Indian Journal of Agricultural Sciences **91** (5): 718–22, May 2021/Article
https://doi.org/10.56093/ijas.v91i5.113070

Seed longevity and vigour studies in different types of Indian mustard

ARCHANA H R[1], SANGITA YADAV[1], SHIV K YADAV[1]*, NAVINDER SAINI[1], ANIL DAHUJA[1], SUJATA VASUDEV[1], RAVISH CHOUDHARY[1] and D K YADAVA[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 26 July 2020; Accepted: 16 December 2020

ABSTRACT

An experiment was conducted at ICAR-Indian Agricultural Research Institute, New Delhi (2017-18) to study the possible reason for reduced seed vigour in Indian quality mustard genotypes during storage for the first time. Freshly harvested seeds, one year, two year aged seeds and controlled deteriorated seeds of freshly harvested seeds of conventional and quality Indian mustard were used for the study. Reactive oxygen species (ROS), i.e. hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$**)** were estimated along with enzymatic antioxidants such as catalase and peroxidase and redox couples ratio (ASC/DHA and GSH/GSSG) in all the nine genotypes of different age group and correlated with germination percentage, mean germination time and seed vigour indices. The experiment revealed that during storage, the amount of ROS accumulation goes on increasing and both enzymatic and non-enzymatic antioxidants goes on decreasing. In comparison to conventional genotypes, quality genotypes were found to contain significantly lower level of antioxidants and significantly higher level of ROS content during the storage period. Germination percentage as well as seed vigour indices were negatively correlated with ROS and positively correlated with enzymatic and non-enzymatic antioxidants. This experiment revealed that reduced vigour in quality genotypes could be due to faster deterioration during the storage due to increased production of ROS.

Keywords: Antioxidants, Canola, Indian mustard, Longevity, ROS, Storage, Vigour

The plant family Brassicaceae is one among the top 10 economically important plant families with about 3500 species and 350 genera (Christopher *et al.* 2005). Among the *Brassica* oilseed species in India, *B. juncea* L. (Indian mustard) occupies around 80% of the production acreage and is predominantly used for consumption in most parts of the country. The major drawback of conventional Indian mustard is high concentration of erucic acid (35-55%) in oil and glucosinolate (49.9–120.3 µmol/g defatted meal) in defatted seed meal. With increasing consumer awareness about these anti-nutritional factors, breeders developed single zero 'canola quality' Indian mustard having low erucic acid content (<2%) and double zero canola quality Indian mustard having both low erucic acid and low glucosinolate content (<30 µmol/g defatted meal) (Yadav *et al.* 2020). Although these quality mustard genotypes are nutritionally enriched they have been reported to suffer from low vigour problem (Biswas *et al.* 2020).

Seed deterioration is an undesirable and detrimental attribute of agriculture which causes loss of around 25% of the harvested crop annually. During seed ageing, loss of seed vigour precedes the loss in germinability (Eksi and Demir 2011). Loss of vigour and viability is accompanied with solute leakage and excessive accumulation of ROS (superoxide radical and H_2O_2) in seeds (Bailly 2011, Swami *et al.* 2016). Protection against ROS is offered by battery of anti-oxidases, such as peroxidase (POD) and catalase (CAT) and their impairment is the key determinants in the loss of vigour in seeds (Sahu *et al.* 2017). Non-enzymatic antioxidants like ascorbic acid (ASC) and glutathione (GSH) can donate electrons to form dehydroascorbic acid (DHA) and glutathione disulphide (GSSG) respectively (Kumari *et al*. 2017). The GSH /GSSG and ASC/DHA are the most abundant redox couple in cells which determines the redox state of the seeds. The present work is the first report that unraveled the potential causes of seed ageing by monitoring the levels of ROS and its detoxifying enzymes to explain the mechanism of seed ageing and loss of seed vigour during storage of Indian quality mustard.

