In vitro Regeneration of Wild species of Guar
(Cyamopsis serrata and Cyamopsis senegalensis)

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ABSTRACT
Wild relatives of Cyamopsis i.e. C. serrata is an early maturing (40-50 days), slow growing and branched species, while the other species i.e. C. senegalensis is a slow growing annual herb and matures in 120-130 days. Both these wild relatives possess some desirable attributes like drought resistance, photo-and thermo-insensitivity and disease resistance.

Seedling explants like cotyledon, cotyledonary node and hypocotyl taken from 7-10 d old aseptically grown seedlings and immature embryos (10-12 d after pollination) were cultured on MS medium with B5 vitamins and fortified with different concentrations of growth regulators. Maximum callus induction from cotyledon explant was evident in C. serrata and C. senegalensis on MS medium with B5 vitamins and supplemented 2, 4-D (2mg/l). Hypocotyl explants of both the tested species of Cyamopsis showed very good callus induction response in media supplemented with 2, 4-D @2mg/l. BAP at concentrations of 0.5 and 2.0 mg/l supported indirect multiple shoot regeneration via callusing in C. senegalensis whereas 1 mg/l BAP supported direct multiple shoot regeneration. On the other hand, BAP induced differentiation of multiple shoots in C. serrata and the number of shoots per hypocotyl explant increased with the increasing concentration of BAP. Indirect shoot regeneration from hypocotyl explants was evident in C. serrata at MS medium supplemented with 1mg/l each of NAA and BAP, whereas NAA (2mg/l) with BAP(1mg/l) and NAA(2mg/l) with BAP(2mg/l) showed callusing in both the wild species. Cotyledonary node explant was the most responsive explant for plant regeneration in both the wild species of Cyamopsis under investigation. Indirect shoot regeneration in C. serrata was observed in MS medium supplemented with NAA alone and response increased at 2mg/l. NAA induced only callusing from cotyledonary nodes in C. senegalensis. 2,4-D (1mg/l) induced callusing and indirect shoot regeneration in C. serrata and C. senegalensis while its higher dose (2mg/l) induced callusing only from cotyledonary node explant in both the species tested. Supplementation of MS medium with BAP alone lead to indirect shoot regeneration via callusing and its frequency decreased with increasing its concentration for 0.5 mg/l to 2.0 mg/l. Interestingly, 2mg/l BAP supported multiple shoot formation from cotyledonary explants in C. serrata. Supplementation of 1.0 mg/l BAP to the medium containing 2.0 mg/l NAA lead to indirect shoot regeneration in C. senegalensis while it induced only callusing in C. serrata. The frequency of shoot regeneration however, decreased with the increase in concentration of BAP to 2mg/l. Half strength of MS with 0.1 mg/l and 0.5 mg/l IBA supported best rooting in C. senegalensis and C. serrata, respectively and plantlets were successfully transferred to paper cups. 

Key words: Callus induction, Cluster bean (Cyamopsis tetragonoloba), Differentiation, Regeneration.

INTRODUCTION
Guar (Cyamopsis tetragonoloba (L.) Taub.) is one of the most important kharif legume and is well adapted to arid and semi-arid regions of the world. However, one of its wild relatives i.e. C. serrata is an early maturing (40-50 days), slow growing and branched species, while the other species i.e. C. senegalensis is also slow growing annual herb and matures in 120-130 days. Both these wild relatives possess some desirable attributes like drought resistance (Menon, 1973), photo-and thermo-insensitivity and disease resistance.

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resistance (Orellana, 1966). The crop is mainly grown in the dry habitat of Rajasthan, Haryana, Gujarat and Punjab and to limited extent in U.P. and M.P. Guar is important source of guar gum (guar galactomanans) which is used as viscosity enhancer for both food and non-food purposes. Galactomanans are the major storage food reserve of endosperm of guar seeds and endosperm constitutes about 30-35 per cent of the whole seed.

The application of tissue culture technology as a central tool or an adjunct to other methods is at the vanguard in plant modification and improvement of agriculture (Brown et al., 1995). Efficient regeneration protocol is a pre requisite for use of tissue culture technology and is lacking in legumes. Legumes, in general, are considered as recalcitrant (Kaviraj et al., 2006; Chakarbarti et al., 2006). Very few studies have been conducted on tissue culture in guar till date.

