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Effect of Aging and Priming on Physiological and Biochemical Traits of Common Bean (*Phaseolus vulgaris* L.)

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Abstract

Aging and deterioration (artificial aging) are the most effective factors on the seed vigour. In order to study the changes in physiological and biochemical characteristics of common bean under aging and priming treatments a factorial experiment based on completely randomized design conducted with three replications. Seed aging (control, 90 and 80% of control germination) and seed invigoration with priming including control, hydro (distilled water), osmo (PEG 6000), hormone (gibberellic acid) and halo (NaCl) priming were considered as experimental factors. Results showed that osmo-priming had the ability to relatively ameliorate the aging effect and recover some of the seed aspects like germination rate, protein and phytin content for invigorate germination and seedling establishment. Priming indirectly increased seed vigour via germination rate and it can provide homogeny of emergence in the field and obtaining appropriate plant population.

Keywords: aging, common bean, germination rate, phytin, priming

Introduction

Legumes are dry edible grains from fabaceae family that ripened ones have valuable nutritional reserves and good storage capability under suitable conditions. Common bean (*Phaseolus vulgaris* L.) is one of the most important plant of this family due to high protein content and other agronomic traits that is more cultivated than other legumes (Torabi *et al*., 2008). Consumption of legumes protein is more economic than animal protein especially in developing countries and approximately 50% of pulse cultivated lands in I.R. Iran have been specified to common bean (Habibi *et al*., 2006).

Common bean is used as dried or fresh for nutritional purposes. Nutritional value of bean is high so that 100 g of its grain has 130 kj energy, 3-4 g dietary fiber, 0.1 g lipid, 1.8 g protein, 34 µg vitamin A, 1.6 mg vitamin C and 37 mg calcium (Nahar *et al*., 2009). Seed viability and vigour decrease under long storage condition due to aging. It is the reason of declining in germination, emergence and seedling growth (Soltani *et al*., 2008). All organisms undergo aging and it enhances under unfavourable or stress environments. Maximum germination potential of seeds achieves immediately after harvesting and gradually decreases with storage time. Aging is one of the key factors in plant yield loss especially in vegetables. Seed aging is recognized by some parameters like delay in germination and emergence, slow growth and increasing of susceptibility to environmental stresses (Walters, 1998). Biotic and abiotic factors affect seeds vigour and quality till they are on mother plants. Genetic structure, environmental condition during seed development and condition of stores mainly affect on seed vigour (Copland and McDonald, 2001). Using vigorous seeds at planting may increase crop yield in two ways: firstly, higher percentage of seedlings emergence than aged or weaken seeds that gives optimum density even under stressful conditions and secondly, high growth and emergence rate in comparison to seedlings produced from aged seeds (Ghasemi-Golezani *et al*., 2010).

Priming is a seed improving and invigoration technique applied before planting and can increase rate and percent of germination and emergence especially under stress conditions like salinity, drought and high temperature stresses (Sedghi *et al*., 2010a). At priming, seeds uptake water and progress into the imbibition (second stage in water absorption curve) but, radicle is not sprout, then seeds were redried and stored at suitable conditions (low temperature and humidity) until planting time (McDonald, 1999). Sing and Amritphale (1993) used GA_3 and benzyladenine for priming soybean seeds and observed vigor increase in artificially and naturally aged seeds. Sedghi *et al*. (2011) indicated that the loss of seed vigor can cause the reduction of germination percentage and priming with phytohormones improved and invigorated the poor vigor seed lots. In the present study it has been investigated the effect of seed aging and the ability of different priming materials for returning seed vigour of common bean and some physiological and biochemical properties.

