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Correlation Between Superoxide Content and Radicle Length During Seed Germination in Different Types of Indian Mustard

NIPA BISWAS[1], SANGITA YADAV[1]*, NAVINDER SAINI[2], SHIV K YADAV[1], ANIL DAHUJA[3], SUJATA VASUDEV[2] AND DK YADAVA[1]

[1]Division of Seed Science and Technology, [2]Division of Genetics, [3]Division of Biochemistry
ICAR-Indian Agricultural Research Institute, New Delhi 110 012
*sangitaydv@gmail.com

ABSTRACT: Reactive oxygen species (ROS) are natural products of metabolism. They originate from either the incomplete or partial reduction of oxygen, which ultimately leads to the formation of superoxide anion and hydrogen peroxide. The involvement of superoxide anion has been shown by many workers to regulate cell proliferation and differentiation during growth of radicals. The experiment was conducted to study the changes in content of superoxide anion and explore the possible role of superoxide anion in increasing radicle length during the germination which could substantiate the vigour differences in various Indian mustard genotypes. The results showed that the superoxide anion was positively correlated with radicle length which in turn was positively correlated with seed vigour index-I. The single zero and conventional genotypes of Indian mustard were having higher content of superoxide anion during 36 hours of germination when radicle length was more than 2 mm. In case of double zero type of Indian mustard both radicle length and superoxide content was significantly lower. Thus, the lower content of superoxide could be considered as a possible reason for insignificant increase in radicle length in double zero type of Indian mustard which further may be the cause for their lower seed vigour index-I than the conventional and double zero genotypes of Indian mustard.

Keywords: Indian mustard, Germination, Radicle length, Seed vigour indices, Superoxide anion

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the most important oilseed crops, which occupy 85% area among the four oleiferous brassicas (*B. rapa, B. napus, B. juncea* and *B. carinata*). There are several uses but it mainly serves as a good source of edible oil. The oil content generally varies from 30-35%. Although the crop accounts for nearly one-third of the oil produced in India but there is presence of undesirable substances like erucic acid (35.7–51.4%) in oil and glucosinolates (49.9–120.3 ìmole/g) in defatted seed meal. High erucic acid in edible oils has been reported to impair myocardial conductance, and causes lipidosis in children and increases blood cholesterol [1]. The cleavage products from hydrolysis of glucosinolates are detrimental to animal health because they reduce the feed palatability, interfere with the iodine uptake and thus, reduce the feed efficiency especially in non-ruminants such as pigs and poultry. Therefore, lowering the content of erucic acid in oil and glucosinolates in defatted oil cakes has been one of the major objectives in brassica breeding programmes. The Indian mustard genotypes developed with low erucic acid (<2%) are popularly called as single zero, while double zero term is used to indicate genotypes with lower content of both anti-nutritional factors *i.e.*, low erucic acid (<2%) and glucosinolates content (<30 µmoles/g of defatted cake). The single zero and double zero genotypes of Indian mustard are known as quality mustard genotypes. Although these quality mustard genotypes are nutritionally enriched but they have been reported to suffer from low vigour [2].

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Seed germination is a complex process which starts from imbibition and terminate with the elongation of embryonic axis. It is influenced by a number of factors like reactive oxygen species (ROS) and nitric oxide (NO), hormone like ABA, gibberellic acid, ethylene and other factors like HCN, Ca^{+2} etc. ROS are continuously produced during seed development from embryogenesis to germination [3]. Despite the well-known deleterious effect of ROS, a model called "oxidative window" has been suggested in which closely regulated ROS generation is required for seed germination [4].

The involvement of both superoxide anion and hydrogen peroxide (H_2O_2) content has been shown by many workers to regulate cell proliferation and differentiation during growth of radicals [5]. Superoxide anion and H_2O_2 are distributed differentially within the root tissues of *Arabidopsis*. Superoxide principally accumulates in the cells of expanding meristem, while H_2O_2 accumulates in the elongation zone of the root tissues. An overlap of both types of ROS is observed within the "transition zone". A gradient of superoxide to H_2O_2 in the root controls the transition between cell proliferation and differentiation. The daughter stem cells divide in the proliferation zone, where the high level of superoxide has been characterized. Once after reaching into transition zone, they come to the increased H_2O_2 level and stop dividing. After that they initiate to elongate and differentiate in the differentiation zone. So the size of the meristem is highly regulated by balancing superoxide/ H_2O_2 content. This study hypothesized the relationship between content of superoxide anion and radicle length which in turn could be correlated with seed vigour indices.

