Vegetable Science (2013) 40 (2) : 169-173

Investigations on seed viability and vigour of aged seeds by priming in French bean (*Phaseolus vulgaris* L.)

G Sarika, GV Basavaraju, K Bhanuprakash, BC Chaanakeshava, R Paramesh and BN Radha

Received : February, 2013 / Accepted : June, 2013

Abstract: French bean seeds are more sensitive to storage conditions.The factors like high temperature, relative humidity, seed moisture content, light exposure and an extended storage period have been found to adversely affect seed quality. These factors may cause color darkening and hard-to-cook defect. A procedure that can ameliorate the detrimental effects of seed ageing is seed priming. Chemo priming using GA3 and ethrel improved the seed quality and showed improved seedling length, seedling dry weight which in turn improved higher seedling vigor index, germination speed, mein germination time etc. During seed priming the process of hydration initiates the earliest physiological stages of germination and perhaps physiological repair of membranes and organelles damaged during seed storage resulting in more rapid and uniform seedling emergence.

Keywords: Seed viability, seed priming, french bean

Introduction

Phaseolus vulgaris, the common bean is popular for both dry and green bean as well. The leaf is occasionally used as a leaf vegetable and the straw is used forfodder. They are good source of proteins, carbohydrate, vitamin C, carotene and a variety of minerals, some fibre but very little protein. French bean seeds are more sensitive to storage condition.A procedure that can ameliorate the detrimental effects of seed ageing is seed priming. Priming is accomplished by imbibing seeds in an aqueous solution. During priming seeds are permitted to enter the lag stage of germination (stage with little or no fresh weight increase prior to radicle emergence), but are then desiccated back to approximately the original moisture content before the radicle emerges. Upon subsequent rehydration, seeds show improved germination characteristics which include (1) reduced time to radicle emergence, (2) synchronization of germination within a seed lot, (3) greater percentage germination, and (4) improved seed vigour in deteriorated seed lots. Recently many scientists have concluded that the post storage priming treatments reduced the chromosomal aberrations, increased the rate of root growth, and decreased the frequency of morphologically abnormal seedlings and more or less complete repair of DNA lesions which are occurring during the storage. Seed priming is not only useful for regeneration purposes but also reduces the incidence of heritable genetic damage.

Significant changes in enzyme activities were noticed in primed seeds compare to un primed seeds, desiccation and storage of seeds has been suggested to result in progressive loss of integrity of the membrane components of the seeds, which in turn contribute to seed deterioration as measured by loss of seed vigor and viability (Agrawal and Dadlani 1995). During imbibition prior to germination the integrity of cell membranes need to be reestablished. Rapid imbibition by the seed at this time, possibly reverse the damage and cell will attain maximum vigor by repair mechanism.Proteins detected by the proteomic analysis, whose abundance specifically increases during hydropriming, is a catalase isoform. Catalase is a free-radical scavenging enzyme. It is presumed that hydropriming initiates an oxidative stress, which generates reactive oxygen species, and catalase is synthesized in response to this stress to minimize cell damage. In addition to catalase, levels of superoxide dismutase, another key enzyme quenching free radicals also increases during priming. Increased levels of these free radical scavenging enzymes due to the oxidative stress during priming could also protect the cell against membrane damage due to lipid peroxidation occurring naturally (Anuradha *et al.,* 2010).

G. Sarika, G.V. Basavaraju, K. Bhanuprakash,
B. C. Chaanakeshava, R Paramesh and B.N. Radha
Department of Seed Science and Technology, GKVK, UAS, Bangalore-560 065, India
Indian Institute of Horticultural Research, Hesaragatta, Bangalore-560 065, India

Materials and Methods

A lab experiment to study various physiological and biochemical were carried out as outlined below in French bean seeds, which were subjected to both ageing (Natural & accerlated) were under taken in Indian Institute of Horticultural Sciences, Hessarghata, Bangalore. Freshly harvested French bean seeds (var. ArkaKomal) were collected from IIHR, Bangalore and stored up to one year to carry out studies on ageing induced changes at evenly 3 months interval. Fresh seeds (untreated) were subjected to artificial ageing as per ISTA procedure for a period of 15 days at 45°C + 75% RH. Samples were collected at 3 days interval for seed quality studies. The seed lots(fresh and aged) are treated with different chemicals(KH2PO4; K2HPO4; PEG; KNO3; KCl; KBr; K2SO4;GA3; ethrel; dry seed; Hydro primed) and at different temperatures(30 ^{0}C &15 ^{0}C), these treatments are subjected for seed quality analysis.

