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Imbibition behaviour and germination response in conventional and quality of Indian mustard (*Brassica juncea*) seeds

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ABSTRACT

Indian mustard [*Brassica juncea* (L.) Czern and Coss] seeds are well known for their multipurpose uses, such as oilseeds, spices etc. Being extensively cultivated in northern and western part of the country it constitutes major share in country's oilseeds production. Developing cultivars having low erucic acid and glucosinolates (single zero and double zero) is a major objective of worldwide *Brassica* breeding. Such cultivars are also known as Quality Indian mustard. Seed; being vital input in agriculture, its quality determines the performance of a given genotype and efficiency of all other inputs too. Color variation among the seed coat is known to affect the rate of water uptake and thereby the vigor of seeds and these color variation is mainly because of pigments such as melanin and phenolics compounds. No such studies are reported in Indian mustard, though there is variability with respect to seed coat colour in both conventional and quality type of Indian mustard seeds. Thus the present investigation was carried out with a view to evaluate the imbibitional behaviour in black and yellow seeds of Indian mustard. The material had eight varieties of Indian mustard of which four genotypes each were of conventional and quality types. The seed of these genotypes varied for seed coat color, i.e. four black seeded and four yellow seeded genotypes. Thus, the material has two genotypes each with black and yellow seed coat from conventional and quality groups. We studied the rate of imbibition in black and yellow seeds and correlated them with the melanin and phenol content. We found that the black seeds were having higher amounts of melanin and phenol content and thus imbibing slowly than yellow seeds. On the other hand, faster imbibition in yellow seeds leads to imbibitional injury which is known to reduce the vigor in *Brassica*. Use of controlled deterioration revealed the proneness of yellow seeds to ageing and thus reduction in vigor of such seeds. Few yellow seeded cultivars showed resistance to such deterioration and thus could be used in improvement programme in *Brassica*.

Key words: Black and yellow seed coat color, Germination, Imbibition, Indian mustard, Melanin, Phenol

There are seven different edible oilseeds cultivated in India; among these nearly 24.02% of the total oilseeds production is contributed from rapeseed and mustard. The area and production of rapeseed-mustard in India is 6.7 mha and 7.87 mt, respectively (DAC 2013-14) and its contribution to world acreage and production is 19.29% and 11.27%, respectively (USDA 2012-13). The role of seed in agriculture sector is of prime importance in developing countries like India where the population and GDP (Gross Domestic Product) considerably depend on agriculture sector (Tyagi 2012). Water uptake (imbibition) is first and critical phase of seed germination. Imbibition is followed by initiation of biochemical changes and subsequently emergence of radical (Bewley and Black 1994). Imbibitional rate is one of the most critical steps in seed germination in which rapid uptake of water can cause imbibitional damages to the germinating seeds, whereas slower imbibitional rate may delay in germination. Therefore, rate of imbibition must be regulated to avoid the imbibitional damage as well as achieving timely emergence. The role of seed coat and its components such as coat color, phenol and melanin content in regulating the rate of imbibition and prolonging storability is well documented by various authors such as Duran and Retamal (1989) in *Sinapsis*, Kantar *et al.* (1996) in faba bean etc. Although it's well established fact that the quality and quantity of oil can be improved by the development of yellow-seeded cultivars as they contribute 5–7% more oil in the seeds of Indian mustard [*Brassica juncea* (L.) Czern and Coss] than the black seeded cultivars (Xu *et al*. 2010), the black colored seeds are known to have better resistance against imbibitional damage and better longevity as compared to yellow seeded cultivars (Zhang *et al*. 2006). It was found that the testa of yellow-seeded rapeseed lacked some abilities to protect the embryo against adverse environmental conditions and during artificial ageing leading

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to the poor storability. Such variation for seed coat color is also present in Indian mustard. The color of mature seeds varies from yellow to black. Considering the existence of variability for seed coat color in Indian mustard the study was undertaken to find out the differences for phenol, melanin content and its effect on rate of imbibition and vigor properties.

MATERIALS AND METHODS

Experiment was carried out during 2013-2015 in the laboratories of the Division of Seed Science and Technology and Division of Biochemistry, ICAR- Indian Agricultural Research Institute (IARI), New Delhi. The material had eight genotypes of Indian mustard. The material was selected on the basis of seed moisture and percentage germination. Among these eight genotypes, four genotypes were having black colored seed coat - Pusa Vijay, Pusa Mustard (PM) 28 (conventional type), PM 21 and PM 30 (quality type) and four having yellow colored seed coat - Navgold, NPJ189 (conventional type), Pusa Karishma, LES48 (quality type).

