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REGENERATION SWITCHES IN RECALCITRANT GRAIN LEGUMES: CHICKPEA A CASE STUDY

Richa Chauhan and *N. P. Singh

Assistant Professor, Department of Agriculture,

Phonics Group of Institutions, Roorkee,

Hardwar, UK, India-247667

*Biotechnology Unit

Indian Institute of Pulses Research,

Kanpur – 208 024

Corresponding Author Email: meenu2ki@rediffmail.com

ABSTRACT:

The main pre-requisite for the utilization of biotechnological approaches to the genetic manipulation of chickpea is availability of simple, highly reproducible, efficient and genotype neutral regeneration protocol. There are several factors which influence the frequency and efficiency of regeneration via. organogenesis and somatic embryogenesis. The sucrose is the main factor which induces embryogenic callus and subsequently shoots are differentiated. Further, growth regulators like BAP and IBA when used in low concentrations were found to be most suitable for multiple shoot induction. Similarly, low concentration of 2, 4-D (1.25 mg/l) was found to be best growth regulator for induction of somatic embryos. However, 2, 4-D free medium was required for maturation of somatic embryos. The rooting percentage can be improved by reduction the strength of medium and sucrose concentration. All the physical and

chemical factors responsible for switching on different regeneration pathways are being discussed in detail.

Keywords: Organogenesis, somatic embryogenesis, regeneration pathways, growth regulators.

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Introduction

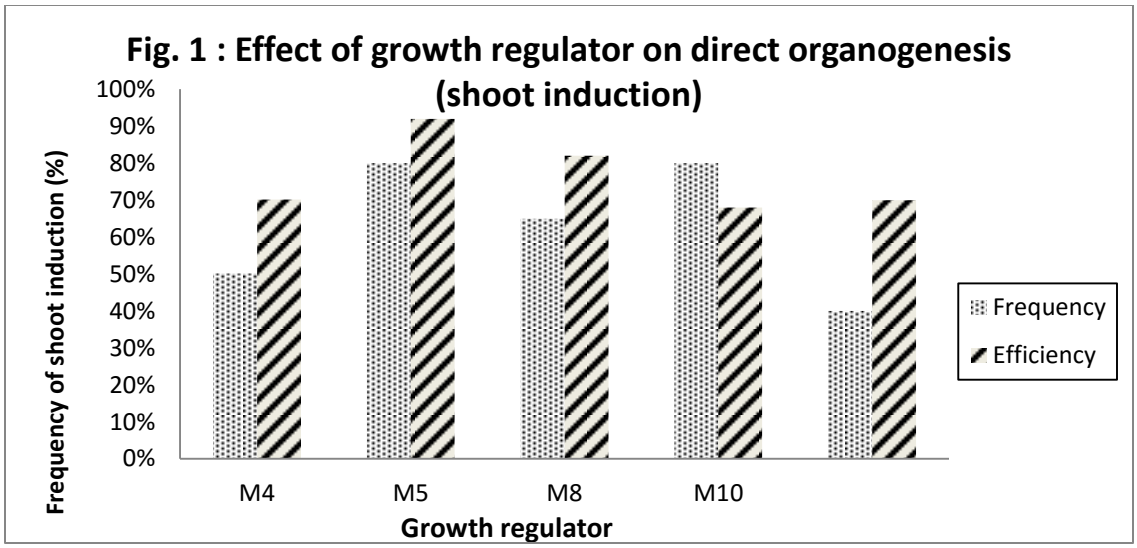
During last financial year India has experienced unpredictable high price in pulses due to low production, black marketing and high demand pressure. Pulse crops are very important edible crop for Indians and majority of Indians depends on different types of pulses for food supplement. Pulse crops are important in Indian Agriculture due to their ability to fix atmospheric nitrogen. Conventional methods are not very successful in increasing the productivity of pulses. Tissue culture and genetic engineering has shown promise for genetic enhancement of crop plants. One of the pre-requisite for the exploitation of tools of biotechnology is regeneration of complete plant from cell/tissue. However, grain legumes in general and chickpea in particular is considered to be recalcitrant species. As such, no single medium can be suggested as being entirely satisfactory for all genotypes, tissues and organs (1). Regeneration is considered to be polygenic trait and results from chain reaction of number of events. Each step in the chain is governed by one or more gene(s) (1). The expressions of these genes are induced by several physical and chemical factors. Therefore, no single medium or growth regulator combinations can be satisfactory for induction of different regeneration pathways. There are number of reports on regeneration of chickpea (2-6). However, most of these protocols have low reproducibility and are genotype dependent. We report here a simple, highly reproducible and genotype neutral protocol for regeneration of chickpea from embryonic axes. Based on relative effect of various factors on frequency and efficiency of regeneration, critical factors/switches responsible for different regeneration pathways were worked out.

