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Effects of heavy metals on *Eleusine coracana* (L.) Gaertn

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Heavy metal (Abiotic Factor) pollution is one of the most troublesome environmental problems faced by mankind nowadays. Lead and Nickel in particular, pose serious problems due to its widespread industrial and agricultural use. Plants in general are very sensitive to Pb and Ni toxicity, displaying metabolic disturbances and growth inhibition. *Eleusine coracana* (L) Gaertn (var. PR202) which mainly grows in dry condition faces the toxicity of Pb and Ni which may have negative effect on its yield. Some inorganic nutrients can overcome the toxicity of heavy metals and addition of these inorganic nutrients in media can increase the resistance (tolerance level) of plant against heavy metal toxicity. Embryogenic sectors of seed callus from control (MS + 2 mg/l 2,4-D + 0.5 mg/l Kn) were sub cultured on regeneration medium (MS + 1mg/l NAA) supplemented with of toxic level of heavy metals and varied concentrations of inorganic nutrients ($MgSO_4$ and $ZnSO_4$) to minimize the toxicity of heavy metals. Callus induction obtained only in the medium containing 100 µM concentrations of Pb and Ni but there was better growth obtained in Ni containing medium than Pb. These results show that Pb and Ni above 100 µM concentration were toxic for callus induction in *Eleusine coracana* but Pb was more toxic than Ni and same results was observed during plant regeneration.It was concluded that $ZnSO_4$ at the four times of MS level best to minimize the toxicity of Nickel and $MgSO_4$ at three times of MS level best to minimize the toxicity of Lead.

Keywords-Heavy metals, Lead, Nickel, Magnesium Sulphate, Zinc Sulphate.

Eleusine coracana (var. PR202 45) was used as a model cereal crop. Looking to the scope and realizing its importance, the present study was undertaken to investigate the effects of different concentrations of Pb and Ni elements on callus induction and plant regeneration with the following Objectives- To standardize a medium with growth regulators for tissue culture of *Eleusine coracana.* To study the influence of Pb and Ni in the morphogenesis of *Eleusine coracana* tissue culture. To optimize the culture condition with an objective of minimizing Pb and Ni toxicity and maximizing the yield .To Implantation in field to increase the productivity of cereals. Levels of different heavy metals were varied taking MS as the standard medium. Today Abiotic stress is a major global problem limiting crop productivity. Stress factors are a serious problem limiting the yield potential of modern cultivars. Stress causes nutritional imbalances in the plant causing reduction in water uptake and toxicity, decreasing the production. Abiotic stresses like salinity, heavy metals and pesticides are the primary cause of crop failures in India. It has been estimated that about 8.6 million ha of land is affected by salinity in India (Pathak, 2000). Stresses are increasing drastically today because of pollution, declining availability of quality water and land degradation. Ragi is used as cereal,

