Bangladesh J. Bot. **50**(2): 199-208, 2021 (June)

EFFECTS OF SEED PRIMING ON ROOT- SHOOT BEHAVIOUR AND STRESS TOLERANCE OF PEA (*PISUM SATIVUM* L.)

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Keywords: Seed priming, Pea varieties, Root architecture, Water stress tolerance

Abstract

A pot culture experiment was carried out under controlled conditions for the detailed study of morpho-physiological and advanced stress response adaptive mechanisms along with yield performance of pea varieties under the influence of seed priming substances. The performances of selected seed priming substances in different pea varieties were tested with water stress. Growth and physiological parameters documented at the stress period. From the experiment, it can be inferred that seed priming substances like KH_2PO_4 (1.5 and 3%), H_2O_2 (10 mM) and PEG (5%) were significantly outperformed in inducing higher growth with positive physiological changes.

Introduction

North-Eastern Hill Region (NEHR) of India is located in most vulnerable and fragile ecosystem experiencing the invariable occurrence of major abiotic stresses such as low moisture stress, soil acidity and low temperature under changing the climate. These abiotic stresses relentlessly restrict the crop production during post-monsoon season in *rabi* crops. Moreover, the attaining potential yield and successful cultivation of *rabi* crops are determined by vigorous germination and crop establishment under this stressful environment. In order to increase the productivity of these crops, pre-sowing seed invigoration techniques have proven promising technologies for sustaining productivity with minimum stress damage. Drought and soil acidity being edaphic and meteorological events exerts an irreversible adverse effect on seed physiological and biochemical reactions thereby reducing the overall germination and establishment of several legume and vegetable crops during *rabi* season. This is mostly due hindrance to initial steps of germination like inhibiting the absorption of adequate water, or by exposing to moderate to severe toxic effects of Al and Fe which are abundant in acidic soil. Altogether, plant water relations and osmotic regulation under imminent low moisture and soil acidity substantially hamper seed germination resulting in improper crop establishment.

Germination process is associated with many metabolic, cellular and molecular events that are well coordinated by an array of complex regulatory framework of biochemical reactions. Seed priming is a pre-sowing approach to enhance seed physiological performance (Yanglem *et al.* 2016), thereby enabling better crop establishment in many fields crops (Hussain *et al.* 2006) and several vegetables (Rouhi *et al.* 2011). For attaining proper seed germination, a suitable temperature and presence of sub-optimal oxygen is very much required along with appropriate hydration environment. In natural situations, dormancy eventually required to control precocious germination according to seasonal variation and plant programming. In an ideal crop production

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DOI: https://doi.org/10.3329/bjb.v50i2.54074

system, plant stand establishment determines the density, uniformity and management options of cultivation. Chemical compounds having both positive and negative effects on germination, can alter the germination percentage in greater way and thereby manifests their seed behaviour for enhanced crop performance (Abbasi *et al*. 2012). Variation in the results depends on ambient temperature, priming duration, the concentration of the priming substances and crop type (Jeong *et al*. 2000 a,b). Seed priming may increase or decrease the activity of many metabolic enzymes that counteracts the effects of lipid peroxidation and cellular stress damage. Thus, Jeng and Sung (1994) found that free-radical scavenging enzymes were increased by increasing hydration of peanut seeds upon seed invigoration.

The soil of north-eastern region of India is inherently acidic and undergo sinimical water stress condition during the active growth period of pea crop as farmers of the region mainly cultivate pea from the second fortnight of October or first fortnight of November. Therefore, the simple and reliable techniques like seed priming are very pertinent for having a substantial plant stand. Keeping the above points in view, this study was formulated to investigate the effect of seed priming on the performance of root and shoot growth and other morpho-physiological behaviours of pea crop under moisture stress condition of acidic soil.

