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Pattern of seed development in sunflower (*Helianthus annuus* L.) as influenced by seed priming

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Abstract
The physiological and biochemical changes in developing seeds of sunflower (*Helianthus annuus* L.) were studied in predicting the particular date of harvesting for getting maximum quality of seeds. Seeds at different position of sunflower heads were harvested, cleaned properly and evaluated as per standard procedure in the laboratory of Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal. Seven priming treatments along with control were applied in sunflower variety- WBSH-2021. Observations on physiological parameters like germination percentage (%), seedling length (cm), seedling dry weight (mg seedling^{-1}), vigour index-I, vigour index-II and biochemical parameters like oil content percentage, soluble protein content (mgg^{-1}), peroxidase activity (ΔA min^{-1} g^{-1}), alpha amylase activity (µg min^{-1} g^{-1}) were measured. The pattern of seed development was studied in various stages starting from 10 days old seed to 40 days old seed at an interval of 5 days. Considering all the physiological characters, germination percent, seedling length, vigour index-I and vigour index-II showed a steady increase upto 30 days after anthesis (DAA) of 40 days old seed irrespective of treatments. Except for soluble protein content, other biochemical activities were gradually increased up to 30 days after anthesis (DAA) i.e 40 days old seed. From 25 days old seed to 35 days old seed, the vigour index-I and vigour index-II values drastically increased, indicating quick seed filling at this stage and optimal physiological maturity stage for seedling quality. Based on this T_6 (Vermi priming) followed by T_7 (Bio priming) appeared to be the best treatments for quality seed production in sunflower.

Keywords: Seed development, seed priming, seed production, seed quality, sunflower

1. Introduction
Sunflower (*Helianthus annuus* L., 2n=34) is one of the world's four primary edible oilseed crops (Soybean, Groundnut, Rapeseed-Mustard, and Sunflower) that originated in North America (Shamshad *et al*., 2016; Yamgar *et al*., 2018) [21]. It is grown on 26 mha. Around the world, yielding 45 mMt. In India, Sunflower is grown on 2.5 lh with a yield of 2.2 lt and a productivity of 886 kgha^{-1} (Anonymous, 2018-2019) [2]. The need for high-yielding, high-quality edible oil is growing day by day, necessitating the expansion of the crop's area, production, and productivity, which can be accomplished through crop enhancement measures. The seeds of a single capitulum of sunflower starts accumulation of dry matter from the peripheral position seed. The seeds in the centre position of capitulum sometimes do not develop properly due to less accumulation of storage food and remains chaffy during harvesting. In present experiment seeds of single capitulum from different positions *viz*. centre (C_3), middle (C_2) and peripheral (C_1) positions were separated and studied for physiological and biochemical parameters. The changes in developing seeds has been reported in several crops like mustard (Saxena and Kumar, 1981) [20], but survey of literature indicates that research related to seed development is lacking in sunflower. Hence, the present experiment was conducted to assess the changes in physiological and biochemical characters during the developing and maturing seeds of sunflower to fix the correct stage of harvesting.

2. Materials and Methods
The field experiment was conducted during *rabi* season of 2019-2020 (November-March) and 2020-2021 (November-March) at C-Block Farm (Incheck Farm), Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The research station is located in West Bengal's Gangetic alluvial zone (latitude: 22.57°N, longitude: 88.20°E, elevation: 9.75 MSL). The experiment was laid out with seven seed priming treatments, *viz.* [T_1- Hydro priming (Bennett and Waters, 1987) [3], T_2- Solid matrix priming (Bennett and Waters, 1987 and Khan

et al., 1992) [3, 13], T_3- Osmotic priming (Bennett and Waters, 1987) [3], T_4- Halo priming (Khan *et al*., 1992) [13], T_5- Hormonal priming (Sundstrom *et al*., 1987) [24], T_6- Vermi priming, T_7- Bio Priming with Azatobactor) along with control (T_8- Dry Seed). Seeds of variety WBSH-2021, collected from Pulses and Oilseed Research station, Berhampore, West Bengal was treated by maintaining standard protocol of seed priming.