MATERIALS AND METHODS

Experiment in triplicate with nine genotypes of Indian mustard (*B. juncea*) was conducted during 2017-18 at Division of Seed Science and Technology, ICAR-IARI, New Delhi. These included three conventional genotypes; Pusa Vijay, PM 25 and PM 28 and six quality genotypes that were obtained from Division of Genetics, ICAR-IARI, New Delhi. Among quality genotypes, three genotypes each

Present address: [1]ICAR-Indian Agricultural Research Institute, New Delhi. *Correspoding author e-mail: sky_sst@yahoo.com.

were of single zero; Pusa Karishma, PM 24 and PM 30 and double zero; PM 31, PDZ 4 and PDZ 5. Seeds were harvested, cleaned and air dried. The moisture content of dry seeds was 8.5%.

The germination test was evaluated on the 7th day according to the ISTA rule, 2018 and germination percentage was calculated. Seeds with more than 2 mm radicle emergence were counted on every day. Mean germination time (MGT) was calculated using the method of Nicholas and Heydecker (1968). The seed vigour indices were calculated adopting the method of Abdul Baki and Anderson (1973) relative to the control. Freshly harvested seeds were treated with high temperature and humidity. Briefly, seeds moisture content was increased by 20% and were kept overnight in evacuated foil packet at 7°C. These were subjected to high temperature at 45°C for 24 h and dried to bring back to its original moisture content. Seeds stored at room temperature in sealed plastic bag, at dry conditions and used as controlled deteriorated (CD) seeds. The spectrophotometric assay of total superoxide radical content was done following Chaitanya and Naithani (1994), while hydrogen peroxide (H_2O_2) content was estimated by following Teranishi *et al.* (1974) methods. CAT activity was measured spectrophotometrically at room temperature, whilst POD activity was determined using guaiacol as substrate for assay following the procedures as described by Braber (1980) and Castillo *et al.* (1984), respectively. Estimation of Ascorbate (ASA) and Dehydroascorbate (DHA) pool was done by Kampfenkel *et al*. (1995) and estimation of reduced glutathione (GSH) and oxidized glutathione (GSSG) content was measured by Rahman *et al*. (2006) methods. The data was analyzed for test of significance and correlation coefficient between parameters using SPSS 16.0.

RESULTS AND DISCUSSION

Germination percentage (G %): Germination is a complex phenomenon which is affected by both external (water, air, light etc.) and internal factors (viability, vigour etc.). One of the major causes for reduction in germination is seed ageing where deteriorative changes accumulate in the seed (Kumar *et al*. 2019) and it ultimately leads to loss of seed viability. The nine genotypes of Indian mustard belonging to four different age groups showed significant differences between the different age groups as well as different types of Indian mustard in terms of germination percentage and mean germination time. Among different age groups, maximum germination was observed in freshly harvested seeds and it decreased with increase in storage period. Oxidation of saturated and unsaturated fatty acids during ageing may be the possible reason for decrease in germination. It has been reported by (Sahu *et al.* 2017) that oxidation protection system gets disturbed during storage resulting in decreased germination percentage. Among the different types of Indian mustard, both conventional type and single zero genotypes have significantly higher mean germination percentage, i.e. 95.11% and 90.7 % respectively. Significantly lower germination percentage was observed for double zero genotypes (81.5%) of Indian mustard (Table 1).

Mean germination time (MGT): There was no significant difference between single zero and conventional type in mean values of MGT and these were statistically at par (Table 1). But these two were significantly different from double zero type which had significantly higher (1.98) MGT. Over the aging period MGT significantly increased. Higher MGT value of double zero represents a longer pre-radical protrusion period which could be due to less equipped repair mechanism. This suggests that they were affected more during storage which resulted in delayed radicle emergence.

Vigour indices: Among the different storage treatments, in the freshly harvested seeds, significantly higher seed vigour index I and II was observed in all the genotypes and in controlled deteriorated genotypes significantly lower value was observed (Table 2). When different types of mustard were compared, significantly higher values for SVI-I and SVI-II was observed in conventional genotypes and significantly lower values were observed in double zero genotypes and single zero genotypes were found to be intermediate. According to Kumar *et al*. (2018) reduced SVI-I and SVI-II are the indicators of poor storability in soybean. Since double zero genotypes also showed reduced seed vigour indices they can be considered as poor storers. Decrease in seed vigour may be due to the non-enzymatic attack by the reducing sugars (Maillard's reaction) on amino groups of proteins and nucleic acids and resulting in deteriorative changes during storage.

