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Effect of ROS Inhibitors and Scavengers on Seed Quality Parameters of Indian Mustard

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ABSTRACT: The present study was conducted to evaluate the effect of different ROS inhibitor and scavenger on the seed quality parameters of Indian mustard. Different concentrations of DPI, $ZnCl_2$, $CuCl_2$ and KI were tested. The concentrations which reduced the seed vigour index-I (SVI-I) by 50% of the normal were selected for further comparison with control. 0.01mM DPI, 20mM $ZnCl_2$, 0.5mM DPI and 5mM KI concentrations were selected and compared with the control (distilled water). The results showed that there was no effect on physiological seed germination at these concentrations but other parameters of seed quality were reduced significantly. The reduction in root growth was more than the shoot growth and such reduction was significantly higher in DPI and $CuCl_2$ treatment. Reduction in shoot and root length resulted in reduction in total seedling length and hence the SVI-I got reduced. At the concentrations where SVI-I got reduced by 50%, the reduction in seed vigour index-II was not much significant. Minimum reduction in SVI-II and dry weight was observed in KI treatment which may be associated with scavenging of hydrogen peroxide (H_2O_2), since H_2O_2 stimulate reserve mobilization process and KI is a scavenger of H_2O_2. So it can be concluded that inhibition in ROS production or scavenging of ROS during germination affect the seed quality parameters and restrict the growth and development of seedling. Such inhibitory effect is more on root growth than the shoot growth and SVI-I get more hampered than SVI-II.

Keywords: Germination, Indian mustard, ROS, Seed vigour indices

Reactive Oxygen Species (ROS) which were long being considered as only deleterious molecule are now considered to play important role in various physiological activities in plant life [1]. Seed germination is the most important phase in plant life cycle which assures an independent existence of the plant. It is a complex process which starts from imbibition and terminate with the elongation of embryonic axis. Strictly regulated concentrations of ROS are currently viewed as being essential for germination [2]. It was proposed that the germination is completed only when the ROS content is within an oxidative window that allows ROS signalling. Cellular antioxidant systems maintain intracellular redox homeostasis, preventing the accumulation of toxic amounts of ROS while allowing ROS mediated signalling to occur [3]. At the same time the changes in ROS homeostasis within cell would play an important role in the perception of external environmental factors by the seeds and proceed towards germination, and hence act as signalling molecule for the completion of seed germination. The production of ROS during seed germination in fact represented an active, beneficial biological reaction that was connected with high germination capacity and vigorous seedling development. This was proved by observing a strong rise in ROS released in the healthy and actively germinating seed, which coincided with the period in which the expanding embryo ruptured the coat and becomes exposed to environmental factors [4]. In situ localization of ROS also revealed the location specific production of different ROS which are important for a successful seed germination and post germination seedling growth [5].

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the most important oilseed crops, which occupy nearly 80% area in *rabi* season among the four oleiferous brassicas. The present study was done to investigate the effect of different ROS inhibitors (either inhibit the production of ROS or scavenge the produced ROS) on seed quality parameters of Indian mustard.

MATERIALS AND METHODS

One of the conventional genotype of Indian mustard Pusa

Vijay was used for the study. The seeds were stored dry under laboratory condition until the experiment began.

Selection of inhibitors concentrations

Four chemicals namely zinc chloride ($ZnCl_2$), copper chloride ($CuCl_2$), potassium iodide (KI), and diphenyleneiodonium chloride (DPI) were used for the study. Different concentrations of the selected chemical were used to treat the seed. For a chemical a single concentration was selected for further comparison and analysis which could reduce the seed vigour index-I by 50 per cent (Table 1 to 4) than the control.

Germination test

Standard germination test was conducted following ISTA rules with control (distilled water) and inhibitors [6]. Three 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 20°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.

Shoot length, root length, seedling length and root to shoot ratio

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. The root to shoot ratio was also calculated using the root length and shoot length of the selected 10 seedlings.

Seedling fresh weight and dry weight

Leaves and cotyledons of ten normal seedlings which were used to measure length were removed and their weight (g) was recorded. After recording the fresh weight, the seedlings were placed in wax paper and put for drying in a hot air oven at 70 ± 1°C 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).

Data analysis

The results of growth parameters presented are average of three replications. Data were statistically analysed by subjecting to ANOVA and probability values were determined for significance using MSTAT C. Standard errors (SE) around the mean value are shown in the figures as vertical error bars.

