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Effect of Priming on Physiological Seed Quality in Fresh and Aged Seeds of Sunflower (*Helianthus annuus* L.) Hybrid KBSH-53

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A B S T R A C T

Priming on seed quality was tested on two seed lots L_1: Fresh seeds (> 91 % Germination) and L_2: Aged seeds (< 70 % Germination). The seeds were treated with priming chemicals 12 hours of soaking *viz.*, C_1: Control, C_2: Hydropriming, C_3: KH_2PO_4 (1 %), C_4: GA_3 (400 ppm), C_5: NaCl (1 %), C_6: KCl (2 %), C_7: KNO_3 (2 %), C_8: $CaCl_2.2H_2O$ (2 %), C_9: PEG (1Mpa), C_{10}: K_2HPO_4 (1 %). The results of the study showed that among the seed lots L_1 has recorded higher seed quality parameters *viz*., germination (93.00 %) and seedling length (26.70 cm) compared to L_2 (70.67 % and 19.30 cm). Among the various seed priming treatments KH_2PO_4 (1 %) is the suitable priming chemical to improve the marginal quality sunflower seed lots *viz*., germination (89.67 %), speed of germination (19.22), days to 50 % germination (2.15), seedling length (28.83 cm), seedling dry weight (64.44 mg) and seedling growth ratio (1.44) compared to control (81.83 %, 15.91, 2.74, 23 cm 55.03 mg and 1.20 respectively). Priming treatments are more effective in marginal seed lots compared to fresh seeds.

Introduction

Sunflower (*Helianthus annuus* L.) belongs to the family Astreaceae and it is one of the world's most important sources of vegetable oil. The native of sunflower is reported to be southern parts of USA and Mexico.

Sunflower ranks third, next to groundnut and soybean in total production. In world it is cultivated in an area of 25.56 million hectares with an annual production of 40.64 million tonnes with productivity of 1590 kg ha^{-1} during 2015 (Anon., 2016).

Sunflower was introduced to India during 1969 as a supplement to traditional oilseed crops to bridge the gap of recurring edible oil shortage in the country. The commercial cultivation of sunflower was started in India during 1972-73 with an introduction of Russian varieties from USSR and Canada. Now, the crop is well adopted because of attributes such as short duration, photoperiod insensitivity, adoptability to wide range of soil and climatic conditions, drought tolerance, higher seed multiplication ratio (1:50) having high percentage of edible oil (45-50 %), which contains polyunsaturated fatty acid (PUFA). In

recent years, India has emerged as second major sunflower producing country in Asia after China. In India, it is being grown in an area of 0.52 million hectares with annual production of 4.2 million tonnes having a productivity of 750 kg ha^{-1} (Anon., 2014).

Priming in its traditional sense, soaking of seeds in water before sowing, has been the experience of farmers in India in an attempt to improve crop stand establishment but the practice was without the knowledge of the safe limit of soaking duration (Harris 1996). Moreover, Harris *et al.,* (1999) promoted a low cost, low risk technology called 'on farm seed priming' that would be appropriate for all farmers, irrespective of their socio economic status. On-farm seed priming involves soaking the seed in water, surface drying and sowing the same day. The rationale is that sowing soaked seed decrease the time needed for germination and allow the seedling to escape deteriorating soil physical conditions. According to Khan (1992), osmotic conditioning in its modern sense, aims to reduce the time of seedling emergence, as well as synchronize and improve the germination percentage, by subjecting the seeds to a certain period of imbibition using osmotic solutions. The seeds normally begin water uptake on contact with this solution and stop the process as soon as they become balanced with the water potential of the solution. Aged seeds are less vigours and decreased germination percentage. Therefore, the present investigation was carried out to study the Effect of priming on physiological seed quality in fresh and aged seeds of sunflower hybrid KBSH-53.