Table 1 Germination percentage (G %) and mean germination time (in days) in fresh and aged seeds of different type of Indian mustard

Age/Types	Freshly harvested		One year aged		Two year aged		Controlled deteriorated		Mean# (Age)	
	G %	MGT	G %	MGT	G %	MGT	G %	MGT	G %	MGT
Conventional	100	1.67	100	1.70	99.5	1.79	80.9	2.03	95.11^{a}	1.80^{b}
Single zero	99.1	1.63	95.7	1.70	92.2	1.89	75.63	1.97	90.7^{b}	1.79^{b}
Double zero	98.4	1.98	83.3	2.06	75.1	2.21	69.2	2.60	81.5^{c}	2.22^{a}
Mean# (Type)	99.2^{a}	1.76^{d}	93.0^{b}	1.82^{c}	88.9^{c}	1.97^{b}	75.3^{d}	2.21^{a}		
CD (P=0.05)	G %	Age = 1.61; Type = 1.26; Type *Age = 2.79								
	MGT	Age = 0.059; Type = 0.048; Type *Age = 0.095								

#Mean values with same alphabet do not differ significantly based on Turkey's Post Hoc test

Table 2 Seed vigour index-I and II of fresh and aged seeds of different type of Indian mustard

Age/Types	Freshly harvested		One year aged		Two year aged		Controlled deteriorated		Mean# (Age)	
	SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II	SVI-I	SVI-II
Conventional	1336	1.53	1250	1.27	984	0.91	524	0.1	1023^{a}	0.95^{a}
Single zero	1177	1.17	971	0.84	870	0.72	461	0.05	870^{b}	0.70^{b}
Double zero	1097	1.14	720	0.46	535	0.38	236	0.03	647^{c}	0.50^{c}
Mean# (Type)	1203^{a}	1.28^{a}	980^{b}	0.86^{b}	796^{c}	0.67^{c}	407^{d}	0.06^{d}		
CD (P=0.05)	SVI-I	Age = 61.1; Type = 52.9; Type *Age = 105.9								
	SVI-II	Age = 0.056; Type = 0.048; Type *Age = 0.095								

#Mean values with same alphabet do not differ significantly based on Turkey's Post Hoc test

SVI-I: Seed vigour index- I and SVI-II: Seed vigour index- II

Reactive oxygen species content: Among the different types of Indian mustard, Hydrogen peroxide (H_2O_2) and Superoxide radical content ($O_2^{\cdot-}$) were significantly higher in double zero genotypes (Fig 1). In both single zero and conventional genotypes, the ROS content was significantly lower. Over the storage period ROS accumulation have increased in all the genotypes. However, the rate of accumulation was higher in double zero genotypes followed by single zero genotypes. Reactive oxygen species that are produced during lipid peroxidation are the major cause of seed ageing (Bailly 2011). Accumulation of H_2O_2 during ageing cause programmed cell death and this could be one of the reasons of reduced vigour (Xin *et al*. 2014). Lower seed vigour indices (Table 2) in double zero genotypes could also be due to increased ROS accumulation as correlation analysis also revealed that ROS are negatively correlated with seed vigour ($O_2^{\cdot-}$: -0.801** and H_2O_2: -0.861**).

Enzymatic antioxidants: On comparison between mean values of different types of IQM, conventional genotypes (0.1017 µM/min/g FW) showed significantly higher catalase activity and significantly lower value was obtained in case of double zero type (0.0559 µM/min/g FW) and single zero type (0.0774 µM/min/g FW) was found to be intermediate. Mean values of peroxidase activity was significantly lower in double zero genotypes (0.118 µM/min/g FW) and significantly higher in conventional genotypes (0.924 µM/min/g FW). Over the storage period both the enzymes were found to be decreasing and least values were observed in controlled deteriorated seeds. These antioxidant enzymes scavenge the ROS and reduce the oxidative stress to aid the seed for better seedling establishment. They were positively correlated with seed vigour (Catalase: 0.674**; Peroxidase: 0.647**). Reduced activity of these enzymes in quality genotypes could be due to age induced rapid deterioration by ROS, as ROS was found to be significantly higher in these genotypes. These enzymes used in protection and repair systems of seeds and their loss causes reduction in viability and vigour (Demirkaya *et al*. 2010, Kibinza *et al*. 2011). Reduced activity of enzymatic antioxidants during storage could also be the cause for poor vigour in quality genotypes.

