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In vitro callus formation in cultivated and wild species of Cyamopsis

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2,4-Dichlorophenoxy acetic acid (2, 4-D) and benzylaminopurine (BAP) induced callusing from cotyledons in all three species of *Cyamopsis.* The maximum callus induction from cotyledon explant was evident in *Cyamopsis serrata* and *Cyamopsis senegalensis* on a medium supplemented with 2,4-D (2 mg/l). On the other hand, *Cyamopsis tetragonoloba* showed poor callus formation on the same medium. The callus however, proliferated well on Murashige and Skoog (MS) medium adjuncted with naphthalene acetic acid (NAA) (2 mg/l) + BAP (2 mg/l). Hypocotyl of all the tested species of *Cyamopsis* showed very good callus induction response in the medium supplemented with 2 mg/l 2,4-D. As the concentration of BAP increased from (1 mg/l) to (2 mg/l) in combination with NAA (2 mg/l) callus formation was also increased. From cotyledonary node explant, when NAA (2 mg/l) is combined with BAP (1 mg/l), then good callusing was observed in *C. serrata* whereas no callusing was found in other species. 2,4-D induce callusing in all the three species of *cyamopsis* at (2mg/l) concentration and both the wild species have more callus formation then cultivated species. In *C. serrate*, good callusing was observed at BAP (1 mg/l) from immature embryo explant. When the concentration of NAA is increased to 1 mg/l and concentration of BAP is decreased with 0.5 mg/l, response was decreased in wild species of *C. senegalensis* whereas no change in response was found in the other two species.

Key words: Callus induction, cluster bean (*Cyamopsis tetragonoloba*), *Cyamopsis serrata*, *Cyamopsis senegalensis*.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba* (L.) Taub. syn. *C. psoraliodes* (lamk; D.C. 2n = 2x = 14) (family leguminaceae), is one of the most important kharif legume crop and is well adapted to arid and semi-arid regions of the world. India accounts for 80% of the total guar produced in the world enabling its export to more than 65 countries recording export turnover of ₹ 1126 crore during 2006-2007 (Pahuja et al., 2009). Cluster bean is also an important crop of South-western Haryana, which is second largest producer having area, production and productivity of 3.0 lakh ha, 3.6 lakh tons and 1200 kg ha-1, respectively in 2010-2011 (Anonymous, 2010). Rajasthan accounts for about 75% of area and 55% of total production in the country. There are possibilities for further increasing the production of this crop by improving the productivity and quality. The crop is mainly grown in the dry habitat of Rajasthan, Haryana, Gujarat and Punjab and to limited extent in Uttar Pradesh (UP) and Madhya Pradesh (MP), India. India has the largest area under guar cultivation in the world, 75% of the guar gums or their derivatives produced in India are exported mainly to USA and European countries enabling its export to 65

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Abbreviations: NAA, Naphthalene acetic acid; **2, 4-D,** 2, 4- dichlorophenoxy acetic acid; **BAP,** benzylaminopurine.

countries. Considering guar seed and guar gum's domestic and export demand, it is necessary to increase guar production and productivity.

A critical requirement for crop improvement in general, is the introduction of new genetic material in the cultivated lines of interest, whether through conventional or non-conventional breeding or plant tissue culture technologies. Interspecific hybridization among the *C. tetragonoloba* and its wild relatives is anticipated to produce hybrid with trait of early maturity. Presently, the cultivated species of guar is late maturing crop which needs 80-120 days for its maturation whereas the wild species needs only 40-50 days for its maturation. Unfortunately, conventional plant breeding technique has so far failed to yield desired result. Such a failure may be due to the presence of pre-and/or post-fertilization barriers. Supplementing conventional plant breeding with unconventional less popular methods along with plant biotechnological techniques is anticipated to go headway in resolving the issue.

