Modes of application	Seedling emergence (%)	Plant height (cm)	Number of leaves•plant^{-1}	Number of pods•plant^{-1}
Seed treatment	73.59 ± SD B	61.51 ± SD B	162.17 ± SD B	29.53 ± SD B
Seed + Foliar treatment	73.68 ± SD A	71.50 ± SD A	202.31 ± SD A	37.60 ± SD A
Foliar treatment	51.82 ± SD C	53.45 ± SD C	136.83 ± SD C	22.58 ± SD C
LSD (0.01)	0.93	0.79	1.37	0.99
	Salicylic acid levels (ppm)			
0	55.44 ± SD D	45.11 ± SD D	130.00 ± SD D	18.00 ± SD D
50	69.20 ± SD B	63.30 ± SD C	180.57 ± SD B	33.31 ± SD B
100	73.90 ± SD A	71.80 ± SD A	193.76 ± SD A	38.70 ± SD A
150	66.90 ± SD C	68.40 ± SD B	164.09 ± SD C	29.60 ± SD C
LSD (0.01)	1.66	1.57	1.89	1.16
Interaction	**	**	**	**

Means followed by different letters are significantly different. Standard deviations of the mean are depicted by "±"
NS = Non-significant; * = Significant at 0.05; ** = Significant at 0.01

