ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro Plant Regeneration Studies on *Cicer arietinum* L. (Chick pea) for its Heavy Metal Tolerance ($K_2Cr_2O_7$).

P Krishna Chaitanya, BVM Santhi Priya, NVH Sailesh Babu, P Avinash, S Asha, and R Bharath Kumar*.

Department of Biotechnology, Vignan's University, Vadlamudi, Guntur-522213 Andhra Pradesh, India.

ABSTRACT

Chickpea (*Cicer arietinum* L) is most important grain legume, occupying first position both in area and production among the Indian pulse food crops. There is also a high reported mineral content: phosphorus (340 mg/100 g), calcium (190 mg/100 g), magnesium (140 mg/100g), iron (7 mg/100 g), zinc (3 mg/100 g) Recent studies by government agencies have also shown that they can assist in lowering of cholesterol in the bloodstream. Due to its wide industrial use, chromium is considered a serious environmental pollutant. Toxicity of Cr to plants depends on its valence state: Cr (VI) is highly toxic and mobile whereas Cr (III) is less toxic. Since plants lack a specific transport system for Cr, it is taken up by carriers of essential ions such as sulfate or iron. Toxic effects of Cr on growth of Chick pea *(Cicer arietinum* L*)* and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield. The absorption capacity of the Chick pea *(Cicer arietinum* L*)* depends on the ph of the chromium solution. The absorption capacity increases with increase in rate of dilution and minimum at the control conditions.

Keywords: *In-vitro* Plant Regeneration, *Cicer arietinum,* Heavy metal torelance, $K_2Cr_2O_7$.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chick pea

The chickpea (*Cicer arietinum*) (also garbanzo bean, Indian pea, ceci bean, Bengal gram) is an edible legume of the family Fabaceae. Chickpea (*Cicer arietinum* L) is most important grain legume, occupying first position both in area and production among the Indian pulse food crops[1,8]. Fortunately, due to its wide adaptation it is cultivated in all the continents in more than 50 countries.The plant grows to between 20 and 50 cm high and has small feathery leaves on either side of the stem[1].

Chickpeas are a helpful source of zinc, folate and protein. They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes[1,9]. Chickpeas are low in fat and most of this is polyunsaturated. Nutrient profile of desi chana (the smaller variety) is different, especially the fibre content which is much higher than the light coloured variety. One hundred grams of mature boiled chickpeas contains 164 calories, 2.6 grams of fat (of which only 0.27 grams is saturated), 7.6 grams of dietary fiber and 8.9 grams of protein. Chickpeas also provide dietary calcium (49–53 mg/100 g), with some sources citing the garbanzo's calcium content as about the same as yogurt and close to milk. Recent studies by government agencies have also shown that they can assist in lowering of cholesterol in the bloodstream [27].

Chromium

Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin.It is a transition element located in the group VI-B of the periodic table with a ground-state electronic configuration of Ar 3d54s1. The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species, although there are various other valence states which are unstable and short-lived in biological systems[9,15]. Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions.

Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer et al., 2003). Contamination of soil and ground water due to the use of Cr in various anthropomorphic activities has become a serious source of concern to plant and animal scientists over the past decade. Its complex electronic chemistry has been a major hurdle in unraveling its toxicity mechanism in plants[4,12].

Diseases of Chick pea and their control measures in *in-vivo* and *in-vitro* conditions

Virus Diseases

The major virus diseases in chick pea Stunt is caused by leaf roll virus,due to the aphid vector activity (*Aphis craccivora, Myzus persicae*), Mosaic [alfalfa mosaic virus,cucumber mosaic virus], Narrow leaf [bean yellow mosaic virus]- Yellowing and drying of the plants it also effect on growth and development of plant s are necrosis, leaf discoloration,stuting, shortened internodes, which may cause damage for crop yield[11,28].

Mosaic [alfalfa mosaic virus, cucumber mosaic virus], Narrow leaf [bean yellow mosaic virus]- Yellowing and drying of the plants[28].

Nematode Diseases

Several nematodes are parasitic to Chick pea. These are root-knot nematodes *(Meloidogyne arenaria, Meloidogyne hapla, Meloidogynejavanica)*, root lesion nematode *(Pratylenchus brachyurus)*, ring nematode *(Macroposthonia ornata)*, sting nematode *(Belonolaimus longicaudatus)*, and testa nematode *(Aphelenchoides arachidis)*[29].

Bacterial Diseases

Bacterial blight [*Xanthomonas campestris* pv.*cassiae* Kulkarni et al.] Leaves dry up and are shed. The disease may show up as post emergence damping off, killing the seedlings within 3-4 days.

Seed rots and seedling diseases are the main bacterial diseases. Many of them are soil habituated fungi, which infects the seed and germinating seedlings of groundnut .They may be identified by fungal spores that give characteristic colorations to the seed, e.g., gray spores indicate *Rhizopus arrhizus*[29,33] .

Foliar diseases

The major foliar diseases caused by fungi are rust *(Puccinia arachidia* Speg.) late leaf spot *(Cercosporidium personatum* recently renamed *Phaeoisariopsis peraonata* Berk & Curt) , and early leaf spot(*Cercospora arachidicola* Hori) . Rust and late leaf spot are important diseases in India and most of the semi-arid tropic (SAT) regions[28,30].