MATERIALS AND METHODS
The present plant tissue culture investigations were aimed to optimize medium recipe and cultural conditions for plant regeneration in wild Cyamopsis species viz. C. serrata and C. senegalensis. Seedling explants like cotyledon, cotyledonal node and hypocotyl taken from 7-10 d old aseptically grown seedlings and immature embryos (10-12 d after pollination) were cultured on MS medium with B5 vitamins and fortified with different concentrations of growth regulators i.e. naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy acetic acid (2, 4-D) and 6-benzyl aminopurine (BAP) alone and in combinations.

Seeds of C. serrata and C. senegalensis were washed thoroughly with tap water containing a drop of teepol for 5-10 minutes. Subsequently the seeds were surface sterilized with 70 per cent alcohol for 1 min and then with 0.1 per cent 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 per cent sucrose, 8 per cent agar under aseptic conditions initially under dark condition until germination and then shifted to light conditions. Different explants measuring 4-5mm obtained from aseptically grown seedlings were inoculated on the surface of culture medium. 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 16h light and 8h darkness.

RESULTS AND DISCUSSION
Cotyledon explants:
It was observed that NAA (1mg/l) failed to induce any morphogenic response from cotyledons while its higher dose (2mg/l) induced rooting directly from the explant which was coupled with callus formation in C. serrata and C. senegalensis (Table 1). 2, 4-D and BAP on the other hand, induced callusing from cotyledons in both the species of Cyamopsis (Fig1) 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 (2mg/l) and the callus was yellowish white in color. Regeneration of shoots however, could not be achieved from callus on any of the medium tried from cotyledonal explants.

Hypocotyl explants:
Hypocotyl of all the tested species of Cyamopsis showed very good callus induction response in the medium supplemented with 2, 4-D (Table 2). BAP at concentration of 0.5 and 2.0 mg/l supported indirect multiple shoot regeneration via callusing in C. senegalensis whereas 1 mg/l BAP supported direct multiple shoot regeneration. On the other hand, BAP induced differentiation of multiple shoot in C. serrata and the number of shoots increased with the increasing concentration of BAP. Combination of 2,4-D and NAA with BAP induced good callusing in C. serrata and C. senegalensis except NAA(1mg/l) + BAP (1mg/l) which induced shoot regeneration from hypocotyls of C. serrata. Cheema and Bawa (1991) reported de novo formation of shoot buds from hypocotyl explants of pigeonpea on the medium containing BAP and IAA.
### Table 1. Morphogenic response of cotyledons taken from 7-10 day old aseptically grown seedlings of wild species of *Cyamopsis* to plant growth regulators.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Adjuvants to MS Medium</th>
<th>Morphogenic Response</th>
<th>C. serrata</th>
<th>C. senegalensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS basal medium</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>2</td>
<td>NAA (1 mg/l)</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>3</td>
<td>NAA (2 mg/l)</td>
<td>Callusing + adventitious root formation from explants</td>
<td>Callusing + adventitious root formation from explants</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2,4D (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Swelling of explants</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,4D (2 mg/l)</td>
<td>Callusing (+++)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BAP (0.5 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>BAP (1 mg/l)</td>
<td>Swelling of explants</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BAP (2 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2,4D (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2,4D (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>NAA (1 mg/l) + BAP (1 mg/l)</td>
<td>Swelling of explants</td>
<td>Swelling of explants</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NAA (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Swelling of explants</td>
<td>Swelling of explants</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>NAA (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>NAA (2 mg/l) + BAP (2 mg/l)</td>
<td>Callusing (+++)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
</tbody>
</table>

+ = Low(10-30% Callusing), ++ = Medium(40-60% Callusing), +++ = Good (70-90% Callusing)

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Adjuvants to MS Medium</th>
<th>Morphogenic Response</th>
<th>C. serrata</th>
<th>C. senegalensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS basal medium</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>2</td>
<td>NAA (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NAA (2 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2,4D (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,4D (2 mg/l)</td>
<td>Callusing (+++)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BAP (0.5 mg/l)</td>
<td>Callusing + multiple shoot formation</td>
<td>Callusing + multiple shoot formation</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>BAP (1 mg/l)</td>
<td>Multiple shoot formation</td>
<td>Multiple shoot formation</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BAP (2 mg/l)</td>
<td>Callusing + multiple shoot formation</td>
<td>Callusing + multiple shoot formation</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2,4D (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2,4D (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>NAA (1 mg/l) + BAP (1 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NAA (2 mg/l) + BAP (0.5 mg/l)</td>
<td>No response</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>NAA (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>NAA (2 mg/l) + BAP (2 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
</tbody>
</table>

