

Effect of temperature at various growth stages on seed quality in *Brassica* species

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ABSTRACT A field experiment was conducted with eleven rapeseed and Indian mustard varieties grown in 4 dates of sowing in *rabi* 2011-12 to evaluate the emergence of seeds. The mature seeds of the varieties were collected as and when the varieties matured. The activity of enzymes (peroxidase and catalase) and seed germination at five temperature (20, 25, 30, 35 and 40°C) conditions in the laboratory were recorded. The varieties showed lower germination at 20°C and 35°C in comparison to that in 25°C and 30°C temperature while it was nil at 40°C. The seeds maturing at lower temperature in early sowing conditions showed higher seed germination as compared to those matured at higher temperature in case of late sowing conditions. The results also indicated that higher temperature at seed filling and maturity enhanced the enzyme activities. A comparison among early, timely and late sown varieties indicated that timely and late sown varieties had higher activities of peroxidase and catalase enzymes.

Keywords: Seed quality, seedling emergence, germination, catalase, peroxidase, rapeseed-Indian mustard

Seed germination is defined as the developmental period that begins with water uptake and ceases with radicle emergence through the seed coat. Before water imbibition, mustard seeds rest in a quiescent state with very low moisture (5-6%) and exhibit low metabolic activity. After imbibition, germination process begins and ends in emergence of plumule and radicle and their elongation. Seedling emergence in a field condition is a function of seed germination and early seedling growth. Soil temperature is the major factor promoting germination once seeds imbibe water. Germination time varied with temperature from 11 to 14 days at 2°C to 1 day at 21 to 25°C. Low temperature slows the rate of imbibitions [1].

Under moist seed bed conditions crop emergence can usually be detected after 4-5 days, whereas, seedling emergence is delayed by 10-12 days under moisture deficit and higher soil temperature conditions .

Brassica is an important oilseed crop of winter season and its early sowing is very

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advantageous. Seeds attain maximum vigour on the mother plant at the end of seed filling phase [2-4] or slightly after this phase [5-10] after which they begin to deteriorate on the mother plant or during storage, losing viability and vigour [7, 11]. The rate of seed deterioration is positively related to ambient temperature, relative humidity and seed moisture content [12-13]. Heat stress often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. It's a raising issue and poses a serious threat to crop production worldwide [14] in the present scenario of climate change. High temperature increases the rate of reproductive development, which shortens the time for photosynthesis to contribute to fruit and seed development [15]. In response to both biotic and abiotic stresses, plants synthesize a wide set of proteins including chitinases and peroxidases [16].

Enzyme activity, particularly peroxidase is considered as biochemical marker of stress [17-19] and varies with temperature during germination. Higher temperature provoked

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damage at the physiological, biochemical and molecular level [20]. Though literature is available on effect of various temperature conditions on crop growth and yield, scanty information is available on the effect of growing conditions on seed quality and its subsequent effect in seed germination. In order to diversify areas under Indian mustard, new varieties suitable for specific agro-ecological conditions particularly in eastern India were developed. These varieties are sown under high temperature conditions and mature under low temperature conditions. In the present study, Indian mustard and rapeseed varieties were grown under four different dates of sowing, seeds harvested at different times on maturity and their seed quality parameters including activities of few important enzymes were evaluated. The results of the study are presented in this paper.

MATERIALS AND METHODS

Four early sown (Pusa Mahak, Pusa Agrani, NPJ-112 and EJ-17), two timely sown (Pusa Vijay and Pusa Bold) and two late sown (RGN-145 and NPJ-113 (Pusa Mustard 26) varieties of Indian mustard and three varieties (BBS-1, T-9 and YID-1) of *Brassica rapa* were sown at field of IARI in winter season of 2011-12. New Delhi has a semi-arid and sub-tropical climate characterized by extreme hot summers and extreme cool winters. The seeds were sown in plots with sufficient soil moisture on four dates of sowing (D1:19th September, D2:12th October, D3:2nd November and D4:22nd November, 2011). Two hundred seeds of each variety were sown in 2 rows of 5 m length with a spacing of 50 cm between rows. Spacing between plants within a row was about 15- 20 cm after thinning of additional plants. Recommended dose of fertilizers were applied to grow a normal and healthy crop. Need based plant protection measures, particularly to control aphid attack was taken. Irrigation was provided as and when required.

