Indian Journal of Agricultural Sciences **92** (7): 882–5, July 2022/Article
https://doi.org/10.56093/ijas.v92i7.113603

Biochemical responses to natural and accelerated ageing on seed quality in Indian mustard (*Brassica juncea*)

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Received: 06 August 2021; Accepted: 06 September 2021

ABSTRACT

Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] is an important oilseed crop of many tropical and sub-tropical countries of the world. The present study was carried out at research farm of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during winter (*rabi*) season 2017–18 and 2018–19. The objective of present study was to evaluate the effect of natural and accelerated aging on biochemical constitutes in 3 Indian mustard varieties, viz. RH-30, RH-9304 and RH-0406 with three seed lots (fresh seed, 1-year old seed, 2-year-old seed). Naturally aged seed lots of variety RH-30 was found to meet out Indian Minimum Seed Certification Standard after 1-year under ambient condition. Tetrazolium test indicated that the viability of Indian mustard seeds decreased after increasing the ageing duration. Maximum seed viability recorded in the variety RH-30 and minimum was recorded in variety RH-0406. Activities of all the enzymes, viz. superoxide dismutase, dehydrogenase and peroxidase significantly decreased with natural ageing; RH-30 was found to be a good storer variety. The maximum performance for standard germination and enzymes activity was observed in fresh seed lots. Results showed that among different Indian mustard varieties, RH-30 and RH-9304 were found best responsive with minimal seed deterioration effects, and better germination and seedling establishment potential.

Keywords: Accelerated aging, Mustard, Quality, Superoxide Dismutase, Viability, Vigor

Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] belongs to the family cruciferae is a perennial herb grown as an annual or biennial throughout India. In India, Brassica oilseed is collectively referred to as rapeseed and mustard, which is the most important *rabi* oilseed crop and occupies an important position in the rainfed agriculture. In India, Central Organization for Oil Industry and Trade (COOIT) has estimated 89.5 lakh tonnes of mustard seed to be produced in the country in the ongoing season of 2020–21 against 75 lakh tonnes recorded last year, which brings a growth of about 19% over last year. Among the major oilseed producing states, Haryana has the highest yield (1533 kg/ha) followed by Rajasthan (1170 kg/ha) and Uttar Pradesh (1121 kg/ha) during 2020–21 (Singh and Bansal 2020). The crop is globally gaining importance relative to other crops due to higher oil content in the seed along with higher yield potential and highly remunerative. Seed quality plays an important role in the agricultural production as well as in national economy (Punia *et al*. 2019, 2021a,b,c). Therefore, good quality seed is necessary to enhance the production and productivity. Biochemical traits, genetic structure and environmental condition during seed development mainly affect the seed quality (Punia *et al.* 2021). Seed being a living entity, deterioration in its quality occurs with the advancement in ageing which is natural, inevitable, irreversible and continuous process (Ghasemi-golezani *et al*. 2010). Seed deterioration is related to loss of membrane integrity, changes in enzymatic activities, reduction in protein and nucleic acid synthesis, and lesions in DNA. Deteriorative change is a reverse correlation between antioxidant and lipid peroxidation. Thus, aim of the present study was to assess the effect of natural ageing on biochemical traits on seed quality of Indian mustard.

MATERIALS AND METHODS

Plant material and growth conditions: The present study was carried out at research farm of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during winter (*rabi*) season 2017–18 and 2018–19. The seeds of 3 Indian mustard varieties, viz. RH-30, RH-9304 and RH-0406 were procured from Oil Seed Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. All 3 seed lots of each variety were laid out in factorial Complete Randomized Design (CRD) for laboratory studies and factorial Randomized Block Design (RBD) for field studies. The observations were recorded at every six months interval up to two year on the above seed lots.

