

Full Length Research Paper

Effect of environmental factors on hybrid seed quality of Indian mustard (*Brassica juncea*)

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The trinity of parental line superiority, climatic conditions during crop growth and effective cross-pollination is the decisive factor to reap best quality hybrid seed. In order to study the effect of environmental factors during sowing time on hybrid mustard seed quality, freshly harvested hybrid seeds from three plots sown on 21st October (D1), 30th October (D2) and 18th November (D3), 2009-2010 were tested for quality traits before and after nine months of ambient storage. Percent germination had strong correlation with T_{max} at vegetative ($r^2=0.881$) and seed filling stage ($r^2=0.88$). Sunshine hours at vegetative and seed filling stage had significant correlation with percent germination (0.957 and -0.957 respectively), shoot length (0.898 and -0.870 respectively) and electrical conductivity of seed leachate after accelerated ageing (-0.880 and 0.856 respectively). No significant difference for germination and vigour indices was found between 1st (D1) and 2nd (D2) dates of sowing as the weather conditions during these two sowing periods was favourable and did not fluctuate much. Percent decrease in germination from D1 to D2 was 0.83/°C temperature decrease at vegetative stage and 1.33/°C temperature increase in seed filling stage. But percent decrease in germination from D2 to D3 was 2.31/°C temperature decrease at vegetative stage and 3.66/°C temperature increase in seed filling stage. A predictive model was developed as- $Y = 234.545 - 1.688 \times (\pm 1.013) \times RH_{pf} - 15.359 (\pm 1.335) \times SH_{sf}$, where, Y= Percent germination, RH_{pf} = relative humidity at peak flowering stage and SH_{sf} = sunshine hours at seed filling stage. Seeds were subjected to accelerated ageing followed by electrical conductivity test after storage. D3 seeds had minimum germination percentage at 96 h ageing (5.1); significantly lower than that from D1 seeds (52.4). Grow out test result suggested standard isolation distance may be reconsidered to check out-crossing in optimum date of sowing. Seeds harvested from 21st October sowing (optimum time of sowing) gave the best result regarding seed quality parameters than that in hybrid seeds from 30th October and 18th November sowings.

Key words: Indian mustard, hybrid seed quality, environmental factors, percent germination, predictive model, correlation coefficient.

INTRODUCTION

Indian mustard (*Brassica juncea*) is an important oilseed crop in India, China and in south-western areas of the former Soviet Union. Indian mustard is cultivated

worldwide as a vegetable, condiment and for oilseed. Due to its relatively greater tolerance to drought and heat that is, abiotic stress, pod shattering and blackleg

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(*Leptosphaeria maculans*) than that of *B. napus*/*B. rapa*, interest in growing Indian mustard (*B. juncea*) as an alternative to canola (*B. napus*) has recently increased worldwide (Anonymous, 2005; Banga et al., 2013). *B. juncea* is widely used for vegetable production particularly in Asia. Recently, it is being cultivated for biodiesel production. Since varieties with higher yield and enhanced oil content are becoming available through conventional breeding programs, it has found its growing way. In a significant effort, Directorate of Rapeseed-Mustard Research, Bharatpur, India successfully developed the first ever hybrid (released in 2008), NRC Sankar Sarson (NRCHB 506) of *Brassica juncea* (Indian mustard) through heterosis breeding using *Moricandia* CGMS system for cultivation primarily in Rajasthan and U.P., India to mitigate the ever-increasing demand of oilseed. This hybrid is of medium maturity duration, medium tall with high oil content and tolerant against diseases and pests.

How higher the seed yield is, its quality precisely depends on prevailing environmental factors. Environmental variation as a function of planting date is an important factor which significantly affects the timing and duration of the vegetative and reproductive periods as well as yield, its attributes and seed quality (Bhuiyan et al., 2008; Dornbos, 2002). Temperature, effective sunshine hours and light intensity differ with varying planting dates. Early date of planting results in significant increase in vegetative growth and produces more pods per plant consequently increases yield and quality of seeds. Delay in planting after October results in drastic reduction in seed quality (Thakur and Singh, 1998).