Materials and Methods

Effects of seed priming on root, shoot and plant growth were evaluated by soaking surface sterilized seeds in two sets of each working solution *viz.*, 2.5 and 5% PEG (Polyethylene glycol-6000), 0.05 and 0.075% $ZnSO_4$ (Zinc sulphate), 10 and 50 mM H_2O_2 (Hydrogen peroxide), 1.5 and 3% KCl, 1.5 and 3% KH_2PO_4, 2 and 2.5% KNO_3, 1 and 2 µM ABA (Abscisic acid), 10 and 20% of 596-1 (bioextract-1), 10 and 20% of RF-79 (bioextract-2) for 24 hrs and hydro priming as control carried out with two pea variety I-10 (V_1) and GS-10 (V_2) produced and certified by Durga Seed Co. and NSCO Co., respectively which was procured from commercial outlets based at Shillong, Meghalaya (India). All the seed priming chemicals used for study are manufactured by Himedia Pvt. Ltd. except PEG (Polyethylene glycol-6000) which was procured from Sisco Research laboratory Pvt. Ltd. and ABA from Sigma-Aldrich while bio-extract formulations were produced by weed science laboratory of Division of Crop Production, ICAR Research Complex NEH Region, Umiam. For control, seeds were soaked in distilled water for the same period and growing condition.

Primed seeds were transferred to standard size plastic pots filled with sieved (2 mm size) acid soil and the whole set of experiment was undertaken under growth condition of plant growth chamber of ICAR RC NEH Region, Umiam, Meghalaya (India) in 2014 and 2015 (for two seasons) and replicated thrice. This pot culture experiment was conducted for 60 days by maintaining standardized environmental conditions *viz.*, temperature at 20^0C, relative humidity of 65% and 11 hours day length for optimum growth of the test crop. The recommended dose of FYM and fertilizers were applied as basal and half dose of urea was applied at 45 days after transplanting. Pots were watered at same day's interval according to field capacity of the soil to check the ability of different primed treatments and pea varieties.

Initial soil data pertaining to soil moisture parameters like the field capacity, permanent wilting point, bulk density, particle density and porosity were 38.25 and 17.17%, 1.27 g/cm^2, 2.24 mg/m^2 and 43.43%, respectively recorded before sowing of crop while water holding capacity recorded was 31.24%. The soil chemical composition for available nitrogen was high (263.78 kg/ha), low in available phosphorus (16.56 kg/ha) and low in available potassium (165.15 kg/ha) with moderate level acidity in reaction (pH 4.49). The electrical conductivity of soil was 0.18 dS/m at 25^0C and initial organic carbon content was 2.82 per cent.

Plant height of pea was measured in cm from ground level up to the terminal node of the main shoot. The numbers of functional leaves plant-1 were counted from each pot treatment. For chlorophyll estimation, 0.5 g of matured leaves from the tip was sampled and grounded with 25 ml of 80 : 20 acetone : water solution. The homogenate was filtered through Whatman 42 filter paper. After filtration within 30 min, absorbance readings were measured at 648, 652 and 663 nm in Spectronic-20 spectrophotometer for deriving chlorophyll 'a', chlorophyll 'b' and total chlorophyll. Further calculations were done by using a modified method of Misyura *et al.* 2013. However, leaf carotenoid was measured by Kirk and Allen, 1965 formula from the same aliquot of chlorophyll was assessed by taking absorbance at 480, 645 and 663 nm.

Chlorophyll 'a' (mg/g FW) = ((12.72 * A663) – (2.58 * A645)) * V/1000 * 1/W
Chlorophyll 'b' (mg/g FW) = {(22.87 * A645) – (4.67 * A663)}* V/1000 * 1/W
Chlorophyll 't' (mg/g FW) = {(20.29 * A645) + (8.05 * A663)}* V/1000 * 1/W
Car 480 (µg/g FW) = (A480 + 0.114*A663 – 0.638*A645)*(v/w)

RWC was calculated by using the formula given by Barrs and Weatherley (1962) and expressed in percentage

$$\text{RWC} = \frac{\text{Fresh weight (g)} - \text{dry weight (g)}}{\text{Turgi weight (g)} - \text{dry weight (g)}} \times 100$$

Lipid peroxidation was calculated in terms of amount of melondialdehyde (MDA) production as a decomposition by-product under the impact of different stresses. The MDA content was determined by the reaction of thiobarbituric acid (TBA), as described by Yagi (1987) with minor modifications. Briefly, leaf tissue was collected and homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 rpm for 5 min, 1 ml of solution containing (4% (w/v) TCA + 0.5% TBA (w/v) was added to 0.5 ml of supernatant. The mixture was heated at 95^0C for 1 hr, then cooled down to room temperature and centrifuged at 10000 rpm for 5 min. The absorbance of clear solution was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA content was calculated by using its absorption coefficient of 155 nmol/cm.