Estimation of Total Phenols was carried using the Folin-Catechu Reagent (Singleton and Rossie 1965) and melanin content was estimated as per the method given by Ye *et al.* (2001).

Before starting imbibitional studies all seeds were brought to 8% moisture in order to avoid any initial differences due to differential moisture content in genotypes. Five-hundred seeds were weighed and placed in 50 ml of distilled water at 25°C. Water uptake was measured as a percentage weight increase with mean of three replicates after 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 8^{th} and 24^{th} hours of imbibition (Chachalis and Smith 2000). For EC determination seeds were brought to 10% moisture from initial 8% before soaking in water (ISTA 2014) and then three replicates of 1 g seed were immersed in 100 ml de-ionized water at 25^0C. Observations were made at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th} and 24^{th} hour of soaking.

50 seeds were placed equidistantly on top of two layers of moist filter paper in Petri plates at 25^0C with eight replications (ISTA 2014). Radical emergence (2mm) were recorded on every day basis to calculate mean germination time using prescribed formula MGT= " (n*d)/ " (N) where; n= no. of seeds germinated on "d" days, d= day and N= Total germinated seeds (Nicholas and Heydecker 1968). Percentage of normal seedling was used to calculate standard germination. The vigor indices were computed adopting the method of Abul Baki and Anderson (1973).

All studies were carried out before and after controlled deterioration treatment. Initial moisture content of seeds was calculated to compute the weight of seeds at desirable moisture content (i.e. 20%). Seeds were then placed on moist germination paper, allowed to imbibe water and weighed repeatedly till it reaches to 20%. Seeds were then packed in foil packet and kept overnight at 7°C for uniform distribution of moisture. These packets were then placed in water bath at 45°C for 72 hr, cooled under running water and kept for drying in shade to bring it to original moisture (Powell 1995).

The data collected from the experiments on all parameters studied were subjected to statistical analysis using statistical package namely, SPSS package (version 10) and LSD were obtained. The contrast analysis (using lmatrix in SPSS), instead of multi-factorial CRD analysis, was performed for genotype for comparisons between black and yellow seeded genotypes. The variance ratio was calculated for significance at 5% level of probability in all cases.

RESULTS AND DISCUSSION

Pigments and imbibitional rate

There are various reports indicating the role of seed coat color in determining the rate of imbibition (Zhang *et al*. 2008, Duran and Retamal 1989, Kantar *et al*. 1996) in various crops. In *Brassica napus*, the pigment content of testa such as melanin greatly affects the imbibition properties and flooding tolerance of seeds. Duran and Retamal (1989) emphasized that white seeds imbibe more rapidly than colored seeds of legumes. Percentage imbibition rose more rapidly in yellow coated than in dark brown seeds of Honey locust (Ertekin and Kirdar 2010). In soybeans, seed with dark colored testa have been associated with a slower rate of water uptake (Tully *et al.* 1981, Kuo 1989). Such relation of slower water uptake is correlated with pigments such as phenol and melanin. Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids and play important roles in various activities in plants. The phenolic compounds or the oxidized flavonoids which are impermeable to water can reduce seeds solute leakage and imbibition damage with slow water uptake during imbibition stage. Pigmentation of the seed coat in *Brassica* species occurs mainly due to deposition of polyphenols. Studies on turnip rape indicated that yellow seed coats have reduced palisade layers and smaller cells thus decreased the proportion of testa to whole seed causing faster imbibition. Similar results were found in our studies wherein the average phenol content in black seeded genotypes was about 61% more (11.119 mg GAE/g of seeds) over the yellow seeded genotypes (6.867 mg GAE/g of seeds). The melanin content in black colored genotypes (58.80 U) was almost twice as compared to yellow colored seeds (29.15 U) (Table 1). We found that there was slower uptake by black seeded and conventional seeds. Following controlled deterioration, the rate of imbibition increased in black seeded genotypes but not in yellow seeded genotypes. However, the imbibition rate in black seeded genotypes was still much lower than that of yellow seeded genotypes. Decrease in imbibition of deteriorated yellow seeded genotypes could be attributed to lowered ability of its testa to withhold moisture. This could also be correlated with increased values of electrical conductivity in yellow seeded genotypes from 95.54 µS/cm/g (in fresh seeds) to 170.82 µS/cm/g (in CD seeds) at 24^{th} hour of soaking. Thus, it might be that seed coat of yellow seeded genotypes was more susceptible to

