Regeneration studies 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 considerable variation in umber of multip0le shoot production by different varieties. Profuse rooting was obtained on the medium containing 0.5 and 1.0 mg/1 IBA. This protocol is optimized plant regeneration of chickpea for genetic transformation.*

INTRODUCTION-

Chickpea (*Cicer arietinum* L.) is an important legume, which has worldwide acceptance as major source of protein for vegetarians. Many wild sp0ecies posses the wealth of agronomically desirable genes which are sexually incompatible to cultivated varieties and difficult to transfer. In the present report describe an approach for inducing a high frequency de novo shoot regeneration of four genotypes of C. arietinum. Seeds of four varieties viz. Vijay, Vishal, ICCV-10, PG 9702, were collected from All India Co-Coordinated Research Project on Pulses Mahatma Phule Krishi Vidyapeeth, Rahuri – 413 722, India. Out of these Vihay and Vishal are commercially grown high yielding varieties and latter two are promising genotypes. Those varieties are susceptible for pod borer and wilt. Earlier attempts are made to develop some resistance using conventional breeding Methods involving wild relatives of chickpea, but without much success [1]. 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] .Despite in vitro plant regeneration in chickpea has been reported through organogenesis 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.

RESULTS AND DISCUSSION

Seeds were surface sterilized by quick rinse of 70% alcohol followed by 0.1 % Mercuric chloride for 6 to 7 minutes with continuous shaking and finally rinsed with 4time sterile double distilled water to remove all traces of sterilant. Surface sterilized seeds soaked aseptically for 12-14 her. 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 (1962) (MS) medium [9] and Gamborg (1968) (B5) medium[10] supplemented with growth hormones (Table-1) along with 3% sucrose and solidified with 0.8 per cent agar. 5.8 pH was adjusted before autoclaving Cultures were incubated at 25 +[0] C under diffused light for one week and transferred under 3000 lux intensity with 16 hrs photoperiod . The experiment repeated thrice to confirm the results.

Table l : Different media used for regeneration and rooting of chickpea varieties .

Media	Regeneration		Rooting
M1	MS +0.5 mg BAP/1mg/1AA+0.1mgKinetin	R1.	½ MS+0.5mg/1IBAI
M2	MS+1.0mgBAP/NAA+0.1mg/Kinetin	R2	½ MS+0.1mg/1TBA
M3	B5+0.5mgBAP/1+1mg/!NAA+0.1mg/1 Kinetin	R3	½ B5+0.5mg/1IBAI
M4	B5+1.0mgBAP/1+1mg/1NAA+0.1mg/1 Kinetin	R4	½ B5+0.5mg/1 IBA

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 (Figure 1). De novo regeneration reported using Thiadiazuron [11]. Thus in the present experiment we found combination of BAP +NAA suitable for regeneration [12].

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	M1	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	M1	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
ICCV 10	M1	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	3.39 0.71
PG9702	M1	34(94.4)	23.07 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

The varieties Vijay Vishal and ICCV-10 gave hundred per cent explants regenerative ability when the cultured on M3 medium (B5+0.5mg/1BAP+1mg/1NAA+0.1mg). In general this medium proved better for regeneration capacity is all varieties except PG 9702. This confirms that the in vitro regeneration capacity is genotype specific [4]. Initiation of shoots started after 11thdays on M3 medium for the variety Vijay (Table 1) .

Further it is revealed that regeneration varieties Vijay and IPG 9702 for within 11-16 days on when basal medium containedB5 salts irrespective plant hormone content while genotype ICCV-10 started initiation of shoots after 13 days which was about on week earlier than other media . This suggested different media given 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 3-5cm and were transferred on half strength basal. Salts of MS[9] and B5[10] medium supplemented with two levels (0.5, 1.0mg/l) of Indol buteric acid (IBA) in each medium. The induction lf roots was noticed within 3 weeks 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 rootin was seen in all genotypes (Figure 1). The number 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 note worthy 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.

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