Mycopath (2014) 12(2): 123-127 **Research Article**

Response of maize to hydrogen peroxide priming under chromium stress

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Abstract

Chromium (Cr) is a heavy metal and has toxic effects on plants. Pretreatment of seeds with hydrogen peroxide (H_2O_2) can improve stress tolerance. Present investigation was made to study the response of maize (*Zea mays* L.) to H_2O_2 (80 µM) priming under chromium stress (100 and 200 µM) at seed germination and seedling stage. Experiments were conducted in Petri plates in completely randomized block design with three replications. A significant decrease was found in photosynthetic pigments, and germination and tolerance index of seedlings under chromium stress. Sugar and proline content were increased under chromium treatment. Tolerance was improved by the application of hydrogen peroxide (80 µM). present study concludes that pretreatment of maize seeds with optimum dose of H_2O_2 can help the plant in improving the tolerance to chromium stress at germination and seedling growth stage.
Keywords: Chromium, germination, hydrogen peroxide, maize, priming.

Introduction

Heavy metals are kept under environmental pollutant category due to their toxic effects on plants, animals and human beings. Heavy metals are persistent in nature, therefore get accumulated in soils and plants. Heavy metals interfere with physiological activities of plants such as photosynthesis, gaseous exchange and nutrient absorption, and cause reductions in plant growth, dry matter accumulation and yield. Dietary intake of many heavy metals through consumption of plants has long term detrimental effects on human health (Sharma and Agrawal, 2005). In line with other heavy metals like As, Cd, Co, Cu, Ni, Sn and Zn, the Cr is a broad line heavy metal, and is phytotoxic (Nieboer and Richardson, 1980). Cr is widely used in industries like steel, leather, textile, etc. (Dixit *et al*., 2002). Cr phytotoxicity can result in degraded pigment status, inhibition of seed germination, nutrient imbalance, antioxidant enzymes and oxidative stress in plants (Panda, 2003). Coupled with these effects, Cr can change chloroplast and membrane ultrastructure in plants (Choudhury and Panda, 2004).

Maize enjoys an important position in the existing cropping systems of Pakistan. It ranks third after wheat and rice in Pakistan for its grain production. It is also gaining importance due to being a commercial/industrial crop, where a large number of products are being manufactured out of its grain. Maize contribution in total food grains production of the country is about 6.4%. The average grain yield of maize is not only substantially lower compared with other important maize growing countries but also less than the production potential of existing genotypes (Anonymous, 2005). Although maize is considered to be tolerant to heavy metals, phytotoxic lesions on maize plantlets in response to heavy metals have been described in the literature (Sharma *et al*., 2003).

Hydrogen peroxide (H_2O_2) is one of the main chemical which is induced to elevate in plants by biotic and abiotic stresses. Higher levels of H_2O_2 usually result in damages to plant cells and toxicity to cellular membrane system (Kathiresan *et al*., 2006). However, the increased data indicate the biological activity of H_2O_2 as a stress signal molecule in plants (Hung *et al*., 2005). Application of H_2O_2 at low concentration has been shown to induce stress tolerance in plants. In Arabidopsis or tobacco H_2O_2 protects the plant from oxidative damage caused by high light intensity. H_2O_2 can serve as a second messenger in signal transduction pathways, leading to stress acclimation. The information available suggests that H_2O_2 directly regulates the expression of numerous genes involved in plant defense and the related pathways such as antioxidant enzymes, defense proteins and transcription factors (Hung *et al*., 2005). Hence, H_2O_2 signaling functions importantly in plant growth and development. H_2O_2 also help in defense against environmental stresses. The present investigation was made to study the effect of H_2O_2 pretreatment on the growth and metabolism of maize at seed germination and seedling growth stage under different concentrations of chromium.

Materials and Methods

The experiment was carried out in the Plant Physiology laboratory, Department of Botany, Lahore College for Women University. Seeds of local variety of maize were collected from Punjab seed corporation Lahore. Seeds were germinated in laboratory conditions in glass Petri dishes containing Whatman No. 1 filter paper. In each Petri dish 20 seeds were grown.

