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Effective Callus Formation and Plant Regeneration of Chromium Induced Mutants in Eleusine Coracana(L.) Gaertn.

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ABSTRACT

In the present work two accessions of *Eleusine coracana* (E1, E2) were tested for callus formation and plant regeneration. The seedlings were raised in 10 different molar concentrations of Cr (10^{-1} to 10^{-10}M $K_2Cr_2O_7$). For dedifferentiation various seedling parts like hypocotyl and cotyledons or whole seedlings were used. Three growth hormones (auxin 2, 4-D and cytokinins, kinetin and BAP) were used for callus induction. 2, 4-D was used in combination with kinetin (KN) and BAP in ratios 3:1, 4:1 and 5:1. The callus was observed on the 5^{th} day from inoculation. The callus formation was found in all treatments except 10^{-2}M and 10^{-3}M Cr concentration. The callus formation was observed maximum in MS with 2, 4 –D and BAP in 5:1 in both the accessions. The callus was white brownish in colour and granular in nature. The MS medium with 2, 4-D and kinetin didn't respond. In E1, 10^{-10}M and 10^{-6}M showed better and in E2 10^{-8}M and 10^{-6}M showed good callus formation.

The calli obtained from above experiments were further subjected to shoot and root induction experiments. For shoot induction two cytokinins BAP and KN were used in concentrations 1, 2 and 3 µl in solid MS medium. Maximum shoots were observed in control and medium having 1µl KN in all concentrations except 10^{-4}M and 10^{-3}M, in both accessions. MS with BAP showed poor results. For root formation auxins 2, 4-D and NAA were used in concentrations 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl/L in solid MS. Maximum roots were observed in control and lower Cr concentrations of NAA in both the accessions. When NAA was used root formation was observed in all treatments except 10^{-3} and 10^{-2} M, whereas when 2, 4 –D was used no root formation was observed in all treatments.

Keywords: chromium, mutants, dedifferentiation, regeneration.

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INTRODUCTION

The genera *Eleusine* has 9 species in tropics and subtropics, whileonly3 species *(E. compressa, E. indica* and *E. coracana)* are found in India and belongs to the family Poaceae. It is commonly cultivated to 2000m, rarely met as an escape. It is widely cultivated in the tropics, and is introduced intothe new world. *Eleusine* flowers and fruits from August to October. The *E. coracana* (Finger millet) is commonly known as **mandwa** or **kodu** in Garhwal. *Eleusine coracana* a species native to tropical East Africa is a short stemmed, dry land adapted, milletwith excellent storage characteristics and an outstanding mineral content (Gaur, 1999).

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. Tissue culture techniques offer an easy and important tool in developing stress tolerant variants.

In the present work *Eleusine coracana* was exposed to heavy metal chromium (10^{-1}M to 10^{-10}M $K_2Cr_2O_7$) treatment. The mutants which survived in the heavy doses were exposed to *in vitro* tests for callus formation and plant regeneration. For this purpose earlier *in vitro* works in this crop were considered. Rangan (1976) did experiments on growth and plantlet regeneration in tissue cultures of *E. coracana*. Mohanty *et al.* (1985) then worked on callus initiation and plant regeneration of the plant. High frequency plant regeneration through somatic embryogenesis in finger millet*(E. coracana*)was observed by Eapen and George (1989). High frequency embryoid and plantlet formation from tissue cultures of the finger millet was achieved by Sivadas *et al.* (1990). Kumar *et al.* (2001) worked on the tissue culture of *E. coracana* and found that in cultured embryos first an enlarged apical dome forms and then shoot buds get differentiated on the entire surface of the dome. In the transformation of the millet callus and leaf explants were used. *Uid A* was used as a vector and the expression observed was transient (Gupta *et al.* 2001).

Kothari *et al.* (2004) worked out inorganic nutrient manipulation for highly improved *invitro* plant regeneration in finger millet. Kamble *el al.* (2004) studied the variability for weight of callus in finger millet.*In vitro* culturing of *Eleusine coracana* was also worked out by a number of workers by using a number of growth promoting hormones 2,4-D, NAA, IBA, BAP and KN.

