

SEED PRIMING WITH SALICYLIC ACID IMPROVE SEED GERMINATION AND PHYSIOLOGICAL RESPONSES OF CARROT SEEDS

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Carrot is a cool season vegetable and belongs to family Apiaceae. It is very popular because of its high nutritional value along with many health benefits. Usual time to grow carrots in Punjab-Pakistan is October; however, due to profitable outcomes, carrot production is preferred in August-September in some areas. The high temperature during these months however, reduces seed germination and poses significant threat to early seedling growth of carrot which directly affect crop yield due to insufficient plant population. Thus, a comprehensive study was conducted at Institute of Horticultural Sciences, University of Agriculture Faisalabad to boost carrot seed germination at higher temperature. Recent studies have suggested the significant role of salicylic acid (SA) in seed priming to improve seed germination and subsequent growth of the plants under various environmental conditions. That's why, SA was employed for carrot seed priming to achieve the benefits. Initially, in a laboratory trial, carrot seeds (cultivar T-29) were primed with various concentration of SA (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mM and distilled water) for 12 h at room temperature while unprimed seeds served as control. Seeds were dried and then placed in Petri dishes for evaluation in terms of germination assay at high temperature (35±2°C) in the incubator. Results revealed that maximum germination (79%) was attained with 0.1 mM SA priming followed by 0.2 mM SA while, unprimed seeds showed least germination (55%) under high temperature (35±2°C). Based on the results of laboratory trial, 0.1mM and 0.2mM SA priming doses were tested in field trials along with distilled water priming and (unprimed) control. Seeds primed in 0.1 mM SA solution resulted in considerably higher antioxidants and total phenolic contents in carrot seedlings and also enhanced other enzymatic activities in terms of superoxide dismutase, peroxidase and catalase. Seed emergence and some of the physiological responses were also improved along with carrot yield attributes during field trials. Keeping in view the results of this study, it is concluded that lower concentration of SA (0.1mM) can be effectively used to boost carrot seed germination even under high temperature conditions.

Keywords: *Daucus carota*, abiotic stress, salicylic acid, seed germination, seed invigoration, enzymatic activities.

INTRODUCTION

Carrot cultivation is a profitable practice for the farmers in Punjab province of Pakistan. Mostly, farmers grow it on small scale i.e., around 1-5 acres, indicating that majority of them grow it for the local market (Ahmad *et al.*, 2012). Although it is a winter crop, usually sown during October, but to earn higher profit, farmers sow it during August-September as early crop. High temperature during August-September causes germination problem. To get optimum plant population, farmers use high seed rate (1.5 to 2 times more) as compared to normal seed rate.

Abiotic stresses delays or inhibits the germination process through reducing water availability, affecting the structural organization of proteins, and changing or creating imbalance for mobilization of stored reserves inside the cells (Ibrahim, 2016). Carrot crop is usually established by direct seeding and therefore, high or low temperature may lead to poor stand. In tropics, carrot production is vulnerable to loss due to high temperature during germination of seed and stand

establishment (Vieira *et al.*, 2005). High temperature $\geq 35^{\circ}\text{C}$ during or after seeding may completely inhibit or delay carrot seed germination as well as reduce uniformity ultimately resulting in poor stand establishment (Nascimento and Pereira, 2007). At advanced stages of plant life, high temperature could influence photosynthesis, respiration, water relations or stability of membrane and can alter endogenous levels of plant hormones and primary/secondary metabolites (Wahid *et al.*, 2007). Most of the commercial carrot cultivars exhibit very low seed germination at high temperature (Pereira *et al.*, 2007). An alternating day and night temperature (30 and 20°C) for the period of 8 h and 16 h is recommended for proper germination (AOSA, 1993). Seedling emergence rate and vigor of seedling directly affects yield and grades of carrot crop, therefore rapidness and uniformity in emergence of seedling is prerequisite (Kaur *et al.*, 2005) and such characters are influenced by the quality of seed and adverse conditions of environment e.g., drought, high temperature and high concentration of salts in the soil (Sarlikioti *et al.*, 2010). A considerable decrease was

observed in water uptake during the imbibition process under such stresses.

Seed priming is a technique that involves in hydration of seeds under aerated conditions to persuade all those metabolic activities which are very essential for germination process but emergence of radical from seed coat is forbidden. Priming has association with increased protein synthesis, repairing and building up of membrane and nucleic acid (McDonald, 2000). Under a range of environmental conditions (stressful) seed priming encourages uniformity and rapidness in germination of seed (Nascimento, 2003), and benefits also have been found for subsequent seedling growth (Calışkan *et al.*, 2012). Primed seeds usually show better germination (Hardegree and van Vactor, 2000) or emergence parameters, particularly under unfavorable or stressful regimes (Ghassemi-Golezani *et al.*, 2012).