+ = Low(10-30% Callusing), ++ = Medium(40-60% Callusing), +++ = Good (70-90% Callusing)

### Cotyledonary node explants:

Indirect shoot regeneration in *C. serrata* was observed in MS medium supplemented with NAA alone and response increased at 2mg/l NAA. NAA, on the other hand, induced only callusing from cotyledonary nodes in *C. senegalensis*. 2, 4-D (1mg/l) induced callusing and indirect shoot regeneration in *C. serrata* and *C. senegalensis* while its higher dose (2mg/l) induced callusing from explant in both species tested (Table 3). The callus was yellowish green in *C. serrata* and *C. senegalensis*. Supplementation of MS medium with BAP alone lead to indirect shoot regeneration via callusing and its frequency decreased with **Journal of Krishi Vigyan**
Table 3. Morphogenic response of cotyledonary nodes taken from 7-10 day old aseptically grown seedlings of wild species of *Cyamopsis* to plant growth regulators.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Adjuvants to MS Medium</th>
<th><em>C. serrata</em> Morphogenic Response</th>
<th><em>C. senegalensis</em> Morphogenic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS basal medium</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>2</td>
<td>NAA (1 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing (++)</td>
</tr>
<tr>
<td>3</td>
<td>NAA (2 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing (++)</td>
</tr>
<tr>
<td>4</td>
<td>2,4D (1 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>5</td>
<td>2,4D (2 mg/l)</td>
<td>Callusing (++)</td>
<td>Callusing (++)</td>
</tr>
<tr>
<td>6</td>
<td>BAP (0.5 mg/l)</td>
<td>Callusing + multiple shoot formation</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>7</td>
<td>BAP (1 mg/l)</td>
<td>Callusing + multiple shoot formation</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>8</td>
<td>BAP (2 mg/l)</td>
<td>Multiple shoot formation</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>9</td>
<td>2,4D (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>10</td>
<td>2,4D (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
</tr>
<tr>
<td>11</td>
<td>NAA (1 mg/l) + BAP (1 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>12</td>
<td>NAA (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>13</td>
<td>NAA (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (++)</td>
<td>Callusing + shoot regeneration</td>
</tr>
<tr>
<td>14</td>
<td>NAA (2 mg/l) + BAP (2 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing + shoot regeneration</td>
</tr>
</tbody>
</table>

+ = Low (10-30% Callusing), ++ = Medium (40-60% Callusing), +++ = Good (70-90% Callusing)

**Fig 1:** *In vitro* response of cotyledons of *Cyamopsis senegalensis* (A) and *C. serrata* (B) to 2,4D and BAP X = 2mg/l 2,4-D + 0.5 mg/l BAP, Y = 2mg/l 2,4-D +1mg/l BAP

**Fig 2:** Morphogenic response of cotyledonary nodes of *C. serrata* (A) and *Cyamopsis senegalensis* (B) to different concentrations of BAP (X=0.5mg/l, Y=1.0mg/l, Z=2mg/l)
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Adjuvants to MS Medium</th>
<th>Morphogenic Response</th>
<th>C. serrata</th>
<th>C. senegalensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS basal medium</td>
<td>No response</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>NAA (0.5 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NAA (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NAA (2 mg/l)</td>
<td>swelling of cotyledons</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,4D (0.5 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2,4D (1 mg/l)</td>
<td>Callusing (+)</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2,4D (2 mg/l)</td>
<td>Callusing (+)</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BAP (0.5 mg/l)</td>
<td>Root formation</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>BAP (1 mg/l)</td>
<td>Callusing (+++)</td>
<td>Callusing</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>BAP (2 mg/l)</td>
<td>Callusing + shoot regeneration</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>NAA (0.5 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+++)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NAA (0.5 mg/l) + BAP (2 mg/l)</td>
<td>No response</td>
<td>Callusing + shoot regeneration</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>NAA (1 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing (+)</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>NAA (1 mg/l) + BAP (2 mg/l)</td>
<td>No response</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>NAA (2 mg/l) + BAP (0.5 mg/l)</td>
<td>Callusing + adventitious root formation from explant</td>
<td>Callusing (+)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>NAA (2 mg/l) + BAP (1 mg/l)</td>
<td>Callusing (+)</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>CH (500 mg/l)</td>
<td>swelling of explant</td>
<td>swelling of explants</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>NAA (0.2 mg/l) + BAP (0.2 mg/l) + CH (500 mg/l)</td>
<td>Direct shoot formation</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>NAA (0.2 mg/l) + BAP (0.001 mg/l) + CH (500 mg/l)</td>
<td>Direct shoot formation + root formation</td>
<td>No response</td>
<td></td>
</tr>
</tbody>
</table>