Interaction graphs

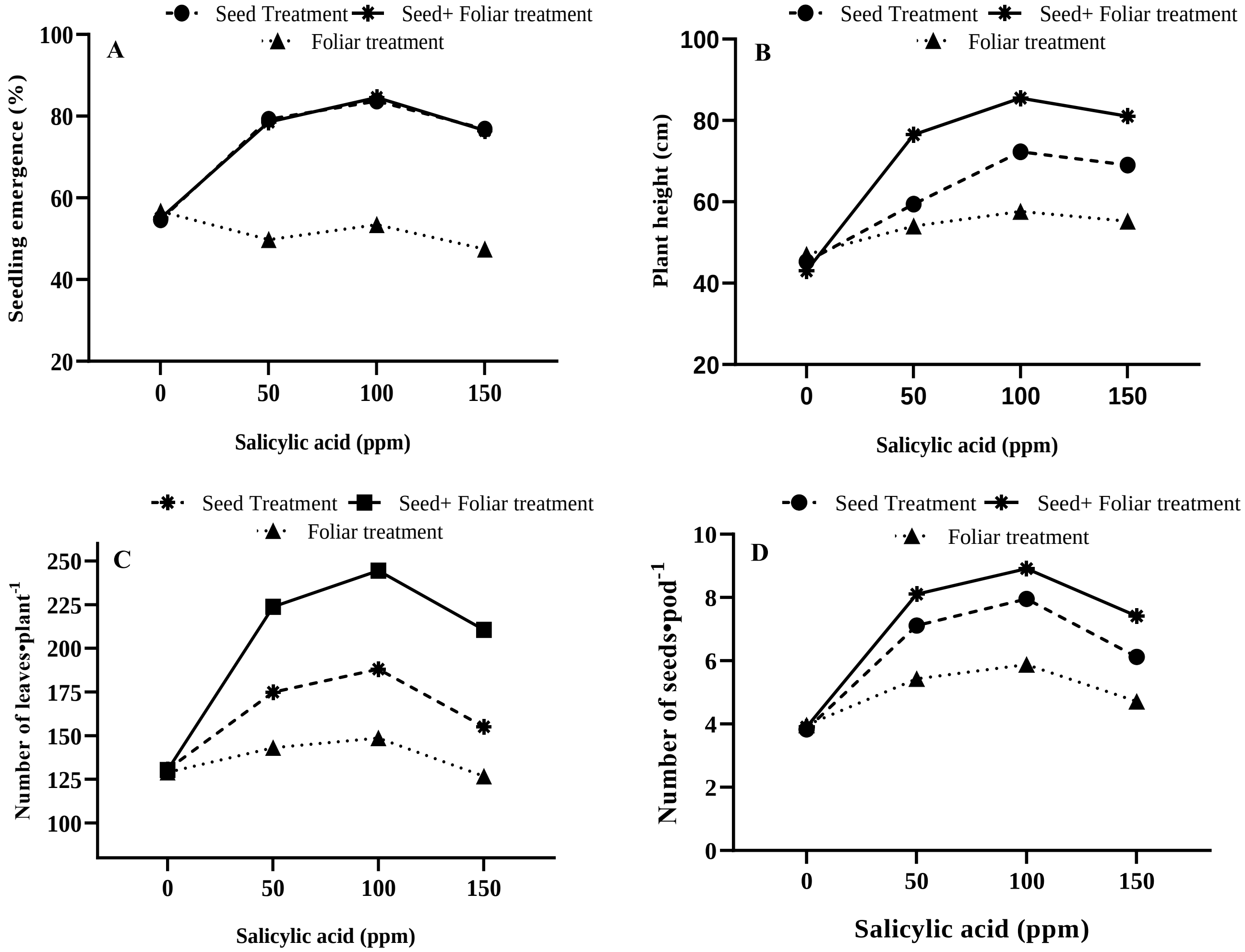

Fig. 2. Seedling emergence (%), plant height (cm), number of leaves plant^{-1} and number of seeds pod^{-1} of pea as affected by different modes of application and salicylic acid levels.

Plant height (cm): Table 2 shows mean data plus and minus the standard deviation, of plant height as affected by different modes of application and SA levels. Their interactive effect was found to be significant (Fig. 2B). Among modes of application greatest plant height (71.50 ± SD cm) was found for the combined seed plus foliar SA treatment while the shortest height (53.45 ± SD cm) was recorded for the plants which received salicylic acid only as a foliar treatment. Among the SA concentrations, the greatest plant height (71.80 cm) was noted in plants treated with 100 ppm while the lowest plant height (45.11 ± SD cm) was observed in the control. The interactive effect of modes of application and SA concentrations revealed that maximum plant height (85.50 ± SD cm) was noted in plants which received 100 ppm salicylic acid in seed plus foliar SA treatment, as compared to the plant height (43.00 ± SD cm) in plants which received 0 ppm of SA in seed plus foliar treatment.

Number of leaves plant^{-1}: Table 2 shows mean data plus and minus the standard deviation, of number of leaves plant^{-1} as affected by different modes of application and SA levels. Their interactive effect was found to be significant (Fig. 2C). Maximum number of leaves•plant^{-1} (202.31 ± SD) was noted for plants which were given seed plus foliar treatment and the minimum number of leaves•plant^{-1} (136.83 ± SD) was noted for plants which were given the foliar treatment. Concerning the levels of SA, the highest number of leaves plant^{-1} (193.76 ± SD) was noted in plants treated with 100 ppm and least number of leaves plant^{-1} (130.00 ± SD) was noted in control. The interaction of modes of application and SA concentrations showed that highest number of leaves•plant^{-1} (244.50 ± SD) was found in plants which received 100 ppm salicylic acid in seed plus foliar treatment and lowest number of leaves•plant^{-1} (129.00 ± SD) was noted in plants which received 0 ppm of SA in foliar treatment.

Number of seeds pod^{-1}: Table 3 shows data pertaining to number of seeds•pod^{-1} in response to modes of application and SA levels which were affected significantly by modes of application and SA levels and their interaction (Fig. 2D). It is apparent from the mean data that the greatest number of seeds pod^{-1} (7.08 ± SD) was recorded for seed plus foliar treatment, while the lowest number of seeds pod^{-1} (5.00 ± SD) was recorded for plants which got the foliar treatment. In case of SA concentrations, 100 ppm showed maximum number of seeds•pod^{-1} (7.58 ± SD), followed by number of seeds pod^{-1} (6.88 ± SD) in plants treated with 50 ppm while least number of seeds•pod^{-1} (3.90 ± SD) was noted in control. The interaction of modes of application and SA concentrations showed that greatest number of seeds•pod^{-1} (8.91 ± SD) was found in plants which were applied with 100 ppm SA in seed plus foliar treatment, while minimum number of seeds pod^{-1} (3.83 ± SD) was noted in plants which received 0 ppm of SA in seed treatment.

Number of pods plant^{-1}: Its data are shown in Table 3. Modes of application and SA levels significantly affected the number of pods plant^{-1}. Their interaction was also found to be significant (Fig. 3E). Greater numbers of pods•plant^{-1} ‘37.60 ± SD) were recorded for plants that had been subjected to both seed plus foliar treatments while fewer pods plant^{-1} (22.58 ± SD) was recorded for foliar treated plants. Concerning SA levels, 100 ppm concentration showed maximum number of pods plant^{-1} (38.70 ± SD), in relation to the control (18.00 ± SD). It is clear from modes of application and SA interaction that maximum numbers of pods plant^{-1} (50.40 ± SD) was found in plants which received 100 ppm SA in seed plus foliar treatments, followed by the number of pods plant^{-1} (42.90 ± SD) in plants which received 50 ppm SA in seed plus foliar treatment while minimum number of pods plant^{-1} (17.00 ± SD) was noted in plants which received 0 ppm of SA in seed treatment.

