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Physiological and Biochemical Changes during Seed Development and Maturation in Sunflower (*Helianthus annuus* L.) Hybrid KBSH-1

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ABSTRACT. A study on physiological and biochemical changes during seed development and maturation in sunflower (Helianthus annuus L.) hybrid KBSH-1 revealed that seed attained physiological maturity at 42 days after anthesis with maximum rate of accumulation of dry weight and oil content between 18 and 24 days after anthesis. Protein content decreased during seed maturation and an appreciable decrease in protein content and start of oil biosynthesis coincided at the same phase indicating that synthesis of these nutritional factors are interrelated. Anti-nutritional factors, phenol and chlorogenic acid content decreased with stages of development. The enzyme activities were high up to hydrical step (18-24 DAA), thereafter it starts to decrease. Genotypic differences with reference to physiological and biochemical characteristics were evident during seed development and maturation.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) (2n = 34) is the second most important oil seed crop of the world next to soybean. In India it is grown over an area of 2.09 million ha with a production of 13.15 million tons and productivity of 640 kg ha^{-1} (Singhal, 1999). With a modest beginning of 1.5 million ha in 1972-73, the area under sunflower in India increased to 2.9 million ha during 1998-99. The increase was mainly due to development of hybrids.

In sunflower, many varieties and hybrids have been developed with varying concentrations of nutritional factors (oil and protein) and anti-nutritional factors (phenol and chlorogenic acid). The presence of these biochemical compounds and secondary metabolites play a major role in deciding the seed quality. Seed maturation refers to morphological, physiological and functional changes that occur from the time of fertilization until the matured seeds are ready for harvest (Delouche, 1973).

In case of hybrid seed production involving CGMS system (three line breeding) the genetic make up of each line is different and it is inherited in the hybrid in varying degrees which decides the hybrid seed quality. The parental lines vary with biochemical and physiological potential, nutritional quality and seed storability. This differential nature of parental lines of hybrid and inheritance of these characters to the hybrid, influence the hybrid seed quality. Information on the changes in both nutritional and anti-nutritional

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quality of seed during seed development and maturation in important oil seeds like sunflower is lacking. Hence a detailed study on physiological and biochemical changes during seed development and maturation in sunflower hybrid KBSH-1 was undertaken.

MATERIALS AND METHODS

A crop was raised during September, 2000 with the parental lines of sunflower hybrid KBSH-1 *viz.*, CMS 234 A × 6 D-1, CMS 234 A × CMS 234 B and 6 D-1 in separate blocks with a planting ratio of 4:1 and isolation of 600 m from each block. In CMS 234 A × 6 D-1, male parent was sown six days earlier to female and simultaneous sowing was undertaken in CMS 234 A × CMS 234 B. Hand pollination to the female parent at alternate days was given during 8:00-11:00 am from initiation to completion of flowering by means of palm covered with muslin cloth. In each block (A × B, A × R and R) 25 plants randomly selected were tagged for collection of seeds at six days interval up to 42 days after anthesis. The various physiological observations *viz.*, seed fresh weight, dry weight, seed moisture content, germination, root and shoot length, dry weight of seedling and vigour index were evaluated as per ISTA (1999) method. The electrical conductivity of seed leachate (Presley, 1958), and other biochemical observations *viz.*, oil content (AOAC, 1945), protein content (Lowry *et al.*, 1951), phenol content (Malick and Singh, 1980), chlorogenic acid, peroxidase activity (Malik and Singh, 1980), α - amylase activity (Paul *et al.*, 1970), catalase activity (Povolotskaya and Sedenka, 1956) and polyphenol oxidase activity (Esterbaner *et.al.*, 1977) were also recorded from the seeds collected during different stages of development and maturation.

RESULTS AND DISCUSSION

Significant differences due to genotypes, stages of maturation and their interaction were observed in all the parameters studied except protein content in which the interaction effect was not significant.