Soaking of seeds with optimal concentration of plant growth regulators (hormones) is shown to effectively enhance germination, crop stand establishment and yield performances of many crops under normal as well as stress conditions (Lee *et al.*, 1998; Hurly *et al.*, 1991; Jaskani *et al.*, 2004, 2006). Plant growth hormones which normally used for pre-sowing seed soaking are salicylic acid, auxins (IAA, TBA and NAA), gibberellins, kinetin, polyamines, ethylene and ascorbic acid. Singh and Singh, (2016) primed tomato seeds with SA and evaluated under high temperature stress conditions. They revealed that seed priming with SA enhanced germination percentage and lower the time for germination under heat stress conditions. Moreover, Pre-sowing application of salicylic acid significantly affected vitamin C, TSS, TA, of tomato fruit. So, SA ameliorated the yield contributing aspects which increased the fruit yield of tomato. Rehman *et al.* (2015) conducted a seed priming study for early planted spring maize and stated that hormonal priming with salicylic acid took minimum time to emergence and has high vigor for seedling. Meanwhile, hormonal priming, improved the leaf relative and chlorophyll contents, while reduced the electrical conductivity EC of seed leachates. Moreover, 1000-grain weight, plant height, harvest index, grain and biological yield were also enhanced by seed priming. Rehman *et al.* (2011) evaluated germination and earlier growth of seedling in cucumber in response to salicylic acid seed priming and reported that seed priming with lower concentration of SA could be a good source to improve the germination rate and earlier seedling growth especially in cucumbers. Ahmad *et al.* (2017) conducted experiments to evaluate the effect of hormonal priming with SA, AsA, and H₂O₂ on hybrid maize. Plant growth regulators applied as a seed priming agent, improved the biochemical, physiological, morphological along with grain yield, quality and yield related attributes for late sown spring maize under high temperature conditions. They recorded higher activities of enzymes i.e., catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in those plants which were primed. Moreover, chlorophyll

contents, relative water contents, membrane stability, grain oil contents and grain yield were also enhanced through seed priming. So, seed priming with PGRs, i.e., SA could be employed to diminish the heat stress effect on yield losses in spring maize.

Therefore, a study was planned to evaluate the influence of hormonal seed priming with salicylic acid to enhance carrot early germination and better subsequent seedling growth under high temperature condition.

MATERIALS AND METHODS

Seeds of carrot variety 'T-29' were collected and this research was conducted at Institute of Horticultural Sciences (IHS), University of Agriculture, Faisalabad, Pakistan.

Hormonal priming with salicylic acid (SA): Seeds of carrot variety 'T-29' were primed in aerated solutions of SA with various concentrations (T₂), 0.1 mM (T₃), 0.2 mM (T₄), 0.4 mM (T₅), 0.8 mM (T₆), 1.6 mM (T₇) and 3.2 mM of SA along with distilled water priming (T₁) for 12 h in darkness at room temperature, while unprimed seeds served as control (T₀). Primed seeds were retrieved to the original moisture contents under shady condition. Seed weight to solution ratio was 1:5 (g/mL) (Ruan *et al.*, 2002).

Germination test: There were four replications of each treatment and one hundred seed per treatment (primed and unprimed) were allocated for germination test in Petri dishes on double layer of filter papers which were already moistened with 4 ml of distilled water. Petri plates were kept in incubator with temperature 35±2°C. Those seeds were considered as germinated which showed radicle protrusion up to 2 mm. Data recording were on daily basis for germination process up to seven days on various aspects viz., germination percentage (FGP), time to 50 percent germination (T₅₀ (days), mean germination time MGT (days), energy of germination (GE), germination index (GI), seedling length (cm), seedling fresh and dry weight (g, gm), and vigor index (SVI) following the rules of AOSA (1990). Five normal seedlings per replication were used to calculate seedling related parameters. Germination test was performed to optimize the best seed priming treatment that could be evaluate under field conditions.

The experiment related to optimization of priming treatments at germination stage was conducted in the laboratory according to completely randomized design and replicated four times while, two consecutive field trials were performed under RCBD with three replications to evaluate the optimized seed priming treatments (which were SA 0.1 mM (T₂) and SA 0.2 mM (T₃) along with control (T₀) and hydro-priming T₁) and following enzymatic, physiological and yield parameters during both years were recorded.

Enzymes and related activities: After germination, seedlings for enzyme extraction were collected from field within ten days of sowing.