MATERIALS AND METHODS

With the objective to optimize medium recipe and cultural conditions for plant regeneration in *Cyamopsis* species *viz. C. tetragonoloba* cv. HG 563, *Cyamopsis serrata* and *Cyamopsis senegalensis*, seeds of three different species of *Cyamopsis viz. C. tetragonoloba* HG 563, *C. serrata* and *C. senegalensis* were washed thoroughly with tap water containing a drop of teepol for 5 to 10 min. Subsequently, the seeds were surface sterilized with 70% alcohol for 1 min and then with 0.1% mercuric chloride solution for 5 min. The seeds were then washed thoroughly three to four times in sterile distilled water on the hood of laminar flow to remove all traces of mercury. These sterilized seeds were germinated on germination medium containing 3% sucrose and 8% agar under asceptic conditions initially under dark condition until germination and then shifted to light conditions. Different explants viz. cotyledon, coteledonary node and hypocotyl measuring 4 to 5 mm obtained from asceptically grown seedlings were inoculated on the surface of different culture medium. Murashige and Skoog (MS) salts supplemented with B_5 vitamins was used as basal medium and fortified with different concentrations of growth regulators. Embryo explants were excised from surface sterilized 10 day old green pods taken from net house and three explants per flask were cultured. Inoculated flasks were kept in culture room at 25±1°C temperature, under photoperiod of 16 h light and 8 h darkness.

RESULTS AND DISCUSSION

In the present study, callus induction was tried from several explants in *Cyamopsis* species viz. *C. tetragonoloba* cv.HG563, *C. serrata* and *C. senegalensis*. Seedling explants like cotyledon, hypocotyls, cotyledonary node taken from 7-10 day old aseptically grown seedlings and immature embryos (10 to 12 days after pollination) were cultured on MS medium with vitamin B_5 and fortified with different concentrations of growth regulators, that is, naphthalene acetic acid (NAA), 2, 4- dichlorophenoxy acetic acid (2, 4-D) and benzylaminopurine (BAP) alone and in combinations. The genotype and the source of the explants played an important role in deciding callus induction frequency. 2, 4-D was observed to be the most efficient source of auxin for callus induction. Several reports for callus induction in legumes using 2, 4-D as an auxin source have also been proposed in which 2,4-D (1-2 mg/l) concentration was the most suitable for callus induction (Kiran et al.,2005; Kaviraj et al.,2006; Kumari et al., 2006).

Cotyledon explant

The morphogenic response of cotyledon explants of the three species of *Cyamopsis* is shown in Table 1. NAA (1 mg/l) failed to induce any morphogenic response from cotyledons while its higher dose (2 mg/l) induced rooting directly from the explant which was coupled with callus formation in *C. serrata* and *C. senegalensis*. 2, 4-D and BAP on the otherhand, induced callusing from cotyledon in all three species of *Cyamopsis* while NAA (1 and 2 mg/l) in combination with BAP (0.5-1 mg/l) induced only swelling of explant uncoupled with callusing. The maximum callus induction was evident in *C. serrata* and *C. senegalensis* on a medium supplemented with 2, 4-D (2 mg/l) and the callus was yellowish white in color. On the other hand, *C. tetragonoloba* showed very less callus formation on the same medium and the callus was brown in color. The callus however, proliferated well on MS medium adjuncted with NAA (2 mg/l) + BAP (2 mg/l) and it was whitish brown in color. Addition of 0.5 mg/l BAP to 2,4-D supplemented medium yielded green fragile callus in wild species. It shows that BAP acts as a trigger at the level chloroplast functioning which subsequently lead to production of green photosynthetic callus cells (Polanco and Ruiz, 1997). So, this activation of choloplast or rather chlorophyll may be considered as a preliminary response towards differentiation. According to Mathiyazhagan (2007) and Mathiyazhagan et al. (2013), the best response for callus induction was observed on CI_6 (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium for all guar cultivars except guar cultivar HG 563 and in case of wild species, the best response was observed on CI_5 (2,4-D 3 mg/l + BAP 3 mg/l) medium. The genotypic differences observed in callus initiation response of various guar genotypes at different media compositions indicate clearly that callus induction being highly variable genetically controlled traits and these results are not surprising since significance of genotype in determining the probability of *in vitro* culture response has been documented in several legumes such as pigeon pea (Surekha and Arundhati, 2007; Reichert et al., 2003; Van et al., 2002). Singh (2008) did not observed any shoot regeneration in cotyledon explants in *C. tetragonoloba* and *C. serrata.*

Hypocotyl explant

Hypocotyl of all the tested species of *Cyamopsis* showed very good callus induction response in the medium

Table 1. Morphogenic responses of cotyledons taken from 7-10 day old aseptically grown seedlings of three species of *Cyamopsis* to plant growth regulators.