Physiological parameters

Seed germination: Done as per ISTA rules, the germination counts were recorded on 5th and 9th day and per cent germination was expressed on normal seedling basis.

Speed of germination:The daily germination count was taken up to final count. The germination speed index (GSI) was calculated by using the following formula.

$$GSI = \frac{G1}{T1} + \frac{G2}{T2} + \text{————} + \frac{Gn}{Tn}$$

Where,

G1, G2, ———— Gn = Number of seeds germinated

T1, T2, ———— Tn = Number of days taken for germination

Root length, shoot length, seedling dry weight, seed vigour index-I, seed vigour index-II were recorded as per ISTA guidelines.

Biochemical parameters

Electrical conductivity (ms/cm):1g of seed sample was soaked in 25 ml of distilled water for 24 hours at 25±1°C. The EC at 25±1°C was measured using conductivity meter.

Membrane injury index:Membrane injury index (MII) was calculated by the formula given by Blum and Ebercon(1981).

$MII = C_1/C_2$

$C_{1=}$ Electric conductivity at 40^{o}C for 30 min.

C_2=Electric conductivity at 100^{o}C for 10 min.

Protein content : The protein content of seeds was estimated by using alkaline copper and folin method (Lowry *et al.*, 1951) with crystalline bovine serum albumin as standard curve. The soluble protein in the

Table 1. Effect of priming treatments on germination (initial count), no. of normal seedlings, at different temperatures on fresh an aged seed lots

Priming treatments	Initial germination				No. of normal seedlings				No. of abnormal seedlings			
	High vigour(B1)		Low vigour(B2)		High vigour(B1)		Low vigour(B2)		High vigour(B1)		Low vigour(B2)	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
A1	97.6	97.6	76.6	70.0	95.6	95.6	72.3	66.3	1.66	2.00	3.66	3.66
A2	98.6	98.6	75.0	73.6	95.3	96.6	69.6	68.0	3.33	1.66	4.66	5.33
A3	97.0	97.0	65.6	60.3	95.0	95.6	61.0	56.6	2.00	1.33	4.66	3.66
A4	96.6	97.6	71.6	70.0	93.6	95.6	67.0	66.3	3.33	2.33	4.66	3.66
A5	97.6	96.6	70.0	68.0	95.0	93.6	65.6	63.6	2.66	3.66	4.33	4.33
A6	97.6	97.6	71.6	69.3	94.6	94.0	66.6	64.6	3.00	3.33	5.00	4.66
A7	97.6	97.6	71.6	69.3	95.0	95.0	66.6	64.0	2.66	2.66	5.00	5.33
A8	98.6	98.6	82.3	70.3	97.3	97.3	80.6	66.6	1.33	1.33	1.66	3.00
A9	99.0	98.6	78.3	71.0	97.3	97.3	75.0	67.3	1.66	1.33	3.00	2.66
A10	98.0	97.3	60.0	60.0	96.0	95.6	56.6	55.3	2.00	1.66	3.33	4.66
A11	98.0	97.3	75.0	71.0	95.0	95.3	70.0	67.0	3.00	2.00	5.00	3.00
	C.D. @1%				C.D. @ 1%				C.D. @1%			
A	2.27				2.75				1.58			
B	0.96				1.17				0.67			
C	0.96				1.17				0.67			
A*B	3.21				3.89				2.23			
A*C	3.21				3.89				2.23			
B*C	1.37				1.65				0.95			
A*B*C	4.54				5.50				3.16			

A_1-KH_2PO_4;A_2-K_2HPO_4; A_3– PEG; A_4- KNO_3; A_5–KCl; A_6–KBr; A_7- K_2SO_4;A_8- GA_3; A_9-ethrel; A_{10}- dry seed; A_{11}- Hydro primed; B_1-Fresh seed lot; B_2- Aged seed lot; C_1-temperature at 30 ^{0}C;C_2- temperature at 15^{0}C

sample was expressed as mg/ml of protein extract.