Enzyme extraction: Carrot seedlings (1 g) from each treatment were homogenized in 2 mL phosphate buffer (pH 7.2) with the help of mortar and pestle. After thorough homogenization, sample was taken in Eppendorf tube, and centrifuged at 10,000 rpm for 6 min using micro-centrifuge machine (235-A, Pegasus Scientific Inc., USA)

Superoxide Dismutase (SOD): Activity of SOD was assessed by following the method of Stajner and Popovic (2009).

Catalase (CAT) ($U\ Kg^{-1}$ protein): Catalase (CAT) enzyme activity for the carrot seedling was assessed according to Liu *et al.* (2009).

Peroxidase (POD) ($U\ Kg^{-1}$ protein): Peroxidase (POD) enzyme (EC 1.11.1.7) activity in carrot seedling was calculated as described previously by Liu *et al.* (2009).

Total phenolic contents (%): Total phenolic contents were measured according to the method described by Ainsworth and Gillespie (2007).

Total antioxidants: Total antioxidants from carrot seedlings were recorded by following the method of Razzaq *et al.* (2013) using DPPH assay.

Malondialdehyde contents ($\mu\text{mol/g FW seed}$): To estimate lipid peroxidation in carrot seedlings, malondialdehyde content was determined by following Heath and Packer (1968).

Photosynthetic characteristics: Three mature leaves per plant (four plants in each replication per treatment) were selected (four weeks old) and placed one by one in the chamber of portable apparatus Infra-Red Gas Analyzer (IRGA) (LCi-SD, ADC Bio-scientific, UK) for data of transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$), photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance to water (Moya *et al.*, 2003).

Water use efficiency ($\text{pmol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$): It was measured by following the below mentioned equation.

$$\text{Water use efficiency (WUE)} = \frac{\text{Photosynthetic rate (A)}}{\text{Transpiration rate (E)}}$$

Field evaluation in term of yield response:

Final emergence percentage (%): For each treatment, seeds were sown on both sides of small beds (2.5 ft wide). After emergence of first seedling of every treatment, the no. of emerged seedlings was counted daily up to 10 days after sowing. Emergence percentage was calculated as:

$$\text{Emergence (\%)} = \frac{\text{Total no. of emerged seedlings} \times 100}{\text{Total no. of seed sown}}$$

Seedling vigor index (SVI): Vigor index (SVI) of the carrot seedlings in response to various pre-sowing seed treatments was calculated according to (Abdul-Baki and Anderson, 1973).

Seedling Vigor Index = Final Emergence (%) $\times$ Seedling Length (cm)

Carrot root weight (g): At harvesting, randomly five plants were selected for carrot roots. Plants were dug out and roots were washed. Weight for each root was calculated and averaged.

Carrot root length (cm): In individual replication of a treatment, five normal roots were taken at random during harvest. Root length (RL) was measured and their mean was calculated.

Root yield ($\text{kg}/12.5 \text{ ft}^2$): Total yield was calculated by harvesting carrot roots from small beds having area of 12.5 ft^2 for each replication of a treatment. Their weight was recorded by using digital weighing balance (786 BBCI, ACS).

Data analysis: Least square means and standard errors (SE) were calculated to show the differences between control and treatments the using “lsmeans” function of “lsmeans” package in R software. Furthermore, Tukey-HSD adjustments for multiple-comparisons between control and treatments were applied to show the significant mean differences between groups using “CLD” function of “lsmeans” package in R software. To estimate the variance between response variables (Optimization at germination stage, Enzyme and related activities, Physiological responses and Field performance) against T_0 , T_1 , T_2 , T_3 , T_4 , T_5 , T_6 , T_7 treatments, a multivariate linear analysis was performed using “candisc” function for computing and visualizing generalized canonical correlation analysis in R-software (version-3.5). The “candisc” generalizes the 'MANOVA' designs for all factors in a multivariate linear model, computing canonical scores and vectors for each variable. The graphic functions provide biplot visualizations of both response and effect variables via 'heplot.candisc' methods.

RESULTS

Optimization of SA priming treatments: Table 1 shows of the LS-means differences among control and seven other treatment considering the performance of crop biophysical parameters. Two out of seven seed priming treatments (0.1 mM salicylic acid and 0.2 mM salicylic acid) showed a significant difference for most of the biophysical parameters. Final germination percentage (FGP) and seedling vigour index (SVI) were highly and positively correlated with the effects of 0.1 mM SA (T_2) seed priming treatment (Fig. 1). On the other hand, Time taken to 50% germination (T_{50}) and mean germination time (MGT) were linked with control which means, maximum time to reach 50% germination and mean germination time was taken by unprimed seeds. Overall, improvement in important parameters like SVI, FGP, SL (seedling length), GI (germination index) and GE (germination energy) showed that seedling vigour was enhanced by T_2 (0.1 mM SA) and T_3 (0.2 mM SA) priming treatments. Two axes of candisc exhibited maximum (92%) variation in germination and seedling related parameters.