Adjuvants to MS Medium	Morphogenic response		
	C.tetragonoloba	***C.serrata***	***C.senegalensis***
MS basal medium	No response	No response	No response
NAA (1 mg/l)	No response	No response	No response
NAA (2 mg/l)	Adventious root formation from explant	Callusing + adventious root formation from explant	Callusing + adventious root formation from explant
2,4D (1 mg/l)	Adventious root formation from explant	+	Swelling of explant
2,4D (2 mg/l)	+	+++	+++
BAP (0.5 mg/l)	Swelling of explant	+	+
BAP (1 mg/l)	+	Swelling of explant	+
BAP (2 mg/l)	+	+	++
2,4D(2 mg/l) + BAP (0.5 mg/l)	+	+	++
2,4D (2 mg/l) + BAP (1 mg/l)	+	+	+
NAA(1 mg/l)+ BAP (1 mg/l)	Swelling of explant	Swelling of explant	Swelling of explant
NAA (2 mg/l) + BAP (0.5 mg/l)	Swelling of explant	Swelling of explant	Swelling of explant
NAA (2 mg/l) + BAP (1 mg/l)	+	++	++
NAA(2 mg/l) + BAP (2 mg/l)	++	+++	+++

-, No callusing; +, low amount of callus formation; ++, medium amount of callus formation; +++, good amount of callus formation.

supplemented with 2 mg/l 2, 4-D (Table 2). BAP induced only little brown callusing from the base of the explant which did not proliferate further in *C. tetragonoloba* whereas no callus formation was found in both the wild species at the same concentration of the medium. Combination of 2,4-D and NAA with BAP did not induce any significant response in *C. tetragonoloba* but induced healthy callusing in *C. serrata* and *C. senegalensis* except NAA(1 mg/l) + BAP (1 mg/l) which induced no response in both the wild species. As the concentration of BAP increased from (1 mg/l) to (2 mg/l) in combination with NAA (2 mg/l) callus formation was also increased. According to Mathiyazhagan (2007) and Mathiyazhagan et al. (2013) among the media tried, the best responses for callus induction were observed on CI_6 (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium for all guar cultivars except FS 277 and in wild species, best response was observed on CI_5 (2,4-D 3 mg/l + BAP 3 mg/l) medium whereas, Singh (2008) observed that direct shoot regeneration from hypocotyls explants of *C. serreta* was observed on MS medium supplemented with BAP (2 mg/l).

Cotyledonary node explant

Cotyledonary node of *C. senegalensis* showed medium quantity of callus formation in a medium having NAA. When NAA (2 mg/l) is combined with BAP (1 mg/l), healthy callusing was observed in *C. serrata* whereas no callusing was found in other species. 2,4-D induce callusing in all the three species of *Cyamopsis* at (2 mg/l) concentration and both the wild species hadmore callus formation than cultivated species. The callus was yellowish green in *C. serrata* and *C. senegalensis* whereas it was brownish in *C. tetragonoloba*. When 2,4-D (2 mg/l) was combined with BAP (1 mg/l), response of callus formation was dropped in both the wild species whereas no change was observed in *C. tetragonoloba.* In *C. serrata,* little amount of callus formation was also found at 2,4-D (2mg/l) with BAP (0.5 mg/l) (Table 3). However, Singh (2008) observed that for shoot regeneration from cotyledenory nodes of guar, the best response was observed on MS medium supplemented with 1.5 mg/l BAP and maximum number of shoots per explant was observed on MS medium containing 1 mg/l TDZ.

