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Effect of ageing and hormonal priming on different physiological attributes on French bean (*Phaseolus Vulgaris*)

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Abstract

The experiments were carried out with an objective of studying the effect of hormonal priming treatments on germination and seedling growth on aged seed of *Phaseolus vulgaris* variety Pant - Anupama. We collected the seed from Garhwal seed store, Srinagar Garhwal. The laboratory experiments were conducted on blotter at 25°C and 30°C temperature respectively. We have taken IAA, kinetin and GA₃ with three concentration 5ppm, 10ppm, and 15ppm. The planting value parameters assessed were germination %, speed of germination, germination value, root length, shoot length, seedling length, seedling fresh weight, seedling dry weight, seedling vigor index -1 and seedling vigor index-2.

For accelerated ageing seeds were treated with 1% solution of sodium hypo chloride for 5min. After that seeds placed in desiccators for 7 days at 45°C for accelerated ageing. Each alternate day we take 20 seed and placed in germinator at 25°C. After that seeds were primed with different doses of growth regulators, which were GA₃, IAA and kinetin. Seeds primed with 5 ppm solution of GA₃ showed the highest germination percentage, speed of germination, germination value, root length, shoot length, seedling length and seedling vigor index-1 and vigor index -2.

Growth regulators have been used to reduce the affect and to initiate the process of invigoration. This indicates that the lower concentration of growth regulators favors the increased enzymatic activity which leads to the favorable environment for the germination. Marked reduction in germination and other traits might have been due to solute leakage and lipid per oxidation which limit the necessary material for germination and seedling growth. This present study showed that growth regulator in higher concentrations inhibits the seed germination. It can be concluded that significant variation was found in GA₃5ppm.

Keywords: ageing and hormonal priming, physiological attributes, French bean

Introduction

Accelerating ageing is a method in which the seed's natural ageing is accelerating by exposure to heat, sometimes in combination with high humidity. The quality parameters related to longevity and viability can quickly be estimated. Seed aging has to be recognized as the major cause of reduced seed vigor and viability, and involves the process of deterioration. Until eventually the ability to germinate is lost (Powell, 2006). Most crop seeds lose their viability at relative humidity approaching 80% and temperatures of 25 to 30°C (Copeland and McDonald, 1995) [2].

French bean is a cool weather crop but develop well in optimum temperature ranging between 20°C to 25°C. French bean is sensitive to frost; high temperature and high rainfall destroy the young growing seedlings of *Phaseolus vulgaris*. One of the most popular test used to evaluate French bean seed vigor is the Accelerated ageing test (Spears, 1995), which is based on increase seed deterioration after exposure to high temperature and relative humidity. This test has been standardized to evaluate the physiological potential of bean seeds (Hampton and Tekrony, 1995) [10]. Seed ageing and priming with growth regulators can affect vigor of seeds, the present study aims to observe the impact of accelerated ageing for different time and treatment of aged seeds by different growth regulators concentrations on seeds and seedlings development.

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Methodology

Present studies work carried out at the seed science and technology laboratory, H.N.B Garhwal (Central) University during academic session 2014-2015. The details of the material used and method adopted in carrying out the studies are presented below:-

A) Plant material: - French bean (*Phaseolus vulgaris* L.) seeds were obtained from Garhwal Seed Store Srinagar Garhwal Uttarakhand.

B) Variety:- Pant- Anupama

C) Seed treatment: Seeds were treated with 1% sodium hypo chloride solution. The seeds were soaked in the solution for 5-10 min at room temperature. After that seeds washed two to three times with the help of distilled water and the seed were dried properly. Twenty seeds were placed in the blotter paper used as a control. Then seeds were placed in desiccators, covered and maintained in an oven at 45°C for 7 days of ageing treatments. After this ageing period, three replicates of 20 seeds were treated with different concentration of growth regulators (IAA, Kinetin, and GA₃).

	Germination%
control without ageing	100%
control with ageing	
After 2 days	75%
after 4 days	50%
after 6 days	30%



Experimental Method

Treatment details:-

T₀ – Control (aged)

T₁- Seed primed with 5ppm solution of IAA, Kinetin, and GA₃.

T₂- seed primed with 10ppm solution of IAA, Kinetin, and GA₃.

T₃- seed primed with 15ppm solution of IAA, Kinetin, and GA₃.

Towel Paper Test: Moist towel paper used as a substratum to conduct all the experimental trials. Twenty seeds were placed on the moist towel paper in three replicates. The towel paper was placed in seed germinator at cylindrical position for 7 days at 25°C and daily observations were taken up to the completion of experiment (ISTA, 2010).