After sexual fusion, the developing seed begins to increase in weight as a result of nutrient and water intake associated with rapidly accelerating cell division and elongation. The fresh weight of developing seed increased continuously up to physiological maturity. The attainment of high seed fresh weight indicated the cessation of cell division during seed maturity (Noggle and Fritz., 1991). Differences in fresh weight among the genotypes were also observed. Hybrid seed accumulated more fresh weight at all stages of development followed by its maternal parent (Table 1). The differences in fresh weight of seeds among the genotypes could be of genetic control rather than other factors (Egli, 1988). Such differences in accumulation of fresh weight also existed in maize (Carter and Poneleit, 1973), pearl millet (Fussell and Pearson, 1978) and balsam (Arunachalam, 1991).

Seed dry weight continued to increase rapidly during all developmental stages. This increase in seed dry weight might be due to synthesis and deposition of storage materials like protein and oil bodies in cotyledonary tissues. The maximum dry weight accumulation which started between 18-24 days after anthesis (DAA) was maintained until physiological maturity stage (Table 1). The genotypic differences were obvious in dry weight. Maximum dry weight was noticed in hybrid followed by female parent indicating

its ability to synthesize more storage material from the precursor molecules available in the mother plant.

Table 1. Changes in seed fresh weight (g), dry weight (g) and moisture content (%) during seed development and maturation of sunflower hybrid KBSH-1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Fresh weight (g)								
A line	2.89	4.53	5.19	5.64	5.97	6.25	6.46	5.28
B line	2.99	4.72	5.48	5.87	6.17	6.43	6.56	5.46
R line	2.65	4.34	5.05	5.47	5.79	6.08	6.21	5.08
Hybrid	3.26	4.85	5.58	6.77	7.59	7.76	7.83	6.23
Mean	2.95	4.61	5.33	5.94	6.38	6.63	6.77	
	L		S		L × S			
S.E. (difference)	0.04		0.03		0.05			
LSD (p=0.05)	0.09		0.05		0.10			
Dry weight (g)								
A line	0.48	0.93	1.92	3.42	4.51	5.25	5.75	3.18
B line	0.47	0.91	1.86	3.36	4.69	5.43	5.83	3.22
R line	0.45	0.94	1.28	2.22	3.51	3.91	4.15	2.35
Hybrid	0.57	1.03	2.12	3.99	6.08	6.44	6.90	3.88
Mean	0.49	0.96	1.95	3.50	4.95	5.58	6.02	
	L		S		L × S			
S.E. (difference)	0.10		0.01		0.03			
LSD (p=0.05)	0.02		0.03		0.06			
Seed moisture content (%)								
A line	83.58	79.03	63.06	40.06	24.13	16.06	11.06	45.29
B line	83.63	80.79	66.29	42.32	23.97	15.68	11.06	46.25
R line	82.53	78.39	62.81	41.26	22.05	16.00	10.04	44.73
Hybrid	3.19	79.15	63.57	42.02	22.81	16.76	10.80	45.49
Mean	83.04	79.35	63.49	41.15	23.90	16.18	10.97	
	L		S		L × S			
S.E. (difference)	0.23		0.29		0.58			
LSD (p=0.05)	0.44		0.58		1.16			

Developing seeds started to lose moisture content continuously as they matured. Rapid loss of moisture which was observed between 18-30 DAA, coincided with the faster accumulation of dry weight (Table 1). When seed loses moisture and reaches minimum, the vascular connection between the developing seed and mother plant is broken so that no water or solutes could enter into the seed. The maturing seed attains a minimum of 11%

moisture content. It was interesting to note that a low seed moisture content was recorded at physiological maturity stage. Similar results of decreased seed moisture content at physiological maturity was also reported by Rao *et al.* (1993) in different cultivars of sunflower.

Results of electrical conductivity (EC) revealed that from the initial stage onwards EC decreased as the maturation advanced in all the genotypes which could be attributed to an alteration in membrane structure (Table 2). Loss of water from the seed at later stages of maturity turned the seed coat hard which did not permit the leaching of internal solutes (Roberts, 1972). Here also, differences in genotypes were observed. At all stages of development, hybrid maintained low EC when compared to others.

