Expression of GSH Gene Related to Heavy Metals Tolerance and Accumulation in Brassica sp. Plant Genotypes

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Abstract

The present study included two experiments related to heavy metals tolerance and accumulation in Brassica sp plant genotypes Brassica juncea and Brassica nigra. The first one concerned with the study of two genotypes for Seedling characters. The second experiment was carried out under different molecular markersto characterize the relationship between treatment and over expression of GSH gene. The effect of treatment on the level of GSH hence, the highest expression as 5.3 fold of GSH was obtained in treatment with Cd, and 3.5 fold of GSH was obtained in treatment with Ni in B. juncea. While 1.4 fold was obtained in treatments Pb. The same results were obtained with treatment in B. nigra.

Keywords

Heavy Metals Tolerance, Accumulation in Brassica sp.

Introduction

The Brassica genus is very essential agricultural crops in all the world and are also recognized to be heavy metal accumulators. There had been a big number of research regarding the tolerance, uptake and protection mechanism in numerous of those species, considerably Brassica juncea and B. napus, against to the stress caused by heavy metals (Miguel et al., 2015).

Phytoremediation uses green plant to clean up poisonous amount of inorganic and organic pollutants from the environment. The rapid industrialization and urbanization of many evolved and recently growing international locations (as an example China, India, Brazil etc.) have extended heavy metal and natural pollution inside the environment (Memon and Schroder, 2009). Metal infected soils pose an excessive danger to environment, agriculture and therefore thru the meals chain to human and animal health (Schwitzguébel et al., 2011). plants that hyper acquire heavy metals such as Zn(2+) and Cd(2+) are currently of notable medical hobby as they can be used for cleaning up contaminated soils, a process known as phytoremediation (Salt et al., 1995; Raskin et al., 1997). Environmental
pollution from business effluents and other natural pollutants has turned out to be a primary environmental and human situation international (Li and Yi (2012); Dong et al., (2013).

Metals are essential components of all ecosystems and arise evidently inside the earth’s crust (Pinto et al., 2003). They appear in a huge variety of oxidative states and coordination numbers, influencing their chemical characteristics and for this reason their bioavailability and toxicity (Pinto et al., 2003; Verbruggen et al., 2009). Positive metals along with iron (Fe), copper (Cu) and zinc (Zn) are taken into consideration vital nutrients to flora and are wished for photosynthesis and as cofactors for many enzymes (e.g. Kovacik et al., 2010; Shanmugam et al., 2011). Plant life soak up vital elements from their environment, however they may be also in a position to accumulate factors, which haven’t any recognized organic feature, which include heavy metals like cadmium (Cd), chromium (Cr) or lead (Pb) Peralta-Videa et al., 2009). Those nonessential metals are capable of input plant cells through metallic transporters and vendors for the uptake of crucial metals (Clemens, 2001; Shanker et al., 2005).

Phytoextraction is based on the use of pollutant-accumulating plants for trace element removal from soils by concentrating them in the harvestable parts (Salt et al., 1998). An ideal plant for trace element phytoextraction should possess the following characteristics: (a) tolerance to the trace element concentrations accumulated, (b) fast growth and highly effective trace element accumulating biomass, (c) accumulation of trace elements in the above ground parts, (d) easy to harvest (Vangronsveld et al., 2009). Plant species generate a range of defense mechanisms to resist Cd induced toxicity and to recover the subsequent damages (Meharg, 1993; Mohamed et al., 2012). Among heavy metals cadmium (Cd), a soil pollutant with a strong toxicity (Waalkes, 2000), inhibits photosynthesis (Qian et al., 2009) and prevents the growth of roots and stem. Cd inactivates some enzymes by a strong affinity with the thiol group (Mendoza-Cozatl et al., 2005) and it forms the active oxygens such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and hydroxyl radical (·OH) (Romero-Puertas et al., 2004) together with lipid peroxide, causing damage to biopolymer and cell membrane by inducing the oxidative stress (Heyno et al., 2008).