+= Low (10-30% Callusing), ++ = Medium (40-60% Callusing), +++ = Good (70-90% Callusing)

Table 5. Combination of growth regulators used for rooting and flowering in wild species of *Cyamopsis*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Medium</th>
<th>C. serrata</th>
<th>C. senegalensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>½ MS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>¼ MS</td>
<td>-,- Flowering</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>½ MS + IBA 0.1 mg/l</td>
<td>-</td>
<td>-,- Flowering</td>
</tr>
<tr>
<td>4.</td>
<td>½ MS + IBA 0.5 mg/l</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>½ MS + IBA 1 mg/l</td>
<td>+</td>
<td>+,- Flowering</td>
</tr>
<tr>
<td>6.</td>
<td>½ MS + IBA 1.5 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>½ MS + IAA 1 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>½ MS + NAA 2 mg/l + IAA 1 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>½ MS + NAA 2 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>¼ MS + IBA 0.1 mg/l</td>
<td>-</td>
<td>-,- Flowering</td>
</tr>
<tr>
<td>11.</td>
<td>¼ MS + IBA 0.5 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>¼ MS + IBA 1 mg/l</td>
<td>-</td>
<td>-,- Flowering</td>
</tr>
<tr>
<td>13.</td>
<td>¼ MS + IBA 1.5 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>¼ MS + IAA 1 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15.</td>
<td>¼ MS + NAA 2 mg/l + IAA 1 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16.</td>
<td>¼ MS + NAA 2 mg/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17.</td>
<td>MS + NAA 0.2 mg/l + BAP 0.001 mg/l + 500 mg/l CH</td>
<td>+</td>
<td>-</td>
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-= Nil + = Rooting
increasing its concentration from 0.5 mg/l to 2.0 mg/l (Fig 2). Interestingly 2mg/l BAP support multiple shoot formation from cotyledonary node explants in *C. serrata*.

Supplementation of 1.0 mg/l BAP to the medium containing 2.0 mg/l NAA lead to indirect shoot regeneration in *C. senegalensis* while it induced only callussing in *C. serrata*. The frequency of shoot regeneration however, decreased with the increase in concentration of BAP (2mg/l). Lower concentration of BAP (0.5mg/l) with 2mg/l NAA induced indirect shoot regeneration through callussing in both the species of *Cyamopsis* studied. Shoot regeneration from cotyledonary node has also been reported in other legumes on BAP supplemented medium like *Cajanus cajan* (Prakash et al., 1994), *Phaseolus vulgaris* (McClean and Graftan, 1989 and Mohamed et al., 1992), *V. radiate* (Mathew, 1987 and Gulati and Jaiwal, 1992, 1994) and chickpea (Subhadra et al., 1998). Surekha and Arundhati (2007) also observed regeneration from cotyledonary node of peanut cultured on BAP and in combination with kinetin.

**Immature embryo explants:**

NAA induced callussing in *C. serrata* and *C. senegalensis* except 0.5mg/l NAA which induced indirect shoot regeneration from callus in *C. serrata* (Table 4). Similarly, indirect shoot regeneration was delayed until 60-70 days of inoculation in MS medium supplemented with 1mg/l NAA. On the other hand, indirect regeneration was observed at NAA 0.5 mg/l in *C. senegalensis* and callus formation was found at NAA 1mg/l after 60-70 days of inoculation. Like NAA, 2,4-D also induced callussing in *C. serrata*. Immature embryos of *C. senegalensis* did not respond to 2,4-D. Immature embryos however, responded best at 2.0 mg/l BAP which induced callussing and differentiation of shoot from callus in *C. serrata*. Callus growth of *C. serrata* was highest on MS medium adjunted with 1mg/l BAP (Fig 3).