Table 2. Changes in electrical conductivity (dSm^{-1}), seed germination (%), root length of seedling (cm) during seed development and maturation of sunflower hybrid KBSH-1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Electrical conductivity (dSm^{-1})								
A line	0.257	0.248	0.232	0.217	0.213	0.203	0.190	0.224
B line	0.294	0.284	0.275	0.253	0.236	0.218	0.200	0.252
R line	0.265	0.254	0.243	0.230	0.218	0.205	0.190	0.230
Hybrid	0.267	0.254	0.249	0.230	0.213	0.205	0.180	0.230
Mean	0.271	0.260	0.250	0.233	0.220	0.208	0.196	
		L		S		L × S		
S.E. (difference)		0.002		0.002		0.004		
LSD (p=0.05)		0.003		0.004		0.008		
Seed germination (%)								
A line	0.00	0.00	0.00	2.67 (5.48)	73.00 (58.81)	93.67 (75.57)	98.00 (83.44)	38.19 (31.90)
B line	0.00	0.00	0.00	5.33 (10.87)	79.67 (63.24)	97.33 (82.57)	99.66 (88.08)	40.29 (34.97)
R line	0.00	0.00	0.00	0.00	46.00 (42.71)	81.67 (64.82)	96.00 (80.67)	31.95 (26.89)
Hybrid	0.00	0.00	0.00	8.00 (16.08)	82.67 (65.49)	95.67 (80.53)	98.00 (85.27)	40.62 (35.34)
Mean	0.00	0.00	0.00	4.00 (8.11)	70.33 (57.56)	92.08 (75.87)	98.00 (84.37)	
		L		S		L × S		
S.E. (difference)		0.92		1.72		2.41		
LSD (p=0.05)		1.82		2.40		4.81		
Root length seedling (cm)								
A line	0.00	0.00	0.00	7.23	22.10	22.93	23.23	10.79
B line	0.00	0.00	0.00	12.70	22.00	21.53	23.10	11.33
R line	0.00	0.00	0.00	0.00	17.70	18.47	19.13	7.90
Hybrid	0.00	0.00	0.00	23.00	23.27	23.70	24.10	13.47
Mean	0.00	0.00	0.00	10.78	21.27	21.66	23.39	
		L		S		L × S		
S.E. (difference)		1.08		1.37		1.25		
LSD (p=0.05)		2.08		2.75		2.50		

Values in parentheses indicate arcsine values

The germination is undoubtedly an important indicator of seed quality. In fully developed seed, germination was maximum and it not only depends upon the nutritional status of plant but also on several other factors (Hodgkin, 1980), besides the biochemical and physiological process during seed development and maturation (Tekrony *et al.*, 1980). The developing seed acquired maximum percentage of germination at 36-42 DAA in all genotypes (Table 2), whereas, it was 20 DAA in soybean (Sabirahamed, 1989), 56 DAA in cashew (Renganayaki and Karivaratharaju, 1993) and 40 DAA balsam (Arunachalam, 1991).

Seedling length is the best indicator of seed vigour. The relative length of root and shoot of seedlings would predict their subsequent growth and performance. The root length increased with increase in days after flowering (Burris *et al.*, 1973). The genotypic variability also existed and hybrids produced longer seedlings than the pollen parent. It indicated that the amount of nutrients or storage materials present in seed had a significant role in producing longer seedlings with more dry weight. The maximum seedling length, dry weight and vigour index were recorded at 42 DAA, coinciding with the accumulation of maximum dry weight of seed (Table 2-3). Similar associations of seed dry weight and vigour were reported by Pollock and Roos (1972) and Delouche (1973).

The protein and oil content had an inverse relationship with reserve material accumulation during seed development and maturation. The lipid accumulation started when the storage protein synthesis lowered. Murphy (1993) studied the relationship between storage protein and lipid accumulation in oil seeds and observed that lipid related m-RNA found at an early stage of embryo development was degraded at the same phase. Accumulation of oil would start at the end of storage protein accumulation and at the beginning of seed dehydration. In the present investigation, invariably, all the genotypes recorded the highest protein content at early stages of maturity, which decreased throughout the period of development with a sudden decrease at 18-24 DAA (Table 4) which might have coincided with the lipid m-RNA decomposition encoding for protein synthesis (Cummins and Murphy, 1990; Bewley and Black, 1994).

