Journal of Pure and Applied Agriculture (2021) 6(3): 20-30
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Priming with growth regulators stimulates germination, growth and physiological characteristics of Indian squash (*Praecitrullus fistulosus*)

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Abstract

Indian squash (*Praecitrullus fistulosus*) is a summer vegetable crop in which higher temperature limits its production during sowing period. The present experiment 1 and 2 elucidated the possible effect of plant growth regulators on germination, growth and physiological characteristics of Indian squash (cv. Sahavi) under laboratory conditions. A completely randomized design with fourteen treatments (non-primed dry seeds (control), priming with distilled water, indole acetic acid @ 100, 150, 200 mg L^{-1}, salicylic acid @ 50, 100, 150 mg L^{-1}, ascorbic acid @ 50, 100, 150 mg L^{-1} and thiourea @ 500, 1000, 1500 mg L^{-1}) was used. Results of experiment 1 and 2 indicated that priming treatment of ascorbic acid @ 100 mg L^{-1} significantly ($P \leq 0.05$) improved germination (93 and 94%) and seedling vigor index (1515.4 and 1531.2). Moreover, ascorbic acid @ 100 mg L^{-1} produced heavier shoot (2012.5 and 2014.1 mg) and root (424.7 and 425.4 mg) fresh weight and longer shoot (8.58 and 8.61 cm) and root (7.65 and 7.67 cm) length per seedling. Furthermore, results depicted that ascorbic acid @ 100 mg L^{-1} enhanced photosynthesis (3.72 µmol m^{-2} s^{-1}), chlorophyll (30.98 and 30.99 SPAD), total soluble proteins (1.53 and 1.54 mg g^{-1} F. wt.), proline (7.94 and 7.95 µmol g^{-1} f. wt.), rate of transpiration (3.30 and 3.32 mmol m^{-2} s^{-1}) and stomatal conductance (3.90 and 3.91 mmol m^{-2} s^{-1}) during experiment 1 and 2. Additionally, ascorbic acid @ 100 mg L^{-1} was shortened in time to 50% germination (2.99 and 3.00 days) and mean germination time (3.00 and 3.03 days). It is concluded that a low dose of ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} can effectively improve the germination characteristics, growth and physiological attributes of Indian squash.

Keywords: Gas exchange attributes, Germination, Indian squash, Plant growth regulators, Priming

To cite this article: Qamar, R., Khan, S., Safdar, M. E., Rehman, A., Rehman, A.-U., Javeed, H. M. R., & Khan, B. A. (2021). Priming with growth regulators stimulates germination, growth and physiological characteristics of Indian squash (*Praecitrullus fistulosus*). *Journal of Pure and Applied Agriculture, 6*(3), 20-30.

Introduction

Indian squash (*Praecitrullus fistulosus*) is a summer season vegetable that belongs to family Cucurbitaceae and its fruit is edible (Tyagi et al., 2012; Birbal et al., 2014). In Pakistan, Indian squash was cultivated on the area of around 0.01 million hectares with average production of 103 million tonnes (Govt. of Pakistan, 2017-2018). In Pakistan and India, it has particular names i.e. Tinda, spherical gourd, apple gourd, Indian baby pumpkin and especially called Dilpasand (Sridhar et al., 2002). Optimum temperature for growth of Indian squash is 23 ºC (Maynard & Hochmuth, 2006). However, sowing time significantly affects its growth and fruit setting which is primarily due to low temperature (Khan et al., 2001). In addition, the lower temperature leads to a delay in seed germination. Therefore, farmers who want to grow Indian squash during February cannot get higher production. The lower temperature leads to a delay in seed germination and is seen as a critical hindrance in production (Lu et al., 2020). However, treating seeds with growth chemicals improved germination, resulting in healthy development and performance at lower temperature (Lu et al., 2020).

In the current scenario, seed priming is known as an effective strategy that can control seed water content to partially activate the germination processes such as metabolism but prevents full germination of the seeds (Sano & Seo, 2019). It will improve plant beginning and development that leads to higher production. Different chemicals like plant hormones and antioxidants are applied to protect the cell death at apical meristem, especially plant DNA damage (Hammoudi, 2021) applied as production agents to ensure higher germination and plant development during abiotic stresses (Mahmoud & Abdelhameed, 2021). Priming is a very beneficial technique through interaction of abiotic and biotic systems and increased soil organic carbon in the detritus sphere of arable ecosystems for improving seed germination and seed plant formation on various vegetables and food crops (Xu et al., 2019). Priming could activate the metabolic rates that are crucial for germination and improve the consistency in germination rate, so priming techniques substantially increased production of crops (Afzal et al., 2011).

In plant growth stimulators, indole acetic acid is an auxin that improves the plant foliage and development (Schaller et al., 2015) through enhancing the seed sprouting, encouragement of rooting cell, internodal elongation, and cell division at shoot top (Khamna et al., 2010; Zhang et al., 2020). Ascorbic acid is primarily distributed in the plant cytosol, which facilitates plants to withstand stress by reducing the oxygen containing radicals. In addition, it is also essential for the bio-production of various plant hormones like gibberellins and ethylene which enhance the germination process (De Tullio & Arrigoni, 2003). Similarly, salicylic acid is recognized as an endogenic plant regulator that is involved in many physiological and biochemical developments of plants under stress (Yu et al., 2020). Growth hormones work as a signaling molecule that influences a variety of different physiological processes (Han & Kahmann, 2019; Blázquez et al., 2020), like enzyme performance (Chen et al., 2020), the rate of photosynthesis (Khan et al., 2003) and plant development (Hussein et al., 2007). Moreover, it reduces detrimental effects of different types of stresses through modification of internal processes of plants (Méndez-Hernández et al., 2019). Thiourea has ability to improve nutrient uptake, controls the biosynthesis of important secondary metabolites, osmolytes, hormones and stabilizes different metabolic activities to enhance tolerance against abiotic stresses. Thiourea benefits are not limited to the stress environment, but also enhance plant growth in a normal abiotic environment, by employing the same mechanisms (Waqas et al., 2019).

Numerous have been executed concerning their effects on germination, growth and physical traits of Indian squash. The present study was conducted in order to find out the role of seed priming with concentrations of growth regulators on germination, growth, physiology and gaseous exchange traits of Indian squash under laboratory conditions.

Materials and Methods

Experimental Site

The experiment of both experiments 1 and experiment 2 were conducted in a Post-Graduate Laboratory in the Department of Agronomy, College of Agriculture, University of Sargodha. Indian squash (*Praecitrullus fistulosus*) (cv. *Sahavi*) was used as a test crop. Indian squash seeds were taken from the Horticultural section, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds contained 5.3% moisture content on a dry weight basis.