Table 1. Effect of Salicylic acid seed priming on germination related attributes of carrot seeds under high temperature regimes.

Treatments	FGP (%)	T ₅₀ (%)	GE	GI	MGT (days)	SL (cm)	SFW (mg)	SDW(mg)	SVI
Control	55.25±0.75a	5.12±0.04b	38.5±0.96a	10.4±0.26a	5.56±0.07d	5.89±0.22ab	27.5±0.51abc	2.1±0.06cd	326.00±9.40a
ddH ₂ O	64.75±1.25c	3.14±0.08a	58.5±1.32c	15±0.57b	5.2±0.03c	6.49±0.28abc	29.3±0.48bc	2.25±0.05de	421.00±9.15c
0.1 mM SA	79±0.58e	2.77±0.08a	70±1.08e	31.41±1.59e	4.26±0.04b	6.85±0.47bc	26.55±1.53ab	1.95±0.06cd	539.15±15.2d
0.2 mM SA	72±0.91d	3.13±0.07a	58±1.15c	25.33±0.98d	4.17±0.04ab	7.51±0.34c	32.55±1.72c	2.6±0.14e	538.25±12.0d
0.4 mM SA	62±0.82bc	3.1±0.11a	51±0.91b	21.27±0.5cd	4±0.08ab	6.04±0.18ab	27.45±0.99abc	1.95±0.1cd	372.05±11.0abc
0.8 mM SA	66±1.41c	2.89±0.11a	64±0.82d	24.67±0.76d	4.06±0.06ab	5.99±0.16ab	23.25±0.43a	1.4±0.08a	395.68±4.19bc
1.6 mM SA	57±1.29ab	2.72±0.15a	52±0.82b	23.14±0.7cd	3.89±0.04a	5.53±0.23a	26.45±1.37ab	1.8±0.04bc	318.03±8.16a
3.2 mM SA	61±1.29bc	3.13±0.04a	58±1.78c	20.32±1.01c	3.99±0.11ab	5.82±0.13ab	24.4±1.19ab	1.55±0.1ab	360.20±20.38ab

Note: Standard error indicates the 95% confidence interval of LSM. Means sharing the similar letters are not significantly different. (FGP = final germination percentage, T₅₀ = time taken to 50% germination, GE = germination energy, GI = germination index, MGT = mean germination time, SVI = seedling vigour index, SFW = seedling fresh weight, SDW = seedling dry weight and SL = seedling length).

Table 2. Effect of Salicylic acid seed priming on enzymes and related activities of carrot seedlings under high temperature regimes.

Treatments	TPC (mg/GAE 100 g)	TA (%) inhibition-DPPH)	POD (U Kg ⁻¹ protein)	CAT (U Kg ⁻¹ protein)	SOD (U kg ⁻¹ protein)	MDA (μmol/g FW seed)
Control	210.5±6.67a	60.00±3.70a	2031.5±50.46a	567.46±22.33a	350.27±19.44a	7.1±0.28c
ddH ₂ O	236.5±10.69ab	68.97±2.56ab	2593.2±81.78c	650.95±29.51ab	427.59±15.43ab	5.47±0.35b
0.1 mM SA	288.1±15.36c	87.98±1.68c	2761.1±39.06c	833.17±18.67c	543.04±24.78c	3.13±0.18a
0.2 mM SA	261.8±13.99bc	72.88±1.36b	2300.9±27.14b	668.04±17.62b	432.69±18.03b	4.94±0.2b

Note: Standard error indicates the 95% confidence interval of LSM. Means sharing the similar letters are not significantly different. (TPC=total phenolic contents, TA=total antioxidants, POD=peroxidase, CAT=catalase, SOD=superoxide dismutase and MDA= malondialdehyde).