MATERIALS AND METHODS

The seeds of Indian mustard were collected from the Division of Genetics, Indian Agricultural Research Institute, New Delhi. These included three conventional genotypes; Pusa Bahar, PM 28 and BEC 144 and six quality genotypes. Among the quality mustard, three genotypes each were of single zero; Pusa Karishma, PM 24 and PM 30 and double zero; PDZ 1, PDZ 4 and PDZ 5 types.

Germination test: Standard germination test was conducted following ISTA rules [6]. Four replications of 50 seeds each of all genotypes were placed at equidistance on top of two layers of moist filter paper in Petri plates and kept at 25±0.5^{0}C. First count was taken on 5th day and final count done on 7th day. The evaluation was done by categorizing them into normal seedling, abnormal seedling, hard and dead seeds. Average numbers of normal seedlings was used to calculate standard germination and expressed in percentage.

Seedling length: Ten normal seedlings were randomly selected from the germination test on the day of final count. The length between the collar region and the tip of the primary shoot was measured as shoot length and the length between collar region and tip of the primary root was measured as root length. Total seedling length (cm) was calculated by adding shoot and root lengths.

Seedling fresh weight: Cotyledons of ten normal seedlings which were included to measure length were removed and their weight (g) was recorded.

Seedling dry weight: After recording the fresh weight, the seedlings were placed in wax paper and put for drying in a hot air oven at 70 ± 1ºC for 48 h. Seedling dry weight (g) was measured after cooling for 30 minutes in a desiccator with silica gel.

Vigour indices: Seedling vigour indices were calculated using the formula [7]:

Vigour index-I (SVI-I) = Germination (%) × Total seedling length (cm).

Vigour index-II (SVI-II) = Germination (%) × Seedling dry weight (g).

Mean germination time (MGT): For calculation of mean germination time (MGT), four replications of 50 seeds each of all genotypes were placed at equidistance on top of two layers of moist filter paper in Petri plates as in germination. Counts were made on every day for seeds with more than 2 mm radicle coming out of it. Results were calculated with mean of replicates using formula [8]:

MGT= Σ (N*d)/ Σ (N) where; N= no. of seed germinated on'd' days, d= day number.

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Radicle length: The radicle length of the germinated seeds was recorded after 24 hours until 48 hours of germination. Ten normal seeds were selected randomly from the petri plate. The length of the protruded radicles was taken using vernier caliper and the average radicle length was measured (mm).

Superoxide anion ($O_2^{\bullet-}$) analysis: The content of superoxide anion was measured at 0 hour and from 24 hours to 48 hours at an interval of 6 hours of imbibition. 1g seeds were homogenized in pre-cooled 0.2M phosphate buffer containing 1 mM diethyl dithio carbamate (pH 7.2). The homogenate was centrifuged at 10000 rpm for 20 minutes. Supernatant was used immediately for the estimation of superoxide radical. The superoxide anion ($O_2^{\bullet-}$) was measured by its capacity to reduce nitrobluetetrazolim and formation of blue coloured formazone. After incubating the reaction mixture at 30°C for 10-15 min, superoxide content was measured spectrophotometrically at 540nm and the result was calculated as ΔA_{540}/min/gFW [9].

Data Analysis: The test of significance and *post-hoc* analysis *of data* was done using SPSS 10.0 software.

RESULTS AND DISCUSSION

Germination and vigour

The independent existence of a plant starts with seed germination. It is a complex phenomenon of development involving a number of morphogenetic as well as physiological changes occurring under a tight regulation [10]. The phenomenon of germination and establishment has been considered as the most critical phase in the life of plant by many scientists [11]. The nine genotypes of Indian mustard belong to three different types of Indian mustard viz., conventional type, single zero type and double zero type. There was no significant difference among the genotypes as well as different types of Indian mustard in terms of germination percentage (Table 1).