Dehydrogenase activity:Dehydrogenase activity was estimated as per the procedure given by (Agarwal and Dadlani, 1995). The dehydrogenase activity was measured by reading the absorbance at 510 nm and OD values were expressed.

Catalase activity: Catalase activity measured by an assay of hydrogen peroxide based on formation of its stable complex with ammonium molybadate (Goth, 1991).

a-amylase activity: The a-amylase activity in developing seeds were estimated according to the method of Sadasivam and Manickam (1996). The enzyme activity was calculated by measuring OD at 560 nm. a-amylase activity was expressed as mg maltose released/ml/min.

Total soluble sugars in seed leachate: It was estimated by the method Dubois *et al.*, (1951). The intensity of the color was recorded at 490 nm in spectrophotometer and values were expressed in ug/ml.

Results and Discussion

There was significant difference between germination per cent, normal seedlings, abnormal seedlings, dead seeds, mean germination time, speed of germination, vigour index-I & vigour index-II, seedling length, dry weight of the seedlings between fresh seed lot and aged seed lot. All fresh seed lots should better seed quality attributes in all chemical treatments. The temperature treatment at 15^0C good quality attributes compare to 30^0C. It was noticed that there was significant increase in the seed quality parameters in case of aged seed lot undergone priming with GA_3(1000ppm), the per cent improved in germination (82.3), number of normal seedlings increased (97.3), no. of abnormal seedlings decreased (1.66), decrease in the number of ungerminated seeds(6.66), at 15^oc, at 30^oc the germination per cent (69.66), no of abnormal seedlings decreased to (3.00), no of ungerminated seeds reduced to (7.33) followed by ethrel(1%), KH_2PO_4 and hydro priming treatments.

Significant increase in the seed quality parameters in case of aged seed lot undergone priming with GA_3 (1000ppm), the increase in initial root length (6.02cm), initial shoot length (8.13cms), final root length (11.5cms), final shoot length (17.56cms), seedling vigour index-I (3056), dry weight of 10 seedlings increased (1038.6mg) in 15^oc, at 30^oc the initial root length (5.77cm), initial shoot length(7.8cms), final root length (10.63cms), final shoot length (16.59cms), seedling vigour index (2966), dry weight of seedlings increased (1022) was observed followed by ethrel (1%), KH_2PO_4 and hydro priming treatments (Tables 2, 3 & 4).

There was significant difference between catalase activity in fresh (0.296) and aged (0.176) seed lots at 15^oC as well as the catalase activity in fresh (0.283) and aged (0.173) seed lots at 30^0C, with respect to fresh seed lot, the catalase activity among the treatments are on par with each other at 15^oC and 30^oC.

It was noticed that there was significant increase in the catalase activity in case of aged seed lot undergone priming with GA_3 (1000ppm) which resulted in

Table 2. Effect of priming treatments on seedling vigour index-I, seedling vigour index-II, dry weight of seedlings, at different temperatures on fresh an aged seed lots

