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SHORT COMMUNICATION

DE NOVO REGENERATION IN CHICKPEA GENOTYPES *(CICER ARIETINUM* L.)

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ABSTRACT

Direct regeneration from mature embryo axes was achieved without intervening of callus phase in four chickpea varieties on the Media MS and B5 supplemented with combination of BAP, NAA and Kinetin. Hundred percent regeneration capacity was exhibited by commercially grown Vijay and Vishal varieties. There was considerable variation in umber of multiple shoot production by different varieties. Profuse rooting was obtained on the medium containing 0.5 and 1.0 mg/l IBA. This protocol is optimized for complete plant regeneration of chickpea for genetic transformation.

Keywords: Regeneration, Chickpea, Genotypes

INTRODUCTION

Chickpea *(Cicer arietinum* L.) is one among the highly cultivated and consumed legume throughout the world due to its high protein contents. Many wild species possess the wealth of agronomically desirable genes which are sexually incompatible to cultivated varieties and difficult to transfer. Earlier attempts are made to develop some resistance using conventional breeding methods involving wild relatives of chickpea, but without much success [1].

Despite *in vitro* regeneration from shoot meristem [2,3], immature cotyledons [4] and through embryo genesis from immature cotyledons [5], leaflet callus [6-8], influence of genotype regeneration capacity poorly studied. Present paper describes the complete regeneration in chickpea varieties.

MATERIALS AND METHODS

Seeds of four varieties viz. Vijay, Vishal., ICCV-10, PO 9702, were collected from All India Coordinated Research Project on Pulses Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, India. Out of these Vijay and Vishal are commercially grown high yielding varieties and latter two are promising genotypes. Those varieties are susceptible for pod borer and wilt. In the present report describe an approach for inducing a high frequency *de novo* shoot regeneration of four genotypes of C. *arietinum*.

It' is therefore proposed to put these varieties in genetic transformation. One of the prerequisites for successful genetic transformation is the availability of efficient reproducible protocol compatible with *in vitro* plant regeneration method of target tissue [2].

Seeds were surface sterilized by quick rinse of 70% alcohol followed by 0.1 % mercuric chloride for 6 to 7 min with continuous shaking and finally rinsed with 4-time sterile double distilled water to remove all traces of sterilant. Surface sterilized seeds soaked aseptically for 12-14 h. to excise mature embryo. Mature embryos were excised from 20-22 seeds by splitting the halves of the cotyledons. At least 30 explants of each genotypes were used for inculcation on culture medium. Two basal culture media contained salts of the Murashige and Skoog [9] (MS) medium 9 and Gamborg [10] (B5) medium supplemented with growth hormones (Table-I) along with 3% sucrose and solidified with 0.8 per cent agar. 5.8 pH was adjusted before autoclaving. Cultures were incubated at 25 °C under diffused light for one week and transferred under 3000 lux intensity with 16 h photoperiod. The experiment was repeated thrice to confirm the results.

RESULTS AND DISCUSSION

Mature embryos cultured on two basal media supplemented with cytokine (BAP), Kinetin and growth regulator (NAA) could give direct regeneration of chickpea without intervening the callus phase. Initially embryo pretend to callus formation with green spots under defused light. But when kept under light all green spots elongated in to shoots. *De novo* regeneration reported using Thiadiazuron [11]. Thus in the present experiment we found combination of BAP+NAA suitable for regeneration [l2]. The varieties Vijay Vishal and ICCV-IO gave hundred per cent explants regenerative ability when the cultured on M3 medium (B5+0.5 mg/l BAP+I mg/l NAA+0.1 mg/l. In general, this medium proved better for regeneration capacity in all varieties except PG 9702. This confirms that the *in vitro* regeneration capacity is genotype specific4.

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Initiation of shoots started after 11th days on M3 medium for the variety Vijay (table 1). Further it is revealed that regeneration varieties Vi jay and IPG 9702 for within 11-16 d on when basal medium contained B5 salts irrespective plant hormone content while genotype ICCV-10 started initiation of shoots after 13 d which was about one week earlier than other media. This suggested different media gave different response for genotype. There was considerable variation for shoot produced per explants. the range was 3.60 to 21.16 shoots/explants. It is interesting to note that variety PG 9702 had least response for regeneration capacity but produced maximum number shoots per explants. This may be due to indigenous hormone content of genotype. Vijay had produced least number of shoots on all media tested though it gave high percentage response for regeneration.