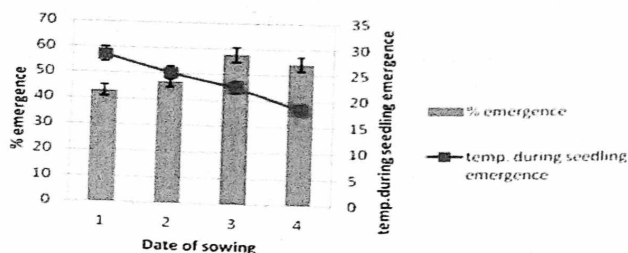
The emergence of the seeds sown in all the four dates of sowing was observed. The harvested seeds from various dates of sowing were kept at ambient conditions for 3 months and evaluated for germinability at different temperature (20°C, 25°C, 30°C, 35°C and 40°C) conditions in the laboratory using standard ISTA methodology [21]. Activity of two antioxidant enzymes namely, peroxidase and catalase were estimated on 3 days old seedlings by using method suggested by Aebi and Castillo respectively [22 and 23]. Daily weather parameters for the growing period starting from 1st sowing till last harvesting were collected from the meteorological observatory, Division of Agricultural Physics, I.A.R.I., New Delhi, India. Statistical analysis of the recorded data was carried out using STPR and OPSTAT software.

RESULTS AND DISCUSSION

Seedling emergence

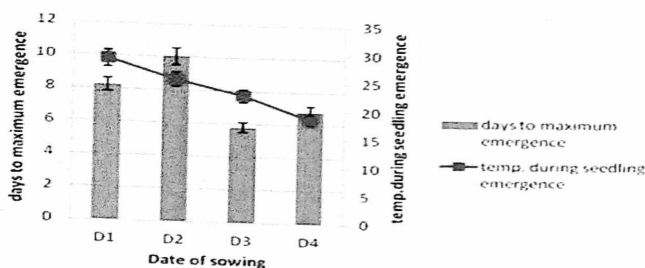
A significant difference in seedling emergence among the varieties and date of sowing was recorded (Fig. 1). The highest seedling emergence was obtained in D3 (57.9%) and lowest in D1 (43.1%) among the dates of sowing. The early varieties showed higher emergence in all the dates of sowing, indicating their suitability under varied sowing dates encompassing temperature regimes. The varieties except Pusa Bold, RGN-145 and YID-1 were similar in emergence pattern. However, early varieties showed better emergence in early sowing conditions than varieties of other maturity groups (Fig. 3).

There was a significant difference in number of days taken to achieve maximum per cent field emergence in the sowing dates (Fig. 2). However, the varieties did not differ significantly for this trait across the dates of sowing. In D1, the maximum emergence was reached in 8.2 days while it took 10.7 days in D2, but in later dates (D3 and D4) it took 5.8



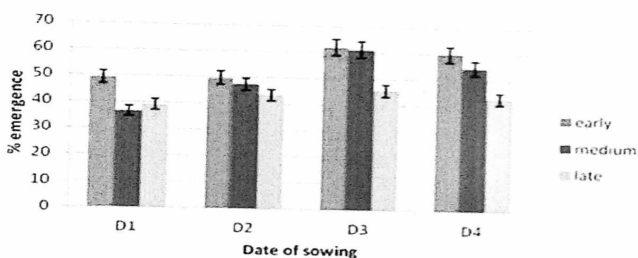
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Fig.1. Effect of date of sowing on seedling emergence in rapeseed - mustard varieties



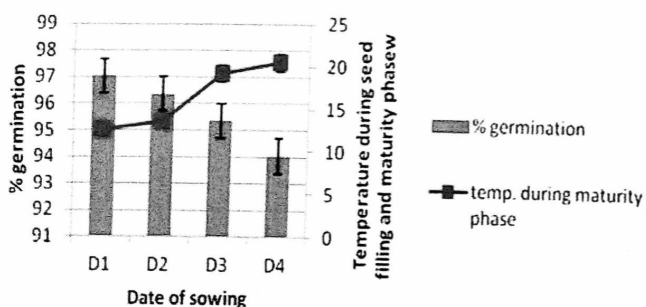
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Fig. 2. Effect of date of sowing on day to maximum emergence in rapeseed-mustard varieties.



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Fig. 3. Comparison between varieties of different maturity groups for seedling emergence in different dates of sowing



Error bar indicates standard error

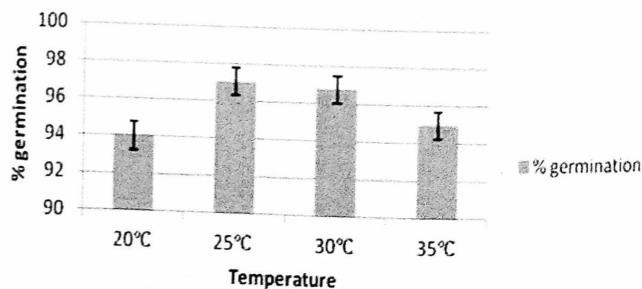
Fig. 4. Effect of date of sowing on per cent germination of seeds of rapeseed -mustard varieties

and 6.8 days, respectively. The results indicated that low temperature as available in D3 and D4 is required to get early emergence in rapeseed-mustard seeds.