Immature embryo explant

NAA (1 mg/l) supported callusing of immature embryos in all the three species. *C. tetragonoloba* and *C. serrata* showed little amount of callusing at 1 mg/l and 2 mg/l 2,4-D and no response was found in *C. senegalensis* at the same medium. In *C. serrate*, good amount of callus was observed at BAP (1 mg/l) and little response was observed in case of *C. senegalensis* whereas the cultivated species does not show any callus formation. When BAP is combined with NAA, callusing was observed in all the tested species. At NAA (0.5 mg/l) with BAP (1 mg/l) medium quantity of callus was found in *C. tetragonoloba*

Table 2. Morphogenic responses of hypocotyls taken from 7-10 day old aseptically grown seedlings of three species of *Cyamopsis* to plant growth regulators.

Adjuvants to MS medium	Morphogenic response		
	C. tetragonoloba	***C. serrata***	***C. senegalenisis***
MS basal medium	No response	No response	No response
NAA (1 mg/l)	Callus + adventious root formation from explant	+	+
NAA (2 mg/l)	Callus + adventious root formation from explant	+	+
2,4D (1 mg/l)	+	+	+
2,4D (2 mg/l)	+	+++	+++
BAP (0.5 mg/l)	+	-	-
BAP (1 mg/l)	+	-	-
BAP (2 mg/l)	+	-	-
2,4D (2 mg/l) + BAP (0.5 mg/l)	No response	+	++
2,4D(2 mg/l) + BAP (1 mg/l)	+	+	++
NAA(1 mg/l)+ BAP (1 mg/l)	Browning of explant	-	+
NAA(2 mg/l) + BAP (0.5 mg/l)	No response	No response	No response
NAA (2 mg/l)+ BAP (1 mg/l)	No response	++	++
NAA(2 mg/l) + BAP (2 mg/l)			

-, No callusing; +, low amount of callus formation; ++, medium amount of callus formation; +++, good amount of callus formation.

and *C. serrate*, whereas *C. senegalensis* showed good quantity of callusing at the same medium. When the concentration of NAA was increased to 1 mg/l and concentration of BAP was decreased, 0.5 mg/l response was decreased in wild species of *C.senegalensis* whereas no change in response was found in other two species. In *C. serrate*, adventitious roots were formed from the explant along with little callusing in NAA (2 mg/l) + BAP (0.5 mg/l) whereas other species showed little callus formation only (Table 4).

According to Mathiyazhagan (2007), the best responses for callus induction from immature embryo were observed on CI_6 (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium and callus induction was also found to be good on CI_4 (2,4-D 2 mg/l + BAP 2 mg/l) medium. But in case of wild species, the per cent callus induction increased with increase in the concentration of both the growth regulator that is, 2,4-D and BAP. Callus induction at media supplemented with 2,4-D alone occurred from the radical end of the embryos within 5-7 days of incubation, the plumule end, however failed to show any callusing even after prolonged incubation of 20-30 days. The callus induced on this media was white and friable, but could not be maintain for sub culture due to excessive browning. Prem (2005) reported that on MS culture medium containing 2,4-dichlorophenoxyacetic acid (10.0µM) in combination with 6-benzylaminopurine (5.0µM) with embryo explants is most suitable for induction of green and friable morphogenic callus. However, Singh (2008) observed somatic embryogenesis in *C. serrata* hypocotyls explants in MS medium containing TDZ (0.5 mg/l). He also observed direct shoot morphogenesis in *C. tetragonoloba* (HG 563) from immature embryo axis explants upon culturing on MS medium supplemented with BAP and TDZ and NAA combinations. Rooting was also observed in surgically separated regenerated shoots from immature embryo axis and somatic embryos on half strength MS medium containing IBA (1.0 mg/l) + GA3 (0.1 mg/l). Whereas with the view to obtain extra early maturing hybrids, Somvir (2013) further attempted crosses between *C. tetragonoloba* (Var. HG 563) and *C. serrata* and reported best rooting response of hybrids on half strength MS medium supplemented with IBA (2.0 mg/l) + GA3 (0.5 mg/l).

