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EFFECT OF SEED PRIMING ON GERMINATION AND INITIAL SEEDLING GROWTH OF PEA (*PISUM SATIVUM* L.) CULTIVARS

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ABSTRACT

To assess the effect of various seed priming substances on pea germination and seedling growth, a laboratory investigation was carried out at mid altitude of Meghalaya (India). The treatments consist of three elite pea cultivars and nine priming substances at two concentrations each and the treatment combinations replicated thrice. Germination percentage, seed vigour index, germination rate, seedling dry weight and seedling fresh weight were varied from 19.11 to 87.10%, 564.23 to 10516.99, 29.48 to 88.89%, 25.44 to 119.72 mg and 0.46 to 0.63 g, respectively. In addition, root length and shoot length data recorded showed the range of 0.68 to 4.08 cm and 0.41 to 1.46 cm respectively, with significant growth changes across the priming substances. There was significantly positive and highly correlation recorded between germination percentage and seedling vigour index with others parameters *viz*., root length, shoot length, seedling dry weight and seedling fresh weight. However, seeds of I-10 pea cultivar responded significantly superior for seed priming over rest two pea cultivars. Among various priming materials tested both concentration of PEG, $ZnSO_4$, H_2O_2, KH_2PO_4 and bio-extract RF-79 were found more efficient for enhancing germination, seedling vigour and initial seedling growth promotion in pea.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the important *rabi* season pulse crops of the world which acts as very good source of vegetative protein for human and animal nutrition. In crop production, field stand establishment determines the plant density, uniform growth, crop management options and productivity of land. For expensive crop like pea, it is particularly important that seed germinate rapidly and uniformly, tolerate adverse germination conditions and produce healthy seedlings (Zhiyuan and Kent, 1999). Seed priming is one of the techniques widely employed to ensure good crop establishment with optimum plant population as several research studies on priming treatments have been reported with positive advantage of enhancement of germination percentage under adverse conditions (Peyvast *et al.,* 2010).

Germination is complex process with regulatory network of many metabolic, cellular and molecular events inside the seed residing at below ground. Seed germination is usually the most critical stage in seedling establishment, determining the success of crop production especially under adverse field conditions (Almansouri *et al.,* 2001). Seed priming is one the improved seed invigoration techniques are used in the world to reduce the germination time, to synchronize germination, to improve germination rate and increase overall plant stand (Lee and Kim, 2000). Seed priming adopted as pre-sowing strategy technique by various farmers to enhance the seed performance, notably with respect to rate and uniformity of germination and seedling performance (Bradford, 1986; Taylor and Harman, 1990), thereby enabling better crop establishment in several seed species (Singh 1995), in many field crops (Chavan *et al.,* 2014; Donaldson *et al.,* 2001; Farooq *et al.,* 2006; Hussain *et al.,* 2006) and in horticulture crops (Dutta *et al.,* 2015; Janmohammadi *et al.,* 2009; Parma *et al.,* 2016; Rai and Basu, 2014; Rouhi *et al.,* 2011).

Most priming treatments involve imbibing seed with restricted amount of water to allow sufficient hydration and advancement of metabolic processes but preventing immediate germination and loss of desiccation tolerance. This process generally causes faster germination and quicker field emergence which has significant practical agronomic implication particularly under adverse germination and soil condition (Black and Bewley, 2000). So therefore, present study has been undertaken with the aim to assess suitability priming substances with right concentration and to select good pea cultivar for higher germination percentage and seedling vigour index.