Non-enzymatic antioxidants: Ascorbate-Glutathione cycle plays a key role in cell wherein the ascorbate gets converted into dehydroascorbate to reduce hydrogen peroxide. Reduced forms are regenerated back by utilizing glutathione (GSH) yielding its oxidized form (GSSG). GSSG

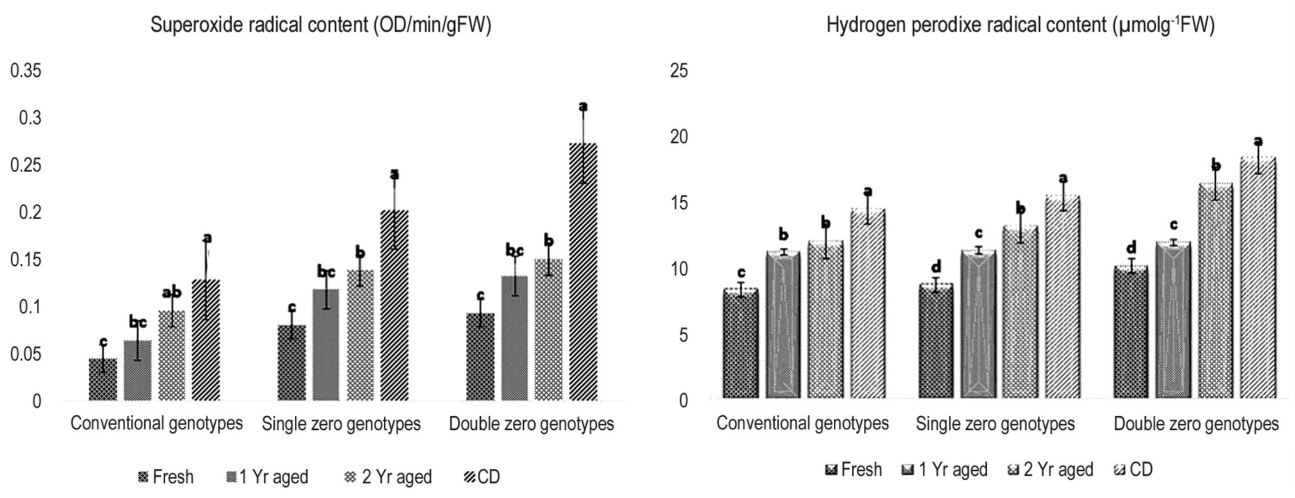

Fig 1 Superoxide radical content ($O_2^{\cdot-}$) and Hydrogen peroxide (H_2O_2) in fresh and aged seeds of different type of Indian mustard.