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Anjaneyulu *etal*. (2011) worked out an efficient protocol for callus Induction and plant regeneration in Finger Millet.

MATERIALS AND METHODS

In the present investigation two accessions of *Eleusine coracana* were procured from different places of Garhwal (Table 1). The seeds of each accession of *Eleusine* (E1 and E2) were first surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 3-4 minutes and washed thoroughly and then with distilled water. The seeds were placed on double layered filter papers (3 mm, Whattman, filter papers) with cotton pads 'sandwiched' between them equidistantly in various treatments in petri dishes and incubated under white light at 22°C. Ten molar solutions of $K_2Cr_2O_7$ ranging between 10^{-10} M to 10^{-1} M were prepared. The control sets were raised in distilled water and treated sets were raised in various molar concentrations of $K_2Cr_2O_7$. Only a fixed amount (about 50 ml.) of distilled water or Cr solutions was added in petri dishes. The various molar concentrations of Cr were prepared in Hoagland's solution (Table 2). The seedling parts were then exposed to tissue culture methods for callus formation and plant regeneration.

Standard tissue culture techniques were used as given below.

1. Explant preparation.
2. Tissue culture media preparation.
3. Methodology.
4. Sterilization of Medium.
5. Aseptic conditions.
6. Surface sterilization of plant material.
7. Inoculation.
8. Incubation and
9. Observations.

In present work MS medium was used. The pH of the medium was adjusted to about 5.8.

Seven stock solutions (A, B, C, D, E, F, and G) of 200 ml containing minerals and organic adjuvant were first prepared. Each and every time 20ml of these stock solutions were taken for preparing a liter of MS medium. Sucrose and agar were dissolved in DDW (double distilled water)

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separately and then the final volume was raised to 1000ml. The composition of stock solutions is given in table 3.

The medium after adding agar was poured in test tubes and flasks and autoclaved (15 min at 15 psi, 121^{o}C). Around 1 cm^2 of plant tissue was cut with a sharp blade. These explants were first washed with soapy water and running water for 15-20 minutes, and then transferred to a mercuric chloride solution (0.1%) for 5-6 minutes and then washed with DDW to remove any trace of $HgCl_2$. When the explant was surface sterilized, it was rinsed several times in sterile, distilled water. This last step was performed inside the laminar flow hood to maintain the aseptic condition of the explant and to prevent the re-introduction of contaminating microbes.

DDW and the implements such as scalpel, forceps, needles etc. were irradiated by ultraviolet germicidal light for 30 min before starting the inoculation and sub culturing procedures. All the implements were also sterilized by keeping them immersed in 90% alcohol and flamed on the spirit lamp before use. Inoculation was carried out in laminar air flow and all precautions were taken while doing this experiment. The vessels were properly labeled.

In the accessions E1 and E2 of *Eleusine coracana* all the control and treated seedlings were subjected to callus formation and regeneration attempts. For callus formation in *E. coracana* various seedling parts like hypocotyl and cotyledons or whole seedlings were also taken from precultured condition (fig. 1, 2). Three growth hormones (1 auxin and 2 cytokinins) were taken for callus induction, 2, 4-D, KN and BAP respectively. In the present work solid MS medium supplemented with growth hormones was used. The 2, 4-D was used in combination with KN and BAP in ratios 3:1, 4:1 and 5:1. In 4:1 ratio, the concentration of auxin and cytokinin used was 2ml: 0.5ml per liter of MS medium. The minor quantities of hormones were taken with the help of micropipette from the prepared stock solutions of growth hormones.

The calli obtained from above experiments were further subjected to shoot and root induction experiments. For shoot induction two cytokinins: BAP and KN were used in concentrations 1, 2 and 3 µM in solid MS medium. For root formation auxins: 2, 4-D and NAA were used in concentrations 0.1, 0.2, 0.3, 0.4 0.5 and 0.6 mg/L in solid MS medium.

