



Seed quality differences in diverse seed colored Indian mustard (*Brassica juncea*) genotypes

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Received: 08 March 2019; Accepted: 15 July 2019

ABSTRACT

‘Canola quality’ Indian mustard (*Brassica juncea*) with varying seed color are a new kind of breeding resources with single zero genotypes having low level of erucic acid (<2%) while double zero genotypes have both low erucic and glucosinolate content (<30 $\mu\text{mole/g}$ defatted meal). In order to know the variation in seed quality of different types of Indian mustard, in the present study nine genotypes consisting of conventional as well as ‘canola quality’ having either black or yellow seed testa colour were subjected to multivariate analysis including principal component (PCA) and cluster analysis of physiological and biochemical parameters. Data for physiological, biochemical and seed vigour trait was recorded and analysed during 2017–18 at ICAR-Indian Agricultural Research Institute, New Delhi. The first three axes of the PCA captured 85% of the total variation encountered and identified water uptake ratio, electrical conductivity (EC), PUFA/MUFA ratio and Malondialdehyde (MDA) values positively contributing to most of variation while melanin content, total phenol content, germination percentage, vigour indices and glucosinolate content contributed negatively. Cluster analysis classified these genotypes into two distinct groups based on seed testa colour. Black seeded genotypes clustered in group I and yellow seeded genotypes clustered in groups II. Moreover, these techniques also differentiated canola from conventional genotypes among their testa groups. The majority of yellow-seeded genotypes had low melanin and total phenol content in testa causing rapid water uptake and higher leakage than the black-seeded genotypes, which led to imbibition damage resulting in lower germination percentage and seed vigour indices.

Key words: Canola, Cluster analysis, Principal component analysis (PCA), Seed quality, Testa colour

Indian mustard (*Brassica juncea* (L.) Czern & Coss), occupying more than 80% of the total area of rapeseed-mustard in India, is predominantly used for oil extraction and consumption in most parts of the country. The major disadvantage of conventional Indian mustard is high concentration of erucic acid (35-55%) in oil and glucosinolate (49.9–120.3 $\mu\text{mol/g}$ defatted meal) in defatted seed meal (Saini *et al.* 2016). To overcome the limitations of these anti-nutritional factors, breeders developed single zero ‘canola quality’ Indian mustard (*B. juncea*) having low erucic acid content (<2%) and double zero ‘canola quality’ Indian mustard having both low erucic acid and low glucosinolate content (<30 $\mu\text{mol/g}$ defatted meal).

The seed coat is a multifunctional organ which helps in embryo nutrition during seed development but also protects the seed from adverse climatic conditions. Moreover, it is one of the major barriers to water movement during the initial stages of imbibition and provides mechanical resistance to radical protrusion due to its germination-restrictive action by being less permeable to water and/or oxygen (Weber *et al.* 1996). The color of mature seeds varies from yellow to black in *B. juncea*. Yellow-seeded *Brassica* genotypes have thinner and transparent or semi-transparent testa with less unwanted crude fiber and pigments. Thus, yellow seeded genotypes have better nutritional value because of higher protein and oil content as compared to brown or black seeded forms (Ye *et al.* 2001). Previous investigations have shown that the seed testa color has been associated with seed quality in various crops: mustard (Swami S 2016) and *Arabidopsis thaliana* (Debeaujon I 2000). Multivariate statistical methods are in extensive use for summarizing and describing the inherent variation in a population of crop genotypes for exploiting them in breeding programmes. There is no published information about seed coat characteristics that may affect the seed quality in Indian ‘canola quality’ mustard using these exploratory techniques. Thus, the aim of the present

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study was to analyze the physiological and biochemical differences between yellow-seeded and black-seeded 'canola quality' Indian mustard along with the conventional Indian mustard (*Brassica Juncea* L.) genotypes and to establish their relationship with seed quality traits using multivariate techniques.