Selected disease Control Measures for chick pea:

- Follow the crop rotation practices, i.e., cereal-cereal-groundnut.
- So we get good quality and disease-free seed.
- Avoid damage to the seed testa and deep placement of seed at sowing.
- Treat the seed w i t h thiram e 3 g kg-1 seeds or with carbendazim *% 2*g kg-1 seeds.
- Use cereal-cereal-groundnut crop rotation and seed treatment with thiram.
- Harvest at proper maturity and discard the wilted and dead plants as such plants are likely to have seeds infected by *Aspergillusflavus.*
- Sprays of Bordeaux mixture and dithiocarbamate have been found effective to control rust and late leaf spots. Chlorothalonil 0.2% spray has been found effective against rust and late leaf spot, when sprayed 30 days after germination till 15 days before harvesting at regular 10-15 day intervals. However, this schedule could be modified using a suitable disease forecast system based on temperature, humidity, cloudy weather, and rainfall pattern to save the fungicide and reduce the spray cost. Calixin is effective against rust but not against leaf spots, whereas benomyl is effective against leaf spots but not against rust (Subrahmanyam et.al. 1984).

MATERIALS AND METHODS

Plant Profile

The chickpea (*Cicer arietinum*) (also garbanzo bean, Indian pea, ceci bean, Bengal gram) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpeas are high in protein and one of the earliest cultivated vegetables. 7,500-year-old remains have been found in the Middle East region[27].

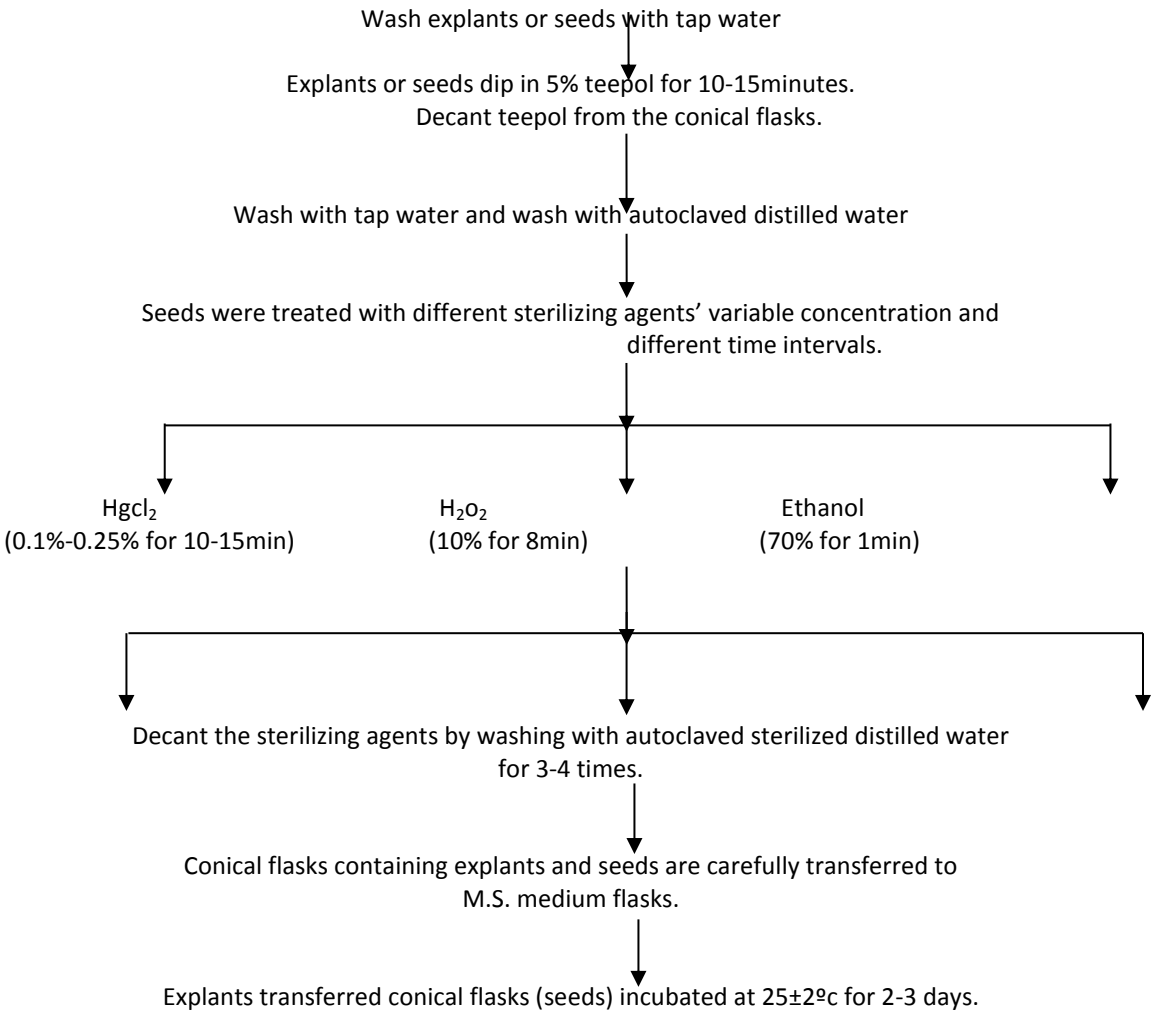
Scientific classification

Binomial name	***Cicer arietinum* Linn.**
Family:	Fabaceae
Ln./En. :	Bengal gram

Collections of Explants/seeds

Fresh and clean seeds or explants of ground nut and chick pea are taken and subjected to surface sterilization . The surface sterilizations technique is shown in the below flow chart[25].