Materials and Methods

In the present work two species of the Indian mustard were used. The first species (Brassica juncea) was obtained from the Agriculture Research Center (A.R.C.), Giza, Egypt, while the second species (Brassica nigra) was kindly obtained from (IPK), Germany.

Treatments and experimental design

The experiment was arranged in factorial experiment in Randomized Complete Block Design (RCBD) with three replications. The experiment included two different Indian mustard which were gotten the first species (Brassica juncea), while the second species (Brassica nigra).

Four different concentrations of lead (Pb) i.e. 0, 100, 200 and 400 ppm, cadmium (Cd) i.e. 0, 10, 20 and 40 ppm and nickel (Ni) i.e. 0, 20, 50 and 150 ppm were used. Fifty seeds of uniform size in each cultivar were allowed to germinate to primary roots of 2 mm length in a Petri dish containing a filter paper of 9 cm diameter; the Petri dishes were placed in a growth chamber for 7 days at ± 28°C, germinated seeds were selected and transferred to pots (diameter 10 cm)
containing quartz sand. Then, these pots were transferred to the green house with day and night temperature of 25°C and grown in plastic pots. Seeds were germinated, in the presence of lead (Pb), cadmium (Cd) and nickel (Ni). Each dish contained fifty seeds and a total of three dishes were used for each treatment. After One week from the initiation of germination, selected seedlings (stressed and non-stressed) were carefully transferred to suitable pots containing quartz sand to continue their growth in the greenhouse. They were irrigated with Hoagland’s solution, containing macro and micro nutrient. After 21 days of sowing, plantlets were harvested and were homogenized in liquid nitrogen; the plant material was stored in liquid nitrogen until used.

**Electrophoresis and isozyme techniques**

Peroxidase (Prx.) E.C.1.111.1.7 was determined by using polyacrylamide gel electrophoresis according to the method of Davis (1964).

**SDS Protein Electrophoresis**

SDS – Polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970).

**Total RNA isolation**

Total RNA was isolated from leaves of both species of the treated and untreated Indian mustard. Plants using the SV Total RNA isolation kit (Promega) according to the technical manual-TM 048.

**RT-PCR amplification**

Total RNA was subjected to reverse transcriptase polymerase chain reaction RT-PCR by using specific primers for the GSH gene (www.ncbi.com). RT-PCR Ready To Go kit (Bio Basic Inc.) was used to isolate the cDNA fragment according to the manufacture system as following:

1- The following reagents were added to the tubes which contained the kit reagents pellet. These reagents contained 1 µg. of total RNA and 10 µM of each specific primer.

5’ - CCC GGA TCC ATG GAG TCT TCA AGT CCC - 3’

5’ - CCC CTC GAG TTA GCG CTC GAT TTG TAT - 3’ a total volume of 50ul. Reaction was completed using nuclease free water.

The PCR conditions were as follows; the tubes were incubated at 42°C for 15 minutes to activate the reverse transcriptase enzyme, and 94°C for 5 minutes to inactivate the enzyme, then followed by 35 cycles of 94°C for 1 min, 67°C for 1min, and 72°C for 1min. Finally 72°C for 10 minutes.

The PCR products were electrophoresed in 1% agarose gel. A 100 bp DNA marker was used with the samples and photographed.

**Results and Discussion**

The present study for both Indian mustard *Brassica nigra* and *Brassica juncea*. study the response of germinate under heavy metals; Lead (Pb), Cadmium (Cd) and Nickel (Ni) stress to confirm the seedling growth performance to examine a range of genetic variability for heavy metals tolerance among both Indian mustard *Brassica nigra* and *Brassica juncea*.

**Seedling characters**

**Lead tolerance evaluation**

The results shown in Table (1) revealed highly significant differences for root/shoot
ratio (R/S), germination index (GI), root length (RL), shoot length (SL) and germination % (FGP) traits between the genotypes and Lead application as well as their interaction.

With respect to root/shoot ratio, significant differences were detected among the two studied Indian mustard species (Table 2).

