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RESEARCH ARTICLE

Effect of hydro priming on biochemical properties sunflower hybrid and its parental line during seeds storage

■ J. SHANTHALA, R. SIDDARAJU AND PARSHIVAMURTHY

SUMMARY

Priming of seeds enhances the physiological quality through activation of the biochemical events, which advances seed germination processes without radical emergence, enabling rapid and uniform germination of seeds coupled with high vigour. This has helped the farmers to achieve better plant stand in the field that leads to uniform crop stand and improve in crop yields. This in view, an experiment on biochemical and molecular basis of seed priming was initiated to identify seed specific markers to achieve precision in priming in sunflower hybrids and its parental lines. The seed samples of the sunflower hybrids and its parental lines were subjected to hydro priming treatment at 25 ^{0}C for 18 hr and evaluated for seed quality, biochemical parameters, enzyme activity and molecular analysis and compared with control. The result revealed that primed seeds manifested maximum mean seed germination (92%), speed of emergence, mean seedling dry weight (21.0 mg), vigour index, total dehydrogenase (2.69) activity of sunflower hybrid KBSH-53 and lowest electrical conductivity was noticed in KBSH-41 (73.4 dSm^{-1}), total DNA content increased in primed seeds (998 ng/µl). It is concluded that the hydro-priming treatment enhanced seed quality parameters leading to better crop stands with higher yields in sunflower hybrid KBSH53 and its parental lines.

Key Words : Biochemical parameters, Enzyme activity, Molecular analysis, Sunflower hybrids, Seed priming

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Rapid and uniform field emergence is two essential pre-requisites that increase final yield, quality and ultimately the market gains. Germination and subsequent seedling growth could be inhibited by various environmental and genetic factors depending on species and varieties. The modern approaches of novel seed technologies combines classical genetics, plant molecular biology and other seed treatment methods *viz*., priming, pelleting, coating of seeds to improve seed quality, which enhances physiological quality and vigour. Priming is beneficial for seedling establishment under drought conditions of irregular rainfall reducing the risk of poor stand and permits more uniform growth conditions. Off which hydro-priming is the simplest approach and the most important physiological seed enhancement method, which allows controlled imbibitions' and induction of the pre-germinative metabolism, simultaneously preventing radical emergence. Germination speed and synchrony of primed seeds are enhanced in turn enhancing seed vigour. Behtari *et al.* (2012) in his studies on all germination characteristics and seedling growth between storage and non-storage of primed tall fescue seed concluded that among all priming treatments, hydro-priming known to improve germination performance without affecting the longevity.

The beneficial effects of priming have also been demonstrated to improve germination and emergence for many field crops such as wheat, sugar beet, maize, soybean and sunflower. An experiment on biochemical and molecular basis of seed priming was, therefore, initiated to identify seed specific markers to achieve precision in priming in sunflower hybrids

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
J. SHANTHALA, Department of Genetic and Plant Breeding, College of Agriculture, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA
Email: shanthala16@gmail.com

Address of the Co-authors:
R. SIDDARAJU AND PARSHIVAMURTHY, National Seed Project, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

and its parental lines.

MATERIAL AND METHODS

The seed samples of the sunflower hybrids of KBSH-41, KBSH-44 and KBSH-53 and its parental lines CMS-234A, RHA 95C-1, CMS-17A, CMS-17B and CMS-335A were procured from AICRP on Sunflower, Zonal Agricultural Research Station, University of Agricultural Sciences, Bangalore.

Seed priming :

Seeds of different germination levels were subjected to hydro priming treatment at 25 ^{0}C for 18 hr and dried under shade for 2 days until they attained original seed moisture content. While untreated seeds served as control.

Priming level	Treatments
C	No priming treatment, used as a control.
P	Hydro priming done for 18 hr at 25 ^{0}C.

