

Tolerance against ageing in different varieties of *Brassica rapa* var *toria*, *B. rapa* var yellow sarson and *B. juncea*(L.)

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Abstract: Seed deterioration is a serious problem in developing countries where seeds are stored in places usually without a proper control of humidity and temperature. In this investigation, an effort was made to identify crops as well as varieties of *brassica spp.* tolerance to ageing and predicting loss of seed viability at regular interval through standard germination under laboratory condition. Analysis of variance showed that mean squares due to crops, ageing periods and crop× ageing period were significant for % seed germination. Result revealed that standard germination declined from 95.63 to 37.54% in toria, 92 to 28.96 in yellow sarson, and 93.79 to 30.92% in mustard. Similar changes in germination % were observed at 9 and 12 months of observation. After 6 months, germination % declined from 95.13 to 35.50% in toria, 91.87 to 28% in yellow sarson and 93.33 to 29% in mustard. Analysis of variance showed significant differences for %seed germination due to varieties, ageing period and interaction of varieties × ageing periods. At varietal level AP2, AP3 and AP4 aged seed, Bhawani (82.66%, 68.16% and 28.17%) showed higher seed germination % respectively than other three varieties of toria. In case of yellowsarson and mustard least deterioration for standard germination were observed in B-9 and Kranti respectively. This study indicates that toria seeds have better storability than yeloowsarson and mustard. At varietal level good storage potential was found in Bhawani, B-9 and Krantivariety of toria, yellow sarson and mustard respectively than other three varieties of studied crops.

Keywords: Ageing, Brassica juncea, B. rapa var toria, B. rapa var yellow sarson, Standard germination

INTRODUCTION

High-quality seeds are of great socio-economic significance because seeds provide the majority of our food supply and are important sources of animal and industrial feedstock. High quality seeds could be characterized by maintaining a high germination rate and stable content after storage. Seed germination, seedling emergence and crop establishment are important aspects of canola production, and are the main components of seed/seedling vigour (Devaiah*et al.*, 2007). A major concern of growers is that deterioration of some seed cultivars, leading to loss of vigour, may be undetected before planting.

However seeds gradually deteriorate during prolonged storage and lose viability (McDonald, 1999). In addition, incorrect sowing date or harvesting period under warm and humid conditions can induce seed ageing. This ageing is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling (Veselova and Veselovsky, 2003). The survival of various seed lots or cultivars may differ

when seeds are stored under identical condition. Availability of genetic diversity in the gene pool of cultivated species is keys for a planned genetic improvement and variety development programme. In Brassica, availability of genetic diversity has been one of the major constraints. This is because the sizable collection available in the country does not represent the total spectrum of variability, and that despite recognition of the importance of genetic diversity, it is being eroded because of poor conservation efforts. In case of cultivated plant species, where most of the genetic diversity is confined to the farmer's fields, ex situ conservation of plant genetic resources is the most common method for conservation. Appropriate conditions have to be identified to restrict the deterioration of seed during processing and to prolong the life of the seed in storage. Frequent fluctuation in temperature and relative humidity make the processing and storage of seeds difficult to minimize the loss of viability and change in genetic integrity of the seed, thereby conservation of genetic resources.

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Seed deterioration is a serious problem in developing countries where seeds are stored in places usually without a proper control of humidity and temperature. Temperature and seed moisture content (and/or relative humidity) are the main factors influencing seed deterioration and viability reported by Barton (1964) James (1967) and Roberts (1972). Seed ageing is generally marked by reduction in vigour reported by Trawathaet al., (1995); Agrawal and Sinha (1980); Saxena, 1987 and Gupta and Aneja, 2004). Chhetriet al., (1993) and Arefi and Abdi (2003) reported that viability and rate and capacity of germination declined during ageing. Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Atici et al., 2007). Hsu et al. (2003), Goel et al.(2003), Pukacka and Ratajczak (2007) reported that due to activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase were decreased that lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency. Loycrajjouet al. (2008) reported that ageing induced deterioration increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes. Ghasemi- Golezaniet al. (2010) reported that decrease in germination percent and other indexes can be related to physiological and biochemical changes during seed aging.