The species Brassica juncea gave highly significant mean (33.481), while second species Brassica nigra provide decreased mean (29.456). These differences among two studied Indian mustard species might be attributed to their genetic background.

The analyses of variance indicate that root/shoot ratio was significantly influenced by Pb application levels. As shown in Table (3), the treatment without Pb application produced the highly mean of root/shoot ratio (75.449), while the application Lead with 200 ppm gave the lowest value for root/shoot ratio (6.145). The change in root growth due to the consequences of the exposure of the radical to toxicity and accumulation of metals in the roots followed by slow mobility to the plant shoots (Godzik, 1993 and Fargasova, 1994). According to (Chaignon and Hinsinger 2003), higher concentrations of heavy metals can inhibit root growth before shoot growth and can accumulate in the roots without any efficient increase in its content of the aerial parts. Heavy metals are found to be more toxic for root growth because they accumulate on roots and retard cell division and cell elongation.

Cadmium tolerance evaluation

The results shown in Table (4) revealed highly significant differences for final germination percentage (FGP), germination index (GI), root length (RL), shoot length (SL) and root/shoot ratio (R/S) traits between the genotypes and Lead application as well as their interaction.

Regarding to root/shoot ratio, significant differences were detected among the two studied Indian mustard species (Table 5).

The species Brassica juncea gave highly significant mean (35.240), while, second species Brassica nigra provide decreased mean (25.546). These differences among two studied Indian mustard species might be attributed to their genetic background.

The analysis of variance indicates that final germination percentage was significantly influenced by Cadmium application levels. As shown in Table (6), the untreated seedling (control) gave the highly mean value of final germination percentage (91.88%), while the application Cadmium with 400 ppm gave the lowest mean for final germination percentage (68.36%).

Nickel tolerance evaluation

In Table (7), the analysis of variance revealed highly significant differences among the genotypes tested for final germination percentage (FGP), germination index (GI), root length (RL), shoot length (SL) and root/shoot ratio (R/S) traits by the genotypes and nickel application as well as their interaction.

 Estimates of final germination percentage, significant differences were detected among the studied cultivars (Table 8), Indian mustard species Brassica juncea gave highly significant mean (76.833%), while Brassica nigra species provide decreased mean (75.00%).

As shown in Table 9, the untreated seedlings (control) produced the highly mean of final germination percentage (91.889%), while the application nickel treated with 150 ppm gave the lowest mean for final germination percentage (29.611%).
Biochemical and Molecular Genetic Analysis

Isozyme Analysis

The biochemical methods of investigation, especially isozyme studies, have provided valuable tools for breeders. Isozyme can serve as unique molecular genetic markers for biochemical characterization of genotypes (Tanksley and Orton, 1983). In the present study an attempt was made to assay the variation of number and activity of one isozyme pattern; peroxidase, in the leaves crude extract of three weeks old seedlings. Two varieties; three heavy metals Pb, Cd, Ni and three different concentration were chosen for isozymes studies of all the materials. Variations in number and activity of bands are shown in Figures 1, and 2 for the peroxidase.

Figure one of peroxidase isozyme patterns of Brassica juncea as well as their treated with different concentrations of lead, cadmium and nickel. In total, Figure 1 shows a total number of 68 bands detected in the present materials. The untreated genotype showed seven bands as a total. The treated concentration with 40 ppm Cd showed the lowest number of peroxidase isozyme bands (Prx 2, Prx 3, Prx 4 and Prx 6). While the treated concentration with 200 ppm Pb, 20 ppm Ni and 150 ppm Ni showed the highest number of bands (Prx 1, Prx 2, Prx 3, Prx 4, Prx 5, Prx 6, Prx 7 and Prx 8). The band (Prx 1) was present in the treated with heavy metals; 200 ppm Pb, 400 ppm Pb, 10 ppm Cd, 20 ppm Cd, 40 ppm Cd, 20 ppm Ni, 50 ppm Ni and 150 ppm Ni and was absent in the control and 100 ppm Pb so, it could be considered as potential markers associated with heavy metals tolerance under treatment. While the bands (Prx 1 and Prx 2) were present in the control and were absent in their treated with some heavy metals; 100 ppm Pb, 200 ppm Pb, 400 ppm Pb, 10 ppm Cd, 20 ppm Cd, 40 ppm Cd, 20 ppm Ni and 50 ppm Ni.