Seven treatments were applied and each treatment had three replicates. Treatments were arranged in a randomized complete block design (RCBD). Control (1st treatment) contained just distilled water. In 2nd treatment, seeds were first soaked in distilled water for 8 hours succeeded by application of distilled water. In 3rd treatment, seeds were first soaked in 80 µM H_2O_2 solution for 8 hours followed by application of distilled water. Treatment 4th comprised of 100 µM Cr and 5th one consisted of 200 µM Cr. In 6th treatment, seeds were first soaked in 80 µM H_2O_2 solution for 8 hours and then 100 µM Cr was applied. In 7th treatment, seeds were first soaked in 80 µM H_2O_2 solution for 8 hours and then 200 µM Cr was applied. Cr was applied in the form of $K_2Cr_2O_7$ solution. Seeds were allowed to germinate in laboratory conditions. Data were collected for about 2 weeks. Germination data was collected daily and from this following parameters were calculated.

Germination percentage was calculated by following formula as given by Close and Wilson (2002):

$$\text{Germination percentage} = \frac{\text{number of seeds germinated}}{\text{total number of seeds grown}} \times 100$$

Germination rate was estimated by using a modified Timson index of germination velocity.

Germination rate = $\sum G/t$

Where *G* is the percentage of seed germination every day, *t* is the total germination period (Khan and Ungar, 1984).

Seed vigour was calculated by the following formula given by Baki and Anderson (1973).

Seed vigour = Seedling length x germination percentage

The lengths of root and shoot of the seedling were measured and recorded after 10 days. The tolerance index was calculated by the following formula (Iqbal and Rahmati, 1992).

$$\text{TI} = \frac{\text{Mean root length in treatment applied}}{\text{mean root length in control}} \times 100$$

The amount of chlorophyll was calculated as per standard method (Arnon, 1949) and carotenoid content was estimated by using the formula of Lichtenthaler and Wellburn (1983) by following formulae:

Chlorophyll a = 12.21(A663) - 2.81(A645)
Chlorophyll b = 20.13(A645) - 5.03(A663)
Total chlorophyll = 20.2(A645) + 8.02(A663)
Carotenoid content=[1000A470 - 3.27(chlorophylla) - 104(chlorophyll b)] / 227

For determining the concentration per gram of plant tissue following formula was used:
C x V/g

Where, C is the amount of the pigment (mg L^{-1}), V is the volume of acetone used and g is the weight of maize leaves taken for the test.

Proline content was measured for leaves of maize by following the method described by Bates *et al.* (1973). The actual concentration of proline was calculated with reference to a proline standard curve. Using Dubois *et al.* (1956) method sugar content was estimated. The amount of sugar was estimated by using standard curve for glucose solution of known concentration. Statistical analysis was performed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Results and Discussion

Data presented in Table 1 show that the effect of all the treatment on germination percentage and rate of maize was non-significant. This might be due to tolerance of maize seeds to chromium stress at germination stage. Since seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of some tolerance to this metal (Peralta *et al.*, 2001).

The shoot length was significantly less under chromium stress as compared to control and H_2O_2 pretreated seeds (Table 1). Adverse effects of Cr on plant height and shoot growth have been reported earlier (Rout *et al.*, 2000). Reduction in plant height due to Cr on *Curcumas sativus*, *Lactuca sativa* and *Panicum miliaceum* was reported by Joseph *et al.* (1995). Barton *et al.* (2000) also observed that Cr addition inhibited shoot growth in lucerne cultures. The root growth decreased under chromium stress (Table 1). Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Goldbold and Kettner, 1991; Tang *et al.*, 2001). General response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle in the roots. The reduction in root growth of maize seedlings under high concentration of Cr could be due to the direct contact of seedlings roots with Cr in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium (Barcelo *et al*., 1986). The

results of present investigation confirm the findings of increased root and shoot growth when seeds were subjected to priming with H_2O_2 under stress (Wahid *et al*., 2007). This positive effect might be due to the fact that H_2O_2 enhance cell division and promoted the secondary wall formation (Potikha *et al.*, 1999).

The effect of pretreatment of H_2O_2 on tolerance index of maize is represented in Table 1. The tolerance index was minimum under chromium treatment. The tolerance index was higher when seeds were pretreated with H_2O_2 and chromium was applied as compared to chromium treatment. Seed vigour decreased significantly under chromium stress while pretreatment of seeds with H_2O_2 under chromium stress and presoaking in distilled water showed increase in seed vigour as compared to chromium treatment. The increased stress tolerance might be due to an enhanced level of antioxidant defense system induced by H_2O_2 pretreatment, which alleviates reactive oxygen species accumulation and oxidative damage during the subsequent stress conditions (Hu *et al.*, 2005).