Plants were separated from each treatment by reversing and gentle tapping the pot to loosen the whole soil without disturbing intact root system of plant and were allowed for smooth flush of water flow with hand washing and removing adhering soil. After washing and shade drying for 30 min under laboratory condition, the root was separated from shoot and evenly spread on a transparent fibre tray (30 cm × 20 cm) and taken inside the scanner (Epson v 700 perfection) for root imaging at a resolution of 200 dpi (dots per inch). The resulting images were imported and digitized by using the WinRHIZO professional software program manufacturer's Regent Instrument Canada Inc., Quebec, Canada. Root images were analyzed for root surface area (RSA), total root length (TRL), root volume (RV) and root diameter (RD). Both Regent's non-statistical method and Tennant's statistical method were chosen to perform root morphology measurements in WinRHIZO. The large root sample was subdivided into smaller sub-samples before scanning, to avoid a high scanning density (Bauhus and Messier 1999).

After determining fresh weight of plant sample (root and shoot separately) was first dried for some time and kept inside a hot air oven at 60^0C or till attainment of constant weight. The roots were rinsed for 3 - 4 times with deionized water before subjected to drying. Dry matter weight for root and shoot part was recorded separately and total dry matter accumulation was also calculated per plot basis.

The required statistical analysis of data recorded in the above investigation which was based on RCRD for standardization parts has been undertaken using SPSS software (version 19). Treatment means were compared using Duncan's multiple comparison tests at 5% level of probability.

Results and Discussion

The pooled data from both years of investigation were averaged for every parameter and interpreted. Firstly plant height and functional leaf number were recorded at 60 days after sowing (at flower initiation) was significantly influenced by different varieties and seed priming substances (Table 1). Among the pea varieties, I-10 (V_1) were recorded higher plant height and number of functional leaves than GS-10 (V_2). Seed priming with KH_2PO_4 at 3% and PEG at 5% significantly enhanced plant growth parameters whereas seed priming with KCl and hydropriming reduced the plant growth parameters compared to other seed priming substances. It could be due to the fact that priming seeds with the above-mentioned substances would vary the minute levels of cytokinin and auxin contents and may cause auxiliary bud dominance and increased plant growth. These results are in agreement with the findings of Yucel (2012), Cokkizgin (2013) and Kaur *et al.* (2015).

The experimental data on chlorophyll a, b, total chlorophyll and carotenoid content as influenced by pea varieties and seed priming substances are presented in Table 1. However, it was not influenced significantly among pea varieties. In seed priming treatment, significantly higher chlorophyll a content was found at 1.5% KH_2PO_4 which was followed by both the concentrations of ABA. However, chlorophyll b content was recorded significantly higher at 1 µM ABA which was found at par with 2 µM ABA, 5% PEG, 10 mM H_2O_2, 1.5% KH_2PO_4 and bio-extracts. Moreover, significantly lower total chlorophyll content was recorded at 1.5% KH_2PO_4 which was found at par with both the concentrations of ABA and 10 mM H_2O_2. The pooled data on carotenoid content of seed priming treatment as indicated significantly influenced. The significantly higher carotenoid content was recorded at 1.5% KH_2PO_4 and found at par with 1 µM ABA. However, significantly lower carotenoid content was recorded at both the concentrations of $ZnSO_4$ and hydropriming. Chlorophyll and carotenoid contents were also found higher under primed seed with 1.5% KH_2PO_4 and ABA treatments. This might be due to increasing nitrogen translocation and greater assimilation of light which leads to increase in the number of chloroplasts in leaves. Due to water deficit condition, chlorophyll and carotenoid content were reduced. In fact, chloroplast thylakoid membranes became more sensitive under stress as delicate envelope is damaged causing leakage of chloroplast content and early senescence or chlorophyll degradation. Din *et al.* (2011) reported that due to water stress chlorophyll content of leaves are reduced. Bejandi *et al.* (2009) also reported that relative water content as well as chlorophyll content is affected by seed priming in soybean.

