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ORIGINAL ARTICLE

Effect of Accelerated Aging and Growth Regulators on Germination and Vigour of *Phaseoluse vulgaris* L.

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Legumes play very important role in agriculture as they form associations with bacteria that, fix nitrogen from the air. French bean is used in all over of the world as an edible vegetable crop. Seed ageing is the main problem of seed storage. Changes of bio-chemical and reduction of seedling growth are consequence of seed deterioration. An experiment was conducted to evaluate the effects of accelerated ageing on French bean seed germination and vigour indexes and hormone activity. Seeds of *Phaseolus vulgaris* L. were treated by accelerating treatments for 4, 6 and 8 days and the influence of ageing, GA_3 (20, 30 & 40 ppm) and IBA (5, 10 & 20 ppm) under different concentrations in 8 days aged seeds of French bean were observed. Various parameters were taken for consideration such as germination percentage, along with the mean daily germination, speed of germination, dry weight of seedling and vigour index-I & II. Seeds of *P. vulgaris* showed 96.66% germination without any treatment (control condition) but during the exposure of specific temperature (42^0C) with 100% relative humidity, seeds showed 30% reduction in germination just after 8 days of ageing treatments. While the treatment for 4 and 6 days did not show any remarkable changes in percent germination. So the seeds treated for ageing for 8 days were taken to enhance the germination by treatment of different hormones i.e. GA_3 and IBA with different concentrations. Aged seeds treated with GA_3 20 ppm showed high germination (82.99%) in comparison to GA_3 40 ppm (66.32%).

Key words: French bean, Seed germination, Vigour, Accelerated ageing, plant growth regulators

Legumes play very important role in agriculture as they form associations with bacteria that, fix nitrogen from the air. This quality affects the internal fertilization and it may be main reason that legume is richer in proteins than all other plants. Similar to other beans, the common bean is high in starch, protein and dietary fibber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamins B_6 and folic acid. French bean is used as a vegetable and pulses in India it contains 94% edible portion of the pods. It is a cool weather crop but thrives well in the optimum temperature ranging between 20 degree Celsius to 30 degree Celsius French bean is sensitive to frost; high temperature and high rain fall. High rain fall destroy the young growing seedlings of *Phaseolus vulgaris*. French bean is used in all over of the world as an edible vegetable crop. Seed ageing is the main problem of seed storage. Changes of bio-chemical and reduction of seedling growth are consequence of seed deterioration. An experiment was conducted to evaluate the effects of accelerated ageing on French bean seed germination and vigour indexes and hormone activity. Seeds were incubated in closed plastic boxes for the accelerated ageing treatments. As seeds age each seed in a, they deteriorate and eventually die. The reasons for the deterioration are complex and difficult to study since seed lot behave uniquely (Copeland & McDonald, 2001). The ability of a seed to germinate can only be measured by a germination test that in its nature is destructive.

The ageing of seeds is characterized by a sigmoid relationship between viability and storage time (Walters *et al.*, 2010 and Ellis & Roberts, 1980a). A long period in which very few seeds die is followed by a breaking point, and continued by a period of rapid decline of viability until most seeds are dead (Ellis & Roberts, 1980a).

Ageing is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling (Veselova and Veselovsky, 2003). Seed ageing is the main problem of seed storage. Unsuitable storage condition which has high temperature and moisture reduces seed vigour and viability (Sveinsdottir *et al.,* 2009). Accelerated ageing of seed is a treatment uses to assess storage quality, germination characteristics by simulation natural ageing conditions for different crops (Galleschi *et al.,* 2002 and Moradi *et al.,* 2009). Accelerated ageing of French bean seeds, which consists of placing seeds at high temperature and relative humidity, is associated with a progressive decrease in seed germinability (Bailly *et al.*, 1996).

One of the most popular tests used to evaluate French bean seed vigour is the accelerated ageing (AA) test (Spears, 1995), which is based on increased seed deterioration after exposure to high temperature and relative humidity. This test has been standardized to evaluate the physiological potential of bean seeds (Hampton & TeKrony, 1995).