ISSN: 0975-8585



***In vitro* Culture Techniques**

Sterilization of Equipments and Glass ware and other necessary materials

All operations for *in vitro* culture were carried out inside a laminar air flow cabinet under aseptic conditions using sterilized plant materials, equipments, glass materials and chemicals. A horizontal laminar flow cabinet with HEPA filter was used. The hood surface was wiped clean with paper towel soaked in 70 % ethanol and sterilized by germicidal ultraviolet light for at least 10 min prior to use. All surgical instruments, glassware and other accessories were sterilized in autoclave at 121 ºC with15 psi for 30 min and then dried in oven. Surgical instruments like scalpel, forceps, and scissors were sterilized by dipping in 100 % ethyl alcohol and flaming prior to use[20,25].

Culture Room

The explants were incubated in a culture room where the temperature was maintained at 25-26 ^{0}C, humidity at 85 % and either under continuous dark or under a photoperiod of 16 h light(25 µmol s-1m-1) and 8 h dark.

Preparation of Culture Media

Media-making can be time-consuming. Nowadays the plant tissue culture media most commonly used are available in the market as dry powders. The simplest methods of preparation media is to dissolve these powders contain inorganic and organic nutrients in some quantity of distilled water. After the content

has been thoroughly mixed in water, sugar and agar other organic supplements are added. Finally, the volume is made up to one liter. The p^H is adjusted and the medium autoclaved[19,30].

Powdered media are useful for propagation of plant species requiring nutrients according to the recipe of standard media. In experiments in which changes in the quantity and quality of media constituents become necessary, it is desirable to weight and dissolve each ingredient separately before mixing them together. Another convenient procedure is to prepare stock solutions which, when mixed together in appropriate quantities, constitute a basal medium. Four stock solutions are prepared, consisting of

- Major salts
- Minor salts
- Iron
- Organic nutrients except sucrose

MS (Murashige and Skoog 1962) inorganic salts, organic supplements and vitamins they were used as basal media for seed germination, callus induction, callus multiplication, shoot and root induction. The formulation and composition of MS medium is given in below[25,30].

Preparation of Stock Solution/s

Stock solutions of the major components, such as macronutrients, micronutrients, vitamins, and plant growth regulators of the media were prepared and stored in refrigerator

Stock solution-1		**Stock solution-2**	
$MgSo_4$ .7 H_2O	- 370mg/lt	$MnSO_4.4H_2O$	- 22.3mg/lt
$CaCl_2$.2 H_2O	- 440mg/lt	$ZnSO_4$	- 8.6mg/lt
KNO_3	- 1900mg/lt	$CuSO_4$.5 H_2O	- 25µg/lt (0.025mg/lt
NH_4NO_3	- 1650mg/lt	H_3BO_3	- 6.2mg/lt
KH_2PO_4	- 170mg/lt	Na_2MoO_4.2 H_2O	- 0.25mg/lt

Stock solution-3
Add -83mg/lt (or) 0.83g/lt -KI into 100ml Dist.H2O.

Stock solution-4
Dissolve - 373mg Na_2 EDTA/279mg of $Fe.SO_4$.7 H_2O - in 50ml of dist.H_2O.

Stock solution-5

Vitamins	Actual Conc.(mg/lt)	100X(mg)
Glycine -	2mg	200
Thymine -	0.1mg	10
Pyridoxine HCL -	0.5mg	50
Nicotinic acid -	0.5mg	50

Stock solution-6
Dissolve 1000mg in 50ml distilled water
Use 5ml to prepare 1000ml of culture medium

Media
- Sucrose -20to30gm/lt
- PH -5.6 to5.8
- Agar –Agar -4to8 (as per the requirement)

To prepare 1lt of media has stocks to be taken as –stock1-50ml, stock2-1ml, stock3-1ml, stock4-5ml, stock5-1ml, stock6-2ml.

Growth Regulators

Auxins and cytokinins were the two major phytohormones are taken in different concentrations and combinations in various media for induction and growth of callus, root and shoot[23,26].

Auxins

Powders of auxin were dissolved in 1N NaOH and made up the volume with sterilized distilled water and then used or stored in freezer as stock for further use. One auxin/s is used in the present study were 2, 4-

Dichlorophenoxyacetic Acid (2, 4-D) and Two different concentrations (2 and 4µdM) of 2, 4-D were tested in MS medium[18,20].

Cytokinins

Two different cytokinins 6-Benzyl adenine (6-BA) and Kinetin (Kn) are taken and they were dissolved in 1N NaOH and make in to them for the required volume solution. They were kept in refrigeration and stored for further utilization[20,23].

Two concentrations of 6 BAP (2 and 4 µM) and two concentrations of Kn (5µM and 10µM) were added to the basal medium of MS salts[9,25].

Seed Germination

The sterilized seeds were given a cut at the non-embryo side and placed in petri dishes, test tubes embryo side up in a hormone-free MS medium solidified with agar 0.8 % (w/v). For shoot multiplication one accession was used.

Inoculation of Explants & Seed Material:

The seeds of Chickpea (*Cicer arietinum*) are inoculated in the M.S medium of about 30 test tubes and incubated at 25±2ºc. The seeds were germinated in a conical flask containing MS basal medium in dark hypocotyls, epicotyls, axillary bud, immature leaves and coteyledonary nodes of 10 days old seedlings were used as explants. Immature embryos were dissected out from sterilized pods 35-45 days after pollination[11,14].

Seedlings explants were cultured on MS salts supplemented with B5 vitamins and various growth harmones.The nutrient media consists of major and minor salts, according to Murashige and Skoog(1962),B5 vitamins(Gamborg et al.1968),3.0%(W/V)Sucrose and 0.7%(W/V)Agar. It was variously supplemented with 6-Benzyl adenine (6-BA) and Kinetin (Kn)[20,25].