which is also called finger millet and scientific name is ***Eleusine coracana.****Eleusine coracana* belonging to family Poaceae. Cereals constitute a major source of food for the human population of the world. Important cereals in day to day use are rice, wheat and millets. Of all the millets *Eleusine* contains more percentage of different nutrients as compared to rice and wheat. It is a rich source of calcium, magnesium and potassium. *Eleusine* is nutritionally very rich cereal and is a staple food for poor and invalids (Panda, 1999). Due its nutritional superiority and requirement by poor people production needs to be improved. *Eleusine coracana* mainly growing in dry condition faces heat, salinity and heavy metal stress which may cause negative effect on its yield. Stress tolerant plants can also be developed by breeding and by transgenesis which are complex processes. (Babu *et al*, 2007) Tissue culture techniques offer an easy and important tool in developing stress tolerant variants (Nabors and Dykes, 1985.). Finger millet contains more fiber, minerals and vitamins, which are normally deficient in the Indian diet, and has eight times more calcium than other cereals The high calcium, high soluble fiber, low fat and low glycemic index of malted grains is effective in controlling the blood glucose levels of diabetics. Studies were conducted on diabetics (male and female) living in different rural and urban locations in 2008 in different countries (Adikant prahan *et al*, 2008). Finger millet is especially valuable as it contains amino acid methionine, which is lacking in the diets of millions of the poor people. Finger millet can be ground and cooked into cakes, puddings or porridge. The grain is made into a fermented drink (or beer) in many parts of Africa. The straw from finger millet is used as animal fodder. It is also used for as a flavored drink in festivals. It has been shown that the nutrient level in the medium has profound effect on callus induction and subsequent plant regeneration (Ramage and Williams 2002). There also occurs interplay between nutrients and plant growth regulators (PGRs) during the course of in vitro growth and differentiation (DeFossard 1974). Appropriate levels of nutrients may also partially substitute the requirement of PGRs in the plant regeneration medium (Preece 1995; Poddar et al. 1997). Bregitzer et al. (1998, 2000) and Dahleen and Bregitzer (2002) investigated the effect of various micronutrients on barley culture and reported several fold better regeneration of green plants by combining the various improvements. Optimized levels of copper lead to improved plant regeneration from callus cultures of barley (Bregitzer et al. 1998;Castillo et al. 1998; Chauhan and Kothari 2004), rice (Sahrawat and Chand 1999), finger millet (Kothariet al. 2004), sorghum (Nirwan and Kothari 2003) and wheat (Tahiliani and Kothari 2004). Sahasrabudhe et al. (1999), found an increase in the embryogenic response of indica rice on higher concentrations of boric acid. These nutrient levels which were adequate for tobacco tissue culture may not necessarily be optimum for the culture of other plant species like graminaceous monocots (Dahleen 1995).

Materials and Methods

An important plant *Eleusine corcana* (L.) Gaertn of family Poaceae was taken as the model plant for the study (Fig 2) Seeds of agronomically superior and released variety (PR 202) of ragi were procured from Agricultural University, Bangalore (Fig1) and this study was conducted in 2011 at IIS University Department of Life Sciences, Jaipur.

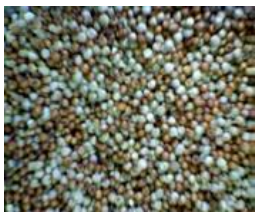

Figure 1. (Left) Seeds of *Eleusine coracana*, Figure 2. (Right) Mature Plant of *Eleusine coracana* in field.

Common Name: Fingermillet
Vernacular Name: Ragi
Explants Taken: Seeds

Sterilization and Preparation of Explants
Explants taken were aseptically sterilized. Sterilization process of seeds was carried out in the laminar airflow cabinet. Seeds were surface sterilized in 0.1 percent mercuric chloride solution for 3 minutes. These explants (seeds) were inoculated on MS medium supplemented with auxin 2, 4-D (2 mg/l) and cytokinin Kn (0.5 mg/l).

Basal Medium Preparation
Basal medium used in the present study was MS (Murashige and Skoog, 1962) medium. Sucrose: 3% (w/v), Agar: 0.8-1% (w/v) and pH: 5.8.

Aseptic Manipulations
Aseptic culture was carried out in laminar air flow chamber.

Incubation
During the entire work, cultures were incubated in growth chamber equipped with two air conditioners and temperature controller to maintain temperature at26±1°c. A photoperiod of 16 hours alternating with 8 hours of darkness was maintained.

Preparation of Medium with Varied Concentration of Heavy Metals (Lead and Nickel)
All the constituents of the MS medium i.e. inorganic and organic nutrients, growth regulators and sucrose were mixed in required amount and the Volume of medium was raised by adding distilled water to attain the final concentration of nutrients in the medium as per requirement. The medium was poured in beakers and then the different amounts of heavy metals (the concentration of which is to be varied i.e. Pb or Ni) were added separately in each beaker. The beakers were marked for the concentration of heavy metal **(Table 1-2).**

Statistical Analysis
The observations recorded for the various experiments were subjected to following statistical analysis. The average (mean) was calculated by dividing the sum of values of observations for a particular treatment by the total number of observations for that treatment.

Standard Deviation
This is a measure of dispersion which was calculated by squaring the deviation of each observation from the mean, adding the squares, dividing by the number of observation and extracting the square.

Table 1- Callus induction medium for *E. coracana* with various concentrations of lead.