Table 1 Seed total phenol content and melanin content in fresh and controlled deteriorated (CD) black and yellow seeded Indian mustard genotypes

Genotype	Total phenol (μg GAE/g seed)		Melanin (U)	
	Fresh seeds	CD seeds	Fresh seeds	CD seeds
Black				
Pusa Vijay	10.56	10.63	60.38	62.02
PM 28	12.37	14.37	59.38	65.76
PM 21	9.75	13.03	56.30	56.51
PM 30	11.8	14.73	59.15	63.11
Mean	11.12	13.19	58.80	61.85
Yellow				
Navgold	7.21	11.13	32.13	33.29
NPJ 189	6.00	6.53	28.69	29.44
P. Karishma	7.51	13.03	27.88	28.39
LES 48	6.74	9.3	27.91	35.56
Mean	6.87	10.00	29.15	31.67
Grand Mean	8.99	11.6	43.98	46.76
CD (P=0.05)	1.21	1.08	3.9	7.24

deterioration and loose its ability to regulate water flow and withhold moisture under free available water. Our results are in support with previous findings and suggest the definite role of phenols in determining seed coat color and imbibitional rate in different types of Indian mustard genotypes. Following controlled deterioration the phenolic content was found to be increased in all different types of Indian mustard genotypes and detailed investigation is needed to know the reasons for increase in total phenols during seed deterioration.

Electrical conductivity

The result for imbibitional rate and damage could be substantiated by EC results. EC was highest for yellow seeded quality genotype and lowest for black seeded conventional genotypes in fresh as well as in CD seeds. The maximum value was recorded in yellow seeded genotype Pusa Karishma (63.27 μS/cm/g) at initial stages which continued till 24 hr whereas black seeded genotype PM 28 recorded the lowest EC value of 47.83 μS/cm/g. In CD seeds, the yellow seeded quality genotypes recorded highest EC over conventional black and yellow as well as quality type black seeded genotype. There is a negative linear relationship between electrolyte leakage and seed vigour as reported in many crops like in wild species of *Brassicaceae* (Sara *et al*. 2011); in garden pea (Matthews and Bradnock 1967); in legumes (Sivritepe and Dourado 1995); in *B. napus* (Zhang *et al.* 2006). The electrical conductivity was found to be higher in yellow seeded and quality type genotypes than in black seeded and conventional genotypes.

Germination, mean germination time and seedling vigor

Efficient seed germination, early, rapid and uniform seedling emergence are determinant of effective plant stand in field (Harris 1996). Based on contrast analysis, it was observed that germination percentage in fresh seeds shows non-significant differences between black and yellow seeded genotypes. In controlled deteriorated seeds, germination percentage was higher in black seeded genotypes (85%) as compared to yellow seeded genotypes (61%) (Table 2). The percent germination in fresh seeds of conventional and quality type of genotypes was non-significant. Whereas, in CD seeds the conventional type of genotypes recorded 95.0% germination and found to be significantly higher than quality type of genotypes (62.0%). The ANOVA revealed significant differences between conventional and quality types of genotype in fresh as well as CD seeds where conventional were found to be superior (Table 2). Vigour index I in controlled deteriorated seeds was significantly higher in black seeded genotypes (1189.14) than in yellow seeded

Table 2 Germination, mean germination time and vigour index in fresh and CD black and yellow seeded Indian mustard genotypes

Genotypes	Total germination percentage		Mean germination time (days)		Vigour index-I		Vigour index-II	
	Fresh seeds	CD seeds	Fresh seeds	CD seeds	Fresh seeds	CD seeds	Fresh seeds	CD seeds
Black								
Pusa Vijay	96.0(78.5)#	82.0(64.93)	1.62	2.10	1366	917	1.47	1.26
PM 28	95.0(77.12)	89.0(70.67)	1.54	2.35	1117	1173	1.55	1.56
PM 21	90.0(71.60)	78(62.06)	1.43	2.09	1189	1267	1.11	0.98
PM 30	92.0(73.61)	91.5(73.09)	1.91	2.24	1327	1078	1.78	1.64
Mean	93.3	85.1	1.63	2.20	1250	1108.7	1.48	1.36
Yellow								
Navgold	94.0(75.86)	92.5(74.14)	1.2	2.13	1557	1407	1.66	1.58
NPJ 189	90.0(71.30)	75.5(60.36)	1.33	2.22	1156	1000	1.10	1.04
P. Karishma	96.0(78.50)	60.5(51.09)	1.05	2.22	1037	772	0.93	0.81
LES 48	91.0(72.58)	15.0(22.80)	1.26	2.60	1133	151	1.01	0.17
Mean	92.8	60.9	1.21	2.29	1221	832.5	1.18	0.90
Grand Mean	93.0(74.70)	73.0(58.72)	1.42	2.24	1236	971	1.33	1.13
CD (P=0.05)	4.0	6.2	0.18	0.21	115	200	0.30	0.25