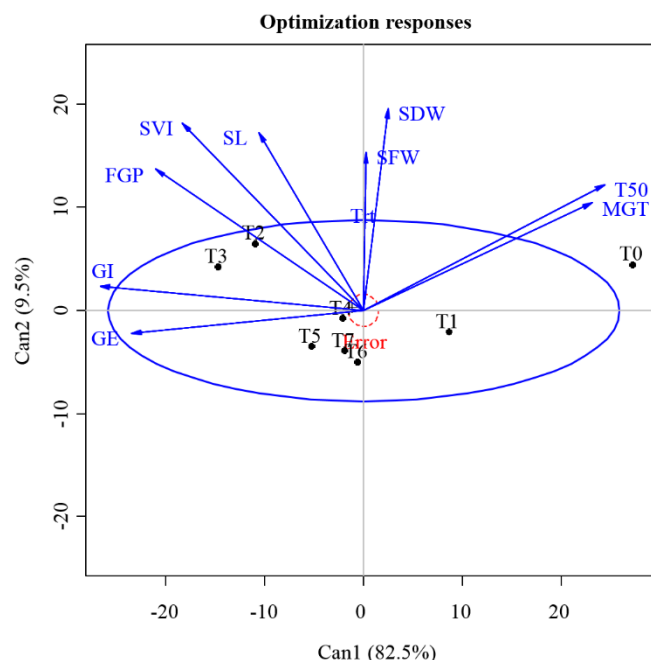


Figure 1. Influence of hormonal priming with Salicylic acid on germination and seedling vigor of carrot under high temperature regimes. The candisc orientation results showing the association

between germination and related parameters ("FGP"= Final germination percentage, "GI"= Germination index,

"GE"= Germination energy, "T₅₀"= Time taken to 50 % germination, "MGT"= Mean germination time, "SL"= Seedling length, "SFW"= Seedling fresh weight, "Seedling dry weight and "SVI"= Seedling vigour index in response to seed priming treatments ("T₀"= Control-unprimed, "T₁"= distilled water priming, T₂= SA 0.1 mM, T₃= SA 0.2 mM, T₄= SA 0.4 mM, T₅= SA 0.8 mM, T₆= SA 1.6 mM, T₇= SA 3.2 mM). The length of the vectors represents the magnitude of the relationship between variables. The angles between explanatory and response variables showed positive correlation (angle < 90°) and negative correlation (angle > 90°).

Enzyme and related activities: Table 2 shows the LS-means differences among the control, hydropriming and two best treatments from optimization experiment calculated based on the enzymatic activities. 0.1 mM salicylic acid (T₂) seed priming treatment showed a significant difference considering most of the enzymatic activities.

Most of the enzymes and related parameters were strongly correlated with 0.1 mM SA priming treatment except malondialdehyde (MDA) contents which were associated with control and it showed that maximum degradation was occurred in unprimed seeds (Fig. 2). First two axes of candisc explained maximum variation in enzymatic activities. MDA negatively correlated with the activities of all other enzymes and related parameters. Highest enzymatic activity parameters like catalase, total antioxidants and superoxidase dismutase

were exhibited in response to SA 0.1 mM seed priming treatment.

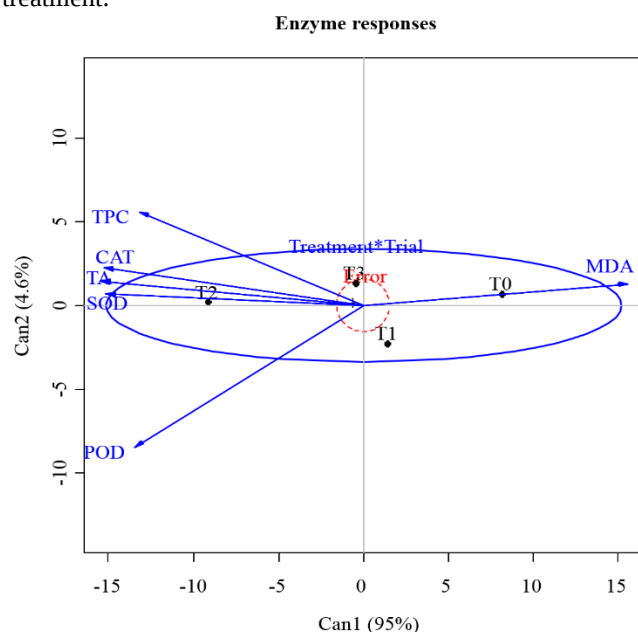


Figure 2. Modulation of enzymes and related activities of carrot seedlings in response to salicylic acid seed priming under high temperature regimes. The candisc orientation results showing the association between enzyme and related activities ("SOD"= Superoxidase dismutase, "CAT"= Catalase, "POD"= Peroxidase, "TA"= Total antioxidants, "TPC"= Total phenolic contents and "MDA"= Malondialdehydes in response to seed priming treatments ("T₀"= Control-unprimed, "T₁"= distilled water priming, T₂= SA 0.1 mM, T₃= SA 0.2 mM). The length of the vectors represents the magnitude of the relationship between variables. The angles between explanatory and response variables showed positive correlation (angle < 90°) and negative correlation (angle > 90°).