Medium	Concentration of Pb (µM)
MS + 2,4-D + Kn (Control)	Nil
MS + 2,4-D + Kn+Pb	100 µM
MS + 2,4-D + Kn +Pb	300 µM
MS + 2,4-D + Kn+Pb	500 µM

Growth regulators added: *Auxin- 2, 4D,Cytokine- Kinetin, Agar - 8gms/ liter.*

Table 2- Callus induction medium for *E. coracana* with various concentration of nickel.

Medium	Concentration of Ni (µM)
MS + 2,4-D + Kn(Control)	Nil
MS +2,4-D + Kn+ ($NiSO_4$)	100 µM
MS + 2,4-D + Kn+($NiSO_4$)	300 µM
MS + 2,4-D + Kn+($NiSO_4$)	500 µM

Steps of Experiment -1)

Primary Callus Induction A) Seeds was inoculated on MS Medium supplemented with 2mg/l 2,4-D+0.5mg/l Kinetin (control) After 3-4weeks induced callus of the control seeds were inoculated on MS medium with lower concentration of 2,4-D(0.2mg/l).

B) Seeds was inoculated on MS Medium supplemented with 2mg/l 2, 4-D+0.5mg/l Kinetin (control) In addition to this in one set of experiment lead concentration were varied in callus induction medium and in second set Nickel was varied, the amount of callus formed in each case was record and after 3-4 weeks all the calli were transferred to (MS+1mg/l NAA) regeneration media. Approximately 4-5 seeds were cultured in each flask and approx 5 replica of each concentration were made.

2) Embryogenic callus cultures and Plant Regeneration: Approx 250mg of callus was transferred to each flask for regeneration. The embryogenic callus obtained from primary subculture control, as well as the variation of Pb/Ni were transferred on regeneration i.e.MS+1mg/l NAA media.

3) Effect of Inorganic Nutrient in Overcoming Toxicity Third set of experiment was planned to study effect of some inorganic nutrients in overcoming the toxicity of Pb and Ni. Out of all these concentration of Pb/Ni toxic level was chosen.

Now regeneration media was prepared which was MS+NAA and addition of only nontolerant concentration of Pb/Ni with various concentration of inorganic nutrients ($MgSO_4$,$ZnSO_4$).

On this media embryogenic callus obtained from control after 3-4 subculture, approx 250 mg of callus(control) was transferred to each flask of regeneration media and addition of toxic concentration of Pb/Ni (300µM) with various concentration (0x, 1/2x, 1x, 2x, 3x, 4x) of inorganic nutrients ($MgSO_4$,$ZnSO_4$).

Results

A) Seed Culture (Table 3)After 2-3 weeks of inoculation of seeds, it was observed that the callus so developed was healthy, compact, nodulated, darkgreen embryogenic sectors along with watery translucent non embryogenic sectors (Fig 3a). Amount of callus formed was recorded (Fig 3b).Only the embryogenic sectors were transferred to maintenance medium; the non embryogenic portion of the callus was excluded from being subculture.

Table 3. Callus formation from seeds of *Eleusine coracana* L. cultured on MS medium Supplemented with 2, 4-D and Kn.

Media	Amount of callus formed per seed(mg)
Callus Induction Medium(CIM)	
MS +2,4-D (2mg/l)+Kn (0.5mg/l)	250
Maintenance Medium(MM)	
MS+2,4-D (0.2mg/l)	296

C.I.M=Callus Induction Medium, C=Control,S.D= Standard deviation
R.M=Regeneration Medium and M.M=Maintenance Medium

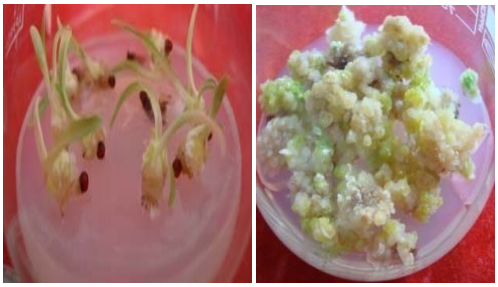

Figure3. Response of seeds on Callus Induction Medium and Response after this Primary callus was subculture on 2, 4-D (0.2 mg/l). a. Callus induced from seeds of *Eleusine coracana* on MS + 2, 4-D (2 mg/l) +Kn (0.5 mg/l). b. Embryogenic callus after subculture on MS medium with 2, 4-D (0.2 mg/l).