After the priming treatments, the seeds were evaluated for quality parameters and observations were recorded on seed germination, seedling dry weight, seedling vigour index, speed of germination, rate of water uptake, biochemical analyses *viz*., electrical conductivity, enzyme activity, total dehydrogenase activity and molecular analysis of electrophoretic profile of total soluble seed proteins by SDS-PAGE (Blum, 1987), total DNA content through nano drop was carried out in the laboratory as per ISTA. Seed storage studies were carried out for the seeds that were stored after priming at ambient condition and samples were drawn in three intervals (at initial stages, 3 months and 6 months) for testing germination, seedling vigour index, electrical conductivity and total dehydrogenase.

Seed quality parameters

Germination (%) :

The seed germination test was carried out in the laboratory by following the 'between paper' method as per ISTA. One hundred seeds in four replications were placed equidistantly between moist kraft paper towels. The rolled towels were placed in a germination chamber maintained at 25$\pm$1^{0}C and 100 per cent relative humidity. The seedlings were evaluated on fourth and tenth day of incubation and cumulative percentage of germination was expressed based on normal seedlings. The data collected were processed by analysis of variance (ANOVA) and analyzed with SAS software. Then treatment means were compared using the least significant difference (LSD) at 5 per cent probability levels.

Dry weight of seedlings :

Ten normal seedlings were selected randomly and dried under shade for 24 hours and then dried in a hot-air-oven maintained at 82$\pm$1^{0}C for 24 hours. After cooling the seedlings in a desiccator containing silica gel, the seedlings dry weight was recorded and expressed in mg.

Seedling vigour index (SVI) :

Based on mean seedling dry weight, the seedling vigour index (SVI) was calculated as per Abdul-Baki and Anderson (1973) using the following formula :

SVI = Mean seedling dry weight (mg) x germination (%)

Speed of germination (SG) :

Speed of germination or rate of germination represents the population of fast germinating seeds was tested for germination. Normal seedlings were counted every day till the end of test period. The speed of germination was calculated using the formula suggested by Magurie (1962).

$$SG = \frac{\text{No. of seed germinated first day}}{1} + \frac{\text{No. of seed germinated second day}}{2} + \ldots\ldots\ldots + \frac{\text{No. of seed germinated}}{\text{Final day}}$$

Rate of water uptake :

The characteristics of water absorption in control and primed seeds during the germination process have been studied as per Takahashi (1995). This was to know the pattern of water absorption and the mechanism of water intake in the deterioration of seeds. 25 seeds were selected randomly in each variety and placed in between moistened filter paper and incubated at 25 $\pm$ 1^{0}C to avoid the possible effects of temperature variations on water absorption during imbition. Seeds were incubated up to 18 hrs. After incubation period the seeds were taken out, surface water on the seed was removed before weighing by gentle pressing between 2 - 3 layers of filter paper and fresh weight was recorded.

Biochemical analyses :

Seed leachate analyses: Electrical conductivity (*EC*) :

Twenty seeds of uniform size in three replications were soaked in 25 ml of distilled water for 24 hours at 25$\pm$1^{0}C. After collecting the seed leachate, the soaked seeds were surface washed with additional 5 ml of water and again collected the leachate. Both the leachates were pooled and filtered through Whatman No.1 filter paper. The filtrate was made up to 25 ml with water and EC was measured using a digital Conductivity Meter. The EC was expressed as dSm^{-1} at 25$\pm$1^{0}C (ISTA, 2003).

Enzyme activity: Total dehydrogenase activity :

The total dehydrogenase activity (TDH activity) of seeds was determined (Todaka *et al*.,2000) with slight modification. Ten randomly selected seeds were preconditioned by keeping overnight between moist filter paper and their seed coat was removed carefully and soaked in 0.5 per cent TTC (2, 3, 5- triphenyl tetrazolium chloride) in a test tube. They were incubated at 30$\pm$1^{0}C overnight in dark and then washed thoroughly with distilled water. The red colour (formazan) developed was eluted from the stained embryo by soaking in five ml of 2-methoxy ethanol for one day in air tight screw capped vials. The extract was decanted and the colour

was measured using a spectrophotometer at 480 nm against 2-methoxy ethanol as blank. The TDH activity was expressed as absorbance values.