The tetrazolium viability test (Moore 1973) based

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on three replication of 100-seeds each was followed. The accelerated aging test was performed for germination according to ISTA (1999) and expressed in percentages. The electrical conductivity of the seed leachates was measured from each seed lots and was expressed as dS/cm/seed. Peroxidase activity (POX) was determined by the method of Shannon *et al.* (1966), following the oxidation of o-dianisidine in the presence of hydrogen peroxide (H_2O_2). DHA test was performed as per Kittock and Law (1968) by monitoring the formation of formazan and absorbance was read at 480 nm and expressed as change in OD/g/ml. Superoxide dismutase activity (SOD) activity was assayed by the method of Giannopolitis and Ries (1977) with minor modification by the photochemical reduction of NBT.

The statistical analysis was carried out for each observed character and the mean values of data were subjected to analysis of variance as described by Sheoran (2006) for Factorial Randomized Block Design (RBD) and factorial Completely Randomized Design (CRD) through online (*http://www.hau.ernet.in/opstat.html.*) statistical tool.

RESULTS AND DISCUSSION

Tetrazolium salts stains all living embryo red, thus enabling trained researchers to determine the seed viability. The present investigation resulted that the viability of Indian mustard seeds decreased after increasing the ageing duration. Maximum seed viability (90.78, 84.22, 76.44 and 65.33%) recorded in the variety RH-30 and minimum (78.11, 70.00, 60.33 and 47.56%) seed viability was recorded in variety RH-0406 during the year 2015–16 and 2016–17. Significantly, maximum seed viability (92.89, 88.22, 84.22 and 78.44%) was recorded with the seed lot L_1 while, minimum seed viability (73.33, 62.78, 46.22 and 27.11%) was reported in seed lot L_3 during the period of study, respectively. Seeds lost their viability during storage, due to the limited water uptake during imbibition (Balesevic Tubic *et al*. 2007).

Accelerated ageing of seeds is an important method to regulate vigour and viability changes during seed storage conditions (Tian *et al*. 2008). The stress condition was provided by the artificial ageing for 72 h in accelerated ageing chamber and data on per cent normal seedling was recorded (Table 1). At initial months of storage, significantly maximum germination (68.56%), then after six months (54.89%), successively after 12 months (45.33%), then after 18 months of storage (31.89%) was recorded in freshly harvested seed lot L_1 which were freshly harvested in 2015–16, while minimum (0.11%) was reported in three year and six months old seed lot L_3 which were harvested in 2013–14 during the period of study. Similar findings were also observed in chickpea by Kapoor *et al*. (2010); in maize by Hussein *et al*. (2012); in bitter gourd by Kanwar *et al*. (2014); in coriander by Kumar *et al*. (2017) and in sesame by Kavitha *et al*. (2017).

The permeability of membrane has been reported to increase upon seed storage which is expressed by increased loss of electrolytes. The electrical conductivity (Table 2) was

Table 1 Effect of naturally aged seed lots on accelerated ageing test (%) of Indian mustard

Year	2017–18								2018–19							
	0 month				After 6 months				After 12 months				After 18 months			
Seed lot	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean
Variety																
RH-30	75.33	49.33	23.67	49.44	61.00	37.33	11.00	36.44	50.67	24.33	1.33	25.44	36.00	9.33	0.33	15.22
RH-9304	68.33	44.33	19.67	44.11	53.67	31.67	7.67	31.00	44.67	19.00	1.00	21.56	32.33	6.00	0.00	12.78
RH-0406	62.00	41.67	16.67	40.11	50.00	25.67	5.00	26.89	40.67	14.67	0.33	18.56	27.33	4.33	0.00	10.56
Mean	68.56	45.11	20.00		54.89	31.56	7.89		45.33	19.33	0.89		31.89	6.56	0.11	

CD at 5% level of significance; M = 0.82; L = 0.71; M × L = 1.42; V = 0.71; M × V = N/A; L × V = N/A; M × L × V = N/A.

*Figures in the parenthesis are arc sine transformed; L_1, freshly harvested seed lot; L_2, one year old seed lot; L_3, three year and six month old seed lot.