The activity of isoenzyme in the two cultivars genotypes treated with heavy metals (Pb, Cd and Ni) and control determined electrophoretically the intensity of activity of the peroxidase. Results summarized in Figure 2 where the appearance band of Prx 7 under treatment with Pb, Cd and Ni and in Brassica nigra could be considered as a potential marker for Pb, Cd and Ni tolerance.

An increase in peroxidase activity probably represents an induced protective reaction delaying senescence (Birecka et al., 1977). Since, as we know the importance of peroxidase isoenzyme to catalyze the reaction that protects the plants, against damage by free radicals. Populations showing low peroxidase activity indicated that it may not adapt them at wider range because plants may lose the permeability of membrane and proceed toward the end of life due to the harmful action of free radicals. Lipid of membranes where a peroxidation of
unsaturated fatty acids takes place is main cellular components susceptible to damage by free radicals (Monk et al., 1989).

**SDS Protein Electrophoresis**

Electrophoretic analysis was carried out using SDS – PAGE for water soluble protein fraction and was stained to detect the whole protein banding pattern SDS – PAGE was achieved to screen the water soluble leaf protein extracted from the two species of the Indian mustard *Brassica juncea* and *Brassica nigra* under the treatments of heavy metals (Pb, Cd, and Ni) and control. The protein banding patterns showed a maximum number of 20 bands which were characterized with molecular weights (MW) ranging from 116 to 12.5 KDa, and shown in Figure 3 and 4 and Tables 10 and 11.

Concerning protein banding pattern treated of *Brassica juncea* for the heavy metals (Pb, Cd, and Ni) are shown in Table 10 and Figure 3. The control without any treatment has 15 bands ranging from 116 to 14 KDa. The treatment with 100 ppm Pb metal showed 15 bands ranging from 116 to 14 KDa, and also the treatment with 200 ppm Pb showed 15 bands ranging from 116 to 14 KDa. For the treatment with 400 ppm Pb showed 13 bands ranging from 116 to 14 KDa. On other hand the treatment with 10 ppm Cd exhibited 13 bands which ranged from 116 to 12.5 KDa, also the treatment with 20 ppm Cd showed 12 bands ranging from 116 to 14 KDa, and also the treatment with 40 ppm Cd showed 13 bands ranging from 116 to 12.5 KDa. While, the treatment with 20 ppm Ni exhibited 14 bands which ranged from 116 to 14 KDa, also the treatment with 50 and 150 ppm Ni showed 13 bands ranging from 116 to 12.5 KDa. The band with MW 29.8 KDa which was present in Pb treatments except the control can be considered as potential markers associated with lead tolerance under treatment. While, the band with MW 17.5 KDa which was present in all treatments except the control can be considered as potential markers associated with heavy metals tolerance under treatment.

Concerning protein banding pattern treated of *Brassica nigra* for the three heavy metals (Pb, Cd, and Ni) are shown in Table 11 and Figure 4. The control without any treatment has 14 bands ranging from 116 to 12.5 KDa. The treatment with 100 ppm Pb showed 16 bands ranging from 116 to 12.5 KDa, also the treatment with 200 ppm Pb showed 16 bands ranging from 116 to 12.5 KDa. Treatment with 400 ppm Pb showed 15 bands ranging from 116 to 12.5 KDa. On other hand the treatment with 10 ppm Cd exhibited 13 bands which ranged from 116 to 14 KDa, also the treatment with 20 ppm Cd showed 12 bands ranging from 116 to 14 KDa, and also the treatment with 40 ppm Cd showed 13 bands ranging from 116 to 12.5 KDa. While, the treatment with 20 ppm Ni exhibited 14 bands which ranged from 116 to 12.5 KDa, also the treatment with 50 and 150 ppm Ni showed 13 bands ranging from 116 to 12.5 KDa. The band with MW 29.8 KDa which was present in Pb treatments except the control can be considered as potential markers associated with lead tolerance under treatment. While, the band with MW 17.5 KDa which was present in all treatments except the control can be considered as potential markers associated with heavy metals tolerance under treatment.