This study has attempted to identify the effect of ageing on germination of rapeseed-mustard seeds over a period of four years, in order to compare tolerance to ageing and predicting loss of seed viability at regular interval through standard germination among three oilseed crops and their varieties.

MATERIALS AND METHODS

This study was carried out during 2011-12 at GBPUAT, Pantnagar using four varieties of three oilseeds *Brassica viz*. toria (*Brassica rapa Var toria*) yellow sarson (*Brassica rapa var yellow sarson*) and mustard (*Brassica juncea L*). Varieties of *Brassica rapavartoria viz* PT-303, PT-507, T-9 and Bhawani varieties of (*Brassica rapavar yellow sarson*) PPS-1, B

-9, NDYS-2, and Ragani and the varieties of *Brassica juncea viz* kranti vardan varuna and vardan were used to evaluate the seed viability. For ageing, seeds of all the varieties of *brassica* spp. were stored in cloth bag for one, two, three and four years under natural conditions and deginated by AP1, AP2, AP3 and AP4 respectively. Standard germination was evaluated after 3, 6 9 and 12 month interval.

Seed viability was tested through standard germination % test. First of all about 25 seeds in four replication from each treatment was treated with the Thiram (Tetramethyl thiramdisulphide) to remove dust particle that adhere on the seed surface. Then after seeds washed with distill water 2 to 3 times and placed for standard germination test was conducted on 100-seed samples of each crop at 20 ± 1 °C for 7d on moistened Whiteman papers in dark growth chamber. After 7 days, percent germination was calculated on the basis of number of normal seedlings in relation to total number of seeds placed for germination as prescribed in International Rules for Seed Testing (ISTA, 1976). Percent germination was calculated by the following formula:

Germination percentage = Number of normal seedling / Total number of seed X 100

Statistical analysis: Performance of crops over ageing period was determined by using split- split plot design. Performance of varieties over ageing periods was determined by applying two- factor ANOVA. Test of significance were recorded on basis of CD differences at 5% level of significance.

RESULTS AND DISCUSSION

Effect of ageing on standard germination in rapeseed-mustard: Analysis of variance showed that mean squares due to crops, ageing periods and crop× ageing period were significant for % seed germination (Table -1). Prolonged ageing periods caused reduction in germination % in all the three *Brassica* oilseeds. After 3, 6, 9 and 12 month ageing periods, toria showed the highest mean germination % then mustard and yellow sarson (Fig-1). After 3 month, percent reduction in seed germination from AP1 to AP4 declined from 95.63 to 37.54% in toria, 92 to 28.96 in yellow sarson, and 93.79 to 30.92% in mustard. Similar changes in

Table 1. ANOVA for germination % over ageing periods and crops in *Brassica rapavartoria, B. rapavar* yellow sarson and *B. juncea* (L.).

Sauras of Varianas	Degree of		Mean	square	
Source of variance	freedom	3 month	6 month	9 month	12 month
Crop	2	510.38**	437.53**	799.51 **	971.81 **
Error(a)	6	0.39	0.229	0.434	0.145
Varieties	3	59.76**	15.93	19.01	73.82
Error (b)	24	14.55	23.29	77.85	146.38
Ageing periods	3	26032.9**	27229.3**	31042.0**	35328.3**
Crops ×AP	6	28.81**	24.79*	37.69**	79.42 **
Varieties ×AP	9	28.15**	6.94	28.22*	29.65
Error(c)	90	4.07	6.84	9.99	15.22

*, **, Significant at 5% and 1% probability level



Fig 1. Effect of ageing periods on seed germination in toria, yellow sarson and mustard at different time intervals. Note:-AP1- One year aged seed, AP2- Two year aged seeds, AP3-Three year aged seeds and AP4- Four year aged seeds.

germination % were observed at 9 and 12 months of observation.