Molecular analyses :

Electrophoretic profile of total soluble seed proteins :

The SDS-PAGE of total soluble seed protein was performed in slab gels containing 10 per cent resolving at 5 per cent stacking gels. The method was based on the procedure described by Laemmli (1970).

Sample preparation :

The powder prepared from decorticated single seed is defatted with three changes of a mixture of chloroform: methanol: acetone (2:1:1 v/v) at an intervals of three hours. Soluble proteins from defatted materials were extracted with 25 mM Tris-glycine buffer pH 8.3. The extract was centrifuged at 8000 rpm for 15 min at 4^0C. The supernatant was used for characterization of soluble seed protein profiles by SDS-PAGE.

SDS-page :

The protein sample was boiled with sample buffer for 5min in water bath. A sample containing approximately 50 mg of protein was loaded per gel track along with bromophenol blue as a tracking dye. Electrophoresis was initially carried out at 30mA of current and later increased to 35mA. After the protein had entered the resolving gel, the current was maintained constantly until the dye moved to the bottom of the gel. The gel was staining by the method described by Blum (1987).

Total DNA content: DNA extraction :

Seedlings were used for DNA extraction. DNA was prepared as per the modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Cao and Oard, 1997). The protocol for DNA extraction is illustrated in figure below. However, the procedure adopted is outlined briefly as follows:

- Fresh and healthy leaves (0.5g) of about sixteen day's old seedling were collected.
- The leaves were cut into pieces and homogenized completely by adding 400µl of extraction buffer.
- Again added 400 µl of extraction buffer and mixed well and from this around 400 µl homogenized solution was taken into an eppendorf tube.
- 400 µl of chloroform was added and the mixture was centrifuged at 14,000 rpm for one minute.
- The supernatant was transferred through mira cloth into another centrifuge tube.
- To the supernatant obtained, 800 µl of absolute alcohol was added and centrifuged for another 3 minutes at 14,000 rpm.
- The supernatant was discarded and the DNA pellet was washed with 70 per cent alcohol and dried to remove the residues of alcohol.
- Finally, the DNA pellet was dissolved in 50 µl of TE buffer and stored at –20 °C for further use.