On the other hand, after harvest, seeds also deteriorate during prolonged storage and gradually lose viability (McDonald, 1999). Accelerated ageing test can give a simulated estimation of natural ageing. Its effect is manifested as reduction in percentage germination and if it germinates, produce weak seedling as in of case of natural ageing (Veselova and Veselovasky, 2003). Seed germination of the sunflower lines submitted to accelerated aging for three days was approximately equal to the seed germination rate measured after 12 months of natural aging ($R^2=0.93$). The soybean seed subjected to extreme conditions of accelerated aging for five days suffered stress that was comparable to that suffered by 12 months of natural aging (Balešević-Tubić et al., 2010).

A few literatures are available regarding storage life of mustard varieties. Devi and Dadlani (2003) reported that mustard cv. Pusa Bold can last up to thirty one months in storage, in Delhi. Sharma and Singh (1997) suggested twelve months for cv. varuna in Palampur, Himachal Pradesh. Teari et al. (1998) reported eighteen months for cv. varuna and vardan in central plain zone of Uttar Pradesh. Kurdikeri et al. (2000) for thirteen months in Dharwad and Verma et al. (2003) reported three years for mustard variety RH-8113.

Some preliminary studies have been conducted to assess the effect of environmental factors during crop growth period on subsequent seed quality. But till now, limited information is available regarding hybrid seed quality and how the environmental factors influence the hybrid seed storability. The most marked and maximum values of seed storability expressed through percentage germination, shoot and root length, germination after ageing, electrical conductivity etc. differ in response to different planting dates (Veselova and Veselovasky, 2003). Hence, the objective of our experiment was to study the effect of different sowing dates and weather conditions on hybrid seed quality of first hybrid of Indian mustard (*Brassica juncea* (L.) Czern. and Coss.) and to compare the quality of hybrid seeds with that of an open pollinated variety, Pusa Bold.

MATERIALS AND METHODS

The study area is situated at 28.38° N, 77.20° E and with an altitude of 228.7 m above the mean sea level in the IARI Research Farm, New Delhi, India. The climate of the area is semiarid and sub-tropical with hot summer and cool winters. The mean monthly maximum and minimum temperature during the year ranges from 21.3 to 40.5°C and 7.3 to 28.7°C, respectively. The annual rainfall is 708.6 mm of which on an average 597 mm (84%) is received during the month from June to September. The female (MJA5) and male line (MJR1) seeds of the hybrid (NRCHB 506) were sown with a planting ratio of 16:2 (A: R) in plots with sufficient soil moisture (1 week after irrigation), in three different dates that is, 21st of October (D1), 30th of October (D2) and 18th of November (D3) in 2009 and was harvested in 3rd and 4th week of March and 1st week of April respectively. The experiment was laid out in a Randomized Block Design (RBD) with three replications each with three sets of plots. Weather data were recorded during the growing season from the agro-met observatory under Division of Agricultural Physics, Indian Agricultural Research Institute, New Delhi and have been presented in Figure 1.

Seed quality parameters were tested soon after harvest and after nine months of ambient storage. Seed moisture content (on fresh wet basis) was measured using an air- oven method at 103°C for 17 h, a thousand seed were weighed at 5-6% seed moisture content and germination test was conducted in a laboratory using paper methods in 8 replicates of 50 seeds following top of paper method at 20°C (ISTA, 2008). Seedling vigour was evaluated by taking seedling length (in cm for Vigour index I) and dry weight (in mg for Vigour index II after drying at 90°C for 18-24 h) of ten randomly selected 7-day old normal seedlings from each replication (Abdul-Baki and Anderson, 1972). According to available literature, effect of accelerated ageing for three consecutive days at around 41°C and 100% relative humidity (RH) gives same average effect of one year of ambient storage. Hence, accelerated ageing was achieved by treating the seeds at 41°C and ~ 100% RH for 24 h, 48 h, 72 h and 96 h followed by standard germination test (ISTA, 2008). The hybrid seeds collected from the female plants in each date of sowing were sown with at least 400 plants in 2010-2011 for grow out test. An authentic sample of NRCHB 506 was grown for comparison. Plants with male fertility were counted as pure and were compared with the authentic hybrid seed sample to check genetic purity. All the data were analysed using SAS (SAS Institute Inc., 1989). The model on germination and environmental factors was developed by SPSS software based on stepwise regression. Stepwise regression includes regression models in which the