Conclusions

In the present study, maximum callus induction from cotyledon explant was evident in *C. serrata* and *C. senegalensis* on MS medium with B_5 vitamins supplemented with 2, 4-D (2 mg/l). Callus however, proliferated well on MS medium adjunct with 2 mg/l each of NAA + BAP in *C. tetragonoloba*. Hypocotyl explants of all the tested species of *Cyamopsis* showed very good amount of callus induction response in media supplemented with 2 mg/l 2, 4-D. Hypocotyl explants on NAA (2 mg/l) with BAP(1 mg/l) and NAA (2 mg/l) with BAP (2 mg/l) showed nice callusing in both wild species. NAA induced only callusing from cotyledonary nodes in *C. senegalensis.* When NAA (2 mg/l) was combined with BAP (1 mg/l) good quantity of callus was observed in *C. serrata.* 2,4-D (2 mg/l) induced callus from cotyledonary node explant in all three species tested. From immature embryo in *C. serrate*, good quantity of callusing was observed with

Table 3. Morphogenic responses of cotyledonary nodes taken from 7-10 day old aseptically grown seedlings of three species of *Cyamopsis* to plant growth regulators.

Adjuvants to MS medium	Morphogenic response		
	C. tetragonoloba	***C. serrata***	***C. senegalensis***
MS basal medium	No response	No response	No response
NAA (1 mg/l)	-	-	++
NAA (2 mg/l)	-	-	++
2,4D (1 mg/l)	Browning of explant	-	-
2,4D (2 mg/l)	+	++	++
BAP (0.5 mg/l)	-	-	-
BAP (1 mg/l)	-	-	-
BAP (2 mg/l)	-	-	-
2,4D(2 mg/l) + BAP (0.5mg/l)	-	+	-
2,4D (2 mg/l) + BAP (1 mg/l)	+	+	+
NAA(1 mg/l)+ BAP (1 mg/l)	Swelling of explant	-	-
NAA (2 mg/l) + BAP (0.5 mg/l)	-	-	-
NAA (2 mg/l) + BAP (1 mg/l)	-	+++	-
NAA(2 mg/l) + BAP (2 mg/l)	-	-	-

-, No callusing; +, low amount of callus formation; ++, medium amount of callus formation; +++, good amount of callus formation.

Table 4. Morphogenic responses of immature embryos excised 10-12 days after anthesis (DAA) in three species of *Cyamopsis* to plant growth regulators and other adjuvants.

Adjuvants to MS medium	Morphogenic response		
	C.tetragonoloba	***C.serrata***	***C.senegalensis***
MS basal medium	No response	No response	No response
NAA (0.5 mg/l)	Swelling of explant	-	+
NAA (1 mg/l)	+	+	+
NAA (2 mg/l)	No response	Swelling of cotyledons	+
2,4D (0.5 mg/l)	-	-	No response
2,4D (1 mg/l)	+	+	No response
2,4D (2 mg/l)	+	+	No response
BAP (0.5 mg/l)	No response	Root formation	No response
BAP (1 mg/l)	-	+++	+
BAP (2 mg/l)	-	-	No response
NAA (0.5 mg/l) + BAP (1 mg/l)	++	++	+++
NAA (0.5 mg/l) + BAP (2 mg/l)	No response	No response	-
NAA (1 mg/l) + BAP (0.5mg/l)	++	++	+
NAA (1 mg/l) + BAP (2 mg/l)	++	No response	+
NAA (2 mg/l) + BAP (0.5 mg/l)	+	Callusing + adventious root formation from explant	+
NAA (2 mg/l) + BAP (1 mg/l)	No response	+	No response
CH (500mg/l)	+	swelling of explant	swelling of explant
NAA (0.2 mg/l) + BAP (0.2 mg/l) + CH (500 mg/l)	+	-	No response
NAA (0.2 mg/l) + BAP (0.001 mg/l) + CH (500 mg/l)	No response	-	No response

-, No callusing; +, low amount of callus formation; ++, medium amount of callus formation; +++, good amount of callus formation.

BAP (1 mg/l) and little response was observed in case of *C. senegalensis,* whereas the cultivated species did not show any callus formation. At NAA (0.5 mg/L) with BAP (1 mg/L) medium *C. senegalensis* shows good quantity of

callusing from immature embryo.

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