MATERIALS AND METHODS

Experimental materials

To find out the effect of priming material/s and their concentration a instead of laboratory-satandardisation study was undertaken at Plant Physiology Laboratory of ICAR Research Complex for NEH Region, Umiam using circular petridish of 9 cm diameter. Truthfully labelled seeds of three pea cultivars viz., Imported-121 (V_1), GS-10 (V_2) and I-10 (V_3)

commercially available (Sultan seed Co, NSCO Co and Durga Seed Co.) at farm outlets of Shillong, Meghalaya. The effects of seed priming was evaluated by soaking surface sterilised seeds in solutions of 2.5% and 5% PEG (Polyethylene glycol-6000), 0.05 % and 0.075% $ZnSO_4$ (Zinc sulphate), 10 mM and 50 mM H_2O_2 (Hydrogen peroxide), 1.5 and 3% KCl (Potassium chloride), 1.5 and 3% KH_2PO_4 (Potassium dihydrogen orthophosphate), 2% and 2.5% KNO_3 (Potassium nitrate), 1 µM and 2 µM ABA (Abscisic acid), 10% and 20% bio-extract 596-1, 10% and 20% bio-extract RF-79 for 24 hours and hydro priming treated as control. All the seed priming chemicals are manufactured by Himedia Pvt. Ltd. except PEG (Polyethylene glycol-6000) by Sisco Research Laboratories Pvt. Ltd and ABA (Abscisic acid) from Sigma-Aldrich while bio-extract solutions were produced at weed science laboratory of Division of Crop Production, ICAR Research Complex NEH Region, Umiam. For hydro-priming seeds were soaked in distilled water for the same period and under same conditions. All priming solutions were prepared in appropriate glassware with distilled water and stored under refrigerated conditions for further use.

The treated seeds after seed priming treatments were then washed with distilled water and transferred to sterilised petri dish containing moistened germination paper. About 15 treated seeds were spread on each petriplate and each treatment replicated thrice. Electrical conductivity (EC) of all the priming solutions at initial level and after 24 hours of seed priming was measured by using EC Bridge (ELICO). Everyday all of the Petri dish is watered by distilled water.

Germination test

Primed seeds were allowed to germinate and grow at 25°C for 7 days under laboratory conditions. Radical protrusion of 2 mm length has been scored as germination (Kaya *et al.,* 2006). Observations on germination was counted in 24 hours interval and continued til no further germination occurred. Total germination percentage (%), seedling vigour index, germination rate, seed dry weight and seed fresh weight changes, root length and shoot length variations have been recorded at the end of incubation (7days).

Germination percentage

Germinated seeds were counted routinely according to the germination evaluation procedure mentioned in the Handbook of Association of Official Seed Analysts (AOSA, 1983). The number of germinated seeds was recorded and germination percentage (after 7 days of seed placement) was calculated according standard formulae as mentioned below (Tanveer *et al.,* 2010).

$$\text{Germination percentage (\%)} = \frac{\text{No. of Germinated seeds}}{\text{No. of Germinated seeds at 120 hrs}} \times 100$$

Seedling Vigour index

The Seedling Vigour Index (SVI) was calculated using AOSA (1990) formula as given below-

Seedling vigour index (SVI) = [Seedling dry weight (mg) × Germination percentage]

Germination rate (%)

The germination rate was calculated by using following formula as indicated below by Krishnasamy and Seshu (1990)-

$$\text{Germination rate (\%)} = \frac{\text{No. of Germinated seeds at 48 hrs}}{\text{No. of Germinated seeds at 120 hrs}} \times 100$$

Seedling fresh weight (SFW) and dry weight (SDW)

Randomly five seeds were selected at 7 days after seed priming for recording fresh weight. Before weighing excess moisture present on the surface of the seed was removed using tissue paper. The mean values were calculated and expressed as fresh weight per seeds. Then, seed were kept for oven drying at 60°C for 48 hr. Dry weight of tissue has been recorded when there is no change in dry weight upon further drying. Then the mean value was calculated for all 25 seeds present in petri plate.

Root length (RL), shoot length (SL) and root to shoot length ratio (RSLR)

Five normal seedlings were selected randomly in each treatment from all the replications on seventh day from germination test to record seedling growth parameters *viz.,* root length, shoot length and root to shoot length ratio. Root length recorded from base of radical till the tip of root vertically. Shoot length also recorded from base of coleoptiles till the tip of shoot vertically. Root to shoot length ratio calculated by dividing root length from shoot length of respective seedling.