Table 3 Correlation between vigour attributes in fresh seeds of Indian mustard genotypes

	MGT	Ger	SV I	SV II	Melanin	Phenol
MGT	1	-0.06	0.24	0.56**	0.78**	0.73**
Ger		1	0.12	0.24	0.11	0.2
SV I			1	0.59**	0.18	0.11
SV II				1	0.50	0.54**
Melanin					1	0.91**

Table 4 Correlation between vigour attributes in CD seeds of Indian mustard genotypes

	MGT	Ger	SV I	SV II	Melanin	Phenol
MGT	1	-0.67**	-0.60**	-0.57**	-0.14	-0.13
Ger		1	0.95**	0.93**	0.43	0.36
SV I			1	0.93**	0.30	0.32
SV II				1	0.50**	0.44
Melanin					1	0.59**
Phenol						1

genotypes (852.02). In fresh seeds, the vigor index II was significantly higher in black seeded genotypes (1.48) than in yellow seeded genotypes (1.18) (Table 2) as well as in conventional types (1.45) than quality types of genotypes (1.20). After controlled deterioration, reduction in vigor index II was lower in black seeded genotypes compared to yellow seeded genotypes with significantly higher in conventional types (1.36) than in quality types (0.89). The time of seedling emergence to seedling establishment is cited as most sensitive part of plants life cycle by many scientists (Reisdorph and Koster 1999, Buitink *et al*. 2004). Mean germination time refers to time taken by seeds to germinate and is one of the physiological indicators of seed vigor. In fresh seeds, the black seeded genotypes (mean= 1.63 days) recorded higher MGT than yellow colored seeded genotypes (mean= 1.21 days). After controlled deterioration, MGT increased for all genotypes but increase in MGT was relatively higher for yellow seeded genotypes (Table 2). Whereas, MGT of conventional and quality types of genotype were found to be at par both in fresh seeds and controlled deteriorated seeds. Longer MGT is an indication of longer Phase II thus poor seed quality in maize (Hosseini *et al*. 2009, Matthews *et al*. 2011), in milk thistle (Parmoon *et al*. 2013). In fresh seeds of Indian mustard, MGT was positively correlated to pigments and seed vigour (Table 3). In fresh seeds, although mean values showed no significant differences between black and yellow seeded genotypes. That means in black seeded genotypes, the rate of imbibition was slow and therefore, it has longer phase I (as suggested by imbibitional data) resulting in less imbibitional injuries and seed leachate (as shown by EC values). However, in yellow seeded genotypes, the MGT observed was considerably less, as a result the seeds underwent faster completion of phase I and greater imbibitional damage. Repairing such damages takes more time and causes longer MGT. In CD seeds, MGT was negatively correlated to seed vigour (Table 4). Following deterioration increase in MGT was higher in yellow seeded genotypes revealing that the effect of ageing (controlled deterioration) was more pronouncing on yellow seeds which takes more time to recover such damages (in addition to imbibitional injury) resulting in delayed germination and poor vigour. Correlation suggested that it was negatively correlated with germination and vigour. In our study, we found that yellow seeded and especially quality type genotypes showed drastic reduction in germination percentage and vigour following controlled deterioration. This differential response for germination and vigour shows the superiority of black seeded and conventional genotypes in mediating imbibitional damages by slower rate of water uptake over yellow seeded and quality type genotype. Although in general, the yellow seeded genotypes showed higher viability loss and low vigour. The yellow seeded variety, Navgold was found to be more resistant to such damages. Thus, further studies are needed to distinguish the mechanism operating in this yellow seeded variety in order to have their role in breeding programme for improving yellow seeded genotypes.

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