100 seed weight (g): Mean data given in (Table 3) indicated significant alterations among different modes of application and SA levels on 100 seed weight. One hundred seed weight was significantly affected by modes of application and by SA levels and their interaction (Fig. 3F). The highest 100 seed weight (34.32 ± SD g) was noted for the seed plus foliar treatment, while minimum 100 seed weight (22.43 ± SD g) was recorded for foliar treatment. Regarding SA levels more 100 seed weight (37.78 ± SD g) was found in plants treated with 100 ppm, followed by 100 seed weight (32.89 ± SD g) in plants treated with 50 ppm while less 100 seed weight (15.10 ± SD g) was observed in the control. The interaction of M x S showed that maximum 100 seed weight (46.67 ± SD g) was found in plants which received 100 ppm SA in seed plus foliar treatment while minimum 100 seed weight (14.93 ± SD g) was noted in plants which received 0 ppm of SA in seed plus foliar treatment.

Yield (kg ha^{-1}): Table 3 shows its mean data. Yield was affected significantly by different modes of application and SA levels and their interaction (Fig. 3G). Data showed that a greatest yield (5569.48 ± SD kg) was noted for the seed plus foliar treatment, while a minimum yield (3867.19 ± SD kg) was recorded for foliar treatment. Concerning SA levels, it was detected that SA applied at a rate of 100 ppm showed maximum yield (6268.61 ± SD kg), while a minimum yield (1025.11 ± SD kg) was observed in control plants. It is also obvious from the interaction of modes of application and SA levels that a rising trend in yield was observed from 100 ppm SA application in seed plus foliar treatment which was greater than the control treatment (no SA).

Chlorophyll content (SPAD): Chlorophyll content was affected significantly by different modes of application, SA levels (Table 4) and their interaction (Fig. 3H). Among modes of application, the seed plus foliar treatment revealed significantly greater chlorophyll content (50.13 ± SD SPAD) compared to the foliar treatment which produced a low chlorophyll content (35.69 ± SD SPAD). The significant variation among SA concentrations indicated that the plants treated with 100 ppm SA exhibited more chlorophyll content (52.03 ± SD SPAD) followed by 50 ppm (46.07 ± SD SPAD), while less chlorophyll content (31.00 ± SD SPAD) was measured in the control treatment. The interaction of M x S showed that the highest chlorophyll content (64.33 ± SD SPAD) was found in plants which received 100 ppm SA in seed plus foliar treatment while the lowest chlorophyll content (30.55 SPAD) was noted in plants which received 0 ppm of SA in foliar treatment.

Protein content (%): Protein content of peas was significantly affected by different modes of application, SA treatments (Table 4) and their interaction (Fig. 4I). In the matter of modes of application greater protein content (20.94 ± SD %) was observed from plants treated with both a seed and subsequent foliar treatment, while the least impact was seen from plants undergoing a foliar treatment (16.11 ± SD %). An examination of the data made it obvious that SA of 100 ppm concentration showed the greatest protein content (21.52 ± SD %), followed by 150 ppm (19.25 ± SD %), while the least protein content (14.89 ± SD %) was noted in control. The interaction between modes of application and SA concentrations showed that greatest protein content (24.90 ± SD %) was noted on 100 ppm SA concentration in seed plus foliar treatment and fewer protein content (14.83 ± SD %) was observed on 0 ppm SA concentration on seed treatment.

Total soluble solid (^{0}Brix): Total soluble solids were considerably affected by the modes of application, SA concentrations (Table 4) and their interaction (Fig. 4J). Among the treatments tested in this project, the foliar treated plants revealed the maximum total soluble solids (17.15 ± SD ^{0}Brix) while the minimum total soluble solids (15.27 ± SD ^{0}Brix) were measured for seed plus foliar treated plants. The effect of SA concentrations was also significant on total soluble solids. SA at rate of 0 ppm showed more total soluble solids (18.82 ± SD ^{0}Brix) as compared to 100 ppm SA concentration which gave less total soluble solids (14.73 ± SD ^{0}Brix). The interaction between modes of application and SA levels revealed that both modes of application and SA concentration effect were significant on total soluble solids. The greatest Brix was recorded for plants treated with 0 ppm SA in foliar treatment (19.07 ± SD ^{0}Brix) while the least Brix was seen in total soluble solids on 100 ppm of SA in seed plus foliar treatment (12.7 ± SD ^{0}Brix).

