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Modification in Physiological Performances of French Bean (*Phaseolus vulgaris* L.) Seed Through Seed Priming

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ABSTRACT

Background: The three priming modes using various organic and inorganic compounds for hydro-priming, osmo-priming (PEG 6000, $CaCl_2$, KNO_3) and halo-priming (thiourea, GA_3) with control were considered, to study the improvement in physiological performances of French bean seed.

Methods: The seeds were primed for 6 hours duration and observations associated to seven parameters considering seedling and biochemical activities were considered for getting the real exposure in physiological performances of seed for seedling establishment.

Result: The treatment, T_2 (thiourea, 750 ppm) was best performing with significant variation among diverse treatments except in peroxidase activity. In genotypes, V_3 (RCM-FB-62) showed high significant demarcation for maximum parameters though, V_2 (phyrngop) and V_1 (RCM-FB-18) were highest for peroxidase activity and speed of germination. The treatment-genotype interactions showed significant demarcation with exemption in speed of germination and vigour index, however, T_2V_3 interaction was highest considering all parameters. Considerable parameters were pronounced contributors for cumulative upgradation in physiological performances of seed with exceptions in percent of germination and peroxidase activity. Diverse treatments specified the extent of effects on seedling or enzyme activity to upgrade the quality. Consequently, the treatment T_2 resulted in improved physiological performance in French bean seed production.

Key words: Enzyme activity, French bean, Priming, Seedling parameters.

INTRODUCTION

French bean is an important cool season (15-20°C) annual legume crop which is popular for human consumption for its utilization as both eatable dry seed and unripe fruit (green beans). In India, French bean production is emerging due to its adequacy in communities, simple cultivation methodology and distinct dietary status. The high nourishing value of French bean comprises 17.5-28.7 per cent protein in the dry seeds and about 1.0-2.5 per cent protein in the green pods, about 61.4 per cent carbohydrates, 3.2-5.0 per cent mineral matter, 340-450 kcal energy and about 11 per cent water (Fabbri and Crosby, 2016) although, it may vary from region to region due to developmental variation in plant or seed. The wild forms of small black seeds are found in Tropical America from where it was disseminated to West Africa and later to India. The various genotypes have been categorized into ‘pole beans’ and ‘bush beans’ according to its differential climbing habit. The production and dissemination of quality seed is one of the indispensable factors to achieve anticipated yields in addition to optimum quality of the cultivar. The quality of seed exclusively, is responsible for at least 10-15% rise in productivity (Monalisa *et al*., 2017). The utilization of quality seeds is one of the prime factors that can minimize the vast yield gap between research and farmers’ plot.

Seed priming is one of the invigoration techniques where germination procedure is commenced but appearance of radicle is arrested. Presently, this technology is used for the different crops predominantly in horticultural crops, but crop specific modes of priming need to be developed to further enhance seed production. Seed priming gives rise to greater percentage of final germination, where the seedling parameters, speed of germination are extensively improved. Moreover, a promising reflection specified the decline of dormancy with upgradation of seedling quality (Anese, 2011). The earliness and uniformity in seed germination is habitually attained in application of priming which is vital for establishing a good crop stand (Khan *et al*., 2008). The biochemical activities required to initiate the germination process were also enhanced as a result of priming (Afzal *et al*., 2011). The procedure of priming can be beneficial to farmers because it is used as safeguards escaping additional cultivation time, fertilizer applications,

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re-seeding, weak plants in addition to money. On-farm seed priming can be carried out amongst cooperating farmers because it is simple, cheap and additionally effective for qualitative progression of seedling. Lentil seeds primed with hydro or osmo-priming (KNO_3 or PEG-6000) showed hopeful outcomes in seedling performances (Ghassemi-Golezani *et al.,* 2008 and Saglam *et al*., 2010).