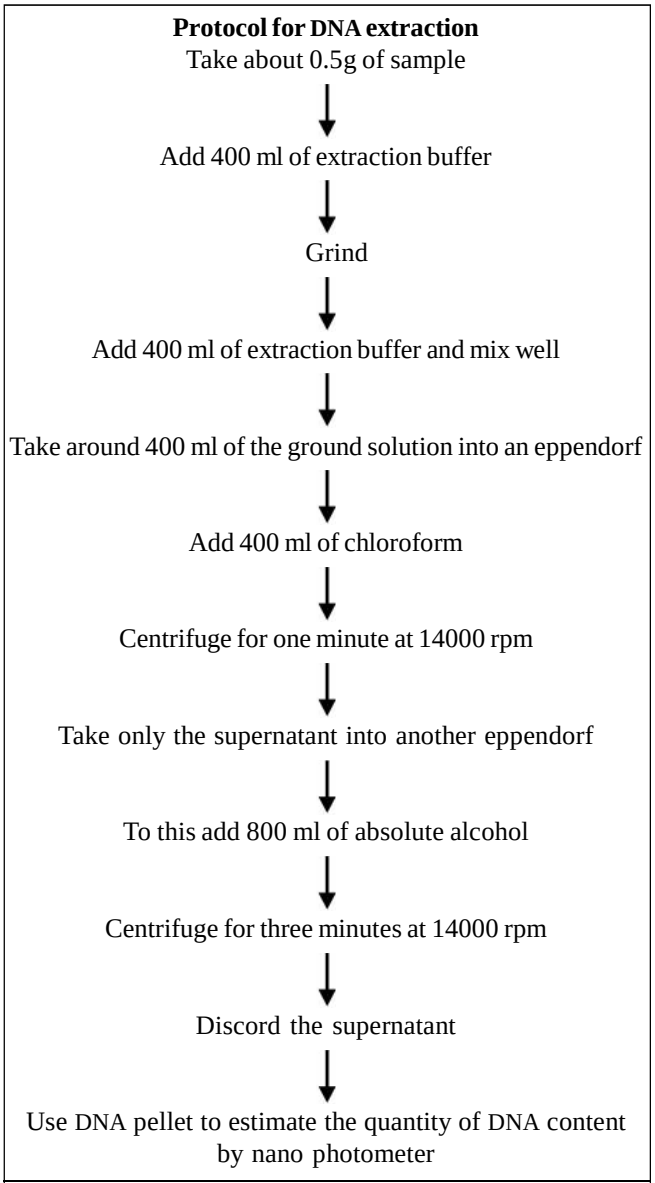

Fig. A : Flow chart for DNA extraction

RESULTS AND DISCUSSION

Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Bradford, 1986). The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini *et al.,* 2003) and Dharmalingam and Basu (1990) reported beneficial effect of a hydration-

dehydration seed treatment on germination of sunflower. According to McDonald (2000), primed seeds can rapidly imbibe and revive the seed metabolism, enhancing germination rate and uniformity. However, Singh and Rao (1993) stress that KNO_3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties with low germination.

Seed priming is one of the precautions that can be taken to counteract the adverse effects of drought stress. The general purpose of seed priming is to partially hydrate the seeds to a point where germination process is initiated but not completed. Treated seeds are usually dehydrated before use, causing rapid germination when re-imbibed under normal or drought stress conditions. Various seed priming techniques have been developed, but in this study we used hydropriming.

Seed quality parameter :

Germination (*%*) :

Significant variations in seed germination were noticed between the hydro primed seeds and the control. Germination was the maximum in most of the lines of primed seeds compared to control. The highest germination was observed in primed seeds of KBSH-41 (92, 91 and 88 %) followed by KBSH-53 (90, 87 and 85%), respectively at initial, 3 and 6 months of storage periods of sunflower. Among the controls, the highest germination was observed in KBSH-53 (89 and 88%) at initial and 3 months of storage, while KBSH-41 (85%) showed the highest germination at 6 months of storage.

Although hydro primed seeds recorded the highest germination at all storage periods in most of the sunflower hybrids, but there was gradually reduction in germination percentage over period of storage (Table 1). The overall results revealed that the KBSH-41 treated with hydro-priming for 18 hrs showed the highest seed germination over others. Germination for primed seeds stored at 25°C for 1 year decreased significantly compared to the un-stored primed seeds in most of the treatments, but the control seeds (stored non-primed seeds) in comparison with hydro-priming for 1 day had the lowest germination value. The results reveal that there were significant decreased in the germination percentage in stored seeds. The benefits of seed priming have been well documented in several reviews *viz.*, Khan (1992), Taylor *et al*. (1998) and Bradford (1986). The reported that the seeds are primed for the following reasons, to overcome or alleviate phytochrome induced dormancy (Lettuce and celary), to decrease the time necessary for germination and subsequent emergence. To improve the crop stand uniformity, in order to facilitate production, management and enhance synchronization at flowering. From a mechanical point of view, priming enables seeds of several species to germinate and emerge at supra-optimal temperature, increase in speed of germination and uniform field emergence. Emergence occurs before soil becomes fully detrimental. Crops compete more efficiently with weeds. Increased control can be exercised over water usage and scheduling. Priming has been commercially used to eliminate or to greatly reduce the amount of seed borne fungi and bacteria.