B) Callus Culture- Experiments for evaluating the effects of different heavy metals (Pb and Ni) were conducted in three sets. In the first set, seeds were used as the plant material and the heavy metal concentration was varied in callus induction medium (MS + 2mg/l 2, 4-D and 0.5 mg/l Kn). These calli were then transferred to regeneration medium (MS + 1mg/l NAA). In the second set of experiments primary callus was subcultured on maintenance medium (MS + 0. 2 mg/l 2, 4-D) and then subcultured on regeneration medium (MS+1mg/lNAA) supplemented with varied concentrations of heavy metals. In the third set of experimental embryogenic sectors of primary callus from control (MS + 2 mg/l 2,4-D + 0.5 mg/l Kn)were sub cultured on regeneration medium (MS + 1mg/l NAA) supplemented with toxic concentration of heavy metals and varied concentrations of nutrients ($MgSO_4$,$ZnSO_4$) (Fig3a-b).

Table 4. Variation in callus induction medium with varied concentration of lead regeneration response

Concentrations of Pb in Callus Induction Medium (µM)	Amount of callus formed / explants (mg)	Number of shoots per seed callus on plant regeneration medium ± S.D.
0 µM[c]	250	5.4 ±2.1
100µM	198	3.3±0.4
300µM	0	0
500µM	0	0

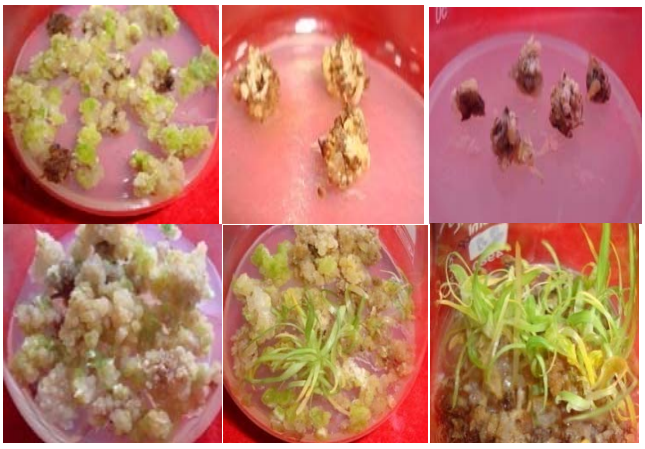

Figure 4. Effects of different concentrations of Pb on primary callus induction and plant Regeneration in *Eleusine coracana* as compare to control. a) MS+ (2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Lead (100µM); b) MS+(2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Lead (300µM); c)MS+ (2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Lead (500µM); d)MS+ (2 mg/l) 2, 4-D and (0.5 mg/l) Kinetin (control) e) Primary callus obtained on callus induction Medium with (100µM) Pb when sub cultured on MS medium with 1mg/l NAA (Regeneration medium); f) Primary callus sub cultured on MS medium with1mg/NAA(control).

Effects of Heavy Metals on Callus Induction and Plant Regeneration (lead and nickel)

1. Effects of Pb on primary callus induction and plant regeneration

In *Eleusine coracana*, regeneration was recorded via formation of shoot buds on enlarged apicaldomes and meristematic nodules (Kumar et al.2001). Here seeds of *Eleusine coracana* were inoculated on modified callus induction medium (2 mg/l 2, 4-D and 0.5 mg/l kinetin) with varied Pb levels (100µM, 300 µM and 500 µM). Calli were formed in treatments with 100 µM

concentration of lead-nodulated, compact, embryogenic and soft, watery non embryogenic callus. The primary callus from 100 µM concentration of lead was then transferred to normal regeneration medium (MS + 1mg/l NAA). After 2-3 weeks deep green and well developed regenerates appeared (Fig 4a-f) **(Table 4).**