MATERIALS AND METHODS

The experiment was conducted at 2016 and 2017 in RKVY Laboratory, Deptt. of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal using six months old stored seed under ambient condition considering three (3) French bean genotypes namely RCM-FB-18 (V_1), Phyrngop (V_2), RCM-FB-62 (V_3). The twelve (12) varied priming treatments comprised of various organic and inorganic chemicals *viz*. T_1 (500 ppm), T_2 (750 ppm Thiourea), T_3 (50 ppm Gibberellic Acid), T_4 (100 ppm Gibberellic acid), T_5 (0.1 Mpa KNO_3), T_6 (0.2 Mpa KNO_3), T_7 (0.1 Mpa $CaCl_2$), T_8 (0.2 Mpa $CaCl_2$), T_9 (0.1 Mpa PEG 6000), T_{10} (0.2 Mpa PEG 6000), T_{11} (Distilled Water), T_{12} (Control, without treatment). The dissimilar varietal seeds were soaked separately for 6 hours in aqueous solutions of these treatments followed by drying back to the original seed moisture content, except control. The five seedling parameters (germination percentage, speed of germination, seedling length, seedling dry weight and vigour index) were projected through Glass-Plate method (Chakraborti, 2010). The effect of priming on two important enzymes (alpha amylase and peroxidase) after 24 hours of imbibition was also studied. The assessment was done using OPSTAT software following CRD of two factors with three replications for each character. The outcome was achieved at one percent level of significance.

RESULTS AND DISCUSSION

Considering the diverse seed priming with 12 treatments (T_1-T_{12}) including control (without treatment), the observations were related to physiological performances of seed on three genotypes (V_1, V_2 and V_3) of French bean. The explanations were regulated to facilitate the seed quality associated to seedling parameters and biochemical activity in two successive years and pooled value. The various treatments showed significant variances for all considerable parameters which were clustered into two different kinds like seedling parameters and biochemical parameters. In application of diverse priming mode, the variable significant distinction showed advancement, similar to observation of other researchers (Ghobadi *et al*., 2012 and Abnavi *et al*., 2012) predominantly the pooled values. The superior treatment values over control emphasized the positive effect of priming, as seen in T_2 (thiourea @700 ppm) though it was variable in peroxidase activity and first year values of germination percentage (Table 1). The observed values on germination percentage were transformed into arcsine value for statistical calculation. In percent of germination, T_6 (KNO_3@0.2 Mpa) was highest only for 1st year and the peroxidase activity showed a positive response when treated with T_3 (50 ppm gibberellic acid) followed by T_{11} (distilled water), T_5 (0.1 Mpa KNO_3) and T_2 (750 ppm thiourea) under both year with pooled analysis. The anti-oxidative nature of peroxidase may favour the physiological aspect of seed, predominantly in germination. Normally, the role of bio-regulators is known for augmenting seedling expansion, mounting photosynthetic efficiency (Ramaswamy *et al.*, 2007) *etc*. for emerged seedling.

Analysis of variance showed that all parameters were significantly influenced by seed priming for all the genotypes where speed of germination, vigour index and the enzyme, alpha amylase were predominant. The highest values were seen in V_3 (RCM-FB-62) genotype for vigour index, alpha-amylase and non-significant deviation with the highest for seedling dry weight considering both years and pooled analysis (Table 2). In germination percentage, the V_2 (Phyrngop dwarf) genotype showed highest for 1st year but, V_3 (RCM-FB-62) indicated its dominance for 2nd year and pooled assessment (Table 2). The priming treatment might have an effect on peroxidase activity where V_3 recorded lowest value as compared to other genotypes as specific genotypic nature was the most dominant factor. Considering the findings on speed of germination and seedling length, the genotype V_1 exposed greater values among various genotypes. The important parameters vigour index and alpha-amylase under physiological performances of seed showed greater performance in V_3 genotype, though it was not truly followed in seedling dry weight. In peroxidase activity, the observation was contrary to previous two parameters. Hence, these parameters may be considered for genotype evaluation.

In interaction of treatment and genotype, the significant enhancement was noticed for parameters only in enzymatic actions considering both years and pooled analysis, but the non-significant trend was followed for most of the seedling parameters in full or partial manner except in seedling length (Table 2). Considering all parameters, the observed interacted values showed a general trend for upgradation in interaction of various priming over control (Lamichaney *et al*., 2018) irrespective of genotypes.

In Fig 1, the effect of treatment was visibly progressive for scheduled parameters considering their percent of enhancement over the control (T_{12}). But, the activity of peroxidase demonstrated variable nature, highlighting highest effect in T_3 with intermediate action in T_2, T_6 and T_{11}. The most promising effect was observed in speed of germination, seedling dry weight and alpha amylase activity.