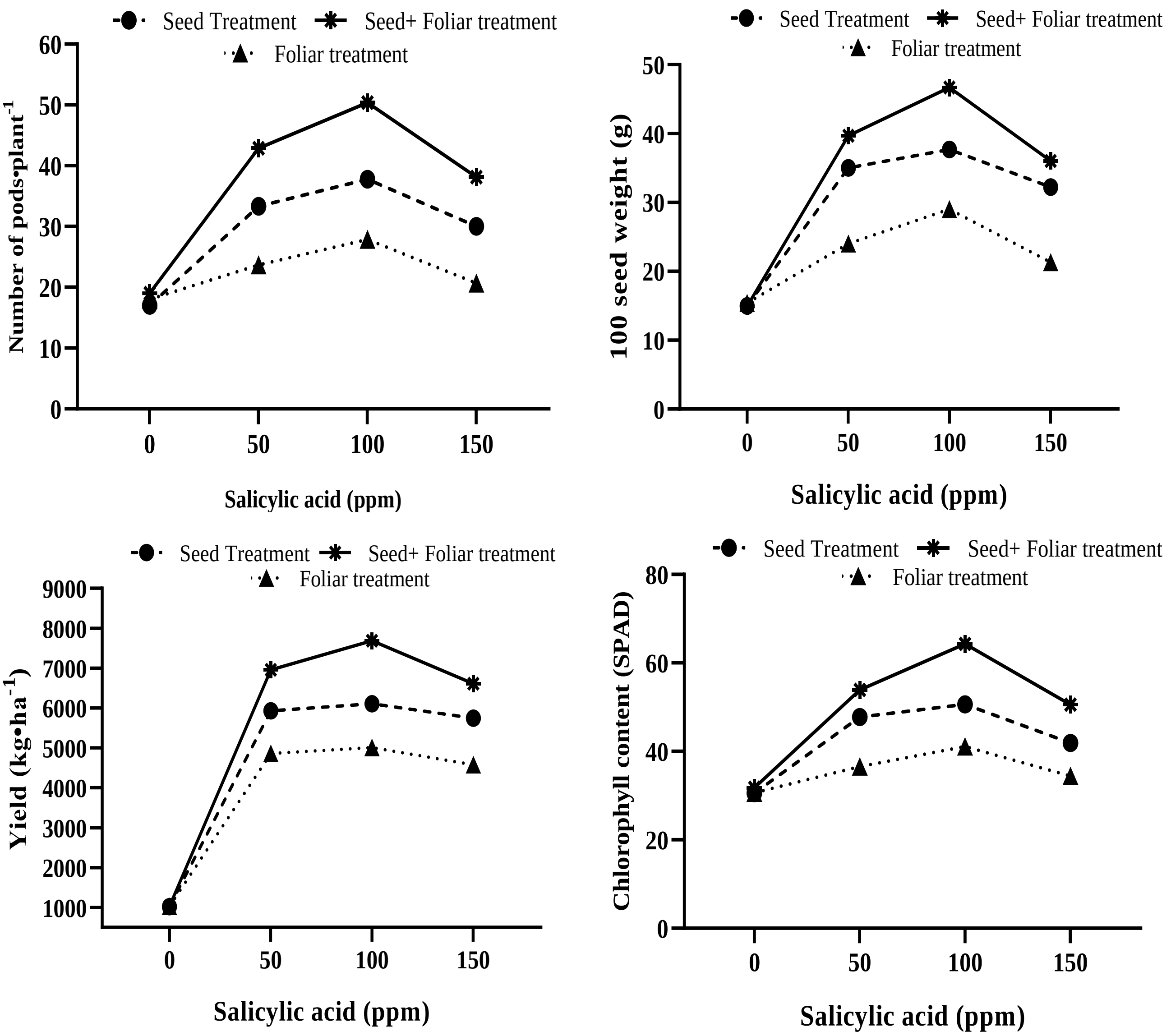

Fig. 3. Number of pods plant^{-1}, 100 seed weight (g), yield (kg ha^{-1}) and Chlorophyll content (SPAD) of pea as affected by different modes of application and salicylic acid levels.