2. Effects of $NiSO_4$ on primary callus induction and plant regeneration

Seeds of *Eleusine coracana* were inoculated on modified callus induction medium (2 mg/l 2, 4-D and 0.5 mg/l kinetin) with varied $NiSO_4$ and levels (100µM, 300 µM and 500 µM). After about 2-3 weeks of incubation, two types of calli were formed in treatments with 100 µM concentration of nickel- nodulated, compact, embryogenic and soft, watery non embryogenic callus. While there was no callus formation in treatments with 300 and 500 µM concentrations of nickel. Callus growth was better on nickel containing medium than lead containing medium. The primary callus from 100 µM concentration of nickel was then transferred to normal regeneration medium (MS + 1mg/l NAA). After 2-3 weeks of sub culture, deep green and well developed regenerants appeared. Nickel was considered less toxic than lead but callus and regeneration was worse than control. (Fig 5a-f) **(Table 5).**

Table 5.Variation in callus induction with varied concentrations of Nickel and regeneration response

Concentrations of $NiSO_4$ in Callus Induction Medium(µM)	Amount of callus formed / explants (mg)	Number of shoots per seed callus on Plant Regeneration Medium ± S.D
0 µM[c]	**250**	**5.2 ± 1.4**
100µM	**206**	**3.8 ± 0.7**
300µM	**0**	**0**
500µM	**0**	**0**

Figure 5. Effects of different concentrations of $NiSO_4$ on primary callus induction and plant regeneration in *Eleusine coracana* as compare to control. a) MS+(2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Nickel 100µM; b) MS+(2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Nickel300µM; c)MS+(2 mg/l) 2, 4-D and (0.5 mg/l) kinetin +Nickel500µM; d) MS+(2 mg/l) 2,4-D and (0.5 mg/l) kinetin. e) Primary callus obtained on callus induction medium with (100µM) $NiSO_4$ when subcultured on MS medium with 1mg/l NAA (Regeneration medium). f) Primary callus sub cultured on MS medium with 1mg/l NAA (control)

3.Effects of Pb on Plant Regeneration

Embryogenic callus from maintenance medium (MS + 0. 2 mg/l 2,4-D) were further transferred on regeneration medium (MS + 1mg/l NAA) supplemented with varied concentrations of Pb (100µM, 300 µM and 500 µM) to see the effects of Pb concentrations on

regeneration of *Eleusine coracana*. After 2-3 weeks, differentiation of shoot buds took place in medium containing 100µM concentration of Pb. Number of shoots per callus piece was counted (Fig 6a-d) **(Table6).**

Table 6. Embryogenic callus (MS+2, 4-D 0.2mg/l) response in variation in plant regeneration medium with different concentration of Lead.

Concentrations of Pb in plant Regeneration Medium (µM)	Mean number of shoot formed / Callus Piece ± S.D.
0 µM[c]	**5.2 ± 1.1**
100µM	**3.8 ± 2.2**
300µM	**0**
500µM	**0**

Initial weight of callus=296mg

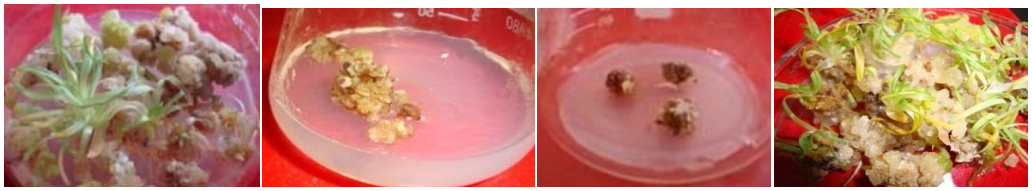

Figure 6. Effects of Pb variations on Plant Regeneration from embryogenic callus.
a) Plant regeneration from embryogenic callus on MS + NAA containing with Pb (100µM).
b) Plant regeneration from embryogenic callus on MS + NAA containing with Pb (300µM).
c)Plant regeneration from embryogenic callus on MS + NAA containing with Pb (500µM)
d)Plant regeneration from embryogenic callus on MS + NAA containing without Pb .

Table7. Embryogenic callus (MS+2, 4-D 0.2mg/l) response in variation in plant regeneration medium with different concentration of Nickel sulphate.