In general, protein bands which induced in SDS-PAGE banding patterns of the two species of the Indian mustard after treatment with three heavy metals (Pb, Cd and Ni). Meanwhile, these bands were absent from the control. This clearly suggests that these protein bands might represent an important enzyme and or structural protein that could be involved either directly or indirectly in the process of heavy metals tolerance of Indian mustard plants. The excess of toxic heavy metal ions induces several cellular stress responses and damage to different cellular components such as membranes, proteins and DNA (Waisberg et al., 2003 and Jimi et al.,...
2004). SDS-PAGE for total soluble proteins and isozyme analysis revealed moderate and moderate to high degree of polymorphism, respectively (Patra and Chawla, 2010), studied Eighteen traditional and improved basmati rice (Oryza sativa L.) varieties for morphological descriptors, total soluble proteins as biochemical markers for determining distinctive features.

**Gene expression and Gene screening analysis**

**RT-PCR and PCR**

cDNA of Glutathione GSH gene which might be induced after treatment with heavy metals was amplified from the two Indian mustard genotypes (Brassica juncea and Brassica nigra) after treatment with heavy metals; Cd, Ni and Pb. Many strategies have been conducted to isolate genes from plants. All techniques for gene isolation exploit one or more of the characteristics that define genes. Some techniques may permit the isolation of genes from any plant, while others are only applicable to one or few. Isolation of plant genes can be done via construction of cDNA library and protein purification. Based on the mentioned clues our was to take advantage of the heavy metal tolerant genotype of Indian mustard plants and to use it for identifying one of the heavy metal tolerance genes using RT-PCR .Pure preparations of the isolated total RNA were found to have 260/280 ratio between 1.8 and 2.0 RNA integrity was examined on 1.2% agarose-formaldehyde denaturing gels before proceeding in RT-PCR. The total RNA (2µg.) showed sharp and clear 28S and 18S rRNA bands. This indicates that the total (poly A-RNA) isolated for both Brassica juncea and Brassica nigra have no serious signs of degradation.

The expression activity was detected for glutathione GSH gene from the two species (B. juncea and B. nigra) using RT-PCR. The RT-PCR product of GSH gene of Brassica juncea and Brassica nigra treatment with heavy metals separated at about 240 bp on the 1.5% agarose gel. Signals intensities of the separated fragment were different and the band intensity will be indicating the variation in GSH gene expression as a response to different treatments with heavy metals.

**Table.1** Analysis of variance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Pb.

| S.O.V      | df | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|------------|----|-----------------------------|------------------------|------------------|-------------------|------------------|
| Replication| 2  | 3.215 ns                     | 0.0001 ns              | 0.002 ns         | 0.284 ns          | 2.044 ns         |
| Genotypes  | 2  | 205.704 **                   | 0.017 **               | 2.604 **         | 7.893 **          | 175.329 **       |
| Treatments | 3  | 614.414 **                   | 0.070 **               | 391.523 **       | 195.902 **        | 37990.940 **     |
| G x T      | 3  | 24.404 **                    | 0.003 **               | 2.738 **         | 3.195 **          | 200.603 **       |
| Error      | 24 | 4.534                        | 0.001                  | 0.081            | 0.133             | 17.410           |

ns: Not significant; *, **: Significant at 5% and 1%, respectively.
Table.2 Mean performance of Lead for final germination percentage, germination index, root length, shoot length and root/shoot ratio of the twelve rice cultivars.

| Genotypes        | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|------------------|-------------------------------|------------------------|------------------|-------------------|------------------|
| Brassica juncea  | 91.000 ab                      | 0.968 ab               | 2.933 ab         | 7.250 ab          | 33.481 a         |
| Brassica nigra   | 90.333 ab                      | 0.943 ab               | 2.450 ab         | 6.350 b           | 29.456 ab        |