Material and methods

A factorial experiment based on Completely Randomized Design (CRD) with three replications was conducted

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to evaluate the effects of priming on the germination and growth parameters of rapidly aged common bean seeds. Treatments were rapid aging (control, 90% and 80% germination) and priming (hydro, osmo, hormone and halo priming with control). Seed aging was performed according to rapid aging method. Firstly, seed moisture content has been raised to 15-16% in a growth chamber with high relative humidity (RH= 80±5 %) and measuring moisture content in one day intervals for confidence of considered moisture content. Then, seeds placed into an oven at 40°C. A preliminary experiment was done to determine the aging levels considering to 80 and 90% germination of control. On the basis of this experiment and probit analysis of data, the required time for achieving 80 and 90% germination was 277 and 256 hours, respectively. Dry seeds were soaked 12 h in aerated priming solutions and then rinsed several times by distilled water and re-dried at laboratory temperature (25±2°C). Concentrations of priming solutions were 253 g lit^{-1} Poly Ethylene Glycol (PEG 6000) for osmo priming, 3% NaCl for halo priming and 20 mg lit^{-1} gibbarllic acid (GA_3) for hormone priming. Standard germination test was done according to ISTA (2008) with paper towel as planting bed for 8 days at 20°C with 50 seeds and three replications. Size of paper towels (Boeco-Germany) was 58×58 cm and seeds were planted on papers that folded like sandwich. Paper sandwich were covered by plastic bags and vertically placed in an incubator. On the 8th day, germination percentage was calculated by counting the normal and abnormal seedlings. Ten days after germination period at 20°C and after developing cotyledon leaves, 5 seedlings from each plot were selected for the measurement of plumule and radicle length (cm), dry fresh weights (g) of radicle and plumule (after oven drying at 72°C to constant value).

Germination test was conducted again at 15°C for calculating the germination rate according to the method described by Ellis and Roberts (1980). Sprout counting was began on a day after planting and continued to the 10th day. Sprouting 2 mm of radicle was assumed as germination. Germination rate was calculated as below:

$$Gr = \sum_{i=1}^{N} \frac{Si}{Di}$$

That, *Gr* was germination rate (germinated seed day^{-1}), *Si* number of germinated seeds in each day, *Di* number of days of counting and *N*, number of counting.

Seed reserves using rate (SRUR, mg seed^{-1}), seed reserve using efficiency (SRUE, mg mg^{-1}) and fraction of seed reserves (FUSR) were calculated by formulas below according to Sedghi *et al.* (2010a) and Soltani *et al.* (2008):

SRUR= SDW-RSDW
SRUE= SFDW/SRUR
FUSR= SRUR/SDW

In these formulas, SDW was seed dry weight, RSDW, Dry weight of seed residue (without radicle and plumule) and SFDW, sum of dry and fresh weight of seedling (radicle + plumule).

Seed total nitrogen percentage was measured by Kjeldahl method and multiplied by 6.25 to convert the nitrogen percent to protein percent. Latta and Eskin (1980) method was used for phytin measurement. A 500 mg sample was extracted with 20 ml of 2.4% HCl (0.65 N) for 2 h at room temperature on a rotatory shaker (200 rpm). The extract was centrifuged (10,000 g, 15 min) and the supernatant was decanted and filtered through Whatman number 1 filter paper. A 3 ml aliquot of the filtrate was diluted to 18 ml with distilled water and the diluted sample was passed through a 200-400 mesh AG1-X8 chloride anion exchange resin. Phytate was determined colorimetrically based on the pink color of the Wade reagent, which was formed upon the reaction of ferric ion and sulfosalicylic acid, and had an absorbance maximum at 500 nm.

Phosphorous content was determined according to Warraich *et al.* (2003). One gram of seed was digested in 20 ml of concentrated HNO_3 and then 10 ml of 72% $HClO_4$ was added to heat and give colorless end point, then was cooled and transferred to a flask, and 5 ml H_2SO_4, 5 ml ammonium vendate (0.25%) and 5 ml ammonium molybadate (5%) were added and allowed to stand for 3 min. Reading was recorded on Backman Photometer using blue filter paper. From standard curve actual reading was calculated and reported as µmol g^{-1} fresh weight.

Data analyses were performed by SAS 9.1 statistical software after normality test and with considering to mathematical expectations of sources of variation in experimental design.

Results and discussion

Interaction of two factors of the experiment (aging × priming) was significant on all traits (Tab. 1).