RESULTS AND DISCUSSION

Selection of inhibitors concentrations

Diphenyleneiodonium chloride (DPI) and $ZnCl_2$ are inhibitor of NADPH oxidase [8, 9] which is a plasma membrane embedded enzyme catalyse the production of superoxide radicals. $CuCl_2$ is a scavenger for superoxide radical [10] and KI is H_2O_2 scavenger [11]. Various concentrations of the mentioned chemicals were used for the experiment, but those concentrations at which there was physiological germination but the germinated seeds failed to grow into a seedling were not used. Only those concentrations at which germinated seeds could grow into a seedling were shown in the tables. The concentrations at which the physiological germination (protrusion of radicals) were at par with the control only used for the experiment.

For $ZnCl_2$ five different concentrations were used to soak the filter paper for germination (Table 1). Among these five concentrations, 20mM concentration of $ZnCl_2$ was able to reduce the SVI-I below 50% of the control. So this concentration was selected for further comparison. Among eight different concentrations of DPI, 0.01mM was showing SVI-I below 50% (Table 2). The value was 0.5mM for $CuCl_2$ (Table 3) and 5mM for KI (Table 4).

Effect of ROS inhibitors on seed quality parameters

Germination

Seed germination is a complex phenomenon of development involving a number of morphogenetic as well as physiological changes occurring under a tight regulation [5]. There was no effect on physiological seed germination by the used concentrations of DPI, $ZnCl_2$, $CuCl_2$ and KI (Data not shown). The germinated seeds were able to grow into seedling but with reduced growth.

Table 1. Standardization of $ZnCl_2$ concentration based upon reduction in seed quality in comparison to control

$ZnCl_2$ concentrations	Fresh wt	Dry wt	Shoot length (cm)	Root length (cm)	Seedling Length (cm)	Root/ Shoot ratio	SVI-I	SVI-II
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
5 mM	81.92	98.68	77.67	69.67	71.53	92.34	71.53	98.68
10 mM	78.63	96.69	66.99	54.73	57.57	89.2	57.57	96.69
20 mM	76.53	88.08	65.78	37.73	44.23	57.02	44.23	88.08
30 mM	54.83	82.12	61.65	24.69	33.26	40.48	33.26	82.12
40 mM	54.32	80.79	58.5	19.63	28.64	34.46	28.64	80.79
50 mM	40.18	67.55	38.83	12.16	18.35	31.73	18.35	67.55

Table 2. Standardization of DPI concentration based upon reduction in seed quality in comparison to control

DPI concentrations	Shoot length	Root length	Seedling length	Root/ Shoot ratio	Fresh Weight	Dry Weight	SVI-I	SVI-II
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.005 mM	72.45	58.21	61.51	81.79	98.08	90.4	61.51	90.4
0.01 mM	69.9	27.51	37.34	40.39	92.88	89.07	37.34	89.07
0.02 mM	60.68	17.47	27.49	29.08	71.84	87.75	27.49	87.75
0.03 mM	60.07	16.59	26.67	28.38	63.23	86.42	26.67	86.42
0.04 mM	57.16	14.65	24.51	26.19	64.93	84.77	24.51	84.77
0.05 mM	54.73	11.25	21.33	21.53	62.82	79.47	21.33	79.47
0.075 mM	45.39	8.83	17.3	20.25	51.84	72.85	17.3	72.85
0.1 mM	33.01	5.53	11.9	16.9	27.76	69.87	11.9	69.87

Table 3. Standardization of $CuCl_2$ concentration based upon reduction in seed quality in comparison to control

$CuCl_2$ concentrations	Fresh wt	Dry wt	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Root/ Shoot ratio	SVI-I	SVI-II
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.25 mM	85.57	90.26	98.79	37.29	51.52	38.18	51.52	90.26
0.5 mM	70.78	82.12	78.16	25.93	38.04	37.41	38.04	82.12
1 mM	66.69	77.48	73.54	13.41	27.35	19.14	27.35	77.48
2 mM	62.89	68.21	64.08	9.08	21.83	13.37	21.83	68.21

Table 4. Standardization of KI concentration based upon reduction in seed quality in comparison to control

KI concentrations	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root/ Shoot ratio	Fresh weight	Dry weight	SVI-I	SVI-II
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
5 mM	58.13	44.95	48.00	82.71	72.93	95.36	48.00	95.36
10 mM	49.64	30.92	35.26	67.38	65.78	85.76	35.26	85.76
20 mM	45.87	23.26	28.50	51.61	58.63	77.48	28.50	77.48
30 mM	43.69	21.14	26.36	50.80	56.15	72.19	26.36	72.19

Above these concentrations physiological germination was there but germinated seeds failed to grow into a seedling.