Data analysis:

Assessment of planting value: Parameters used for assessing the planting value as follows.

No. of seeds germinated

$$1. \text{ Germination \%} = \frac{\text{No. of seeds germinated}}{\text{No. of seed sow}} \times 100 \quad (\text{ISTA Rules, 2010})$$

2. **Speed of germination:** Speed of germination calculated by using the formula given by Maguire 1962, Bradbeer 1988; Wardle *et al.*, 1991).

$$(N_1/1+N_2-N_{1/2}+\dots+N_n-N_{n1})$$

Where N₁=Total Number of speed germinated on one day 1;
N₂= Total Number of speed germinated on day 2 and so on till
N_n= Total Number of seed germinated on final day.

3. **Germination value:** Germination value calculated by using the following formula.

$$\sum \text{DGS}/N \times \text{GP} \times 10$$

Where, DGS = Daily germination speed, N =Total number of daily count, GP= Germination per cent.

4. **Seedling length:** total length of seedling was obtained by adding root and shoot length (cm).

5. **Shoot length:** Length of shoot from collar to the apical tip (cm).

6. **Root length:** Length of root from collar to the apical tip (cm) (ISTA Rules, 2010).

7. **Fresh weight of seedling:** Weight of uprooted seedling.

8. **Dry weight of seedling:** Weight of dried seedling.

9. **Seedling vigour index -1:** Seedling length × Germination % (Abdul-Baki and Anderson, 1973).

10. **Seedling vigour index -11:** Seedling dry weight × Germination % (Abdul-Baki and Anderson, 1973).

Statistical analysis: The data's were analyzed by following analysis of CRD. Analysis of variance (ANOVA) was performed and when significant difference existed (CD at 1% and 5%) was calculated to compare the mean of different treatments.

Result and Discussion

1. **Germination Percentage:-** Seeds primed with different growth regulators showed a increase of 94% germination percentage over control recorded in GA₃ 5ppm, 11% in 10ppm followed by IAA (5ppm(-58%), 10ppm(-64%), 15ppm(-64%) and kinetin (5ppm(-52%), 10ppm(-64%), 15ppm (-52%). However the maximum germination percentage results were significantly higher over control. Chauhan *et al.*, (2009) [1] worked on horse gram and black gram, the seeds were soaked in different concentrations (10, 50, and 100 ppm) of GA₃ and IAA for 24 hours. Four replicates of each treatment with 20 seeds per replicate were arranged for precise analysis. Significant variation was found between the Black gram and Horse gram in all aspects. GA₃ 10 ppm showed highest germination percentage as well as the higher radicle and plumule length in contrast to other treatments. But when considered particularly on the radicle and plumule elongation, these did not show any significant effect on both the crop species

2. **Speed of Germination:-** Best speed of germination over control was found in GA₃5ppm 96%. This was followed by IAA (5ppm (-60%), 10ppm (77.5%), 15ppm (-67%), and kinetin (5ppm (-53%), 10ppm (-63%), 15ppm (-

- 53%).
- Germination Value:-** An increase of 96.25% was observed for germination value in aged seeds treated with GA₃5ppm, followed by IAA (5ppm (-24.06%), 10ppm (-77.50%), 15ppm (-67.50%) and kinetin (5ppm (-53.75%), 10ppm (-63.75%), 15ppm (-53.75%) over control. GA₃5ppm showed highly significant over control. Results of seed ageing studies in *Brassica napus* by **Jannohammadi et al.** (2008) showed that with increase in the time of accelerated ageing treatment, all the germination parameters decreased, however the sensitivity of cultivars to ageing periods was different environment condition.
 - Root Length:-** Best performance was observed an increase in root length over control by GA₃ 5ppm that was 19.88% over control. While the seeds treated with IAA and kinetin showed non-significant results over control. This was followed by IAA5ppm, 10ppm and 15ppm -55.71% -73.69%, -49.64% respectively and in kinetin 5ppm-39.28%, -40.11%, -19.88% respectively. Positive effect of GA₃ was found.
 - Shoot Length:-** Maximum percentage increase in shoot length 22.24% over aged control was observed in GA₃5ppm. Minimum percentage increase was recorded in kinetin5ppm(-23.21%). Kinetin and IAA showed negative effect.
 - Seedling Length:-** Aged Seeds primed with GA₃5ppm was rated best with 23.43% increase in seedling length over aged control. This was followed by IAA 5ppm (-53.60%), 10ppm(-70.45%), 15ppm(-38.94%) and kinetin 5ppm(-23.71%), 10ppm(-35.66%), 15ppm(-19.09%).
 - Fresh Weight:-** Best performance was observed an increase in fresh weight over control by GA₃5ppm (142.17%), 10ppm (72.78%), 15ppm (32.65%). Minimum result was showed in kinetin 15ppm (-6.80%).
 - Dry Weight:-** Although the best performance was recorded an increase in dry weight over control by GA₃5ppm(405%), 10ppm(330%), 15ppm(145%). Minimum dry weight was recorded in kinetin 5ppm(10%). All GA₃ treatments were recorded highly significant. In IAA& kinetin results were non-significant.
 - Vigour Index-1:-** Aged Seed primed with GA₃5ppm an increase 133.53% vigour index-1 over control. Minimum vigour index-1 was recorded in kinetin 15ppm (-62.87%). Maximum result showed a significant increase in vigour index-1 over control. The accelerated aging conditions of 41°C for 72 hrs internationally accepted for seed vigor test of soybean could also separate pea seeds in different vigor levels (Hampton *et al.*, 2004) [9].
 - Vigour Index-2:-** Maximum vigour index-2 was recorded in the treatment GA₃5ppm 872.49%, 10ppm (382.87%), 15ppm (60.89%). This was followed by IAA and kinetin which were non-significant over aged control. Ashraf *et al.* (2002) found that GA₃ treatment enhanced the vegetative growth of two wheat cultivars under but caused a slight reduction in their grain yield. As per Basra *et al.* (2006) [6], rice seeds primed with 10 ppm GA₃ promote early germination and vigor enhancement.