Priming treatments	Seedling vigour index-I				Seedling vigour index-II				Dry weight of seedlings			
	High vigour(B1)		Low vigour(B2)		High vigour(B1)		Low vigour(B2)		High vigour(B1)		Low vigour(B2)	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
A1	2879	2798	1942	1709	99.6	99.4	70.3	64.5	1022	1019	925	921
A2	2886	2820	1907	1791	100	99.8	69.2	67.7	1016	1013	924	921
A3	2815	2714	1640	1437	99.0	98.4	60.6	55.5	1021	1015	925	922
A4	2771	2695	1804	1649	98.5	98.8	66.1	64.0	1018	1011	922	915
A5	2797	2676	1763	1680	99.1	98.0	64.6	62.0	1017	1013	923	913
A6	2544	2535	1813	1719	98.8	98.5	66.0	63.5	1013	1011	924	918
A7	2798	2698	1802	1713	99.9	99.2	65.9	63.4	1024	1018	922	915
A8	3056	2967	2371	1948	102	100	77.4	64.3	1038	1022	941	925
A9	3059	2906	2239	1919	102	101	72.1	64.6	1030	1026	928	923
A10	2966	2847	1527	1490	100	99.4	55.3	55.1	1026	1022	922	919
A11	2867	2847	1948	1745	100	99.1	69.1	64.3	1026	1019	922	919
	CD @1%				CD @1%				CD @1%			
A	100.9				2.16				3.89			
B	43.05				0.92				1.66			
C	43.05				0.92				1.66			
A*B	142.7				3.06				5.51			
A*C	142.7				3.06				5.51			
B*C	60.88				1.30				2.35			
A*B*C	201.9				4.32				7.79			

Table 3. Effect of priming treatments on final root length, final shoot length, seedling length (final count), at different temperatures on fresh an aged seed lots

Priming treatments	Final root length				Final shoot length				Seedling length (final count)			
	High vigour (B1)		Low vigour (B2)		High vigour (B1)		Low vigour (B2)		High vigour (B1)		Low vigour (B2)	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
A1	12.1	11.9	10.2	10.0	17.4	16.8	15.3	14.3	29.5	28.7	25.6	24.4
A2	12.0	11.9	10.1	10.0	17.2	16.7	15.2	14.3	29.3	28.6	25.4	24.3
A3	11.9	11.6	10.0	9.76	17.1	16.3	15.0	14.1	29.0	28.0	25.0	23.8
A4	11.7	11.3	9.8	9.50	16.9	16.2	15.3	14.0	28.6	27.5	25.1	23.5
A5	11.6	11.2	9.86	9.40	17.0	16.4	15.3	15.3	28.7	27.6	25.2	24.7
A6	11.1	11.2	10.0	9.93	14.9	14.8	15.3	14.9	26.1	26.0	25.3	24.8
A7	11.6	11.2	9.86	9.93	17.0	16.4	15.3	14.8	28.7	27.6	25.2	24.7
A8	13.4	13.2	11.5	11.1	17.5	16.9	17.2	16.9	31.0	30.1	28.3	28.0
A9	13.5	13.0	11.5	10.6	17.4	16.4	17.2	16.7	30.9	29.5	28.8	27.3
A10	12.8	12.1	10.1	9.89	17.4	17.1	15.3	14.9	30.2	29.3	25.4	24.8
A11	12.0	11.7	10.1	10.0	17.2	17.5	15.8	14.9	29.2	29.2	25.9	24.9
	CD @1%				CD @1%				CD @1%			
A	0.53				0.59				0.84			
B	0.22				0.25				0.36			
C	0.22				0.25				0.36			
A*B	0.75				0.84				1.19			
A*C	0.75				0.84				1.19			
B*C	0.31				0.36				0.50			
A*B*C	1.06				1.19				1.69			

improved catalase activity (0.31) at 15°C, at 30°C the catalase activity (0.293), followed by ethrel(1%)(0.30), KH_2PO_4 and hydro priming treatments.

With respect to priming treatments at fresh and aged seed lot, the GA_3 treatment given highest catalase activity (0.23) compare to control (0.176) followed by ethrel, the remaining treatments were on par with each other.The activity of catalase was very less in aged seeds, but the priming induced more catalase activity significantly in GA3 and followed by ethrel, priming also reduces lipid peroxidation during subsequent seed storage. In onion seeds (Bassu, 1976) it has been demonstrated that hydro priming treatments effectively showed physiological deterioration under natural and accerlated ageing conditions, with the effect being dependent on seed vigor, this improved stability was associated with greater dehydrogenase activity and appreciabiliy lowered the catalase activity, increased hydration enhanced membrane repair in seeds attributed this to the stimulation of free radical scavenging enzymes such as superoxide dismutase, catalase, peroxidase and glyoxysomes enzymes, these enzymes control ageing