Growing conditions

Before the beginning of study, seeds were treated with a solution having 1% sodium hypochlorite for five minutes, then three washings were done with distilled water. Seeds were dried under shade and then primed with different concentrations of indole acetic acid, salicylic acid, ascorbic acid and thiourea. Priming was done at 25°C for 24 h with constant gentle agitation. For this purpose, an aquarium pump was used for adequate supply of oxygen to the priming solution. The ratio 1:5 was kept for seed weight to solution volume (w/v). The priming solution was replaced with a new solution after 12 h. Autoclaved distilled water was utilized for hydropriming. The primed seeds were washed with distilled water after 24 h for 2 min. For surface drying, blotting paper was then shifted to an air-drying oven for 48 h at 25°C to lower the moisture content less than 10%. Non-primed dry seed had been taken into consideration as a control. After drying, 12 seeds for each treatment in four repeats were placed in plastic pots (L×W, 30×30 cm). Plastic pot of treatments was kept at the Post-Graduate Laboratory of Agronomy at temperature 25±3°C for 30 days.

Experimentation

Experiments were arranged in completely randomized design (CRD). There were fourteen treatments viz., T1 (Control; non-primed dry seeds), T2 (priming with distill water), T3 (indole acetic acid @ 100 mg L^{-1}), T4 (indole acetic acid @ 150 mg L^{-1}), T5 (indole acetic acid @ 200 mg L^{-1}), T6 (salicylic acid @ 50 mg L^{-1}), T7 (salicylic acid @ 100 mg L^{-1}), T8 (salicylic acid @ 150 mg L^{-1}), T9 (ascorbic acid @ 50 mg L^{-1}), T10 (ascorbic acid @ 100 mg L^{-1}), T11 (ascorbic acid @ 150 mg L^{-1}), T12 (thiourea @ 500 mg L^{-1}), T13 (thiourea @ 1000 mg L^{-1}) and T14 (thiourea @ 1500 mg L^{-1}) and replicated four times. Germination data were recorded on daily bases. After one month, growth, physiology and gaseous exchange traits were recorded.

Data collection

Germination characteristics

For calculation of germination percentage, procedure of the Association of Official Seed Analysts (1990) was used and further calculation done by using below formula:

$$GP = \frac{\text{Germinated/emerged seeds}}{\text{Total seeds}} \times 100$$

Time taken to 50% Germination (E50) [Days] was calculated by using following formula given by Coolbear et al. (1984) and modified by Farooq et al. (2005):

$$T_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i}\right](t_j - t_i)$$

N for total germination; n_i and n_j for the combined number of seeds sown by next add up at times t_i and t_j, in that order of $n_i < N/2 > n_j$.

Mean germination time (MGT) [Days] was calculated by adopting formula of Ellis & Roberts (1981):

$$MGT = \sum(D_n)/\sum n$$

Here, n for total germinated on day D, and D for number of days noted from the first of germination and germination (%) on a daily basis up to the constant level.

Data of germination % and seedling height (cm) were applied for determining seedling vigour index (SVI) by adopting formula as designated by Orchard (1977).
SVI = [seedling length (cm) × germination/emergence percentage]

Growth attributes

After careful removal of seedlings from the sand, shoot and root fresh weight from three (03) seedlings in every experimental pot in each replicate were taken instantly after harvest with the help of electrical weight balance (PL 3200+L Japan) in mg and then their averages were computed. For shoot and root length, three seedlings from every experimental pot were selected. Shoot and root length recorded with measuring scale. Then their averages were calculated.

Physiological characteristics

Chlorophyll meter (Model, SPAD-502: Konica Minolta Sensing: Inc, Japan) was utilized to measure chlorophyll contents from completely flourish 3rd to 4th younger leaf (Khan et al., 2003). Bates et al. (1973) procedure was adopted for measurement of proline contents. According to this method, 0.5 g leaves were used and their absorbance were recorded at 520 nm with the help of a spectro-photometer (Hitachi-120; Japan). For blank value toluene reading was used. Then proline was calculated by using a standard curve and computed on a fresh weight basis. For determination of total soluble protein, seeds were blended into a medium having buffer solution (pH 7.0) of 50 mM potassium phosphate. Bradford (1976) procedure was executed for recording quantitative protein. To determine the protein in the sample, 5 µL of liquid and 0.1 N NaCl were add into 1.0 mL Bradford dye. Whole solution was kept for 5 min for formation of protein dye complex. Then use a spectrophotometer to calculate absorbance at 595 nm.

Gaseous exchange traits

On the 30th day, selected four younger leaves and placed them in the chamber of an Infrared Gas Analyzer (IRGA) in order of one by one. The stomatal conductance, photosynthesis and transpiration rate were recorded during 11.00 to 12.00 am. During readings, IRGA chamber was set according to guideline of Zekri (1991) and Moya et al. (2003) at 403.3 mmol m^{-2} S^{-1} molar flow rate; 99.90 KPa atmospheric pressure; 6.0 to 8.9 mbar vapor pressure; 1711 µmol m^{-2} S^{-1} photosynthetically active radiation; 28.40 to 32.40°C leaf temperature; 22.40 to 27.90°C ambient temperature; 352 µmol mol^{-1} ambient CO_2 concentration.

Data analysis

The experimental processing follows a completely randomized design. SAS software (Version 9.1; SAS Institute, Cary, NC, USA) was assigned for statistical analysis of variance (ANOVA) on all traits and means were distinguished by applying Duncan's multiple range test at 5% probability (Steel et al., 1997). Analysis of correlation was executed by using Minitab (Version 14, State College, PA, USA) (Minitab, Inc. 2006) to evaluate the effect of experimental traits designated on the existing results.

Results

Germination characteristics

In laboratory experiment 1 and 2, seed priming with ascorbic acid @ 100 mg L^{-1} for six hours showed considerable ($P \leq 0.05$) results on different germination characteristics of Indian squash (Table 1 and 2). Statistically ($P \leq 0.05$) higher germination percentage (93 and 94% in experiment 1 and 2, respectively) was recorded in ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} compared to lower germination percentage in thiourea @ 1500 mg L^{-1} in both experiments (24 and 25%, respectively). Maximum seedling vigour index (1515.4 and 1531.2 in both experiments, respectively) was obtained in ascorbic acid @ 100 mg L^{-1} while minimum seedling vigour index (81.7 and 85.0 during both experiments, respectively) was noted in thiourea @ 1500 mg L^{-1} (Table 1). Statistically ($P \leq 0.05$) shorter time to 50% germination (2.9 and 3.0 days in both experiments, respectively) and mean germination time (3.0 and 3.0 days in both experiments, respectively) were resulted due to priming with ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} while longer time to 50% germination (4.4 days in both experiments) and mean germination time (5.2 days in both experiments) were noted in thiourea @ 1500 mg L^{-1} during both experiments (Table 2). Briefly, ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} were found to be more useful in enhancing germination characteristics of Indian squash than thiourea @ 1500 mg L^{-1} treatment during both experiments (Table 1 and 2).

