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PLANTLET REGENERATION FROM HYPOCOTYL AND COTYLEDON EXPLANTS OF CLUSTER BEAN *(CYAMOPSIS TETRAGONOLOBA L)*

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Callus cultures of *Cyamopsis tetragonoloba* were established using seedling explants on MS medium supplemented with different auxins alone or in combination with cytokinin at various levels.Regeneration of shoots/and whole plants with multiple shoots, were obtained from cotyledon and hypocotyl segments on various combinations of plant growth regulators. Such *in vitro* regenerated plants are being tested for survival upon transfer to soil.

Keywords: Cluster bean; *Cyamopsis tetragonoloba;* Tissue culture.

Introduction

Grain legumes (sub family: Papilionoideae; Family: Leguminosae) constitute an important part of human and animal diet.[1,2] Plant cell, tissue and organ culture offers the possibility of their production and improvement. Successful application of these novel techniques in crop improvement largely depends on their ability to regenerate *plants in vitro*[3] But grain legumes have shown low competence for plantlet formation in vitro[4,5]. This report describes a successful and efficient plantlet regeneration schedule in Cluster bean or Guar (*Cyamopsis tetragonoloba L.).*

Material and Methods

The seeds of *C.tetragonoloba* var.Pusa navbahar were initially treated with 0.1% teepol (10 min) and dettol (10 min) with a water wash in between, then with 70% ethanol (3 min) and were finally surface sterilized with 0.1% $HgCl_2$ (3-5 min). They were then thoroughly washed with sterile distilled water (3-5 times) and finally kept for germination either in sterile petriplates having moist sterile filter paper or in erlenmeyer flasks (250 ml) containing moist sterile cotton. Four to six day old seedlings were used to provide the explants i.e. hypocotyl (1cm) and cotyledon (0.5 x 1 cm^2). Explants were aseptically inoculated onto Murashige and skoog's medium[6] containing various concentrations (0.1, 1, 5 and 10 mg/l) of auxins viz. Indole 3-acetic acid (IAA), Indole 3-butyric acid (IBA) and 2, 4-dichlorophenoxy acetic acid (2,4-D) alone as well as in combination with cytokinin viz.6-benzylamino purine (BAP). Cultures were incubated at 25±2°C temperature, 60-70% RH and 1000-1500 lux irradiance. Parallel control experiments (i.e. basal medium without any growth regulator) were set up for each treatment.

Results and Discussion

Callusing : Neither of the explants inoculated on BM initiated any *in vitro* response.

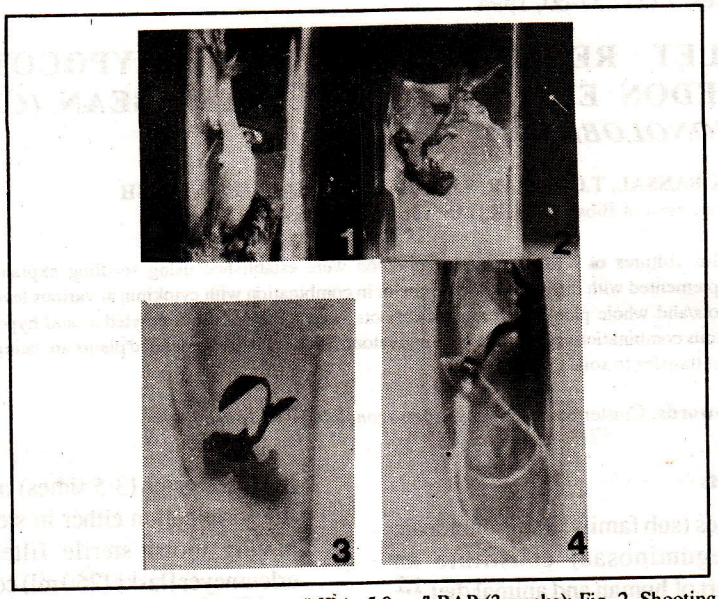

Fig. 1. Shoot budding from hypocotyl on 0.1 mg/l IBA+5.0mg/l BAP (3 weeks); Fig. 2. Shooting from cotyledon (IAA 0.1 mg/l, 3 weeks); Fig. 3. Plantlet formation from cotyledon (IAA 5.0 mg/l, 12 weeks); Fig. 4. Polar regeneration from hypocotyl (IBA 10.0 mg/l, 7 weeks).