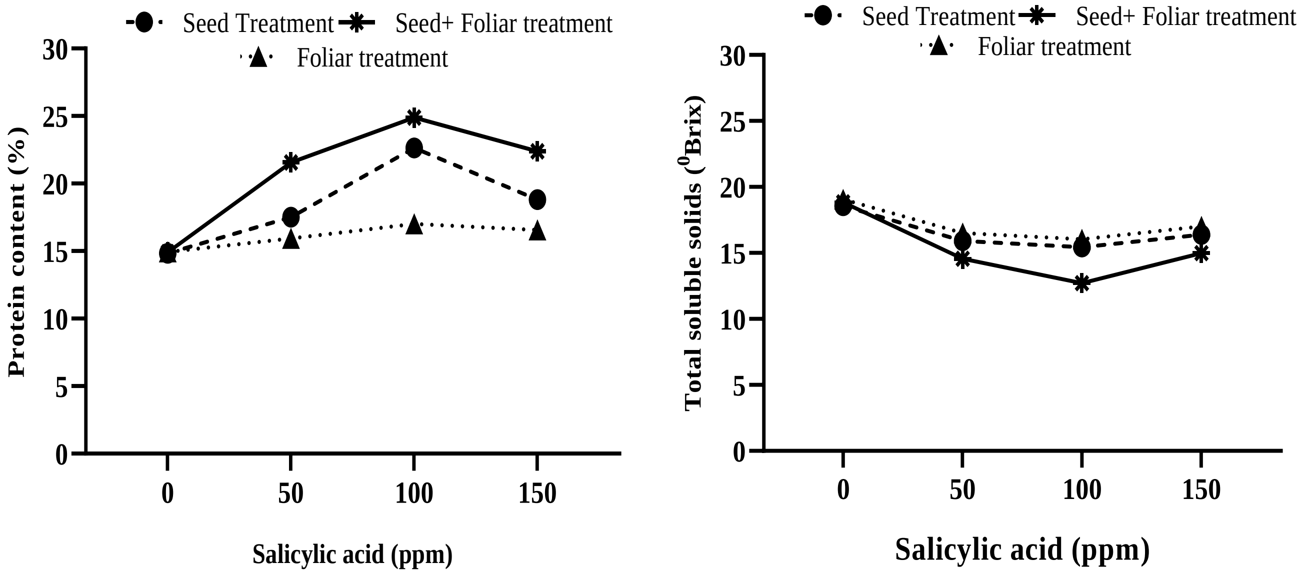

Fig. 4. Protein content (%) and total soluble solids (^{0}Brix) of pea as affected by different modes of application and salicylic acid levels.

Discussion

Morphological and yield attributes: Seed germination is regulated by two phytohormones namely abscisic acid (ABA) and gibberellic acid (GA) (Yang *et al.*, 2003). Pea seeds treated with SA before sowing had an increased percentage of germination. Also the seedling growth of the SA pre-treated seeds was superior to that of the control seeds (Table 2). Similar results were reported by (Kumar *et al.*, 1999) who, when working with soybean, found that seed application of SA improved the final percentage germination. Seed germination is affected by soil moisture content and temperature (Eraslan, 2007). Seed treatment (priming) by SA had improved the performance of pea under abiotic stresses (Chiu *et al.*, 2002). During seed treatment (priming), seeds hydrated sufficiently to commence germination associated metabolic processes. However, seeds primed in SA did considerably better than those primed with water so there must be additional beneficial effects imparted to the seed by SA than simple priming.

The increase in the height of the plants might be due to the enhanced elongation and division in the cells of the plant stem. SA is known to increase plant height by optimizing the activity of Rubisco and rate of photosynthesis (Nagasubramaniam, 2007). SA results in more uptake of nutrients (Khan *et al.*, 2003) which improves the plant growth. SA has also been shown to improve the antioxidant system of plants which increased the oxidative damage and ion leakage from membranes and this ultimately reduced the abiotic stress in the plants (Yusuf *et al.*, 2008). One study has found that the treatment of SA also enhanced the indole acetic acid and cytokinine levels which are themselves important growth promoters and they increased the cell division and cell elongation (Sakhabutdinova *et al.*, 2003). As a result, taller plants were produced compared to plants which were not given SA (Sakhabutdinova *et al.*, 2003).

SA is also responsible for the regulation of cell growth as it affects the enlargement of cells as well as by increasing cell numbers available to expand through cell division (Vanacker *et al.*, 2001). It also has a role in physiological and biochemical process of plants (Maity & Bera, 2009). The importance of SA in plant growth was described by El-Shraiy & Hegazi (2009) who applied 100 mg l^{-1} SA to pea plants and noted increased in their height. The plants which were treated with SA twice i.e seed- and foliar-treatment gained more height than that of plants which were treated only once, regardless of mode (seed soak or foliar spray), presumably due to the maximum absorption of SA by double exposure. As SA has a key role in growth and development and the plants needed it at different stages of life cycle therefore its maximum absorption by the plants produced pea plants of the greatest height.