After 6 months, germination % declined from 95.13 to 35.50% in toria, 91.87 to 28% in yellow sarson and 93.33 to 29% in mustard. At AP1 stage, yellow sarson (91.87%) was statistically at par with mustard (93.33%) in % germination but lower than toria (95.13%). At AP2, toria (85.58%) had significantly higher germination% followed by mustard (83.45%) and yellow sarson (81.04%) whereas in AP and AP4 stage, yellow sarson (62.37% and 28%) being statistically at par with mustard (64.42 and 29%). These results are in agreement with the finding of Mohammadiet al. (2011) who reported that rate of germination percentage depends on the ability of the seeds to resist degradation changes and protection mechanisms, which are specific for each plant species In the present study drastic reduction in mean germination was occurred yellow sarson than toria and mustard. Zhang et al. (2006) reported that seed germination in yellow seeded Brassica napus as compared to brown seeded toria. This could be attributed to high oil and protein content owing to the thinner and transparent testa in yellow seeded B. napus and yellow sarson compared with black or brown-seeded varieties of toria. Transparenttesta of yellow-seeded rapeseed lacked some abilities to protect he embryo against adverse environmental conditions which caused poor storability. Seed coat pigment of brown seeded varieties of toria and mustard may be playing a protective role against seed deterioration during ageing.

Effect of ageing on standard germination in different varieties of toria: Analysis of variance showed that mean squares due to varieties, ageing period and varieties× ageing period interaction in all three brassicas crop showed significant differences for % seed germination (Table-2).

The mean value of seed germination (%) was declined with increase in ageing periods from AP1 to AP4 in all

Source of variation	Degree of Free-						Mean	square					
	dom		To	ria			Yellow	v sarson			Mu	istard	
		3month	6month	9month	12month	3month	6month	9month	12month	3month	6month	9month	12month
Varieties	e	26.61**	25.58**	91.36**	154.82**	41.28**	48.64**	248.35**	363.67**	104.03**	124.02**	297.04	723.06**
A.P	c,	7746.77**	8212.65**	9577.09**	11387.74**	9172.77**	9411.13**	11221.73**	12372.46**	9171.05**	9655.10**	10318.61**	11726.98**
Varieties x AP	6	8.53**	13.37**	39.81**	24.79**	17.87**	15.42**	32.86**	53.19**	33.98**	38.69**	51.25**	99.22**
Error	32	1.03	0.79	0.39	0.51	0.94	0.77	0.57	0.46	0.88	1.08	0.78	065

