REGENERATION IN CULTIVATED (CYAMOPSISSTETRAGONOLOBA L.) AND WILD SPECIES (C. SERRATA) OF GUAR

S. Mathiyazhagan, S.K. Pahuja and Anju Ahlawat*

Forage Section, Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar -125 004, India

Received: 19-04-2012 Accepted: 19-10-2012

ABSTRACT

Different guar cultivars and the wild species of explants such as the hypocotyls, cotyledon, and mature embryo were used for callus induction and these explants along with cotyledonary node explants were used for direct shoot morphogenesis. It was established that Murashige and Skoog culture medium containing 2,4-D (2.5 mg/l) and 6-benzylaminopurine (0.5 mg/l) gave the highest frequency of callus induction using mature embryo explants in guar cultivars as well as wild species. Over 2-3 shoots per explants were observed via somatic embryogenesis on MS medium supplemented with NAA 2.0 mg/l + BAP 0.5mg/l + Charcoal 3g/l in cultivars HG 563 and FS 277 using mature embryo as explants. Direct shoot regeneration was observed using cotyledonary node explants in guar cultivars and its wild species on MS medium containing Kinetin (1.0 mg/l) + BAP 0.5 (mg/l) and maximum 2-3 of shoots per explants were observed on medium supplemented with Kinetin (1.0 mg/l) + BAP 0.5 (mg/l) and Zeatin (1.0mg/l). No shoot regeneration was observed in cotyledon, mature embryo, hypocotyl explants of the guar cultivars and the wild species. In vitro rooting was observed on somatic embryo derived shoots on a half strength concentration of Murashige and Skoog's culture medium fortified with indole-3-butyric acid (0.5 mg/l) + charcoal (3g/l).

Key words: Guar, Cyamopsis tetragonoloba, C. serrata, Callus, Plant regeneration.

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). Clusterbean is also an important crop of South-western Haryana, which is second largest producer having area, production and productivity 3.0 lakh ha, 3.6 lakh tons and 1200 kg ha⁻¹, respectively in 2010-11 (Anonymous, 2010). Rajasthan accounts for about 75 per cent of area and 55 per cent 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 U.P and M.P.

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 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 Cyamopsis 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 results. Such a failure may be

*Corresponding author’s e-mail: pahuja66@gmail.com, ahlawatanju19@gmail.com
due to 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

The present study was conducted in the tissue culture lab off Fogg section of department of Plant breeding CCS HAU, Hisar for the two consecutive years of 2008-2009.Two species of guari.e C. tetragonoloba (L.)Taub. (Var: - FS277, HG 563 HG 2-20 and HVG 2-30) C. serrata Schinz.(Wild) were taken into consideration for present study. Seedling explants cotyledons, hypocotyls and seed explants of mature embryos were used for callus induction followed by regeneration and the above explants along with cotyledonary node explants were used for direct shoot regeneration in the experiments.

Various explants were cultured on MS medium with B5 vitamins and fortified with different concentrations of growth regulators like auxins viz.indole 3-butryic acid (IBA), naphthalene acetic acid (NAA), Indole acetic acid (IAA) cytokinins viz. 6-benzyl amino purine (BAP), zeatin and kinetin were used alone or in various combinations with basal media.

Seeds of three different species of Cyamopsis were washed thoroughly with tap water containing a drop of teepol for 5-10 minutes. Subsequently the seeds were surface sterilized with 70% alcohol for 1 minutes 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 mercuric. These sterilized seeds were germinated under aseptic conditions initially under dark condition until germination and then shifted to light conditions MS media (1962) containing charcoal (3 g ml/l). Mature embryo explants were excised from surface sterilized seeds soaked overnight in distilled water. Approximately 15 explants were cultured in petriplates containing different media compositions in each replication. Each treatment was replicated thrice. Culture was kept in culture room at 25 ± 1°C temperature, under photo period of 16h light and 8h darkness.

Callus induction and maintenance

MS salts supplemented with B5 vitamins (Table 1) was used as basal medium. Various concentrations of 2,4-D alone and in combination with BAP were tried for callus induction and subsequent maintenance of callus (Table 2).

