



STUDIES ON EFFECT OF SOME HEAVY METALS ON *IN VITRO* CALLUS INDUCTION AND RHIZOGENESIS IN *VIGNA MUNGO* (L.) HEPPER

Sreelatha. Thallapally

Head & Assistant Professor of Botany

Department of Botany, Govt. Degree College,

Jammikunta Dist. Karimnagar

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. Heavy metals represent a growing threat for ecosystems worldwide. In India, several studies have searched the amounts of heavy metals in soils at roadsides of high ways and soils of litter dumps. The conducted studies found that these soils contained worrying levels of heavy metals that have exceeded in many cases the average world safe limit. For example, Chromium (Cr) level was 79 mg/ kg in soils closed to some highways while the world safe limit is only (25 mg/kg). In other studies, Nickel (Ni) concentration was (5.9 mg/kg) while the world safe limit for Mn is only (0.03 mg/kg). Also in some litter dumps in India, soil Magnesium (Mg) average content was (6.0 mg/kg) while the world safe limit was only (0.1 mg/kg). *Vigna mungo* (L.) Hepper is a flowering plant that has recently attracted the attention of researchers due to its novel phytoremediation powers against some heavy metals, which encouraged some countries to grow them in roadsides of highways and litter dumps. Tissue culture is an excellent approach for studying responses of *Vigna mungo* (L.) Hepper to heavy metals without interference of other factors. In addition, tissue culture is the main method for producing elite plants lines from callus and cell suspension cultures with superior characteristics by genetic transformation.

Stress causes nutritional imbalances in the plant causing reduction in water uptake and toxicity, decreasing the production and Seeds of *Vigna mungo* (L.) Hepper T-9 var were taken as cotyledonary explants here and inoculated on callus induction medium with varied concentration of MS+ 2.4-D (1.0-3.0mg/L). (2.5mg.L 2.4-D) Induced best callus induction. This calli were formed in treatments with (0.5, 1.0, 2.0, 3.0, 5.0, 7.5 and 10 mg/1) concentration of Mn, Cr, Ni and Mg. Callus induction and rhizogenesis was studied with incorporation of some heavy metals individually at different concentrations in MS medium

supplemented with MS+ 2,4-D (2.0 mg/L) each. The study was carried out to see the overall effect of selected metals *in vitro* on variations in induction, nature and growth of callus and rhizogenesis produced in relation to metals and discussed in detail. The study was carried out to see the overall effect of selected metals *in vitro* on variations in induction, nature and growth of callus and rhizogenesis produced in relation to metals and discussed in detail.

Key Words: *Vigna mungo* (L.) Hepper, Heavy metal, MS media, 2,4-D, Mn, Cr, Ni and Mg

Introduction:

Stresses are increasing drastically today because of pollution, declining availability of quality water and land degradation. Heavy metals have played a great role in the genesis of present day civilization. Heavy metals are deposited on soils, the study of heavy metal pollutions specifically in agro system, because more important due to their non-biodegradable nature. Toxic heavy metals are mostly absorbed and get accumulated in various plant parts and free metals, which adversely effect in plant growth and metabolism. Human beings are effected when these metals are incorporated into the food chain. Though many of these are essential plant nutrients, but they become phyto toxic at higher concentrations.

Stress tolerant plants can also be developed by breeding and by transgenesis which are complex processes (Babu *et.al.*, 2017). Tissue culture techniques offer an easy and important tool in developing stress tolerant variants (Nabors and Dykes 1985). Studies were conducted on diabetics (male and female) living in different rural and urban locations in 2008 in different countries (Pradhan Adikant *et.al.*, 2010). Mature Plant of *Vigna mungo* (L.) 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 (Van *et.al.*, (1998). 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 (Bazzaz *et. al.*, (1974), Lee *et. al.*, (1976) and Lamoreaux and Chaney (1978).