MATERIALS AND METHODS

The present study was conducted during 2017 and 2018 in the laboratories of the Division of Seed Science and Technology, ICAR-Indian Agriculture Research Institute (IARI), New Delhi situated at 28° 35' N latitude and 77° 12' E longitudes and at an altitude of 228.6 m amsl. Nine genotypes of Indian mustard (*B. juncea* L.) were obtained from Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi. It consisted of four black seeded genotypes {two conventional genotypes (Pusa Vijay and Pusa Mustard 28) and two single zero canola genotypes (Pusa Mustard 21 and Pusa Mustard 30)} and had five yellow seeded genotypes {(one conventional genotype (NPJ189), two single zero canola genotypes (Pusa Karishma and LES48) and two double zero canola genotypes (Pusa Mustard 31 and PDZ-2)}. Seeds were harvested, cleaned and air dried. The moisture content of dry seeds was 8.5%. All experiments on these genotypes were done in triplicate.

The melanin contents were estimated from 0.1 g of dried testa powder by incubating in 2% NaOH at 70°C for 48 h and OD measured at 290 nm (Shimadzu UV 1800) (Ye *et al.* 2001). Total phenol content in seed testa was analysed using Folin-Ciocalteu method (Singleton and Rossie 1965).

The germination test was evaluated on the 7th day on *Brassica* seeds according to the ISTA rule, 2015. The percentage of germination and radicle length of germinated seeds were recorded. The seed vigour indices were calculated adopting the method of Abdul Baki (1973) relative to the control.

Seeds (1 g) were soaked in 75 ml deionised water and kept in the incubator at 25°C for 24 h. Leachate conductivity was determined with a conductivity meter (Goel *et al.* 2003).

Five-hundred seeds of each genotype were weighed and placed in a 200 ml beaker containing 50 ml distilled water at 25°C. After 24 h of imbibition, seeds were removed from the water, blotted dry and weighed again. Water uptake was measured as a percentage weight increase (Chachalis and Smith 2000) taking mean of three replicates.

Seeds (0.5 g) from each genotype were homogenized manually with 5.0 ml trichloroacetic acid (TCA) at 4°C and centrifuged at 8000 rpm for 15 min. The supernatant was used for MDA determination according to the methods of Heath and Packer (1968).

Fatty acid content of the seeds of genotypes was analyzed by gas liquid chromatography (Perkin Elmer Claurus 600) using Flame Ionization Detector (FID) and Column temperature: 150–270°C, Injector temperature: 250°C and Detector temperature: 250°C. GLC was programmed for the temperature at the rate of 10°C per minute increase and finally it was maintained at 270°C

(Sujata *et al.* 2008). After analysis, Polyunsaturated fatty acid (PUFA) / Monounsaturated fatty acid (MUFA) was calculated as sum of Linoleic acid + Linolenic acid/ Oleic acid + Eicosenoic acid + Erucic acid.

The data was analysed for correlation coefficient between parameters using SPSS 16.0. The dissimilarity matrix was used to construct dendrogram by unweighted pair group method for arithmetic mean (UPGMA) based Sequential Agglomerative Hierarchical and Nested (SAHN) clustering. The Principle Component Analysis (PCA) based on the Euclidean coefficients of dissimilarity was done. All these calculations were done using NTSYS-pc version 2.11 (USA) (Rohlf 2000).

RESULTS AND DISCUSSION

Seed characteristics of yellow-seeded and black-seeded 'Canola quality' and conventional type Indian mustard: Irrespective of testa colour, the conventional genotypes have high erucic acid (35-42%) and glucosinolate content (144-185 µmol/g defatted meal). The single zero varieties had low erucic acid content of <2% but high content of glucosinolates (158-180 µmol/g defatted meal) while double zero genotypes had both low erucic acid (<2%) and low glucosinolate content (21.1-25.2 µmol/g defatted meal). The black-seeded genotypes differed from yellow-seeded genotypes with different seed coat characteristics and seed quality (Table 1).

Germination and seed vigour indices: More pronounced decrease in germination percentage and seed vigour indices was observed in the yellow-seeded 'canola quality' genotypes compared to the black seeded genotypes (Table 1). Compared to the conventional genotypes, black seeded 'canola quality' genotypes (single zero genotypes) were at par with conventional genotypes in terms of germination percentage and seed vigour indices. However, no significant differences were observed among yellow seeded single and double zero 'canola quality' genotypes. Thus, germination of 'canola quality' Indian mustard genotypes was significantly affected by seed coat color. Zhang *et al.* (2013) have also reported rapid decline from darker to lighter seed coat colours in *B. napus*.