Accelerated ageing is a method in which the seed's natural ageing is accelerated by exposure to heat, sometimes in combination with high humidity. Since the ageing process occurs during a few days up to some weeks, the quality parameters related to longevity and viability can quickly be estimated. The technique is based on the assumption that accelerated ageing mimics natural ageing. However, very few studies has been made comparing seeds aged naturally and accelerated (Galleschi *et al.*, 2002).

Knowing that, seeds ageing and priming with growth regulators can affect vigour 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 with different concentrations on seeds and seedlings development.

MATERIALS AND METHODS

The Experiment was carried out in Laboratory at the Department of Seed Science and Technology, Chauras Campus HNB Garhwal University, Srinagar, Uttarakhand. French bean (*Phaseolus vulgaris* L.) seeds were obtained from Local farmers of Srinagar Garhwal Uttarakhand. The experiment was lay out in a Randamized block design (RBD) and four replication with Ten treatment. The treatment consisted of **T_1-** Control, **T_2 -**4DAA, **T_3 -**6 DAA, **T_4-**8 DAA, **T_5 -**GA_3-20 PPM, **T_6 -**GA_3-30 PPM, **T_7 -**GA_3-40PPM, **T_8 –** IBA-5 PPM, **T_9** -IBA-10PPM, **T_{10} –**IBA-20PPM. Seeds were incubated in sealed containers at temperatures of 42ºC ±1º C with 100% humidity for 4, 6, and 8 days to accelerate the aging process.

Accelerated aging treatment

The test was conducted according to the procedure described by Hampton and TeKrony (1995). The desiccators were covered and maintained in an oven at 42 ± 2ºC for 4, 6, and 8 days of ageing treatments. After this aging period, four replicates of 25 seeds each were submitted for the standard germination test as described above.

Germination test

Four replications with twenty five seeds in each replication were sown on towel paper. Seeds were placed on the surface of double sheets of towel paper, which were moistened with distilled water. The seeds were covered with another sheet of paper towel. The sheets were rolled and placed vertically in a plastic beaker, covered with a butter paper at 25ºC in a seed germinator (ISTA, 1993). Seed germination & seedling development were observed daily. Such as Germination (%), mean daily germination, Speed of Germination, Seedling length (cm), Seedling Fresh and Dry weight, Seedling vigour index-1, Seedling vigour index-2 were measured and recorded.

Statistical Analysis

The collected data for various parameters were statistically analyzed by using ANOVA. The significance of comparison was tested. The Standard Error of Means (SEm±) and significance difference values were computed for 5 percent probability of error. Wherever the variance ratio (F value) was found significance, critical difference (CD) values were computed for the comparison among the treatment means.

RESULTS AND DISCUSSION

Seeds of *Phaseolus vulgaris* L. were treated by accelerating treatments for 4, 6 and 8 days and the influence of ageing, GA_3 (20, 30 & 40 ppm) and IBA (5, 10 & 20 ppm) under different concentrations in 8 days aged seeds of French bean were observed. Various parameters were taken for consideration such as germination percentage, along with the mean daily germination, speed of germination, dry weight of seedling and vigour index-I & II. (Table -1)

Germination percentage

Seeds of *P. vulgaris* showed 96.66% germination without any treatment (control condition) but during the exposure of specific temperature (42^{0}C) with 100% relative humidity, seeds showed 30% reduction in germination just after 8 days of aging treatments. While the treatment for 4 and 6 days did not show any remarkable changes in percent germination. So the seeds treated for aging for 8 days were taken to enhance the germination by treatment of different hormones i.e. GA_3 and IBA with different concentrations. Aged seeds treated with GA_3 20 ppm showed high germination (82.99%) in comparison to GA_3 40 ppm (66.32%).

However in case of IBA, highest germination (76.32%) was observed in 20 ppm in comparison to 10 & 5 ppm. In case of IBA effects on aged seeds, as the concentration increased germination percent also increased. But all the experiment on aged seeds did not achieve the actual germination capacity of seeds, which was observed in control condition (96.66%).