Germination Percent (GP)

The highest GP was observed in both control and PEG priming with control aging treatment, but in the third aging level the lowest level of GP was seen by hormone priming (Tab. 2). Increasing seed age decreased GP and this result is in accordance with Jan-Mohammadi *et al.* (2008) and Ghassemi-Golezani *et al.* (2010) in rapeseed, Bhattacharjee *et al.* (2006) in common bean and sunflower and Saha and Sultana (2008) in soybean. Akhtar *et al.* (1992) suggested that decreasing in GP was related to chromosomal aberrations that occur under long storage conditions. Decreasing of GP in aged seeds can be due to reduction of α-amylase activity and carbohydrate contents (Mitra *et al.*, 1974) or denaturation of proteins (Nautiyal *et al.*, 1985). Bourland *et al.* (1988) reported that low quality of seed lot is the main reason for decreasing GP. Ali *et al.* (2003) demonstrated that differences in quality and vigour of rice cultivars after deterioration are the source of difference in GP.

Tab. 1. Analysis of variance for germination traits in common bean

		Mean of square								
S.O.V	DF	GP	GR	EC	Length		Fresh weight		Dry weight	
					Plumule	Radicle	Plumule	Radicle	Plumule	Radicle
Priming (P)	4	44.922 **	0.518	19656.631 **	47.96 **	105.373 **	1.286 **	0.157 **	0.009 **	0.001 **
Aging (A)	2	765.8 **	301.487 **	7038.287 **	95.599 **	297.702 **	1.305 **	0.18 **	0.01 **	0.001 *
P×A	8	58.106 **	23.416 **	100.166 **	15.735 **	29.836 **	0.259 **	0.024 **	0.001 **	0.001 **
Error	30	15.622	0.365	13.785	1.082	3.719	0.020	0.001	0.0002	0.0001
CV (%)		10.15	14.22	6.25	11.75	9.10	11.91	10.11	9.12	13.39

* and ** indicating the significant differences at 5 and 1 percent probability levels. DF: Degree of freedom; GP: Germination Percent; GR: Germination Rate; EC: Electrical Conductivity

Tab. 2. Analysis of variance for common bean grain biochemical characteristics

		Mean of square					
S.O.V	DF	SRUR	SRUE	FUSR	Protein	Phytin	Phosphate
Priming (P)	4	0.035 *	2.112 *	0.259 **	11531.671 **	67.404 **	0.001 **
Aging (A)	2	0.0315 *	2.479 *	0.348 **	736.986 **	0.912 **	0.0008 **
P×A	8	0.027 *	1.841 *	0.191 **	49.643 **	0.072 **	0.0004 **
Error	30	0.009	0.683	0.057	4.518	0.008	0.0001
CV(%)		16.19	10.41	11.24	1.36	1.47	0.34

* and ** indicating the significant differences at 5 and 1 percent probability levels. DF: Degree of freedom; SRUR: Seed reserves using rate; SRUE: Seed reserve using efficiency; FUSR: Fraction of seed reserves

Germination Rate (GR)

The maximum rate of germination obtained from hormone priming at control aging treatment and the minimum rate was observed in hydro priming at the second level of aging. GR is an indicator of seed vigour, and vigorous seeds have greater GR. Priming can enhance germination metabolism and allow radicle to emergence earlier. Aging or deterioration can affect seed vigour and quality that lead to decreasing GR (Basra *et al*., 2003).

Electrical Conductivity (EC)

The highest EC achieved by halo priming at three levels of aging and lowest EC was obtained at control level of both experimental factors (Tab. 3). It seems that priming by NaCl can increase the electrolyte leakage of seeds due to osmotic potential. Aging of seeds can increase the electrical conductivity that is in accordance with Kaewnaree *et al*. (2010). They demonstrated that cell membrane damage decreases the ability of carrier proteins and causes to increasing electrolyte leakage (Kaewnaree *et al*., 2010). Electrical leakage is an indicator of relative permeability of plasma membrane and transition of liquid-crystal phase of membrane to solid-gel state maybe the first phenomenon that affect membrane transporters (Boonasiri *et al*., 2007). After this transition, phospholipid layer of membrane is destroyed by lipid peroxygenase and ROS (Torres and Androw, 2006) leading to de-construction of membrane integrity.