Membrane permeability and water uptake ratio (%): Significant differences were found associated with the leakage of electrolytes (Table 1). The lowest EC was found in black seeded conventional (54.7 µs/cm/g of seed) Indian mustard genotypes compared to single zero black seeded genotypes (73.4 µs/cm/g of seed). Highest EC was found in yellow seeded double zero genotypes (165.6 µs/cm/g of seed) followed by single zero yellow seeded genotypes (142.3 µs/cm/g of seed) when compared with conventional yellow seeded Indian mustard genotypes (81.7 µs/cm/g of seed).

The genotypes with yellow seed coat had the highest water uptake followed by black seeded genotypes. Similar pattern was observed in electrical conductivity of seed leachates. However, black seeded single zero genotypes were at par with conventional black seeded genotypes in

Table 1 Average value of variables in different clusters

Sub-group of Clusters	Black seeded genotypes (Cluster I)			Yellow seeded genotypes (Cluster II)			
	Cluster IA	Cluster IB	Average values	Cluster IIA1	Cluster IIA2	Cluster IIB	Average values
Type of Mustard	Conventional genotypes	Single zero canola genotypes		Conventional genotypes	Single zero canola genotypes	Double zero canola genotypes	
<i>Seed Coat Characteristics</i>							
Melanin content (U)	655.3	657.1	656.2	87.1	72.3	29.4	62.9
Total Phenol content (mg/g seed)	26.5	31.0	28.8	10.9	16.1	13.4	13.5
PUFA/MUFA (SC)	0.75	1.19	0.97	0.96	1.17	1.48	1.20
<i>Seed quality parameters</i>							
Germination (%)	95.8	93.8	94.8	91.0	93.2	90.8	91.7
Seed Vigour Index-I	1223.2	1260.9	1242.0	1173.4	1086.3	949.6	1049.8
Seed Vigour Index-II	1.5	1.4	1.5	1.04	0.96	0.83	0.94
Water uptake ratio (%)	69.9	68.8	69.4	103.2	121.6	121.9	115.6
Electrical conductivity ($\mu\text{s}/\text{cm}/\text{g}$ of seed)	54.7	73.4	64.1	81.7	142.3	165.6	129.9
MDA content ($\mu\text{mol g}/\text{FW}$)	11.0	7.5	9.3	20.7	22.6	37.8	27.0
Erucic acid content (%)	37.8	0.3	19.0	42.1	0.2	0.3	14.2
Glucosinolate content ($\mu\text{g g}/\text{defatted meal}$)	156.3	163.0	159.6	185.8	177.6	26.7	130.0

terms of water uptake ratio. The yellow-seeded 'canola quality' Indian mustard genotypes rapidly absorb more water with 121% increase of fresh weight while the black seeded genotypes absorb water slowly with only 69% increase in fresh weight after 24 h of imbibitions.

Thus, the average percentage weight increase and electrical conductivity indicated that water uptake and leachate conductivity of seeds was associated with different seed colours. The yellow-seeded genotypes had a faster water uptake rate and higher leachates conductivity leading to greater imbibitional damage than the black-seeded genotypes (Legesse and Powell 1992, Kantar *et al.* 1996, Chachalis and Smith 2000), indicating that these genotypes are more susceptible to membrane damage leading to reduced seed vigour.

Seed testa parameters: The content of melanin in seed testa was found to be higher in black seeded genotypes (656.2U) compared to yellow seeded genotypes (58.1U) (Table 1). Among yellow seeded genotypes significant differences were present between conventional (87.1U) and 'canola quality' genotypes. The single zero canola genotypes have higher melanin content (72.3U) compared to double zero 'canola quality' genotypes (29.4U).

Similar pattern was observed in total phenol content in seed testa. The black seeded genotype had higher total phenol content of 28.8 mg/g seed compared to 14.0 mg/g seed in yellow seeded genotypes. The total phenol content was higher in 'canola quality' black seeded genotypes (31.0 mg/g seed) compared to conventional black seeded genotypes (26.5 mg/g seed). The single zero yellow seeded

'canola quality' genotypes have higher total phenol content (16.1 mg/g seed) compared to double zero genotypes (13.4 mg/g seed) of Indian mustard.