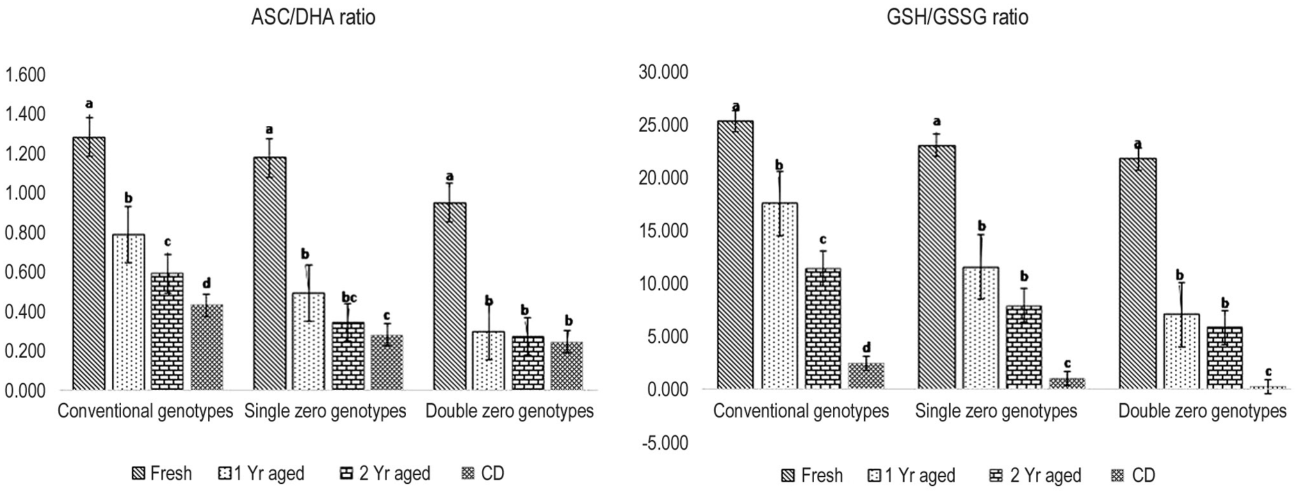

Fig 2 ASC/DHA and GSH/GSSG ratio in fresh and aged seeds of different type of Indian mustard.

is again reduced using NADPH as electron donor. Thus, both ascorbate and glutathione are regenerated back in this pathway. Thus, the ratio of ASC/DHA and GSH/GSSG varies under different condition and can be used as a measure to estimate seed vigour. Similar to enzymatic antioxidants, non-enzymatic antioxidants ratios (i.e. Ascorbate (ASC)/ Dehydroascorbate (DHA); and Glutathione (GSH) / Glutathione disulphide (GSSH)) was significantly higher in conventional genotypes in comparison to quality genotypes and it goes on decreasing over the storage period (Fig 2).

As ROS content was higher, the redox ratio shifted to more oxidized form resulting in lower ratio in quality genotypes. Seed deterioration due to seed ageing has been linked with oxidative modifications, including the overall cellular redox state. Both ascorbate and glutathione are important cellular redox buffer. During ageing, they got used up and their reduced state will be converted into oxidized state and their ratio decreases. Similar results were also observed when soybean seeds were subjected to artificial ageing (Xin *et al*. 2014). It could be also due to reduced enzymatic antioxidant involved in ascorbate-glutathione cycle such as, dehydroascorbate reductase. Schausberger *et al*. (2019) reported that GSSG got accumulated in the seed in the process of reducing the reactive oxygen species, resulting in decreased ratio. They have also observed reduction in germination speed and seed vigour along with reducing ratio. Reduced vigour in quality genotypes could also be due to reduced ratio of redox couples.

In conclusion, results substantiated the fact that that accumulation of ROS (superoxide radical and H_2O_2) resulted in loss of vigour and viability in Indian quality mustard especially in double zero genotypes seed during ageing. In addition, impairment of antioxidative enzymes (enzymatic and non-enzymatic) in double zero seeds leads to excessive build-up of damaging levels of ROS that eventually could lead to massive lipid peroxidation and membrane damage. Thus, our study also established the vital role of antioxidative enzymes (catalase and peroxidase along with redox couple) in maintaining seed quality, i.e. high viability and vigour in the conventional mustard seeds during storage.

ACKNOWLEDGEMENTS

The authors are grateful to the Indian Agricultural Research Institute, New Delhi for providing required funds and necessary facilities to conduct the studies.

REFERENCES

Abdul-Baki A and Anderson J D. 1973. Vigour determination in Soybean seed by multiple criteria. *Crop Science* **13**: 630–33.

Bailly C and Kranner I. 2011. Analyses of reactive oxygen species and antioxidants in relation to seed longevity and germination. *Seed Dormancy*: 343-67.

Biswas N, Yadav S, Yadav S, Choudhary R, Saini N, Dahuja A, Vasudev S and Yadav D K. 2020. Vigor difference during storage and germination in Indian mustard explained by reactive oxygen species and antioxidant enzymes. *Turkish Journal of Agriculture and Forestry* **44**(6): 577-88.