Table 4. Effect of priming treatments on seedling growth rate, speed of germination, catalase activity, at different temperatures on fresh an aged seed lots

Priming treatments	Seedling growth rate				Speed of germination				Catalase activity			
	High vigour (B1)		Low vigour (B2)		High vigour (B1)		Low vigour (B2)		High vigour (B1)		Low vigour (B2)	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
A1	5.90	5.74	5.11	4.88	74.6	72.6	24.7	24.5	0.31	0.30	0.18	0.17
A2	5.86	5.72	5.08	4.87	72.1	71.2	24.5	23.2	0.31	0.30	0.18	0.17
A3	5.80	5.60	5.00	4.77	72.9	70.8	24.2	23.4	0.31	0.29	0.18	0.17
A4	5.72	5.51	5.02	4.71	72.8	71.8	23.1	22.0	0.31	0.30	0.17	0.17
A5	5.74	5.53	5.04	4.94	73.1	71.6	23.8	22.0	0.30	0.28	0.17	0.16
A6	5.22	5.20	5.07	4.96	73.5	72.5	23.5	20.3	0.29	0.27	0.16	0.16
A7	5.74	5.53	5.04	4.94	72.6	71.7	23.2	21.9	0.29	0.26	0.16	0.15
A8	6.20	6.02	5.76	5.60	74.7	72.4	31.2	26.2	0.31	0.29	0.23	0.20
A9	6.18	5.90	5.76	5.47	72.7	70.7	25.8	21.3	0.30	0.28	0.23	0.20
A10	6.05	5.86	5.09	4.96	71.6	70.4	25.9	21.3	0.29	0.28	0.17	0.17
A11	5.85	5.85	5.19	4.98	71.3	70.4	25.3	24.6	0.29	0.26	0.17	0.17
	CD @1%				CD @1%				CD @1%			
A	0.16				1.16				0.0171			
B	0.07				0.49				0.0073			
C	0.07				0.49				0.0073			
A*B	0.23				1.64				0.0242			
A*C	0.23				1.64				0.0242			
B*C	0.10				0.70				-			
A*B*C	0.33				2.32				-			

process by counteracting with lipid peroxidation.

Alteration in the isozyme profiles in primed and unprimed seeds was also noticed. In the present investigation, when compared to dry seeds, primed seeds exhibited more activation of enzyme and more of isoforms. The increase in the germination of primed seeds might be due to increase in the synthesis of these enzymes. Similar results were reported in various crops (Mc Donald, 1999).

Thus based on the findings, it was concluded that in French bean, the loss of viability in aged seeds was due to impaired metabolism as evident from lower levels of enzyme synthesis and higher membrane injury. Priming allowed repair system to combat subcellular damage activated enzyme synthesis and thus restored deterioration process started due to ageing. The changes in the activities of the enzymes, upon priming, suggest that mobilization of storage material may be responsible for increased germination and vigor in primed seeds when compared to unprimed –aged seeds.

Acknowledgements

Authors would like to thank Indian Institute of Horticultural Research, Hesaragatta, Bangalore, for providing seed and lab facilities for conducting biochemical and molecular work.

References

Agrawal PK and Dadlani M (1995) Techniques in Seed Science and Technology. Second Edition. South Asian Publishers New Delhi International Book Company Absecon Highlands: 109–113.

Anuradha V, Alice KV and Malavika D (2010) The subcellular basis of seed priming. Current Science 99:450-456.

Bassu RN (1976) Physico-chemical control of seed deterioration. Seed Res 4:15–23.

Blum A and Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21 (1):43-47.

Dubois KG, Hamilton JA, Rebersand PA and Smith F (1951) A Colorimetric Method for the Determination of Sugars. Nature, 167-168.

Goth L (1991) A simple method for determination of serum catalase activity and revision of reference range, Clinica Chimica Acta 196:143-151.

Lowry OH, Rosebrough JN, Lewisand FA and Randall JR (1951) Protein measurement with the folin phenol reagent. The Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri.

Donald MC, MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Tech 27:177-237.