Shoot Multiplication and Maintenance:

The explants were sub cultured onto fresh media every 15 days. When the explants started to multiply, well grown axillary shoots were separated with the help of a sterile scalpel under the hood and put in the same media for further multiplication. The shootlets derived from each seed were tracked individually to determine the total number of plants produced from single seed and their subsequent genetic identity[6,21]. The explants were inoculated in different concentrations of growth hormones of MS basal medium combinations are given here:

1. MS medium+6-BA
2. MS-medium+2, 4-D
3. MS-medium+ (Kn)
4. MS-medium+6-BA+2,4-D
5. MS-medium+2,4-D+ (Kn)
6. MS-medium+6-BA+ (Kn)
7. MS-medium+6-BA+2, 4-D+ (Kn)

2, 4-D- 2, 4 Dichloro pheonoxyacetic acid, 6-BA-6 benzyl adenine, (Kn)- Kinetin

Uptake and Estimation of chromium in shoots

The germinated shoots are sub cultured in different dilutions of Chromium (Chromium is prepared at 1molar with dilutions of 10^{-1} to 10^{-9}) in above mentioned combinations(A,B,C,D,E & F).

The subculture samples are subjected to A.A.S to determine the toxic effect on the selected plant species (chick pea)[24,26].

ISSN: 0975-8585

RESULTS AND DISCUSSION

The present study was conducted to investigate optimal concentrations and combinations of plant growth regulators in the medium for efficient micro propagation in ground nut & chickpea via nodal shoot culture. To determine the effect of Chromium in the selected plant species.

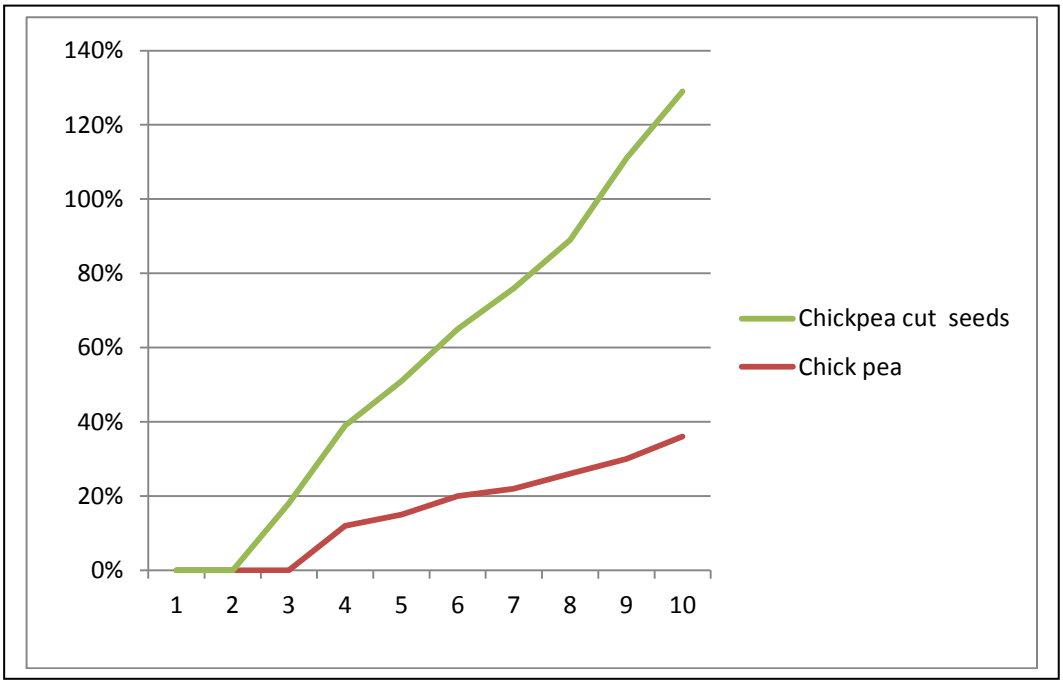
Seed Germination

Seeds of one accessions of chickpea were used for germination test in the below table. The seeds were placed in MS medium without any plant growth regulator. When a cut was given to the seed at the opposite end to the embryo, there was remarkable increase in the percentage of germination. For example, 93 % of cut seeds of chick pea germinated after 10 days, whereas only 36 % germination was noticed for uncut seeds[11,12].

Growth percentage of Chick pea for 10days

S.No.	Name of the plant species	Part of the plant	Day of inoculation	Percentage of Germination(days)										Contaminated		
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Petri dishes	Test Tubes	Conical flasks
1.	Chick pea	Seed	25.01.10	--	--	--	10%	12%	15%	18%	21%	25%	30%	3	---	----

Growth curve of Chick pea



X-axis represents number of days; Y-axis represents the growth percentage

Shoot Multiplication in Chick pea explants/seeds

In a preliminary experiment of shoot multiplication from cotyledonary nodal explants, two accessions were tested with seven different M.S medium-combinations. There was no response in 2, 3, 5 (detailed in material and methods), where only vertical growth was observed. On the other hand, 1, 4, 6 and 7 media responded well to shoot multiplication .Eventually, two accessions were used for shoot multiplication in three media i.e., M.S+1, M.S+4, and M.S+6&7.The Responses of Shoot Multiplication in Chick pea are show in the table below[19,25].