Concentrations of $NiSO_4$ in plant Regeneration Medium(µM)	Mean number of shoots formed / callus piece ± S.D
Control	5.6 ± 1.7
100µM	4.2 ± 0.9
300µM	0
500µM	0

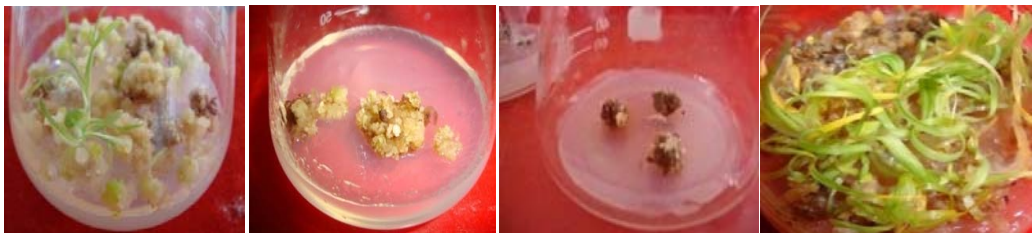

Figure 7) Effects of $NiSO_4$ variations on Plant Regeneration from embryogenic callus.
a)Plant regeneration from embryogenic callus on MS+ NAA containing with $NiSO_4$ (100µM).
b)Plant regeneration from embryogenic callus on MS +NAA containing with $NiSO_4$ (300µM).
c)Plant regeneration from embryogenic callus on MS+ NAA containing with $NiSO_4$ (500µM).
d)Plant regeneration from embryogenic callus on MS +NAA containing without $NiSO_4$ (control).

4. Effects of $NiSO_4$ on Plant Regeneration
Embryogenic callus from maintenance medium (MS + 0. 2 mg/l 2,4-D) were further transferred on regeneration medium (MS + 1mg/l NAA) supplemented with varied concentrations of $NiSO_4$ (100µM, 300 µM and 500 µM)After 2-3 weeks, differentiation of shoot buds took place in medium containing 100µM concentration of $NiSO_4$ and number of shoots per callus piece was counted (Fig7a-d) **(Table7).**

Effects of inorganic nutrients on Lead and Nickel toxicity

5. Effects of $MgSO_4$ on lead toxicity for Plant Regeneration
It was observed that $MgSO_4$ at 2x concentration of the MS medium was able for overcoming lead toxicity. The callus induced on callus induction medium with 100µM Pb when cultured on regeneration medium with MS level of magnesium produced 3.3 shoots per callus piece. When 300 µM Pb was increased to two times the normal level of MS medium $MgSO_4$ 4.2 shoots were obtained per callus piece suggesting that $MgSO_4$ at this level can overcome lead toxicity. This number was almost similar to regeneration under control conditions i.e. callus induced on normal callus induction medium cultured on normal regeneration medium. However it was seen that on increase of the $MgSO_4$ to four times of normal level improved regeneration (Fig8a-f) **(Table 8).**

Table 8.Variation in plant regeneration, response from embryogenic callus of *Eleusine coracana* on MS with toxic level of Pb (300µM) and varied concentrations of $MgSO_4$

The toxic level of Pb in Regeneration Medium	Concentrations of $MgSO_4$ (mM) added in Regeneration Medium	Mean number of shoots formed / callus ±S.D
	0mM	**0**
	0.75mM	**0**
300µM	1.5* mM	**0**
	3mM	**4.2 ± 0.9**
	4.5mM	**5.2 ± 1.1**
	6mM	**5.6 ± 0.4**