Mean seedling dry weight :

The mean seedling dry weight also showed significant variations among the priming treatments (Table 2). Among the treatments hydro priming of KBSH-53 recorded maximum mean seedling dry weight (21.0, 19.6 and 18.7 mg) at initial stages, 3 months of storage and 6 months of storage, respectively. However, the unprimed seed (control) of RHA-95C-1 recorded maximum mean seedling dry weight at initial

Table 1: Germination (%) of control and primed seed lots at different storage periods of sunflower

Lines	Germination (%)					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA- 95C-1	71	72	68	71	63	68
CMS-17A	76	78	73	77	69	72
CMS-17B	78	90	76	86	73	84
CMS-234A	66	67	65	64	59	61
KBSH-41	88	92	87	91	84	88
KBSH-53	89	90	88	87	82	85
KBSH-44	82	82	79	79	74	74
CMS-335A	78	78	76	75	73	74
Mean	78.6	81.1	76.5	78.8	72.1	75.8
S.E.±	4.74		3.22		4.72	
C.V. %	9.0		5.1		6.6	

stages and 6 months of storage (19.4 and 18.5 mg), respectively whereas, at 3 months of storage CMS-17B recorded maximum mean seedling dry weight.

The overall results revealed that the highest mean seedling dry weight was observed in the KBSH-53 over its parents upon priming treatments.

Seedling vigour index (SVI) :

Significant variations in SVI based on mean seedling dry weight was noticed among the genotypes and priming treatments. The SVI was the highest in KBSH-53 (1890) followed by KBSH-53 (1705) at initial observations as well as at three months of storage which was gradually reduced at six months of storage. At six months of storage KBSH-41 (1601) showed the highest seedling vigour index. Among the control KBSH-41 showed the highest seedling vigour index at all the three storage levels (1645, 1513 and 1360), respectively (Table 3).

The overall results indicated that the highest SVI based on mean seedling dry weight was observed in KBSH-53 and KBSH-41 treated with hydro priming for 18 hrs.

Priming had increased germination activity and growth of seedlings under stressed conditions in sunflower seeds. Rao *et al.* (1987) reported that primed *Brassica* seeds may enhance the establishment of good crop stands in cold and moist soils. Primed seeds had higher final germination percentages, germination rates, seedling length and dry weight measurements than unprimed seeds (Rouhi *et al*., 2011).

Table 2: Seedling dry weight of control and primed seed lots at different storage periods of sunflower

Lines	Seedling dry weight (mg)/10 seedling					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA-95C-1	19.4	20.8	18.9	19.6	18.5	18.3
CMS-17A	15.8	19.8	14.8	18.9	14.2	17.4
CMS-17B	19.0	20.7	19.6	18.2	18.3	17.8
CMS-234A	18.5	19.1	17.4	18.6	16.3	17.9
KBSH-41	18.7	19.3	17.4	18.7	16.2	18.2
KBSH-53	16.1	21.0	15.8	19.6	15.2	18.7
KBSH-44	17.2	19.2	16.8	18.9	15.4	18.5
CMS-335A	10.4	10.2	9.8	9.8	8.7	9.5
Mean	16.88	17.10	16.31	17.79	15.4	17.0
S.E.±	1.18		1.02		1.15	
C.V. (%)	9.8		4.8		5.2	

Table 3 : Seedling vigour index of control and primed seed lots at different storage periods of sunflower

Lines	Vigour index					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA-95C-1	1377	1497	1285	1391	1165	1244
CMS-17A	1200	1544	1080	1455	979	1252
CMS-17B	1614	1710	1489	1565	1335	1495
CMS-234A	1221	1279	1131	1190	961	1091
KBSH-41	1645	1775	1513	1701	1360	1601
KBSH-53	1449	1890	1390	1705	1246	1589
KBSH-44	1410	1574	1327	1493	1139	1369
CMS-335A	811	795	744	735	635	703
Mean	1341	1508	1245	1404	1103	1293
S.E.±	124		98		112	
C.V. (%)	9.1		5.2		8.1	

Speed of emergence :

Speed of emergence showed significant variations among the treatments (Table 4). The highest speed of germination was recorded in primed seeds of KBSH-41 (130.27) followed by CMS 335A (125.5) and it was lowest in the control seeds of RHA95C-1 (68). The overall results revealed that the primed seeds of KBSH-41 for 18 hrs @ 25^0 C showed the highest speed of germination over others.