Physiological responses: Table 3 shows of the LS-means of difference among the control, hydropriming and two best treatments from optimization experiment calculated based on the crop physiological parameters. Salicylic acid @ 0.1 mM (T₂) seed priming treatment showed a significant difference

considering most of the crop physiological parameters, except stomatal conductance.

Photosynthetic rate (A) and water use efficiency (WUE) were positively correlated with T₂ while T₀ (control) was positively correlated with transpiration rate (E) and stomatal conductance (G) but negatively correlated with A and WUE. Photosynthetic rate (A) and water use efficiency (WUE) were highest in treatment with 0.1 mM SA (Fig. 3). First two axes of candisc explained maximum 99.8% variation in physiological responses.

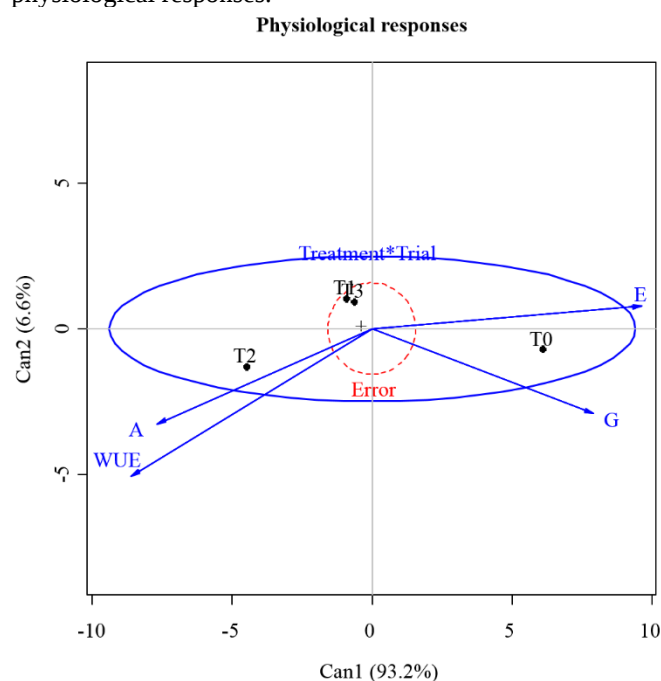


Figure 3. Physiological modulation of carrot plants in response to salicylic acid seed priming under high temperature regimes. The candisc orientation results showing the association between plant physiological parameter ("A"=Photosynthetic rate, WUE=Water use efficiency, E=Transpiration rate, and G=Stomatal conductance) in response to seed priming treatments ("T₀"=Control-unprimed, "T₁"=distilled water priming, T₂=SA 0.1 mM, T₃=SA 0.2 mM). The length of the vectors represents the magnitude of the relationship between variables. The angles between explanatory and response variables showed positive correlation (angle<90°) and negative correlation (angle >90°).

Table 3. Effect of Salicylic acid seed priming on photosynthetic characteristics of carrot plant under high temperature regimes.

Treatments	E (mmol m ⁻² s ⁻¹)	G (mmol m ⁻² s ⁻¹)	A (μmol m ⁻² s ⁻¹)	WUE (pmol CO ₂ mmol ⁻¹ H ₂ O)
Control	9.12±0.40c	0.25±0.01b	6.41±0.64a	0.70±0.06a
ddH ₂ O	6.31±0.43c	0.15±0.01a	9.01±0.38b	1.47±0.12b
0.1 mM SA	4.62±0.45a	0.14±0.02a	12.57±0.60c	2.84±0.29c
0.2 mM SA	6.66±0.27b	0.14±0.01a	9.00±0.85b	1.37±0.17ab

Note: Standard error indicates the 95% confidence interval of LSM. Means sharing the similar letters are not significantly different. (E = transpiration rate, G = stomatal conductance, A = photosynthetic rate and WUE= water use efficiency).

Table 4. Effect of Salicylic acid seed priming on field performance and yield parameters of carrot.

Treatments	FEP (%)	SVI	CRL (cm)	CRW (g)	RY ($\text{Kg}/12.5 \text{ ft}^2$)
Control	15.88 $\pm$ 0.94a	114.44 $\pm$ 7.04a	15.85 $\pm$ 0.33a	58.67 $\pm$ 2.01a	1.91 $\pm$ 0.06a
ddH ₂ O	20.63 $\pm$ 0.77b	171.89 $\pm$ 4.47b	17.3 $\pm$ 0.23ab	65.33 $\pm$ 3.20a	2.08 $\pm$ 0.08a
0.1 mM SA	29.77 $\pm$ 0.88c	283.40 $\pm$ 11.47d	20.73 $\pm$ 0.36c	106.83 $\pm$ 6.35b	2.68 $\pm$ 0.07b
0.2 mM SA	28.75 $\pm$ 1.05c	238.15 $\pm$ 6.08c	17.98 $\pm$ 0.51c	72.00 $\pm$ 5.29a	2.13 $\pm$ 0.09a

Note: Standard error indicates the 95% confidence interval of LSM. Means sharing the similar letters are not significantly different. (FEP = final emergence percentage, SVI = seedling vigour index, CRL = carrot root length, CRW = carrot root weight, RY= root yield).