One of the important factors regulating the rate of water uptake is the presence of pigments of phenolic compounds localized in seed coat especially in the palisade cells of the epidermis in diverse species (Kuo 1989, Debeaujon *et al.* 2000, Rahman *et al.* 2001). Many authors have reported that the main pigment in the testa of *Brassica napus* L. is melanin specially proanthocyanidins (condensed tannins), as seed matures, these oxidize resulting in species specific seed color and characteristic seed-coat pigmentation (Hu 1988, Marles *et al.* 2003, Ye *et al.* 2001). Thus, in the present study, reduced water uptake by black seeded genotypes due to presence of melanin and phenol could be the reason for higher seed quality traits in these genotypes on the other hand; the yellow-seeded genotypes have significantly reduced testa pigments resulting in increased water uptake and imbibition damage by solute leakage.

Fatty acid ratios and lipid peroxidation: PUFA/MUFA ratio was found to be lower in black seeded genotypes (0.97) when compared to yellow seeded genotypes (1.25). However, 'canola quality' genotypes had higher PUFA/MUFA ratio compared to conventional Indian mustard genotypes. The black seeded conventional genotypes had 0.75 PUFA/MUFA ratios while 'canola quality' genotype had 1.19 PUFA/MUFA ratios. Yellow seeded conventional genotypes had lower PUFA/MUFA ratio of 0.96 but higher PUFA/MUFA ratio of 1.17 in single zero genotype which increased to 1.48 in double zero genotypes (Table 2).

Malondialdehyde (MDA) content of the yellow-seeded Indian mustard genotypes was significantly higher (28.2 $\mu\text{mol/g}$ FW) than the black-seeded genotypes (9.2 $\mu\text{mol/g}$ DW). MDA content increased in yellow seeded canola quality genotypes with conventional genotypes having MDA content of 20.7 $\mu\text{mol/g}$ DW to 22.6 $\mu\text{mol/g}$ DW in single zero yellow seed genotype and 37.79 $\mu\text{mol/g}$ DW in double zero genotypes.

Fatty acids that compose the membrane determine their susceptibility to oxidative damage as membrane lipids become primary targets of oxidative damage. Additionally, the PUFA content of phospholipids are extremely sensitive to oxidation due to presence of 2 or more double bonds per fatty acid molecule. Thus, PUFA are much more easily attacked by free radicals than MUFA (with one double bond) side chain. High PUFA/MUFA ratio in seed coat of yellow seeded quality genotypes make them more susceptible to oxidative damage. Membrane damage either through lipid peroxidation or free radical accumulation were found to play a key role in this degradation process (Khan *et al.* 1996). When membranes are disturbed due to lipid peroxidation, then this alteration in the membranes would lead to electrolyte leakage during seed imbibition, especially in oil rich seeds having linoleic and linolenic acid content (Chang and Sung, 1998).

Cluster analysis: The genotypes could be grouped into two major groups i.e. Group I and II (Fig 1) on the basis of Manhattan coefficient (1.46) of set parameters/variables. The group I belonged to black seeded genotypes while group II included yellow seeded genotypes. The group I was further divided into two sub-groups i.e. IA and IB which differentiated black seeded conventional and 'canola quality' genotypes while group II was divided into: IIA1, IIA2 and IIB which also differentiated yellow seeded conventional and yellow seeded single zero and double zero 'canola quality' genotypes. These groups showed reasonably significant results i.e. ordering/grouping the seed on basis of testa colour, physiological and biochemical characters. The outcome of this analysis was consistent with the results obtained through PCA, whereby the major differences between the clusters were attributed to the same traits that contributed most to PC1 and PC2.

Principal component analysis: Three of the principal components accounted for more than 85% of the total variation encountered (Table 2). Figure 2 shows two dimensional PCA resulting from principal component analysis.

The discriminatory power of the variables in each principal component is measured by correlation between each variable and a principal component. The water uptake ratio variable, electrical conductivity, PUFA/MUFA ratio and MDA content were responsible for discrimination of group I, located towards right side of PCA plot. Thus, group I was characterized with black seeded genotypes with low water uptake ratio, low EC, low PUFA/MUFA ratio and low MDA values, indicating that these genotypes have less problem of membrane integrity and thus, performed better than yellow

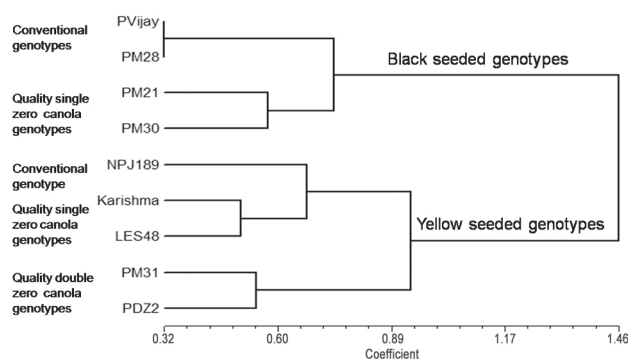


Fig 1 Dendrogram based on Manhattan distance in 9 mustard genotypes.

seeded genotypes in terms of germination.