Among the three auxins used 2,4-D turned out to be the most effective for callusing from cotyledon at high (5 and 10 mg/l) concentrations. Similar concentrations of IAA ans IBA proved less effective but their low (0.1 mg/l) concentrations supported callusing. This was attributed to more rapid absorption and metabolic inactivation of IAA and IBA in growing tissue.[7] Similarly in combined treatments no significant callus initiation was recorded in IBA + BAP combinations in both hypocotyl and cotyledon explants. Only 2,4-D exhibited a tendency for callusing at its high (10mg/l) concentrations in conjunction with both low (0.1 mg/l) as well as high (10 mg/l) concentrations of BAP.

Shoot differentiation: Despite the presence of good callus growth, in no case calli exhibited, any tendency for organogenesis from any explant in any hormone concentration or combination suggesting the differential expression of the genome in various tissues of a plant. Cotyledon was found to be suitable for direct shoot differentiation on IAA supplemented media 4 weeks after its original placement on the culture medium. Shoots differentiated in the form of light green shoot buds (Figs. 1 and 2) leading to the plantlet formation (Fig.3). However, this explant failed to differentiate shoots with any concentration of IBA and 2, 4-D.

Hypocotyl explant, on the contrary, proved better for direct shoot morphogenesis with IAA (0.1 mg/l, 2,4-D (0.1 mg/l and IBA (5, 10 mg/l) (Table 1). The differentiation of shoots on auxin rich

Table 1. *Explants of Cyamopsis tetragonoloba* showing effective PGR requirements for shooting and/or plantlet regeneration.

Sr. No.	Growth Media mg/l	Explant	Shoot differentiation	Plantlet Regeneration
1.	Basal MS	H	-	-
		C	-	-
2.	MS + IAA 0.1	H	13.6	-
		C	30.0	-
3.	IAA 5.0	C	6.6	6.6
4.	IBA 5.0	C	12.5	8.0
5.	IBA 10.0	H	12.5	-
6.	2, 4-D 0.1	H	3.0	-
7.	IBA+BAP 0.1+1.0	H	15.0	-
8.	0.1 + 5.0	H	8.0	-
9.	0.1 + 10.0	H	18.0	-
10	1.0 + 1.0	H	5.0	-
11.	1.0 + 5.0	H	10.0	-
12.	1.0 + 10.0	H	15.0	-
13.	5.0 + 0.1	H	5.0	-
14.	5.0 + 1.0	H	10.0	5.0
15.	5.0 + 5.0	H	15.0	15.0
16.	5.0 + 10.0	H	10.0	5.0
17.	10.0 + 1.0	H	5.0	-
18.	10.0 + 5.0	H	10.0	-
19.	10.0 + 10.0	H	10.0	-
20.	2, 4-D+BAP 1.0 + 1.0	H	9.8	3.5
21.	1.0 + 5.0	H	28.0	16.0
22.	1.0 + 10.0	H	3.5	1.5
23.	5.0 + 0.1	H	12.7	10.8
24.	5.0 + 1.0	H	2.3	-
25.	5.0 + 5.0	H	13.7	10.0
26.	5.0 + 10.0	H	7.1	1.9

H-Hypocotyl, C-Cotyledon.

medium in the present study is in sharp contrast to most earlier reports where a cytokinin was found essential for the differentiation of shoot buds.[8] In some cases shoots and roots emerged from the opposite poles (Fig.4) as reported in *Acacia nilotica*.[9]

For complete plantlet formation IBA (5, 10 mg/l) proved best. Multiple shoot differentiation also occurred from this explant on a combination of different concentrations of IBA with BAP and different concentrations of BAP with moderate-high (1, 5 mg/l) concentrations of

2,4-D. Most of the combinations of auxin and cytokinin evoked a growth response indentical to that observed with auxin alone. Higher suitability of hypocotyl for shoot regeneration in this study is supported by similar findings in other leguminous species[10,11]. The requirement of different levels of auxin alone or auxin and cytokinin combination to obtain regeneration is probably due to the differential endogenous levels of plant growth regulators[12].

Rooting: Rooting of the *in vitro* differentiated shoots occurred simultaneously with shooting in the shoot induction medium (2,4-D + BAP) and no special medium was required in most cases for rhizogenesis. Rooting, however easily occurred on media containing high concentrations (5-10 mg/l) of IBA and BAP. This indicates that IBA at high levels is very favourable for root formation in regenerated shoots. Such suitability of higher concentrations of IBA for root formation has been reported in *Prosopis cineraria.*[13]

The results reported here highlight the morphogenic potential of somatic tissue of *C.tetragonoloba*. This report also supports the observation that the role of 2,4-D is somewhat intriguing. It has been considered to be a strong auxin[14] metabolized more slowly in the tissue and, therefore, more active but generally antagonistic to shoot differentiation.[15,16]

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