Shoot length, root length and seedling length

At the concentrations where SVI-I reduced by 50%, significantly lower shoot length was observed in case of KI treatment (Figure 1) and the reduction was 41.87% from the control. All the treatment showed significantly lower root length than the control. This is possibly because the effect of ROS is more pronounced in root growth than shoot length [12]. The highest reduction in root length was observed in $CuCl_2$ treated seedlings which was statistically at par with the DPI treatment (Figure 2). In terms of seedling length also $CuCl_2$ and DPI treatments were statistically at par and the highest reduction in seedling length was observed in DPI treated seeds (Figure 3). DPI is a potent inhibitor of NADPH oxidase. So NADPH oxidase is the most important source for ROS production at least during initial days of seedling growth [13]. Inhibition in NADPH oxidase activity resulted in lower production of superoxide radicals which may be

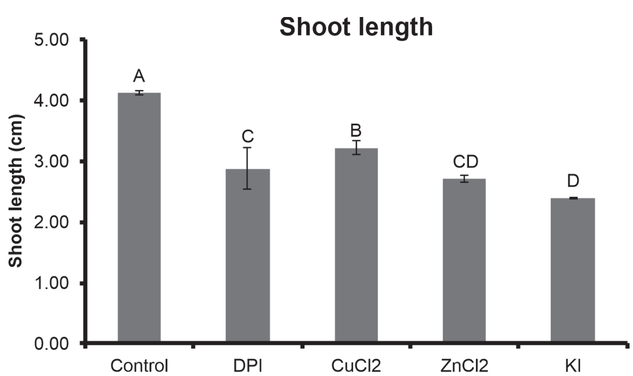

Figure 1. Effect of ROS inhibitors and scavengers on shoot length of Indian mustard

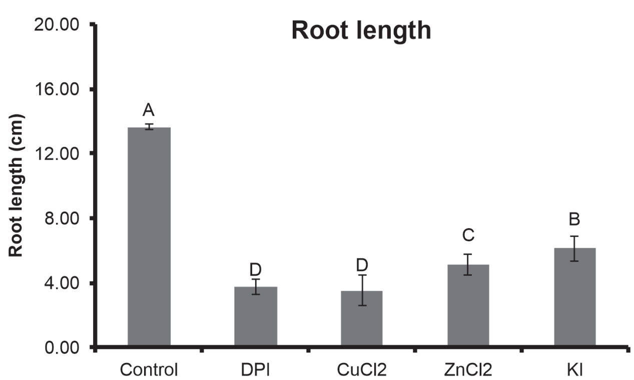

Figure 2. Effect of ROS inhibitors and scavengers on root length of Indian mustard

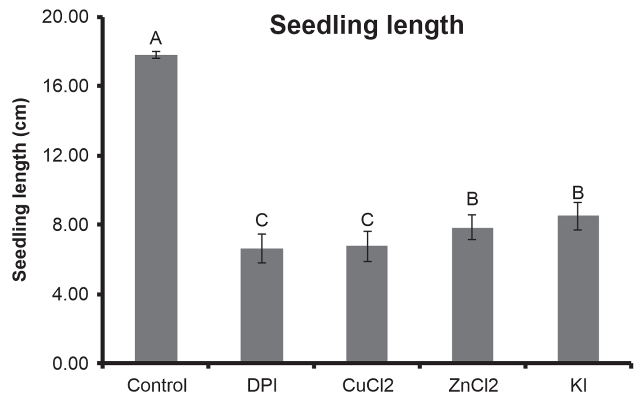

Figure 3. Effect of ROS inhibitors and scavengers on **s**eedling length of Indian mustard

resulted in restricted growth of the seedlings. It mainly effects the post-germinative growth of the seedlings in Indian mustard. Similar observation was in moong bean [5]. Accumulation of superoxide radicals in the growing tip of the radical is very important. The initial burst of superoxide production is very critical for the breakage of cell wall and protrusion of radical whereas the second burst is associated with the radical elongation [14, 15]. So scavenging of superoxide by $CuCl_2$ may supressed the level of superoxide radical below the oxidative window level which was not sufficient to stimulate the physiological role and the growth got returded. At the same time there was a burning symptom at the tip of the radical which was more intense in higher concentrations (Figure 4).