Statistical analysis

The required statistical analysis of data recorded in the above investigation which was based on completely randomized block design (CRD) has been undertaken using SPSS software (version 19). Treatments means were compared using Duncan's multiple comparison tests at 5% level of probability.

RESULTS AND DISCUSSION

Data pertaining seed germination percentage, seed vigour index, germination rate, seedling dry weight, fresh weight per seed, root length, shoot length and root to shoot ratio as influenced by different treatments are presented in Table 1 and graphically depicted in Fig. 1.

Germination percentage

Germination percentage was significantly affected by different priming treatments in three elite pea cultivars. Maximum germination percentage of all cultivars was recorded higher in the priming treatment with PEG at 2.5% followed by $ZnSO_4$ at 0.075%. Minimum germination percentage was found in both concentration of KNO_3 followed by both concentration of KCl priming treatment. The germination response of I-10 pea cultivar was recorded superior than other two pea cultivars which were at par with each other. In the similar way, Kaur *et al.* (2015) also revealed that osmo-priming with 5% PEG for 24 hours for enhancing seedling emergence and better seedling growth in okra. Further, McDonalds (2000) reported that seed priming with PEG would be more suitable and preferable as it is inert molecule with minimum adverse effects on germination and subsequent seedling growth. Beneficial effect of seed priming with PEG 300 g $litre^{-1}$ in sorghum was also observed by Tiryaki and Buyukcingil (2009) resulted in better germination percentage. In addition, few priming compounds may also have negative or inhibition effects on

Table 1: Effect of seed priming substances on germination and seedling growth of pea cultivars

Treatment	Germination percentage (%)	Seed vigour index	Germination rate(%)	Seedling dry weight per seed (mg)	Fresh weight per seed (g)	Root length (cm)	Shoot length (cm)	Root shoot length ratio
A. Pea Cultivar								
V_1	68.03b	6524.36b	73.43b	87.85b	0.55b	2.86a	1.06a	2.58a
V_2	67.12b	6556.95b	74.19b	90.27b	0.57a	2.87a	1.09a	2.44ab
V_3	78.70a	9107.03a	81.87a	107.06a	0.58a	2.77a	1.19a	2.16b
B. Priming								
PEG (2.5%)	87.11a	10516.99a	87.49ab	119.72a	0.63a	3.92ab	1.46a	2.71ab
PEG (5.0%)	79.56 abc	8646.94abc	87.23ab	106.44abc	0.59ab	3.34abcd	1.30abc	2.61abc
$ZnSO_4$ (0.05%)	84.89 a	9295.71abc	88.89ab	107.88abc	0.60a	3.12abcd	1.19abcd	2.69ab
$ZnSO_4$ (0.075%)	87.11 a	9835.90ab	81.23abc	111.36ab	0.62a	3.43abcd	1.20abcd	2.97ab
H_2O_2 (10mM)	82.67 ab	9771.23ab	82.94abc	116.19ab	0.62a	4.08a	1.26abc	3.24a
H_2O_2 (50mM)	82.67 ab	9436.69abc	77.13abc	110.91ab	0.61a	3.49abc	1.34abc	2.59abc
KCl (1.5%)	66.67 cd	6991.33cd	69.38abcd	93.35bc	0.53bcd	2.36cde	0.96cd	2.17bcd
KCl (3.0%)	52.44 e	4234.13e	29.48e	69.58d	0.52cde	1.15efg	0.59ef	1.26de
KH_2PO_4 (1.5%)	80.00 abc	8661.35abc	77.68abc	106.22abc	0.59ab	3.29abcd	1.29abc	2.45abc
KH_2PO_4 (3.0%)	77.78 abcd	8507.10abc	72.40abc	107.16abc	0.59ab	3.35abcd	1.34abc	2.48abc
KNO_3 (2.0%)	37.56 f	1989.49f	89.16ab	44.49e	0.47de	0.96fg	0.45f	1.08e
KNO_3 (2.5%)	19.11 g	564.23f	51.62d	25.44e	0.46e	0.68g	0.41f	1.65cde
ABA (1 μM)	76.89 abcd	7596.60bcd	69.11bcd	96.14abc	0.57abc	3.75abc	1.33abc	2.78ab
ABA (2 μM)	68.00 bcd	6809.40cd	65.68cd	97.98abc	0.58abc	3.07abcd	1.18abcd	2.65abc
Bio extract 596-1 (10%)	64.44 de	5745.11de	66.55cd	86.00cd	0.52bcde	2.06def	0.82de	2.68ab
Bio extract 596-1 (20%)	72.89 abcd	7148.15cd	83.65abc	96.92abc	0.56abc	2.54bcd	1.01bcd	2.53abc
Bio extract RF-79 (10%)	79.11 abc	8759.25abc	90.14a	108.97abc	0.61a	2.88abcd	1.39ab	2.18bcd
Bio extract RF-79 (20%)	77.33 abcd	7875.09bcd	80.05abc	98.07abc	0.58abc	3.52abc	1.34abc	2.63abc
Hydro priming (Control)	78.20 abcd	8141.48abcd	79.71abc	103.42abc	0.58abc	2.84abcd	1.33abc	2.12bcd

