ISSN No:-2456-2165

Protocol Optimization for Development of Multiple Shoots Initiation & Peroxidase Enzyme Activity in *Cicer arietinum L.*

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Abstract:- In the present investigations the effect of growth regulators for callus initiation was studied in India's important Pulse crop Cicer arietinum to observe its response. Six genetic lives of Cicer arietinum were screened for studying morphogenic potential. The intact seedlings were regenerated and develop into shoots from cotyledonary-node on B_1 basal medium. However, there was variation in response from different cultivars. Experimental study proved that excellent growth in cytokinin supplemented with benzylaminopurine. The response of regeneration was observed good in combination of cytokinin and benzylaminopurine. When cotyledonary node were isolated, the number of multiple shoots did not increase than on intact seedlings. There was little swelling observed in different explants like roots, hypocotyl and epicotyl.

Keywords:- *Cicer arietinum, auscius, cytokinin, cotyledon.*

I. INTRODUCTION

Cicer arietinum L. is an important grain legume cultivated throughout the world. But the productivity of Cicer arietinum has not increased satisfactory over the years. (Singh and Kataria, 2012). Conventional breeding methods for improving productivity of any legumes are limited, and great interest has been developed in biotechnological methods (Gamborg 2002; Mallikarfuna and Muehlbauer 2011). Regeneration in chick pea via direct shoot induction has been reported by various explants. (Batra et al 2002; Rich and Singh, 2002; Kiran et al 2005; Krishna and Shanu 2008). Somatic embryogenesis has also been reported from cotyledon explant. (Shri and Davis 1992; Sajare et al 1993). Successful shoot regeneration of pre-conditional mature embryo and embryonic axis explant was achieved with benzylaminopurine (BA) (Aasim et al 2011). Maximum number of shoots was produced with shoot tip and cotyledons explants. (Sujatha et al, 2007; Rekha and Thiruvengadam, 2009; Ugandhar et al, 2012)

II. MATERIALS AND METHODS

Seeds of six genetic lines of Kabuli gram (L-550, Pusa 267, BG-315, BG-316, BG-333) were collected from the directorate of Pulses, IARI, New Delhi. The collected seeds were surface sterilized for 5 minutes by using sodium hypochlorite solutions or chlorine water, rinsed thoroughly with sterilized distilled water for 2 minutes. The seeds of Cicer arietinum were further transferred aseptically into Basal medium supplemented with cytokinin and BAP. The pH of medium was adjusted top 5.6-5.8. The culture vial were incubated in a culture room at $25+2^0$C in diffuse light of 11-12 hours.

Half of the seedlings formed were retained for observing the response of intact seedlings. The remaining 3-4 day old seedlings served as source for different explants of root, hypocotyl, stem leaves, cotyledons and cotyledonary node. The explants were cultured onto B_5 BM or BM enriched with cytokinin or auxins of different concentration. For intiating differentiation of calli, auxin and cytokinin either alone, or in different combinations were employed.

III. RESULTS

After two weeks the intact seedlings of all six genetic lines of white or kabuli gram produced multiple shoots successfully onto the basal medium. There were significant differences in multiples shoots ranged from 3.3 in Pusa 267 to 6.8 in BG-331. After excision of various explants from the seedlings and culturing on basal medium, only cotyledonary node explants responded well. When it is isolated the number of multiple shoots decreased (Fig.-1B). The different explants such as root, hypocotyl and cotyledons showed little swelling and formal callus followed by rhizogenesis.

When seeds were culture in B5 medium, supplemented with benzylaminopurine, it was observed that number of multiple shoots were increased in cytokinin medium. The large number of multiple shoots were formed in BAP where the average number of multiple shoots varied from 8.5 in BG-315 to 10.3 in BG-316. Moreover on isolation of cotyledonary node, the number of multiple

ISSN No:-2456-2165

shoots did not increase than on intact seedlings. The isolated node responded well in BAP.

The effect of auxins, NAA, Picoloram, 2, 4-D and 2,4,5-I were isolated from cotyledonary node of BG-331. The shoots bud regeneration was obtained in picloram. Callus formation, regeneration of shoot buds and rhizogenesis were observed in medium of NAA. A well develop callus was formed in the combination of 2,4-D and 2,4-5T. The highest number of regeneration of multiple shoots and callus formation were produced in optimum concentration of BAP from both sides of cotyledons. Rhizogenesis was observed only in media containing IBA. When regenerated shoots transferred to 2ppm IBA, develop plantlets.

