*Research Journal of Recent Sciences* _____ ISSN 2277-2502
Vol. **2(ISC-2012),** 24-28 **(2013)** *Res. J. Recent. Sci.*

# Effects of heavy metal stress on callus induction and regeneration of Finger millet (*Eleusine coracana*) (L.) Gaertn

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**Available online at: www.isca.in**
Received 20th October 2012, revised 31st December 2012, accepted 30th January 2013

## Abstract

*Today Abiotic stress is a major global problem limiting crop productivity and Stress factors are a serious problem limiting the yield potential of modern cultivars faced by mankind now a days. Stress causes nutritional imbalances in the plant causing reduction in water uptake and toxicity, decreasing the production and Seeds of Eleusine coracana (L.) Gaertn variety PR202 were taken as explants here and inoculated on callus induction medium with varied ($Cd(NO_3)^2$ levels (100µM, 300 µM and 500 µM) Calli were formed in treatments with 100 µM concentration of Cd. Induced Callus were sub cultured on maintenance media and then on regeneration medium (MS + 1mg/l NAA) supplemented with toxic level of heavy metals. Cd above 100 µM concentration was inhibitory for callus induction as well as for plantlet regeneration*.

**Keywords:** Heavy metal, MS media, Cd $(NO_3)^2$.

## Introduction

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 **Figure-2**. 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 **Figure1**. 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[1]. 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[2]. Tissue culture techniques offer an easy and important tool in developing stress tolerant variants[3]. Studies were conducted on diabetics (male and female) living in different rural and urban locations in 2008 in different countries[4]. Finger millet is especially valuable as it contains amino acid methionine, which is lacking in the diets of millions of the poor people. 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[5]. Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations[6]. They present a risk for primary and secondary consumers and ultimately humans[7].

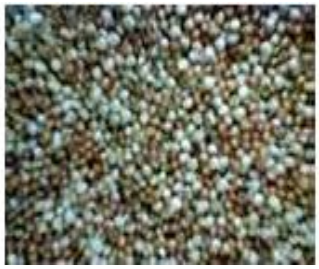

**Figure1** **Figure2**
**Figure1-Seeds of Eleusine coracana**
**Figure2-Mature Plant of Eleusine coracana**

Cadmium is an industrial pollutant in constant rise in the environment, due to activities such as mining, smelting and refinement of zinc, manufacturing and use of fungicide and phosphorous fertilizers, metallurgy, among others[8]. Although Cd is not an essential mineral nutrient for plants, it is easily absorbed by the root system, causing a decrease in transpiration and photosynthesis and an increase in the respiratory rates[9,10,11]. These effects seem to be related to Cd induction of premature senescence in plants[12]. Cadmium, being a highly toxic metal pollutant of soils, affects nutrient uptake and homeostasis, inhibits root and shoot growth and yield. Zn supplementation efficiently reduced lipid peroxidation, electrical conductivity and lipoxygenase activity induced by Cd[13]. Stress-induced enzyme, peroxidase, in the regenerating tissues of *Linum usitatissimum* showed less activity in cadmium and zinc combinations[14]. Looking to the scope and realizing its importance, the present study was undertaken to investigate the effects of different concentrations of Cd elements on callus induction and plant regeneration with the following Objectives- To standardize a medium with growth regulators for tissue

culture of *Eleusine coracana and* to study the influence of Cd in the morphogenesis of *Eleusine coracana* tissue culture and Implantation in field to increase the productivity of cereals. Levels of different heavy metals were varied taking MS as the standard medium.

## Materials and Methods

Important plant Eleusine *corcana* (L.) Gaertn of family Poaceae was taken as the model plant for the study. Seeds of agronomically superior and released variety (PR-202) of Ragi were procured from Agricultural University, Bangalore and this study was conducted in 201-12 at International College for girls, Department of Life Sciences, Jaipur. Common Name: Finger millet- Vernacular Name: Ragi, Explants Taken:- Explants taken for the experiment-Seeds. Sterilization and Preparation of Explants -Explants taken were aseptically sterilized. 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 -Cultures were incubated in growth chamber equipped with two air conditioners and temperature controlled at 26±1°c.A photoperiod of 16 hours alternating with 8 hours of darkness was maintained. Statistical Analysis- The observations recorded for the various experiments were subjected to following. Average: 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.