Regenerated shoot separated out when they attained the height about 3-5 cm and were transferred on half strength basal salts of MS9 and B510 medium supplemented with two levels (0.5, 1.0 mg/l) of Indol Buteric Acid (IBA) in each medium. The induction of roots was noticed within 3 w in all genotypes under study and on all genotypes under study and on all four media used. This indicated concentration of IBA did not influence the rooting [12,13]. Only average over four rooting media was considered (table 1). The profuse rooting was seen in all genotypes. The numbers of roots are crucial for hardening of *in vitro* regenerated seedlings. Virtually there was not much difference in number of roots produced by different genotypes on different media. The results obtained through this experiment confirm the influence genotype on the behavior of *in vitro* culture of chickpea. A noteworthy aspect of this regeneration protocol is the direct differentiation of shoot from mature embryo of chickpea. This suggested that this regeneration protocol could be optimized for each genotype.

Table 1: Different media used for regeneration and rooting of chickpea

Media	Regeneration		Rooting
M1	MS+0.5 mg/l BAP+1 mg/l NAA+0.1 mg/l kinetin	R1	½ MS+0.5 mg/l IBA
M2	MS+1.0 mg/l BAP+1 mg/l NAA+0.1 mg/l kinetin	R2	½ MS+1.0 mg/l IBA
M3	B5+0.5 mg/l BAP+1 mg/l NAA+0.1 mg/l kinetin	R3	½ B5+0.5 mg/l IBA
M4	B5+1.0 mg/l BAP+1 mg/l NAA+0.1 mg/l kinetin	R4	½ B5+1.0 mg/l IBA

Table 2: *De novo* regeneration of chickpea genotypes from mature embryo axes

Genotype	Medium	No of explants regenerated (%)	Days required for. regeneration	Average number of shoots	Average. number of roots
Vijay	Ml	32 (88.9)	16.36±2.13	3.60±1.06	5.26±0.56
	M2	36 (94.74)	14.62±1.18	4.16±0.6	7.22±0.68
	M3	40 (100.0)	11.39±2.10	3.50±0.33	4.32 ± 0.98
	M4	35 (97.2)	13.33±0.98	4.83±0.56	6.67±0.33
Vishal	Ml	28 (87.5)	18.32±1.33	9.18±0.52	5.39±0.06
	M2	26 (72.2)	26.43±2.16	11.13±0.98	7.00±0.13
	M3	40 (100.0)	21.43±3.11	16.28±1.33	6.68±0.18
	M4	30 (85.7)	21.48±2.17	17.33±1.16	4.23±0.37
ICCVI0	Ml	30 (90.9)	12.18±1.68	8.16±98	3.28±0.52
	M2	28 (93.3)	13.17±1.68	11.26+1.36	4.49±0.31
	M3	38 (100.0)	17.19±3.12	13.29+0.67	4.31±0.39
	M4	31 (96.8)	18.17±2.14	15.18±0.39	3.93±0.71
PG9702	Ml	34 (94.4)	23.07:t 1.93	14.23±1.69	6.26±0.18
	M2	32 (88.9)	27.36±2.16	13.22±1.08	6.62±0.17
	M3	29 (74.4)	14.12±2.12	18.26±1.06	8.16±0.09
	M4	26 (68.4)	15.13±1.19	21.16±0.92	8.29±0.13