It is revealed from the present study that different priming techniques can enhance the germination and early seedling growth in hybrid sunflower. Primed achenes had higher vigour, which resulted in earlier start of emergence (Basra *et al.*, 2002).

Rate of water uptake (%) :

Significant variations in rate of water uptake were noticed among the genotypes and priming treatments (Table 5). The highest rate of water uptake was recorded in hydro primed seeds of CMS 335A for 18 hrs (140.57), followed by KBSH-53 (77.12) and it was lowest in the control seeds of RHA 95C-1 (6.43).

Biochemical parameters :

Seed leachate analyses: Electrical conductivity (EC) :

Significant variations in EC were noticed among the priming treatments (Table 6). The lowest EC was recorded in

Table 4: Speed of emergence of control and primed seed lots of sunflower

Lines	Speed of emergence					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA-95C-1	68.00	70.80	66.80	69.11	60.14	63.80
CMS-17A	59.57	84.09	56.70	80.01	51.63	76.31
CMS-17B	56.95	93.77	55.08	90.60	50.60	81.95
CMS-234A	54.86	78.78	53.01	76.21	48.72	72.11
KBSH-41	72.25	130.27	70.18	126.61	67.59	119.30
KBSH-53	77.12	84.00	76.01	81.07	71.30	78.06
KBSH-44	67.88	79.18	66.36	78.26	62.01	76.01
CMS-335A	94.88	125.50	92.06	120.11	90.63	118.20
Mean	68.94	93.29	67.03	90.25	62.83	85.72
S.E.±	4.25		4.81		5.61	
C.V. (%)	7.9		8.9		9.0	

Table 5: Rate of water uptake of control and primed seed lots of sunflower

Lines	Vigour index					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA-95C-1	6.43	16.54	6.16	16.28	6.01	15.11
CMS-17A	41.96	46.38	40.06	45.61	38.96	44.80
CMS-17B	50.55	56.95	50.00	55.11	48.96	54.94
CMS-234A	36.61	36.86	36.06	36.6	35.82	36.10
KBSH-41	27.37	72.25	26.36	70.11	25.61	66.71
KBSH-53	19.35	77.12	10.06	70.81	16.09	68.11
KBSH-44	45.43	67.88	42.00	60.61	40.31	56.89
CMS-335A	94.88	140.57	92.63	130.6	90.11	126.30
Mean	40.35	66.82	39.04	60.72	37.73	58.62
S.E.±	2.72		28.63		28.36	
C.V. (%)	7.6		7.7		8.0	

primed seeds of KBSH-41 (73.4 dSm^{-1}) followed by KBSH-53 (76.00 dSm^{-1}) and it was highest in the control seeds of KBSH-53 (385 dSm^{-1}) indicating higher degree of membrane integrity in primed seeds at initial stage which was gradually increased by end of sixth month.

Further, the EC was negatively correlated with seed germination percentage, mean seedling dry weight and seedling vigour index.

Enzyme activities: Total dehydrogenase (TDH) activity :

A significant variation in the total dehydrogenase activity was noticed among the Priming treatments (Table 7). The TDH activity was highest in primed seed lots CMS-335 A (4.00 and 3.76) followed by KBSH-53 (2.69 and 2.31), KBSH-44 (2.49 and 2.24) and KBSH-53 (2.28 and 2.24) and it was lowest in controlled seed lots of CMS-335A (0.651 and 0.42) at initial stage and at the end of sixth month, respectively. Further, the TDH activity was positively correlated with the seed germination percentage, seedling vigour index and mean seedling dry weight, while it was negatively correlated with the EC. The overall results revealed that the highest TDH activity was observed in primed seeds of KBSH-53 compared to other lines.