Field evaluation responses: Table 4 shows the of LS-means differences between the control, hydropriming and two best treatments from optimization experiment calculated based on crop yield and related attributes under field condition. Salicylic acid 0.1 mM (T₂) seed priming treatment showed a significant difference considering all of the crop field performance and yield parameters.

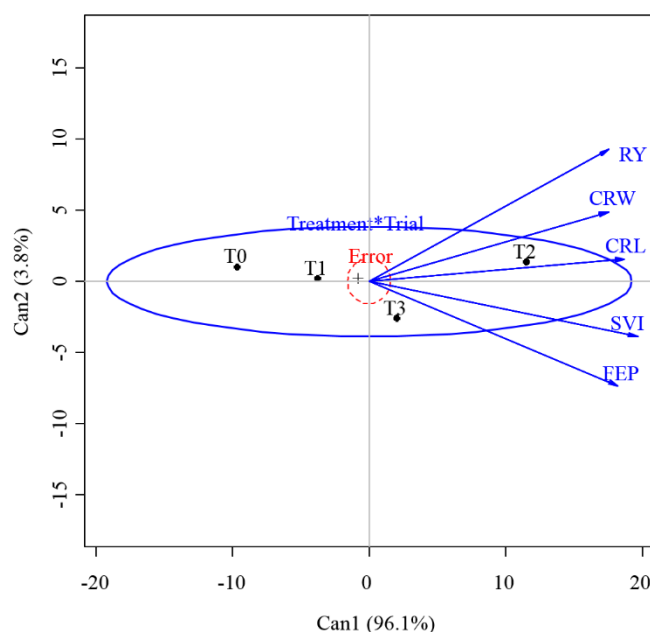
Field_performance responses

Figure 4. Field performance of carrot plants related to yield attributes in response to salicylic acid seed priming. The candisc orientation results showing the association between Field performance response ("FEP"= Final emergence percentage, "SVI"= Seedling vigour index, "CRL"= Carrot root length, "CRW"= Carrot root weight, "RY"= Root yield in response to seed priming treatments ("T₀"= Control-unprimed, "T₁"= distilled water priming, T₂= SA 0.1 mM, T₃= SA 0.2 mM). The length of the vectors represents the magnitude of the relationship between variables. The angles between explanatory and response variables showed positive correlation (angle < 90°) and negative correlation (angle > 90°).

During field trials, all studied parameters exhibited positive correlation with the priming treatment T₂ (Fig. 4). Unprimed seeds showed very negative correlation with all of the studied parameters. Carrot root length (CRL), root weight (CRW), yield (RY), vigour index (SVI) and emergence percentage (FEP) were positively enhanced by SA @ 0.1 mM priming treatment. Here, two axis of candisc explained 99.9% of variation.

DISCUSSION

Priming induce various biochemical changes in seeds, which are required to initiate the germination process i.e., hydrolysis and metabolism of inhibitors, imbibition and enzyme activation, hence primed seeds rapidly imbibe and retrieve seed metabolism, resulting in faster germination rate and higher germination percentage. After germination, it also helps the subsequent seedlings to cope with stress induced challenges (Rowse, 1995; Zheng *et al.*, 2015).

It is evident from our study that seed priming with salicylic acid positively improved germination percentage and time taken to 50% germination, which could be due to faster production of germination metabolites as a result of priming (Saha *et al.*, 1990; Basra *et al.*, 2005). Plant growth hormones (PGR) is a group of germinating metabolites which affect extensive range of processes and functions in plants under normal and stressful conditions. Exogenous application of plant growth regulators (PGR) have proven to ameliorate the damaging effect of stress i.e., abiotic stresses (heat, drought or salinity) on germination as well as on plant growth (Javid *et al.*, 2011; Finch-Savage *et al.*, 2004).