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OBSERVATIONS

In *Eleusine* the callus induction was observed on the 5th day from the inoculation date (fig. 2). The callus formation was found to be in all treatments except 10^{-2} M and 10^{-3} M Cr concentration (table 4). The frequency of callus formation was maximum in combination 2, 4 –D and BAP at 5:1 ratio in both the accessions. The callus was white brownish in colour and granular in nature (Fig. 3, 4, 5). No results were found in combination 2, 4-D and KN. In E1, 10^{10} M and 10^{-6} M were showing more callus formation than other concentrations, similarly in E2, 10^{-8} M and 10^{-6} M were showing good callus formation.

The calli obtained from above experiments were further subjected to shoot and root induction experiments. The experiment of redifferentiation was carried out in 1000 lux light. For shoot induction two cytokinins BAP and KN were used in concentrations 1, 2 and 3 µM in solid MS medium. Maximum shoots were observed in control and medium having 1µM kinetin in all concentrations except 10^{-4}M and 10^{-3}M in both E1 and E2. Though shoots were observed in some concentrations of BAP also. No results were found in other concentrations as well as with BAP (table 5).

For root formation two auxins 2, 4-D and NAA were used in concentrations 0.1, 0.2, 0.3, 0.4 0.5 and 0.6 mg/l in solid MS. Maximum roots were observed in control and lower Cr concentrations (table 6) of NAA in both the accessions. When NAA was used root formation was observed in all treatments except 10^{-3} and 10^{-2} M, whereas when 2, 4 –D was used no root formation was observed in all treatments.

RESULTS AND DISCUSSIONS

The plant biotechnology has emerged as an important aid for rapid genetic improvement. Success with culture formation and plant regeneration has given impetus for further work in pulses and cereal crops. In the present work hilly crop *Eleusine coracana* was exposed to various treatments of heavy metal chromium (Cr). Cr being an industrial pollutant is very toxic. The main attempt of the present work was to raise plants in high Cr concentrations, so that they may be grown in Cr rich soils in plain areas.

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In the present investigation callus was observed in low concentrations of Cr but no callus was found in high Cr concentrations showing sensitivity of the plant to heavy metal. Similarly when organogenesis attempts were made, root induction and shoot induction was found only in low Cr concentrations. In 10^{-2}M and 10^{-3}M no root and shoot formation took place.

The cereals have received maximum attention in the past few decades in plant regeneration by tissue culture methods due to their contribution to the diet of man and livestock. Most of the major cereal crops can be regenerated from tissue culture. A large number of tissue culture works can be found in *Eleusine coracana* but to the best of our knowledge no such attempts have been made to culture the high Cr concentration plants, seedlings and seeds.

Eleusine is nutritionally very rich cereal and is a staple food for poor and invalids. *Eleusine* mainly growing in dry condition faces heat, salinity and heavy metal stress which may cause negative effect on its yield. Tissue culture techniques offer an easy and important tool in developing stress tolerant variants. In *Eleusine coracana* callus induction was found to be best in MS medium along with 2, 4-D and Kinetin in 4:1 (Kumar *et al*., 2001). They also found multiple shoot formation 1 mg/L NAA. Anjaneyulu *et al*. (2011) observed highest degree of callus formation in 2, 4-D and BAP (5:1). Shoot buds were also observed more in MS medium with 1 mg/L Kinetin and 0.5 mg/L NAA.

Krishna and Agarwal (2012) found MS medium with 2 mg/L 2, 4-D and 0.5 mg/L Kinetin to be more effective in callus formation. They observed the effect of heavy metal Pb and Ni on the callus formation and plant regeneration. Yemets *et al*. (2013) found that 2, 4-D caused callus formation, and also along with KN it gave better results. In the present investigation for shoot induction kinetin and BAP were used. But multiple shoots were observed in 1 µl in all control and treated sets. No results were obtained in BAP. For root formation NAA and 2, 4-D was used. Maximum rooting was found in 0.5 µl NAA and no rooting was found in 2, 4-D. no rooting and shooting was observed in high doses of Cr.