The effect of primed seeds exposed a pronounced demarcation over control for most of the traits in the current study. It was reported that priming modified the metabolic processes involved in germination and earlier establishment of seedlings (Ghassemi-Golezani *et al.,* 2008) as well as

Table 1: Seed priming influence on different parameters under physiological performances of seed.

	# Germination			Speed of germination			Seedling length (cm)			Seedling dry weight (g)			VI			Alpha amylase (µg $min^{-1}g^{-1}$)			Peroxidase (ΔA $min^{-1}g^{-1}$)		
	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P
T1	75.30 (92.86)	74.87 (92.08)	75.08 (92.47)	21.86	23.23	22.55	43.78	44.68	44.23	0.83	0.87	0.85	4062.9	4153.3	4108.1	73.70	86.51	80.11	1.13	0.99	1.06
T2	75.64 (92.83)	75.75 (92.97)	75.69 (92.90)	23.73	24.77	24.25	45.76	47.10	46.43	0.96	0.97	0.97	4243.0	4446.6	4344.8	75.84	88.71	82.28	1.21	1.04	1.13
T3	75.75 (93.08)	74.15 (91.98)	74.95 (92.53)	20.73	23.21	21.97	41.99	44.34	43.17	0.81	0.85	0.84	3908.0	4049.7	3978.8	71.83	84.63	78.23	1.72	1.56	1.64
T4	75.57 (93.66)	72.46 (90.31)	74.02 (91.99)	21.48	22.14	21.81	41.64	45.02	43.33	0.85	0.83	0.84	3896.9	4067.4	3982.2	69.88	82.71	76.29	0.93	0.79	0.86
T5	72.64 (90.91)	73.37 (91.08)	73.00 (91.00)	22.35	22.99	22.67	42.91	43.90	43.41	0.78	0.88	0.83	3897.2	4053.1	3975.2	70.92	83.77	77.34	1.10	0.97	1.03
T6	76.71 (95.12)	72.82 (90.65)	74.76 (92.89)	22.94	23.59	23.27	43.93	45.04	44.49	0.91	0.90	0.91	4170.9	4093.2	4132.1	72.37	85.19	78.78	1.20	1.06	1.13
T7	75.70 (93.15)	71.56 (89.29)	73.63 (91.22)	22.70	22.87	22.78	44.45	46.93	45.69	0.80	0.91	0.86	4139.3	4217.5	4178.4	70.13	82.94	76.54	1.06	0.93	0.99
T8	73.12 (91.02)	72.02 (89.75)	72.57 (90.39)	21.58	21.82	21.70	42.94	43.76	43.35	0.83	0.87	0.85	3905.8	3916.8	3911.3	67.74	80.54	74.14	1.10	0.93	1.02
T9	75.01 (94.18)	69.76 (87.49)	72.39 (90.84)	21.61	21.40	21.51	42.85	43.25	43.05	0.86	0.87	0.87	4031.9	3866.6	3949.3	68.17	81.03	74.60	1.00	0.84	0.92
T10	74.61 (91.93)	71.32 (89.19)	72.96 (90.57)	21.55	21.60	21.57	39.86	42.60	41.23	0.83	0.88	0.86	3658.9	3818.6	3738.8	68.09	80.92	74.51	1.10	0.96	1.03
T11	73.36 (90.77)	71.23 (88.90)	72.29 (89.84)	21.49	22.27	21.88	42.89	45.51	44.20	0.82	0.84	0.83	3891.8	4000.0	3945.9	70.43	83.24	76.84	1.27	1.11	1.19
T12	72.39 (90.19)	71.44 (89.33)	71.91 (89.76)	21.48	21.69	21.59	42.06	44.05	43.06	0.81	0.88	0.85	3789.7	3930.9	3860.3	64.27	77.10	70.68	1.10	0.92	1.01
Mean	74.65	72.56	73.61	21.96	22.63	22.30	42.92	44.68	43.80	0.84	0.88	0.86	3966.4	4051.1	4008.8	70.28	83.11	76.70	1.16	1.01	1.08
SEm(±)	1.00	0.79	0.61	0.50	0.53	0.38	0.66	0.59	0.50	0.02	0.02	0.01	68.57	79.36	53.43	0.65	0.73	0.69	0.01	0.01	0.01
LSD (0.01)	2.83	2.22	1.72	1.41	1.49	1.06	1.88	1.67	1.40	0.05	0.05	0.04	193.71	224.19	150.93	1.83	2.07	1.94	0.03	0.04	0.03