Means followed by the same letter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table.3 Mean performance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Pb application levels.

| Treatment | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|-----------|-------------------------------|------------------------|------------------|-------------------|------------------|
| Control   | 91.889 a                      | 1.000 a                | 7.297 a          | 9.667 a           | 75.449 a         |
| 100 ppm   | 86.833 b                      | 0.948 b                | 2.158 b          | 7.450 b           | 29.499 b         |
| 200 ppm   | 84.611 c                      | 0.922 c                | 0.350 c          | 5.758 c           | 6.145 c          |
| 400 ppm   | 82.194 d                      | 0.897 d                | 0.292 c          | 4.231 d           | 6.937 c          |

Means followed by the same letter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table.4 Analysis of variance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Cd.

| S.O.V       | df | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|-------------|----|-------------------------------|------------------------|------------------|-------------------|------------------|
| Replication | 2  | 0.444 ns                      | 0.006 ns               | 0.057 ns         | 0.001 ns          | 9.337 ns         |
| Genotypes   | 1  | 438.310 **                    | 0.062 **               | 1.872 **         | 5.555 **          | 183.429 **       |
| Treatments  | 3  | 3782.840 **                   | 0.410 **               | 386.433 **       | 275.628 **        | 36730.476 **     |
| G x T       | 3  | 284.759 **                    | 0.038 **               | 2.575 **         | 2.094 **          | 266.640 **       |
| Error       | 24 | 4.48                          | 0.006                  | 0.072            | 0.125             | 22.369           |

ns: Not significant; *, **: Significant at 5% and 1%, respectively.
Table.5 Mean performance of Cadmium for final germination percentage, germination index, root length, shoot length and root/shoot ratio of the twelve rice cultivars.

| Genotypes       | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|-----------------|-------------------------------|------------------------|------------------|------------------|-----------------|
| Brassica juncea | 84.083<sup>ab</sup>          | 0.972<sup>a</sup>     | 3.033<sup>a</sup> | 5.942<sup>b</sup> | 35.240<sup>a</sup> |
| Brassica nigra  | 84.500<sup>b</sup>           | 0.851<sup>b</sup>     | 2.442<sup>b</sup> | 6.850<sup>a</sup> | 25.546<sup>b</sup> |

Means followed by the same latter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table.6 Mean performance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Cd application levels.

| Treatment     | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|---------------|-------------------------------|------------------------|------------------|------------------|-----------------|
| Control       | 91.889<sup>a</sup>           | 1.000<sup>a</sup>     | 7.297<sup>a</sup> | 9.667<sup>a</sup> | 75.449<sup>a</sup> |
| 10 ppm        | 87.528<sup>b</sup>           | 0.929<sup>b</sup>     | 2.053<sup>b</sup> | 4.878<sup>c</sup> | 43.863<sup>b</sup> |
| 20 ppm        | 84.194<sup>c</sup>           | 0.919<sup>b</sup>     | 0.533<sup>c</sup> | 5.278<sup>b</sup> | 10.506<sup>c</sup> |
| 40 ppm        | 68.361<sup>d</sup>           | 0.749<sup>c</sup>     | 0.236<sup>d</sup> | 3.172<sup>d</sup> | 7.654<sup>d</sup> |

Means followed by the same latter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table.7 Analysis of variance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Ni

| S.O.V          | df | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|----------------|----|-------------------------------|------------------------|------------------|------------------|-----------------|
| Replication    | 2  | 1.799<sup>ns</sup>           | 0.001<sup>ns</sup>     | 0.051<sup>ns</sup> | 0.051<sup>ns</sup> | 32.565<sup>ns</sup> |
| Genotypes      | 1  | 1127.035<sup>**</sup>        | 0.195<sup>**</sup>     | 2.504<sup>**</sup> | 3.944<sup>**</sup> | 1614.818<sup>**</sup> |
| Treatments     | 3  | 29967.30<sup>**</sup>        | 3.467<sup>**</sup>     | 364.392<sup>**</sup> | 719.956<sup>**</sup> | 39857.13<sup>**</sup> |
| G x T          | 3  | 759.453<sup>**</sup>         | 0.081<sup>**</sup>     | 2.470<sup>**</sup> | 1.902<sup>**</sup> | 1312.858<sup>**</sup> |
| Error          | 24 | 3.054                         | 0.0001                 | 0.060            | 0.054            | 27.708          |