Nickel is required by legumes (Eskew *et al.*, 1983) and cereals (Brown *et al.*, 1987) to complete their life cycle for a long time. The reductions in yield might be due to reduced uptake of nutrients and undesirable effects on growth and development and metabolism (Dahiya *et al.*, 1990) have observed the depressing effect of higher levels of P and Mn on Pea. (Bruce *et al.*, 1978) showed inhibitory effects of Mn, Zn, Co and Cd on tomato seedlings. At higher concentrations of chromium (192 and 384 μ M) the root became blunt and cotyledons did not expand properly. It is due to the inhibitions of cell division and cell elongation (Corradi *et al.*, 1991). Heavy metals ions such as Pb^{+2} , Mg^{+2} , Zn^{+2} when present at elevated level in the environment are taken up by the plants and get accumulated in different parts of the plant.

Materials and Methods:

Seeds of *Vigna mungo* (L.) Hepper T-9 var were soaked in concentrated Sulphuric acid (H_2SO_4) for 24 hours. These seeds were surface sterilized with 0.1% (W/V) Mercuric chloride ($HgCl_2$) solution for 3-5 minutes followed by three rinses with sterile distilled water.

These explants (seeds) were inoculated on MS medium supplemented with auxin 2, 4-D (1.0-2.5mg/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 $(26 \pm 1^\circ\text{C})$. 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

Cotyledon segments were surgically excised and inoculated in to culture tubes containing MS (Murashige and Skoog, 1962) media supplemented with (IBA + BAP 2.0 mg/L) each and different concentrations of Mn, Cr, Ni and Mg (0.5, 1, 2, 3, 5, 7.5 and 10 mg/l). After the addition of sucrose pH of the media was adjusted to 5.6 – 5.8 with 0.1 N NaOH / 0.1 N HCl. Then agar was added and heated gently with constant stirring till the added agar was dissolved. Media without the addition of heavy metals was constituted as control. Explants were inoculated and incubated at a temperature of $(25 \pm 2^\circ\text{C})$ under 16 h photo period. Callus induction and Rhizogenesis response were recorded after 4 – 6 weeks of culture.

The effects of treatments were qualified on the basis of percentage of cultures showing response, type of callus and rooting of response. All the experiments were carried out in triplicate. Each replicate consist of 40 culture tubes.

Preparation of Medium with Varied Concentration of Heavy Metals Mn, Cr, Ni and Mg.

Callus Induction and rhizogenesis, seeds of *Vigna mungo* (L.) were inoculated on callus induction medium with varied Mn, Cr, Ni and Mg (0.5, 1, 2, 3, 5, 7.5 and 10 mg/l). Cotyledon explants inoculated on MS Medium supplemented with (2.0 mg/L 2,4-D (Control). In addition to this Mn, Cr, Ni and Mg (0.5, 1.0, 2.0, 3.0, 5.0, 7.5 and 10 mg/l). Cr 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/Heavy metal) maintenance media and then on rhizogenesis media with (MS+1.0 mg/L NAA + heavy metal). In this experiment heavy metal was added in each step of sub culturing in compare to control.

Results and Discussion:

In control cultures, cotyledonry explants on MS media supplemented with (2.0 mg/L 2,4-D) (Table-1) showed direct rhizogenesis from light blood reddish callus from leaf explants. The initiation of callus and rhizogenesis took place with in one week and cent percent response was seen. Different concentrations of heavy metals were added to MS media to see their response.

The percentage of callusing response and rooting of response decreased as increasing concentrations of all the heavy metals added to the media individually (Table-2). Lowest concentration (0.5 mg/l) of Magnesium showed maximum percentage of response i.e. 85% compared to other heavy metals studied.

Ni (10mg/L) added cultures exhibited development of friable blood reddish and light green yellowish callus with few roots formation (Table-2) (Fig-1). In higher concentrations (7.5, 10 mg/L) of Cr and Mn treated samples observed friable blood reddish callus and friable blood reddish and yellowish callus free from root formation respectively. Friable blood red and yellowish callus from leaf explants on (MS

media + 2.0mg/L) (2mg/l each) + Cr 5.0mg/L, Ni 10mg/L and Mg 10mg/L exhibiting different response giving rhizogenesis formation and their growth respectively. Induction of callus and high response of rhizogenesis was first observed in Mg (0.5 mg/L) added media compared to other heavy metal treated samples.