Braber J M. 1980. Catalase and peroxidase in primary bean leaves during development and senescence. *Journal of Plant Physiology* **97**(2): 135-44.

Castillo F J, Penel C and Greppin H. 1984. Peroxidase release induced by ozone in sedum album leaves involvement of Ca^{2+}. *Plant Physiology* **74**(4): 846-51.

Chaitanya K K and Naithani S C. 1994. Role of superoxide, lipid peroxidation and superoxide 5 dismutase in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaertn. f. *New Phytologist* **126**(4): 623-27.

Christopher G L , Andrew J R, Geraldine A C L, Clare J H, Jacqueline B, Gary B, German C S and David E. 2005. Brassica ASTRA: an integrated database for Brassica genomic research. *Nucleic Acids Research* **33**: 656-59

Demirkaya M, Dietz K J and Sivritepe H O. 2010. Changes in antioxidant enzymes during ageing of onion seeds. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **38**(1): 49-52.

Eksi C and Demir I. 2011. The use of shortened controlled deterioration vigour test predicting field emergence and longevity of onion seed lots. *Seed Science and Technology* **39**: 190-98.

ISTA. 2018. *International Rules for Seed Testing*. International Seed Testing Association, Bassersdorf, Switzerland.

Kampfenkel K, Vanmontagu M and Inze D. 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry* **225**(1): 165-67.

Kibinza S, Bazin J, Bailly C, Farrant J M, Corbineau F and El-Maarouf-Bouteau H. 2011. Catalase is a key enzyme in seed recovery from ageing during priming. *Plant Science* **181**(3): 309-15.

Kumar G, Olekar N S and Dingal D K. 2018. Ageing induced biochemical and physiological changes during seed deterioration of groundnut (*Arachis hypogaea* L.). *International Journal of Chemical Studies* **6**(6): 1125-30.

Kumar M V, Evera T, Ramesh D and Masilamani P. 2019. Seed deterioration in long lived sesame seeds under accelerated ageing conditions. *Journal of Pharmacognosy and Phytochemistry* **8**:706-09.

Kumari A, Duhan S, Sheokand S and Kaur V. 2017. Effects of short and long term salinity stress on physiological and oxidative metabolism in chickpea (*Cicer arietinum*) and its possible alleviation by nitric oxide. *Indian Ecological Society* **44**(2): 250-58.

Nicholas M A and Heydecker W. 1968. Two approaches to the study of germination data. *(In) Proceedings of the International Seed Testing Association* **33**: 531–40.

Rahman I, Kode A and Biswas S K. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature Protocols* **1**(6): 3159.

Sahu B, Sahu A K, Chennareddy S R, Soni A and Naithani S C. 2017. Insights on germinability and desiccation tolerance in developing neem seeds (*Azadirachta indica*): role of AOS, antioxidative enzymes and dehydrin-like protein. *Plant Physiology and Biochemistry* **112**: 64-73.

Schausberger C, Roach T, Stoggl W, Arc E, Finch-Savage W E and Kranner I. 2019. Abscisic acid-determined seed vigour differences do not influence redox regulation during ageing. *Biochemical Journal* **476**(6): 965-74.

Swami S, Yadav S, Yadav S K, Dahuja A and Yadava D K. 2016. Imbibition behaviour and germination response in conventional and quality of Indian mustard (*Brassica juncea*) seeds. *Indian Journal of Agricultural Sciences* **86**(12): 1625–29.

Teranishi Y, Tanaka A, Osumi M and Fukui S. 1974. Catalase activities of hydrocarbon-utilizing Candida yeasts. *Agricultural and Biological Chemistry* **38**(6): 1213-20.

Xin X, Tian Q, Yin G, Chen X, Zhang J, Ng S and Lu X. 2014. Reduced mitochondrial and ascorbate–glutathione activity after artificial ageing in soybean seed. *Journal of Plant Physiology* **171**(2): 140-47.

Yadav S, Yadav S K, Swami S, Saini N, Hussain Z, Vasudev S and Yadava D K. 2020. Seed quality differences in diverse seed colored Indian mustard (*Brassica juncea*) genotypes. *Indian Journal of Agricultural Sciences* **90** (2): 401-06.