Seed germination

No significant differences in percent germination was recorded between the seeds harvested in D1 (97.04) and D2 (96.36) sowing dates and between D3 (95.36) and D4 (94.04) sowing dates (Fig. 4). The varieties differed significantly for germination percentage in the seeds harvested from plants grown in a particular date of sowing.

No significant difference between temperatures at 20°C and 35°C for percent germination among the varieties was observed indicating sensitivity of seeds towards very low and high temperatures (Fig. 5). Similarly, seed germination did not vary significantly between 25°C and 30°C. Early varieties (EJ-17, NPJ-112, Pusa Agrani) and varieties of *Brassica rapa* showed significantly higher germination at 25-30°C. BBS-1, YID-1 (*Brassica rapa*) and RGN-145 did not show significant response in germination test conducted at different temperature conditions. Pusa Agrani showed least germination (86.25%) at 35°C (Table 1). Significant difference in germination of seeds was observed among varieties belonging to different maturity groups.



Error bar indicates standard error

Fig. 5. Effect of germination temperature on per cent germination of seeds of rapeseed - mustard

Table 1. Effect of temperature on per cent germination of seeds of rapeseed -mustard varieties

Varieties	Germination temperature				Mean
	20°C	25°C	30°C	35°C	
NPJ-112	90.75 (74.2)	93.00 (78.3)	96.50 (82.5)	93.50 (78.3)	93.43 (78.3)
Pusa Agrani	95.25 (80.0)	98.25 (85.5)	98.50 (84.5)	86.25 (68.8)	94.56 (79.7)
Pusa Mahak	92.87 (78.2)	98.00 (83.7)	99.25 (87.5)	97.00 (80.8)	96.78 (82.6)
EJ-1	97.62 (83.3)	100.0(90.0)	96.50 (83.6)	98.75 (85.5)	98.21 (85.6)
Pusa Bold	90.37 (74.1)	96.75 (83.7)	99.00 (87.1)	98.00 (83.7)	96.03 (82.2)
Pusa Vijay	92.62 (75.4)	97.50 (82.2)	96.50 (80.3)	91.25 (73.3)	94.46 (77.8)
RGN-145	94.50 (78.4)	92.50 (77.5)	97.00 (81.5)	92.00 (75.2)	94.00 (78.2)
NPJ-113	95.00 (80.0)	97.75 (84.1)	99.75 (88.9)	95.75 (78.3)	97.06 (82.9)
BBS-1	97.25 (82.7)	99.00 (86.5)	99.00 (86.5)	95.25 (80.3)	97.62 (84.0)
YID-1	96.50 (82.6)	98.75 (85.5)	91.87 (75.2)	99.25 (86.9)	96.59 (82.6)
T-9	91.25 (73.4)	96.12 (82.1)	90.75 (75.6)	97.75 (83.3)	93.96 (78.6)
Mean	94.00 (78.4)	97.05 (83.6)	96.78 (83.0)	94.97 (79.5)	
CD (p=0.05)					
Variety		2.64			
Germ. Temp.		1.59			
Var. X Germ. Temp.		5.28			

DOS: date of sowing; D1:19.9.2011; D2:12.10.2011; D3:2.11.2011; D4:22.11.11

Figures in parenthesis are transformed values

Seeds produced at relatively low temperature showed significantly higher germination at 25°C (99.45% and 96.95% and 30°C (97.27% and 98.22%) in D1 and D2, respectively, than those produced at higher temperatures (D3 and D4) (Table 2). However, no variety showed any germination of seeds irrespective of its growing and maturity conditions when germination test was carried out at 40°C.

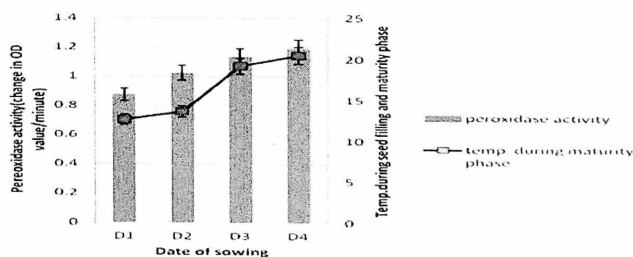
Enzyme activity

Peroxidase: Peroxidase enzyme activity was measured in the seeds of varieties harvested from different sowing date plots and results showed a significant variation for the enzyme activity in different varieties and sowing dates. The seeds of varieties maturing at lower temperature showed lower enzyme activity and increased in seeds maturing under high temperature condition (Fig. 6). Maximum peroxidase activity was noted in *B. rapa* cultivar, T-9 indicating its tolerance to terminal heat stress. In Pusa Bold and NPJ-113, higher

enzyme activity was recorded across the sowing dates. A relatively lower activity was recorded in varieties Pusa Agrani and Pusa Mahak (*B. juncea*) and variety BBS-1 (*B. rapa*) (Table 3).