Y1- 1st year; Y2- 2nd year; P- pooled value; # - arcsine-transformed value (figures presented in parenthesis are original value).

plants which may elicit the early development in comparison to control (Emongar, 2007). Moreover, the enzymatic action at initiation of germination may also be beneficial to advance seed vigour through sharing its expanding dry weight and length of the seedling (Arun *et al.,* 2017).

In correlation matrix of the different physiological performances of seed, the R^2 value of 0.7352 (Table 3) denoted the usual significant positive relationship for considerable characters. The positive significant mode was observed for most of the characters though the exception was revealed in peroxidase activity. The non-significant relationship was also followed in per cent of germination with seedling length, seedling dry weight and alpha-amylase activity though other parameters were highly significant within them in positive mode. All values of negative correlation showed non-significant differentiation particularly in case of peroxidase. However, peroxidase activity only showed a positive correlation with germination percentage, similar to a study by El-Araby *et al.,* (2004). Therefore, the activity of peroxidase may favour germination by reducing antagonistic effect in seed, while alpha-amylase activity is closely related to germination activity. The adaptation of seed quality through priming and its effect on seedling parameters was studied by different scientists (Abnavi *et al.*, 2012; Lamichaney *et al.*, 2018). The dissimilar physiological performances of seed may diminish or increase in various ways through utilization of diverse treatments on seed in different crops, particularly under legumes and cereals. But seedling dry weight always showed an increase on application of PEG 6000 (Ghobadi *et al.*, 2012), thiourea (Khan and Shah, 2010) KNO_3 (Kumar *et al.*, 2018), GA_3 (Abnavi *et al.*, 2012; Arun *et al.*, 2017; Mahnaz and Masomeh, 2016) similar to our study and sometimes in presence of $CaCl_2$ (Afrayeem *et al.*, 2018) and water as hydropriming (Mahnaz and Masomeh, 2016). Thiourea was the most capable sulphydryl group (-SH) of bio-regulator helpful in seedling growth (Anese *et al.,* 2011 and Premaradhya *et al.*, 2018). The intensity in activity of super-oxide dismutase (SOD) and quantity of soluble protein was amplified in radish owing to the effect of thiourea (LiQin *et al.*, 2001). Growth promoters and nutrients directly influence metabolic activities in leaves during plant development and are thus responsible for improving the quality of the seed at the time of seed development. The potency of priming may enhance the activity of peroxidase, catalase, SOD and minimize electrolyte leakage under soaking.

Generally, germination and interrelated parameters involve the activities of enzymes/isozymes, where anti-oxidative activity of specific isozymes is closely related to germination. A few reports relating to the valuable priming effects on seed indicated a build-up of more metabolites or osmotic adjustments at germination through the restoration of metabolic processes (Elkoca *et al.,* 2007), or upgraded membrane integrity and superior

Table 2: Deviation in genotypes considering physiological performances of seed and interactions of considerable two factors.