Root to shoot ratio may decrease either due to decrease in root length or increase in shoot length. In treated seeds, both shoot length and root length decreased where

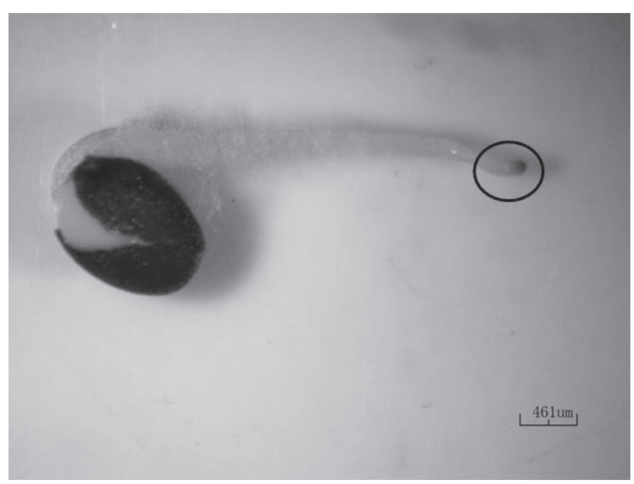

Figure 4. Burning symptom at the radical tip of $CuCl_2$ treated seed of Indian mustard

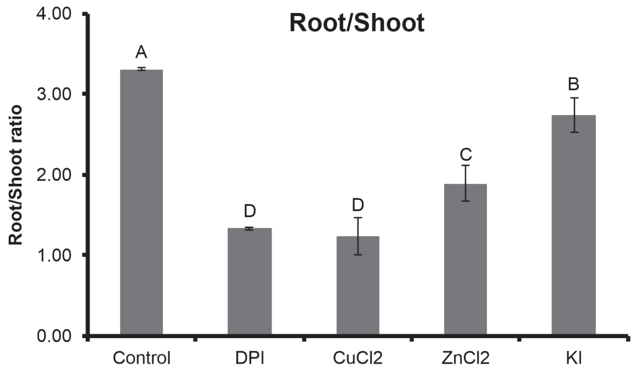

Figure 5. Effect of ROS inhibitors and scavengers on root/shoot ratio of Indian mustard

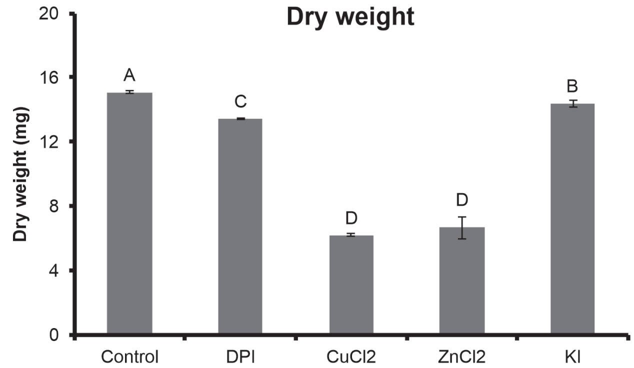

Figure 7. Effect of ROS inhibitors and scavengers on dry weight of Indian mustard

percentage of root length was more than the shoot length (Figure 5). Significantly lower root to shoot ratio was observed in DPI and $CuCl_2$ treated seedlings which were statistically at par. Their reduction was 59.61 and 62.59% than the control respectively. In $ZnCl_2$ and KI treatment, this reduction was less than 50% of the control. This implies that DPI and $CuCl_2$ were more capable to restrict the root growth.

Seedling fresh weight and dry weight

Highest percentage of reduction in fresh weight was observed in $CuCl_2$ treatment which was statistically at par with $ZnCl_2$ treatment (Figure 6). Similar observation was also there in terms of dry weight (Figure 7). CuCl2 treated seedlings showed highest reduction in dry weight of the untreated seeds (58.94% of the control). Reduction in dry weight was very low in KI treated seedlings. On the final count day the KI treated seedlings were short in length but they were thicker. It may be because of the unavailability of H_2O_2 which is important for initiation of seed reserve mobilization to take place [16, 17] Maintenance of ROS at a high level causes oxidative modifications that are perceived as "hunger" signs by