Materials and Methods

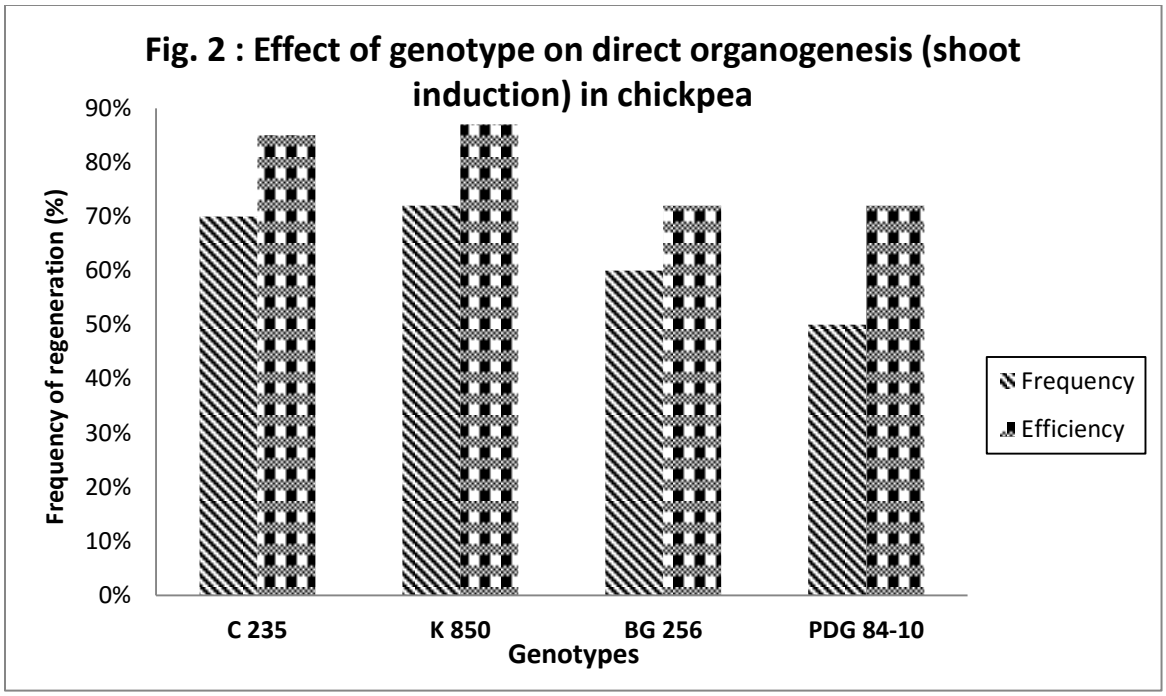
Embryonic axes (explant) from mature seeds were excised from four genotypes of chickpea viz., C 235, K 850, BG 256 and PDG84-10. Explants were double surface sterilized with 10% sodium hypochlorite solution followed by 70% ethyl alcohol for 15 minutes and rinsed thoroughly three times with sterile distilled water. Explants were inoculated on MS medium augmented with ε – naphthalene acetic acid (NAA), Indole-3-butyric acid (IBA), benzyl amino purine (BAP), dichlorophenoxy acetic acid (2,4-D) and kinetin with different doses of sucrose (10-50 g/l) and pH of the medium was adjusted to 5.8 prior to adding 8 g/l agar-agar and autoclaved at 1.4 kg cm^{-2} for 20 minutes. The cultures were incubated in a culture room at 3000 Lux intensity. Somatic embryos induced from callus were subculture after 5 weeks interval on maturation medium. Elongated shoots obtained through direct or indirect organogenesis were excised from culture and transferred on rooting medium. Plantlets with well-developed roots were transferred to pot. The present study was carried out during 2000 to 2001 year.