ISSN: 0975-8585

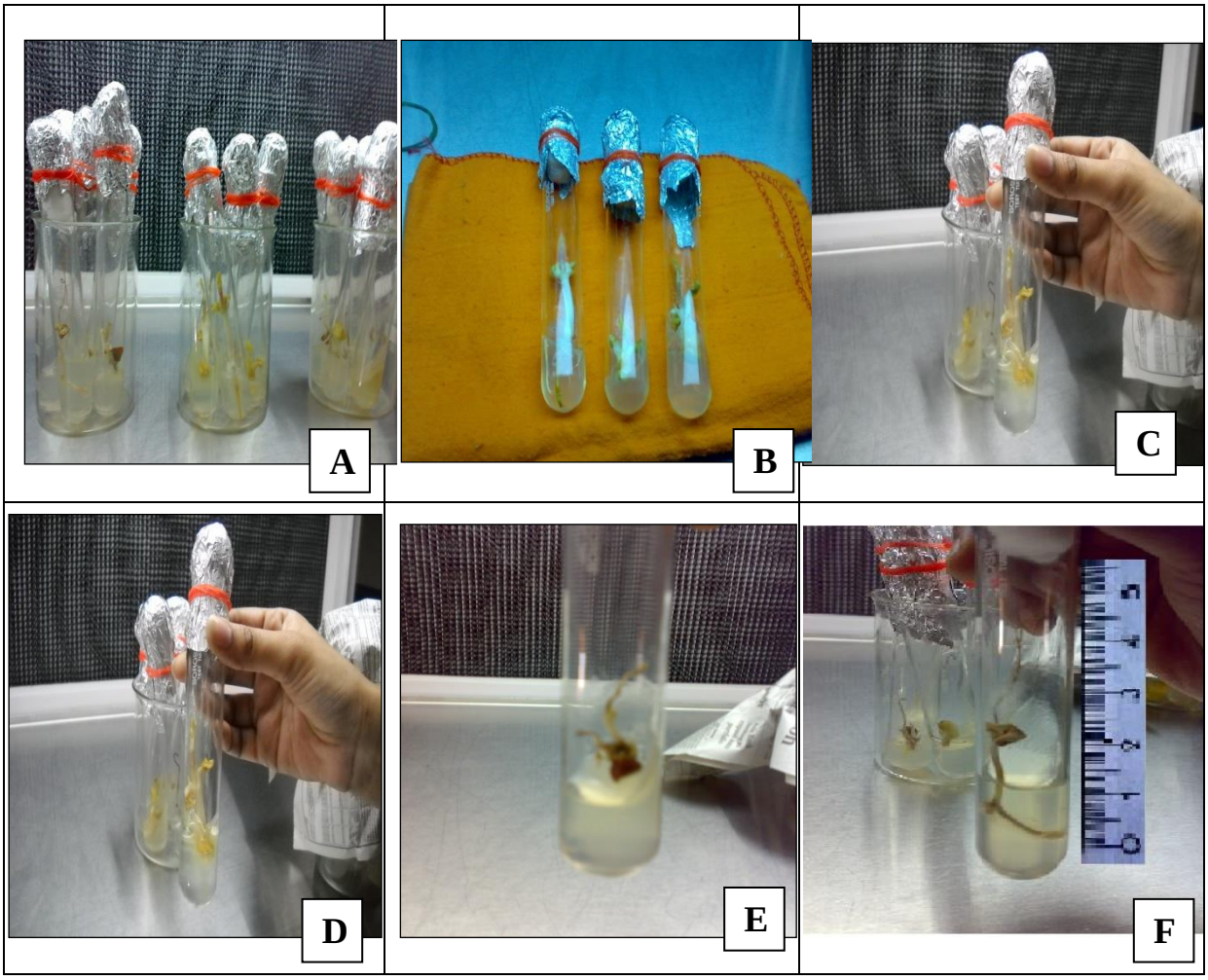
Growth response of Ground nut and Chick pea in different media Compositions

S.No.	Name of the plant species	Part of the plant	M.S+1	M.S+4	M.S+6	M.S+7	No of test tubes Inoculated	Response plant species of different combinations				Contami-nated
								M.S+1	M.S+4	M.S+6	M.S +7	
1.	Chick pea	Germinated seeds	2ml	1:1	1:2	1:2:4	10	---	60%	30%	80%	3

[Where as M.S+1= MS medium+6-BA, M.S+4= MS-medium+6-BA+2,4-D, M.S+6=MS-medium+6-BA+(Kn), M.S+7=MS-medium+6-BA+2,4-D+(Kn)].

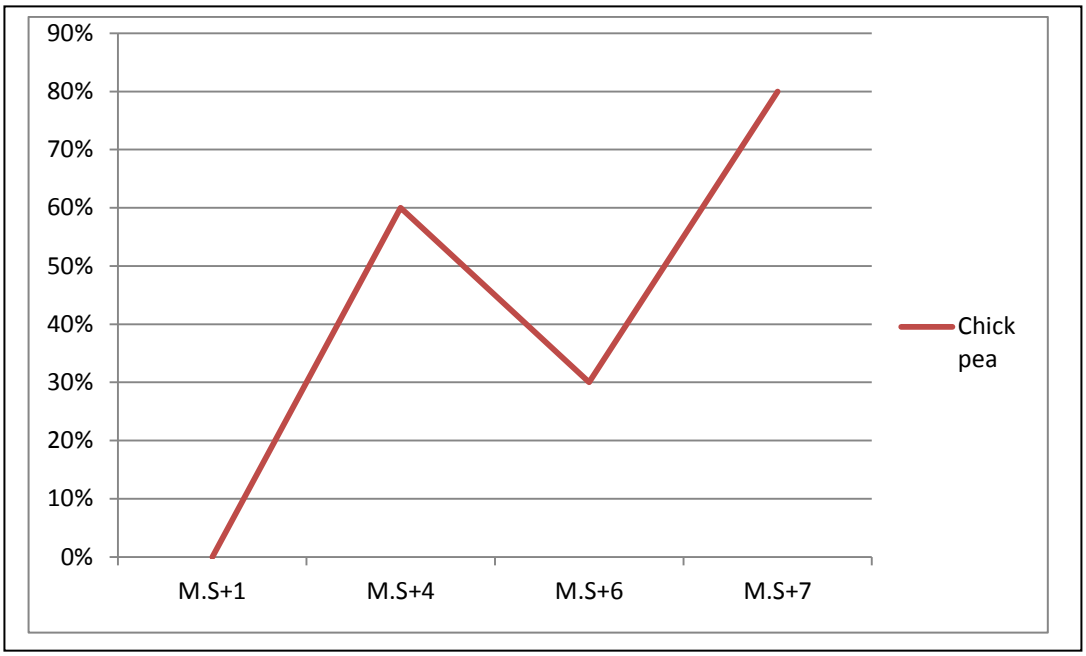
The germination & growth response of chickpea are shown here with pictorial representation.