Mustuoka and Hinata (1979) observed both embryogenesis and organogenesis in hypocotyls derived calli maintained in the presence of NAA. Frequency of shoot bud regenerations varied with dose of plant growth regulators in the MS medium. Highest frequency of shoot buds obtained at a concentration of 2 mg/l BAP (Nagarajan *et al.*, 2006), (Sanjeev Kumar *et al.*, 2003) performed the regenerating cultures subjected to different levels of CuSO₄ and ZnSO₄ to monitor morphogenetic events in *Tinospora cordifolia* and noted that CuSO₄ proved beneficial but higher level caused decline in growth.

Neelima and Jaganmohan Reddy (2005) reported enhancement of callus and regeneration at lower levels of heavy metals such as Hg, Cd and Zn but failed to produce shoot at higher concentrations of them. Multiple shoots were induced by culturing nodal explants excised from aseptic seedlings of *Capsicum annum* (Ahmad *et al.*, 2005). The callus induction from cotyledon explants as observed by Gosal and Bajaj (1979) in chickpea, lentil, mung bean and pea where in cotyledon explants showed callusing on MS basal medium supplemented with 2, 4-D (2 mg/l). Kumar *et al.*, (1983) observed callusing from cotyledon explants in pigeon pea on Blaydes medium supplemented with 2, 4-D (2 mg/l) + Kn (0.5 mg/l). Wali and Siddiqui (1999) reported callusing response from cotyledon explants of *Cajanus cajan* on MS basal medium supplemented with IAA + BAP (0.5 mg/l each). Differential response of excised embryo in induction of multiple shoots in different genotypes of *Cicer arietinum* observed by Vani Devi and Jaganmohan Reddy (1997). Similarly, the callus induction from different explants was also observed by them.

Table – 1

Morphogenetic response of Cotyledonary explants of *Vigna mungo* (L.) Hepper T-9 var on MS medium with different concentrations of 2, 4-D.

Hormone concn (mg/L)	% of cultures responding	Morphology	Callusing response
2.4 – D			
1.0	53	White compact	++
2.0	60	White compact	+++
3.0	80	FBRYC	+++
4.0	70	Creamy compact	++
5.0	58	Creamy compact	+

Relative amount of Callus formation: - - = No, + = low, ++ = moderate, +++ = high

*** Embryogenic callus**

Table-2 Effect of some heavy metals individually on Callus induction and Rhizogenesis from cotyledon callus cultures of *Vigna mungo* (L.) Hepper T-9 var

Concentrations in mg/L				Percentage of response	Rooting of response	Response of callus induction
Control				100	++++	BRC
Concentrations in mg/L						
Mn	Cr	Ni	Mg			
0.5	-	-	-	85	+++	FBRYC
1.0	-	-	-	80	++	FBRYC
2.0	-	-	-	72	++	FBRYC
3.5	-	-	-	60	++	FBRYC
5.0	-	-	-	53	++	FBRYC
7.5	-	-	-	40	+	FBRYC
10.0	-	-	-	24	A	FBRYC
-	0.5	-	-	89	+++	FBRC
-	1.0	-	-	80	+++	FBRC
-	2.0	-	-	76	++	FBRC
-	3.5	-	-	68	++	FBRC
-	5.0	-	-	55	++	FBRC
-	7.5	-	-	42	+	FBRC
-	10.0	-	-	35	A	FBRC
-	-	0.5	-	87	+++	FBRYGC
-	-	1.0	-	82	+++	FBRYGC
-	-	2.0	-	75	++	FBRYGC
-	-	3.5	-	68	++	FBRYGC
-	-	5.0	-	52	++	FBRYGC
-	-	7.5	-	41	+	FBRYGC
-	-	10.0	-	30	A	FBRYGC
-	-	-	0.5	90	+++	FBRC
-	-	-	1.0	85	+++	FBRC
-	-	-	2.0	82	+++	FBRC
-	-	-	3.5	76	++	FBRC
-	-	-	5.0	62	++	FBRC
-	-	-	7.5	60	++	FBRC
-	-	-	10.0	55	++	FBRC

BRC – Blood reddish callus, FBRYGC – Friable blood reddish yellow green callus, FBRYC – Friable blood reddish yellow callus, FBRC – Friable blood reddish callus, A – Absence.