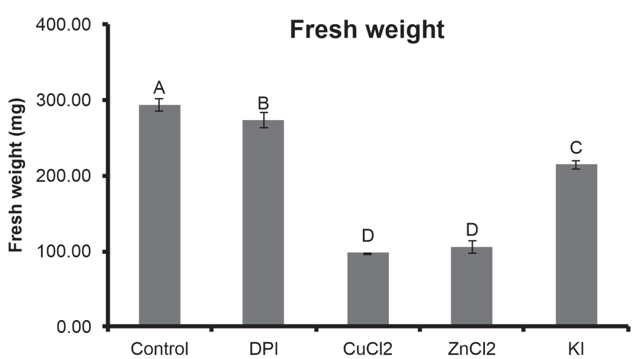

Figure 6. Effect of ROS inhibitors and scavengers on fresh weight of Indian mustard

the storage organs and prompt them to mobilize reserves to the rapidly growing axis. Superoxide radical gets converted to H_2O_2 either enzymatically or spontaneously. This H_2O_2 get accumulated in the elongating region of the radical whereas the superoxide radical get accumulated in the expanding meristem. A gradient of superoxide to H_2O_2 in the root controls the transition between cell proliferation and differentiation and so the size of the meristem is highly regulated by balancing superoxide/ H_2O_2 [18]. KI is a scavenger of H_2O_2. Hence in the KI treated seeds, the process of reserve mobilization perhaps got disturbed which resulted in higher dry weight of the seedling on final day of count.

Seed vigour indices

The seed vigour indices give a better prediction of field emergence than the germination percentage of seeds under laboratory condition alone. Although seed germination is the most tangible manifestation of seed quality, however, is not closely related with field emergence particularly under unfavourable environmental conditions. Thus, the development of seed vigour concept is a more promising seed quality character reflecting potential seed germination and field emergence under different conditions than standard germination [19].

50% reduction in seed vigour index-I was the criteria for selection of treatment concentration in the study. At these concentrations, DPI and $CuCl_2$ was statistically at par whereas $ZnCl_2$ and KI were categorised under same group (Figure 8). At these concentrations the highest reduction in seed vigour index-II was observed in $CuCl_2$ treated seedlings (1.24) and lowest decrease was observed in KI treated seedlings (Figure 9). SVI-I consider germination percentage and total seedling length whereas SVI-II considers germination percentage and

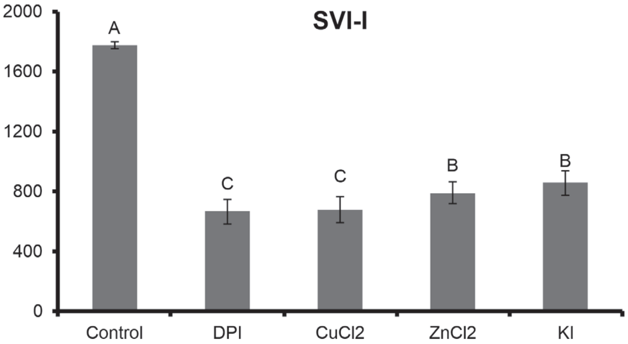

Figure 8. Effect of ROS inhibitors and scavengers on seed vigour index-I of Indian mustard

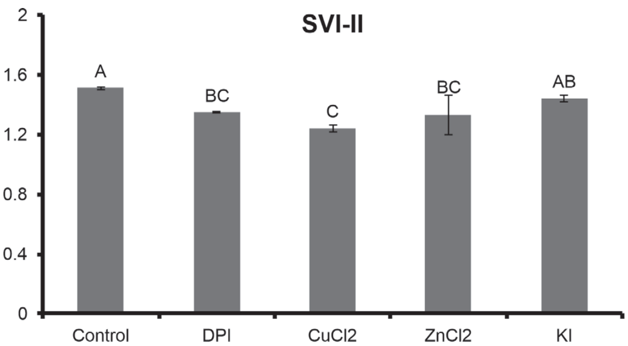

Figure 9. Effect of ROS inhibitors and scavengers on seed vigour index-II of Indian mustard

dry weight. Hence SVI-I was more effected due to this treatment of seeds with ROS production inhibitors and scavengers. Due to inhibition in ROS production and scavenging of ROS resulted in its sufficient accumulation which caused reduction in seedling growth. The reduction in seedling length was more than reduction in dry weight at a particular concentration in all the treated seedlings. Hence the SVI-I was reduced in great percentage and effected more than SVI-II in comparison to control.

CONCLUSION

Thus, this study conclude that the inhibition in ROS production and scavenging of ROS result in suppression of growth and development of seedling and reduce in seed quality parameters. At a particular concentration of ROS production inhibitors or scavengers, root growth is more effected that the shoot growth and as a whole seedling length gets reduced much more than the reduction in dry weight. Hence SVI-I is more effected than the SVI-II.

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