Growth attributes

Ascorbic acid seed priming @ 100 mg L^{-1} increased ($P \leq 0.05$) shoot fresh weight (2012.5 and 2014.1 mg in experiment 1 and 2, respectively) and root fresh weight (424.8 and 425.4 mg in experiment 1 and 2, respectively), while lower shoot fresh weight (496.7 and 498.3 mg in experiment 1 and 2, respectively) and root fresh weight (22.4 and 23.1 mg in both experiments, respectively) were recorded in thiourea @ 1500 mg L^{-1} in both experiments (Table 3). Shoot and root length per seedling significantly ($P \leq 0.05$) improved through priming of ascorbic acid @ 100 mg L^{-1} during both experiments of study when compared with thiourea @ 1500 mg L^{-1} (Table 4). Significantly ($P \leq 0.05$) longer shoot length per seedling (8.6 cm during experiment 1 and 2, respectively) and root length per seedling (7.6 and 7.7 cm during experiment 1 and 2, respectively) were obtained by application of ascorbic acid @ 100 mg L^{-1} while shorter shoot length per seedling (2.8 cm during experiment 1 and 2, respectively) and root length per seedling (1.4 cm during experiment 1 and 2, respectively) were recorded in thiourea @ 1500 mg L^{-1} (Table 4). Briefly, ascorbic acid @ 100 mg L^{-1}

followed by salicylic acid @ 50 mg L^{-1} were concluded useful in enhancing growth attributes of Indian squash than thiourea @ 1500 mg L^{-1} treatment during both experiments (Table 3 and 4).

Table 1 Role of growth promoting substances on germination and seedling vigor index of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Germination %		Seedling vigor index (SVI)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	47^{h}	48^{gh}	321.6^{hi}	328.4^{gh}
Distilled water	68^{e}	69^{de}	651.2^{e}	660.9^{d}
Indole acetic acid @ 100 mg L^{-1}	75^{d}	76^{cd}	886.3^{d}	901.9^{c}
Indole acetic acid @ 150 mg L^{-1}	42^{hi}	43^{hi}	258.7^{ij}	264.8^{hi}
Indole acetic acid @ 200 mg L^{-1}	29^{k}	30^{j}	114.7^{k}	118.8^{jk}
Salicylic acid @ 50 mg L^{-1}	86^{b}	87^{ab}	1195.6^{b}	1214.0^{b}
Salicylic acid @ 100 mg L^{-1}	54^{g}	55^{fg}	415.6^{gh}	423.1^{fg}
Salicylic acid @ 150 mg L^{-1}	41^{i}	42^{hi}	231.9^{ij}	239.5^{h-j}
Ascorbic Acid @ 50 mg L^{-1}	81c	82^{bc}	1024.3^{c}	1041.0^{c}
Ascorbic Acid @ 100 mg L^{-1}	93^{a}	94^{a}	1515.4^{a}	1531.2^{a}
Ascorbic Acid @ 150 mg L^{-1}	62^{f}	63^{ef}	566.3^{ef}	575.4^{de}
Thiourea @ 500 mg L^{-1}	58^{fg}	59^{ef}	495.4^{fg}	504.1^{ef}
Thiourea @ 1000 mg L^{-1}	35^{j}	35^{ij}	178.4^{jk}	183.5^{i-k}
Thiourea @ 1500 mg L^{-1}	24^{k}	25^{j}	81.7^{k}	85.0^{k}
SE (M)	1.03	2.04	20.09	27.27
$LSD_{0.05}$	5.31	10.53	103.99	141.12

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Table 2 Role of growth promoting substances on time to 50% germination and mean germination time of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Time to 50% germination (days)		Mean germination time (days)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	4.3^{ab}	3.9^{c}	4.1^{d-f}	4.2^{b-e}
Distilled water	3.5^{gh}	3.6^{ef}	3.8^{gh}	3.7^{e-g}
Indole acetic acid @ 100 mg L^{-1}	3.5^{hi}	3.4^{g}	3.7^{hi}	3.7^{e-g}
Indole acetic acid @ 150 mg L^{-1}	3.5^{gh}	4.3^{ab}	4.2^{de}	4.1^{c-e}
Indole acetic acid @ 200 mg L^{-1}	4.3^{bc}	4.3^{ab}	4.7^{b}	4.7^{ab}
Salicylic acid @ 50 mg L^{-1}	3.2^{j}	3.5^{e-g}	3.2^{j}	3.2^{gh}
Salicylic acid @ 100 mg L^{-1}	3.8^{de}	3.2^{h}	4.0^{e-g}	3.8^{d-f}
Salicylic acid @ 150 mg L^{-1}	3.7^{ef}	3.5^{fg}	4.3^{cd}	4.4^{b-d}
Ascorbic Acid @ 50 mg L^{-1}	3.4^{i}	3.5^{e-g}	3.5^{i}	3.5^{f-h}
Ascorbic Acid @ 100 mg L^{-1}	2.9^{k}	3.0^{i}	3.0^{j}	3.0^{h}
Ascorbic Acid @ 150 mg L^{-1}	3.6^{fg}	3.8^{cd}	3.9^{f-h}	4.0^{d-f}
Thiourea @ 500 mg L^{-1}	3.8^{d}	3.7^{de}	3.7^{hi}	3.9^{d-f}
Thiourea @ 1000 mg L^{-1}	4.2^{c}	4.2^{b}	4.6^{bc}	4.6^{bc}
Thiourea @ 1500 mg L^{-1}	4.4^{a}	4.4^{a}	5.2^{a}	5.2^{a}
SE (M)	0.02	0.03	0.05	0.11
$LSD_{0.05}$	0.11	0.16	0.28	0.59

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Table 3 Role of growth promoting substances on shoot and root fresh weight of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Shoot fresh weight (mg)		Root fresh weight (mg)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	937.9^{i}	939.6^{i}	75.4^{i}	76.1^{g}
Distilled water	1403.2^{e}	1404.9^{e}	156.5^{e}	157.2^{e}
Indole acetic acid @ 100 mg L^{-1}	1513.7^{d}	1515.3^{d}	204.6^{d}	205.2^{d}
Indole acetic acid @ 150 mg L^{-1}	847.1^{j}	848.8^{j}	66.2^{i}	66.9^{g}
Indole acetic acid @ 200 mg L^{-1}	525.0^{m}	526.3^{l}	25.8^{k}	26.4^{ij}
Salicylic acid @ 50 mg L^{-1}	1847.2^{b}	1848.8^{b}	312.4^{b}	313.1^{b}

Salicylic acid @ 100 mg L^{-1}	1176.9^{h}	1178.6^{h}	103.2^{h}	103.8^{f}
Salicylic acid @ 150 mg L^{-1}	801.4^{k}	802.9^{k}	45.8^{j}	46.4^{h}
Ascorbic Acid @ 50 mg L^{-1}	1737.6^{c}	1739.3^{c}	265.4^{c}	265.7^{c}
Ascorbic Acid @ 100 mg L^{-1}	2012.5^{a}	2014.1^{a}	424.8^{a}	425.4^{a}
Ascorbic Acid @ 150 mg L^{-1}	1351.9^{f}	1353.6^{f}	142.5^{f}	143.2^{e}
Thiourea @ 500 mg L^{-1}	1224.3^{g}	1225.9^{g}	118.6^{g}	119.3^{f}
Thiourea @ 1000 mg L^{-1}	767.3^{l}	768.3^{k}	41.5^{j}	42.1^{hi}
Thiourea @ 1500 mg L^{-1}	496.7^{n}	498.3^{l}	22.4^{k}	23.1^{j}
SE (M)	4.53	7.44	2.45	3.60
$LSD_{0.05}$	23.48	38.52	12.66	18.62