Results indicated that H_2O_2 application under chromium stress reduced the toxic effect of chromium on photosynthetic pigment content of maize shoot (Table 2). Cr can reduce chlorophyll content and there by inhibit growth (Panda *et al.*, 2003). Decrease in total chlorophyll, chlorophyll a and b have been well documented under Cr stress in plants (Choudhury and Panda, 2004). Chlorophyll a/b ratio decrease under chromium treatment perhaps due to faster breakdown or decreased synthesis of chl *a* as compared to chl *b*, although chl *b* also decreased (Appenroth *et al.*, 2003). Carotenoid content decreased under chromium treatment (Table 2) was in accordance with Rai *et al.* (1992) who determined that Cr can induce degradation of carotenoids in plants. Seeds pretreated with H_2O_2 overcame the toxicity of chromium treatment applied, as there was no decrease found in caroteniod content under chromium treatment.

Proline content increased under chromium treatment and H_2O_2 alone in shoot (Table 2). Accumulation of free proline in response to heavy metal was determined in non-tolerant and metal-tolerant *Silene vulgaris* (Moench) Garcke; the constitutive proline concentration in leaves was 5 to 6 times higher in the metal-tolerant ecotype than in the non-tolerant ecotype (Schat *et al*., 1997). H_2O_2 under chromium stress showed opposite effect which might be due to the increase in proline dehydrogenase enzyme and caused proline degradation under H_2O_2 plus chromium stress.

Sugar content increased significantly under chromium treatment (Table 2) which is in agreement with the findings reported by Jayakumar *et al.* (2007) in radish under zinc application and is also in consonance with the findings of Mahadeswaraswamy *et al.* (1983) in *Phaseolus mungo* under chromium application.

Conclusions

It was concluded that both concentrations (100 and 200 µM) of chromium have negative effects on germination and seedling growth of maize. High Cr concentration (200 µM) caused more damage. While pretreatment of seeds with H_2O_2 overcame the adverse effects of chromium. Therefore, pretreatment of maize seeds with H_2O_2 could help the plant to cope with chromium stress at germination and seedling stage.

Table 1: The effect of pretreatment of H_2O_2 on germination and seedling growth of maize under chromium stress.

Treatments	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Tolerance index	Seedling Vigour
T1	96.66 a	38.88 a	4.06 a	19.53 a	-	2280 a
T2	98.33 a	61.11 a	4.16 a	18.5 a	94.7	2230 a
T3	95.00 a	61.11 a	4.03 a	18.8 a	96.245	2171 a
T4	98.33 a	44.44 a	2.03 b	13.7 b	70.13	1547 b
75	98.33 a	44.44 a	2.83 b	12.66 b	64.84	1519 b
T6	98.33 a	61.11 a	4.86 a	18.83 a	96.41	2328 a
T7	98.33 a	61.11 a	4.4 a	18.16 a	93	2218 a

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by DMR Test.
T1: Control; T2: Seeds Presoaked in distilled water; T3: Presoaked in H_2O_2; T4: $K_2Cr_2O_7$ (100 µM); T5: $K_2Cr_2O_7$ (200 µM); T6: $K_2Cr_2O_7$ (100µM) + Seeds Presoaked in H_2O_2; T7: $K_2Cr_2O_7$ (200µM) + Seeds Presoaked in H_2O_2

Table 2: The effect of pretreatment of H_2O_2 on pigments, proline and sugar content of maize seedlings under chromium stress.

Treatments	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Chlorophyll a/b ratio	Carotenoid (mg g^{-1})	Proline content (µg g^{-1})	Sugar content (µg g^{-1})
T1	199 a	72.44 a	311 a	2.75 a	59.96 a	0.99 bcd	1.2 b
T2	184 ab	61.36 ab	280 a	3.14 a	54.51 ab	0.96 cd	0.53 cd
T3	171 ab	56.46 ab	260 a	3.02 a	50.71 ab	1.21 abc	0.43 d
T4	151 b	68.94 ab	251 a	2.22 bc	45.76 bc	1.23 ab	1.21 b
75	94 c	51.28 b	167 b	1.84 c	35.87 c	1.31 a	1.93 a
T6	160 ab	71.22 ab	264 a	2.26 b	49.93 ab	0.85 d	0.74 c
T7	185 ab	62.84 ab	283 a	2.92 a	58.23 Vab	0.99 bcd	1.49 b

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by DMR Test.
T1: Control; T2: Seeds Presoaked in distilled water; T3: Presoaked in H_2O_2; T4: $K_2Cr_2O_7$ (100 µM); T5: $K_2Cr_2O_7$ (200 µM); T6: $K_2Cr_2O_7$ (100µM) + Seeds Presoaked in H_2O_2; T7: $K_2Cr_2O_7$ (200µM) + Seeds Presoaked in H_2O_2

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