Table 3. Changes in yield attributes of peas as affected by different modes of application and salicylic acid levels.

Modes of application	Number of seeds•pod^{-1}	100 seed weight (cm)	Yield (kg•ha^{-1})
Seed treatment	6.25 ± SD B	29.99 ± SD B	4702 ± SD B
Seed + Foliar treatment	7.08 ± SD A	34.32 ± SD A	5569 ± SD A
Foliar treatment	5.00 ± SD C	22.43 ± SD C	3867 ± SD C
LSD (0.01)	0.09	0.99	1.99
	Salicylic acid levels (ppm)		
0	3.90 ± SD D	15.10 ± SD D	1025 ± SD D
50	6.88 ± SD B	32.89 ± SD B	5913 ± SD B
100	7.58 ± SD A	37.78 ± SD A	6268 ± SD A
150	6.08 ± SD C	28.89 ± SD C	5644 ± SD C
LSD (0.01)	0.09	0.76	2.47
Interaction	**	**	**

Means followed by different letters are significantly different. Standard deviations of the mean are depicted by "±"
NS = Non-significant; * = Significant at 0.05; ** = Significant at 0.01

Table 4. Changes in quality attributes of peas as affected by different modes of application and salicylic acid levels.

Modes of application	Chlorophyll content (SPAD)	Protein content (%)	Total soluble solids (^{0}Brix)
Seed treatment	42.72 ± SD B	18.45 ± SD B	16.57 ± SD B
Seed + Foliar treatment	50.13 ± SD A	20.94 ± SD A	15.27 ± SD C
Foliar treatment	35.69 ± SD C	16.11 ± SD C	17.15 ± SD A
LSD (0.01)	0.54	0.12	0.05
	Salicylic acid levels (ppm)		
0	31.00 ± SD D	14.89 ± SD D	18.82 ± SD A
50	46.07 ± SD B	18.34 ± SD C	15.65 ± SD C
100	52.03 ± SD A	21.52 ± SD A	14.73 ± SD D
150	42.29 ± SD C	19.25 ± SD B	16.12 ± SD B
LSD (0.01)	0.50	0.27	0.13
Interaction	**	**	**

Means followed by different letters are significantly different. Standard deviations of the mean are depicted by "±"
NS = Non-significant; * = Significant at 0.05; ** = Significant at 0.01

Researchers have found that SA improved the activities of various enzymes which are responsible for the effective metabolism of nitrogen. It also improved the mobilization and transportation of nitrogen inside plants. Szepesi *et al.*, (2005) linked SA application with the absorption of CO_2 and increased uptake of mineral in plants which were under abiotic stress. Findings are alike with the work of Dat *et al.*, (1998) and Shah (2003) who stated that SA was helpful in promoting cell division and plant tolerance to various stresses. Some agriculture researchers had also proposed that application of SA activated soluble carbohydrate consumption to form new cells and this mechanism in turn activated the rapid growth (Khodary, 2004). The results are similar to the research done by Agamy *et al.*, (2013), Ratushnyak *et al.*, (2012), and Zamaninejad *et al.*, (2013) who recorded that leaf number in pea crops was increased with the application of SA. The leaf number was enhanced by the use of SA on plants of marigold (Gharib, 2006). In good agreement with these studies, in this work, the plants which received SA in seed plus foliar treatment gave the highest number of leaves.

Heat stress induced the synthesis of abscisic acid (ABA) which reduced the mobilization and translocation of carbohydrates from leaves (source) to the seed (sink). As a result, the size of the seeds remained small and the number of seed per pod remained low. High temperature decreased crop productivity by affecting pollen development (Fahad *et al.*, 2015). These results are alike with the results reported by Sujatha (2001) and Jeyakumar *et al.*, (2008) who reported an increase in the number of seeds per pod in pea crop. Application of SA enhanced different traits in comparison to plants in control and its superiority over control plants (untreated) (Gurbaksh *et al.*, 1980: Sujatha, 2001). Heat stress brought abrupt changes in the activity of various enzymes, development of the leaf and crop productivity (Wahid *et al.*, 2007). Desirable effects of using plant growth regulators on pea included higher growth, which ultimately caused an increase in the total number of seeds. These findings are in accordance with the work of the following researchers (Singh & Singh, 2011), (Shaukat *et al.*, 2012) and (Tiwari *et al.*, 2014). The plants treated with both a seed plus foliar application of salicylic gave a greater number seeds pod^{-1}.