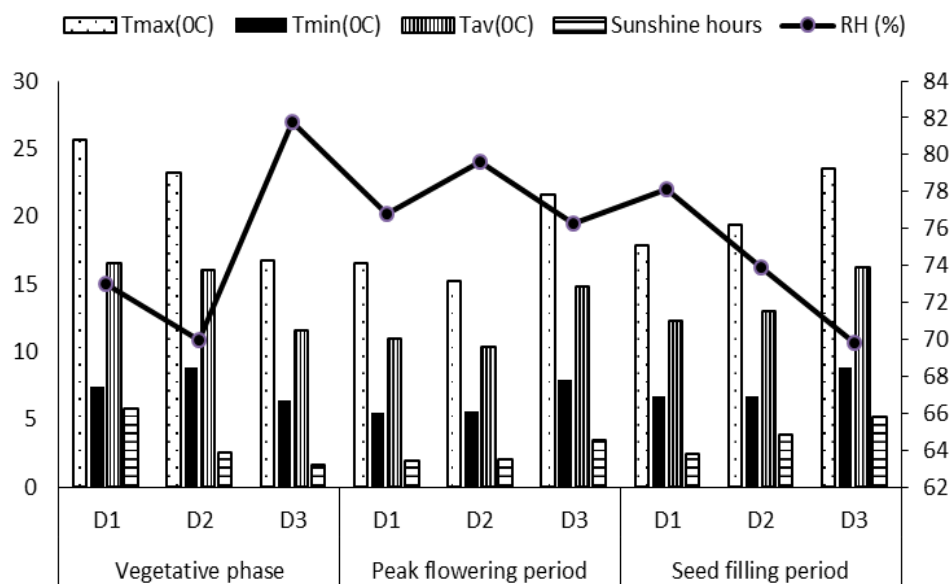


Figure 1. Weather data during crop growth period (winter season, 2009-10), RH (%) is presented by secondary axis.

Table 1. Effect of date of sowing on seed quality before ambient storage.

Quality parameters	21 st Oct, 2009 (D1)	30 th Oct, 2009 (D2)	18 th Nov, 2009 (D3)
Off-type % in Grow out test	15.5	12.5	9.0
Moisture (%)	6.6 ^a	6.9 ^a	8.7 ^b
Germination (%)	96.7 ^a	94.7 ^a	79.7 ^b
Vigour Index I	1357 ^a	1343 ^a	1071.7 ^b
Vigour Index II	480 ^a	512 ^a	394 ^b
Test weight(g)	6.2 ^{ns}	5.7 ^{ns}	5.6 ^{ns}

Alphabets in each cell represent their correspondent group by CD value in all the tables; a, b different group at $p < 0.05$; ns - non significant, D1= 21st October sowing; D2= 30th October sowing; D3= 18th November sowing.

choice of predictive variables is carried out by an automatic procedure.

RESULTS

Hybrid seed purity and quality

Results on grow out test showed non-significant difference for plant morphological characters among the seeds harvested from plants grown under different dates of sowing. Percent off-type plants from seeds produced under different dates of sowing had substantial difference, among these D1 maximum (15.5%) off-type plants in grow out test (Table 1).