Plumule Length (PL) and Radicle Length (RL)

The highest PL and RL were observed by hydro priming in non-aged seeds. The lowest PL and RL were obtained by hydro priming in the third level of aging (Tab. 2). Seed aging decreased PL and RL and it can be concluded that aging has a destructive effect on seedling growth and development. Neto *et al*. (2001) concluded that decreasing of radicle and plumule length and seedling dry weight in common bean is due to decline in seed vigor. Increase in aging time decreased GP, GR and seedling height in *Bromus* (Abdi and Maddah-Arefi, 2001).

Plumule Fresh Weight (PFW) and Radicle Fresh Weight (RFW)

Halo priming in non-aged seeds had the heaviest plumule and radicle. The lightest plumule and radicle was obtained by hydro priming and halo priming respectively at the third level of aging (Tab. 2). Aging decreased PFW and RFW like other seedling parameters.

Plumule Dry Weight (PDW) and Radicle Dry Weight (RDW)

The maximum values of these traits were same of PFW and RFW but the lightest plumule and radicle was concerned to halo priming and hydro and halo priming respectively, in third level of aging (Tab. 2). Sedghi *et al*. (2010a) reported that priming had significant effect on GP, GR and plumule and radicle length in two medical plants including pot marigold and sweet fennel. They reported that priming had significant effect on plumule dry fresh weight of pot marigold and the highest plumule dry weight was seen in hormone priming. In another report about *Silybum marianum*, the highest length of plumule and radicle, and plumule dry and fresh weight achieved from hormone priming (Sedghi *et al*., 2010b).

Seed Reservoirs Using Rate (SRUR)

The highest SRUR was observed in control level of aging by halo priming. The lowest SRUR was obtained in

Tab. 3. Effect of aging (A) and priming (P) on the germination traits of common bean

	GP (%)	GR (Seed day^{-1})	EC (µS cm)	Length (cm)		Fresh weight (g)		Dry weight (g)	
				Plumule	Radicle	Plumule	Radicle	Plumule	Radicle
A1P1	96.667	8.439	16.273	6.733	11.589	1.096	0.39	0.093	0.036
A1P2	95.33	11.272	22.577	10.067	15.956	0.808	0.324	0.099	0.032
A1P3	96.667	9.889	24.75	6.944	12.311	0.944	0.426	0.073	0.027
A1P4	65.33	15.056	20.313	7.678	11.5	0.838	0.258	0.074	0.026
A1P5	95	12.424	114.673	8.45	13.15	1.138	0.43	0.12	0.037
A2P1	76.667	5.428	32.833	7.332	9.578	1.012	0.344	0.064	0.029
A2P2	27.33	0.114	32.99	1.644	2.711	0.044	0.018	0.006	0.012
A2P3	57.33	4.78	36.173	3.878	7.567	0.536	0.178	0.019	0.019
A2P4	22.33	5.624	33.04	3.611	2.311	0.241	0.096	0.022	0.011
A2P5	68.33	1	144.44	3.06	4.1	0.36	0.015	0.009	0.009
A3P1	62.33	3.579	53.383	4.856	7.466	0.158	0.116	0.027	0.012
A3P2	12.667	0.687	67.857	0.5	0.95	0.012	0.016	0.002	0.002
A3P3	21.33	3.538	55.593	1.439	2.145	0.196	0.087	0.006	0.003
A3P4	11.667	0.869	66.65	0.9	0.894	0.10	0.037	0.003	0.006
A3P5	32.42	0.25	169.523	1.1	1.26	0.023	0.008	0.001	0.002
LSD	2.947	0.451	3.547	0.775	1.438	0.104	0.027	0.006	0.011

GP: Germination Percent; GR: Germination Rate; EC: Electrical Conductivity

Tab. 4. Effect of aging (A) and priming (P) on the biochemical traits of common bean