No shoots were produced, when callus formed from cotyledonary node and transfer to medium containing cytokinin as well as in basal medium./

FIGURE -1

FIGURE -2

FIGURE -3

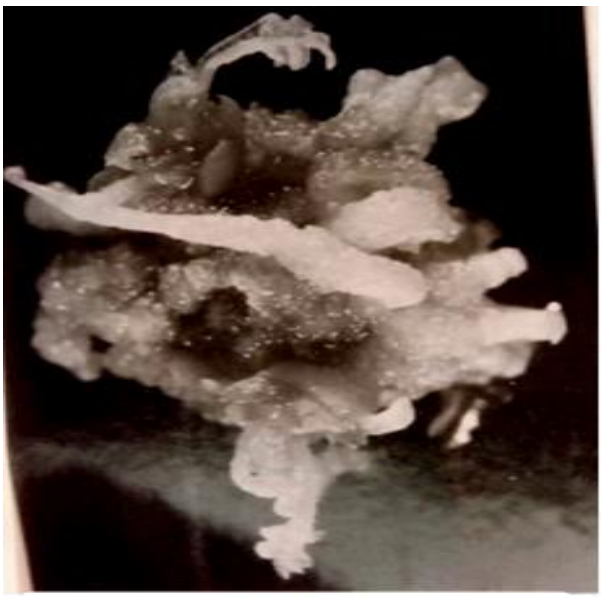
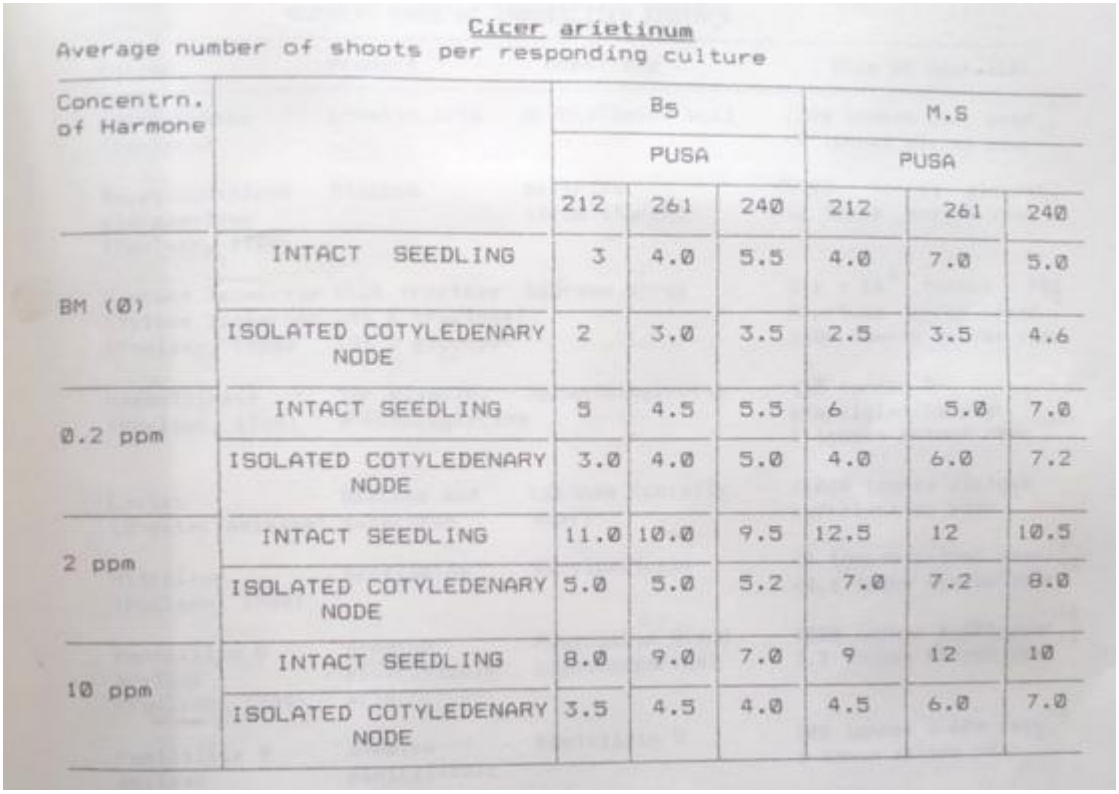


FIGURE -4

LEGENDS OF FIGURES

1. Formation of multiple shoots from the cotyledonary node region of an intact seedling a medium containing 10 ppm BAP (PUSA -240) in Cicer arietinum.
2. Formation of multiple shoots from the Isolated cotyledonary node on medium supplemented with 0.2ppm BAP (PUSA -240) in Cicer arietinum.
3. Formation of multiple shoots from the Isolated cotyledonary node on medium containing 2 ppm BAP (PUSA -261) in Cicer arietinum.
4. Formation of Callus Shoots/shoot buds as well as roots on medium containing NAA (10^{-5} M) (PUSA – 212) from isolated Cotyledonary node of Cicer arietinum.