*Figures not sharing the same letters in the same column differs signiûcantly at $p < 0.05$

Figure 1: Response of seed priming substances on germination of pea cultivars with relative changes in radical and plumule lengths

germination of pea as indicated by present study with relative inhibition of pea seed germination under priming with high concentration of KNO_3 and KCl for 24 hrs which seemed to prolonged time. The above mentioned inorganic priming substances exposure for long time might induce negative effect in terms of non availability of water molecules for growing tissues in the seed like embryo and water molecule for hydration of stored tissue. The germination percentage was decreased with increasing osmotic potentials of some priming substances for prolonged time tested with PEG and hydropriming (Abbasi *et al.,* 2012).

Seed vigour index

Similarly, priming treatments was significantly influenced on seedling vigour index of pea cultivars used for study. Amongst the pea cultivars, commercially available I-10 pea cultivar responded well in terms of higher SVI. Significantly, maximum SVI recorded in seed priming with PEG at 2.5% and bioextract RF-79 as compared to others eight priming substances treatments (at two concentrations) followed by $ZnSO_4$ (0.075%) and H_2O_2 (10 mM) priming treatment. This might be due to significant increase in germination percentage and seedling dry weight and also due to removal of deleterious substances like inhibitory hormones, higher seed leachates at the time of seed invigouration. Similarly, the osmopriming (PEG and H_2O_2) and hydro priming have been reported to have promoted seed germination and seedling growth in rice although higher concentrations of PEG indicated negative effects on seed germination (Yuan-Yuan *et al.,* 2010). Besides Umair *et al.* (2013), found KH_2PO_4 proved to be the most appropriate priming treatments for mungbean and Sathish *et al.* (2011) reported same effect in maize. However, in the present study lowest SVI was observed in both the concentration of inorganic priming substances *viz.,* KCl and KNO_3. In the similar line Agawane and Parhe (2015), revealed that seed priming with 0.5% KNO_3 increase seedling vigour in soybean for 12 hr. While in the present study KCl and KNO_3 reported lowest might be due to either inhibitory effect of higher concentration of KCl and KNO_3 for longer duration of seed soaking in the solution rich in potassium and chloride radicals which might not be suitable for the pea.