Table 1: Effect of hormonal priming on germination%, speed of germination, germination value, fresh weight and dry weight in aged seed of French bean (*Phaseolus vulgaris*).

	Germination %	Germination value	Speed of germination	Fresh weight	Dry weight
IAA					
5ppm	11.66	26.46	0.32	4.10	0.14
10ppm	10	16.98	0.18	1.76	0.33
15ppm	10	13.33	0.26	3.62	0.28
Kinetin					
5ppm	13.33	21.09	0.37	1.21	0.22
10ppm	10	17.62	0.29	3.06	0.30
15ppm	13.33	14.52	0.37	4.11	0.26
GA ₃					
5ppm	55	102.29	1.57	10.68	1.01
10ppm	31.67	56.73	0.81	7.62	0.86
15ppm	20	33.15	0.58	5.85	0.49
Control	28.33	50.52	0.80	4.41	0.20
CD 1%	7.79	17.78	0.20	0.64	0.80
ANOVA					
A	1318.75**	404552.30*	1.06*	51.62**	0.75*
B	424.07**	217818.30*	0.40*	3.94**	0.11*
A*B	345.60**	112597.40*	0.27*	16.17**	0.59*

Table 2: Effect of hormonal priming on shoot length, root length, seedling length, vigour index 1&2 in aged seed of French bean (*Phaseolus vulgaris*)

	shoot length	root length	seedling length	Vigour index-1	vigour index-2
IAA (T1)					
5ppm	4.32	3.72	8.04	94.97	1.63
10ppm	2.91	2.21	5.12	35.58	0.48
15ppm	6.34	4.23	10.58	107.50	3.00
Kinetin(T2)					
5ppm	7.11	5.10	13.22	173.56	2.90
10ppm	5.12	5.03	11.15	112.25	3.10
15ppm	6.24	6.78	14.02	187.00	3.36
GA ₃ (T3)					
5ppm	11.32	10.07	21.39	1176.45	56.21
10ppm	8.29	7.12	15.41	490.70	27.91

15ppm	9.02	8.85	17.88	353.93	9.30
Control	9.26	8.40	17.73	503.75	5.78
CD 1%	0.45	0.78	1.04	139.54	6.92
ANOVA					
A	43.15**	47.48**	179.65**	706779.90**	1860.64**
B	47.48**	20.46**	77.10**	233831.40**	435.45**
A*B	7.69**	7.84**	25.49**	205568.50**	592.11**

A=Growth regulator B=concentration of growth regulator

Conclusion

It was concluded that 5ppm GA₃ showed the highest germination %, speed of germination, germination value, dry and fresh weight and vigor index I and II in *phaseolus vulgaris* as compared to Kinetin and IAA. Germination % is decreases according to increasing concentration of the used hormones regulators, due to the lower concentration of growth regulators favors the increased enzymatic activity for the germination and seedling growth. Marked reduction in germination and other traits might have been due to solute leakage and lipid per-oxidation which limit the necessary material for germination and seedling growth. This present study showed that growth regulator in higher concentration inhibits the seed germination. It can be concluded that significant variation was found in 5ppm GA₃.

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