At the end, all the treated seeds were sealed in airtight container and placed in refrigerator at 8±2ºC till further use. Two seeds were planted treatment wise in three replications with plant to plant spacing of 30 cm and row to row spacing of 60 cm. The surplus seedlings were removed after seven days, leaving one seedling per hill. Recommended package of practices were followed and plant protection measures were taken as per necessity.

One head from each treatment and each replication was harvested at 10 days after 100% flowering (10 DA100F), 20 DA100F and 30DA100F. Seeds separated from heads in 3 concentric rings *viz*. central (C_3), middle (C_2), peripheral (C_1) position. Harvested seeds at 10DA100F was marked as $C_1$20, $C_2$15, $C_3$10. Similarly, 20DA100F was marked as $C_1$30, $C_2$25, $C_3$20 and 30DA100F was marked as $C_1$40, $C_2$35, $C_3$30. At 10 DA100F, altogether 8(T)X3(R)=24 heads were harvested and observations were marked as $C_1$20, $C_2$15, $C_3$10 for each of the 24 heads. Similarly, 20DA100F, 24 heads were harvested and marked as $C_1$30, $C_2$25, $C_3$20. At 30DA100F again 24 heads were harvested and marked as $C_1$40, $C_2$35, $C_3$30. Thus seeds were collected from different positions of head having different age of seeds *viz*. $C_3$10 (10 days old seed), $C_2$15 (15 days old seed), $C_1$20 (20 days old seed), $C_2$25 (25 days old seed), $C_1$30 (30 days old seed), $C_2$35 (35 days old seed), $C_1$40 (40 days old seed). Observations of different physiological and biochemical parameters were taken considering treatments as first factor and age of seeds as second factor.

The physiological parameters like germination percentage (%), seedling length (cm), seedling dry weight (mg seedling^{-1}), vigour index-I, vigour index-II and biochemical parameters like oil content percentage (%), soluble protein content (mgg^{-1}), peroxidase activity (ΔA min^{-1} g^{-1}), alpha amylase activity (µg min^{-1} g^{-1}) were studied at laboratory of department of seed science and technology, faculty of agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia.

Data were statistically analysed following FCRD design (Two factor analysis) by using OP STAT Computer programme developed by CCS, HAU, HISAR (Sheoran *et al*., 1998) [23].

3. Results and Discussion

3.1 Physiological parameters

The physiological parameters of harvested seeds in different positions of sunflower head having different ages of seeds starting from 10 days upto 40 days with an interval of 5 days were evaluated in the laboratory. From (Table-1), 10 days old seed showed no germination of the seed due to incomplete development of embryo and cotyledon. From 15 days old seed upto 40 days old seed, there was progressive increase of germination percentage. Analysis of variance indicated that germination percentage recorded a significant variation among treatments, age of seeds as well as interaction between treatments × age of seeds. In this study, T_7 (Bio priming) showed highest germination percentage during both the years (49.37% and 51.85% respectively). 40 days old seed ($C_1$40) showed maximum mean germination percentage (84.46%) over the years. Seedling length, seedling dry weight, vigour index-I, vigour index-II values were taken from 15 days old seed upto 40 days old seed as germination of seed was attained at 15 days old seed. Seedling length recorded significant variation for the treatments and age of seeds during both the years but they followed non significant variation under interaction between treatments × age of seeds during both the years. Among the treatments, T_7 (Bio priming) followed by T_6 (Vermi priming) and T_5 (Hormonal priming) during first year (14.55 and 13.88 cm respectively) and T_6 (Vermi priming) followed by T_7 (Bio priming) during second year (14.18 and 14.01 cm respectively) recorded maximum seedling length. However in 2 years, average indicated maximum seedling length in case of T_7 (Bio priming) with 14.28 cm. Among different aged seeds, 40 days old seed ($C_1$40) showed maximum seedling length (16.66 cm in first year and 13.81 cm in second year) over the years (15.24 cm). Interaction between treatments × age of seeds recorded non significant variation during both the years for seedling length. Seedling dry weight was maximum in T_6 (Vermi priming) under different treatments during both the years (94.89 mg seedling^{-1} and 93.50 mg seedling^{-1} respectively). Vigour index-I was maximum in T_7 (Bio priming) under different treatments during both the years. Vigour index-II was maximum in T_6 (Vermi priming) under different treatments during both the years. Treatments, age of seeds and as well as interaction between treatments × age of seeds recorded significant variation in case of vigour index I and II during both the years. The improvement in seed germination and vigour were associated with dry matter accumulation in the developing seeds. Germination percent, seedling length, seedling dry weight, vigour index-I and vigour index-II value were maximum at 30 days after anthesis (30DAA) of 40 days old seed ($C_1$40) during both the years. In a similar study, Dharmalingam and Basu (1990) [12] noticed that maximum germination percentage of seeds was observed at 30 days after anthesis (30DAA) in mungbean. The seedling length, seedling dry weight, vigour index-I and vigour index-II showed similar trends of improvement like germination. The results of the study suggested that physiological maturity of sunflower seed attained at 40 days old seed critically indicated the stage for optimum seed quality.