ISSN No:-2456-2165

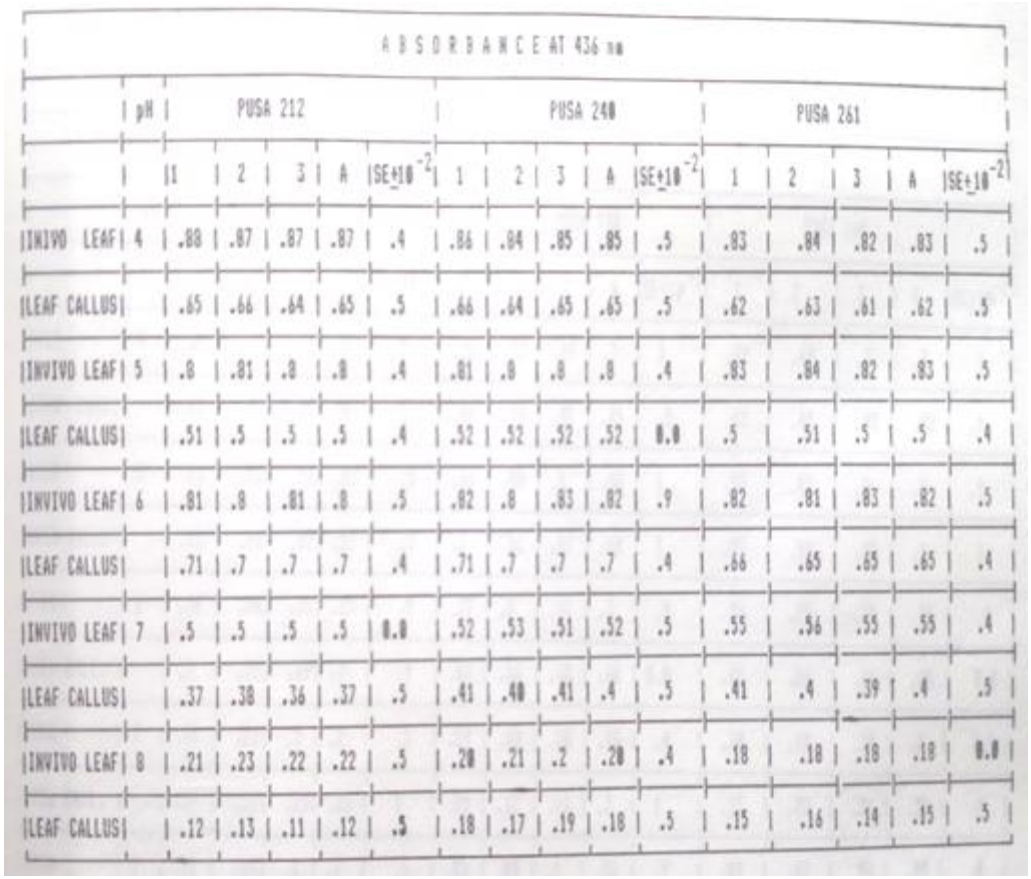


Cicer arietinum

Average number of shoots per responding culture

Concentrn. of Harmone		B_5			M.S		
		PUSA			PUSA		
		212	261	240	212	261	240
BM (Ø)	INTACT SEEDLING	3	4.0	5.5	4.0	7.0	5.0
	ISOLATED COTYLEDENARY NODE	2	3.0	3.5	2.5	3.5	4.6
0.2 ppm	INTACT SEEDLING	5	4.5	5.5	6	5.0	7.0
	ISOLATED COTYLEDENARY NODE	3.0	4.0	5.0	4.0	6.0	7.2
2 ppm	INTACT SEEDLING	11.0	10.0	9.5	12.5	12	10.5
	ISOLATED COTYLEDENARY NODE	5.0	5.0	5.2	7.0	7.2	8.0
10 ppm	INTACT SEEDLING	8.0	9.0	7.0	9	12	10
	ISOLATED COTYLEDENARY NODE	3.5	4.5	4.0	4.5	6.0	7.0