**; Significant at 5% and 1% probability levels

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(years)		PT-507	3 Month T-9	Bha-				1. 1.												
`		PT-507	T-9	Bha-				01001010					MODUL 6					UIUOINI 71		
	PT- 303			wani	A.P Mean	PT-303	507 507	6-1	Bha- wani	A.P Mea	PI- 303	507 507	6-1	Bha- wani	A.P Mean	PT- 303	PT-507	6-1	Bhawani	A.P Mean
AP1	94.84	96.50	96.17	95.00	95.63	94.33	96.33	95.33	94.50	95.12	93.66	95.50	94.16	93.83	94.29	90.33	91.83	91.83	91.33	91.33
AP2	85.66	85.17	86.17	86.67	85.92	86.50	84.33	85.66	85.83	85.58 	84.66	83.17	84.17	84.66 - 20	84.17	76.83	78.17	80.33	82.66	79.50
AP3	68.34	73.00	72.34	74.67	72.08	67.17	71.16	70.16	73.83	70.58	62.83	61.16	68.00	70.83	65.70	56.17	58.33	60.83	68.16	60.87
AP4	34.67	35.83	38.16	41.50	37.54	32.33	33.16	36.33	40.16	35.50	21.83	28.33	31.50	38.66	30.08	14.16	17.00	24.50	28.17	20.95
V.Mean	70.87	72.62	73.20	74.45	72.79	70.08	71.25	71.87	73.58	71.69	65.75	67.04	69.45	72.00	68.56	59.37	61.33	64.37	67.58	79.50
SEm(V)			0.293°					0.257°					0.182°					0.206°		
SEm (A.P)			0.293^{a}					0.257^{a}					0.182^{a}					0.206^{a}		
SEm			0.586^{av}					0.513^{av}					0.363^{av}					0.412^{av}		
(A.PXV)			VALOO					10100					×					V1010		
			0.044					0. /40					676.0					+6C.U		
CD (A.P)			0.844^{a}					0.740^{a}					0.523^{a}					0.594^{a}		
CD (A PXV)			1.688^{av}					1.479 ^{av}					1.046^{av}					1.188 ^{av}		
Ageing	Varie	ties																		
Period	3 Mon	ιth				6Month					9 Mon	th				12 Mc	hth			
(years)	-Sqq	B-9	VUV	Ragi	A.P	-Sdd	B-9	VDY	Ragi	A.P	PPS-1	B-9	VUV	Ragi	A.P	-Sqq	B-9	VDV	Ragini	A.P
	-		S-2	E.	Mean	-	1	S-2	E.	Mea	2		S-2	E	Mean	-		S-2	D	mea
4P1	93.17	91.67	92.50	90.67	92.00	92.50	91.83	92.83	90.33	91.87	92.17	91.33	90.00	88.67	90.54	87.16	87.66	87.00	84.50	86.5
AP2	81.50	82.17	82.00	80.00	81.42	81.17	82.33	81.16	79.50	81.04	80.50	81.33	73.17	72.00	76.75	75.83	78.17	67.16	67.00	72.0
AP3	67.50	66.67	57.16	61.33	63.17	66.17	66.00	56.83	60.50	62.37	53.66	64.50	49.50	47.33	53.75	41.83	58.83	41.00	36.50	44.5
AP4	28.83	32.33	28.16	26.50	28.96	28.17	32.00	26.50	25.33	28.00	18.00	29.00	17.83	17.50	20.58	10.50	23.50	11.50	9.66	13.7
V.Mean	67.75	68.21	64.96	64.62	66.38	67.00	68.04	64.33	63.91	65.82	61.08	66.54	57.62	56.38	60.40	53.83	62.04	51.66	49.41	54.2
SEm (V)	0.780	-				0.7530					0.10		10.00			0 106 ^v				
SEm (A P)	0.200					0.253^{a}					0.219 ^a					0.196				
SEm	0.560ª	٨				0.506 ^{av}					0.437^{a}	v				393	Ň			
(A.PXV)																				
CD (V)	0.807°					0.730°					0.629°					0.566'	,			
CD(A.P)	0.807^{a}					0.730^{a}					0.629^{a}					0.566°	_			
CD	1.614^{a}	×				1.460^{av}					1.258^{a}	٨				1.132^{6}	IV			
(A PXV)																				

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Ageing Peri-										Var	ieties									
od (years)			3 month					6month					9 month					12 month		
	Vardan	Varu-	Krant	NDR	A.P	Var-	Varu-	Krant	NDR	A.P	Var-	Var-	Krant	NDR	A.P	Var-	Varu-	Krant	NDRE-4	A.P
		na	-	E-4	Mean	dan	na	-	4	Mean	dan	una	-	E-4	Mean	dan	na	-		Mean
AP1	93.50	93.67	94.50	93.50	93.79	93.50	93.00	93.66	93.16	93.33	92.83	88.83	92.83	91.00	91.37	91.00	81.83	91.00	87.33	87.79
AP2	85.34	81.00	85.50	83.17	83.75	84.66	80.33	85.50	83.33	83.45	84.33	72.83	84.00	82.00	80.79	81.67	61.16	80.83	77.83	75.37
AP3	62.83	56.83	74.84	68.17	65.67	61.50	55.00	74.66	66.50	64.42	59.00	47.16	71.33	61.66	59.79	50.83	33.83	68.00	50.66	50.83
AP4	31.16	27.67	32.83	32.00	30.92	29.66	25.67	31.50	29.16	29.00	29.00	20.83	28.16	21.50	24.87	24.00	11.50	19.33	12.66	16.87
V.Mean	68.20	64.79	71.91	69.20	68.53	67.33	63.50	71.33	68.04	67.55	66.29	57.41	69.08	64.04	64.20	61.87	47.08	64.79	57.12	57.71
SEm(V)			0.271 ^v					0.300°					0.256^{v}					0.232°		
SEm (A.P)			0.271^{a}					0.300^{a}					0.256^{a}					0.232^{a}		
SEm (A.PXV)			0.542 ^{av}					0.599^{av}					0.512 ^{av}					0.465^{av}		
CD (V)			0.780^{v}					0.863 ^v					0.737^{v}					0.670		
CD (A.P)			0.780^{a}					0.863^{a}					0.737^{a}					0.670^{a}		
CD (A.PXV)			1.560^{av}					1.727^{av}					1.474 ^{av}					1.341 ^{av}		