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Fig. 1. Precultured seedlings of *Eleusine*

Fig. 2. Callus induction in *Eleusine*

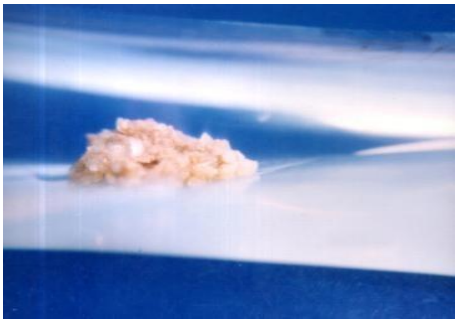

Fig.3. Callus in *Eleusine*

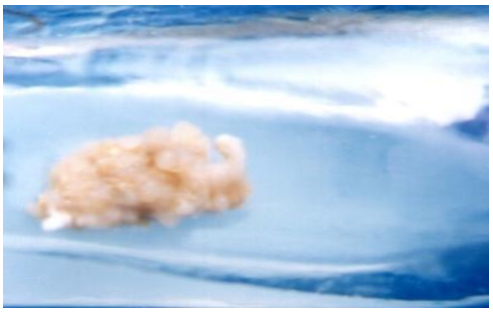

Fig.4. Callus from hypocotyl in *Eleusine*

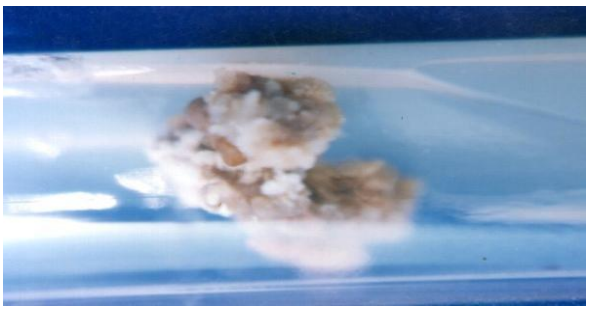

Fig.5. Callus in *Eleusine*

Table 1. List of *Eleusine coracana* accessions

S. No.	Species	Procurement place	Abbreviations used
1.	*Eleusine coracana*	Chaubattakhal, 1800m asl	E1
		Uttarkashi, 1200 m asl	E2

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Table 2. Composition of Hoagland's solution

S.NO.	Component	Volume of stock solutions (ml/L)
1	KNO_3	10
2	$Ca(NO_3)_2.4H_20$	10
3	$MgSO_4.7H_2O$	10
4	KH_2PO_4	10

Table 3. The composition of seven stock solutions containing nutrients.

Stock solution	Constituents	Concentration (g/200 ml)	Volume of stock solutions (ml)
A	NH_4NO_3	16.5	20
B	KNO_3	19.0	20
C	H_3BO_3	0.062	20
	KH_2PO_4	1.70	
	$NaMo.O_4$	0.0025	
	KI	0.0083	
	$CoCl_2.6H_20$	0.00025	
D	$CaCl_2.6H_2O$	4.40	20
E	$MgSO_4.7H_2O$	3.70	20
	$MnSO_4.4H_2O$	0.223	
	$ZnSO_4.7H_2O$	0.086	
	$CuSO_4.5H_2O$	0.00025	
F	Na_2 EDTA	0.373	20
	$FeSO_4.7H_20$	0.278	
G	Thiamine HCl	0.001	20
	Pyrodoxine	0.005	
	Nicotinic acid	0.005	
	Glycine	0.020	
	Myoinositol	100	
	Sucrose	30,000	
	Agar	6000-8000	

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Table 4. Table showing auxin and different cytokinins in different concentrations showing callus in control and treated seedlings in both accessions of *Eleusine coracana.*

Growth hormones	Ratios	E 1									E 2								
		Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M	Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M
2,4-D + BAP	3: 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4: 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5:	+	++	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
	1						+				+			+		+			
2,4-D + KN	3: 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4: 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5: 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. Table showing multiple shoot formation in different cytokinins concentrations in control and treated seedlings of both accessions of *Eleusine coracana.*

Hormone	Conc. mM/L	E 1									E 2								
		Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M	Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M
BAP	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
	3	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
KN	1	+	++	+	+	+	+ +	+	-	-	+ +	+	+ +	+	+	+ + +	+	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Table 6. Table showing multiple root formation in different concentrations of auxins in control and treated seedlings in both accessions of *E. coracana.*