Percent germination and vigour index I and II significantly reduced in hybrid seeds produced in D3 (79.7, 1071.7 and 394, respectively) as compared to those in 1st and 2nd dates of sowing (Table 1). Weather parameters had significant correlation with seed quality

traits. Weather conditions on D1, D2 and D3 have been presented in Figure 1. In case of D1 sown crop, T_{max} was 25.6°C, 16.5°C and 17.9°C at vegetative phase, peak flowering period and seed filling period respectively. In D2 T_{max} was 23.2°C, 15.2°C and 19.4°C and in D3 16.7°C, 21.6°C and 23.5°C at those stages. T_{max} at vegetative stage of D1, D2 and D3 were 16.5°C, 15.6°C and 17.9°C respectively. T_{max} and T_{min} at vegetative phase showed significant correlation with quality traits in almost all the cases (Table 6). Percent germination had strong correlation with T_{max} at vegetative ($r^2=0.881$) and seed filling stages ($r^2=0.88$). Sunshine hours at vegetative and seed filling stages had significant correlation with percent germination (0.957 and -0.957 respectively), shoot length (0.898 and -0.870 respectively) and solute leakage (-0.880 and 0.856 respectively). No significant difference for germination and vigour indices was found between 1st (D1) and 2nd (D2) dates of sowing as the weather conditions during

Table 2. Effect of accelerated aging on percent germination of harvested seeds from different dates of sowing

Accelerated ageing period (h)	Germination percentage			
	(D1)	(D2)	(D3)	Pusa Bold
Control	76.9(61.3)	66.0(54.5)	57.9(49.6)	78.7(62.5)
24	72.1(58.1)	53.7(47.2)	37.4(37.4)	70.5(57.2)
48	67.6(55.4)	38.9(38.5)	20.2(26.4)	59.6(50.6)
72	63.4(52.8)	28.7(32.1)	8.7(17.1)	42.6(40.6)
96	52.4(46.4)	13.9(21.9)	5.1(13.0)	20.7(27.0)
Mean	66.5	40.2	25.9	54.4
	AA period	DOS	Interaction	
CD at 5%	2.5	2.3	5.0	

Values in parentheses are arc sine conversion value; D1 = 21st October sowing; D2= 30th October sowing; D3 = 18th November sowing.

Table 3. Effect of dates of sowing on shoot length (mm) after accelerated ageing.

Accelerated ageing period (h)	Shoot length (mm)			
	(D1)	(D2)	(D3)	Pusa Bold
Control	51.0	43.9	38.6	74.8
24	42.0	29.4	25.0	55.6
48	32.8	20.0	16.5	27.2
72	23.9	15.6	11.4	17.4
96	20.2	10.5	10.3	17.1
Mean	34.0	23.9	20.4	38.4
	AA period	DOS	Interaction	
CD at 5%	3.2	2.9	6.4	

D1= 21st October sowing; D2= 30th October sowing; D3= 18th November sowing.

these two sowing periods were favourable and did not fluctuate much (Figure 1). Percent decrease in germination from D1 to D2 was 0.83°C temperature decrease at vegetative stage and 1.33°C temperature increase in seed filling stage. But percent decrease in germination from D2 to D3 was 2.31°C temperature decrease at vegetative stage and 3.66°C temperature increase in seed filling stage. A predictive model by stepwise regression was developed on the basis of predictive correlation coefficient. Stepwise regression includes regression models in which the choice of predictive variables is carried out by an automatic procedure.

$$Y = 234.545 - 1.688 \times (\pm 1.013) \times RH_{pf} - 15.359 (\pm 1.335) \times SH_{sf}$$

$$N = 24, r = 0.968, r^2 = 0.936, F = 66.254, SE = 24.$$

Where, Y = Percent germination, RH_{pf} = Relative humidity at peak flowering stage, SH_{sf} = Sunshine hours at seed filling stage, N = sample size, r = correlation coefficient, SE = standard error of estimate, F = ratio of correlation. Other environmental predictive variables except RH_{pf} and

SH_{sf} were automatically excluded from the model. Both RH_{pf} and SH_{sf} were negatively correlated with percent germination.