four varieties of toria (Table 3). At 3 month observation, germination (%) declined from 94.84 to 34.67% in PT-303, 96.50 to 35.83 in PT-507, 96.17 to 38.16 in T-9 and 95 to 41.50 % in Bhawani. After 6 months, reduction in seed germination was from 94.33 to 32.33% in PT-303, 96.33 to 33.16% in PT-507, 95.33 to 36.33% in T-9 and 94.50 to 40.16 % in Bhawani. Decline in seed germination at 9 month, 93.66 to 21.83% in PT-303, 95.50 to 28.33%, in PT-507, 94.16 to 31.50 %, in T-9, 93.83to 38.66% in Bhawani respectively. Similarly reduction in 12 months 90.33 to 14.66% in PT-303, 91.83 to 17% in PT-507, and 91.83 to 24% in T-9, and 91.33 to 28.17% in Bhawani. After 3 months Bhawani (95%) was found statistically at par with T-9 (96.17%) and PT-507 (96.50%) being statistically higher in germination% than PT-303 in AP1 seeds. At AP2 all varieties were statistically at par with each other whereas in AP3 and AP4, Bhawani (74.67% and 41.50%) showed higher germination% than other three varieties in which T-9 and PT-507 were at par with each other. After 6 month at AP1 aged seed, Bhawani (94.50%) was statistically at par with PT-303 (94.33%) whereas in AP2 aged seed, PT-303 (86.50%) was statistically at par with T-9 (85.66%) and Bhawani (85.83%) which expressed higher germination % than PT-303. In AP4 aged seed, PT-303 (32.33%) was statistically at par with PT-507 (33.16%) which showed lower germination than other two varieties. Similar trend was observed after 9 months interval.

After 12 month observation, at AP1 PT-507 and T-9 (91.83%) was statistically at par with Bhawani (91.33%) expressed higher germination% than PT-303 (90.33%) whereas in AP2, AP3 and AP4 aged seed, Bhawani (82.66%, 68.16% and 28.17%) showed higher seed germination % respectively than other three varieties of toria.

Effect of ageing on standard germination in different varieties of yellow sarson: Germination percentage declined with an increase in ageing periods from AP1 to AP4. After 3 months, it declined from 93.17 to 28.83 in PPS-1, 91.67 to 32.33% in B-9, 92.50 to 28.16% in NDYS-2 and 90.67 to 26.50 in Ragini (Table-4). Similar declining trend among varieties was observed after 6 months observations. After 9 month, reduction in seed germination was from 92.17 to 18% in PPS-1, 91.33 to 29% in B-9, 90 to 17.83 % in NDYS-2 and 88.67 to 17.50% in Ragini during the corresponding period. After 12 months, germination% was reduced from 87.16 to 10.50% in PPS-1, 87.66 to 23.50% in B-9, 87 to 11.50% in NDYS-2 and 84.50 to 9.60 in Ragini. In all four observations, least deterioration was observed in B-9.

Effect of ageing on standard germination in different varieties of mustard: After 3 month decline in germination from AP1 to AP4 was 93.50 to 31.16% in Vardan, 93.17 to 27.67 in Varuna, 94.50 to 32.83 in Kranti and 93.32 to 32% in NDRE-4(Table-5). Higher mean germination % was observed in Kranti in all ageing periods at 3 and 6 month interval. However, at 9 and 12 month interval % germination in Kranti was found similar to Vardan in AP1 and AP2 seeds but Kranti showed significantly high % germination in AP3 and AP4 seeds. In all ageing periods, Kranti showed lower deterioration whereas rapidly reduction in germination was found in Varuna. Similar declining trend was observed for 12 month observation.