* Normal level in the ms medium and initial weight of callus=250mg

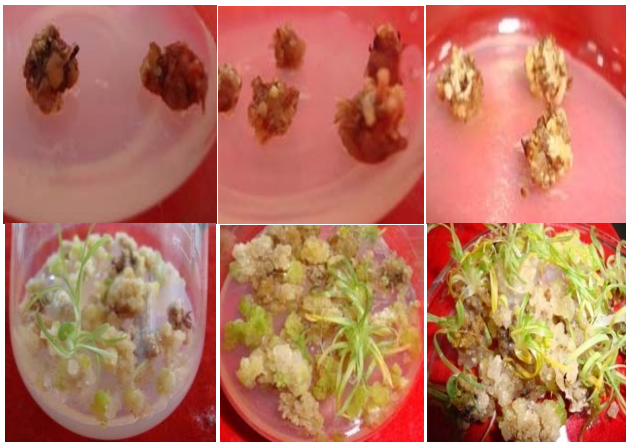

Figure 8. Effects of $MgSO_4$ on Lead toxicity in Plant Regeneration. a)Plant regeneration on MS medium without $MgSO_4$+NAA (1mg/l). b) Plant regeneration on MS medium with 0.75mM $MgSO_4$+NAA (1mg/l). c) Plant regeneration on MS medium with 1.5 mM$MgSO_4$ +NAA (1mg/l). d) Plant regeneration on MS medium with 3mM $MgSO_4$+NAA (1mg/l). e) Plant regeneration on MS medium with 4.5mM$MgSO_4$ +NAA (1mg/l). f) Plant regeneration on MS medium with 6mM $MgSO_4$+NAA (1mg/l).

6. Effects of $ZnSO_4$ on Nickel toxicity for Plant Regeneration

It was observed that $ZnSO_4$ at 2x concentration of the MS medium was able for overcoming nickel toxicity. The callus induced on callus induction medium with 100µM $NiSO_4$ and when subcultured on regeneration medium with MS level of magnesium produced 4.2 shoots per callus piece. When $ZnSO_4$ was increased to two times the normal level of MS medium 4.9 shoots were obtained in regeneration medium. However it was seen that on increase of the $ZnSO_4$ to three times of normal level improved regeneration but further increase of $ZnSO_4$ at Four times decrease the regeneration frequency (Fig 9a-f) **(Table 9).**

Table 9. Variation in plant regeneration response from embryogenic callus of *Eleusine corcana* on MS with toxic level of $NiSO_4$ (300µM) and varied concentrations of $ZnSO_4$.

The toxic level of $NiSO_4$ in Regeneration Medium	Concentrations of $ZnSO_4$ (mM) added in Regeneration Medium	Mean number of shoots formed / explant ±S.D
	0mM	**0**
	14.95mM	**0**
300µM	29.91*mM	**0**
	59.82mM	**4.9 ± 1.7**
	89.73mM	**5.4 ± 0.8**
	119.64µM	**4.4 ± 0.5**

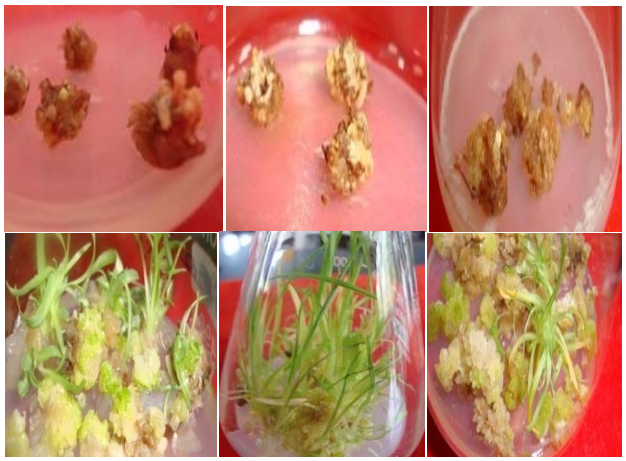

Figure 9) Effects of $ZnSO_4$ on Nickel toxicity in Plant Regeneration. a) Plant regeneration on MS medium without $ZnSO_4$ +NAA (1mg/l). b)Plant regeneration on MS medium14.95mM $ZnSO_4$+NAA (1mg/l). c) Plant regeneration on MS medium 29.91*mM $ZnSO_4$+NAA (1mg/l). d) Plant regeneration on MS medium 59.82mM $ZnSO_4$+NAA (1mg/l). e) Plant regeneration on MS medium 89.73mM $ZnSO_4$+NAA (1mg/l). f) Plant regeneration on MS medium 119.64µM $ZnSO_4$+NAA (1mg/l).