**Preparation of Medium with Varied Concentration of Heavy Metals (Cd):** Callus Induction, Embryogenic callus and Plant Regeneration A) Seeds of *Eleusine coracana* were inoculated on callus induction medium with varied Cd levels (100µM, 300 µM and 500 µM) .Seeds was inoculated on MS Medium supplemented with 2mg/l 2,4-D+0.5mg/l Kinetin (control) .In addition to this Cd concentration were varied in callus induction medium and the amount of callus formed in each case was record and after 3-4 weeks all the calli were transferred to MS medium with lower concentration on (2,4-D(0.2mg/l)+Heavy metal) maintenance media and then on regeneration media with MS+1mg/l NAA + heavy metal. In this experiment heavy metal was added in each step of subculturing in compare to control. B). Seeds was inoculated on MS Medium supplemented with 2mg/l 2,4-D+0.5mg/l Kinetin (control) the amount of callus formed in each case was record and after 3-4 weeks all the calli were transferred to MS medium with lower concentration (2,4-D(0.2mg/l) maintenance media without heavy metal and then on regeneration media (MS+1mg/l NAA) with heavy metal. In control there is no heavy metal in any stage of subculturing. Approximately 4-5 seeds were cultured in each flask and approx 5 replica of each concentration were made. Approx 250mg of callus was transferred to each flask for regeneration. In this experiment heavy metal were added only in regeneration media in compare to control.

## Results and Discussion

Seed Culture **Table-1** Seeds of *Eleusine coracana* were inoculated on callus induction medium with varied Cd levels (100µM, 300 µM and 500 µM) After 2-3 weeks of inoculation of seeds, it was observed that the callus was healthy, compact, nodulated, dark green embryogenic sectors along with watery translucent non embryogenic sectors **Figure 3a**. Amount of callus formed was recorded. Only the embryogenic sectors were transferred to maintenance medium, the non embryogenic portion of the callus was excluded from being subculture and then obtained callus were transferred on Regeneration media **Figure-3b**. Callus Culture and Plant Regeneration **Table- 2** Approx 250mg of maintenance callus was transferred to each flask for regeneration. Experiments for evaluating the effects of heavy metal (Cd) were conducted and regenerated shoots were transferred on soil pots **Figure-3c**.

**Table-1**
**Callus induction medium for *E.coracan* with various concentrations of Cadmium**

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

Growth regulators added: Auxin (2, 4- D), Cytokine (Kinetin/Kn), Agar (8 g/l)

**Table2**
**Callus formation from seeds of *Eleusine coracana* L. cultured on MS medium supplemented with 2, 4-D and Kn. and on Maintenance Medium. (Control)**

| Media | Amount of callus formed per seed (mg) |
|---|---|
| Callus Induction Medium(CIM) MS +2,4-D (2mg/l)+Kn (0.5mg/l | 200 |
| Maintenance Medium(MM) MS+2,4-D (0.2mg/l) | 250 |

C.I.M.=Callus Induction Medium, C=control, S.D= Standard deviation, R.M.=Regeneration Medium and M.M=Maintenance Medium and Number of explants =5 seeds/flask



**Figure 3a** **Figure3b** **Figure3c**

**Figure-3 a-b Response of seeds on Callus Induction Medium and Response after Primary callus transferred on Regeneration media. Figure 3-a Callus induced from seeds of on MS + 2, 4-D (2 mg/l) +Kn (0.5 mg/l), Figure 3-b Embryogenic callus after subculture on regeneration media, Figure3-c Regenerates were transfer to pot from regeneration media**

Effects of Heavy Metal on Callus Induction and Plant Regeneration (Cd), Effects of Cd on primary callus induction and plant regeneration table-3.

**Table-3**

**Response on callus and regeneration with continues varied concentration of Cd in media**

| Concentrations of Cd in Callus Induction Medium(µM) | Amount of callus formed / explant(mg) | Number of shoots per seed callus on plant regeneration medium ± S.D. |
|---|---|---|
| 0 µM | 250 | 12.4 ± 6.4 |
| 100µM | 150 | 3.3±0.4 |
| 300µM | 0 | 0 |
| 500µM | 0 | 0 |

**Table-4**

**Response on plant regeneration with varied concentration of Cadmium only in regeneration media**

| Concentrations of Cd in plant Regeneration Medium (µM) | Mean number of shoot formed / Callus Piece ± S.D. |
|---|---|
| 0 µM | 13.4 ± 1.45 |
| 100µM | 6.8 ± 2.2 |
| 300µM | 0 |
| 500µM | 0 |