Results and Discussion

I. **Direct Organogenesis:** Differences in magnitude of multiple shoot induction among various treatments used under present investigation may be due to kind and concentration of auxin, cytokinin and genotypes being used. Further, these differences among treatments were more pronounced when response of growth regulators were expressed as efficiency (no. of shoots/explant) as compared to frequency (Fig. 1 & 5a).



As evident from Fig. 1, increasing the concentration of BAP from 2 to 3 mg/l, decreased the frequency and efficiency of regeneration. MS +0.125 mg/l, IBA+ 2.0 mg/l, BAP (M_5) gave maximum response in terms of frequency (80%) and efficiency (90%) followed by MS+0.125 mg/l, IBA+ 3.0 mg/l, BAP (M_{10}). The results similar to present investigation showing effect of growth regulators on regeneration have also been reported in chickpea (2-4). Besides, genotypes played the second most important role in shoot induction. Among genotypes, K 850 showed best response (74.86%) followed by C 235 (68.84%) (Fig. 2). Differential response of genotypes to regeneration has also been reported by several workers in chickpea (4 & 5).

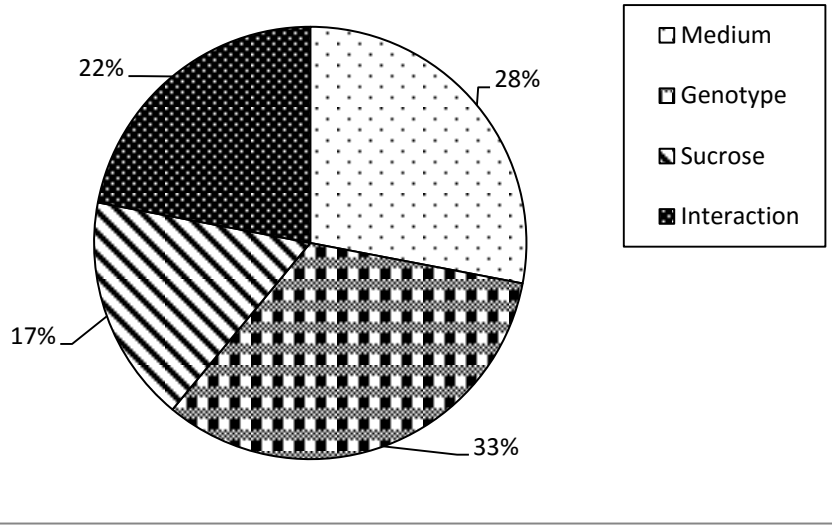


II. Indirect Organogenesis: The auxin and cytokinin ratio is considered crucial factor in callus induction and differentiation. MS salt + B_5 vit. + 0.125 mg/l, IBA + 2.0 mg/l, BAP + 50 g/l, sucrose showed best response (82.81%) followed by MS + 0.5 mg/l, NAA + 0.5 mg/l, BAP (70.0%) to callus induction. Best shoot differentiation (88.74%) was observed on MS salt + B_5 vit. + 0.125 mg/l, IBA + 2.0 mg/l, BAP + 40 g/l, sucrose followed by MS + 0.5 mg/l, NAA + 0.5 mg/l, BAP (59.13%).

a. Relative effect of different factors on callus induction and regeneration :