Discussion

Biotechnology of millets has lagged behind the major cereal crops due to difficulties in plant regeneration and poor transformation efficiencies (Kothari et al. 2005). Inorganic nutrients are major components of MS medium and hence offer to be the best variable to study their effect on the morphogenetic potential of the plant under study. Of all the micronutrients, Magnesium and Zinc has gained utmost importance because it is known to enhance

the embryogenic callus growth, and plant regeneration efficiency in several other plants (Popelka and Altpeter 2001; Nirwan and Kothari 2003; Tahiliani and Kothari 2004; Joshi and Kothari 2007). According to Ramage and Williams (2002) in some cases callus proliferation and plant regeneration can be improved by modifying the salt concentration, irrespective of the growth regulator used, the regenerants grown from such a medium performed better in *Eleusine* and many times there is increase in regeneration response in the presence of elevated levels of Mg and Zn was observed. This observation was supported by various workers in different plants (Dahleen 1995; Bregitzer et al. 1998; Yang et al. 1999; Zhang et al. 1999; Chauhan and Kothari 2004; Joshi and Kothari 2007). In our study an improvement of in vitro response has been discerned when inorganic nutrients was added to the MS medium. However, Witte et al. (2002) completely denied the role of CoCl2 in plant tissue culture. They reported presence of CoCl2 to accumulation of urea which is not good for plant growth. Similarly, higher levels of CoCl2 also stimulated the production of betalains (Trejo-Tapia et al. 2003), which inhibits morphogenesis in both rice and barley at concentrations higher than the optimum. The increasing world population and the unpredictable changes in the world climate pose a threat on the already shrunken food basket. Millets are an excellent alternative cereal for coeliac patients. Coeliac disease is a lifelong intolerance to glutin found in wheat, barley and rye, the prevalence rate of which is found to be 1:100 (Mc Gough and Cummings 2005). Improvement in the millet yield and production is attempting alternate. Various studies with manipulated levels of mineral proved that every species has its particular level of mineral requirement. Catering the need of a particular plant further requires the extensive evaluation of tissue culture medium to optimize the regeneration response.In *Eleusine coracana* (L.) Gaertn which mainly grows in dry condition faces the toxicity of Lead and Nickle, which may have negative effect on its yield and these heavy metals decrease the growth rate of explant in *in vitro* conditions. Some inorganic nutrients can overcome the toxicity of heavy metals. At non-toxic concentration of heavy metals, addition of these inorganic nutrients in media can increase the resistance of explant against heavy metal toxicity. Experiments for evaluating the effects of different heavy metals (Pb and Ni) were conducted in three sets. Callus induction was obtained only in the medium containing 100 µM concentrations of Pb and Ni but there was better growth obtained in Ni containing medium than Pb. These results show that Pb and Ni above 100 µM concentration were toxic for callus induction in *Eleusine coracana* but Pb was more toxic than Ni. Embryogenic sectors of the seed callus obtained from media containing 100 µM concentration of Pb and Ni was sub cultured in the regeneration medium (MS + 1 mg/l NAA) for shoot regeneration. Sub cultured regenerants appear deep green and well developed. Sub culturing of callus obtained from control maintenance medium (MS + 0. 2 mg/l 2,4-D) on regeneration medium (MS + 1mg/l NAA) supplemented with varied concentrations of Pb and Ni also resulted in the shoot regeneration in medium containing 100 µM concentration of Pb and Ni. These results show that Pb and Ni were also toxic for shoot regeneration in *Eleusine coracana* above 100 µM concentration. Embryogenic sectors of seed callus from control (MS + 2 mg/l 2,4-D + 0.5 mg/l Kn) were sub cultured on regeneration medium (MS + 1mg/l NAA) supplemented with of toxic level of heavy metals and varied concentrations of inorganic nutrients ($ZnSO_4$ and $MgSO_4$) to minimize the toxicity of heavy metals. Results obtained show that $ZnSO_4$ at the four times of MS level was best to minimize the toxicity of lead and $MgSO_4$ at the three times of MS level was best to minimize the toxicity of nickel.

Conclusion

These experiments led to a conclusion that lead and nickel above 100 µM concentration are inhibitory for callus induction as well as for plantlet regeneration in *Eleusine coacana*. It can be concluded that magnesium and zinc can minimize the toxicity of Pb and Ni but Zinc at four times of MS level was resulted worse shoot induction three times.

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