Catalase

The varieties differed significantly for catalase activity under various maturity conditions (Table 4). NPJ-113 showed a higher level of catalase activity which enables it to be grown in late sowing condition. *B. rapa* varieties had



Error bar indicates standard error

Fig. 6. Effect of date of sowing on peroxidase activity (change in OD value per min.) of seeds of rapeseed -mustard varieties

Table 2. Effect of date of sowing(DOS) and germination temperature on per cent germination of seeds of rapeseed-mustard

DOS	Germination temperature				Mean
	20°C	25°C	30°C	35°C	
D1	97.45(82.8)	99.45(88.0)	97.27(83.8)	94.00(78.2)	97.04(83.2)
D2	94.54(80.4)	96.95(83.5)	98.22(86.3)	95.72(81.6)	96.36(83.0)
D3	90.18(73.5)	95.54(81.1)	94.90(79.5)	95.54(79.6)	94.04(78.4)
D4	93.81(76.9)	96.27(81.6)	96.72(82.6)	94.63(78.6)	95.36(79.9)
Mean	94.00(78.4)	97.05(83.6)	96.78(83.0)	94.97(79.5)	
CD(p=0.05)					
DOS	1.59				
Germ. Temp.	1.59				
DOS X Germ. Temp.	3.19				

DOS: date of sowing; D1:19.9.2011; D2:12.10.2011; D3:2.11.2011; D4:22.11.11

Figures in parenthesis are transformed values

Table 3. Effect of date of sowing on peroxidase activity (change in OD value per min.) of seeds of rapeseed -mustard varieties

Varieties	Date of sowing(DOS)				MEAN
	D1	D2	D3	D4	
NPJ- 112	0.941	1.191	1.432	1.063	1.156
PUSA AGRANI	0.814	0.697	1.177	1.201	0.972
PUSA MAHAK	0.56	0.773	0.938	1.247	0.879
EJ- 17	1.204	0.954	1.16	0.831	1.037
PUSA BOLD	1.147	0.943	1.243	1.289	1.155
PUSA VIJAY	0.63	0.858	1.305	1.274	1.016
RGN- 145	0.785	0.883	0.624	0.925	0.804
NPJ- 113	1.085	1.115	1.276	1.298	1.193
BBS- 1	0.481	1.36	0.861	1.221	0.98
YID- 1	1.113	1.06	0.863	1.34	1.094
T- 9	0.865	1.461	1.599	1.427	1.338
MEAN	0.875	1.026	1.134	1.192	1.057
CD (p=0.05)					
DOS	0.318				
Variety	0.528				
DOS X Variety	0.105				

DOS: date of sowing; D1:19.9.2011; D2:12.10.2011; D3:2.11.2011; D4:22.11.11

a relatively lower activity of this enzyme, YID-1 having the lowest. Early varieties showed a higher level of enzyme activity when grown under late sowing conditions. Pusa Mahak had the highest activity across the dates of sowing. The seeds of varieties maturing at lower temperature showed lower enzyme activity, which increased

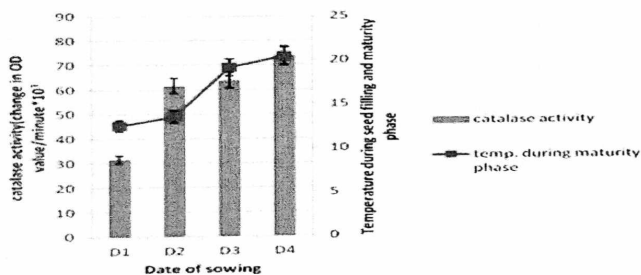
proportionately in seeds maturing under high temperature conditions (Fig. 7).