Relative amount of Callus formation: + = low, ++ = moderate, +++ = high

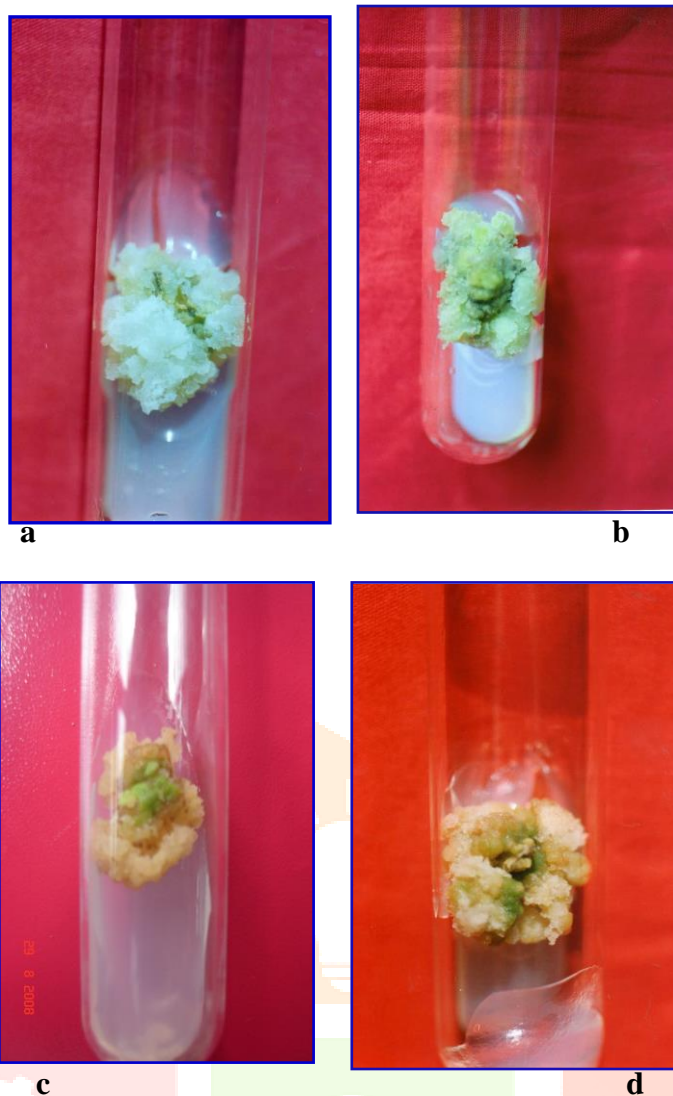


Figure-1 a-b Response of Cotyledon explants Callus Induction Medium and Response after Primary callus transferred on heavy metal treated media. Figure c and d on MS + 2, 4-D (2 mg/l) + Mn, Cr, Ni and Mg (10.0 mg/L)

References:

- Ahmad, N., Siddique, I. and Anis, M. (2005). Improved plant regeneration in *Capsicum annum* L. from nodal segments. *Biologia plantarum*, **2**.
- Babu S., Sheeba A., Yogameenakshi J. and Rangaswamy P., Effect of salt stress in the selection of salt tolerant hybrids in rice (*Oryza sativa* L.) under in vivo and in vitro conditions. *Asn J Plt Sci.*, **6**, 137-142 (2007).
- Bazzaz F.A., Rolfe G.L. and Carlson R.W (1974). Effect of Cd on photosynthesis and transpiration of excised leaves of corn and sunflower. *Physiologia Plantarum*. **32**, 373-376.
- Brown, P.H., Welch, R.M., Cary, E.E. and Checkai, R.T. (1987). Beneficial effects of nickel on plant growth, *J. Plant Nuir.*, **10**: 2125-2135.
- Bruce, S., Cachita Cosma, D. and Trifu, M.C. (1978). Morphological changes induced by microelements Mn, An, Co and Cd in tomato seedling (*Solanum lycopersicum*). *Stud., Univ. Babes. Balgen. Biol.* **2**: 11-18.