Plant growth regulators had a key role in translocating nutrients from lower parts of the plant to the apical parts. Because of SA application, plants entered into reproduction stage earlier than its normal cycle and stimulated the production of flowers. This stimulation then in turn accelerated the synthesis of secondary metabolites (Hayat *et al.*, 2010). At the flower initiation and pod formation stage the application of SA lowered the flower drop because photosynthates are effectively transferred from the source to sink. Because of this process pods per plant have been enhanced as explained by Ganapathy *et al.*, (2008). Such outcomes were also stated by Khalil & Mandurah (1989). Such results were also reported by Kumar *et al.*, (1999) that more pods were formed in those plants treated with SA. SA application had an immense impact on the pods per plant; it is in line with the research of (Sujatha, 2001). These findings are alike with Gurbaksh *et al.*, (1980) who clarified that SA increased the number of pods in gram plants. Sujatha (2001) also reported an increased in number of pods in green gram. The use of plant growth regulators enhanced the plant growth under different abiotic stresses (Singh *et al.*, 2007; Hussain *et al.*, 2006). In the current study, seed application of SA benefited the plant enhancing growth while foliar application of SA increased the number of flowers and the number of pods^{-1}.

High temperature caused more evaporation from the plant and the plants faced water shortage which resulted in poor seed development and small sized seeds having light weight. The increase in 100 seed weight may be because of greater numbers of leaves that formed in SA treated plants. While not measured, it is possible that greater leaf numbers resulted in higher photosynthates being assimilated by the sinks that resulted in greater numbers of pods and seeds per pod. When SA was applied to mustard (*Sinapis alba*) it affected the dry matter accumulation positively and it grew taller than the plants in the control (Fariduddin *et al.*, 2003). Mustard treated in this way had excellent stomatal conductance and effective photosynthesis during heat stress, possibly influencing the amount of assimilate transformed into starch (Hayat *et al.*, 2010). Seed- and foliar-treatment by SA resulted in availability of SA on two occasions during the life cycle of plants which may have enhanced the growth and yield of treated plants compared untreated plants (Bozoglu *et al.*, 2007). Results similar to those described here were also reported by Akram (2007) who did research on broad bean (*Genus speices*). The seed yield of soybean was also enhanced with the treatment of SA (Gutierrez *et al.*, 1998). When SA was applied to soybean foliage at a rate of 100 mg per liter, it enhanced the seed yield (Devi *et al.*, 2011). Superior seed yield may be attributed to the fact that SA enhanced growth vigor of plants and increases plant growth under stressful situations like high temperature and drought conditions. Results are alike with the findings of Babar *et al.*, (2015) and Ahmad *et al.*, (2014) they noted an increased in biological yield under drought conditions with the SA application. The seed and foliar SA treatment may have provided the plant with a more regular supply of nutrients that could be utilized for reproductive growth, it may have decreased heat-induced flower drop, and promoted seed formation, leading to enhanced seed size and weight. Potentially, although this surmise remains to be tested, the seed plus foliar treatment by SA may have resulted in maximum accumulation of photosynthates in the seeds formed on the treated plants because the double treatment maximized absorption of SA.

Growth regulators like SA according to Khodary (2004) brought an increase in the production of biomass in plants which were under different environmental stresses. According to the research of Shakirova *et al.*, (2003) SA had a positive effect on growth and yield of plants because SA regulated other plant growth hormones that positively influence growth and yield. The use of SA increased the rate of photosynthetic water use efficiency which increased the yield (Fariduddin *et al*., 2003). The increase in the yield in late sowing was a positive effect of SA (Cai *et al*., 2015). It was reported by Canakci & Munzuroglo (2000) that when SA was applied to radish, it causes gains in both fresh and dry weight and hence yield attributes were enhanced. Such results have also been reported by Sirwaiya & Kushwah (2018) who found that use of plant growth regulators provided an excellent internal environment for the proper development crops. Seed plus foliar spray with SA might have increased the number of pods because of better nutrient absorption. These outcomes are alike with El-Hak (2012) who reported similar results in pea. SA application twice i.e. first the seed soaking followed by foliar spray potentially resulted in maximum absorption of SA which was positively correlated with a greater number of pods plant^{-1} and maximum yields. Assuming an increased absorption of SA in plants treated in this manner, the plants may be more thermotolerant allowing a greater survival during the harsh temperature of summer while the plants in control were damaged by the heat stress.