This could be because of the fact that the seeds were freshly harvested (2015-2016). But the genotypes showed significant differences in terms of seed vigour and other seed quality parameters. The seed vigour indices give a better prediction of field emergence than the germination percentage of seeds under laboratory condition alone. The genotype PM 30 showed significantly higher SVI-I (1481.71) as well as SVI-II (1.81) whereas the

Table 1. Seed quality parameters of different genotypes of Indian mustard

GENOTYPE	Germination %	SVI-I	SVI-II	MGT (Days)
Pusa Bahar	96.00* (78.76)^{a}	1381.8^{c}	1.66^{bc}	1.35^{b}
PM-28	96.67 (79.64)^{a}	1323.7^{c}	1.52^{b}	1.32^{b}
BEC-144	95.33 (77.88)^{a}	1248.9^{bc}	0.90^{a}	1.56^{c}
Pusa Karishma	96.67 (79.64)^{a}	1255.9^{bc}	0.96^{a}	1.09^{a}
PM-24	96.00 (78.76)^{a}	1467.5^{c}	1.12^{a}	1.32^{b}
PM-30	96.67 (79.64)^{a}	1481.7^{c}	1.81^{c}	1.32^{b}
PDZ-1	94.67 (76.74)^{a}	939.5^{a}	1.03^{a}	1.77^{d}
PDZ-5	96.00 (78.76)^{a}	1195.4^{abc}	1.12^{a}	1.34^{b}
PDZ-4	94.00 (75.99)^{a}	1069.0^{ab}	1.04^{a}	2.10^{e}
Mean	95.78	1262.6	1.24	1.46
CD (p=0.05)	NS	97.43	0.09	0.05

*Figures in parentheses are arcsine transformed values
NS: Non significant
* Figures not sharing the same letters in the same column differ significantly at $p< 0.05$

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genotype PDZ 1 showed the lowest SVI-I (939.5). Conventional genotypes Pusa Bahar and PM 28 also showed higher seed vigour indices. The differences in vigour can also be observed among various types of Indian mustard. The single zero mustard showed a higher SVI-I (1401.70) and conventional mustard showed higher SVI-II (1.36) whereas the double zero mustards were poor both in terms of SVI-I and SVI-II (Table 2).

Table 2. Seed quality parameters of different types of Indian mustard

TYPE	Germination %	SVI	SVII	MGT (Days)
Conventional	96.00 (78.76)*	1318.16	1.36	1.41
Single zero	96.44 (79.35)	1401.7	1.29	1.24
Double zero	94.89 (77.16)	1067.96	1.06	1.74
Mean	95.78 (78.42)	1262.61	1.24	1.46
CD (p=0.05)	NS	97.43	0.09	0.05

*Figures in parentheses are arcsine transformed values
NS: Non significant

Rapid and uniform seed germination decides crop establishment and levels of crop production. The lower MGT indicates high speed of germination of the seeds. The yellow seeded genotype Pusa Karishma showed the least MGT (1.09 days) whereas the genotype PDZ 4 showed maximum MGT (2.10 days). Among the various types of Indian mustard, single zero mustard were having significantly lower MGT followed by conventional type and double zero mustard. A slower rate of germination is an early physiological expression of seed ageing and the major cause of reduced vigour. Moreover, MGT showed a negative correlation with SVI-I (-0.616**) (Table 3).

It may because that the genotypes having more MGT require a longer period for repairing and thus the preparation for radicle protrusion. The similar relationship of SVI and MGT has also been shown in lettuce [12]. The double zero type of mustard which had poor vigour were found to have higher MGT and thus, are possibly more prone to deterioration.

Table 3. Correlation among various traits of mustard

	SVI-I	SVI-II	MGT	36hrAL	36hrSO
SVI-I	1				
SVI-II	0.477(*)	1			
MGT	-0.616(**)	-0.31	1		
36hrAL	0.606(**)	0.17	-0.882(**)	1	
36hrSO	0.29	-0.22	-0.413(*)	0.478(*)	1

(AL: Radicle length; SO: Super oxide radical)

Radicle length

The root axis length of 2mm is essential to consider the seed as germinated. The change in radicle length of germinated seeds can be divided in two phases. During the initial hour of radicle protrusion or the first phase, the increase in radicle length is due to the elongation of cells whereas during second phase the increase in radicle length is supported by the mechanism of cell division [10]. In single zero mustard, it came at 30 hours of germination, while in conventional type Indian mustard it was observed around 36 hours (Fig. 1).