Electrophoretic characterization of soluble seed protein profiles :

The SDS-PAGE banding pattern of soluble seed protein profiles of seeds of sunflower genotypes subjected to hydro priming treatment is depicted in Plate 1. CMS-234A showed a maximum of 11 bands and band No. 5 and 8 was unique where as band no.5 was unique to KBSH-41. Band no.7 was common to all line expect CMS-234A in primed seed lots

Table 6 : Electrical conductivity of control and primed seed lots at different storage periods of sunflower

Lines	Electrical conductivity (dSm^{-1})					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA- 95C-1	320	129.3	360.0	140.6	372.0	152.1
CMS-17A	304	166.7	323.4	184.1	346.3	194.6
CMS-17B	235	175.8	258.0	183.4	269.1	192.3
CMS 234A	347	224.4	363.4	243.6	384.1	259.1
KBSH-41	227	73.4	254.1	92.2	271.0	98.1
KBSH-53	385	76.0	391.3	89.1	408.4	94.3
KBSH-44	294	64.1	314.6	78.4	329.1	86.4
CMS-335A	220	86.0	248.0	96.2	259.4	112.1
Mean	291.5	124.4	314.1	138.4	329.9	148.6
S.E.±	14.8		18.2		16.0	
C.V. (%)	9.7		4.8		5.2	

Table 7 : Total dehydrogenase (TDH) activity of control and primed seed lots at different storage periods of sunflower

Lines	Total dehydrogenase (TDH) activity					
	Initial		3 Months of storage		6 Months of storage	
	Control	Primed	Control	Primed	Control	Primed
RHA- 95C-1	2.00	2.000	1.86	1.91	1.43	1.53
CMS-17A	0.920	1.174	0.84	1.06	0.76	0.93
CMS-17B	1.760	1.917	1.63	1.84	1.43	1.72
CMS-234A	1.237	1.491	1.16	1.36	1.08	1.24
KBSH-41	0.700	2.280	0.61	2.13	0.53	2.06
KBSH-53	2.218	2.695	2.14	2.54	2.03	2.31
KBSH-44	1.381	2.491	1.26	2.36	1.13	2.24
CMS-335A	0.651	4.00	0.53	3.93	0.42	3.76
Mean	1.48	2.38	1.38	2.27	1.2	2.1
S.E.±	1.56		0.62		0.62	
C.V. (%)	8.6		6.2		7.2	

where as in control the maximum number of bans found was 8 and band no.4 for unique to KBSH-41.

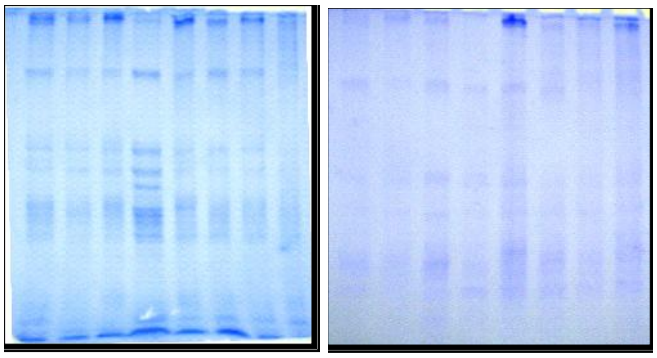

Plate 1: Total soluble seed protein profile of hydro primed (P) and control (C) lines of sunflower

The overall results suggested that upon priming treatments, the number of protein bands and intensity increased in low vigour seeds when compared to unprimed seeds.

Generally, seed storage caused a decrease in the protein content, which may be related to oxidation of the amino acids, due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds. Thus, prolonged seed storage would increase the metabolic activity of the seeds and consequently decrease the reserve substance content and reduce the dry material weight of the seeds (Bewley and Black,1994).The SDS PAGE studies provide clear understanding about the mechanism of priming, variation in the protein expression of sunflower was observed after priming (Wahid *et al*., 2008). Priming showed little improvement in the banding pattern and intensity of protein in normal seeds, while low-vigour seeds had significant improvement in the banding pattern and intensity of protein. They further found that priming in seeds from some oil species has caused significant increases in the soluble protein content, compared with non-treated seeds from these same species. And that the increase in protein synthesis could be related to greater germination capacity and performance under field conditions shown by the osmoprimed seeds (Kausar *et al*., 2009).