Previous studies indicated that seed priming of various crops resulted in improvement of germination, seedling establishment and in some cases became a source of higher crop yield. Present study revealed that seedling raised from seeds primed with different concentration of pre-sowing seed treatment SA exhibited characteristics of morphological responses only at particular concentration of priming agents. Similarly, in our studies only two concentrations of SA @ 0.1 mM and 0.2 mM SA exhibited significant differences for seedling traits (early and higher final germination, emergence percentage, seedling length, higher fresh and dry weight, and less mean germination time). It might be due to the

antioxidants properties of SA which has promoted the metabolites in carrot seedlings. Kumari *et al.* (2017) reported that hormonal priming with GA₃ and SA resulted in highest germination percentage (%), energy of emergence, germination index, seedling fresh and dry weight (g), seedling length (cm) and vigour indices of maize crop. In the same way, Heydariyan *et al.* (2014) reported that seed priming of Caper (*Capparis spinosa*) with gibberellic acid and salicylic acid could enhance the germination percentage, seedling length and seedling vigour index under abiotic stress condition.

A positive correlation between seed vigor and emergence was reported by Yamuchi and Winn (1996) while Farooq *et al.* (2008) reported that seed primed with salicylic acid enhanced the abiotic tolerance in hybrid maize by activation of antioxidant enzymes i.e., SOD, CAT, POD and APX. Different priming strategies significantly improved shoot and root fresh as well as dry weight along with seedling length. Increment in seedling length may be due to early emergence with the benefits of seed priming.

ROS are produced under stressful conditions like salinity, heat or cold. Seed priming with SA increased activities of ROS scavenging enzymes like, catalase (CAT), super oxidase dismutase (SOD), peroxidase (POD), while lowered the malondialdehyde (MDA) contents in carrot seedlings during our study. This might be the cause of increment in the free radicle scavenging enzymes due to the action of SA (Chang and Sung, 1998). Similar studies by Yan (2015) on cabbage revealed that seed priming could enhance seed germination and early seedling growth under abiotic stress conditions, and that enhancement could be associated with modulations of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and levels of soluble sugar. Ara *et al.* (2013) also explained that quantity of such enzymes was increased in heat tolerant genotypes.

The temperature has a pronounced direct effect on carrot seedlings. Vollenweider and Georg (2005) explained that high temperature caused marked reduction in root and shoot growth of seedling probably because of lower in moisture levels due to high temperature stress. In our studies, root growth was improved due to SA even at high temperature conditions in comparison with unprimed-control, while reduction might be the due to root sensitivity to high temperature conditions. Similarly, Porter and Gawith (1999) work showed that root growth was relatively more sensitive to heat stress than vegetative and reproductive part of plant. Improvement in photosynthetic characteristics in our study with SA seed priming might be because of better scavenging activities of antioxidants and defense enzymes against reactive oxygen species under stress or SA could have special role in preventing cell wall by decreasing the concentration of membrane degrading enzymes like lipoxygenase, pectin methylesterase, and cellulase (Zang *et al.* 2003) which might be stabilized and/ or improved photosynthetic rate, stomatal

conductance, transpiration rate and water use efficiency of carrot plants. Our studies are in line with Kaur (2017) who reported significant improvement with seed priming in terms of stomatal conductance, photosynthetic rate, damage to membranes and leaf water status under abiotic stress, and revealed higher mungbeen plant biomass along with yield as compared to control under stress conditions. Similarly, Yang *et al.* (2018) revealed significant improvement in quinoa photosynthetic rate and stomatal conductance under stress condition when seeds were subjected to priming. Our results of higher yield of carrot and other related parameters with SA priming are in line with Singh and Singh (2016) who evaluated tomato seeds primed with SA under high temperature stress conditions, and revealed that seed priming with SA enhanced germination percentage, lowered the time for germination under heat stress conditions and ameliorated the yield contributing aspects which increased the fruit yield of tomato. Highest yield with SA 0.1 mM is also in accordance with Rehman *et al.* (2015) who stated that SA priming was economical method for improving productivity of early sown spring maize by stimulation of early seedling growth at unfavorable temperature. Moreover, Ahmad *et al.* (2017) who stated that heat tolerance encouraged through PGRs, i.e., SA could be credited for enhancement in antioxidant enzyme activities and membrane stability, which could retain chlorophyll and relative water contents in maize crop ultimately, resulting in increased yield of maize grain under high temperature conditions. On the other hand, in our study temperature was bit higher than optimal during the course of field evaluation but pre-sowing seed treatment (particularly) i.e., SA 0.1 mM and SA 0.2 mM enhanced root quality as compared to control in term of higher root length along with increased root fresh weight. Higher yield of carrot and improvement in seedling traits might be because of promoted cell division and cell enlargement by SA priming (Hayat *et al.*, 2010).

Conclusion: It is concluded that seed priming with 0.1 mM SA could be employed to improve early germination along with enhanced enzymatic and physiological responses and better yield parameters of carrot under high temperature. It could be helpful for early carrot crop which may enhance not only the production but also profitability of farmers.

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