Quality attributes: Reactive oxygen species (ROS) are free radicals which produce peroxidation and disintegration of the photosynthetic apparatus of plants in stressful environment. SA acted as a scavenger of these damaging free radicals and neutralized them and their ability to harm plant was diminished, this enhanced the chlorophyll content which was lost due to high temperature stress. Schütz & Fangmeier (2001) reported that the degradation of chlorophyll due to abiotic stresses caused production of the ROS in the cell of the plants. The various stresses in the environment had a negative effect on the plant; it diminished the chlorophyll content and the process of photosynthesis, which led to reduced plant production and yield (Sayyari *et al*., 2013). It has also been documented that SA activated both rubisco and PEP carboxylase activity in the presence of abiotic stresses (Singh & Usha, 2003). Application of SA to plants caused an increase in chlorophyll level relative to plants which were in the control. The SA effect on chlorophyll concentration was also examined and reported by Zhao *et al*., (1995) who worked on soybean and Sinha *et al*., (1993) who did research on maize. The plants which got SA twice i.e. seed soaking followed by foliar spray gained more chlorophyll content than plants which were only treated once. This may be because of greater absorption of the SA over two treatments which might heighten the protection received by, and therefore the integrity of, the chlorophyll preventing its loss by of the harsh sunlight in summer.

SA helped in better uptake of nitrogen, its mobilization inside the plant body and then the assimilation of nitrogen. These nitrogen molecules are the building blocks of different amino acids because these amino acids combine to form proteins. So the application of SA increased the protein content of the pea seeds. SA had a key role in increasing the transport of photosynthates from source to the sink. The increase in photosynthates cause an excellent production of building material of seeds of pea. Abiotic stress produces oxidative damage and this can rupture various membranes from which ion leakage can occur. Growth regulators like SA optimized the defense mechanism of plants and improved its ability to fight various abiotic stresses (Yusuf *et al*., 2008). Also research by Sakhabutdinova *et al*., (2003) confirmed that application of SA enhanced the indole acetic acid and cytokinin levels that ultimately increased the protein content of wheat. It is not impossible that a similar mechanism is functional in pea. The SA application twice i.e. first the seed soaking followed by foliar spray probably resulted in maximum absorption of SA which may maximize protection of plants from senescence due to heat stress, of all the treatments included here, because senescence can cause degradation and deterioration of protein and such degradation may have been more prevalent in the control plants. As heat stress produced free radicals, which are known to cause the degradation of proteins, the application of SA twice, may have reduced the production of reactive oxygen species or, potentially, neutralized the free radicals produced by heat stress and this may have prevented protein degradation which, occurring to a greater extent in the control plants, may have resulted in the altered protein concentrations recorded in the current study.

SA played a key role in the process of osmoregulation which helped the plants to maintain an adequate amount of water inside body for its normal functions. It also helps maintain a balance between the water content and solid content of the seed. TSS is an amalgamated measurement of sugars and other dissolved solutes in the cell sap of fruits and vegetables (Haque *et al*., 2009). Of the treatments tested here, the highest TSS, measured in the control, suggests that heat stress caused more evaporation which may have created a water deficiency and such condition may have triggered an enhanced TSS content in seeds (Nahar & Grezmacher, 2002). Osmoregulation is an adoptive strategy of plants under such stressful condition (Ashraf, 2010). The results of Khan *et al*., (2003) are in line with the above results that the application of SA enhanced the quality parameters of plants which were facing different forms of abiotic stresses like drought, extreme temperatures and saline conditions in fields. Similarly, the total soluble solids also declined, which is one key quality indicator of vegetables, in plants which were treated with the SA (Vanacker *et al*., 2001). SA also promoted other plant hormones in a positive way which caused better quality wheat seeds that might also be a reason for a decrease in total soluble solids and improve quality attributes in pea seeds (Sakhabutdinova *et al*., 2003). The plants which were not treated twice with a SA application i.e., first the seed soaking followed by foliar spray, resulted in maximum TSS possibly because of a minimum absorption of SA. The rate of evaporation from leaves was high in control plants and the solution of their leaves was concentrated (greater TSS). Therefore, they had increased TSS contents in their cell sap and the seeds of the control plants produced as a result were therefore high in TSS.

Conclusions

Salicylic acid is an organic compound found effective in mitigating the heat stress from pea. The results revealed that using proper concentration of salicylic acid can promote the growth, yield and quality of pea during summer season. Based on the findings 100 ppm salicylic acid in seeds plus foliar treatment increased the growth, yield and qualitative parameters of pea and hence recommended for improving the growth, yield and quality of peas during summer season.

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