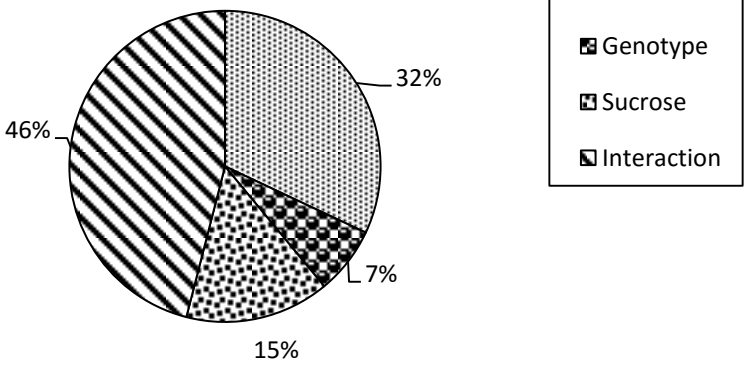
Based on the results of factorial experiment, which is summarized in fig. 3, medium (along with growth regulator) contributed 28%, genotype 2% and sucrose contribution 17% of total callus induction frequency. However, large proportion (33%) of interaction effect was also noticed which is indicative of complex nature of callus induction phenomenon.

Fig. 3 : Relative effect of various factors on callus induction in chieckpea



Regarding shoot induction, medium supplemented with different growth regulators contributed maximum (32%). This was followed by sucrose concentration (15%) and genotype (7.0%). Very interestingly, interaction among these factors contributed to a very large extent (46%) which is the more than any individual factors alone (Fig. 4 & 5b).

Fig. 4 : Relative effect of various factors on shoot induction in chieckpea



III. Rooting: It was observed that concentration of auxin, strength of medium and sucrose concentration are very crucial for rooting of the shoots derived from either of the regeneration routes. Among various auxins, NAA was found to be better for rooting of *in vitro* derived shoots. The concentration of 2.0 mg/l, NAA was found optimum (93.3%) (Table 1 & Fig. 5d).

Table 1: Effect of auxin and sucrose concentration on rooting of regenerated shoots

Concentration of growth regulator (mg/l)	Frequency (%)
1. ¼ MS+ 2.0, NAA+ 2.0, IBA+ 20 g/l, sucrose	66.70
2. ¼ MS+ 2.0, NAA+ 10 g/l, sucrose	80.0
3. ¼ MS+ 2.0, NAA+ 20 g/l, sucrose	93.30

IV. Somatic Embryogenesis: Three different medium with different concentration of growth regulators were required for somatic embryogenesis. Among various factors, growth regulators and physical parameter (light) played more important role in induction of somatic embryos. The frequency of callus induction and efficiency of somatic embryos induction (av.no. of somatic embryos/explant) was found highest (100% & 28.67) on MS+ 1.25 mg/l, 2, 4-D+ 0.25 mg/l, Kin. followed by MS+ 1.4 mg/l, 2, 4-D+ 0.25 mg/l, Kin. (47.44% & 9.33). 2, 4-D in low concentration, helped to induce somatic embryos from callus. Further, maturation of somatic embryo was maximum (21.33) on basal medium (1/2 MS salt+B_5 vit.+40 g/l, sucrose) (Fig. 5c).

Regeneration (40.0%) from somatic embryo was obtained on MS medium containing BAP and IBA in low concentration (Table 2). The dark condition was more suitable for induction of somatic embryos from callus. Further, maturation of somatic embryos and their regeneration was obtained in light condition. There are very few reports on induction of somatic embryogenesis from matured embryonic axes in chickpea (10-11).

The findings of the present study indicated that the concentration of growth regulators, strength of medium sucrose concentration and genotypes are important factor for switching on different regeneration pathways.