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Table 4 Role of growth promoting substances on shoot and root length per seedling of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Shoot length per seedling (cm)		Root length per seedling (cm)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	4.6^{gh}	4.6^{gh}	2.3^{i}	2.3^{i}
Distilled water	5.7^{de}	5.7^{de}	3.8^{e}	3.9^{e}
Indole acetic acid @ 100 mg L^{-1}	6.1^{cd}	6.2^{cd}	5.7^{d}	5.7^{d}
Indole acetic acid @ 150 mg L^{-1}	4.1^{hi}	4.1^{hi}	2.0^{j}	2.1^{i}
Indole acetic acid @ 200 mg L^{-1}	3.1^{j}	3.1^{j}	0.8^{m}	0.8^{k}
Salicylic acid @ 50 mg L^{-1}	7.4^{b}	7.4^{b}	6.5^{b}	6.6^{b}
Salicylic acid @ 100 mg L^{-1}	4.9^{fg}	4.9^{fg}	2.8^{h}	2.8^{h}
Salicylic acid @ 150 mg L^{-1}	3.9^{i}	4.0^{hi}	1.7^{k}	1.7^{j}
Ascorbic Acid @ 50 mg L^{-1}	6.5^{c}	6.6^{c}	6.1^{c}	6.2^{c}
Ascorbic Acid @ 100 mg L^{-1}	8.6^{a}	8.6^{a}	7.6^{a}	7.7^{a}
Ascorbic Acid @ 150 mg L^{-1}	5.6^{de}	5.7^{de}	3.4^{f}	3.5^{f}
Thiourea @ 500 mg L^{-1}	5.4^{ef}	5.4^{ef}	3.1^{g}	3.1^{g}
Thiourea @ 1000 mg L^{-1}	3.8^{i}	3.8^{i}	1.4^{l}	1.4^{j}
Thiourea @ 1500 mg L^{-1}	2.8^{j}	2.8^{j}	0.5^{n}	0.5^{k}
SE (M)	0.11	0.12	0.04	0.05
$LSD_{0.05}$	0.58	0.63	0.20	0.30

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Physiological traits

Photosynthesis rate, chlorophyll contents (SPAD value), total soluble proteins and proline contents of Indian squash deviated significantly ($P \leq 0.05$) among treatments during both experiments (Table 5 and 6). A profound effect of ascorbic acid @ 100 mg L^{-1} was recorded in improved photosynthesis rate and chlorophyll contents in both experiments as compared to thiourea @ 1500 mg L^{-1} as depicted in table 5. Significantly ($P \leq 0.05$) higher photosynthetic rate (3.7 µmol m^{-2} s^{-1} in both experiments, respectively) and chlorophyll contents (30.9 SPAD value in both experiments, respectively) were noted in ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1}, while lower values of photosynthetic rate (2.2 µmol m^{-2} s^{-1} in both experiments, respectively) and chlorophyll contents (20.1 and 20.2 SPAD value in both experiments, respectively) were noted in thiourea @ 1500 mg L^{-1} treatment in both experiments (Table 5). Results of total soluble proteins and proline contents during both experiments of study (Table 6) depicted that ascorbic acid @ 100 mg L^{-1} treatment significantly ($P \leq 0.05$) enhanced total soluble proteins and proline contents in Indian squash seedling. Higher ($P \leq 0.05$) total soluble proteins (1.5 mg g^{-1} F. wt. during experiment 1 and 2) and proline contents (7.9 µmol g^{-1} f. wt. during experiment 1 and 2) were observed in those seedlings, which were treated with ascorbic acid @ 100 mg L^{-1} while lower value of total soluble proteins (0.4 mg g^{-1} F. wt. during experiment 1 and 2) and proline contents (4.6 µmol g^{-1} f. wt. during experiment 1 and 2) were observed in thiourea @ 1500 mg L^{-1} treatment during both experiments (Table 6). Priming of Indian squash seeds with salicylic acid @ 50 mg L^{-1}, ascorbic acid @ 50 mg L^{-1}, indoleacetic acid @ 100 mg L^{-1} and distilled water also improved the physiological traits than control and other treatments (Table 5 and 6).

Gaseous exchange attributes

A significant ($P \leq 0.05$) difference in gas exchange attributes (transpiration rate and stomatal conductance) of Indian squash occurred among different seed priming treatments (Table. 7). Indian squash produced statistically the significant ($P \leq 0.05$) improvement (3.3 mmol m^{-2} s^{-1} during experiment 1 and 2) in transpiration rate and (3.9 mmol m^{-2} s^{-1} during experiment 1 and 2) in stomatal conductance were recorded in response to ascorbic acid priming @ 100 mg L^{-1} (Table 7) while lower improvement (2 mmol m^{-2} s^{-1} during experiment 1 and 2) in transpiration rate and (2.2 mmol m^{-2} s^{-1} during experiment 1 and 2) in stomatal conductance were noted in thiourea @ 1500 mg L^{-1} treatment during both experiments (Table 7). Priming of

Indian squash seeds with salicylic acid @ 50 mg L^{-1}, ascorbic acid @ 50 mg L^{-1}, indoleacetic acid @ 100 mg L^{-1} and distilled water also improved the gaseous exchange attributes than control and other treatments (Table 7).

Table 5 Role of growth promoting substances on photosynthesis rate and chlorophyll contents of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Photosynthesis rate (µmol m^{-2} s^{-1})		Chlorophyll contents (SPAD value)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	3.1^{h}	3.1^{f}	30.0^{g}	30.0^{i}
Distilled water	3.4^{e}	3.8^{d}	30.4^{d}	30.5^{e}
Indole acetic acid @ 100 mg L^{-1}	3.5^{d}	3.5^{c}	30.5^{d}	30.5^{d}
Indole acetic acid @ 150 mg L^{-1}	2.9^{i}	2.9^{g}	20.8^{h}	20.8^{j}
Indole acetic acid @ 200 mg L^{-1}	2.4^{k}	2.4^{i}	20.3^{k}	20.4^{m}
Salicylic acid @ 50 mg L^{-1}	3.6^{b}	3.6^{b}	30.7^{b}	30.7^{b}
Salicylic acid @ 100 mg L^{-1}	3.3^{g}	3.3^{e}	30.2^{f}	30.2^{h}
Salicylic acid @ 150 mg L^{-1}	2.7^{j}	2.7^{h}	20.7^{i}	20.7^{k}
Ascorbic Acid @ 50 mg L^{-1}	3.5^{c}	3.5^{bc}	30.6^{c}	30.7^{c}
Ascorbic Acid @ 100 mg L^{-1}	3.7^{a}	3.7^{a}	30.9^{a}	30.9^{a}
Ascorbic Acid @ 150 mg L^{-1}	3.4^{ef}	3.3^{d}	30.3^{e}	30.3^{f}
Thiourea @ 500 mg L^{-1}	3.3^{f}	3.3^{de}	30.2^{f}	30.3^{g}
Thiourea @ 1000 mg L^{-1}	2.7^{j}	2.7^{h}	20.5^{j}	20.5^{l}
Thiourea @ 1500 mg L^{-1}	2.2^{l}	2.2^{j}	20.1^{l}	20.1^{n}
SE (M)	0.01	0.01	0.014	0.012
$LSD_{0.05}$	0.046	0.092	0.077	0.056