Materials and Methods

Treatment details

The laboratory experiment was conducted in 2016, at Department of Seed Science and Technology, University of Agricultural Sciences, Bengaluru. Treatments consists of sunflower hybrid KBSH-53 seed lots: L_1: Fresh seeds (> 91 % Germination), L_2: 10 months aged seeds (< 70 % Germination) and priming Treatments:C_1: Control, C_2: Hydro priming, C_3: KH_2PO_4 (1 %), C_4: GA_3 (400 ppm) C_5: NaCl (1 %), C_6: KCl (2 %), C_7: KNO_3 (2 %), C_8: $CaCl_2.2H_2O$ (2 %), C_9: PEG (-1 Mpa) and C_{10}:K_2HPO_4 (1 %), seeds are soaked for 12 hours in solutions. The experiment was carried out in factorial completely randomized design in three replications and observations on various seed quality parameters were recorded. One hundred seeds of four replicates were drawn at random from each treatment and the germination test was conducted using between paper (BP) method as per ISTA. The rolled towels were incubated in germination chamber maintained at 25 ± 1°C with 90 per cent relative humidity. The germination of seeds was evaluated on fourth and tenth day as first and final counts, respectively and percentage germination was expressed based on normal seedling percentage. Speed of germination was calculated as Bartlett's Rate Index (Bartlett, 1973), which was worked out from the daily germination counts and calculated as follows:

$$BRI = \frac{P1 + (P1 + P2) + (P1 + P2 + P3) + \ldots + (P1 + P2 + P3 + \ldots + Pn)}{N\,(P1 + P2 + P3 + \ldots + Pn)}$$

Where,

P1 + P2 + P3. ……and Pn are the germination (%) at 1st, 2nd, 3rd…….nth day, respectively and 'N' is the total number of days taken for germination.

Time to get 50 per cent germination was calculated according to the formula of Coolbear *et al.,* (1990). T_{50} was defined as

days needed to reach 50 per cent of final germination percentage.

Ten normal seedlings from each treatment were selected randomly. The shoot and root length were measured from collar region to point of attachment of cotyledons and from the collar region to the tip of primary root respectively. Sum total of the shoot and root length constitute total length of seedling. The mean of ten seedlings in each treatment and replication was calculated and expressed in centimeters. Ten normal seedlings used for measuring root length and shoot length were taken in butter paper and dried in an hot air oven, maintained at 80 ºC temperature for 24 hours. Then the seedlings were removed and allowed to cool in a desiccator for 30 minutes before weighing in an electrical balance. The average was calculated and expressed as seedling dry weight in milligram.

Seedling growth ratio was calculated from the standard germination test. Ten normal seedlings were randomly selected and seedling length was measured from root tip to shoot apex on first and final (4^{th} and 10^{th} day) count and computed by using following formula

$$SGR = \frac{\text{Seedling growth at first count} - \text{Seedling growth at zero days}}{\text{Seedling growth at final count} - \text{Seedling growth at first count}}$$

Results and Discussion

The data on germination percentage as influenced by the seed lots and priming treatments are presented in table 1. The germination percentage exhibited significant variations due to seed lots, priming treatments and their interactions. Between the seed lots, germination percentage was highest (96.37 %) in L_1 and it was lowest in L_2 (77.80 %). Among the priming treatments, highest (89.67 %) germination was recorded in C_3 followed by C_4 (89.58), C_7, C_8, and C_9 (89 % each). However, lowest (81.83 %) germination was recorded in C_1. Germination percentage differed significantly due to L×C. Among the interactions, highest germination was recorded in L_1C_3 (98 %), followed by L_1C_4 (97.83), L_1C_5 (97.33 %), L_1C_9 and L_1C_8 (97 %). However, lowest germination was observed in L_2C_1 (70.67 %).

The increase in seed germination percentage upon priming may be due to increased vigour characteristics, increased peroxide scavenging enzymatic activities and decreased lipid peroxidation. Further, increase in mean seedling dry weight upon priming may be due to increased shoot and root lengths because of higher metabolic activity that leads to the better mobilization efficiency of stored food during the early hours of germination that might have contributed for the better growth of seedlings (Bailly *et al.*, 2002). The similar results were also recorded by Sowmya, (2011) in cucumber and Radha (2013) in maize.

The data on speed of germination as influenced by the seed lots and priming treatments are presented in table 1. The speed of germination exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, speed of germination was maximum in L_1 (19.82) and it was minimum in L_2 (15.10). Among the priming treatments, maximum speed of germination was recorded in C_3 followed by C_4 and C_9 (18.75 and 18.48 respectively).

However minimum speed of germination was recorded in C_1 (15.91). Speed of germination differed significantly due to L×C. Among the interactions, maximum speed of germination was recorded in L_1C_3 (21.43) followed by L_1C_4 (21.17) and L_1C_9 (21.10) However, minimum speed of germination was observed in L_2C_1 (13.67).