In-vitro growth and development of Chick pea seeds (MS Salts + Supplements) on the medium



ISSN: 0975-8585

Growth Curve of Chick pea



[X-axis represents the M.S medium with different combinations (M.S+1= MS medium+6-BA, M.S+4= MS-medium+6-BA+2,4-D, M.S+6=MS-medium+6-BA+(Kn), M.S+7=MS-medium+6-BA+2,4-D+(Kn)) Y-axis represents the growth percentage of chickpea.]

Growth of Chick pea in different dilutions of Cr

S.No.	Name of the plant species	Part of the Explants	M.S medium +6BA+2,4-D +Cr dilutions									
			Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
1.	Chick pea	Roots	-----	----	----	5%	30%	60%	70%	75%	80%	90%
2.	Chick pea	Stem	----	----	----	----	10%	20%	40%	60%	70%	80%
3.	Chick pea	Seeds	----	----	---	20%	40%	60%	70%	75%	80%	95%

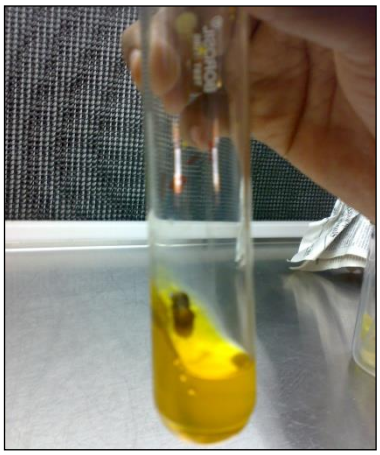
Different phases of growth of Ground nut explants in different dilutions of Chromium is shown in the following figures

Estimation of Chromium

The Germinated plants species of chickpea are surface sterilized and inoculated in to the media having the different dilutions of 10^{-1} to 10^{-9}. Selected media is taken i.e., M.S medium with 6BA and 2,4-D as mentioned in the above graph. Growth observed in the Chromium augmented media is shown in the table below. Chromium levels are estimated my submitting these Chromium Dilutions Samples to A.A.S (Atomic Absorption Spectrophotometer)[24,29].

ISSN: 0975-8585

Standard



10^{-3}

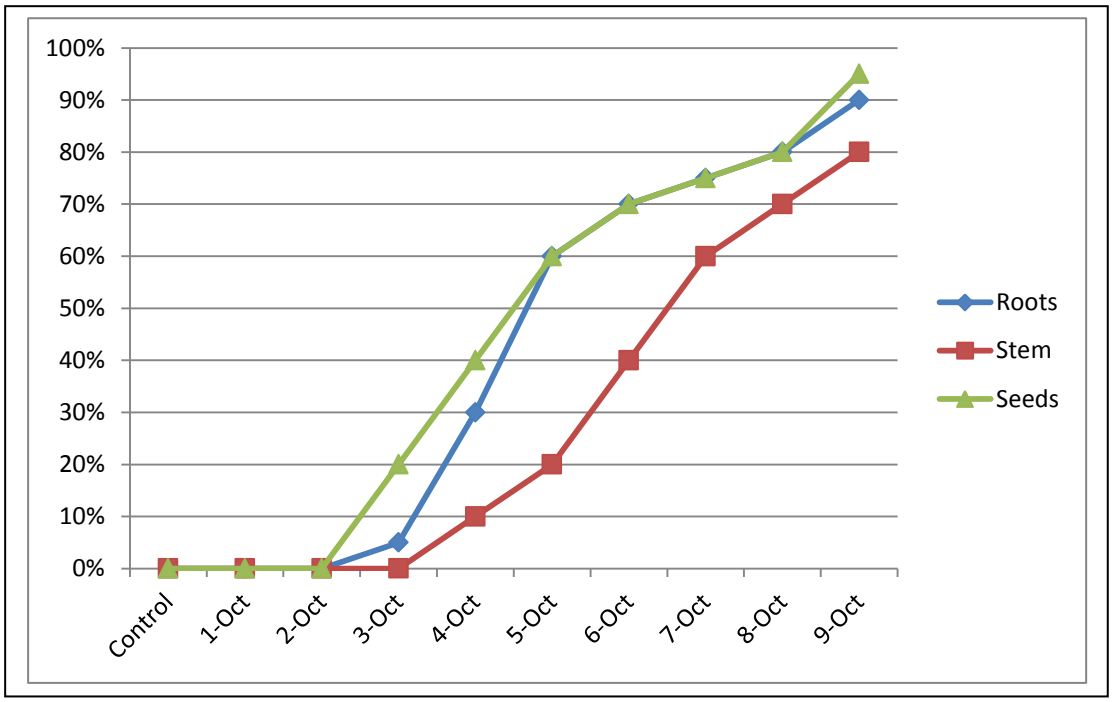
10^{-4}

10-5

10-6

10-9

Growth curve of the Ground nut at different dilutions of Cr



[X-axis represents the Cr dilutions in the selected media (M.S+6BA+2,4-D) the dilutions ranging from 10^{-1} to 10^{-9}.

