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Effect of Hydro and Chemopriming on Seed Reserve Mobilization, Enzyme Activity and Hormone Content of French Bean

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ABSTRACT

Seed vigour is one of the prime attributes of quality seed to ensure better field emergence and disease resistance even under various adverse conditions. Priming is one of the well known methodologies known to improve germination and crop stand in various crops. Seed priming is a pre-sowing treatment that induces a physiological state that promotes more effective seed germination. In this investigation carried out in 2021-2022, Effect of priming on seed reserve mobilization process was studied. Four replication of each treatment was taken for analysis. The results on mobilization efficiency, enzyme activity and hormone content (GA_3, auxin and zeatin riboside) were studied. The results revealed higher seed reserve mobilization rate, use efficiency of seed reserves and use rate of seed reserves upon priming with KH_2PO_4 compared to hydropriming and control. The enzyme activities (amylase and protease) were enhanced significantly and the hormone content during seed germination was also increased due to seed priming treatments over the control.

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INTRODUCTION

French bean scientifically known as *Phaseolus vulgaris*, belongs to the family Fabaceae is one of the most popular and widely grown leguminous vegetables in India. It is an important pulse crop, with high yielding ability. It is grown in almost all states of India. It is also called as kidney bean, common bean, and snap bean.

Seeds are heterogeneous storage reserves with wide array of storage compounds that include various soluble carbohydrates, starch polymer, storage proteins and lipids. These stored reserves comprise 70% of the world's caloric intake in the form of food and animal feed produced through sustainable agriculture, which contributes to food and nutritional security.

During seed germination, the storage tissues become a source of nutrition for the expanding and developing embryo. The cotyledonary reserves are mobilized into the axis, the endosperm and perisperm reserves are degraded and the tissues are utilized by the embryo.

Seed vigour is one of the prime attributes of quality seed to ensure better field emergence and disease resistance even under various adverse conditions. Priming is one of the well known methodologies known to improve germination and crop stand in various crops. Seed priming is a pre-sowing treatment that induces a physiological state that promotes more effective seed germination. In addition to improving the speed and uniformity of germination (Khalil *et al.* 2010), seed priming also stimulates a number of biochemical changes in the seed that are crucial for breaking dormancy, mobilizing or hydrolyzing seed reserves, activating enzymes and promoting the emergence of embryonic tissues. This is may be due to the enhanced mobilization of metabolites/inorganic solutes to germinating plumule/embryo, leading to enhanced growth (Taiz and Zeiger 2006).

Seed reserves and their mobilization play a major role in germination and early seedling development. Although the benefits of seed priming are well documented in various crops but the research work on how primed seed accrue benefits and in what way these benefits are translated to early germination and high seedling vigour is scanty and in particular changes in seed reserves warrants much investigation. With this aim, a research program was proposed to investigate cues and clues related to seed reserve mobilization in primed seeds.

MATERIALS AND METHODS

The experiment was laid out in a factorial Completely Randomised Design with two factors (high vigour seeds and low vigour seeds) and three treatments (control, hydropriming, chemopriming KH_2PO_4 100 ppm) each consisted of four replications under controlled conditions. Fresh seeds (untreated) were subjected to artificial ageing to obtain low vigour seeds as per ISTA procedure (2006). Both fresh seeds (high vigour) and aged seeds (low vigor) were subjected to priming with water (Hydropriming) and KH_2PO_4 100 ppm (chemopriming) for 4 hrs at 25°C and untreated seeds are taken as control.

Seed reserve mobilization rate (SRMR %)

The amount of mobilized seed reserves to the seedling was represented by the seed reserve mobilization rate (SRMR), determined by the ratio between SLDW and SDW and expressed as percentage, according to equation,

$$SRMR = \frac{SLDW}{SDW} \times 100$$

Where,
SLDW=Seedling dry weight
SDW=Seed dry weight

Use efficiency of seed reserves (UESR mg/mg)
The use efficiency of seed reserves (UESR) was determined by the ratio between SLDW and USR, as proposed by Soltani, Gholipoor, and Zeinali (2006), and the result was expressed as mg mg^{-1}, according to Equation,

$$UESR = \frac{SLDW}{USR}$$

Where,

SLDW= Seedling dry weight
USR= Use of seed reserves
Use rate of seed reserves (URSR %)

The use rate of seed reserves (URSR) was calculated by the ratio between USR and SDW and expressed as percentage Soltani (2006), according to Equation,

$$URSR = \frac{USR}{SDW} \times 100$$

Where,
USR = Use of seed reserves
SDW= Seed dry weight

α–amylase activity and alkaline protease activity

The samples for alpha amylase activity and alkaline protease activity was collected after 8 hrs of imbibition. The amylase activity in germinating seeds was estimated according to the method of Sadasivam and Manickam (1996). Amylase activity was expressed as mg maltose released/ml/min. One unit is defined as 1 mg of maltose released/ml/minute under standard condition. The protease activity in germinating seeds was estimated as per the procedure outlined by Thimmaiah (1999). Alkaline protease activity was expressed as **α-**g tyrosine released/ml/min.

