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Induction of callus from cowpea [*Vigna unguiculata* (L.) Walp] through *in vitro* culture

K.D. DADMAL AND V.C. NAVHALE

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SUMMARY

Pulse crops are the most versatile legumes with diversified uses as food, fodder, vegetable and green manuring crop. It is grown for its long green pods as a vegetable, seeds as a pulse and foliage as a fodder. It was observed that, among the surface sterilization treatment for explants, the ethyl alcohol for 5 minutes was found to be most effective to get 87.5 per cent aseptic cultures. The genotype Konkan Safed (83.33%) showed higher response for callus induction when 5.0 mg/l kinetin concentration supplemented with MS medium.

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Key words : *In vitro,* Regeneration, Cowpea, Explant, Callus

The dual purpose cowpea [*Vigna unguiculata* (L.) Walp cultivar group cylindrica] is used as a pulse crop as well as vegetable. It is a nutritious leguminous crop, low in anti- nutritional factor. Pulse crops are the most versatile legumes with diversified uses as food, fodder, vegetable and green manuring crop. This has been recognized as a valuable source of vegetable protein, minerals and vitamins particularly in developing countries like India where majority of population depend on low priced vegetarian food for meeting their dietary requirements. It is grown for its long green pods as a vegetable, seeds as a pulse and foliage as a fodder. It matures in about 65 to 70 days and producing long pods measuring 13 to 15 cm containing 13 seeds.

In vitro culture techniques in cowpea can be used for inducing variability among the cultivars. Callus culture and induction of somaclonal variation have their own advantages in plant breeding. Initially the embryogenic callus is to be induced in genotype which is essential step towards recovering variants. Tissue culture approach might be true effective in gaining phenotypic variation in regenerated plants such as shorter plants and increased number of pods etc. *In vitro* culture technique is useful for developing disease free plants as well as transgenic plants by incorporating desirable genes. Callus initiation and plant regeneration is complex phenomenon, influenced by a number of factors like genotypes, explants, hormones, culture conditions etc.

Correspondence to:
K.D. DADMAL, Department of Agricultural Botany, Vivekanand Agriculture College, Hiwara, BULDANA (M.S.) INDIA
Email : kewal_dadmal888@rediffmail.com

Authors' affiliations:
V.C. NAVHALE, Department of Agricultural Botany, College of Agriculture, AKOLA (M.S.) INDIA

MATERIALS AND METHODS

In the present investigation, the promising 20 genotypes of cowpea [*Vigna unguiculata* (L.) Walp.] selected from the germplasm available at the Research farm of Department of Agriculture Botany, College of Agriculture, Dapoli were used.

Sr. No.	Name of genotypes	Sr. No.	Name of genotypes
1.	Pusa Phalguni	11.	VCP-240
2.	Kunde Local	12.	VCP-16
3.	Konkan Sadabahar	13.	CPD-16
4.	Konkan Safed	14.	DCS-5
5.	Fodder Cowpea	15.	DCP-2
6.	Phule Pandhari	16.	ACP-109
7.	PCP-9757	17.	HC-03-1
8.	PCP-9719	18.	BDN-1
9.	GC-9040	19.	M-10
10.	GC-3	20.	Punjab

Basal medium:

The basal medium developed by Murashige and Skoog (1962) containing 30 g/l sucrose with certain modifications and addition of other desired ingredients and various concentrations and combination of plant growth

substances were used.

Experimental conditions:

All *in vitro* studies were carried out aseptically in laminar airflow chamber. The experiments were conducted under well defined conditions of the culture room maintained at 26 ± 2^0C temperature, uniform light (1000 lux) provided by fluorescent tubes (7200 K) over a light or dark cycle of 16/8 hours during 2007-08.