However, melanin content, total phenol content, germination percentage, SVI-I, SVI-II and glucosinolate content showed a correlation of -0.90, -0.75, -0.76, -0.081, -0.87 and -0.65, respectively, thereby were effectively responsible for discrimination of group II, located to right of PC1 (Fig 2). Thus, group II is characterized by yellow

Table 2 Average range of variation and principle component scores in 9 mustard genotypes

Parameters studied	Range	Mean	SD	First three principal components		
				PC1	PC2	PC3
<i>Seed coat parameters</i>						
Melanin content (U)	22.63-823.5	323.9	326.6	-0.90	-0.31	-0.16
Total Phenol content (mg/g)	10.91-36.34	20.56	8.68	-0.75	-0.51	-0.08
PUFA/MUFA ratio(SC)	0.73-1.532	1.13	0.28	0.79	-0.51	0.15
<i>Seed quality parameters</i>						
Germination (%)	90-96	93.15	2.37	-0.77	-0.04	-0.16
Seed Vigour Index-I	881.7-1366	1135	152	-0.81	-0.02	0.3
Seed Vigour Index-II	0.78-1.78	1.18	0.35	-0.88	-0.12	-0.1
Water uptake ratio (%)	67.33-128.6	96.4	26.6	0.91	0.23	0.21
Electrical Conductivity ($\mu\text{s/cm/g}$ of seed)	52.4-220.7	106	55.02	0.89	-0.13	0
MDA content ($\mu\text{mol/g}$ FW)	5.40-43.04	19.84	12.18	0.96	0.09	-0.26
Erucic acid content (%)	0.21-42.08	13.24	19.55	-0.51	0.75	-0.34
Glucosinolate content ($\mu\text{mol/g}$ defatted meal)	21.21-185.8	136.21	65.20	-0.66	0.32	0.62
Variation						
Component				65.94	12.71	7.14
Cumulative				65.95	78.67	85.81