Table 2: Somatic embryogenesis from embryo axes in chickpea

Concentration of growth regulator (mg/l)	Response	Frequency (%)	Av. No. of somatic embryo/explant	Range
1. MS+1.25 2, 4-D+0.25 Kin.	Callus induction	100	28.67	25-30
2. MS+1.4 2, 4-D+0.25, Kin.	Callus induction	47.44	9.33	8-10
3. ½ MS salt+B_5 vit. + 40 g/l. sucrose	Maturation of somatic embryo	86.35	21.33	20-23
4. MS salt+B_5 vit. + 40 g/l. sucrose	Regeneration	85.00	20.55	20-21
5. MS salt+B_5 vit. + 0.125, IBA+2.0, BAP	Regeneration	40	-	-
6. MS+ 0.519, GA_3+0.020 IBA	Regeneration	20	-	-

References:

1. Bhojwani, S. S. and Razdan, M. K. (1983) : Some aspects of plant regeneration in tissue culture in : Genetic and Crop Improvement (Eds. P.K. Gupta and J.R. Baha), pp : 337 – 385, Rastogi & Com., Meerut, India

2. Rao, J. D. and Reddy, G. M. (1987) *In* vitro morphogenesis and multiple shooting in chickpea (*C.* arietinum L.). In : Proc. Natn. Symp. on Plant Cell and Tissue Culture of Economically Important Plants. (ed. Reddy G M), Hyderabad, India, pp. 355-362.

3. Singh, A. (1997) Genetical and tissue culture studies in chickpea (*Cicer arietinum* L.). Ph.D. Thesis Published, Univ. of Kanpur, India.

4. Sharma, L. and Amla, D. V. (1998) Direct shoot regeneration in chickpea (*Cicer arietinum* L.). *Indian J of Exp Biol*, **36**, (468) : 605-609.

5. Neelam, A., Reddy, C. S. and Reddy, G. M. (1986a): Growth and differences in tissue culture of *Cicer arietinum* (L). *Intl Chickpea News Lett*, **14**, 9-12.

6. Sudhavani, A. K. and Reddy, V. D. (1996): Morphogenesis from callus culture of chickpea (*Cicer arietinum* L.). *Indian J Exp Biol*, **34**, 285-287.

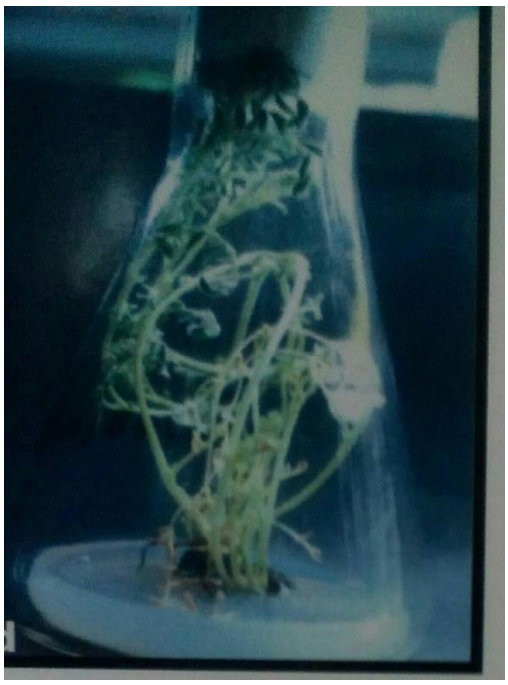
7. Tiwari, A., Renu, Singh, N. P. and Asthana. A. N. (1999): Effect of sucrose concentration on *in* vitro regeneration in pigeonpea. Intl *chickpea and Pigeonpea News Lett*, **6**, 61-62.

8. Wang, X. J. and Hsiao, K.C. (1995) : Sugar degradation during autoclaving : effect of duration and solution volume on breakdown of glucose. *Physiol Plant*, **94**, 415-418.

9. Prakash, S., Chowdhury, J. B., Jain, R. K. and Chowdhury, V. K. (1992): Factors affecting plant regeneration in chickpea (*Cicer arietinum* L.). *Indian J Exp Biol*, **30**, 1149-1153.