Hormone	Conc. mM/L	E 1									E 2								
		Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M	Co	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M
2, 4	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NA A	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.5	+	++	+	+ +	+	+ + +	+	-	-	+ +	+	+ +	+	+	+ +	+	-	-
	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

REFERENCES

1. Anjaneyulu, E. S. Hemlatha, S. Bharath Raj and Balaji M. Callus Induction and Plant Regeneration in Finger Millet (*Eleusine coracana* L.) Libyan Agriculture Research Center Journal International. 2011, 2 **(2):** 57-61.
2. Anjaneyulu, E., I.H. Attitalla, S. Hemlatha, S. Bharath Raj and Balaji.M. An effient protocol for callus Induction and Plant Regeneration in Finger Millet (*Eleusine coracana* L.). World Applied Science Journal2011, **12 (7):** 919-923.

3. Eapen, S. and George L. High frequency plant regeneration through somatic embryo genesis in finger millet*(Eleusine coracana* L. Gaertn.) *Plant Sci.*1989,**61:** 127-130.
4. Gaur, R.D. (1999). Flora of the district Garhwal Northwest Himalaya (with ethno botanical notes)1989,Pp. 302. and 662.
5. Gupta, P., S. Raghuvanshi and Tyagi A.K. Assessment of the efficiency of various gene promoters via biolistics in leaf and regenerating seed callus of millets, *Eleusine coracana* and *Echinochlou crusgalli*. *Plant Biotechnol*. 2001, **18:** 275-282.
6. Kamble, M.S., B.L. Dhonukshe, N.V. Kashid and Gavhane V.N. Variability studies for weight of callusin finger millet [*Eleusine coracana* (L) Gaertn.]*Res. on crops*,2004, **5(1):** 66- 72.
7. Kothari, S.L., K. Agarwal and Kumar S. Inorganic nutrient manipulation for highly improved *in vitro* plant regeneration in finger millet [*Eleusine coracana* (L.) Gaertn]. *In Vitro Cell Dev.Biol Plant.*2004, **40:** 515-519.
8. Krishna S. and AgarwalK. Effects of heavy metals on *Eleusinecoracana* (L.) Gaertn. Research in plant biology. 2012, **2(4):** 43-54.
9. Kumar S, Agarwal K, Kothari S.L. *In vitro* induction and enlargement of apical domes and formation of multiple shoots in finger millet, *Eleusine coracana* (L.) Gaertn and crowfoot grass, *Eleusine indica* (L.) Gaertn. Curr Sci. 2001, **81(11):** 1482-1485.
10. Mohanty, B., D. Dutta,S. Gupta and Ghosh P.D. Callus initiation and plant regeneration in ragi *(Eleusine coracana* Gaertn.). *Plant Cell Tiss. Org. Cult.*1985, **5:** 147-150.
11. Murashige, T. and Skoog E.A. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962, **15:** 473-497.
12. Poddar K, Vishnoi RK, Kothari S. Plant regeneration from embryogenic callus of finger millet *Eleusine coracana* (L.) Gaertn. on higher concentrations of NH4NO4 as a replacement of NAA in the medium. Plant Sci. 1997, **129:** 101–106.
13. Rangan, T.S. Growth and plantlet regeneration in tissuecultures of some Indianmillets: *Paspalum scrobiculatum* L., *Eleusine coracana* Gaertn. and *Pennisetum typhoideum* Pers. Z *Pflanzenphysiol.* 1976,**78:** 208-216.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2015 - 2025 Received
20 October 2020; Accepted 04 December 2020.

14. Sivadas, P., S.L. Kothari and Chandra N. High frequency embryoid and plantlet Formation from tissue cultures of the finger millet *Eleusine coracana* (L.) Gaertn. *Plant Cell Rep.*1990, **9:** 93-96.
15. Yemets, A.I., Galina Ya, Bayer, and Yaroslav B. B. An Effective Procedure for In Vitro Culture of *Eleusine coracana* (L.) and Its Application. Hindawi Publishing Corporation ISRN Botany, Article ID 853121, 2013, 7 pages.