Table 2 Effect of naturally aged seed lots on electrical conductivity of Indian mustard

Year	2017–18								2018–19							
	0 month				After 6 months				After 12 months				After 18 months			
Seed lot	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean	L_1	L_2	L_3	Mean
Variety																
RH-30	0.135	0.355	0.728	0.41	0.265	0.506	0.896	0.55	0.362	0.722	1.045	0.71	0.509	0.892	1.265	0.89
RH-9304	0.216	0.446	0.816	0.49	0.315	0.607	0.989	0.64	0.443	0.823	1.188	0.81	0.599	0.988	1.424	1.00
RH-0406	0.279	0.560	0.903	0.58	0.398	0.725	1.086	0.74	0.572	0.908	1.328	0.94	0.723	1.089	1.607	1.14
Mean	0.21	0.45	0.82		0.33	0.61	0.99		0.459	0.818	1.187		0.610	0.990	1.432	

CD at 5% level of significance; M = 0.003; L = 0.002; M × L = 0.005; V = 0.002; M × V = 0.005; L × V = 0.004; M × L × V = 0.008.
*L_1, freshly harvested seed lot; L_2, one year old seed lot; L_3, three year and six month old seed lot.

found higher in RH-0406 followed by RH-9304 and lower electrical conductivity was recorded in the variety RH-30 during the period of study. Maximum value of electrical conductivity was observed in seed lot L_3 followed by lot L_2 and minimum value of electrical conductivity in seed lot L_1 during the year 2017–18 and 2018–19, respectively. Similar findings were recorded in Indian mustard by Rai *et al*. (2007); in karanj by Kumar *et al*. (2011); in coriander by Kumar *et al*. (2017).

Peroxidase and superoxide dismutase activity (mg/protein/min) decreased as the advancement of natural ageing in all the three varieties and seed lots during the period of study. Among the different varieties, maximum peroxidase and superoxide dismutase activity was observed in RH-30 and minimum in the variety RH-0406. Highest value of peroxidase (0.455, 0.365, 0.282 and 0.223) and superoxide dismutase activity (0.115, 0.102, 0.089 and 0.074) with the seed lot L_1 and lowest value for peroxidase (0.183, 0.127, 0.072 and 0.030) and superoxide dismutase activity (0.067, 0.047, 0.033 and 0.023) with the seed lot L_3 were recorded during the year 2017–18 and 2018–19, respectively. Loycrajjou *et al*. (2008) suggested that ageing induced deterioration, increase the extent of protein oxidation, thus inducing the loss of functional properties of protein and enzymes. These results are also in accordance with the finding of Bhanuprakash *et al*. (2010) in bell pepper; Demirkaya and Sivritepe (2011) in onion; Singh *et al*. (2015) in fenugreek; Rai *et al*. (2017) in Indian mustard and Punia *et al*. (2021b,c) in sorghum.

Effect of naturally aged seed lots on enzyme activity (POX and SOD) in Indian mustard: The range of optical density of formazan was estimated with the help of spectrophotometer. The ageing periods significantly affect the intensity of formazan at initial months of storage then after six months. Among the different varieties, maximum formazan (0.676) was recorded in the variety RH-30 while minimum (0.321) was recorded in RH-0406. The maximum reduction in dehydrogenase activity (0.483) was recorded in variety RH-0406 while minimum (0.388) was observed in variety RH-30 during the period of study. Similar results had also been showed in karanj by Kumar *et al*. (2011); in wheat by Chauhan *et al*. (2011) and by Rai *et al*. (2017) in Indian mustard.

This study explores the impact of seed ageing on the germination and biochemistry of seed and concludes that seed age is negatively correlated with these quality parameters except for EC with which it has a positive correlation. Free radicals in the ageing seeds are responsible for the deterioration of seed membrane, which leads to an increase in seed leachates and consequently EC. In the event of scarcity of antioxidant enzymes, namely DHA, POD and SOD, these free radicals lead to a decline in the seed viability in the ageing seed. This study extends our understanding of the role of antioxidant enzymes in seed ageing as well as their influence on seed physiology in Indian mustard, the aspects which were thoroughly explored only in case of seeds of field crops.

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