Table.1 Influence of seed lots and priming treatments on germination (%) and speed of germination of sunflower hybrid KBSH-53

Treatments	Germination (%)			Speed of germination		
	L_1	L_2	Mean	L_1	L_2	Mean
C_1	93.00	70.67	**81.83**	18.15	13.67	**15.91**
C_2	94.17	72.00	**83.08**	18.43	13.83	**16.13**
C_3	98.00	81.33	**89.67**	21.43	17.00	**19.22**
C_4	97.83	81.33	**89.58**	21.17	16.33	**18.75**
C_5	97.33	74.00	**85.67**	18.57	14.00	**16.28**
C_6	96.67	74.33	**85.50**	18.97	14.17	**16.57**
C_7	96.33	81.67	**89.00**	19.43	15.67	**17.55**
C_8	97.00	81.00	**89.00**	20.07	15.50	**17.78**
C_9	97.00	81.00	**89.00**	21.10	15.87	**18.48**
C_{10}	96.33	80.67	**88.50**	20.83	15.00	**17.92**
Mean	**96.37**	**77.80**	**CV (%)**	**19.82**	**15.10**	**CV (%)**
	S.Em±	**CD (P=0.01)**	**4.20**	**S.Em±**	**CD (P=0.01)**	**3.41**
L	**0.42**	**1.20**		**0.18**	**0.54**	
C	**0.94**	**2.69**		**0.42**	**1.21**	
LC	**1.33**	**3.80**		**0.59**	**1.71**	

Lots:
L_1: Fresh seeds (> 91 % Germination)
L_2: 10 months aged seeds (< 70 % Germination)

Priming Treatments:
C_1: Control
C_2: Hydropriming
C_3: KH_2PO_4 (1%)
C_4: GA_3 (400 ppm)
C_5: NaCl (1 %)
C_6: KCl (2 %)
C_7: KNO_3 (2 %)
C_8: $CaCl_2.2H_20$ (2 %)
C_9: PEG (-1 Mpa)
C_{10}: K_2HPO_4 (1 %)

Table.2 Influence of seed lots and priming treatments on days to 50 % germination and seedling length (cm) of sunflower hybrid KBSH-53

Treatments	Time to 50 % germination			Seedling length (cm)		
	L_1	L_2	Mean	L_1	L_2	Mean
C_1	2.52	2.97	**2.74**	26.70	19.30	**23.00**
C_2	2.44	2.90	**2.67**	27.37	19.53	**23.45**
C_3	2.07	2.23	**2.15**	33.87	23.80	**28.83**
C_4	2.28	2.07	**2.17**	33.33	23.23	**28.28**
C_5	2.43	2.68	**2.56**	27.30	19.77	**23.53**
C_6	2.40	2.88	**2.64**	27.73	19.86	**23.80**
C_7	2.20	2.71	**2.46**	30.00	21.80	**25.90**
C_8	2.09	2.62	**2.36**	30.34	22.43	**26.39**
C_9	2.13	2.61	**2.37**	32.80	23.13	**27.97**
C_{10}	2.20	2.71	**2.46**	31.47	22.80	**27.13**
Mean	**2.28**	**2.64**	**CV (%)**	**29.97**	**21.45**	**CV (%)**
	S.Em±	**CD (P=0.01)**	**3.81**	**S.Em±**	**CD (P=0.01)**	**4.91**
L	**0.03**	**0.08**		**0.20**	**0.57**	
C	**0.06**	**0.19**		**0.45**	**1.29**	
LC	**0.09**	**0.28**		**0.63**	**1.82**	

Lots:
L_1: Fresh seeds (> 91 % Germination)
L_2: 10 months aged seeds (< 70 % Germination)

Priming Treatments:
C_1: Control
C_2: Hydropriming
C_3: KH_2PO_4 (1%)
C_4: GA_3 (400 ppm)
C_5: NaCl (1 %)
C_6: KCl (2 %)
C_7: KNO_3 (2 %)
C_8: $CaCl_2.2H_20$ (2 %)
C_9: PEG (-1 Mpa)
C_{10}: K_2HPO_4 (1 %)

Table.3 Influence of seed lots and priming treatments on seedling dry weight (mg) and Seedling growth ratio of sunflower hybrid KBSH-53