Accelerated ageing

Analysis of variance showed that effect of ageing period on all germination traits was significant. Interactions between ageing period and hybrid seeds were significant for all assessed traits. Mean comparison for percentage germination showed that hybrid D3 had minimum percentage germination at 96 h ageing (5.1), whereas maximum percentage germination was recorded from control seeds of *Pusa Bold* (78.7) followed by D1 (76.9) (Table 2). Evaluation of shoot length of D1, D2, D3 and *Pusa Bold* seeds under different ageing periods followed similar trend, that is, hybrid seeds which had high quality at the beginning of test (control) could maintain this superiority during the different ageing periods (Table 3). Analysing the effect of ageing period on root length (Table 4) showed that during all ageing periods D1 had the longest root (21.3 mm) after 96 h of ageing, while the

Table 4. Effect of dates of sowing on root length (mm) after Accelerated ageing

Accelerated ageing period (h)	Root length (mm)			
	(D1)	(D2)	(D3)	Pusa Bold
Control	64.9	61.0	52.9	60.6
24	48.9	48.6	49.2	41.7
48	43.2	33.0	31.3	30.6
72	28.0	15.4	19.5	19.6
96	21.3	10.4	8.2	15.0
Mean	41.3	33.7	32.2	33.5
	AA period	DOS	Interaction	
CD at 5%	2.9	2.6	5.8	

D1= 21st October sowing; D2= 30th October sowing; D3= 18th November sowing.

Table 5. Effect of dates of sowing on electrical conductivity of seed leachate after Accelerated ageing

Accelerated ageing period (h)	Electrical conductivity (dS/m)			
	(D1)	(D2)	(D3)	Pusa Bold
Control	21.6	29.0	33.5	25.1
24	22.7	32.1	42.6	27.9
48	25.5	37.3	46.8	30.7
72	27.3	44.5	51.3	35.9
96	28.7	60.1	56.8	34.4
Mean	25.2	40.6	46.2	
	AA period	DOS	Interaction	
CD at 5%	4.1	3.7	8.3	

D1= 21st October sowing; D2= 30th October sowing; D3= 18th November sowing.

shortest root length (8.2 mm) was observed in D3. *Pusa bold* had longest shoot followed by D1 during the prolonged ageing periods, but had shorter root than that from seeds of D1.

Electrical conductivity (EC)

Results of EC test showed that maximum solute leakage of the hybrid seeds was recorded for D3 and also revealed that ageing periods lower than 72 h were less efficient to separate hybrid seeds into different vigour levels (Table 5).

DISCUSSION

In later dates of planting, unavailability of compatible pollen source might have limited the extent of outcrossing resulting in better hybrid purity. Tunwar and Singh (1988) in Indian Minimum Seed Certification Standard, suggested the standard isolation distance as 25 m in self-compatible species and 50 m in self-incompatible species

for certified seed production. Some sporadic research results are available suggesting reconsideration of isolation distance for multiplication of hybrid parental lines, but no research is available in hybrid seed production aspect of Indian mustard. In optimum sowing date, minimum isolation distance for certification may certainly be revisited to check out-crossing.

The rate of reduction in seed germination and vigour varied with the different sowing seasons which is associated with changes in temperature and relative humidity conditions during the vegetative, reproductive and harvest stages and this is supported by Tekrony and Egly, 1980. The behaviour of seeds, in terms of percentage of normal seedlings in the germination and accelerated aging tests, as a function of sowing time for hybrid and cropping season, may be a result of physiological quality of seed (Ávila et al., 2003) at the time of harvest.

Mustard, like other angiospermous seeds, when approaching maturity, characteristically accumulates soluble sugars (Amuti and Pollard, 1977) through starch synthase activity and a wide range of Late Embryogenesis Abundance (LEA) proteins. These

Table 6. Correlation of seed quality traits and weather parameters.