Preparation of explants:

Hypocotyls, shoot tips, embryo and embryo with cotyledons were used as initial explants for callus induction. For regeneration of these four explants, the seeds were surface sterilized by 10% teepol for 5 minutes followed by washing under running tap water. After that, seeds were quickly deeped in 70% ethanol for 5, 10 and 15 minutes and further immersed in mercuric chloride (0.1%) for 3 minutes, followed 2-3 washing with double distilled water. The treated seeds soaked for 16 hours and then used for explant like embryo and embryo with cotyledons, respectively. Treated seeds were also inoculated on the 1/2 MS medium without growth regulators and allowed to grow for 7-8 days at 25 ± 2^0 C in the light (1600 lux from cool white fluorescent tubes). These seedlings were used for making explants like hypocotyl and shoot tip. The hypocotyl, shoot tip, embryo and embryo with cotyledon explants were cut by using stainless steel knife and inoculated on culture media containing growth substances for callus induction. About 1 cm lengths of hypocotyls were inoculated on culture media for callus initiation.

Callus induction:

Three segments of followed explants (Hypocotyl, shoot tips, embryo and embryo with cotyledons) were placed in jam bottles containing 20 ml of culture medium. During the course of transfer of explants, all the surgical instruments were first deeped in rectified spirit, flamed on spirit lamp and cooled before use. Five seeds were inoculated for seedling preparation in each culture bottle containing medium. Hypocotyl and shoot tip were inoculated at the rate of 3 explants in each culture vessel. To ensure close contact with the medium, the explants were pressed gently. Culture bottles were covered with suitable caps to avoid contamination. The five media *viz.,* MS + 0.5 mg/l 2,4-D, MS + 1mg/l 2,4-D, MS + 2 mg/l 2,4-D and MS + 3 mg/l 2,4-D were utilized for callus induction. Observation was recorded on callus induction. It was measured on number of explants produced callus out of total culture of explants inoculated and expressed in percentage. An experiment was carried out in factorial completely randomized design and analyzed the data accordingly.

RESULTS AND DISCUSSION

It was observed from the Table 1, among the surface sterilization treatment to explants, the ethyl alcohol (70%) for 5 minutes was found to be most effective which gave 87.5 per cent aseptic cultures followed by 10 minutes (62.50%) while the treatment of mercuric chloride for 3 minutes gave the equal performance.

Table 1 : Chemical sterilization treatment and time for aseptic cultures

Duration of treatment (Minutes)	Ethyl alcohol (70%)	Mercuric chloride $HgCl_2$ (0.1%)
5 min	87.5 %	37.5%
10 min	62.5%	37.5%
15 min	37.5%	37.5%

Genotypic variability in relation to 2,4-D concentrations for callus induction:

In the Present investigation, the per cent of callus induction by using MS medium with 4 concentrations of 2,4-D and 4 explants are presented in Table 2. The maximum (91.67%) response for callus induction was recorded in 3.0 mg/l and 2.0 mg/l 2,4-D concentration by using hypocotyl and embryo explant, respectively, while minimum (12.50%) response callus induction were recorded in 0.5 mg/l 2,4-D concentration by using embryo explant. Among the explants, hypocotyl explant was showed higher (58.33%) for callus induction with 0.5 mg/l 2,4-D concentration, while the hypocotyl, embryo and embryo with cotyledon showed maximum (66.67%) response with 1.0 mg/l 2,4-D concentration. An embryo explant showed higher (91.67%) response for callus induction with 2.0 mg/l 2,4-D concentration, while hypocotyl explant showed highest (91.67%) response for callus induction with 3.0 mg/l 2,4-D concentration. The result are in uniformity with those of Ahmed *et al*. (1986), Li *et al*. (1993) and Li *et al*. (1995) reported maximum callus induction in MS medium with 2,4-D concentration of 2.0 mg/l. Cheema and Bawa (1992) revealed that the range of 2,4-D concentration within 0.5 mg/l to 4 mg/l induced satisfactory callus induction in cowpea. Vazques-Duhalt *et al*. (1991) induced callus and cell suspension cultures of cowpea with 2,4-D grown at different concentrations.