Treatments	Seedling dry weight (mg)			Seedling growth ratio		
	L_1	L_2	Mean	L_1	L_2	Mean
C_1	61.20	48.87	**55.03**	1.34	1.06	**1.20**
C_2	61.73	58.48	**60.11**	1.43	1.06	**1.24**
C_3	69.33	59.54	**64.44**	1.60	1.32	**1.44**
C_4	69.00	59.38	**64.19**	1.57	1.26	**1.42**
C_5	62.47	58.83	**60.65**	1.44	1.12	**1.28**
C_6	62.88	59.23	**61.06**	1.45	1.10	**1.27**
C_7	63.57	60.48	**62.02**	1.47	1.14	**1.31**
C_8	64.75	60.56	**62.66**	1.48	1.16	**1.32**
C_9	65.34	59.50	**62.42**	1.50	1.17	**1.33**
C_{10}	64.84	58.67	**61.76**	1.45	1.15	**1.30**
Mean	**64.51**	**58.35**	**CV (%)**	**1.47**	**1.15**	**CV (%)**
	S.Em±	**CD (P=0.01)**	**2.83**	**S.Em±**	**CD(P=0.01)**	**5.11**
L	**0.71**	**2.03**		**0.010**	**0.031**	
C	**1.01**	**2.88**		**0.023**	**0.069**	
LC	**0.32**	**0.91**		**0.033**	**0.098**	

Lots:
L_1: Fresh seeds (> 91 % Germination)
L_2: 10 months aged seeds (< 70 % Germination)

Priming Treatments:
C_1: Control
C_2: Hydropriming
C_3: KH_2PO_4 (1%)
C_4: GA_3 (400 ppm)
C_5: NaCl (1 %)
C_6: KCl (2 %)
C_7: KNO_3 (2 %)
C_8: $CaCl_2.2H_20$ (2 %)
C_9: PEG (-1 Mpa)
C_{10}: K_2HPO_4 (1 %)

The data on time to 50 per cent germination as influenced by the seed lots and priming treatments are presented in table 2. The time to 50 per cent germination exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, time taken to 50 per cent germination was minimum in L_1 (2.28 days) and it was maximum in L_2 (2.64 days). Among the priming treatments, minimum time taken to 50 per cent germination was recorded in C_3 (2.15 days) followed by C_4 (2.17 days), C_9 (2.37) and C_8 (2.36 days). However maximum time taken to 50 per cent germination was recorded in C_1 (2.74 days). Time taken to 50 per cent germination differed significantly due to L×C. Among the interactions, minimum time taken to 50 per cent germination was recorded in L_1C_3 (2.07), followed by L_1C_8 (2.09) and L_1C_9 (2.13 days each). However, maximum (2.97 days) time taken to 50 per cent germination was observed in L_2C_1 (2.97 days). Genetic damage recuperation and resumption of membrane integrity could result in protein synthesis required for germination, which is possibly responsible for increased germination and seedling growth. Because of active metabolism of the deteriorated seeds improve germination capability, while metabolism velocities are fairly high (Moghanibashi *et al.*, 2012). Priming has no direct effect on cell division, but advances its beginning (G_1 and G_2 phase of mitosis) from phase III to phase II of seed inhibitions. This advance is enabled by an accumulation of e-tubulins in primed seed, which are proteins involved in maintaining the cell cytoskeleton and forming the microtubules necessary to cell division. The accumulation of tubulins is associated with the synchronization of cells on the G_2 phase; in the subsequent phase III, cell division takes simultaneously place in all cells. The similar results were also recorded by Sowmya (2011) in cucumber and Radha (2013) in maize.