Germination rate

An appraisal of data contemplates in Table 1 revealed that germination rate significantly influenced by different pea cultivar and seed priming treatments. Significantly superior germination rate was recorded in cultivar I-10 than rest of the cultivars. However, maximum germination rate was recorded

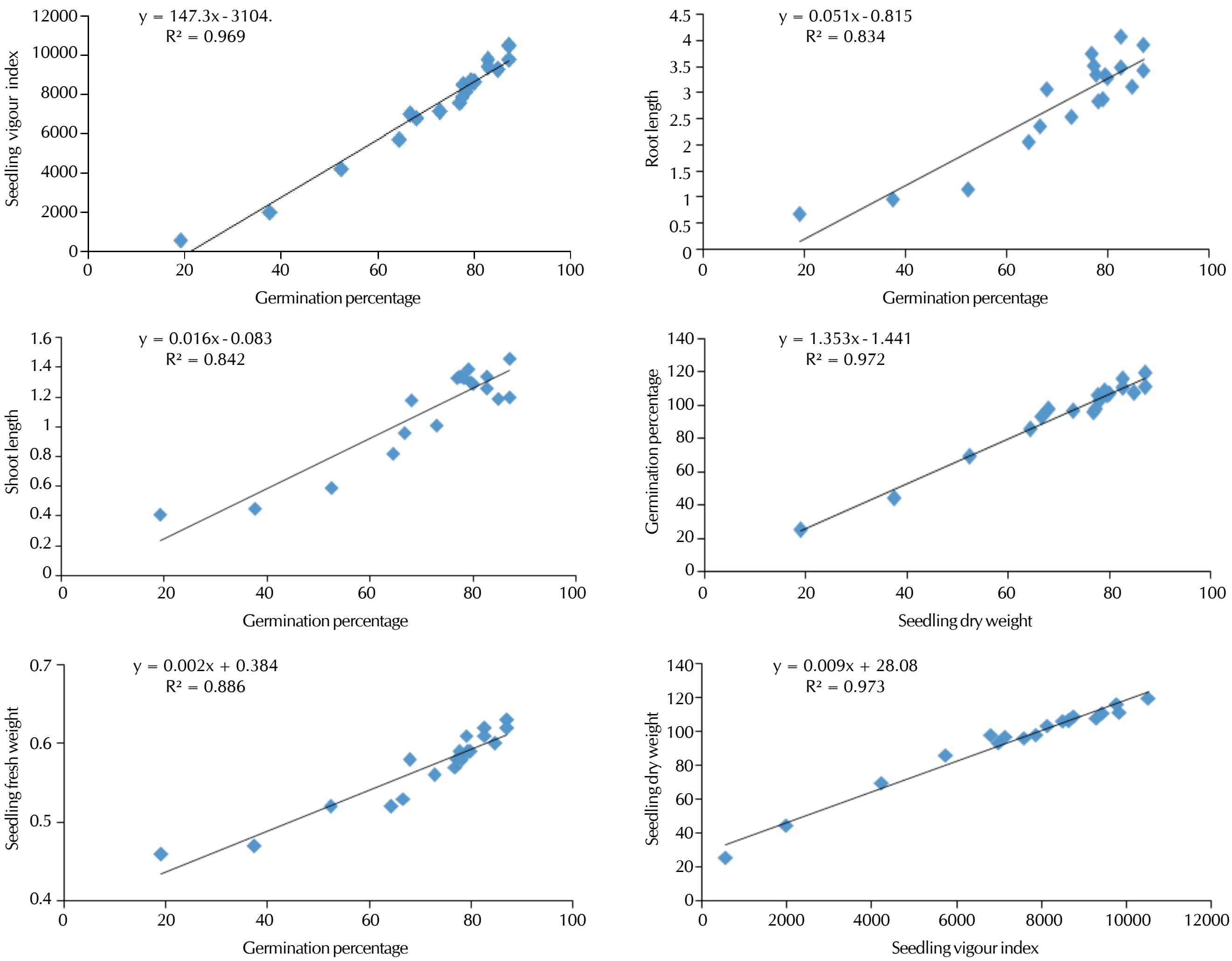

Figure 2: Relationship between different seed germination and seedling parameters: A) GP and SVI; B) GP and RL; C) GP and SL; D) GP and SDW; E) GP and seedling fresh weight; F) SVI and SDW

in seed priming with bio-extract RF-79 (10%) and at par with thirteen others treatments. However, significantly minimum values were was recorded in KCl (3.0%) treatment. Difference in germination rate might be due to variation in temperature, priming duration, concentration of the priming chemical and crop type (Jeong *et al.,* 2000 a & b).