Data were recorded for days to callus induction after incubation and number of explants responding to callus induction. The callusing frequency was worked out as

\[
\text{Callus frequency} = \frac{\text{No of explants showing callus formation}}{\text{Total no. of explants cultured}} \times 100
\]

Indirect regeneration

Regeneration via somatic embryogenesis was attempted on different media compositions using cotyledon, hypocotyl, and embryo axis as explants and also direct shoot morphogenesis was attempted on different media compositions using cotyledon, hypocotyl, cotyledonary node and mature embryo as explants. The shoots thus obtained were further cultured on MS basal medium without any growth regulator. Data on number of shoots per explant and the shooting frequency was worked out as

\[
\text{Shooting frequency} = \frac{\text{No of explants showing \text{Shooting}}}{\text{Total no. of explants cultured}} \times 100
\]

Statistical analysis of data

The experiment was conducted in CRD with three replication, the means and standard errors were worked out from replicated data (in percentages) obtained from various experiments. For analysis of variance the percentage of data was transformed using arc sin transformation to bring it to normal distribution and the comparisons of mean were done using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Legumes exhibit a diversity of responses when cultured in vitro. Depending on several factors, regeneration occurs via organogenesis and or embryogenesis, either directly from explants tissue or indirectly after an intervening callus phase. While several species are limited to either organogenesis or embryogenesis, other regenerate via both. With few exception, legumes are commonly described as
TABLE 1: Effect of various phytohormones on callus induction in mature embryo explants of guar cultivars and wild species (C. serrata)

<table>
<thead>
<tr>
<th>Medium code</th>
<th>HG 563</th>
<th>HVG 2-30</th>
<th>HG 2.20</th>
<th>FS 277</th>
<th>WILD SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl₁ (control)</td>
<td>21.72</td>
<td>25.24</td>
<td>35.35</td>
<td>17.73</td>
<td>16.16</td>
</tr>
<tr>
<td>Cl₁ (2,4-D 1mg/l)</td>
<td>(27.6±2.47)</td>
<td>(30.1±1.19)</td>
<td>(36.4±2.10)</td>
<td>(24.8±0.84)</td>
<td>(23.6±1.13)</td>
</tr>
<tr>
<td>Cl₁ (2,4-D 1mg/l + BAP 1 mg/l)</td>
<td>57.93</td>
<td>62.11</td>
<td>50.73</td>
<td>29.50</td>
<td>45.76</td>
</tr>
<tr>
<td>Cl₁ (2,4-D 2mg/l + BAP 2 mg/l)</td>
<td>(49.5±2.82)</td>
<td>(52.0±1.62)</td>
<td>(46.1±4.64)</td>
<td>(32.8±0.31)</td>
<td>(42.5±1.24)</td>
</tr>
<tr>
<td>Cl₁ (2,4-D 3 g/l + BAP 3 mg/l)</td>
<td>61.66</td>
<td>87.36</td>
<td>45.33</td>
<td>22.05</td>
<td>73.80</td>
</tr>
<tr>
<td>Cl₁ (2,4-D 2.5mg/l + BAP 0.5 mg/l)</td>
<td>(51.7±0.49)</td>
<td>(69.1±0.79)</td>
<td>(42.3±1.38)</td>
<td>(27.3±2.81)</td>
<td>(59.2±0.72)</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at 5% level of significance
Values in parenthesis are transformed (arc sin) mean values of percentage data
N.D.-Not done

recalcitrant species with regard to tissue culture (Kaviraj et al., 2006;).

Callus induction

In the present study, callus induction was tried from several explants in cluster bean cultivars namely, HG 563, HG 2-20, HVG 2-30, and FS 277. The genotype and the source of the explants played an important role in deciding callus induction frequency. Callus induction was tried on various MS basal media supplemented with 2,4-D alone and in combination with BAP. Various explants viz. mature embryos, cotyledon and hypocotyl were tried for callus induction. The results of each explants callus induction are described as under.

(a) Mature Embryo

The best responses for callus induction were observed on Cl₁ (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium and callus induction was also found to be good on Cl₁ (2,4-D 2 mg/l + BAP 2 mg/l) medium (Table 1). But in case of wild species the per cent callus induction increased with increase in the concentration of both the growth regulator i.e. 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.

Addition of BAP in combination with 2,4-D showed remarkable increase in callus induction which was observed from the both ends of mature embryo explants within 5-7 days of incubation and after 21 days entire explants turned into a white friable callus mass.

Genotypic differences in terms of statistical significance were observed on all hormonal combinations (Table 1). Among the guar cultivars, HVG 2-20 and HG 2-30 showed best responses, 100 and 98.66 per cent, respectively on Cl₁ (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium and other guar cultivar HG 563 showed maximum best response, 86.76 per cent on Cl₁ (2,4-D 2 mg/l + BAP 2 mg/l) medium. FS 277 showed poor response in all combinations and maximum callus induction response, 29.50 per cent was observed on Cl₁ (2,4-D 1 mg/l + BAP 1 mg/l) medium. However, the wild species response increased with increase phytohormones and the best response, 73.80 per cent was observed on Cl₁ (2,4-D 3 mg/l + BAP 3 mg/l) medium.