Total DNA content :

A total DNA content increased up on priming when compared to unprimed seed lots of sunflower. The total DNA content was also increased from 24 hrs of germination to 48 hrs of germination indicating there is good improvement in DNA content from G_1 to G_2 phase. The highest DNA content was found in 48 hrs of seed germination in CMS-234A (998 ng/µl) followed by CMS-17A (911 ng/µl) and KBSH-44 (688 ng/µl) and lowest DNA content was found in 24 hrs of seed germination CMS-17A (90.2 ng/µl) in unprimed seed lots (Table 8).

The benefits of seed priming have been well documented in several reviews *viz.*, Khan, 1992, Taylor *et al*., 1998 and Bradford, 1986. Priming is often used as a seed invigouration treatment for improving the seed germination and vigour in low vigour seed lots. Hence, it appears to reverse the detrimental effects of seed deterioration *i.e.*, repair of DNA and protein synthesising machinery.

Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA. Kausar *et al*., 2009 found that twice amino acids were incorporated in proteins during the first 24 h of imbibitions of sweet pepper seeds in PEG solutions (osmotic priming).

Conclusion :

Higher mean seed germination was observed in primed seeds of KBSH-41 (92%). Among the treatments, hydro primed seeds of KBSH-53 registered maximum mean seedling dry weight (21.0 mg) over its parents. The seedling vigour index (SVI) based on mean seedling dry weight was highest in KBSH-53 (1890) followed by KBSH-41 (1775) treated with hydro priming for 18 hrs. The highest speed of emergence was recorded in primed seeds of KBSH-41 (130.27) followed by CMS-335A (125.5). The hybrids KBSH-41 (73.4 dSm^{-1}) followed by KBSH-53 (76.01 dSm^{-1}) recorded the lowest electrical conductivity in primed seeds and was highest in the control seeds of KBSH-53 (385 dSm^{-1}) indicating higher degree of membrane integrity in primed seeds. The total dehydrogenase

Table 8: DNA content (ng/µl) of control and primed seed lots of sunflower

Lines	Control		Primed seeds	
	24 hrs	48 hrs	24 hrs	48 hrs
RHA- 95C-1	244	250	520	546
CMS-17A	90.2	99	885	911
CMS-17B	243	256	890	493
CMS 234A	236	245	900	998
KBSH-41	130.5	141	550	688
KBSH-53	180	190	525	574
KBSH-44	200	220	650	532
CMS-335A	162	170	460	466

activity was highest in primed seeds of KBSH-53 (2.69) compared to other hybrids and parental lines. Upon priming treatments, the number of protein bands and intensity of the bands increased in primed seeds when compared to unprimed seeds. A total DNA content increased up on priming when compared to unprimed seed lots of sunflower. The total DNA content was also increased from 24hrs of germination to 48 hrs of germination indicating there is good improvement in DNA content from G_1 to G_2 phase. The highest DNA content was found in 48 hrs of seed germination in CMS-234A (998 ng/µl) followed by CMS-17A (911ng/µl) and KBSH-41 (688 ng/µl).

Hydro priming treatment done at 18 hr at 25^0 C for medium vigour sunflower seed lots showed an improvement in germination, seedling quality parameters, rate water update and activity of hydrolyzing enzyme. The total DNA content was significantly improved in primed seed lots when compared to un-primed seeds, therefore, indicating that aerated hydro priming treatment could improve seed storage potential. Hence, the present experiment reveals that aerated hydro priming treatment enhances seed quality parameters, germination percentage, mean seedling dry weight, seedling vigour index, TDH activity and DNA content and there by leads to better crop stands and higher yields, however storage of seeds after priming would be detrimental. However, further research is required to identify the specific proteins synthesised and characterization of molecular marker to standardise priming treatment.

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