TABLE - 1

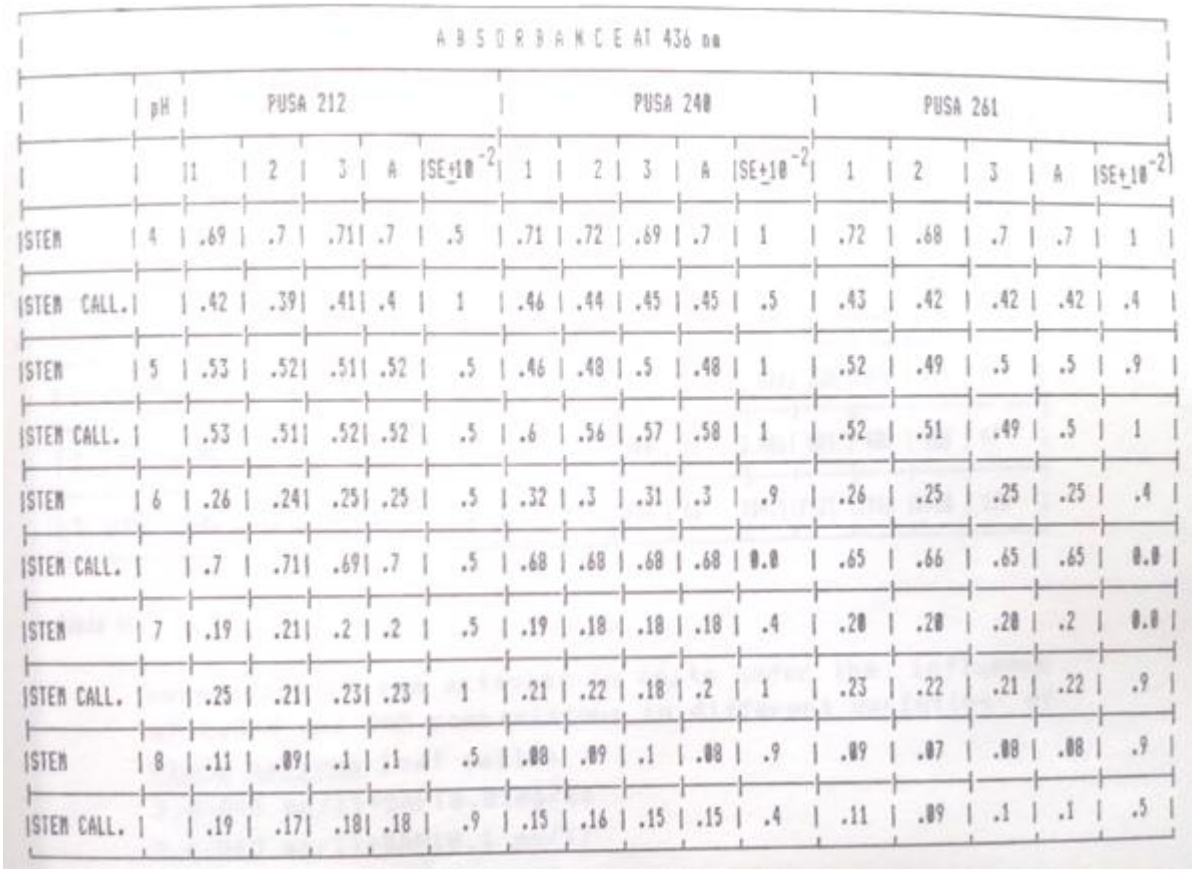


ABSORBANCE AT 436 nm

	pH	PUSA 212					PUSA 240					PUSA 261				
		1	2	3	A	$SE\pm10^{-2}$	1	2	3	A	$SE\pm10^{-2}$	1	2	3	A	$SE\pm10^{-2}$
INVIVO LEAF	4	.88	.87	.87	.87	.4	.86	.84	.85	.85	.5	.83	.84	.82	.83	.5
LEAF CALLUS		.65	.66	.64	.65	.5	.66	.64	.65	.65	.5	.62	.63	.61	.62	.5
INVIVO LEAF	5	.8	.81	.8	.8	.4	.81	.8	.8	.8	.4	.83	.84	.82	.83	.5
LEAF CALLUS		.51	.5	.5	.5	.4	.52	.52	.52	.52	0.0	.5	.51	.5	.5	.4
INVIVO LEAF	6	.81	.8	.81	.8	.5	.82	.8	.83	.82	.9	.82	.81	.83	.82	.5
LEAF CALLUS		.71	.7	.7	.7	.4	.71	.7	.7	.7	.4	.66	.65	.65	.65	.4
INVIVO LEAF	7	.5	.5	.5	.5	0.0	.52	.53	.51	.52	.5	.55	.56	.55	.55	.4
LEAF CALLUS		.37	.38	.36	.37	.5	.41	.40	.41	.4	.5	.41	.4	.39	.4	.5
INVIVO LEAF	8	.21	.23	.22	.22	.5	.20	.21	.2	.20	.4	.18	.18	.18	.18	0.0
LEAF CALLUS		.12	.13	.11	.12	.5	.18	.17	.19	.18	.5	.15	.16	.14	.15	.5

TABLE – 2 : pH optimum of peroxidases of different verities of Cicer arietinum leaf and leaf Callus.
Note : pH optimum of leaf between 4 and 5 and leaf Callus at 6.

ISSN No:-2456-2165



ABSORBANCE AT 436 nm

	pH	PUSA 212					PUSA 240					PUSA 261				
		1	2	3	A	$SE\pm10^{-2}$	1	2	3	A	$SE\pm10^{-2}$	1	2	3	A	$SE\pm10^{-2}$
STEM	4	.69	.7	.71	.7	.5	.71	.72	.69	.7	1	.72	.68	.7	.7	1
STEM CALL.		.42	.39	.41	.4	1	.46	.44	.45	.45	.5	.43	.42	.42	.42	.4
STEM	5	.53	.52	.51	.52	.5	.46	.48	.5	.48	1	.52	.49	.5	.5	.9
STEM CALL.		.53	.51	.52	.52	.5	.6	.56	.57	.58	1	.52	.51	.49	.5	1
STEM	6	.26	.24	.25	.25	.5	.32	.3	.31	.3	.9	.26	.25	.25	.25	.4
STEM CALL.		.7	.71	.69	.7	.5	.68	.68	.68	.68	0.0	.65	.66	.65	.65	0.0
STEM	7	.19	.21	.2	.2	.5	.19	.18	.18	.18	.4	.20	.20	.20	.2	0.0
STEM CALL.		.25	.21	.23	.23	1	.21	.22	.18	.2	1	.23	.22	.21	.22	.9
STEM	8	.11	.09	.1	.1	.5	.08	.09	.1	.08	.9	.09	.07	.08	.08	.9
STEM CALL.		.19	.17	.18	.18	.9	.15	.16	.15	.15	.4	.11	.09	.1	.1	.5

TABLE – 3 : pH optimum of peroxidases of different verities of Cicer arietinum stem and stem Callus.