ns: Not significant; *, **: Significant at 5% and 1%, respectively.
Table 8 Mean performance of Nickel for final germination percentage, germination index, root length, shoot length and root/shoot ratio of the twelve rice cultivars.

| Genotypes     | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|---------------|------------------------------|------------------------|------------------|-------------------|-----------------|
| Brassica juncea | 76.833 a                      | 0.887 a                | 2.958 a           | 3.836 ab          | 41.789 a        |
| Brassica nigra | 75.000 b                      | 0.823 b                | 2.400 b           | 3.963 a           | 22.167 b        |

Means followed by the same latter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table 9 Mean performance for final germination percentage, germination index, root length, shoot length and root/shoot ratio affected by Ni application levels.

| Treatment  | Germination percentage (FGP) | Germination index (GI) | Root length (cm) | Shoot length (cm) | Root/Shoot ratio |
|------------|------------------------------|------------------------|------------------|-------------------|-----------------|
| Control    | 91.889 a                      | 1.000 a                | 7.297 a           | 9.667 a           | 75.449 a *      |
| 20 ppm     | 88.833 b                      | 0.969 b                | 1.361 b           | 3.014 b           | 48.473 b        |
| 50 ppm     | 63.556 c                      | 0.703 c                | 0.374 c           | 18.282 c          |
| 150 ppm    | 29.611 d                      | 0.330 d                | 0.010 d           | 0.00 d            |

Means followed by the same latter in each column or significantly different by the least significant at p 0.05 according to Duncan’s test.

Table 10 Effect of heavy metals (Cd, Ni and Pb) and control on the level of GSH gene transcription in B. juncea using RT-PCR.

| MT 1 Kb | Control | Pb | Ni | Cd |
|---------|---------|----|----|----|
| 240 bp  | 1 fold  | 1.4 fold | 3.5 fold | 5.3 fold |

Fig.1 Peroxidase isozyme pattern Brassica nigra genotype under treatment with heavy metals.
**Fig. 2** Peroxidase isozyme pattern *Brassica nigra* genotype under treatment with heavy metals.

![Peroxidase isozyme pattern](image)

Lane 1: control  – Lane 4: 400 ppm Pb  – Lane 7: 40 ppm Cd  – Lane 10: 150 ppm Ni
Lane 2: 100 ppm Pb  – Lane 5: 10 ppm Cd  – Lane 8: 20 ppm Ni
Lane 3: 200 ppm Pb  – Lane 6: 20 ppm Cd  – Lane 9: 50 ppm Ni

**Fig. 3** SDS-PAGE protein banding pattern for *Brassica juncea* genotype under treatment with heavy metals.

![SDS-PAGE protein banding pattern](image)

Lane 1: control  – Lane 2: 100 ppm Pb  – Lane 3: 200 ppm Pb  – Lane 4: 400 ppm Pb  – Lane 5: 10 ppm Cd  – Lane 6: 20 ppm Cd  – Lane 7: 40 ppm Cd  – Lane 8: 20 ppm Ni  – Lane 9: 50 ppm Ni  – Lane 10: 150 ppm Ni

**Fig. 4** SDS-PAGE protein banding pattern for *Brassica nigra* genotype under treatment with heavy metals.

![SDS-PAGE protein banding pattern](image)

Lane 1: control  – Lane 2: 100 ppm Pb  – Lane 3: 200 ppm Pb  – Lane 4: 400 ppm Pb  – Lane 5: 10 ppm Cd  – Lane 6: 20 ppm Cd  – Lane 7: 40 ppm Cd  – Lane 8: 20 ppm Ni  – Lane 9: 50 ppm Ni  – Lane 10: 150 ppm Ni
**Fig. 5** Agrose gel for RT-PCR amplified fragment on RNA isolated from leaves Indian mustard under treatment with heavy metals