Relative water content of leaves at 60 days after sowing as influenced by different pea varieties and seed priming is presented in Table 1 and expressed in percentage. Significantly higher relative water content was recorded in the leaves of plants of pea variety I-10 (V_1) and seed primed with 10 mM H_2O_2 and 2 µM ABA. However, significantly lower RWC was recorded in plants with hydro-priming, both the concentrations of $ZnSO_4$ and bio-extracts than other priming treatments. The maximum leaf relative water content was found in I-10 pea variety which might be due to positive effect on plant growth and development. In general, relative water content of leaves was reduced due to water stress condition. This might result in reduced water translocation

Table 1. Pooled data on growth parameters, leaf chlorophyll contents, carotenoid content and water stress indicators of pea as influenced by different priming substances and varieties.

Treatment	Plant height (cm)	No. of functional leaves	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (µg/g FW)	Relative water content (%)	MDA content (µ mol/g FW)
Varieties								
I-10	19.98a	3.47a	0.57a	0.32a	0.93a	25.97a	73.82a	35.75a
GS-10	17.81b	2.74b	0.61a	0.34a	1.00a	26.98a	67.52b	37.04a
Priming								
PEG (2.5%)	19.33abc	3.00cde	0.46def	0.28bcde	0.79ef	24.61def	69.49cdefg	33.54cde
PEG (5.0%)	19.50abc	4.00ab	0.44ef	0.33abcde	0.81ef	21.94ef	73.29abcd	33.48cde
$ZnSO_4$ (0.05%)	18.58abcd	3.33abcd	0.37f	0.24de	0.63f	16.59f	67.43defg	38.10bcde
$ZnSO_4$ (0.075%)	17.33cd	3.17bcde	0.38f	0.30bcde	0.73ef	20.97ef	68.64defg	34.04cde
H_2O_2 (10 mM)	19.58abc	3.50abc	0.78abc	0.45ab	1.29abcd	32.85abcd	76.23abc	34.19cde
H_2O_2 (50 mM)	18.33abcd	3.17bcde	0.69bcd	0.31bcde	1.05cde	26.65cdef	70.64bcdef	39.36bcd
KCl (1.5%)	15.75d	2.83cde	0.43ef	0.30bcde	0.78ef	22.80def	66.47defgh	41.72ab
KCl (3.0%)	17.25cd	2.50de	0.57cdef	0.25cde	0.87ef	26.20cdef	59.85h	34.39cde
KH_2PO_4 (1.5%	21.08ab	3.50abc	0.98a	0.41abcd	1.46a	40.99a	73.97abcd	31.43ef
KH_2PO_4 (3.0%)	21.42a	4.17a	0.62bcde	0.27bcde	0.94def	25.19cdef	73.55abcd	26.39f
KNO_3 (2.0%)	19.17abc	2.67cde	0.49def	0.29bcde	0.84ef	20.77ef	64.48fgh	33.43cde
KNO_3 (2.5%)	19.42abc	2.83cde	0.50def	0.26cde	0.81ef	23.63def	76.34abc	32.45def
ABA (1 µM)	19.75abc	3.17bcde	0.82ab	0.49a	1.38abc	37.14ab	77.42ab	38.82bcd
ABA (2 µM)	19.83abc	3.17bcde	0.93a	0.43abc	1.43ab	35.31abc	78.49a	37.69bcde
Bio extract 1 (10%)	18.33abcd	2.33e	0.47def	0.35abcde	0.88ef	23.25def	62.40gh	39.67bc
Bio extract 1 (20%)	18.25bcd	2.67cde	0.63bcde	0.33abcde	1.01def	28.28bcde	67.33defg	46.65a
Bio extract 2 (10%)	18.17bcd	3.17bcde	0.56cdef	0.36abcde	0.97def	25.71cdef	73.02abcde	38.91bcd
Bio extract 2 (20%)	20.17abc	3.33abcd	0.68bcd	0.36abcde	1.09bcde	29.00bcde	78.14ab	37.80bcde
Hydro priming (Control)	17.75cd	2.50de	0.44ef	0.19e	0.66f	21.15ef	65.59efgh	39.35bcd

Figures not sharing the same letters in the same column differs significantly at $p < 0.05$.