Mean Daily Germination

Mean daily germination was reduced in the aged seeds. In case of control (without any treatment) 13.46 mean daily germination was found, followed by accelerated aged seeds for 4 days (7.74) and 6 days (7.67), but lowest (6.32) was found in seeds aged for 8 days. Effect of GA_3 was more in contrast to IBA to enhance the mean daily germination of aged seeds for 8 days. All the treatments showed most mean daily germination in comparison to 8 days aged seeds. Highest enhancement of germination was found in 8 days aged seeds treated with GA_3 20 ppm followed by IBA 20 ppm (11.56), GA_3 30 ppm and IBA 10 ppm (10.37), IBA 5 ppm (9.42) and GA_3 40 ppm (9.18).

Speed of Germination

Speed of germination was highest (24.56) in IBA 10 ppm and lowest (17.15) in 8 days accelerated aged seeds. All other hormonal treatments on 8 days aged seeds showed very positive effect except GA_3 40 ppm (18.55). Speed of germination of 8 days aged seeds treated with IBA 10 ppm (24.56) and GA_3 30 ppm (24.52) were slightly highest than control condition (23.95). Speed of germination was (23.36) in GA_3 20 ppm followed by IBA 20 ppm (21.40) and IBA 5 ppm (21.32).

Seedling Length

Seedling length of control was highest (25.36cm) in comparison to all other treatments. Seedling of 8 days aged seeds showed drastic reduction (15.32cm) in contrast to control condition, positive effect of GA_3 & IBA was found. GA_3 20 ppm treated seeds showed seedling length (24.46) very near to control condition, followed by GA_3 30 ppm (18.38cm) and GA_3 40 ppm (16.46cm) concentration. While in case of IBA maximum enhancement in length was observed in 20 ppm (20.89cm) in contrast to IBA 10 ppm (19.05cm) & 5 ppm (15.87cm).

Dry weight of seedling (mg)

In respect of dry weight, accelerated aging did not show any remarkable variations in seeds aged for 4, 6 & 8 days in contrast to control (78.76) condition. Growth regulator did not seem significant for the enhancement of dry weight of seedling, of seeds aged for 8 days, except IBA 5 ppm (91.96mg) and IBA 10 ppm (75.06).

Table 1. Effect of Accelerated ageing and growth regulators on Germination and vigour of *Phaseolus vulgaris* L.

№	Treatment		Germination %	Mean Daily Germination	Speed of Germination	Seedling Length	Seedling Dry W (mg)	Vigour I	Vigour II
1	T1	Control	96.99±0.33	13.46±0.33	23.95±0.33	25.36±0.33	78.76±0.33	2460.50±37.24	7639.95±50.79
2	T2	4 DAA	96.33±0.33	7.74±0.33	23.51±0.33	25.00±0.32	74.76±0.33	2409.71±23.89	7202.63±49.52
3	T3	6 DAA	93.99±0.33	7.67±0.33	21.64±0.33	24.68±0.33	73.66±0.33	2320.69±39.45	6924.31±28.64
4	T4	8 DAA	66.99±0.33	6.32±0.33	17.15±0.33	15.32±0.31	71.96±0.33	1028.57±17.32	4821.40±40.12
5	T5	GA_3 20 PPM	82.99±0.33	11.56±0.33	23.36±0.33	24.46±0.33	64.76±0.33	2030.87±35.70	5375.30±25.30
6	T6	GA_3 30 PPM	74.66±0.33	10.37±0.33	24.52±0.33	18.38±0.32	72.26±0.33	1373.84±30.91	5395.80±24.57
7	T7	GA_3 40 PPM	66.32±0.33	9.18±0.33	18.55±0.33	16.46±0.33	68.16±0.33	1092.40±27.48	4521.15±22.51
8	T8	IBA 5 PPM	67.99±0.33	9.42±0.33	21.32±0.33	15.87±0.33	91.96±0.33	1079.78±27.84	6253.31±27.67
9	T9	IBA 10 PPM	72.99±0.33	10.37±0.33	24.56±0.33	19.05±0.33	75.06±0.33	1391.29±30.57	5479.50±24.79
10	T10	IBA 20 PPM	76.32±0.33	10.60±0.33	21.40±0.33	20.89±0.33	58.96±0.33	1595.19±32.29	4500.62±23.23
		C.D.	0.865	0.008	0.022	0.023	0.040	20.666	69.000
		SE(m)	0.289	0.003	0.007	0.008	0.014	6.902	23.045
		SE(d)	0.409	0.004	0.010	0.011	0.019	9.761	32.590
		C.V.	0.629	0.048	0.057	0.064	0.032	0.712	0.687