Y-axis represents percentage of ground nut growth at different dilutions of Cr.]

Uptake and accumulation of Cr on plant parts by using AAS:

Different dilutions in Chromium (Cr)	Root	Stem	Seed
Standard	------	------	-------
10-3	0.74	0.68	0.18
10-4	0.84	0.73	0.21
10-5	0.92	0.83	0.23
10-6	0.95	0.90	0.25
10-7	0.99	0.96	0.28
10-8	1.02	0.99	0.32
10-9	1.08	1.06	0.36

Uptake and Accumulation of Cr in

Roots

Chromium content of the root for different dilutions is recorded in the above table. Maximum chromium content of chick pea is observed in 10^{-9}(1.08) dilution and minimum is observed in control plants.

Stem

Chromium content of ground nut for stem is recorded in the above table. Maximum chromium content of ground nut is observed in 10^{-9}(1.06) dilution and minimum is observed in control plants.

Seeds

Chromium content of ground nut for seeds is recorded in the above table. Maximum chromium content of ground nut is observed in 10^{-9}(0.36) dilution and minimum is observed in control plants.

CONCLUSIONS

The chickpea (*Cicer arietinum*) (also garbanzo bean, Indian pea, ceci bean, Bengal gram) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpea (*Cicer arietinum* L) is most important grain legume, occupying first position both in area and production among the Indian pulse food crops[1,2].

The present investigations was carried out on an important legume plant Chick pea (*Cicer arietinum).* The main objectives of the present investigations were: To determine the heavy metal Tolerance (Chromium) of ground nut and chickpea, to get disease-free plants that will lead to qualitative improvement of the crop and to check the toxic levels of chromium in *in-vitro* conditions at different concentrations[3,4].

All operations for *in vitro* culture were carried out inside a laminar air flow cabinet under aseptic conditions using sterilized plant materials, equipments, glass materials and chemicals described in standard literature source. The seeds of Chickpea (*Cicer arietinum*) are inoculated in the M.S medium of about 30 test tubes and incubated at 25±2ºc. The seeds were germinated in a conical flask containing MS basal medium in dark hypocotyls, epicotyls, axillary bud, immature leaves and cotyledonary nodes of 10 days old seedlings were used as explants and they were maintained for under *in-vitro* conditions for further analysis of growth and germination percentage in regular intervals[6,7].

The present study was conducted to investigate optimal concentrations and combinations of plant growth regulators in the medium for efficient micro propagation in chickpea via nodal shoot culture. To determine the effect of Chromium in the selected plant species[5,9].

These explants were observed after incubation of 1to 2 weeks of duration, their % germination results are given respectively in the table (table no. 1&2). The germination of groundnut explants are in the combination of M.S+4 medium, were expressed considerable response(i.e. 98%), when compared with other combinations. Coming to the Chick pea explants are in the combination of M.S+7 medium, were expressed considerable response (i.e. 80%), when compared with the other MS + combinations of Chick pea explants[10,20].

The heavy metal contamination is an alarming issue know a days due to the environmental pollution particularly the various industrial effluents and their residues will causing severe damage to the productivity of lands in at various levels which leads the severe damage to the plants in terms of yield, diseases etc to overcome these problems, phytoremediation is an emerging cleanup technology, can be implemented by using the selected microorganisms (*Chlorobium, Thiobacillus Rhizibium* sp.), grasses (aquatic grasses like *Typa*, *Cynodon* etc)or higher plants can be grown along with the crop plants in the fields, which can able reduce the effects heavy metals on food crops[12,13,14].

As per the modern aspects and developments in the filled of biotechnological methods like Protoplast fusion, genetic engineering and in-vitro propagation technologies, can facilitates for the development of the transgenic strains of the species belongs to the promising food crops against to the heavy metal tolerance, salt tolerance and drought resistance etc. to meet our future needs of adequate food resources.

ACKNOWLEDGEMENTS

Authors are expressing their gratitude to the Chancellor and Vice-Chancellor for their encouragement. Authors are thankful to the Dean, Engineering and Management (Dean E & M), Vignan's University, Vadlamudi, for providing facilities and encouragement. Authors are expressing their sincere thanks to Head, School of Biotechnology for providing necessary facilities to carryout fieldwork and laboratory analysis. Authors are also thankful to the Management for extending financial assistance & providing facilities.