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Table 6 Role of growth promoting substances on total soluble proteins and proline contents of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Total soluble proteins (mg g^{-1} F. wt.)		Proline contents (µmol g^{-1} f. wt.)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	0.9^{g}	0.9^{g}	6.1^{h}	6.1^{h}
Distilled water	1.2^{d}	1.2^{de}	7.1^{e}	7.1^{e}
Indole acetic acid @ 100 mg L^{-1}	1.3^{c}	1.3^{cd}	7.3^{d}	7.3^{d}
Indole acetic acid @ 150 mg L^{-1}	0.8^{h}	0.8^{gh}	5.9^{i}	5.9^{i}
Indole acetic acid @ 200 mg L^{-1}	0.5^{j}	0.5^{j}	4.8^{l}	4.8^{l}
Salicylic acid @ 50 mg L^{-1}	1.4^{b}	1.4^{ab}	7.7^{b}	7.7^{b}
Salicylic acid @ 100 mg L^{-1}	1.0^{f}	1.0^{f}	6.5^{g}	6.5^{g}
Salicylic acid @ 150 mg L^{-1}	0.7^{i}	0.7^{hi}	5.5^{j}	5.5^{j}
Ascorbic Acid @ 50 mg L^{-1}	1.4^{b}	1.4^{bc}	7.5^{c}	7.5^{c}
Ascorbic Acid @ 100 mg L^{-1}	1.5^{a}	1.5^{a}	7.9^{a}	7.9^{a}
Ascorbic Acid @ 150 mg L^{-1}	1.1^{e}	1.1^{ef}	6.8^{f}	6.8^{f}
Thiourea @ 500 mg L^{-1}	1.1^{ef}	1.1^{f}	6.6^{g}	6.6^{g}
Thiourea @ 1000 mg L^{-1}	0.7^{i}	0.7^{i}	5.3^{k}	5.3^{k}
Thiourea @ 1500 mg L^{-1}	0.4^{k}	0.4^{k}	4.6^{m}	4.6^{m}
SE (M)	0.011	0.020	0.019	0.033
$LSD_{0.05}$	0.057	0.103	0.10	0.17

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Table 7 Role of growth promoting substances on transpiration rate and stomatal conductance of Indian squash under laboratory condition during experiment 1 and 2

Treatments	Transpiration rate (mmol m^{-2} s^{-1})		Stomatal conductance (mmol m^{-2} s^{-1})	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	2.5^{h}	2.5^{f}	3.1^{cd}	3.1^{bc}
Distilled water	2.8^{e}	2.8^{de}	3.6^{a-c}	3.6^{ab}
Indole acetic acid @ 100 mg L^{-1}	2.9^{d}	2.9^{cd}	3.7^{ab}	3.7^{a}
Indole acetic acid @ 150mg L^{-1}	2.5^{hi}	2.5^{f}	2.9^{de}	2.8^{cd}
Indole acetic acid @ 200 mg L^{-1}	2.2^{k}	2.2^{g}	2.3^{fg}	2.3^{e}
Salicylic acid @ 50 mg L^{-1}	3.1^{b}	3.1^{b}	3.5^{a-c}	3.5^{ab}

Salicylic acid @ 100 mg L^{-1}	2.7^{g}	2.7^{e}	3.2^{cd}	3.2^{bc}
Salicylic acid @ 150 mg L^{-1}	2.4^{i}	2.4^{f}	2.7^{d-f}	2.7^{c-e}
Ascorbic Acid @ 50 mg L^{-1}	3.0^{c}	3.0^{bc}	3.7^{ab}	3.7^{a}
Ascorbic Acid @ 100 mg L^{-1}	3.3^{a}	3.3^{a}	3.9^{a}	3.9^{a}
Ascorbic Acid @ 150 mg L^{-1}	2.7^{ef}	2.8^{de}	3.5^{a-c}	3.5^{ab}
Thiourea @ 500 mg L^{-1}	2.7^{fg}	2.7^{e}	3.4^{bc}	3.4^{ab}
Thiourea @ 1000 mg L^{-1}	2.3^{j}	2.4^{f}	2.5^{e-g}	2.5^{de}
Thiourea @ 1500 mg L^{-1}	2.0^{l}	2.0^{g}	2.2^{g}	2.2^{e}
SE (M)	0.013	0.032	0.093	0.097
$LSD_{0.05}$	0.066	0.164	0.48	0.50

Different letters in the column point out the statistical variations among treatments according to Duncan's multiple range test ($P \leq 0.05$).

Correlation study

Correlation in different variables clearly directed that germination showed highly significant ($P \leq 0.05$) positive correlation with time to 50% germination, mean germination time, seedling vigor index, shoot fresh weight, root fresh weight, shoot length per seedling, root length per seedling, photosynthesis rate, chlorophyll contents, total soluble proteins, proline contents, transpiration rate and stomatal conductance in both the experiments (Table 8 and 9). Shoot and root fresh weight and length proved highly significant ($P \leq 0.05$) correlation with photosynthesis rate, chlorophyll contents, total soluble proteins, proline contents, transpiration rate and stomatal conductance (Table 8 and 9). Proline contents also had highly significant ($P \leq 0.05$) correlation with transpiration rate and stomatal conductance moreover, it was positively highly significantly ($P \leq 0.05$) correlated with photosynthesis rate, chlorophyll contents and total soluble proteins (Table 8 and 9).

Table 8 *Pearson´*s coefficient correlation among Indian squash parameters experiment 1.
NS – insignificant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$
Germination = GER; Time to 50% germination (Days) = T50; Mean germination time (Days) = MGT; Seedling vigor index = SVI; Shoot fresh weight (mg) = SFW; Root fresh weight (mg) = RFW; Shoot length per seedling (cm) = SLS; Root length per seedling (cm) = RLS; Photosynthesis rate (µmol m^{-2} s^{-1}) = PHR; Chlorophyll contents (SPAD value) = CHC; Total soluble proteins (mg g^{-1} F. wt.) = TSP; Proline contents (µmol g^{-1} f. wt.) = PRC; Transpiration rate (mmol m^{-2} s^{-1}) = TRR; Stomatal conductance (mmol m^{-2} s^{-1}) = STC