It is seen from the Table 2, that the maximum response for callus induction in hypocotyl, shoot tip, embryo and embryo with cotyledon explants showed

Table 2: Genotypic variation in 2,4-D concentration for callus induction

Sr. No.	Genotypes	MS medium											
		Hypocotyl			Shoot tips			Nodes			Embryo with cotyledon		
		2,4-D Concentration (mg/l)			2,4-D Concentration (mg/l)			2,4-D Concentration (mg/l)			2,4-D Concentration (mg/l)		
		0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0
1.	Pusa Barkha	16.67	50.00	66.67	50.00	33.33	16.67	58.33	50.00	16.67	50.00	50.00	50.00	33.33	50.00	62.50	16.67
2.	Kashi Gouri	33.33	16.67	50.00	16.67	16.67	50.00	58.33	16.67	33.33	33.33	31.50	50.00	50.00	31.50	25.00	33.33	16.67	33.33
3.	Kashi Sadabahar	50.00	50.00	66.67	50.00	33.33	50.00	83.33	16.67	16.67	16.67	58.33	75.00	75.00	16.67	33.33	58.33	70.83	16.67
4.	Kashi Sona	58.33	66.67	70.83	66.67	16.67	50.00	66.67	33.33	16.67	16.67	66.67	79.17	79.17	31.50	31.50	66.67	79.17	50.00
5.	Kashi Kanchan	33.33	16.67	50.00	16.67	33.33	16.67	50.00	16.67	31.50	16.67	50.00	50.00	66.67	33.33	33.33	16.67	16.67	29.17
6.	Kashi Kanchan	16.67	50.00	62.50	25.00	29.17	16.67	58.33	66.67	33.33	31.50	50.00	50.00	25.00	25.00	31.50	16.67	16.67	31.50
7.	PCP 9151	33.33	58.33	62.50	50.00	33.33	58.33	75.00	16.67	16.67	16.67	50.00	50.00	91.67	16.67	33.33	16.67	58.33	33.33
8.	PCP 9179	16.67	16.67	58.33	33.33	33.33	16.67	50.00	50.00	33.33	33.33	16.67	66.67	66.67	29.17	29.17	16.67	58.33	16.67
9.	CC 9910	20.83	16.67	50.00	58.33	20.83	33.33	50.00	50.00	20.83	20.83	29.17	16.67	50.00	58.33	16.67	33.33	16.67	20.83
10.	CC 3	16.67	16.67	58.33	25.00	31.50	16.67	50.00	33.33	33.33	33.33	16.67	50.00	50.00	29.17	16.67	16.67	58.33	25.00
11.	VCP 2/6	50.00	58.33	70.83	20.83	16.67	50.00	62.50	29.17	31.50	50.00	50.00	66.67	66.67	16.67	31.50	31.50	50.00	66.67
12.	VCP 16	16.67	50.00	66.67	16.67	16.67	16.67	50.00	16.67	33.33	33.33	31.50	75.00	75.00	29.17	25.00	16.67	50.00	31.50
13.	CCP 16	16.67	16.67	58.33	16.67	33.33	50.00	66.67	16.67	16.67	16.67	50.00	62.50	62.50	58.33	31.50	16.67	58.33	50.00
14.	DCS 5	31.50	58.33	70.83	31.50	16.67	16.67	58.33	16.67	16.67	16.67	31.50	58.33	58.33	31.50	31.50	33.33	16.67	33.33
15.	DCP 2	16.67	50.00	50.00	31.50	16.67	16.67	50.00	16.67	16.67	33.33	16.67	50.00	50.00	29.17	29.17	16.67	16.67	33.33
16.	ACP 09	50.00	50.00	62.50	31.50	16.67	33.33	50.00	29.17	12.50	25.00	31.50	50.00	20.83	33.33	16.67	50.00	58.33	58.33
17.	ICC 03	29.17	50.00	62.50	33.33	31.50	33.33	50.00	16.67	16.67	16.67	50.00	50.00	31.50	33.33	33.33	50.00	62.50	33.33
18.	BDN 1	50.00	50.00	66.67	50.00	29.17	58.33	70.83	50.00	31.50	50.00	58.33	83.33	83.33	16.67	31.50	16.67	50.00	58.33
19.	M 10	31.50	50.00	50.00	33.33	16.67	16.67	50.00	29.17	25.00	25.00	33.33	16.67	16.67	29.17	29.17	33.33	16.67	16.67
20.	Sujata	50.00	33.33	25.00	16.67	16.67	16.67	58.33	29.17	16.67	16.67	29.17	50.00	50.00	31.50	16.67	16.67	50.00	16.67
	Mean	16.67	50.00	62.50	31.29	16.67	50.00	58.13	16.67	31.29	16.67	16.38	59.38	59.38	31.29	32.50	33.75	55.21	16.25
	Minimum	20.83	33.33	25.00	16.67	20.83	33.33	16.67	29.17	12.50	25.00	25.00	31.50	20.83	16.67	33.33	16.67	16.67	20.83
	Maximum	58.33	66.67	70.83	50.00	33.33	58.33	83.33	66.67	33.33	66.67	66.67	91.67	58.33	16.67	33.33	79.17	79.17	66.67
		Genotype 2,4-D			Genotype x 2,4-D			Genotype 2,4-D		Genotype x 2,4-D		Genotype 2,4-D		Genotype x 2,4-D					
	S.E.	2.89	3.12	5.01	5.79	0.67	2.75			2.59	1.89	1.65		1.89	0.72	3.28			
	C.D. (P=0.05)	16.15	17.5	28.03	32.42	3.72	15.26			9.98	3.8	11.30		7.03	2.72	12.8			