Among the explants used for callus induction the performance of embryos and cotyledon was better than hypocotyl. This is so because the embryo contains meristic zones that comprise of dedifferentiated cells, these dedifferentiated cells may proliferate further in response to the exogenous concentration of auxin supplement in the medium. But in case of wild species all explants were found equally responsive. In wild species good response to callus induction was observed which may be because in wild species different potential unexploited genes may be available which may show good response.
TABLE 2: Effect of various phytohormones on callus induction in hypocotyl explants of guar cultivars and wild species (C. serratia).

<table>
<thead>
<tr>
<th>Medium code</th>
<th>HG 563</th>
<th>HVG 2.30</th>
<th>HG 2.20</th>
<th>FS 277</th>
<th>Wild species (C. serratia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl (control)</td>
<td>14.53</td>
<td>17.05</td>
<td>14.90</td>
<td>49.46</td>
<td>13.40</td>
</tr>
<tr>
<td>Cl (2,4-D 1 mg/l)</td>
<td>22.23±2.18d</td>
<td>24.03±3.34d</td>
<td>22.69±0.5b</td>
<td>44.67±2.11b</td>
<td>21.39±1.36d</td>
</tr>
<tr>
<td>Cl (2,4-D 1 mg/l + BAP 1 mg/l)</td>
<td>28.96</td>
<td>45.70b</td>
<td>21.12</td>
<td>67.32</td>
<td>25.26</td>
</tr>
<tr>
<td>Cl (2,4-D 2 mg/l + BAP 2 mg/l)</td>
<td>50.54±1.44b</td>
<td>43.78±1.69b</td>
<td>35.70±1.71a</td>
<td>58.13±0.95a</td>
<td>69.30±1.21b</td>
</tr>
<tr>
<td>Cl (2,4-D 3 mg/l + BAP 3 mg/l)</td>
<td>33.23</td>
<td>27.42</td>
<td>16.96</td>
<td>51.95</td>
<td>94.13</td>
</tr>
<tr>
<td>Cl (2,4-D 2.5 mg/l + BAP 0.5 mg/l)</td>
<td>56.37±0.87a</td>
<td>49.82±2.28a</td>
<td>37.26±2.22a</td>
<td>56.36±0.88a</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at 5% level of significance.
Values in parenthesis are transformed (arc sin) mean values of percentage data.
N.D.: Not done.

(b) Hypocotyl

Amongst all the explants tested for callus induction, the hypocotyls were found least responsive. Among the media tried best responses for callus induction were observed on Cl (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 Cl (2,4-D 3 mg/l + BAP 3 mg/l) medium (Table 2).

Callus initiation was observed from the cut ends of hypocotyls explants both in guar cultivars and wild species after prolonged culture period of 20-25 days. Callus had brown color in guar cultivars and greenish color in wild species.

Genotypic differences in terms of statistical significance were observed on all hormonal combinations (Table 2). Among the guar cultivars, FS 277, showed best response, 72.14 per cent was observed on Cl (2,4-D 2 mg/l + BAP 2 mg/l) medium. Other guar cultivars, HG 563, HVG 2.30, and HG 2.20 showed maximum response i.e. 69.36, 58.33, and 36.77 per cent, respectively on Cl (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium. However, wild species response increased with increase in phytohormones and the best response, i.e. 94.13 per cent was observed Cl (2,4-D 3 mg/l + BAP 3 mg/l) medium.

(c) Cotyledon

The best response for callus induction was observed on Cl (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 Cl (2,4-D 3 mg/l + BAP 3 mg/l) medium. Callus initiation was observed within 5-7 days of incubation and callus had brown color in guar cultivars and greenish color in wild species.

Genotypic differences in terms of statistical significance were observed on all hormonal combinations (Table 3). Among the guar cultivars, HG 563 showed best response, 91.07 per cent on Cl (2,4-D 2 mg/l + BAP 2 mg/l) medium. Other guar cultivars, HVG 2.30, HG 2.20 and FS 277 showed maximum response, i.e. 88.28, 80.28 and 75.23 per cent, respectively on Cl (2,4-D 2.5 mg/l + BAP 0.5 mg/l) medium. However, wild species response increased with increase in phytohormones and the best response, 88.74 per cent was observed Cl (2,4-D 3 mg/l + BAP 3 mg/l) medium.