Seedling Vigour Index (I):

Vigour index-I of artificially aged seeds for 8 days was found very less (1028.57) in contrast to control (2460.50). Remarkable variation in respect of vigour index-I of 8 days accelerated aged seeds was found in GA_3 20 ppm (2030.87) followed by IBA 20ppm, (1595.19), IBA 10 ppm (1391.29) and GA_3 30 ppm (1373.84) treated seeds. All other treatments did not show significant enhancement of vigour index -I in 8 days aged seeds.

Seedling Vigour Index (II)

Vigour index-II was highest (7639.95) in control condition in contrast to all other experimental treatments. All the treatments for enhancement of vigour index-II of 8 days aged seeds was found effective except IBA 20 ppm (4500.62). Highest enhancement (6253.31) was found in IBA 5 ppm treated 8 days aged seeds. This was followed by IBA 10 ppm (5479.50), GA_3 30 ppm (5395.80), GA_3 20 ppm (5375.30) and GA_3 40 ppm (4521.15).

Accelerated aging up to 6 days did not show variation in germination percentage of *Phaseolus vulgaris*, but after 8 days of aging treatment, germination percent decreased by more than 30% in contrast to

control condition. Gay *et al.*, 1991 observed that reduction in sunflower seed germination, when seeds were stored at 45^0C with 100% relative humidity. Seeds, treated for 8 days by accelerated aging treatment, showed marked reduction in respect of all the observed traits were selected for the application of growth regulators to enhance the planting value. GA_3 20 & 30 ppm and IBA 20 ppm found most effective for the enhancement of all the studied traits.

Wheat seeds were primed with 0.5% of KH_2PO_4 showed maximum seed vigour index reported by (Kari *et al.*, 2010). Diaosheng *et al.*, (2009) also reported, enhancement in seeds germination index of draught stressed *Prunella vulgaris* seeds primed with GA_3, PEG, KNO_3 and KH_2PO_4. Abdolachi *et al.*, 2010 reported the invigoration of deteriorated rape seeds by priming with KH_2PO_4 and $CaCl_2$. Seeds primed with growth regulators can rapidly restore the seed metabolism and imbibitions process to enhance the germinability (McDonald, 2000).

In case of *P. vulgaris,* both the vigour index showed marked enhancement in 8 days aged seeds, when treated with GA_3 20 & 30 ppm and IBA 5 & 20 ppm. It showed, significant invigoration in aged seeds may be achieved by the treatment of adequate growth regulators. Vigour test is actually the useful and adequate process to check the seeds performance and identify the vigour seeds lots under wide range of environmental conditions (Rodo and Filho, 2003). Chhabra and Ram (1988) studied ten genotype of each American cotton and desi cotton was tested for eight characters. The accelerated ageing at 40°C for 48 hours significantly reduced the standard germination. However, the reduction was more pronounced when these genotypes were aged for 72 hrs.

CONCLUSION

In India there is a major problem to store the seeds in places with a proper control of humidity and temperature. Both these are the key factor to control the planting value and storability of any seeds. Artificial seed ageing is a process to check the storability of seeds within very short period and to predict about the seeds nature at adverse conditions. It could also help scientists, breeders and agronomists to develop strategies for the reduction of effect of unfavourable conditions.

Effects of accelerated ageing and growth regulators on physiological attributes of *Phaseolus vulgaris* were studied. All the observed parameters have been affected significantly by seed ageing treatment especially for 8 days. Marked reduction in germination and other related traits might have been due to solute leakage and lipid per oxidation which limits the necessary material for germination and seedling growth.

Growth regulators have been used to reduce the effect of ageing and to initiate the process of invigoration. Gibberellic acid (GA_3 20 ppm) and Indole Butyric Acid (IBA 20 ppm) with different concentrations were tried in this experiment and were found effective in almost all the traits. Which showed the invigoration may achieve by the application of proper growth regulators with adequate concentrations.

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