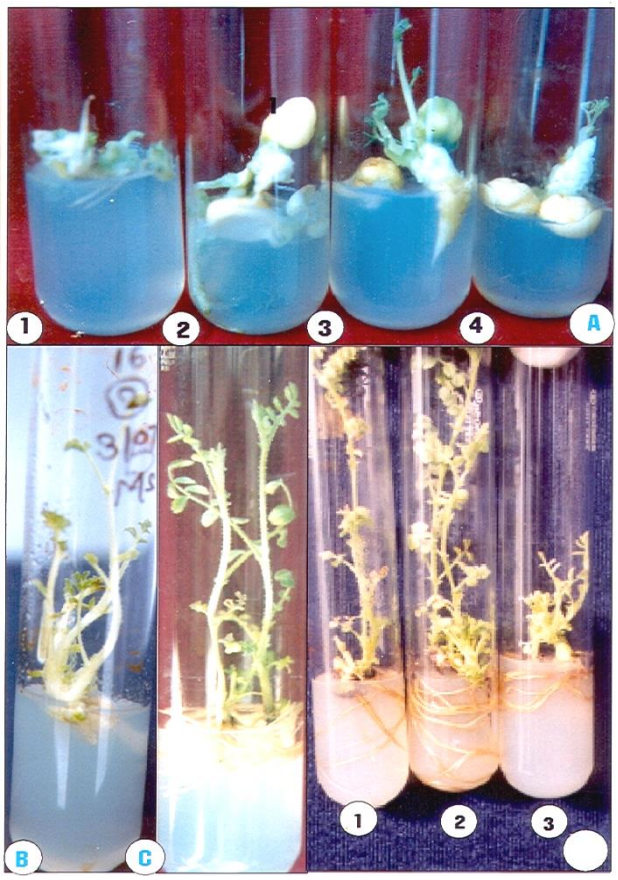

Fig. 1: A. Mature embryo axes cultured and *de novo* regeneration in chickpea, l. Vishal 2. Vijay 3. ICCV-IO 4. PG9702 B. Multiple shoot formation of Vi jay, C. Rooting in regenerated plants in Vishal, 1Vijay, 2. ICCV-IO, 3. PG9702

REFERENCES

1. Anonymous, *Research Review Committee Report of India Coordinated Research Project on Pulses. MPKV, Rahuri,2003.*
2. Kar, S., Johnson, T. M., Nayak, P. and Sen, S. K., 1996 Efficient transgenic plant regeneration through agrobacterium mediated transformation of chickpea (*Cicer arietinum* L.) *Plant Cell Reports,* 1996,16,32-37.
3. Bajaj, Y. P. S. and Danju, M. S. *Curro Sci,* 1979, *48, 906-907.*
4. Kartha, K. K., Pahal., K. Leung, N. L., and Mroginski, L. A., Plant regeneration from grain legumes; soybean, cowpea, peanut, chickpea and bean, Canadian J. Bot. 59, 1671-1679.
5. Shri, P. V., and Davis, T. M., *Plant Cell Tissue organ culture,* 1992,28, 45-51.
6. Sagare, A. P., Suhasini, K., and Krishnmurthy, K. V., *Plant Cell Reports,* 1993. Plant regeneration via somatic embryogenesis in chickpea (*Cicer arietinum* L.) 12, 652-655.
7. Barana, K. S. and Wakhlu, A. K., 1993. Somatic embryogenesis and *Plant Cell Report,* 1993,12,521-524.
8. Barana, K. S. and Wakhlu, A. K., 1994. Whole plant regeneration in *cicer arietinum* L. from callus culture via organogenesis. *Plant Cell Reports,* 13,510-513.
9. Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol, Plant*, 15,473-497.
10. Gamborg, O. L, Miller, R. A. and Oima, K., 1968. Nutrient requirement for suspension culture of soybean root cells. *Exp. Cell Res.,* 50, 151-158.
11. Murthy, B. N. S., Victor, 1., Singh, R. P., Fletcher, R. A Saxena, P. K., 1996. *In vitro* regeneration of chickpea (*Cicer arietinum* L.): Stimulation direct organogenesis and somatic embryogenesis by thiadizuran *Plant Growth Regulation,* 1996, 19:233-240.
12. Arockiasamy, S., Varghese, G. and Ignachimuthu, 2000. High frequency regeneration of chickpea plantlets from leaf callus, *Phytomorphology,* 50:(3-4) *297-307.*
13. Chauhan, R. and Singh, N. P. 2002. Plant regeneration via somatic embryogenesis in chickpea, *Indian J Genet.,* 2002;62: 319-131.