	GER	T50	MGT	SVI	SFW	RFW	SLS	RLS	PHR	CHC	TSP	PRC	TRR	STC
GER	1													
T50	0.98^{**}	1												
MGT	0.98^{**}	0.97^{**}	1											
SVI	0.97^{**}	0.96^{**}	0.97^{**}	1										
SFW	0.99^{**}	0.98^{**}	0.98^{**}	0.97^{**}	1									
RFW	0.95^{**}	0.93^{**}	0.96^{**}	0.99^{**}	0.95^{**}	1								
SLS	0.98^{**}	0.96^{**}	0.99^{**}	0.98^{**}	0.99^{**}	0.97^{**}	1							
RSL	0.98^{**}	0.98^{**}	0.97^{**}	0.99^{**}	0.98^{**}	0.98^{**}	0.98^{**}	1						
PHR	0.96^{**}	0.93^{**}	0.94^{**}	0.87^{**}	0.96^{**}	0.84^{**}	0.94^{**}	0.91^{**}	1					
CHC	0.84^{**}	0.77^{*}	0.79^{*}	0.73^{NS}	0.83^{**}	0.70^{*}	0.81^{**}	0.77^{*}	0.91^{**}	1				
TSP	0.99^{**}	0.97^{**}	0.97^{**}	0.93^{**}	0.99^{**}	0.91^{**}	0.97^{**}	0.96^{**}	0.99^{**}	0.88^{**}	1			
PRC	0.99^{**}	0.97^{**}	0.97^{**}	0.92^{**}	0.98^{**}	0.90^{**}	0.96^{**}	0.95^{**}	0.99^{**}	0.88^{**}	0.99^{**}	1		
TRR	0.99^{**}	0.98^{**}	0.99^{**}	0.96^{**}	0.99^{**}	0.94^{**}	0.99^{**}	0.97^{**}	0.97^{**}	0.82^{**}	0.99^{**}	0.99^{**}	1	
STC	0.95^{**}	0.92^{**}	0.93^{**}	0.86^{**}	0.94^{**}	0.83^{**}	0.92^{**}	0.90^{**}	0.98^{**}	0.91^{**}	0.97^{**}	0.98^{**}	0.95^{**}	1
	GER	T50	MGT	SVI	SFW	RFW	SLS	RLS	PHR	CHC	TSP	PRC	TRR	STC

Table 9 *Pearson´*s coefficient correlation among Indian squash parameters experiment 2. NS – insignificant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$

Germination = GER; Time to 50% germination (Days) = T50; Mean germination time (Days) = MGT; Seedling vigor index = SVI; Shoot fresh weight (mg) = SFW; Root fresh weight (mg) = RFW; Shoot length per seedling (cm) = SLS; Root length per seedling (cm) = RLS; Photosynthesis rate (µmol m^{-2} s^{-1}) = PHR; Chlorophyll contents (SPAD value) = CHC; Total soluble proteins (mg g^{-1} F. wt.) = TSP; Proline contents (µmol g^{-1} f. wt.) = PRC; Transpiration rate (mmol m^{-2} s^{-1}) = TRR; Stomatal conductance (mmol m^{-2} s^{-1}) = STC

	GER	T50	MGT	SVI	SFW	RFW	SLS	RLS	PHR	CHC	TSP	PRC	TRR	STC
GER	1													
T50	0.98**	1												
MGT	0.98**	0.97**	1											
SVI	0.97**	0.96**	0.97**	1										
SFW	0.99**	0.98**	0.98**	0.97**	1									
RFW	0.95**	0.93**	0.96**	0.99**	0.95**	1								
SLS	0.99**	0.96**	0.99**	0.98**	0.99**	0.97**	1							
RSL	0.99**	0.98**	0.97**	0.99**	0.98**	0.98**	0.98**	1						
PHR	0.96**	0.93**	0.94**	0.87**	0.96**	0.84**	0.94**	0.91**	1					
CHC	0.83**	0.77*	0.79*	0.73*	0.83**	0.70*	0.81**	0.77*	0.91**	1				
TSP	0.99**	0.97**	0.97**	0.93**	0.99**	0.91**	0.97**	0.96**	0.99**	0.88**	1			
PRC	0.99**	0.97**	0.97**	0.93**	0.98**	0.90**	0.96**	0.95**	0.99**	0.88**	0.99**	1		
TRR	0.99**	0.98**	0.99**	0.96**	0.99**	0.94**	0.99**	0.97**	0.97**	0.82**	0.99**	0.99**	1	
STC	0.95**	0.92**	0.93**	0.87**	0.94**	0.84**	0.92**	0.90**	0.98**	0.91**	0.97**	0.96**	0.95**	1

Discussion

In both experiments, among priming treatments on Indian squash seeds, ascorbic acid and salicylic acid considerably ($P \leq 0.05$) ameliorated the germination, growth, physiology and gaseous exchange characters. Remarkable improvements in above characteristics were associated with ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} treatments. Minimum temperature is an environmental stress that significantly reduces the plant growth and development of plants (Ding et al., 2020). Plants cope with lower temperature stress by triggering the appearance of resistance genes. However, severity and occurrence of low temperature will restrict the plant's self-regulating system. Application of chemical growth regulators improve the plant defense system which are generally regarded as encouraging tools for plant protection in sustainable crop production (Wang et al., 2020). Priming with growth regulators induces preliminary germination enhances synchronized germination, promotes plant growth (Bryksová et al., 2020). The feasible cause of this effective impact mediated through hormones, its play as main substrate in various reactive oxygen absorption paths (Luo et al., 2020) and protect the plants from any abiotic stress (Wei et al., 2021). The inhibitory results of salicylic acid at maximum application may be characteristics to its function as anti-transparent for the stomatal closure and subsequently its capability to reduce germination, foliage and physiology (Liu et al., 2011).

During experiment 1 and 2, early seed germination and its characteristics verified in ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} may be increase in the vigor of seeds and seedlings through metabolic and biochemical processes occurring during controlled hydration, followed by dehydration that leads to promote radicle to protrude through it and germinated than unprimed seed (Becerra-Vázquez et al., 2020). Such significant ($P \leq 0.05$) modifications were predicted due to timely stimulation of physiological processes (Han & Kahmann, 2019) in germination than non-primed seeds (control). Ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} priming improved ($P \leq 0.05$) mean germination and seedling vigor, which are essential signs of crop consistency, organization of emergence and seedling vigor (Lara et al., 2014). Correlation analysis proposed that germination characteristics were positively significantly ($P \leq 0.05$) correlated with all growth, physiological and gaseous exchange attributes.

The significant ($P \leq 0.05$) improvement in shoot and root fresh biomass and length were observed after priming with ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} treatments in both experiments which could be the result of higher cell division and cell elongation by keeping the hormonal balance in the plant tissues, thereby improving the cell multiplication by increasing the internal level of other regulators of plant growth (Méndez-Hernández et al., 2019). Our results supported the conclusions of Guo et al. (2021) who testified that significant improvement in morphological development and biomass accumulation in the root system due to application of growth regulators. Further, improved shoot and root development in the form of weight and length due to application of salicylic acid can be clarified by the detail that salicylic acid increased cell replication in shoot and root and thus leads to an increase shoot and root biomass and length (Farooq et al., 2006). Correlation analysis proposed that growth attributes were positively significantly ($P \leq 0.05$) correlated with all germination, physiological and gaseous exchange attributes.