Table 1: Effect of seed priming on different age of seeds of sunflower for physiological parameters

	Germination percentage (Tr value)			Seedling length (cm)			Seedling dry weight (mg seedling^{-1})			Vigour Index-I			Vigour Index-II		
Treatments (T)	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**
T_1	46.11(43.06)	50.14(45.37)	48.13(44.22)	13.20	12.65	12.93	86.28	76.83	81.56	650.77	651.84	651.31	4348.65	4203.63	4276.14
T_2	44.16(41.93)	46.59(43.33)	45.38(42.63)	12.56	11.89	12.23	79.11	70.00	74.56	570.58	568.64	569.61	3844.07	3575.07	3709.57
T_3	46.69(43.39)	48.87(44.64)	47.78(44.02)	12.39	12.13	12.26	86.33	83.22	84.78	617.25	606.84	612.05	4347.33	4352.03	4349.68
T_4	47.37(43.78)	50.94(45.83)	49.16(44.81)	13.28	12.35	12.82	83.44	77.61	80.53	684.37	645.94	665.16	4339.22	4324.47	4331.85
T_5	45.65(42.79)	47.67(43.95)	46.66(43.37)	13.88	12.97	13.43	86.78	81.78	84.28	673.50	637.16	655.33	4335.67	4277.63	4306.65

T_6	48.38(44.36)	49.87(45.21)	49.13(44.79)	13.88	14.18	14.03	94.89	93.50	94.20	713.34	735.29	724.32	4942.20	4993.33	4967.77
T_7	49.37(44.93)	51.85(46.35)	50.61(45.64)	14.55	14.01	14.28	92.61	86.89	89.75	746.93	743.42	745.18	4877.18	4807.85	4842.52
T_8	45.37(42.63)	48.58(44.47)	46.98(43.55)	13.04	12.02	12.53	82.94	78.33	80.64	623.36	604.12	613.74	3985.49	4081.62	4033.56
S.Em (±)	0.697	0.628		0.283	0.153		1.175	1.021		19.280	9.735		86.794	70.204	
LSD (P=0.05)	1.959	1.765		0.797	0.429		3.304	2.871		54.208	27.371		244.034	197.388	
Age of seeds (AOS)															
$C_3$10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$C_2$15	12.64(21.25)	13.87(22.28)	13.26(21.77)	11.52	11.47	11.50	66.71	62.29	64.50	146.13	159.08	152.61	849.97	860.16	855.07
$C_1$20	26.46(31.28)	31.32(34.34)	30.39(32.81)	12.33	12.29	12.31	74.46	68.92	71.69	326.37	384.18	355.28	1974.92	2154.95	2064.94
$C_2$25	31.41(34.40)	35.25(36.72)	33.33(35.56)	12.67	12.59	12.63	82.88	76.63	79.76	398.47	444.25	421.36	2603.29	2710.19	2656.74
$C_1$30	55.34(48.35)	56.64(49.10)	55.99(48.73)	13.25	13.07	13.16	91.00	84.92	87.96	733.65	742.03	737.84	5053.24	4822.93	4938.09
$C_2$35	70.63(57.50)	73.23(59.17)	71.93(58.34)	13.64	13.41	13.53	99.17	93.13	96.15	965.23	982.80	974.02	7018.45	6828.81	6923.63
$C_1$40	83.33(66.29)	85.58(68.09)	84.46(67.19)	16.66	13.81	15.24	105.08	100.25	102.67	1390.24	1182.59	1286.42	8764.98	8584.70	8674.84
S.Em (±)	0.603	0.544		0.245	0.132		1.018	0.884		16.697	8.431		75.166	60.798	
LSD (P=0.05)	1.696	1.528		0.690	0.372		2.862	2.486		46.946	23.704		211.339	170.943	
Interaction (T×AOS)															
S.Em (±)	1.706	1.537		0.694	0.374		2.879	2.501		47.226	23.845		212.601	171.964	
LSD (P=0.05)	4.798	4.322		NS	NS		NS	NS		132.782	67.044		597.758	483.501	