	# Germination			Speed of germination			Seedling length			Seedling dry weight			VI			Alpha amylase			Peroxidase		
	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P	Y1	Y2	P
V1	71.45 (89.23)	71.19 (88.98)	71.32 (89.11)	22.88	23.41	23.14	44.81	44.88	44.85	0.72	0.73	0.73	3999.9	3992.6	3996.2	52.15	71.65	61.90	1.14	0.99	1.06
V2	76.35 (93.81)	72.23 (89.94)	74.29 (91.88)	21.84	22.52	22.18	41.42	42.80	42.11	0.92	0.97	0.95	3885.2	3875.9	3880.5	51.53	71.01	61.27	1.27	1.11	1.19
V3	76.14 (94.39)	74.26 (91.83)	75.20 (93.11)	21.15	21.97	21.57	42.53	46.37	44.45	0.89	0.94	0.92	4014.1	4284.9	4149.5	107.17	106.67	106.92	1.07	0.92	1.00
Mean	74.65	72.56	73.61	21.96	22.63	22.30	42.92	44.68	43.80	0.84	0.88	0.86	3966.4	4051.1	4008.8	70.28	83.11	76.70	1.16	1.01	1.08
SE(m)±	0.50	0.39	0.30	0.25	0.26	0.19	0.33	0.30	0.25	0.008	0.009	0.007	34.28	39.68	26.71	0.32	0.37	0.34	0.01	0.01	0.01
LSD (0.05)	1.42	1.11	0.86	0.71	0.74	0.53	0.94	0.84	0.70	0.023	0.026	0.019	96.86	112.10	75.47	0.91	1.04	0.97	0.01	0.02	0.01
V × T	**Interaction effects of Genotype (V) x Treatment (T)**																				
SE(m)±	1.74	1.36	1.05	0.87	0.91	0.65	1.15	1.02	0.86	0.028	0.032	0.024	118.76	137.45	92.54	1.12	1.27	1.19	0.02	0.03	0.02
LSD (0.05)	NS	3.84	2.97	NS	NS	NS	NS	2.89	2.42	0.08	NS	NS	NS	NS	NS	3.17	3.59	3.37	0.04	0.07	0.05

Y1- 1st year; Y2- 2nd year; P- pooled value; NS- Non-significant; # - arcsine-transformed value (figures in parenthesis are original value).

Table 3: Correlation Matrix of the parameters allied to physiological performances of seed.

	Percent of germination	Speed of germination	Seedling length	Seedling dry weight	Vigour index	Alpha-amylase
Speed of germination	0.363*					
Seedling length	0.043^{NS}	0.781**				
Seedling dry weight	0.189^{NS}	0.700**	0.607**			
Vigour index	0.457**	0.857**	0.891**	0.662**		
Alpha-amylase	-0.168^{NS}	0.621**	0.684**	0.544**	0.545**	
Peroxidase	0.439**	-0.009^{NS}	-0.122^{NS}	-0.156^{NS}	0.002^{NS}	-0.131^{NS}

*-Significant; ** -Highly significant; NS-Non-significant.

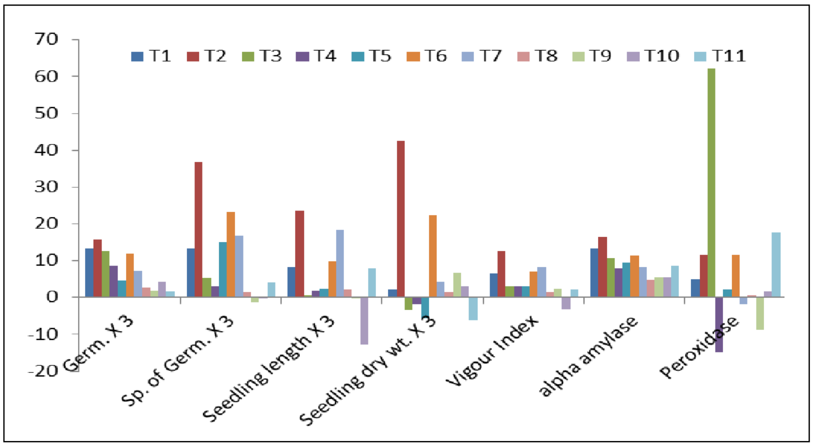

Fig 1: Deviance (in %) of various physiological performances of seed in priming over control.

physiological activities at germination (Sung and Chang, 1993). Soaked seed through hydro-priming or other modes of priming can supplement biochemical action like soluble protein, lipid, sugars, vitamin E, pro vitamin A *etc*. to bear the fundamental capability responsible for germination (Janeczko *et al*., 2015). Estimation of peroxidase activity can be used as an indicator of seed quality as reports have shown a sharp decline during aging (Pallavi *et al.,* 2003). Seed treatment with thiourea has also been reported to enhance germination (Wahid *et al*., 2017) as observed in the present study.

CONCLUSION

The present interpretations showed considerable improvements in physiological performances of seed in French bean genotypes under the influence of priming. The treatment T_2 (thiourea @ 750 ppm) exposed its promising potentiality in seedling irrespective of any genotype. V_3 (RCM-FB-62) can be utilized to explore potentiality in cultivation predominantly in seed production programme to facilitate the quality seed of French bean.

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