Significantly ($P \leq 0.05$) the higher rate of photosynthesis and chlorophyll were recorded in ascorbic acid applied @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} treatments during both experiments. It has been reported that plant growth promoting substances considerably enhanced photosynthetic and chlorophyll contents (Huang et al., 2021). Statistically ($P \leq 0.05$) the lower photosynthesis rate and chlorophyll contents might be due to higher concentrations of growth regulators in treatments which caused stress that induced activity of chlorophyllase and H_2O_2 production that leads to photo-injury of chlorophyll contents (Hussain et al., 2011). Application of plant growth regulators through priming supported the plants for synthesis of osmoprotectants which have lower molecular weight and soluble compounds that are commonly nontoxic at high concentrations (Tekle & Alemu, 2016). Ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} initiated a significant ($P \leq 0.05$) production in total soluble protein contents. The result is in harmony with Mohamed & Akladious (2014). Proline plays an important function as an endogenic osmotic regulation and its concentration in plant tissues improves their resistance against stress (Dos-Reis et al., 2012). Correlation analysis proposed that physiological traits were positively significantly ($P \leq 0.05$) correlated with all germination, growth and gaseous exchange attributes.

In present study, some treatments having higher concentrations of growth regulators (thiourea, indoleacetic acid, salicylic acid and ascorbic acid), distilled water and control imposed adverse effects on gas exchange attributes in Indian squash. Photosynthesis was considerably lower in such treatments, which may be the outcome of oxidative injury to the essential photosynthetic cells (Shahbaz & Zia, 2011). Moreover, reduction in photosynthesis and transpiration rate is related to the decrease in stomatal conductivity, which finally limits the use of carbon dioxide in the leaf tissue. Ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} priming significantly ($P \leq 0.05$) improved Indian squash gas exchange properties. This progress in gas exchange properties may be related to stomatal opening promotes leaf cooling and helps to decrease canopy temperature and higher CO_2 exchange rate under optimum situations (Quintero-Calderón et al., 2021). Correlation analysis proposed that gaseous exchange traits were positively significantly ($P \leq 0.05$) correlated with all germination, growth and physiological attributes.

Conclusion

Priming strategies on Indian squash, ascorbic acid @ 100 mg L^{-1} followed by salicylic acid @ 50 mg L^{-1} were proved more suitable in improving germination characteristics in addition to increase growth traits as well as improved physiological and gas exchange characteristics which investigate its possible effect to reduce any abiotic stress. Therefore, seed priming with a growth regulator is a simple and easy strategy that is recommended to farmers in attaining higher and consistent growth and development of Indian squash in the zaid rabi season under field condition.

Conflict of Interest: The authors declare that they have no conflict of interest.

Acknowledgements: The authors highly acknowledge Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan.

References

Afzal, I., Basra, S. M. A., Ahmad, N., Cheema, M. A., Haq, M. A., Kazmi, M. H., & Irfan, S. (2011). Enhancement of antioxidant defense system induced by hormonal priming in wheat. *Cereal Research Communications*, *39*(3), 334-342.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*(1), 205-207.

Becerra-Vázquez, A. G., Coates, R., Sánchez-Nieto, S., Reyes-Chilpa, R., & Orozco-Segovia, A. (2020). Effects of seed priming on germination and seedling growth of desiccation-sensitive seeds from Mexican tropical rainforest. *Journal of Plant Research*, *133*(6), 855-872.

Birbal, R. V. S., Nathawat, N. S., Soni, M. L., & Yadava, N. D. (2014). Response of squash melon (Citrullus vulgaris var. fistulosus) to FYM and mulching under rainfed condition of hot arid region of Rajasthan. *Indian Journal of Agricultural Research*, *48*(2), 149-153.

Blázquez, M. A., Nelson, D. C., & Weijers, D. (2020). Evolution of plant hormone response pathways. *Annual Review of Plant Biology*, *71*, 327-353.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Bryksová, M., Hybenová, A., Hernándiz, A. E., Novák, O., Pěnčík, A., Spíchal, L., De Diego, N., & Doležal, K. (2020). Hormopriming to mitigate abiotic stress effects: A case study of N9-substituted cytokinin derivatives with a fluorinated carbohydrate moiety. *Frontiers in Plant Science*, *11*, 599228. doi: 10.3389/fpls.2020.599228

Chen, K., Li, G. J., Bressan, R. A., Song, C. P., Zhu, J. K., & Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. *Journal of Integrative Plant Biology*, *62*(1), 25-54.

Coolbear, P., Francis, A., & Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, *35*(11), 1609-1617.

De Tullio, M. C., & Arrigoni, O. (2003). The ascorbic acid system in seeds: to protect and to serve. *Seed Science Research*, *13*(4), 249-260.

Ding, Y., Shi, Y., & Yang, S. (2020). Molecular regulation of plant responses to environmental temperatures. *Molecular Plant*, *13*(4), 544-564.

Dos Reis, S. P., Lima, A. M., & De Souza, C. R. B. (2012). Recent molecular advances on downstream plant responses to abiotic stress. *International Journal of Molecular Sciences*, *13*(7), 8628-8647.

Ellis, R. H., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, *9*, 373-409.

Farooq, M., Basra, S. M. A., Hafeez, K., Asad, S. A., & Ahmad, N. (2005). Use of commercial fertilizers as osmotica for rice priming. *Journal of Agriculture and Social Sciences*, *1*(2), 172-175.

Farooq, M., Basra, S. M. A., Tabassum, R., & Ahmed, N. (2006). Evaluation of seed vigor enhancement techniques on physiological and biochemical basis in coarse rice (*Oryza sativa* L.). *Seed Science and Technology*, *34*, 741-750.

Govt. of Pakistan. (2017-18). Economic Survey of Pakistan. Finance Division Economic Advisor's Wing, Islamabad.

Guo, X., Zhao, J., Wang, R., Zhang, H., Xing, B., Naeem, M., Yao, T., Li, R., Xu, R., Zhang, Z., & Wu, J. (2021). Effects of graphene oxide on tomato growth in different stages. *Plant Physiology and Biochemistry*, *162*, 447-455.

Hammoudi, V. (2021). DNA damage response priming by CK2: a matter of life or cell death in root apical meristems. *The Plant Cell*, *33*, 1099–1100.

Han, X., & Kahmann, R. (2019). Manipulation of phytohormone pathways by effectors of filamentous plant pathogens. *Frontiers in Plant Science*, *10*, 822. doi: 10.3389/fpls.2019.00822

Huang, G., Liu, Y., Guo, Y., Peng, C., Tan, W., Zhang, M., Li, Z., Zhou, Y., & Duan, L. (2021). A novel plant growth regulator improves the grain yield of high-density maize crops by reducing stalk lodging and promoting a compact plant type. *Field Crops Research*, *260*, 107982.