Electrical conductivity

Electrical conductivity of seed was significantly affected due to priming treatment while there was no significant difference among seed cultivars both at initial and final reading (Table 2). Significantly higher EC found in both the concentration of KNO_3 whereas it had recorded still higher in 1.5 and 3.0% of KCl. While minimum initial EC was recorded in hydropriming and PEG (2.5 and 5.0%) solution.

Similar trends were found after 24 hours of priming. Significantly, higher EC were recorded at KNO_3 and KCl. However, lowest were observed at both the concentration of PEG, ABA and hydropring treatment. In the similar way, previous observations by Abdolahi *et al.* (2012) indicate that priming by KH_2PO_4 was effective than $CaCl_2$ in reducing the electrolyte leakage, mean germination time and increase in germination percent, seedling length, root length, seedling dry weight, seedling vigour index in aged seeds of all cultivars. In case of KNO_3 and KCl conductivity values were increased after soaking, which might be due to reduction of vigour index. Similarly, Verma and Singh (2001) showed that increase in conductivity values of the seed leachates at different soaking periods were related to the degree of deterioration of seed lots of Brassica species.

Seedling biomass changes

Seed priming substances have significant effect on seedling biomass related parameters *viz.,* seedling dry weight and fresh weight among all pea cultivars. Maximum response in increase in seed ling biomass changes was observed in I-10 pea cultivar, while response of GS-10 was found to be at par with I-10 in seedling fresh weight. Significantly higher seedling dry weight and fresh weight were recorded in seedlings primed with 2.5% PEG treatment (119.60 mg) and (0.63g) whereas lower values were found in 2.5% KNO_3 (25.60 mg) and (0.46 g), respectively. However, it was found at par with both the concentration of

Table 2: Electrical conductivity of different priming solution before priming and after priming of seed incubation period of 24 hours

Treatment	Initial EC (mS m^{-1})	Final EC (mS m^{-1})
A. Pea Cultivar		
V_1	10.598a	19.53a
V_2	10.598a	18.74a
V_3	10.598a	18.07a
B. Priming		
PEG (2.5%)	0.029r	1.14f
PEG (5.0%)	0.048q	0.79f
$ZnSO_4$ (0.05%)	0.097k	1.39f
$ZnSO_4$ (0.075%)	1.466e	2.26f
H_2O_2 (10mM)	0.608o	1.88f
H_2O_2 (50mM)	0.068l	1.54f
KCl (1.5%)	0.067m	58.52b
KCl (3.0%)	0.118j	105.31a
KH_2PO_4 (1.5%)	26.600d	22.58e
KH_2PO_4 (3.0%)	37.500c	41.92d
KNO_3 (2.0%)	60.800b	50.10c
KNO_3 (2.5%)	71.400a	58.62b
ABA (1 μM)	0.654n	1.17f
ABA (2 μM)	0.056p	1.15f
Bio extract 596-1 (10%)	0.503i	1.59f
Bio extract 596-1 (20%)	0.814g	1.74f
Bio extract RF-79 (10%)	0.644h	1.86f
Bio extract RF-79 (20%)	1.039f	2.05f
Hydro priming (Control)	0.001s	1.23f

*Figures not sharing the same letters in the same column differs signiûcantly at $p < 0.05$