10. Suhasini, K., Sagare, A. P. and Krishnamurthy, K. V. (1994): Direct somatic embryogenesis from mature embryo axis in chickpea (*Cicer arietinum* L.). *Plant Sci*, **102**, 189-194.

11. Adkins, A. L., Godwin, I. D. and Adkins, S. W. (1995): An efficient *in vitro* regeneration system for Australian grown chickpea (*Cicer arietinum* L.) cultivars. *Aust J Bot*, **43**(B), 491-497.



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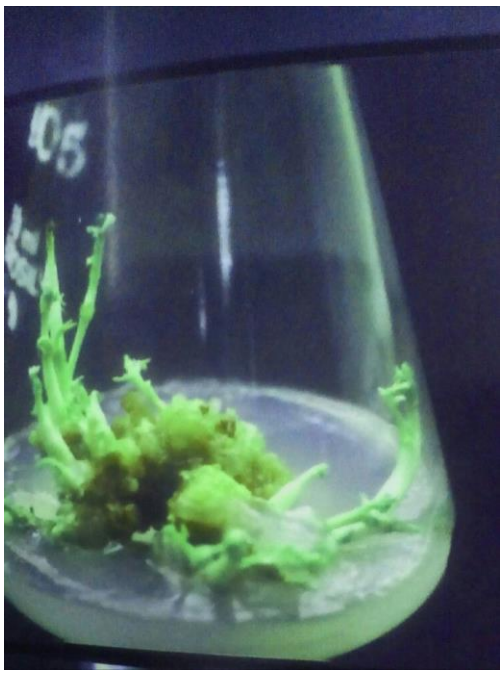

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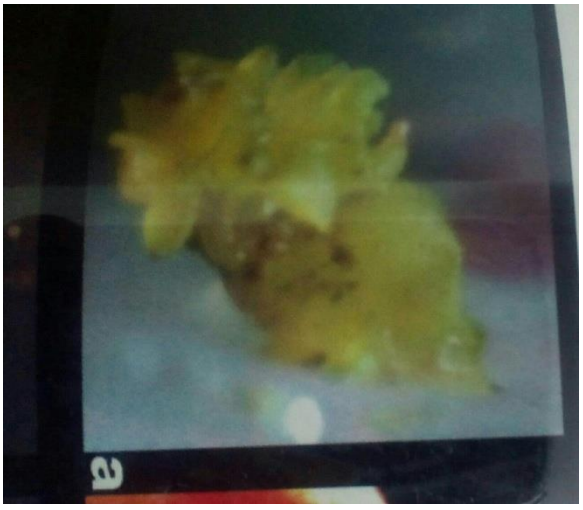

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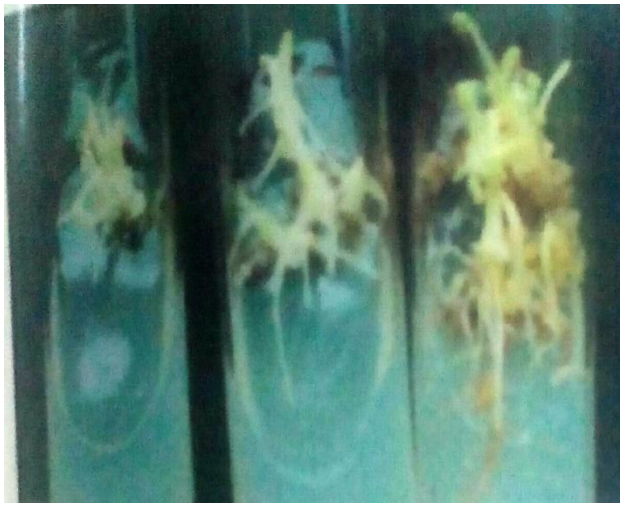

d

Fig 5 : Different regeneration pathway as induced by effect of various factor in Chickpea

(a). Direct shoot induction from explants (Direct organogenesis).

(b). Induction of shoots from callus (Indirect organogenesis).

(c). Somatic embryogenesis

(d). Rooting of shoots obtained from different pathway