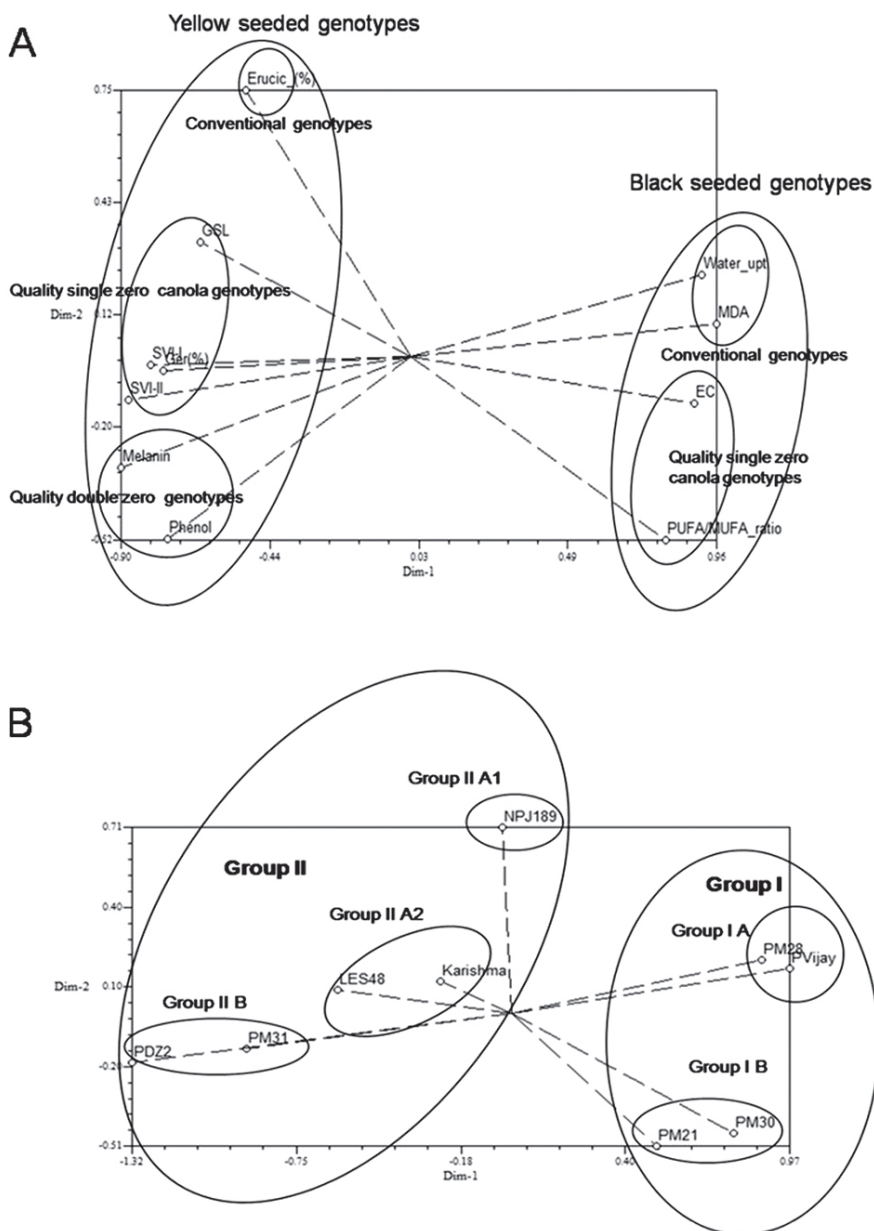


Fig 2 Two dimensional principal components resulting from principal component analysis based on A) 11 traits and B) 9 accessions.

seeded genotypes with compromised membrane integrity due to decreased content of melanin content, total phenol content, increased MDA content, EC, PUFA/MUFA ratio and water uptake ratio. The variation in these parameters also resulted in sub grouping of double zero, single zero and conventional yellow seeded genotypes. The mean values in different subgroups are given in Table 2. By retaining 65.95% of the information contained in the variables, this component has retained most of the information contained in the variables of the experiment.

In the second principal component which accounts for 12.73% variability, erucic acid content showed correlation value of 0.749 and have discriminated the conventional yellow seeded genotype from yellow seeded quality genotypes (Fig 2). In third principal component, erucic

acid showed correlation of -0.34 and glucosinolate showed positive correlation of 0.61, this has helped in discrimination of single zero yellow seeded genotypes. This component contributed 7.21% of the total variability.

The pattern of variations illustrated by the PCA was very well substantiated by the genotypic correlation coefficients determined for pair wise association of the oil quality traits. Consistent to the outputs of the PCA, the traits that contributed most for the 1st principal component (water uptake ratio, electrical conductivity, and MDA content) was negatively correlated with melanin content and total phenol content.

The physiological and biochemical parameters of seed coat and seed quality trait analysed by clustering and PCA have resulted in formation of two distinct groups which has discriminated the genotypes not only based on seed testa colour but also on basis of conventional and quality mustard genotypes. The multivariate analysis has been extensively used in genetic diversity analysis in the areas of genetics and plant breeding; however, there is very little literature regarding use of these techniques in areas of seed technology. Thus, Yellow seeded genotypes had reduced testa pigments, high PUFA/MUFA ratio and MDA content leading to disruption of membranes causing increased water uptake, electrical

conductivity signifying that these genotypes are more prone to imbibitional damage and resulting in low germination and seed vigour compared to black seeded genotypes.

Thus, cluster analysis and principal component analysis is efficient multivariate tool for discriminating the seed quality of genotypes on basis of testa colour. Hence, the breeders may take seed testa colour into consideration while developing varieties for better seed quality in future and can apply these tools to categorize germplasm/genotypes for other qualitative traits.

ACKNOWLEDGEMENTS

The authors are thankful to the ICAR-Indian Agricultural Research Institute, New Delhi for providing required funds for smooth carrying of research works and to the Division

of Seed Science and Technology for providing the necessary facilities to conduct the studies.

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