Irrespective of genotypes, the oil bio-synthesis had started by 24 DAA, and attained maximum oil content at 42 DAA (Table 4). The accumulation of oil and protein followed the same trend in all genotypes but variation in amount of reserves was found among genotypes.

Phenols are the aromatic compounds with hydroxyl groups which are widespread in the plant kingdom. Among the many phenolic compounds, the major polyphenol in sunflower seed is chlorogenic acid, which contributes 70% of the total phenolic compounds. At lower concentrations these phenolic compounds induce a defence mechanism in plants, whereas at higher concentration, they inhibit the germination of the seeds (Noggle and Fritz., 1991).

In the present study, total phenols, chlorogenic acid and polyphenol oxidase showed a significant relationship with each other (Table 4-5). The results were in conformity with Mayer (1987) who stated that the action and biosynthesis of these three compounds were interrelated. The presence of polyphenol oxidase (PPO) confirmed the synthesis and formation of phenols and chlorogenic acid during seed development (Mayer and Harel, 1981). In the present investigation, total phenolic contents didn't show any

marked variation during seed development. However, genotypic differences were noticed. Maintainer line had high amount of phenol compared to other genotypes.

Table 3. Changes in shoot length (cm) and dry weight of seedling (mg seedling^{-1}) and vigour index during seed development and maturation of sunflower hybrid KBSH-1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Shoot length of seedling (cm)								
A line	0.00	0.00	0.00	6.43	19.77	20.77	21.56	9.79
B line	0.00	0.00	0.00	14.20	21.73	20.57	22.23	11.25
R line	0.00	0.00	0.00	0.00	15.03	16.30	17.16	6.93
Hybrid	0.00	0.00	0.00	18.43	20.67	21.58	22.90	11.93
Mean	0.00	0.00	0.00	9.77	19.70	19.79	20.97	
				L		S		L × S
S.E. (difference)				1.01		1.37		2.72
LSD (p=0.05)				2.02		2.67		5.34
Dry weight of seedling (mg seedling^{-1})								
A line	0.00	0.00	0.00	3.33	15.37	22.37	34.46	10.79
B line	0.00	0.00	0.00	5.57	22.83	26.40	33.86	12.67
R line	0.00	0.00	0.00	0.00	7.10	15.47	21.23	6.26
Hybrid	0.00	0.00	0.00	14.93	20.93	24.40	38.26	14.08
Mean	0.00	0.00	0.00	5.96	16.56	22.16	31.96	
				L		S		L × S
S.E. (difference)				0.64		0.86		2.67
LSD (p=0.05)				1.26		1.67		3.35
Vigour Index								
A line	0.00	0.00	0.00	0.00	1124	2095	3380	943
B line	0.00	0.00	0.00	44	1822	2573	3376	1117
R line	0.00	0.00	0.00	0.00	327	1324	2033	526
Hybrid	0.00	0.00	0.00	805	1935	2793	3786	1331
Mean	0.00	0.00	0.00	212	1302	2196	3144	
				L		S		L × S
S.E. (difference)				0.92		1.72		2.41
LSD (p=0.05)				1.82		2.40		4.81

Chlorogenic acid is a complex derivative of phenol. Oxidation of chlorogenic acid by PPO results in chlorogenoquinone, which reacts with amino acids and proteins present in the seed resulting in the production of complex brown polymers (Noggle and Fritz., 1991). The activity of chlorogenic acid decreased with developmental stages and attained minimum at 42 DAA (Table 5). Chlorogenic acid at higher concentration inhibits seed

germination by inhibiting cell division and the attainment of minimum chlorogenic acid at physiological maturity stage which is indicative of the preparation of seed to germinate.