Table 2. Root morphological parameters, mean shoot dry weight, root dry weight and total dry matter of pea as influenced by priming substances and varieties before and after moisture stress period.

Treatment	Surface area (cm^2)	Total root length (cm)	Root volume (cm^3)	Root diameter (mm)	Shoot dry weight (g)	Root dry weight (g)	Total dry matter (g)
Varieties							
I-10	87.54a	94.91a	1.72a	1.19a	0.43a	0.09a	0.52a
GS-10	75.46b	72.74b	1.20b	1.12b	0.27b	0.09a	0.36a
Priming							
PEG (2.5%)	87.00c	100.66bcd	1.67bcd	1.43a	0.34abcde	0.07de	0.41cdef
PEG (5.0%)	95.01b	91.29bcde	2.15b	1.25abc	0.42ab	0.10bcd	0.52abc
$ZnSO_4$ (0.05%)	69.63hi	72.87defgh	1.00d	1.23abc	0.22f	0.08bcde	0.30g
$ZnSO_4$ (0.075%)	68.65i	95.11bcd	1.05d	1.24abc	0.28def	0.09bcd	0.37efg
H_2O_2 (10 mM)	89.71bc	93.38bcde	1.83bcd	1.31ab	0.44a	0.10abc	0.54ab
H_2O_2 (50 mM)	92.70b	81.85cdefg	1.54bcd	1.16bcd	0.39abc	0.10bcd	0.49abcd
KCl (1.5%)	77.42ef	49.03h	1.35bcd	1.07cde	0.29def	0.08cde	0.37efg
KCl (3.0%)	79.39ef	53.67gh	1.04d	1.17bcd	0.32cde	0.07de	0.39defg
KH_2PO_4 (1.5%	114.78a	139.23a	2.05bc	1.04cde	0.42ab	0.11ab	0.53ab
KH_2PO_4 (3.0%)	90.87bc	111.90abc	3.25a	1.03cde	0.43ab	0.13a	0.56a
KNO_3 (2.0%)	74.55fgh	63.28efgh	1.10d	0.99de	0.25ef	0.06e	0.31fg
KNO_3 (2.5%)	68.20i	121.12ab	0.99d	1.00de	0.35abcd	0.09bcd	0.44bcde
ABA (1 µM)	68.75i	96.45bcd	1.46bcd	0.93e	0.28def	0.07de	0.35efg
ABA (2 µM)	85.66cd	82.24cdefg	1.16cd	1.00de	0.39abc	0.11ab	0.50abc
Bio extract 1 (10%)	81.14de	56.19fgh	1.35bcd	1.07cde	0.33bcde	0.10bcd	0.43bcde
Bio extract 1 (20%)	76.08efg	59.58fgh	1.13cd	1.16bcd	0.28def	0.08cde	0.35efg
Bio extract 2 (10%)	71.60ghi	87.04cdef	1.31bcd	1.30ab	0.43ab	0.10bcd	0.52abc
Bio extract 2 (20%)	86.70c	80.59defg	1.46bcd	1.32ab	0.44a	0.09bcd	0.52abc
Hydro priming (Control)	70.54hi	57.25fgh	1.14cd	1.18bcd	0.37abcd	0.07de	0.44bcde

Figures not sharing the same letters in the same column differs significantly at $p < 0.05$.