Hormones analysis

The contents of hormones in four days old seedlings were determined by HPLC according to Kelen *et al.* (2004).

RESULTS AND DISCUSSION

In the present investigation, higher per cent on seed reserve mobilization rate was noticed in fresh seeds (90.28) over low vigor seeds (61.15). Priming treatments also showed significant effect on seed reserve mobilization rate over unprimed. Chemopriming (84.24) followed by hydropriming (80.85) had significantly increased the seed reserve mobilization rate (62.06), (Table 1). The results were in agreement with Tounekti *et al.* (2020) who found that priming with PEG solution improved the weight of utilized (mobilized) seed reserve (WUSR), seed reserve depletion percentage (SRDP) and total seedling dry weight (SLDW) of sorghum under water stress circumstances.

Table 1. Effect of chemo and hydropriming on seed reserve mobilization rate, use efficiency of seed reserves and Use rate of seed reserves of high and low vigour French bean seeds.

Treatments	Seed reserve mobilization rate (%)	Use efficiency of seed reserves (mg/mg)	Use rate of seed reserves (%)
Seed material			
Fresh seeds	90.28	97.89	92.22
Aged seeds	61.15	67.62	90.00
SEM±	0.76	0.83	0.11
CD (5%)	2.28	2.48	0.35
Treatments			
Dry/control	62.06	68.58	89.74
Hydropriming	80.85	88.41	91.38
Chemopriming	84.24	91.27	92.22
SEM±	0.94	1.02	0.14
CD (5%)	2.79	3.04	0.43
Interactions			
Fresh control	87.42	95.36	91.67
Fresh hydroprimed	90.73	98.74	91.88
Fresh chemoprimed	92.69	99.56	93.10
Aged control	36.70	41.80	87.80
Aged hydroprimed	70.97	78.09	90.87
Aged chemoprimed	75.79	82.98	91.34
SEM±	1.32	1.44	0.20
CD (5%)	3.95	4.30	0.61

Similarly, higher per cent of use efficiency of seed reserves was observed in fresh seeds (97.89) over low vigour seeds (67.62). Priming treatments also showed significant effect on use efficiency of seed reserves over unprimed. Chemopriming (91.27) followed by hydropriming (88.41) had significantly increased the use efficiency of seed reserves (68.58) (Table 1). Hydropriming and chemopriming boosted the use efficiency of seed reserves in fresh seeds by 1.03 and 1.04 folds, respectively. In comparison to the control, hydropriming and chemopriming increased the use efficiency of seed reserves by 1.86 and 1.98 folds respectively, in low vigour seeds. The findings are consistent with the findings of Seyyedi *et al.* (2015), who found that KH_2PO_4 priming significantly improved the growth of heterotrophic seedlings in black seed by regulating seed status such as seed weight of mobilized seed reserve utilization.

Similarly, higher per cent of use rate of seed reserves (92.2) was noticed in fresh seeds over low vigour seeds (90.0). Priming treatments also showed significant effect on use rate of seed reserves over unprimed. Chemopriming (92.2) followed by hydropriming (91.3) had significantly increased the use rate of seed reserves (89.74) (Table 1). Hydropriming and chemopriming raised the use rate of seed reserves in fresh seeds by 1.0 and 1.01 folds respectively. In comparison to the control, hydropriming and chemopriming enhanced the use rate of seed reserves in low vigour seeds by 1.03 and 1.04 folds respectively. The results are in accordance with Ansari *et al.* (2012), who reported that osmopriming had a higher weight of utilized (mobilized) seed reserve (WMSR) and seed reserve depletion percentage (SRDP) than unprimed and hydropriming in mountain rye.

Amylase activity

The results revealed that higher amount of amylase activity in fresh seeds (1.23) over low vigour seeds (0.89). Among the priming treatments both hydro and chemopriming has shown significant increase in amylase activity over unprimed seeds (Fig. 1).

The amylase activity was increased by 1.20 and

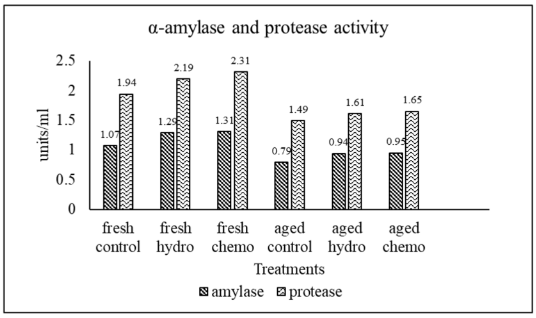

Fig. 1. Effect of chemo and hydropriming on alpha amylase and alkaline protease activity of high and low vigour French bean seeds.