Table 4. Changes in oil content (%), protein content (%) and phenol content (mg 100 g seed^{-1}) during seed development and maturation of sunflower hybrid KBSH-1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Oil content (%)								
A line	0.00	0.00	0.00	4.80	12.46	31.78	41.07	12.84
B line	0.00	0.00	0.00	5.10	12.44	32.17	40.04	12.82
R line	0.00	0.00	0.00	3.91	11.94	31.04	40.84	12.53
Hybrid	0.00	0.00	0.00	4.82	13.11	34.54	42.17	13.52
Mean	0.00	0.00	0.00	4.66	12.49	32.38	41.03	
				L		S		L × S
S.E. (difference)				0.92		1.72		2.41
LSD (p=0.05)				1.82		2.40		4.81
Protein content (%)								
A line	36.27	33.90	31.47	27.57	27.00	26.30	26.63	29.88
B line	37.53	34.53	32.70	28.70	27.10	26.90	25.50	30.43
R line	35.20	32.57	31.53	27.40	25.95	25.30	25.50	29.06
Hybrid	38.80	35.43	33.63	28.40	27.30	26.70	25.86	30.88
Mean	36.95	34.11	32.33	28.02	26.84	26.30	25.88	
				L		S		L × S
S.E. (difference)				0.26		0.34		NS
LSD (p=0.05)				0.51		0.68		NS
Phenol content (mg 100 g seed^{-1})								
A line	3.144	3.757	4.375	5.623	5.332	4.830	3.920	4.426
B line	5.083	5.438	6.123	6.915	6.192	5.320	3.450	5.503
R line	1.311	1.662	1.762	1.885	1.420	1.388	1.250	1.525
Hybrid	4.744	4.924	5.208	5.306	5.680	4.230	3.700	4.828
Mean	3.570	3.945	4.367	4.932	4.656	3.942	3.083	
				L		S		L × S
S.E. (difference)				0.13		0.17		0.33
LSD (p=0.05)				0.25		0.33		0.66

Polypheonol oxidase is a copper protein of wide occurrence in nature which catalyses the aerobic oxidation of phenolic compounds to quinones. This enzyme is assumed to be a single enzyme with broad specificity. The PPO activity was obtained either by acidification or by release of a free fatty acid. Until then the appearance and biosynthesis of this enzyme was not known. The overall evidences for the stage at which PPO was formed is questionable and has many contradictory statements. In sunflower, the

PPO didn't show much difference at the stages of maturation and hybrid had maximum activity followed by seed parent, whereas the male parent registered lesser activity. In cotton, the initiation of fibre formation was associated with an initial increase in PPO activity (Naithani *et al.*, 1981) and PPO activity was decreasing during seed development in sorghum and in apple (Klapp *et al.*, 1989).

Table 5. Changes in chlorogenic acid content (OD), polyphenol oxidase (OD) and peroxidase (OD) during seed development and maturation of sunflower hybrid KBSH- 1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Chlorogenic acid content (OD)								
A line	1.167	1.054	0.653	0.582	0.532	0.308	0.300	0.657
B line	1.547	1.369	1.044	0.942	0.807	0.541	0.520	0.968
R line	0.795	0.766	0.632	0.508	0.458	0.372	0.280	0.546
Hybrid	0.218	1 056	0.764	0.650	0.625	0.454	0.410	0.740
Mean	1.182	1 061	0.773	0.671	0.608	0.419	0.382	
				L		S		L × S
S.E. (difference)				0.006		0.008		0.017
LSD (p=0.05)				0.012		0.016		0.032
Polyphenol oxidase (OD)								
A line	0.056	0.083	0.106	0.163	0.154	0.138	0.120	0.118
B line	0.073	0.114	0.136	0.159	0.164	0.150	0.130	0.132
R line	0.025	0.075	0.082	0.124	0.100	0.090	0.100	0.086
Hybrid	0.063	0.099	0.117	0.157	0.150	0.144	0.130	0.123
Mean	0.054	0.193	0.110	0.151	0.140	0.131	0.123	
				L		S		L × S
S.E. (difference)				0.002		0.002		0.004
LSD (p=0.05)				0.003		0.004		0.008
Peroxidase (OD)								
A line	0.011	0.024	0.032	0.042	0.030	0.027	0.020	0.029
B line	0.008	0.009	0.014	0.019	0.022	0.034	0.020	0.019
R line	0.022	0.026	0.028	0.029	0.031	0.033	0.020	0.0285
Hybrid	0.015	0.018	0.278	0.039	0.040	0.031	0.020	0.028
Mean	0.014	0.020	0.025	0.032	0.031	0.033	0.026	
				L		S		L × S
S.E. (difference)				0.002		0.002		0.004
LSD (p=0.05)				0.003		0.003		0.008