Relationship between temperature and seed quality
A significant correlation between the temperature variables and the enzyme (catalase and peroxidase) activities were recorded ($r=0.419-0.470$ and $0.434-0.444$

Table 4. Effect of date of sowing on catalase activity (change in OD value per min. X 103) of seeds of rapeseed -mustard varieties

Varieties	Date of sowing(DOS)				MEAN
	D1	D2	D3	D4	
NPJ- 112	43.8	72.2	96.1	78.1	72.55
PUSA AGRANI	15	89.9	86	70.9	65.45
PUSA MAHAK	55	98	85.2	85.1	80.825
EJ- 17	46.2	91.8	53.4	50.5	60.475
PUSA BOLD	33.6	87.2	54.3	86.1	65.3
PUSA VIJAY	48.9	53.3	23.3	81.3	51.7
RGN- 145	40	45.5	5.3	67	39.45
NPJ- 113	23.1	61.9	92	69.4	61.6
BBS- 1	11.66	55	96.19	93	63.962
YID- 1	21	10.8	48	36.3	29.025
T- 9	8.4	11.2	62	95.3	44.225
MEAN	31.51	61.52	63.79	73.9	57.68
CD (p=0.05)					
DOS	0.65				
Variety	0.1				
DOS X Variety	0.217				

DOS: date of sowing; D1:19.9.2011; D2:12.10.2011; D3:2.11.2011; D4:22.11.11



Error bar indicates standard error

Fig. 7. Effect of date of sowing on catalase activity (change in OD value per min. X 103) of seeds of rapeseed -mustard varieties

respectively) (Table 5). The results clearly indicated that temperature conditions in the growing period of plant particularly during the seed filling and maturity stage changes the enzymatic activities of seeds leading to differences in seed quality.

Early sowing of *Brassica* implies is very advantageous but high temperature prevailing at the sowing time imposes severe limitations on early germination pattern and subsequent seedling establishment and yield

of *Brassica juncea*. Similarly, low temperature may also cause lower germination. In our study, lower germination was recorded at 20°C which may be attributed to lower enzyme activity at low temperature. No significant difference in *per cent* germination between seeds harvested from D1 and D2 plots and from D3 and D4 plots was observed thereby indicating that seeds maturing in similar temperature conditions showed similarity in germination. High temperature stress decreased germination by reducing the activities of nitrate reductase and amylase enzymes. Further, the activities of antioxidant enzymes like peroxidase and SOD increased substantially due to high temperature stress, but peroxidase and amylase enzyme activities were important in germination [24]. At lower or optimal temperature conditions, plants produce low levels of peroxidase, in chloroplast, mitochondria and peroxisome. A direct result of stress induced cellular changes is the enhanced accumulation of toxic compounds in cells that induced ROS (reactive oxygen

Table 5. Relationship between temperatures at different crop growing periods with enzyme activity in rapeseed - mustard varieties grown under four sowing dates

Character	Growth stage	Tmax	Tmin	Tav
POX	Reproductive	0.338*	0.284NS	0.380*
	Seed filling and maturity	0.434**	0.444**	0.439**
catalase	Reproductive	-0.497**	-0.499**	-0.472**
	Seed filling and maturity	0.419**	0.470**	0.443**

Tmax: Av. of maximum temperature; T min: Av. of minimum temperature; Tav: Average of average temperature; POX: peroxidase

species) [15]. These ROS *viz.*, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O) together constitute the oxidative stress. These overproduced ROS under stress conditions react directly with lipids, proteins and nucleic acids and cause lipid peroxidation mediated membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands [25]. Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non enzymatic defense system. When ROS increases, chain reactions start in which superoxide dismutase (SOD), a metallo enzyme catalyses the dismutation of O_2^- radical to molecular O_2 and H_2O_2 . The H_2O_2 is then detoxified either by catalase/peroxidase or via some other pathway. Catalase (CAT) reduces H_2O_2 into H_2O and O_2 , whereas peroxidase (POX) decomposes H_2O_2 by oxidation of co-substrate such as phenolic compounds. The equilibrium between the production and scavenging of ROS may be perturbed under adverse abiotic stresses thereby results into reduction in crop yield. However, the extent to which heat stress occurs in specific climatic zones is a complex issue. It was suggested that sensitivity of photosynthesis to heat may be mainly due to damage to components of PS-II [26-27].

Also, seed maturing at optimal environmental condition possess better and balanced seed composition as it did not face any stress at seed filling. In our study, it was

found that seeds maturing at relatively lower temperature gave maximum germination at 25-30°C.

In conclusion, rapeseed-mustard varieties being sown in different temperature conditions and seeds harvested at lower and higher temperature conditions indicated a high influence of temperature during growing period, particularly at seed filling and maturity period on the seed germination and enzyme activities. Delay in sowing beyond first week of November deteriorated seed quality directly. Above results suggest that the best quality seeds of early maturing *B. juncea* varieties can be obtained by sowing during third week of September to first week of October in Delhi condition.

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