Hussain, K., Nawaz, K., Majeed, A., Ilyas, U., Lin, F., Ali, K., & Nisar, M. F. (2011). Role of exogenous salicylic acid applications for salt tolerance in violet. *Sarhad Journal of Agriculture*, *27*(2), 171-175.

Hussein, M. M., Balbaa, L. K., & Gaballah, M. S. (2007). Salicylic acid and salinity effects on growth of maize plants. *Research Journal of Agriculture and Biological Sciences*, *3*(4), 321-328.

Khamna, S., Yokota, A., Peberdy, J. F., & Lumyong, S. (2010). Indole-3-acetic acid production by Streptomyces sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsian Journal of BioSciences*, *4*(1), 23-32.

Khan, A. Q., Iqbal, M., Jilani, M. S., Ghaffoor, A., & Waseem, K. (2001). Effect of different sowing dates on the yield of tinda gourd (*Citrullus vulgaris*) var. Fistulosus under the agro-climatic conditions of D. I. Khan. *Journal of Biological Sciences*, *1*(4), 235-237.

Khan, W., Prithiviraj, B., & Smith, D. L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. *Journal of Plant Physiology*, *160*(5), 485-492.

Lara, T. S., Lira, J. M. S., Rodrigues, A. C., Rakocevi, M., & Alvarenga, A. A. (2014). Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. *Journal of Agricultural Science*, *6*(2), 72-80.

Liu, C., Guo, J., Cui, Y., Lü, T., Zhang, X., & Shi, G. (2011). Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. *Plant and Soil*, *344*(1), 131-141.

Lu, J., Guan, P., Gu, J., Yang, X., Wang, F., Qi, M., Li, T., & Liu, Y. (2020). Exogenous DA-6 improves the low night temperature tolerance of tomato through regulating cytokinin. *Frontiers in Plant Science*, *11*, 599111. doi: 10.3389/fpls.2020.599111

Luo, S., Calderón-Urrea, A., Jihua, Y. U., Liao, W., Xie, J., Lv, J., Feng, Z., & Tang, Z. (2020). The role of hydrogen sulfide in plant alleviates heavy metal stress. *Plant and Soil*, *449*(1), 1-10.

Mahmoud, N. E., & Abdelhameed, R. M. (2021). *Superiority* of modified graphene oxide for enhancing the growth, yield, and antioxidant potential of pearl millet (*Pennisetum glaucum* L.) under salt stress. *Plant Stress*, *2*, 100025.

Maynard, D. N., & Hochmuth, G. J. (2006). Knott's handbook for vegetable growers. John Wiley & Sons.

Méndez-Hernández, H. A., Ledezma-Rodríguez, M., Avilez-Montalvo, R. N., Juárez-Gómez, Y. L., Skeete, A., Avilez-Montalvo, J., De-la-Peña, C., & Loyola-Vargas, V. M. (2019). Signaling overview of plant somatic embryogenesis. *Frontiers in Plant Science*, *10*, 77. doi: 10.1016/j.stress.2021.100025

Minitab Inc. (2006) *MINITAB*, Version 14. Minitab Inc., State College, PA

Mohamed, H. I., & Akladious, S. A. (2014). Influence of garlic extract on enzymatic and non-enzymatic antioxidants in soybean plants (*Glycine max*) grown under drought stress. *Life Science Journal*, *11*(3s), 46-58.

Moya, J. L., Gómez-Cadenas, A., Primo-Millo, E., & Talon, M. (2003). Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. *Journal of Experimental Botany*, *54*(383), 825-833.

Orchard, T. J. (1977). Estimating the parameters of plant seedling emergence. *Seed Science and Technology*, *5*(1), 61-69.

Quintero-Calderon, E. H., Sanchez-Reinoso, A. D., Chavez-Arias, C. C., Garces-Varon, G., & Restrepo-Diaz, H. (2021). Rice seedlings showed a higher heat tolerance through the foliar application of biostimulants. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *49*(1), 12120-12120. doi: 10.15835/nbha49112120

Sano, N., & Seo, M. (2019). Cell cycle inhibitors improve seed storability after priming treatments. *Journal of Plant Research*, *132*(2), 263-271.

Schaller, G. E., Bishopp, A., & Kieber, J. J. (2015). The yin-yang of hormones: cytokinin and auxin interactions in plant development. *The Plant Cell*, *27*(1), 44-63.

Shahbaz, M., & Zia, B. (2011). Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions. *Journal of Applied Botany and Food Quality*, *84*(1), 54-60.

Sridhar, S., Arumugasamy, S., Saraswathy, H., & Vijayalakshmi., K. (2002). Organic vegetable gardening. Center for Indian Knowledge Systems, Chenai, India. pp. 1-9

Steel, R. G. (1997). *Pinciples and procedures of statistics a biometrical approach* (No. 519.5 S8).

Tekle, A. T., & Alemu, M. A. (2016). Drought tolerance mechanisms in field crops. *World Journal of Biology and Medical Sciences*, *3*(2), 15-39.

Tyagi, N., Sharma, G. N., & Hooda, V. (2012). Medicinal value of Praecitrullus fistulosus: an overview. *International Journal of Pharma Therapeutics*, *3*(2), 136-142.

Wang, W., Zhou, P., Mo, X., Hu, L., Jin, N., Chen, X., Yu, Z., Meng, J., Erb, M., Shang, Z., & Gatehouse, A. M. (2020). Induction of defense in cereals by 4-fluorophenoxyacetic acid suppresses insect pest populations and increases crop yields in the field. *Proceedings of the National Academy of Sciences*, *117*(22), 12017-12028.

Waqas, M. A., Kaya, C., Riaz, A., Farooq, M., Nawaz, I., Wilkes, A., & Li, Y. (2019). Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. *Frontiers in Plant Science*, *10*, 1336. doi: 10.3389/fpls.2019.01336

Wei, P., Demulder, M., David, P., Eekhout, T., Yoshiyama, K. O., Nguyen, L., Vercauteren, I., Eeckhout, D., Galle, M., De Jaeger, G., & Larsen, P. (2021). Arabidopsis casein kinase 2 triggers stem cell exhaustion under Al toxicity and phosphate deficiency through activating the DNA damage response pathway. *The Plant Cell*, *33*(4), 1361-1380.

Xu, Y., Liu, K., Han, Y., Jiang, H., Yao, S., & Zhang, B. (2019). A soil texture manipulation doubled the priming effect following crop straw addition as estimated by two models. *Soil and Tillage Research*, *186*, 11-22.

Yu, Z., Duan, X., Luo, L., Dai, S., Ding, Z., & Xia, G. (2020). How plant hormones mediate salt stress responses. *Trends in Plant Science*, *11*(25), 1117-1130.

Zekri, M. (1991). Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. *Scientia Horticulturae*, *47*(3-4), 305-315.

Zhang, Y., Wang, Y., Ye, D., Xing, J., Duan, L., Li, Z., & Zhang, M. (2020). Ethephon-regulated maize internode elongation associated with modulating auxin and gibberellin signal to alter cell wall biosynthesis and modification. *Plant Science*, *290*, 110196.

[Received: 22 May 2021; Accepted: 17 September 2021]