In the present study peroxidase activity increased upto a hydrical step and afterwards it started to decrease (Table 5). A similar trend was also observed for PPO, which indicated that the presence and activity of PPO might have induced the peroxidase

activity (Chabanet *et al.*, 1993). The increased activity of peroxidase in the presence of PPO could be attributed as peroxidases catalysed the oxidation of IAA. The presence of PPO inhibits the IAA oxidation which leads to the induced activity of peroxidase to counteract with the inhibitory effect of PPO (Krylov *et al.*, 1994).

The other enzymes, α-amylase and catalase also get reduced during the seed development. The activity of α- amylase attained minimum at 42 DAA in all the genotypes, and more activity was found in hybrid compared to its male parent (Table 6).

Table 6. Changes in catalse activity (μg H_2O_2 g^{-1} min^{-1}) and α-amylase activity (μg maltose g^{-1} min^{-1}) during seed development and maturation of sunflower hybrid KBSH-1 and parental lines.

Genotypes	Days after anthesis							Mean
	6	12	18	24	30	36	42	
Catalse activity (μg H_2O_2 g^{-1} min^{-1})								
A line	68.29	66.90	64.20	63.11	57.30	54.67	53.30	61.11
B line	65.67	63.60	63.33	58.97	55.47	54.83	51.13	59.00
R line	66.87	65.63	64.43	60.97	56.23	52.73	51.60	57.78
Hybrid	67.67	66.60	64.57	63.47	58.90	54.77	53.73	61.39
Mean	67.12	65.68	64.13	61.63	56.98	54.25	52.44	
				L		S		L × S
S.E. (difference)				0.329		0.435		0.872
LSD (p=0.05)				0.656		0.868		1.735
α-amylase activity (μg maltose g^{-1} min^{-1})								
A line	3.338	3.177	2.296	1.343	3.138	0.657	0.650	1.801
B line	2.884	2.418	1.834	1.124	0.884	0.646	0.520	1.474
R line	3.905	3.846	2.206	1.249	1.065	0.669	0.570	1.931
Hybrid	3.338	3.836	2.296	1.190	1.023	0.657	0.650	1.714
Mean	3.366	3.069	2.158	1.226	1.028	0.660	0.606	
				L		S		L × S
S.E. (difference)				0.034		0.045		0.090
LSD (p=0.05)				0.068		0.090		0.180

The activity of α-amylase was induced by the activity of gibberellins, and the same was synthesised and maintained up to hydrical step. Thereafter the action of gibberellins was reduced, thereby it could not induce the activity of α-amylase and get reduced as dehydration advanced (Noggle and Fritz., 1991). The catalase which is an oxidase and a heam protein, appears in free form and is not bound with membrane. In seed development, the catalase activity was reduced and attained minimum at maturity (Table 6). However different genotypes behaved differently and hybrid had more activity than its parental lines.

CONCLUSIONS

While reviewing the results of seed development and maturation, it could be inferred that all genotypes evaluated attained physiological maturity at 42 DAA. Among different stages, the crucial stage of development was between 18-24 DAA. At this stage a sudden decrease in fresh weight, maximum loss of moisture and maximum accumulation of dry weight were noted. It is interesting to note an appreciable decrease in protein content and the start of oil biosynthesis coincided at the same phase indicating that synthesis of these nutritional factors are interrelated. Anti-nutritional factors, phenol and chlorogenic acid content decreased with stages of development. The enzyme activities were high up to hydrical step (18-24 DAA), thereafter it decreased.

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