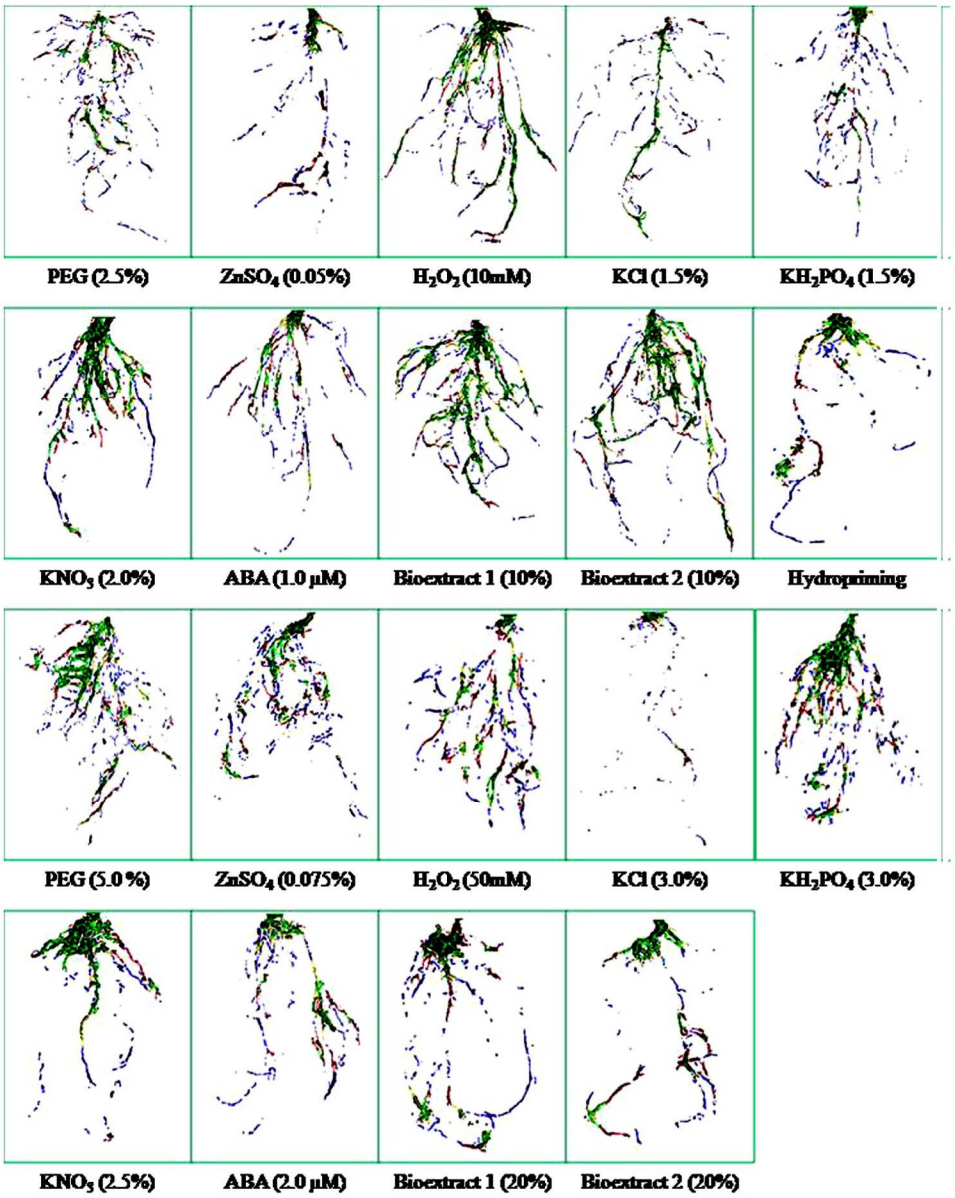

Fig. 1. Root morphology of pea variety V_1 (I-10) as influenced by different seed priming treatments at resolution of 200 dpi (dots per inch).

which leads to reduction of water content of leaves. Many important physiological and morphological processes, such as leaf enlargement, stomatal opening and associated leaf photosynthesis are drastically affected by smaller variation of leaf turgor potential, which accompanies the loss of leaf water and rate of uptake by varied root morphology as influenced by seed priming substances (Abenavoli *et al.* 2015).