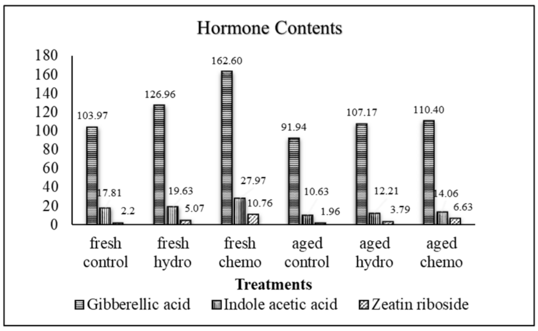

Fig. 2. Effect of chemo and hydropriming on hormone contents of high and low vigour French bean seeds.

1.22 folds in fresh seeds by hydropriming and chemopriming respectively, whereas in low vigour seeds these values were 1.18 and 1.20 folds respectively. The high amount of enzyme activity in both hydro and chemopriming when compared to unprimed, might be due to activation of pre-existing enzymes upon imbibition. During water uptake the glycolytic and oxidative pentose phosphate pathway recommence and already present kreb's cycle enzymes are activated (Botha *et al.*1994). High amylase activity in chemoprimed seeds might be due to the influence of phosphorus salts. High amount of alpha amylase activity was noticed in 4 and 8 day old seedlings that are raised from high phosphorus containing seeds compared to low phosphorus containing seeds (Yugandhar *et al.* 2022). Similarly, seed priming of green gram ensured proper hydration which resulted in enhanced activity of α–amylase activity that hydrolyzed the macro starch molecules to smaller and simple sugars (Basra *et al.* (2005).

Alkaline protease activity

The results revealed that higher amount of alkaline protease activity in fresh seeds (2.15) over low vigour seeds (1.58). Among the priming treatments both hydro and chemopriming has shown significant increase in alkaline protease activity over unprimed seeds. Between treated and untreated seeds, there were noticeable differences in fresh and low vigour seeds alkaline protease activity. Following hydropriming (2.19 units/ml), seeds that had undergone chemopriming (2.31 units/ml) seeds had higher protease activity than control seeds (1.94 units/ml). In contrast, the chemopriming treatment produced the maximum protease activity in low vigour seeds (1.65 units/ml), followed by hydropriming (1.61units/ml), over the control (1.49 units/ml) (Fig.1). The results obtained were in accordance with Varier *et al.* (2010) who reported that priming increased the activity of many enzymes involved in protein metabolism (proteases) that are involved in the mobilization of stored reserves.

Hormone content

In fresh seeds, Hydropriming increased GA_3, IAA and zeatin riboside content by 1.22, 1.10 and 2.2 folds, respectively, while chemopriming increased GA_3, IAA and zeatin riboside content by 1.56, 1.57 and 4.89 folds respectively. Similarly, in low vigour seeds, Hydropriming increased GA_3, IAA and zeatin riboside content by1.16, 1.14 and 1.93 folds, respectively, while chemopriming increased GA_3, IAA and zeatin riboside content by 1.2,1.32 and 3.38 folds respectively. In accordance with our studies increase in GA_3 was observed during 1^{st} 48 h of germination. (Groat and briggs 1969) in barley (Fig. 2).

Our findings are in similarity to Bialek (1992) who reported increase in total content of free IAA steadily from the 2^{nd} day of germination in *Phaseolus vulgaris*. Similarly, Tianlun *et al.* (2020) observed that seed priming significantly improved the seed germination and seedling growth by regulation of the endogenous phytohormone contents in germinating seeds and seedlings. He reported an increase in endogenous IAA and GA contents and decrease in the endogenous ABA content. Auxins (IAA) play key role in regulating cell cycle growth and elongation of radicle tip during and after grain sprouting (Elien *et al.* 2019). In accordance to our results Pino *et al.* (1991) reported supply of cytokinins to the cotyledons by embryonic axis of *Cicer arietunum* after 12 h of imbibition and thus facilitating mobilization of reserve material during this period.

CONCLUSION

Seed vigour in French bean cv Arka komal increased significantly upon seed priming which was due to efficient reserve mobilization, leading to early germination and faster seedling growth. Both hydro and chemopriming enhanced metabolic activity as evident from increase in enzyme and hormone synthesis. Increase in protease and amylase activity might be the cause for growth and reserve mobilization in seeds. Hormone synthesis that was noticed in primed seeds might have a positive role in early seed germination and high vigour seedlings. Based on present studies it is assumed that enzyme activation coupled with hormone synthesis might be responsible for seed reserve mobilization to the growing tips. The data drawn from use efficiency of seed reserves and seed reserve mobilization rate in comparison with less viable further confirmed the fact that seed reserve mobilization is indeed enhanced by seed priming as there were significantly higher rates in primed seeds. The early germination and root elongation in primed seeds might be due to GA_3 and cytokinins stimulated amylases that helps in breakdown of starch to sugars resulting early sprouting and growth.

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