Weather parameter	Seed quality traits				Weather parameter	Seed quality traits			
Tmax (°C) vg	Germination %	Shoot length (mm)	Root length (9mm)	Solute leakage (dS/m)	RH (%) pf	Germination %	Shoot length (mm)	Root length (9 mm)	Solute leakage (dS/m)
Tmin (°C) vg	0.881**	0.774**	0.562	-0.763**	Sunshine hours pf	-0.025	-0.118	-0.158	0.108
Tav(°C) vg	0.250	0.138	0.040	-0.144	Tmax(°C) sf	-0.782**	-0.665*	-0.467	0.658*
RH (%) vg	0.800**	0.685*	0.484	-0.677*	Tmin(°C) sf	-0.880**	-0.773**	-0.561	0.762**
Sunshine hours vg	-0.573	-0.452	-0.290	0.450	Tav(°C) sf	-0.746**	-0.628*	-0.436	0.622*
Tmax(°C) pf	0.957**	0.898**	0.694*	-0.880**	RH (%) sf	-0.839**	-0.727**	-0.521	0.718**
Tmin(°C) pf	-0.614*	-0.493	-0.323	0.490	Sunshine hours sf	0.955**	0.867**	0.650*	-0.852**
Tav(°C) pf	-0.768**	-0.651*	-0.455	0.645*					

Vg = at vegetative stage; pf= at peak flowering stage; sf= at seed filling stage. *significant at (p=0.05); ** significant at (p=0.01).

solutes, enzymes and proteins are known to contribute to the development of grain followed by desiccation tolerance and longevity (Bernal-Lugo and Leopold, 1995). Seed quality is a genetically inherited trait whose intensity is modified by the environment during seed development and maturation. In this study, adverse weather condition during physiological maturity had strong correlation with seed quality parameters (Table 6), hence hampering this processes and conferring more susceptibility to ageing.

The result of accelerated ageing test could be attributed to differential sensitivity of the early growth traits against ageing treatments, the alternation in climatic conditions during entire growing period. As per knowledge, Indian mustard needs higher temperature during vegetative phase and lower temperature during reproductive phase. But in our case the condition was altered. Results of ageing periods on seedling vigour index and percentage germination showed same trend that is, seeds produced in D1 and D2 were better than that in D3. This test classified hybrid

seeds produced in different time of sowing with the best and worst performance and evaluated the seeds vigour and their storability. Therefore, it was observed that D1 had high quality and was resistant against the deterioration, whereas D3 was identified as having a lower physiological potential. However, compared to open pollinated variety *Pusa Bold*, D1 performed worse.

Ripen mustard seed varies in moisture and oil content and this influences how good a seed lot can store. Decrease in oil content with increase in storage period has been reported in mustard and the decrease in oil content has been related to peroxidation of lipid by peroxidase, non-enzymatic lipid autooxidation and also to fungi invasion (Verma et al., 2003). The protective effect of these poly-ols is thought to occur through maintaining the structural integrity of membranes, and providing stability for macromolecules such as proteins (Crowe and Crowe, 1986). Through much of the literature there is an assumption that oxidative reactions are responsible for the deteriorative changes observed in aged seeds.

Four types of oxidations are known which might reasonably contribute to the progress of seed ageing. These include free radical oxidations, enzymic dehydrogenation, aldehyde oxidation of proteins, and Maillard reaction (Bernal-Lugo and Leopold, 1998). Free radicals attack membrane lipids, and cause major disruption of their viscosity and their permeability. Increase of solute leakage at this situation would be due to damaged membrane (Gupta and Aneja, 2004). In addition, as water is withdrawn from a solution of sugars form a glassy state, this can serve as a physical stabilizer. Viscosity obtained in the glassy state, suppresses deteriorative reactions. Different responses of hybrid seeds to accelerated ageing treatments are explained by their differential sensitivity to environmental factors during seed filling period. These chains of interlinked physiological phenomenon are subject to environmental variation and more pronounced in hybrid seeds produced under unfavourable condition than that in case of open pollinated varieties.

In this study, the 72 and 96 h periods had a higher sensitivity in identifying the optimum sowing time to produce hybrid seeds that possessed different levels of physiological potential. Seeds harvested from 21st October sowing (optimum time of sowing) gave the best result regarding seed quality than that in hybrid seeds from 30th October and 18th November sowings in Delhi and adjoining areas.

The effect of climatic variables on the parental lines during hybrid seed production and subsequently on seed quality with respect to its regeneration has not been well studied in Indian mustard. Alarming investigations are being reported now-a-days in this regard. In the changing climatic era, this type of studies is highly needed to adopt some mitigation strategies to supply good quality seeds to the farmers.

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