	SRUR (g seed^{-1})	SRUE (g g^{-1})	FUSR	Protein (mg g^{-1})	Phytin (mg g^{-1} dw)	Phosphate (µmol g^{-1} fw)
A1P1	0.221	0.602	0.511	169.33	7.403	0.04
A1P2	0.189	0.461	0.484	183.6	8.593	0.049
A1P3	0.233	0.58	0.47	185.567	8.17	0.049
A1P4	0.212	1.856	0.565	180.667	8.31	0.049
A1P5	0.280	0.896	0.713	181.1	8.403	0.049
A2P1	0.164	0.389	0.442	140.567	5.527	0.033
A2P2	0.112	0.13	0.215	169.6	6.07	0.037
A2P3	0.108	1.32	0.141	169.9	6.17	0.037
A2P4	0.138	0.329	0.36	170.9	6.1	0.037
A2P5	0.129	0.21	0.21	169.2	6.197	0.037
A3P1	0.090	0.224	0.236	111.767	3.5	0.026
A3P2	0.049	0.085	0.1	127.967	3.927	0.031
A3P3	0.019	0.127	0.083	128.467	4.043	0.031
A3P4	0.041	0.112	0.161	132.033	4.11	0.030
A3P5	0.020	0.073	0.09	130.1	4.103	0.031
LSD	0.072	0.616	0.178	1.585	0.067	0.00004

SRUR: Seed reserves using rate; SRUE: Seed reserve using efficiency; FUSR: Fraction of seed reserves

PEG priming at the third level of aging (Tab. 4). Sedghi *et al*. (2010b), showed that in *Silybum marianum* seeds under salinity, SRUR increased by hormone and halo priming.

Seed Reservoirs Using Efficiency (SRUE)

The highest SRUE was related to non-aged seeds with hormone priming, but the lowest value was seen by halo priming at the third aging level (Tab. 4).

Fraction of Used Seed Reservoirs (FUSR)

The maximum value of FUSR was concerned to control level aging with halo priming. The minimum level was seen by PEG priming at the latest level of aging (Tab. 4). Soltani *et al*. (2008) suggested that by decreasing of GA_3 concentration during wheat seed germination, probably SRUR and FUSR decrease too. Sedghi *et al*. (2011) studied the effect of hormone priming on aged silk tree seeds (*Albizia julibrissin* Durazz) and reported that by increasing seed aging, a reduction in FUSR, SRUE and SRUR was seen, but in hormone priming treatments reduction rate was slower than control. The highest FUSR in *Silybum marianum* obtained by hormone and halo priming, while the highest SRUE was related to priming with manitole (Sedghi *et al*., 2010b).

Protein

The highest amount of protein was achieved by osmopriming in the control level of aged seeds, and the lowest amount concerned to non-primed seeds in the third level of aging (Tab. 4). The results showed that protein is reduced with increasing levels of aging, but priming had positive effect and the lowest protein content was seen in control treatments. Increasing grain total protein affected by priming probably is due to increasing in enzyme pool of seed cells. In other words it seems that *de novo* synthesis of enzyme increased by priming.

Phytin reserves

The maximum value of phytin reserves was seen in hydro priming in non-aged seeds, and the minimum value related to non-primed seeds in the third level of aging (Tab. 4). In all aging treatments the lowest phytin reserves obtained by non-priming that shows enhancing effect of priming on phytin accumulation. Phytin is the main phosphorus storage form in seeds that seen in combination with mineral cations like K^+, Mg^{2+}, Fe^{2+} and Zn^{2+}. Phytin is effective on nutritional quality of seeds and inhibit absorption of Fe^{2+} and Zn^{2+} in mammalians. Also phytin has anti-cancer and antioxidant role in seed and consumers (Murphy *et al*., 2001)

Phosphate content

The highest phosphate content was seen in non-aged seeds and all priming levels except of control. The minimum value for phosphate content was achieved by control priming at the third level of aging (Tab. 4). Increasing Pi concentration to control is an indicator of positive effect of priming and increases the ATP/ADP ratio that positively charges seed energy. Reduction in Pi pool causes by aging and decreases the required energy for growth and emergence of seedling.

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