- Corradi, M.G., Levi, Mussetti, R and Favali, M.A. (1991). The effect of Cr (VI) on different inbred lines of *Zea mays*, Nucleic and cell cycle in the root tip tissue. *Protoplasma* **162**: 12-19.
- Dahiya, D.J., Dhankar, S.S., Dahiya, S.A., Narwal, R.P. and Singh J.P. (1990). Effect of P and Mn on dry matter yield and their concentration in pea. *J. Indian Soc. Soil. Sci.*, **38**: 448-451.
- Eskew, D.L., Welch, R.M. and Cary, E.E. (1983). Nickel, an essential micronutrient for legumes and possibly all higher plants. *Science*, **2122**: 691-693.
- Gosal, S.S. and Bajaj, Y.P.S. (1979). Establishment of callus tissue cultures and the induction of organogenesis in some grain legumes. *Crop. Improv.* **6(2)**: 154-160.
- Jaganmohan Reddy, K. and Vanidevi, K. (1997). Differential response of excised embryo in induction of multiple shoots in different Genotypes of *Cicer arietinum* L. *Ad. Plant Sci.* – **10(2)**: 79-82.
- K. Vani Devi and K. Jagan Mohan Reddy (1997). Induction of calli indifferent explants of chick pea. *J.Swamy Bot. Cl.* (1997), **14**: 118-120.
- Kumar, A.S., Reddy, T.P. and Reddy, G.M. (1983). Plantlet regeneration from callus cultures of pigeon pea. *Plant Sci. Lett.* **32**: 271-278.
- Lamoreaux R.J and Chaney W.R (1978). The effect of cadmium on net photosynthesis transpiration, and dark respiration of excised silver maple leaves, *Physiologia Plantarum*, **43**, 231-236.
- Lee K.C., Cunningham B.A., Paulsen B.M., Liang B.H. and Moore R.B (1976)., Effects of cadmium on respiration' rate and activities of several enzymes in soybean seedlings. *Physiologia Plantarum*. **36**, 4-6.
- Murashige T. & F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Plant Physiol.* **15**: 473-497.
- Mutsuoka, H. and Hinata, K. (1979). NAA induced organogenesis and embryogenesis in radicle cells of *Solanum melongena* L. *J. Exp. Bot.*, **30**: 363-370.
- Nabors M.W. and Dykes T.A., Tissue culture of cereal cultivars with increased salt, drought and acid tolerance. *Biotech in international agri res, IIRI Phillipines*, 121-138 (1985).
- Nagarjan, S.M., Rajasekaran, S., Sathees Kannan, T.M. and Sundaramoorthy, P. (2006). Direct shoot regeneration from nodal explants of *Bougainvillea Spectabilis* willd. *Plant Archives* **6(2)**: 537-539.
- Neelima, P. and Jaganmohan Reddy, K. (2005). *In vitro* callus induction and regeneration with effect of some heavy metals of *Solanum melongena* L. *Ad. Plant Sci.* **18(11)**: 457-466.
- Pradhan Adikant, Nag S.K. and Pati, Dietary management of finger millet (*Eleusine coracana* L. Gaerth) controls diabetes, *Current Science*. **98**, 6-25 (2010).
- Sanjeev Kumar, Alka Narula, Sharma, M.P. and Srivastava, P.S. (2003). Effect of Copper and Zinc on growth, secondary metabolite content and micropropagation of *Tinospora cordifolia*. *Phytomorphology*, **53(1)**: 79-91.
- Van assche F., Cardinales C. and Clijsters J (1998). Induction of enzyme capacity in plants as a result of heavy Doseresponse relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. *Environmental Pollution*, **52**, 103-115.
- Wali, S.A. and Siddiqui, S.A. (1999). Callusing response of cotyledon explant of pigeonpea (*Cajanus cajan* (L.) Millsp.) Var. Upas-120. Abstract: Natinal Symposium on Role of Plant Tissue Culture in Biodiversity Conservation and Economic Development, G.B. Plant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora, June 7-9, pp.27.