It is quite evident that seed ageing caused reduction in germination percentage in all crops including *Brassica* crops. Biochemical and physiological changes in seeds during ageing caused reduction in germinability reported by Ghasemi Golezani *et al.* (2010) and Balešević Tubić *et al.* (2005) in oilseed *Brassica*. Balesevic-Tubic (2001) and Malenčić *et al.* (2003) reported that reduction in germination% was varied in all three oilseed crops due to genetic factors and seed chemical composition influence the expression of seed deterioration and vigour decline indicated by the differential response of various accessions of species to different types of ageing.

Rapid deterioration in germination % in oilseed crops has been attributed to be due to their high oil content in the seeds reported by Beratlief and Iliesku (1997) and Sahoo *et al.* (1998) who noticed that high oil content in seeds is sensitive to autooxidation and increases the content of free fatty acid during ageing period leading to rapid loss in germinability. These processes cause enzyme inactivation, i.e., denaturation of protein and nucleic acids reported by Reuzeau and Cavalie (1995)and Trawatha *et al.* (1995).

Results of present study showed that seed ageing caused significant reduction in seed germination % in oilseed *Brassicas* studied. The magnitude of reduction depended on genetic differences in different varieties. Similar results are reported by Afshari *et al.*, (2009) and Janmohammadi *et al.* (2008) in *Brassica napus*. Afshari *et al.* (2009) and Janmohammadi *et al.* (2008) was reported that reduction in germination% during storage has been ascribed to higher leakage of electrolyte and reduced enzyme activity.

Rabiei and Bayat (2009) reported that significant differences for % germination in different varieties of rapeseed -mustard indicated the existence of genotypic differences for this seed quality trait. Balesevic-Tubic *et al.* (2011) assumed that variation in germination % in soybean and sunflower seeds due to genotype differences.

Reduction in seed germination (%) in different varieties of toria was observed with an increase in ageing period. The germination of seeds stored for 1 to 2 years was greater (85.17-96.50%) than the minimum seed certification standard (85.0%) and rapid reduction was observed in 3 and 4 year old seeds. Similar findings have been reported by Verma *et al.* (2003) in *Brassica campestris.*

Malenčić et al. (2003) and Milošević et al. (1996) sug-

gested that seed longevity is genetically determined, and that significant differences exist among cultivars of the same crop in their ability of quality maintenance during storage. Popović et al. (2006) reported that lipid peroxidation and products resulting from these processes lead to DNA denaturisation, prevent translation and protein transcription, and cause oxidation of the most reactive amino acids that may cause decrease in vigour and seed germination. Job et al. (2005) found that inactivation of antioxidant enzymes, seeds lose their ability to stabilize reactive oxygen species thereby enhancing lipids peroxidation and denaturation causes low germination. Ghasemi- Golezaniet al. (2010) reported that decrease in germination percent and other indexes could be related to physiological and biochemical changes during seed aging. They suggest that during aging, decreases plasma membrane sustainability, changes in molecular structure of nucleic acid, decreases in enzyme activities during seed senescence and increases time for fast germination. Kapoor et al. (2010) showed that accelerated aging in cowpea seeds affected all physiological parameters such as germination percentage and vigour index. They found that decrease in seed viability, germination rate and vigour is correlated with biochemical changes (decrease soluble proteins and sugar content) associated with seed aging. The results are also agreement with the finding of Khajeh Hoseini et al. (2003) in soybean.

Conclusion

On the basis of germination test, the highest mean germination % was observed in Brassica rapa var toria followed by in B. juncea and B. rapa var yellow sarson. Lowest reduction in germination % was observed in Bhawani followed by T-9, PT-507 and PT-303. In yellow sarson minimum percent reduction in germination % was observed in B-9 and maximum in Ragini. Similarly in mustard, Varuna showed high reduction in germination while Kranti showed low reduction. It is significant to mention that results of present study have helped in the better understanding of the effect of ageing periods on seed quality determinants as well as on performance of selected Brassica crops and their varieties studied. Based on the results it can be inferred that during ageing toria showed least reduction in seed germination than other two crops. This study also concluded that seed of toria could be stored for uptotwo years without reduction in germination % under ambient condition because it had greater standard germination for 1 to 2 years (85.17-96.50%) then the minimum seed certification standard (85.0%) and on the basis of varieties, Bhawani of toria, B-9 of yellow sarson, and Kranti of mustard showed better storability then other three varieties of studied crops, respectively.

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