In the present investigation the callus produced on media supplemented with 2,4-D alone was white in color and showed poor growth (presumably due to meiotic death of cells). This suggested that the addition of BAP in the culture media acts as a trigger for cellular level chloroplast functioning and subsequent production of green photosynthetic calli.

The genotypic differences observed in callus initiation response of various guar genotypes at different media compositions indicated clearly that callus induction is highly variable genetically.
TABLE 3: Effect of various phytohormones on callus induction in cotyledon explants of guar cultivars and wild species (C. serrata).

<table>
<thead>
<tr>
<th>Medium code</th>
<th>HG 563</th>
<th>HVG 2-30</th>
<th>HG 2-20</th>
<th>FS 277</th>
<th>WILD SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (control)</td>
<td>37.89</td>
<td>26.88</td>
<td>34.25</td>
<td>20.53</td>
<td>74.71</td>
</tr>
<tr>
<td>C1 (2,4-D 1 mg/l)</td>
<td>(37.95±1.62)</td>
<td>(31.14±2.11)</td>
<td>(35.67±3.41)</td>
<td>(25.85±1.72)</td>
<td>(59.81±1.19)</td>
</tr>
<tr>
<td>C1 (2,4-D 1 mg/l + BAP 1 mg/l)</td>
<td>73.29</td>
<td>33.61</td>
<td>45.75</td>
<td>47.61</td>
<td>81.33</td>
</tr>
<tr>
<td>C1 (2,4-D 1 mg/l + BAP 2 mg/l)</td>
<td>(59.03±2.97)</td>
<td>(35.30±3.08)</td>
<td>(42.54±1.24)</td>
<td>(43.61±1.37)</td>
<td>(64.38±0.65)</td>
</tr>
<tr>
<td>C1 (2,4-D 2 mg/l + BAP 2 mg/l)</td>
<td>91.07</td>
<td>76.39</td>
<td>76.39</td>
<td>63.05</td>
<td>84.57</td>
</tr>
<tr>
<td>C1 (2,4-D 3 mg/l + BAP 3 mg/l)</td>
<td>(75.66±1.16)</td>
<td>(60.97±1.71)</td>
<td>(60.97±1.71)</td>
<td>(52.55±1.15)</td>
<td>(66.95±1.85)</td>
</tr>
<tr>
<td>C1 (2,4-D 2.5 mg/l + BAP 0.5 mg/l)</td>
<td>49.46</td>
<td>59.88</td>
<td>59.88</td>
<td>72.57</td>
<td>88.74</td>
</tr>
<tr>
<td>+ Charcoal 3.0 g/l</td>
<td>(44.67±2.11)</td>
<td>(50.68±0.90)</td>
<td>(50.68±0.90)</td>
<td>(58.41±0.92)</td>
<td>(70.47±1.56)</td>
</tr>
<tr>
<td>+ Charcoal 3.0 g/l</td>
<td>61.99</td>
<td>88.28</td>
<td>80.28</td>
<td>75.23</td>
<td>N.D</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at 5% level of significance. Values in parenthesis are transformed (arc sin) mean values of percentage data.
ND: Not done.

TABLE 4: Shoot regeneration percentage in callus from mature embryo on different combinations of growth regulator.

<table>
<thead>
<tr>
<th>Medium code</th>
<th>Combinations</th>
<th>FS 277</th>
<th>HVG 2-30</th>
<th>HG 2-20</th>
<th>Wild species</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB1</td>
<td>NAA 1 mg/l + BAP 0.5 mg/l</td>
<td>20</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ Charcoal 3.0 g/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NB2</td>
<td>NAA 2 mg/l + BAP 0.5 mg/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ Charcoal 3.0 g/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NB3</td>
<td>NAA 2.5 mg/l + BAP 0.5 mg/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ Charcoal 3.0 g/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

controlled trait 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).

Indirect regeneration

Plant regeneration via somatic embryogenesis or shoot morphogenesis using callus as explants sources was tried on various M S basal media containing BAP, NAA, IAA and Charcoal combinations.

The calli as explants did not show any regeneration response on any of media tried, B1 (BAP 1 mg/l + IAA 0.5 mg/l), B2 (BAP 2 mg/l + IAA 0.5 mg/l), B3 (BAP 2.5 mg/l + IAA 0.5 mg/l) and B4 (BAP 3.0 mg/l + IAA 0.5 mg/l) media. The callus explants showed regeneration response cultured in NB1 (NAA 1 mg/l + BAP 1 mg/l + Charcoal 3 g/l). The guar cultivars namely, HVG 2-30 and FS 277 showed maximum response of 20 and 14 per cent, respectively. The wild species did not show any regeneration response but further multiplication callus was observed. (Table 4).