when 2,4-D concentration was 2.0 mg/l as compared with other concentrations. It was observed that callus induction increased as the concentration increases from 0.5 mg/l to 2.0 mg/l 2,4-D concentration and then reduced in all four explants.

The genotype x 2,4-D concentrations data ranged from 20.83 to 91.67 per cent in hypocotyl, 20.83 to 83.33 per cent in shoot tip, 12.50 to 91.67 per cent in embryo and 16.67 to 79.17 per cent in embryo with cotyledon explant. The maximum response for callus induction in hypocotyl explant was recorded by Konkan Sadabahar (91.67%) with 3.0 mg/l 2,4-D concentration and minimum response by Punjab (16.67%) with 3.0 mg/l 2,4-D concentration. The higher concentration of 2,4-D were detrimental to callus growth (Singh *et al*., 1982). In this way, large genotypic variation in callus induction might have attributed to the differences in the components and concentration of endogenous phytohormones and difference in the level of 2,4-D. In shoot tip explant, higher response for callus induction was observed in Konkan Sadabahar (83.33%) with 2.0 mg/l 2,4-D concentration, while least response by GC-9040 (20.83%) with 0.5 mg/l 2,4-D concentration. In embryo explant, maximum response for this character was recorded by PCP-9757 (91.67%) with 2.0 mg/l 2,4-D concentration and minimum response by ACP-109 (12.50%) with 0.5 mg/l 2,4-D concentration, while the higher response for callus induction was given by Konkan Safed (79.17%) in embryo with cotyledon explant with 2.0 mg/l 2,4-D concentration and least response by GC-9040 (16.67%) with 0.5 mg/l 2,4-D concentration.

The interaction effect of cowpea genotypes for callus induction by using different explants and 2,4-D concentrations are presented in Table 2. The mean response for this interaction significantly ranged from 12.50 to 91.67 per cent for callus induction. The genotype Konkan Sadabahar (91.67%) and PCP-9757 (91.67%) showed higher response for callus induction at 3.0 mg/l and 2.0 mg/l 2,4-D concentration supplemented with MS medium, respectively. The least response for this trait was recorded by ACP-109 (12.50%) when 2,4-D concentration was 0.5 mg/l used in media.

The interaction effect of genotype, explant and 2,4-D concentration also played an important role in callus induction. The mean genotypic response for callus induction in genotype x explant x 2,4-D concentrations interaction in MS medium ranged from 12.50 to 91.67 per cent. The genotype Konkan Sadabahar (91.67%) induced higher percentage of callus by using hypocotyl explant with 3.0 mg/l 2,4-D concentration, while embryo explant of PCP-9757 (91.67%) exhibited higher response for callus induction at 2.0 mg/l 2,4-D concentration over remaining genotypes in MS medium.

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