The pooled data on leaf MDA content (expressed as µmol/g FW) as influenced by different varieties and seed priming are presented in Table 1. The observed data on pea varieties have indicated non-significant changes in leaf MDA content whereas seed priming exerted significant influence on crop. Significantly higher MDA content was noticed in 20% of 596-1(bio-extract-1) which was found at par with 1.5% KCl. However, significantly lower MDA was recorded in both the concentrations of KH_2PO_4. MDA content, a secondary end product of polyunsaturated fatty acid oxidation, was used to measure the extent of lipid peroxidation. Increased MDA is the result of ability to scavengeing reactive oxygen species (ROS). This possible mechanism was later supported by lesser relative water content confirming observations of present study. In general, irrespective of variety, seed priming reduced MDA accumulation. Zheng *et al.* (2008) in wheat plants found seed priming treatment reduced MDA content in salt stress condition.

The averaged root morphology studies like root surface area (RSA), total root length (TRL), root volume (RV) and root diameter (RD) as influenced by different varieties and seed priming are presented in Table 2 and in Fig. 1 for V_1. The priming effect in pea varieties significantly influenced the root morphology parameters. Significantly higher RSA, TRL, RV and RD were recorded at I-10 (V_1). Significantly higher RSA and TRL were recorded at 1.5% KH_2PO_4 while TRL was found at par with 3% KH_2PO_4 and 2.5% KNO_3. However, significantly higher RV was recorded at 3% KH_2PO_4. Moreover, higher RD was recorded at 2.5% PEG and found at par with 5.0 % PEG, both the concentration of 20% RF-79 (bioextract-2) and $ZnSO_4$ and 10 mM H_2O_2. In the present study, root growth was strongly enhanced in the treatments where KH_2PO_4 was primed, whereas PEG and RF-79 (Bioextract-2) also showed the positive effect on root growth while this positive response and the lack of effect on P uptake confirmed the present findings. This might be due to the fact that K uptake did not respond to the spatial allocation of root growth which means that some mechanism involved in K uptake is compensating for the variation in root density. That root length was increased with KH_2PO_4 treatment over untreated control was reported by Ramalal *et al.* (1993). Root morphological traits increased by application of seed priming substances like PEG and KNO_3 solution were reported by the Abdnadani and Ramezani (2012). Similarly, Shehzad *et al.* (2012) reported that the priming treatment significantly increased number of roots and root length. Phosphorus is a major element being limited in acid soil used for the present experiment is equally important in legume nutrition to favour healthy root growth by helping in translocation of carbohydrates, promoting early seed setting and rendering positive effect in achieving increased seed yield (Krishnappa and Hussain 2014).

Dry weight of shoot, root and total dry weight (g/plant) as influenced by different pea varieties and seed priming treatments is presented in Table 2. Pooled data on pea varieties indicated significant effect by shoot dry matter accumulation of plant. Significantly higher shoot dry matter accumulation was recorded in pea variety I-10 (V_1) irrespective of seed priming. Seed priming treatments significantly influenced the dry matter accumulation. Seed priming with H_2O_2 (10 mM) showed higher shoot dry weight and found with ten more priming treatment, namely both the concentration of PEG, KH_2PO_4, RF-79, 50 mM H_2O_2, 2.5% KNO_3, 2 µM ABA and hydropriming. In case of root dry weight, at seed priming with 3% KH_2PO_2 higher values and at par with 10 mM H_2O_2 and 2 µM ABA was recorded. Similar trends were found in total dry matter accumulation, 3% KH_2PO_4 was recorded higher than other priming treatments at par with 5% PEG, both the concentration of H_2O_2 and RF-79, 1.5% KH_2PO_4 and 2 µM ABA. This might be because of efficient mobilization and utilization of seed reserves and better genetic repair, i.e., earlier and faster synthesis of DNA, RNA and protein which leads to increase in cell cycle and cell division processes within apical meristem and cause elongation in growth due to faster production of germination metabolites (Basra *et al.* 2005).

It may be concluded that seed priming enhance the growth of pea crop in acid soil. There is a need to carry out further experiments to understand physiological, shoot and root behaviour and plant growth due to seed priming in pea in future.

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(Manuscript received on 13 June 2018; revised on 25 July, 2020)