![Agrose gel for RT-PCR amplified fragment on RNA isolated from leaves Indian mustard under treatment with heavy metals](image)

**Fig. 6** Database sequences aligned to the query sequence

| Query | Sbjct | Max score | Total score | Query cover | E-value | Ident | Accession |
|-------|-------|-----------|-------------|-------------|---------|-------|-----------|
| ATGAACGGCAATCCATCCTCTTTACATCGCTACAGAGACAAACGCCGCCGATCTAGAAAAA | ATGAACGGCAATCCATCCTCTTTACATCGCTACAGAGACAAACGCCGCCGATCTAGAAAAA | 1397 | 1397 | 100% | 0.0 | 100% | XM_001602501.3 |
| GAGCCGAACCAAGATGCACCACCTAGGCTGGAGGTATTTTCAGTCTTCACAGTCAACCAG | GAGCCGAACCAAGATGCACCACCTAGGCTGGAGGTATTTTCAGTCTTCACAGTCAACCAG | 120 | 120 | 100% | 0.0 | 100% | |
| AAGAGAGCCATGGTGGCACCGGCTCAATGGCGAGCTTTTTCTCGCCTTTGTCTTCAAGC | AAGAGAGCCATGGTGGCACCGGCTCAATGGCGAGCTTTTTCTCGCCTTTGTCTTCAAGC | 180 | 180 | 100% | 0.0 | 100% | |
| ATCTACTTCCCTGCCATGGACACGATCGCAACAGCGCTGAATGTCTCCACATCCAAGATT | ATCTACTTCCCTGCCATGGACACGATCGCAACAGCGCTGAATGTCTCCACATCCAAGATT | 240 | 240 | 100% | 0.0 | 100% | |
| GATTTGACTGTCACGACCTACCTCATAATGCAAGGCATACTCCCATGTTGATCGCCGGA | GATTTGACTGTCACGACCTACCTCATAATGCAAGGCATACTCCCATGTTGATCGCCGGA | 300 | 300 | 100% | 0.0 | 100% | |
| TTCTCAGACACAGCCGCCAGACGTCCCGGCCTACATATCTGTCCACCGTCTATACGCC | TTCTCAGACACAGCCGCCAGACGTCCCGGCCTACATATCTGTCCACCGTCTATACGCC | 360 | 360 | 100% | 0.0 | 100% | |
The untreated control plants (C) of *B. juncea* and *B. nigra* did not show any expression of GSH gene, meanwhile the gene up-regulated in varied levels as a response to stress, is shown in figure 5 and table 12.

Table (12) show the effect of treatment on the level of GSH hence, the highest expression as 5.3 fold of GSH was obtained in treatment with Cd, and 3.5 fold of GSH was obtained in treatment with Ni in *B. juncea*. While 1.4 fold was obtained in treatments Pb. The same results were obtained with treatment in *B. nigra*. These results are in agreement with those obtained by Vido *et al.*, (2001) who reported that (GSH) is strongly increased in response to cadmium treatment, Schützendübel and Polle, (2002), GSH is apparently a critical step in cadmium sensitivity since plants with improved capacities for GSH synthesis displayed higher Cd tolerance. These results also disagreement with those obtained by Liang *et al.*, (1999). In the presence of Cd, the GS enzyme is rate limiting for the biosynthesis of glutathione and phytochelatins.

**Sequence analysis of GSH gene**

A multiple sequence alignment of GSH nucleotide sequence (current study) was carried out with GSH sequences published in the GenBank. Sequence comparison showed that GSH of the current study had sequence homology of about 100% with other GHS isolates, mRNA sequence homology of about 100% with *Aspergillus NIGER* isolates. These results are in agreement with those obtained.
An overview of the Gene bank sequences is shown in figure 6.

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