Initial weight of callus=200mg

Seeds of *Eleusine coracana* were inoculated on callus induction medium with varied Cd levels (100µM, 300 µM and 500 µM) Calli were formed in treatments with 100 µM concentration of Cd Figure-4b .While there was no callus formation in treatments with 300 and 500 µM concentrations of Cadmium Figure4c-d.Calli were nodulated, compact, embryogenic, soft and watery. Control callus was more compact in compare to heavy metal callus Figure-4a. Cd containing media callus was worse than control, only the embryogenic sectors of calli were transferred to maintenance medium with heavy metal, the non embryogenic portion of the callus was excluded from being subculture and then obtained callus were transferred on regeneration media with heavy metal. After 2-3 weeks deep green and well developed regenerates appeared. Less shoots are developed when heavy metal was added continue in media in compare to Heavy metal added only in regeneration media. Effects of Cd on Plant Regeneration Table-4 Embryogenic callus from maintenance medium (MS + 0. 2 mg/l 2, 4-D + without heavy metal) were further transferred on regeneration medium (MS + 1mg/l NAA) supplemented with varied concentrations of Cd (100µM, 300 µM and 500 µM) to see the effects of Cd concentrations on regeneration of *Eleusine coracana*. After 2-3 weeks, differentiation of shoot buds took place in medium containing 100µM concentration of Cd Figure-4f. Number of shoots per callus piece was counted. Regeneration was worse than control Figure-4e. Shoots are better than continue containing heavy metals media. In this experiment heavy metal were added only in regeneration medium Figure-4g *Eleusine Coracana* (L) Gaertn which mainly grows in dry condition faces the toxicity of cadmium which may have negative effect on its yield and these heavy metals decrease the growth rate of explants in *in vitro* conditions. *Holarrhena antidysenterica* L nodal part when treated with $ZnSO_4$ proved to be less inhibitory in comparison to $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$. There is no response on $CdCl_2$ containing medium[15]. Cu and Zn significantly reduced seed germination and root growth in compare to Cd, Pb in *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway), and *Foeniculum vulgare* L. (fennel) [16]. In Cabbage when Zn, Cu, Cd, Pb and Hg presence in excess reduced glutathione (GSH) greatly and increases the soluble and immobilized peroxidase activity[17]. And also observed effects of different heavy metals Ni,Cd,Cu,Zn on different variety of *Eleusine coracana* . [18,19, 20].If low concentration of auxin along with cytokinin increases the rate of shoot multiplication and found that 2iP was the best for shoot elongation[21]. An appropriate concentration of IBA and 2ip was effective on traits of micropropagation of Cymbidium orchid[22]. The value of SSI increases with increasing salinity levels for thirteen sorghum genotypes[23]. In Barley Results showed that paclobutrazol (40 mg l-1) minimizes the negative effects of water stress (60% ETc) with evidence of enhancing leaf water potential by up regulating the endogenous production of proline and antioxidant enzymes like SOD and CAT leading to

maximization of fruit yield accompanied with higher WUE[24].Experiment for evaluating the effects of heavy metal (Cd) was conducted. Callus induction was obtained only in the medium containing 100 µM concentrations of Cd .These results show that Cd above 100 µM concentration were toxic for callus induction in *Eleusine coracana* .Embryogenic callus obtained from 100 µM concentration of Cd was sub cultured in the regeneration medium (MS + 1 mg/l NAA) for shoot regeneration. Sub cultured regenerates appear deep green and well developed. These results show that there is no shoot regeneration in *Eleusine coracana* above 100 µM concentration of heavy metal. Shoots of heavy metals containing media were worst then control. In first Experiment Heavy metal are added in each step of experiment like initiation of callus and then in subculturing on maintenance media and in last in regeneration media to observe the heavy metal stress in all stages of subculturing and survivability of plant in every stage with heavy metals. In experiment second heavy metal was added only in regeneration media to observe the stress only in regeneration media.

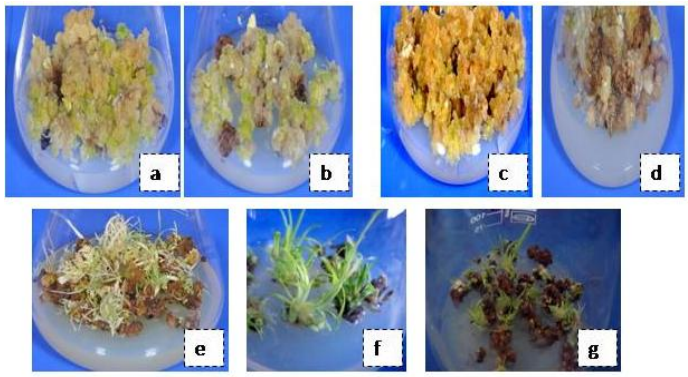


**Figure 4a-f**

**Effects of varied concentrations of Cd on primary callus induction and plant Regeneration *as* compare to control Figure 4a MS+2, 4-D and kn(control), Figure 4b MS+2, 4-D and kn +Cd(100µM) Figure 4c MS+2, 4-D and kn+Cd (300µM)Figure 4d MS+ 2, 4-D and kn +Cd (500µ) Figure 4e Primary callus sub cultured on regeneration media (control) Figure 4f Primary callus obtained from (100µM) Cd concentration media sub cultured on Regeneration medium with heavy metal. Figure 4g No heavy metals containing Primary callus subcultured on regeneration media with heavy metals**

## Conclusion

These experiments led to a conclusion that Cadmium above 100 µM concentration is inhibitory for callus induction as well as for plantlet regeneration in *Eleusine coracana* but callus and regeneration was worse than control.

## Acknowledgments

This work was supported by International College for girls, Jaipur, the IIS University Jaipur-20

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