Legend: T_1: Hydro priming, T_2: Solid matrix priming, T_3: Osmotic priming, T_4: Halo priming, T_5: Hormonal priming, T_6: Vermi priming, T_7: Bio priming, T_8: Dry seed (Control), NS: Non Significant, C_1- Peripheral position, C_2- Middle position, C_3- Central position

3.2 Biochemical constituents

For (Table-2), analysis of variance revealed that effect of seed priming chemicals and age of seeds on oil content, soluble protein content, peroxidase activity, alpha amylase activity recorded significant variation during both the years but the interaction effect of treatments × age of seeds showed non-significant variation in oil content and peroxidase activity during both the years. Among the different treatments T7 (Bio priming) recorded highest oil content (19.38%) followed by T6 (Vermi priming) (18.09%) during first year. During second year T6 (Vermi priming) recorded maximum oil content (17.62%) followed by T7 (Bio priming) (16.67%). The mean value over 2 years was maximum in T7 (Bio priming) with 18.03% oil content. T2 (solid matrix priming) recorded minimum oil content (15.29% and 14.71% respectively) during both the years. At 30 days after anthesis (30DAA) of 40 days old seed ($C_1$40) recorded highest oil content (23.79% and 22.61% respectively) during both the years. The trend of mean value was also same at 30 days after anthesis (30DAA) of 40 days old seed ($C_1$40). Soluble protein content was maximum in T7 (Bio priming) during first year and second year (81.46 mgg^{-1} and 86.26 mgg^{-1} respectively). Protein is an important component of seed where soluble protein is most important. Rate of accumulation on soluble protein varied with the stages of development. The soluble protein content was gradually increased from 10 days after anthesis (10DAA) upto 20 days after anthesis (20DAA) but later on it declined slightly. Reduction of protein content with developing to maturity may be due to denaturation through release in moisture content leading to desiccation. Among the different treatments T7 (Bio priming) recorded highest mean value of soluble protein content (84.11 mgg^{-1}). At 20 days after anthesis (20DAA) of 30 days old seed ($C_1$30) having the maximum soluble protein content (123.73 and 130.76 mgg^{-1} respectively during first year and second year). The isozyme peroxidase is valuable for protection of plant and seed at development stages under different stress condition. The existence of multiple forms of peroxidase in plants has been known for a number of years, but the relationship of individual isozymes to specific biological functions is not clear. Increases in total peroxidase activity are often found during stress condition, with the greatest increases associated with a particular time. Among the treatments, T6 (Vermi priming) recorded highest peroxidase activity during both the years (0.65 and 0.76 ΔA min^{-1}g^{-1} respectively). At 30 days after anthesis (30DAA) of 40 days old seed ($C_1$40) having the maximum mean value (0.82 ΔA min^{-1}g$^{-1)}$ of peroxidase activity. The germination link enzyme α-amylase activities gradually increased with the progression of seed development up to certain point i.e. 30 days old seed ($C_1$30) in significant mode after that the rate of activity was declined at 30 days after anthesis during both the years. Considering the treatments, T7 (Bio priming) indicated superior α-amylase activity (50.36µg min^{-1} g^{-1}) during first year and in T4 (Halo priming) during second year (49.33µg min^{-1} g$^{-1)}$. At 20 days after anthesis (20DAA) of 30 days old seed ($C_1$30) having the maximum α-amylase activity (77.53 µg min^{-1} g^{-1} and 82.36 µg min^{-1} g^{-1}) during first year and second year respectively. The mean value (79.95 µg min^{-1} g^{-1}) was maximum in same stage of 30 days old seed ($C_1$30).