$ZnSO_4$ and H_2O_2 and bio extract RF-79 (10%) in seedling fresh weight. These might be due to more efficiency in utilization of available and stored starch due to seed priming with PEG, $ZnSO_4$ and H_2O_2 as compared with osmopriming substances *viz*., KNO_3 and KCl. Abdnadani and Ramezani (2012) observed Polyethylene glycol solution (2%) has increased the fresh and dry weight of roots and vigour index in maize incubated for 12 h and 18 h. Harris *et al*. (2007), the increase in yield was 13% in case of hydro-priming alone and 26% when primed with ZnSO4 solution of maize. Ramalal *et al*. (1993), reported that soaking of maize seeds in KH_2PO_4 solution significantly increased the germination, speed of germination, mean daily germination, shoot length, root length, seedling vigour index and seedling dry weight over untreated control. In the similar line, Ghassemi-Golezani *et al*. (2008) revealed that hydropriming is also have significant and useful effect for enhancing seedling emergence rate and percentage of lentil.

Root length (RL) and shoot length (SL)

Present investigation shows that there is no significant difference on RL and SL among different cultivars of pea. Maximum was recorded in V_1 while minimum found in V_3. RL and SL were significantly influenced by different priming treatments not following any proper trend. Maximum RL was recorded in H_2O_2 (10 mM) and highest SL was found in PEG (2.5%) seed treatments. The significant increase in RL and SL in primed seeds may be due to priming substance involvement in cell elongation or cell division and the meristematic growth. Significantly, minimum responses were recorded in KNO_3 at 2.0% and 2.5%. This might be due to either higher duration of soaking time which is not suitable for pea or might be higher concentration of osmotic substance. Similarly Barba-Espin *et al*. (2012), reported that priming with hydrogen peroxide (H_2O_2) have effect on anti-oxidative metabolism which has promote on seed germination or seedling growth (measured as fresh weight and length) in pea (*Pisum sativum*) cultivars. H_2O_2 at 20 mM concentration that produced the best response in terms of growth as compared to 0, 40 and 80 mM exposed for 24 h. At 24 h of imbibitions, germination percentage had reached nearly 75%. H_2O_2 which acts as signal molecule at cellular level has significant effect on hydrolytic enzymes which are actively involved in mobilisation of stored reserves. Cokkizgin (2013) reported hydro and osmo-priming with PEG effects on germination in pea (*Pisum sativum L)* seeds on seedling length, germination percentage and vigor index. However, Sarmadi *et al*. (2014), revealed that use of potassium nitrate at rate of 0.35 moles per litre for 10 hour caused increasing the fixed number of root nodules nitrogen in common bean (*Phaseolus vulgaris L*). In this connection, the concentration and duration of KNO_3 treatment used in the present investigation may not be suitable for pea. Shehzad *et al*. (2012) reported that the priming treatments significantly affected the fresh weight, shoot length, root length and vigour index of forage sorghum.

Root to shoot length ratio (RSLR)

Root to shoot length ratio was significantly affected in different treatments of pea cultivars and priming treatments. Highest RSL ratio is found in V_1 cultivar and H_2O_2 (10 mM) priming while lowest in V_3 and KNO_3 (2.0%) treatment. The root length increased higher than the shoot of V_3 might be the reason for increased RSL ratio than others tested cultivars. However, when seeds primed with H_2O_2 (10 mM) showed higher root length as compared to shoot, but in KNO_3 (2.0%) it was reverse. Hence, H_2O_2 (10 mM) yielded maximum RSL ratio.

Relationships between the different seedling parameters are found to be positive in response with increased r_2 value (Fig. 2). There was significantly positive correlation recorded between germination percentage and seedling vigour index, root length, shoot length, seedling dry and fresh weight. This correlation studies clearly elucidates that increased germination percentage increases seedling vigour index, seedling length and biomass.

Interaction among pea cultivars and seed priming substances was found to be non significant on most of the seed ling parameters including germination percentage, SVI, seedling dry and fresh weight, root, shoot length and their ratio.

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