Note : pH optimum of stem at 4 and stem Callus at 6.

IV. DISCUSSION

The protocol of seed sterilization is one of the important aspects for regeneration and growth. Sodium hypochlorite used as a surface sterilization agent, which played significant role in callus formation and regeneration from different explants. (Chaudhary et al 2007).

Callus was obtained from different explants of different varieties of Cicer arietinum on B_5 and MS medium containing different concentration and types of auxins and cytokinin.

Growth regulators were standardized for induction of callus from suitable explants. It was revealed that the synthetic auxin 2,4-D at the concentration of single supplemented individually and 2,4-D in combination with BAP enhanced the production rate of callus of Cicer arietinum varieties. Non photosynthetic nature of callus was obtained in most of the culture especially in response of 2,4-D whereas photosynthetic nature of callus appeared when BAP was included along with 2,4-D in the medium.

Seedlings of Cicer arietinum to form shoots from cotyledonary node on hormone free MS as well as B_5 medium. A variation was however seen in response from within the same genus. Cicer Pusa 240 variety was most responsive.

The optimum pH for peroxidase enzymes were investigated in different varieties of Cicer arietinum and it was concluded that optimum pH differences was variable in case of peroxidase enzymize pH is not fixed.

The high level of peroxidase activity in leaf parts of cicer arietinum when compared to the leaf of callus was evidenced after electrophoresis profiles. It is presumed that decrease in peroxidase activity in leaf callus may be due to some inhibitors present in the medium or it might have been formed during course of interaction among different chemicals.

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