Table 2: Effect of seed priming on different age of seeds of sunflower for biochemical parameters

	Oil content percentage (%)			**Soluble protein content (mgg^{-1})**			**Peroxidase activity (ΔA min^{-1} g^{-1})**			**Alpha amylase activity (µg min^{-1} g^{-1})**		
Treatments (T)	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**	**1st year**	**2nd year**	**Mean**
T_1	17.14	15.79	16.47	77.01	80.90	78.96	0.52	0.69	0.61	46.62	47.75	47.19
T_2	15.29	14.71	15.00	72.60	78.25	75.43	0.48	0.64	0.56	44.89	45.06	44.98
T_3	15.88	16.12	16.00	74.73	80.64	77.69	0.50	0.68	0.59	47.45	46.78	47.12
T_4	17.76	15.59	16.68	76.95	80.12	78.54	0.52	0.67	0.60	48.47	49.33	48.90
T_5	17.96	16.09	17.03	78.23	82.67	80.45	0.55	0.70	0.63	45.86	48.02	46.94
T_6	18.09	17.62	17.86	76.33	82.52	79.43	0.65	0.76	0.71	48.75	49.21	48.98
T_7	19.38	16.67	18.03	81.86	86.36	84.11	0.59	0.75	0.67	50.36	49.24	49.80
T_8	16.01	14.94	15.48	74.55	79.58	77.07	0.51	0.65	0.58	46.99	46.23	46.61
S.Em (±)	0.472	0.405		0.840	0.690		0.011	0.011		0.540	0.480	
LSD (P=0.05)	1.328	1.140		2.356	1.936		0.032	0.030		1.514	1.346	
Age of seeds (AOS)												

$C_3$10	0	0	0	18.95	21.64	20.30	0	0	0	16.69	15.49	16.09
$C_2$15	7.46	8.30	7.88	28.88	31.00	29.94	0.36	0.42	0.39	25.46	23.46	24.46
$C_1$20	14.89	10.77	12.83	58.85	61.69	60.27	0.34	0.46	0.40	26.65	25.09	25.87
$C_2$25	15.81	15.83	15.82	68.47	70.81	69.64	0.48	0.73	0.61	40.69	34.77	37.73
$C_1$30	19.88	18.00	18.94	123.73	130.76	127.25	0.58	0.79	0.69	77.53	82.36	79.95
$C_2$35	21.34	20.14	20.74	119.66	127.76	123.71	0.68	0.85	0.77	73.66	77.93	75.80
$C_1$40	23.79	22.61	23.20	117.19	126.01	121.60	0.74	0.90	0.82	71.27	74.82	73.05
S.Em (±)	0.409	0.351		0.785	0.645		0.010	0.009		0.505	0.449	
LSD (P=0.05)	1.150	0.987		2.204	1.811		0.028	0.026		1.417	1.259	
Interaction (T×AOS)												
S.Em (±)	1.157	0.993		2.221	1.825		0.028	0.027		1.425	1.269	
LSD (P=0.05)	NS	NS		6.233	5.121		NS	NS		4.007	3.561	

Legend: T_1: Hydro priming, T_2: Solid matrix priming, T_3: Osmotic priming, T_4: Halo priming, T_5: Hormonal priming, T_6: Vermi priming, T_7: Bio priming, T_8: Dry seed (Control), NS: Non Significant, C_1- Peripheral position, C_2- Middle position, C_3- Central position

4. Conclusion

It can be concluded that physiological maturity of sunflower seeds attained between 20 to 30 days after anthesis (DAA) where the dry matter, germination and vigour were at their maximum value. Considering germination percent, dry matter and vigour index, at 20 days after anthesis (20DAA) i.e. 30 days old seed appeared to be the most favourable for seed development stages. For up gradation of seed quality parameters T_7 (Bio priming) followed by T_6 (*Vermi priming*) emerges to be encouraging for quality seed production in sunflower.

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