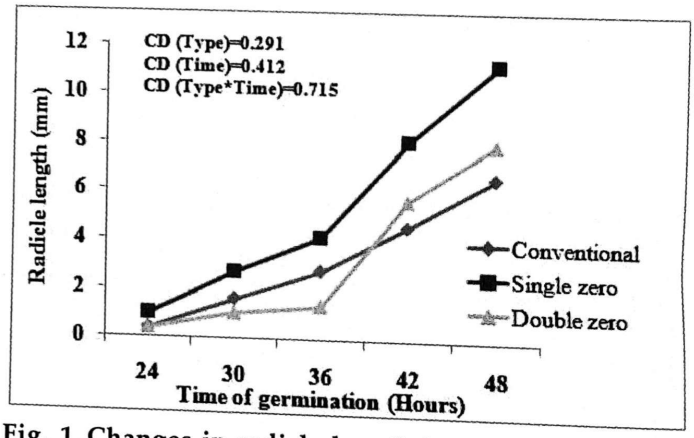

Fig. 1. Changes in radicle length in different types of Indian mustard at different duration of germination

During 30 hours of germination, Pusa Karishma had radicle length of 3.17 mm which was increased to 4.73 mm after 36 hours of germination. The mean radicle length of single zero genotype of Indian mustard after 36 hours was observed to be significantly higher (4.13cm) followed by conventional genotypes (2.78mm). The value was significantly lower for double zero genotypes (1.37mm) (< 2mm). It could be possible that in single zero genotypes both the cell

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elongation and cell division started much earlier than the conventional and double zero genotypes of Indian mustard which is also supported by lower MGT of single zero genotypes. The MGT of single zero mustard was significantly lower and it shows a negative correlation with SVI-I (Table 3), while radicle length showed a positive correlation with SVI-I (0.606**).

Superoxide radical content

Among all types of Indian mustard, superoxide content in dry seed was the highest in double zero type followed by single zero and conventional type (Fig. 2).

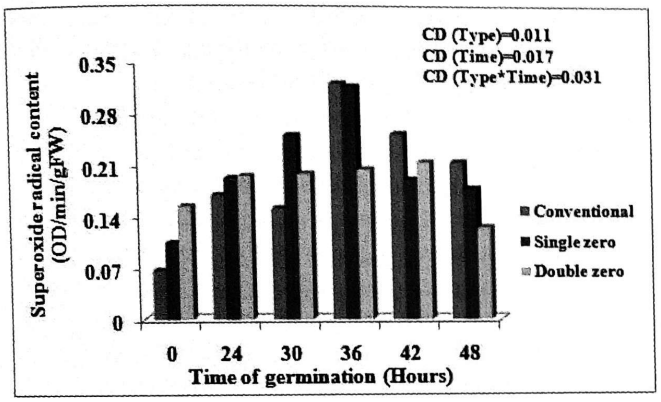

Fig. 2. Change in superoxide content in different types of Indian mustard

Presence of high reactive oxygen species content is involved in seed deterioration in dry state. The genotypes; PDZ 1 and PDZ 4, had high content of superoxide radical in dry seeds and were found to be less vigorous and that was also supported by having maximum MGT. Increased free radicals in dry state could be one of the probable reasons of low vigour and low storability in double zero type mustard seeds [2].

During 30 hours of germination the superoxide radical content was the highest in Pusa Karishma and at the same time the radicle length was also the highest for the genotype. The mean content of superoxide radical was also the highest in single zero type Indian mustard during these hours of germination. The requirement of superoxide radicals for cell division at the terminal part of the radicle during germination has also been reported by other workers [10]. The gradual increase in the ability of germinating seeds to generate superoxide radicals have been shown to positively correlate with radicle growth, in cell division for growth and differentiation [13]. During 36 hours of germination when 2mm radicle protrusion occurred in conventional type mustard, the content of superoxide anion was found to be significantly increased while it was still significantly lower in double zero mustard during this period. However, in double zero genotypes the mean increase in superoxide radical content was found at 42 hours when radicle length increased to 2 mm.

Thus, this study indicates that the increase in superoxide content lead to increase in radicle length (also indicated by positive correlation between superoxide radical and radicle length) and has correlation with SVI-I and MGT. Moreover, the reduced vigour in double zero type Indian mustard compared to conventional and single zero type could be due to reduced superoxide production thereby reduced radicle length.

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