The data on mean seedling length as influenced by the seed lots and priming treatments are presented in table 2. The mean seedling length exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, mean seedling length was highest in L_1 (29.97 cm) and it was lowest in L_2 (21.45 cm). Among the priming treatments, highest mean seedling length was recorded in C_3 (28.83 cm) followed by C_4 and C_9 (28.28 and 27.97 cm respectively). However, lowest mean seedling length was recorded in C_1 (23.00) mean seedling length differed significantly due to L×C. Among the interactions, highest mean seedling length was recorded in L_1C_3 (33.87 cm) followed by L_1C_4, L_1C_9 and L_1C_{10} (33.33, 32.80 and 31.47 cm, respectively). However, lower (19.30 cm) mean seedling length was observed in L_2C_1 (4.10). GA_3 may release the high level of storage protein precursors which is essential for germination and initiates mobilizing storage protein reserves. In the same way lipid storage mobilization initiates. Thus, isocitratelyase (Threo-D-isocitrate glyoxylatelyase) which is the key enzyme in seed lipid mobilization via glyoxylate cycle, increases about 5 - fold in primed seeds. Isocitratelyase plays a crucial role in the synthesis of carbohydrates from storage lipids during seed germination and seedling establishment. Also, it has been proposed that glyoxylate cycle activity is a good indicator of seedling emergence potential and seed vigour (Anil *et al.,* 2011). The similar results were also recorded by Sowmya (2011) in cucumber and Radha (2013) in maize.

The data on seedling dry weight as influenced by the seed lots and priming treatments are presented in table 3. The seedling dry weight exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, seedling dry weight was higher in L_1 (64.51 mg) and it

was lower in L_2 (58.35 mg). Among the priming treatments, highest seedling dry weight was recorded in C_3 (64.44 mg) followed by C_4 and C_8 (64.19 and 62.66 mg respectively). However lowest seedling dry weight was recorded in C_1 (55.03 mg) Seedling dry weight differed significantly due to L×C. Among the interactions, higher (69.33 mg) seedling dry weight was recorded in L_1C_3 followed by L_1C_4, L_1C_9 and L_1C_{10} (69.00, 65.34 and 64.84 mg respectively). However, lower seedling dry weight was observed in L_2C_1 (48.87 mg). These chemicals able to repair the protein damage occurred during oxidative stress and initiates protein *de novo* syntheses. Thus cell will resume the normal metabolic activity *viz*., mobilization of stored proteins, then the stored mRNA and restart of metabolism from stored proteins metabolic transitions to support development. Proteomic evidence for this includes enzymes from energy production pathways in primed seeds: glycolysis [6-phosphofructokinase (PFK), phosphoglycerate kinase (PGK)], gluconeogenesis [PEP carboxykinase (PEPCK)], fermentation [alcohol dehydrogenase (ADH)], pyruvate dehydrogenase (PDH), tricarboxylic acid (TCA) cycle [succinate dehydrogenase, succinyl-CoA ligase, malate dehydrogenase (MDH)], glyoxylate cycle (isocitratelyase) and the amino acid aminotransferases will be recovered. Effective of functioning of metabolism pathways will circulate the energy required for seedling biomass accumulation in essential structures. The produced essential structures were become robust, normal and vigour seedlings thus there was increase in dry weight. The similar results were also recorded by Sowmya (2011) in cucumber and Radha (2013) in maize.

The data on seedling growth ratio as influenced by the seed lots and priming treatments are presented in table 3. The seedling growth ratio exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, seedling growth ratio was higher in L_1 (1.47) and it was lower in L_2 (1.15). Among the priming treatments, highest seedling growth ratio was recorded in C_3 (1.44) followed by C_4 (1.42) and C_9 (1.33). However, lowest seedling growth ratio was recorded in C_1 (1.20). Seedling growth ratio differed significantly due to L×C. Among the interactions, higher seedling growth ratio was recorded in L_1C_3 (1.60) followed by L_1C_4, L_1C_9 and L_1C_{10} (1.57, 1.50 and 1.45 respectively). However, lower seedling growth ratio was observed in L_2C_1 (1.06). Priming allow the activation of repair processes such as the repair of damaged DNA, proteins, membranes, and mitochondria via stored mRNAs and stored proteins (Ilese *et al.,* 2012 and Sallon *et al.,* 2008). Mitochondrial DNA actively divides and increases in number by producing new mitochondria. Thus enzymes encoded on mitochondrial DNA are absolutely essential for oxidative phosphorylation; which in turn increases in the energy (ATP) production required for germination and seedling growth. These were also increases the seedling growth, continued development of the seedling results in increased growth ratio. The results are in line with Radha (2013) in maize. It is evident from the present study seeds primed with KH_2PO_4 (1 %) shows higher germination per cent, speed of germination, days to 50 % germination, seedling length, seedling dry weight and seedling growth ratio. The correlation of physiological parameters correlated with the vigour and viability of sunflower seeds.

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