REFERENCES

[1] Anonymous, 1948-76. *The Wealth of India (Raw Materials).* Vol. 1-11. CSIR, New Delhi, India.
[2] Adriano DC. Trace Elements in the Terrestrial Environment. New York7*Springer* Verlag; 1986. p. 105–23.
[3] Anderson AJ, Meyer DR, Mayer FK. Heavy metal toxicities: levels ofnickel, cobalt and chromium in the soil and plants associated with visual symptoms and variation in growth of an oat crop. *Aust J Agric Res*1972;24:557– 71.
[4] Altpeter, F. and U.K. Posselt. 2000. Improved plant regeneration from cell suspensions of
[5] commercial cultivars, breeding and inbred lines of perennial rye grass (*Lolium perenne* L.). *J. Plant Physiol.* 156:790-796.
[6] Athalye VV, Ramachandran V, D'Souza TJ. Influence of chelatingagents on plant uptake of 51Cr, 210Pb and 210Po. *Environ Pollut*1995;89:47– 53.
[7] Barcelo J, Poschenriender C, Ruano A, Gunse B. Leaf water potential inCr(VI) treated bean plants (Phaseolus vulgaris L). *Plant Physiol Suppl*1985;77:163– 4.
[8] Bera AK, Kanta-Bokaria AK, Bokaria K. Effect of tannery effluent on seedgermination, seedling growth and chloroplast pigment content in mungbean (*Vigna radiata* LWilczek). *Environ Ecol* 1999;17(4):958–61.
[9] Bharath Kumar, R., S.Asha & B. Sarath Babu (2014) A note on Phytodiversity and Phytochemistry of important Plant species of Vignan University Campus, Vadlamudi, Andhra Pradesh. *International Journal of Pharma and Bio Science*.Vol. 5(1): (B) 373 – 386.
[10] Bhaskaran, S. and R.H. Smith. 1990. Regeneration in cereal tissue culture: a review. *Crop Sci*. 30:1328-1336.
[11] Bhojwani, S.S. and Rajdan, "*Plant Tissue Culture: Theory and Practice*", 2004.
[12] Bishnoi NR, Dua A, Gupta VK, Sawhney SK. Effect of chromium on seedgermination, seedling growth and yield of peas. *Agric Ecosyst Environ*.1993b;47:47–57.
[13] Boonyapookana B,Upatham ES, Kruatrachue M,Pokethitiyook P,Singhakaew S. Phyto-accumulation and phytotoxicity of cadmiumand chromium in duckweed *Wolffia globosa*. *Int J Phytoremed* 2002;4:87– 100.

[14] Brauer SL, Hneihen AS, McBride JS, Wetterhahn KE. Chromium (VI)forms thiolate complexes with glutamylcysteine, N-acetylcysteine, andmethyl ester of N-acetylcysteine. Inorg Chem 1996;35:373–81.
[15] Breckle SW. Growth under stress: heavy metals. In: Waisel Y, Eshel A,Kafkafi U, editors. Plant Root: The Hidden Half. NY, USA7 MarcelDekker; 1991. p. 351– 73.
[16] Cary EE, Allaway WH, Olsen OE. Control of chromium concentrations in food plants: 2 Chemistry of chromium in soils and its availability to plants. *J Agric Food Chem* 1977a;25:3059.
[17] Cervantes C, Garcia JC, Devars S, Corona FG, Tavera HL, Torres-guzman JCarlos, et al. Interactions of chromium with micro-organisms andplants. FEMS *Microbiol Rev* 2001;25:33547.
[18] Chandra P, Sinha S, Rai UN. Bioremediation of Cr from water and soil byvascular aquatic plants. In: Kruger EL, Anderson TA, Coats JR, editors.Phytoremediation of Soil and Water Contaminants. ACS SymposiumSeries, vol. 664. Washington, DC7 *American Chemical Society*; 1997.p. 274– 82.
[19] Chaney RL, Malik M, Li YM, Brown SL, Angle JS, Baker AJM.Phytoremediation of soil metals. *Curr Opin Biotechnol* 1997;8:279 – 84.
[20] Chen YX, Zhu ZX, He ZY. Pollution behaviour of organic Cr(III)complexes in soil–plant system. *Chin J Appl Ecol* 1994;5:187–91.
[21] Clijsters H, Van Assche F. Inhibition of photosynthesis by heavy metals. *Photosynth Res.* 1985;7:31–40.
[22] Dodeman, V.L., G. Ducreux and M. Kreis. 1997. Zygotic embryogenesis versus somatic embryogenesis. *J. Expt. Bot.* 48:1493-1509.
[23] Emons, A.M.C. 1994. Somatic embryogenesis: Cell biological aspects. *Acta Botanica* Neerlandica 43:1-14.
[24] Faisal, M., I. Siddique and M. Anis. 2006. An efficient plant regeneration system for *Mucuna pruriens* L. (DC.) using cotyledonary node explants. *In Vitro Cell. Dev. Biol. Plant*. 42:59-64.
[25] Gibbs, A. and Harrison, B. 1976. *Plant virology: The Priniciples*. London, UK: Edward Arnold Publishers.292pp.
[26] Holt, B.L. and Reddy, D.V.R. 1984. Peanut clump. Pages 50-51, in *Compendium of peanut diseases* (Porter, D.M.,Smith, D.H. and and Rodriguez-Kabana,R. eds.). St. Paul, M N, USA, American Phyto pathological Society.
[27] Kalyan Kumar De., "*Introduction to Plant Tissue Culture*", 2nd ed., New Central BookAgency, Kolkata, 1992.
[28] Micheael J.Pelczar JR, E.C.S. Chan & Noel R. Krieg,1993. Microbiology, TATA McGraw –Hill pub. Co. ltd. New Delhi.918pp.
[29] P.D. Sharma, 1991. The Fungi, Rastogi & Company, Meerut, India.540pp.
[30] R. Bharath Kumar, S. Asha, P.Krishna Chaitanya, B.V.M. Santhi Priya, N.V.H Sailesh Babu & P.Avinash - *In-vitro* Plant Regeneration Studies of *Arachis hypogea* L. (Ground nut) for its Heavy Metal Tolerance (K2Cr2O7). in *Research Journal of Pharmaceutical Biological and Chemical Sciences*, November - December 2014, 5(6) Page No. 394-406
[31] Skoog, D.; Holler, J.; Crouch, S. *Principles of Instrumental Analysis*, 6th ed.; Thomson Books/Cole, 2007; pp 238.
[32] Sperling, Michael B.; Welz, Bernhard (1999). *Atomic Absorption Spectrometry*. Weinheim:Wiley-VCH. ISBN 3-527-28571-7.
[33] V. Verma,1991. A Textbook of Economic Botany., Emkay Publications, Delhi. 291 pp.