Culturing of immature embryos 10-12 DAP, yielded callus in both the species of *Cyamopsis* on MS medium adjucted with NAA (0.5 mg/l) + BAP (1 mg/l). Callus showed differentiation of shoot in *C. serrata* in medium supplemented with NAA (0.5 mg/l) + BAP (1 mg/l) after 45-50 DOI. MS medium supplemented with 0.2 mg/l NAA+0.001mg/l BAP+500 mg/l CH showed direct plant regeneration from embryo with very good rooting after 30 days of inoculation in *C. serrata*. Culturing of the nodal and inter-nodal segments of the above regenerated shoots produced multiple shooting after 20-25 days of inoculation on the above described medium. Prem et al. (2005) reported shoot regeneration in guar using embryo as explants via somatic embryogenesis on BAP and NAA supplemented medium.

**Rooting of shoots:**

For rooting of *in vitro* generated shoots, different rooting media were tried. Shoots rooted on a ½ strength MS medium supplemented with 0.5 mg/l and 1.0 mg/l IBA showed rooting in wild species. *C. serrata* also shows good root formation in A6 medium (MS + 0.2 mg/l NAA + 0.001 mg/l BAP + 500 mg/l CH) (Fig 4). Prem et al., (2003) observed rooting response of regenerated shoot of cotyledonary node explants in guar on MS medium supplemented with 4.9 µM IBA.

Flowering was observed from nodal segments in ¼ strength MS medium alone in *C. serrata* while *C. senegalensis* it was evident in both ½ strength and ¼ strength MS medium supplemented with 0.1 mg/l IBA, 1mg/l IBA. *C. senegalensis* flowering was also observed from hypocotyl explants in MS medium supplemented with 2,4-D (2mg/l) and BAP (0.5 mg/l) after longer period of inoculation (explant was shifted to the new medium of same concentration after every 2-3 weeks of inoculation for providing longer period of inoculation to get flowering) (Table 5)(Fig 5).

**Hardening, acclimatization and field transfer:**

The plantlets with sufficient rooting were taken out of the medium and washed properly with tap water. These were then transferred to small cups or pots containing sterilized dune sand and farm yard manure in 3:1 ratio. These were irrigated with ¼ strength MS nutrient solution, covered with polythene bags to maintain high humidity and maintained in culture room at 26±2ºC (Fig 4). Potted cups were irrigated with nutrient solution from time to time.
Fig 3: Morphogenic response of immature embryos (10-12 DAA) of *C. serrata* at BAP 1mg/l (A), BAP 2mg/l (B), NAA 0.5mg/l (C) and *C. senegalensis* at NAA 0.5 mg/l + BAP 1mg/l (D), NAA 0.5 mg/l + BAP 2mg/l (E).

Fig 4A,B: Rooting of shoots on ½ strength MS supplemented with 0.5mg/l IBA (*C. senegalensis*) (A) and MS + 0.2mg/l NAA + 0.001mg/l BAP + 500mg/l CH (*C. serrata*) (B). C,D: Acclimation of rooted plants of *C. senegalensis* and *C. serrata*.

Fig 5: Rooting and flowering of shoots on ½ strength MS medium supplemented with 0.1mg/l IBA in *C. senegalensis* (A) and 0.5 mg/l IBA in *C. serrata* (B). F=Flowering, R=Rooting.
CONCLUSION

In the present study the cotyledon produced only callusing on all the medium tried. BAP at concentrations of 0.5 and 2.0 mg/l supported indirect multiple shoot regeneration via callusing in *C. senegalensis*, whereas 1 mg/l BAP supported direct multiple shoot regeneration from hypocotyl. On the other hand, BAP induced differentiation of multiple shoots in *C. serrata* and the number of shoots per hypocotyl increased with the increasing concentration of BAP. Similar indirect shoot regeneration from hypocotyl was evident in *C. serrata* in medium supplemented with 1mg/l each of NAA and BAP. Whereas NAA (2mg/l) with BAP(1mg/l) and NAA(2mg/l) with BAP(2mg/l) showed callusing. Cotyledonary node explant was the most responsive explant for plant regeneration in both the species of *Cyamopsis* under investigation. Interestingly, 2mg/l BAP supported multiple shoot formation from cotyledonary nodes in *C. serrata*. Medium containing 1.0 mg/l BAP + 2.0 mg/l NAA supported indirect shoot regeneration in *C. senegalensis*. Immature embryos (10-12 DAP) of *C. serrata* showed indirect regeneration at 0.5mg/l NAA after 25 days of inoculation. ½ MS with 0.1 mg/l and 0.5 mg/l IBA supported best rooting in *C. senegalensis* and *C. serrata*, respectively. Plantlets were successfully transferred to paper cups.

REFERENCES