Somatic embryogenesis and shoot morphogenesis were induced from callus grown in high auxin and low cytokinin containing media (Ravi Raj et al., 2006) or by reversing the condition for callus induction i.e. supplementing the regeneration with high cytokinin and low auxin concentrations (Varalaxmi et al., 2007).

However in the present study, NAA in combination with BAP and activated charcoal composition used for regeneration via somatic embryogenesis or shoot morphogenesis were found suitable. Some of the other media did not respond due to rapid death of calli cells for somatic embryogenesis and shoot morphogenesis obviously indicates towards ungenial phytohormones or presence of phenolic compounds compositions in these media.
TABLE 5: Effect of various phytohormones on direct regeneration in cotyledonal node explant of guar cultivars and wild species (*C. serata*).

<table>
<thead>
<tr>
<th>Medium code</th>
<th>HG 563</th>
<th>HVG 2.30</th>
<th>HG 2.20</th>
<th>FS 277</th>
<th>WILD SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_0$ (control)</td>
<td>41.95 (40.30±2.88$^a$)</td>
<td>74.73 (59.82±1.21$^a$)</td>
<td>100 (89.96±0.00$^a$)</td>
<td>93.33 (77.67±1.14$^a$)</td>
<td>63.05 (52.55±1.15$^a$)</td>
</tr>
<tr>
<td>$Z_1$ (Zeatin 1mg/l)</td>
<td>83.02 (59.48±0.49$^a$)</td>
<td>37.77 (37.79±1.56$^b$)</td>
<td>84.25 (66.68±1.66$^b$)</td>
<td>29.61±1.66$^b$)</td>
<td></td>
</tr>
<tr>
<td>$Z_2$ (Zeatin 2.5 mg/l)</td>
<td>82.50 (65.29±1.12$^b$)</td>
<td>40.66 (59.59±0.94$^b$)</td>
<td>37.68 (37.79±2.66$^b$)</td>
<td>48.14 (43.91±1.06$^b$)</td>
<td>24.09 (29.33±1.45$^b$)</td>
</tr>
<tr>
<td>$Z_3$ (Zeatin 3 mg/l)</td>
<td>60.27 (50.91±0.71$^b$)</td>
<td>85.51 (67.63±0.98$^b$)</td>
<td>93.88 (78.22±2.88$^a$)</td>
<td>100 (89.96±0.00$^a$)</td>
<td>87.36 (69.17±0.79$^a$)</td>
</tr>
<tr>
<td>KB$_1$ (Kin 1 mg/l + BAP 0.5 mg/l)</td>
<td>49.59 (44.74±2.57$^b$)</td>
<td>62.22 (52.06±1.32$^b$)</td>
<td>54.84 (47.68±1.43$^b$)</td>
<td>84.49 (68.63±0.63$^b$)</td>
<td>37.51 (69.17±1.62$^b$)</td>
</tr>
<tr>
<td>KB$_2$ (Kin 2 mg/l + BAP 0.5 mg/l)</td>
<td>54.36 (47.49±1.29$^b$)</td>
<td>58.39 (49.82±1.22$^b$)</td>
<td>54.84 (47.77±1.66$^b$)</td>
<td>67.10±3.04$^b$)</td>
<td>(37.73±1.65$^b$)</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at 5% level of significance. Values in parenthesis are transformed (arc sin) mean values of percentage data.

**Direct regeneration**

Direct shoot morphogenesis studies were carried out on various media supplemented with Zeatin alone and kinetin in combination with BAP. The mature embryo, hypocotyls and cotyledon of the four guar cultivars and the wild species failed to show any response in form of shoot morphogenesis on any of the media tried. But cotyledonal node of the four guar cultivars and the wild species showed multiple shoot regeneration shown in Table 5.

Maximum shoot morphogenesis response was observed in low concentration of $Z_1$ (Zeatin 1.0mg/l) and KB$_1$ (Kinetin 1mg/l + BAP 0.5 mg/l) combinations and maximum of 2-3 shoots per explants were observed in both guar cultivars and the wild species. These shoots were surgically separated and sub cultured on same hormonal combinations which showed elongation and normal shoot development within 7 days of incubation and the base of the shoot was surrounded by a small calli portion.

Genotypic differences in terms of statistical significance could be observed on all hormonal combinations (Table 5). The guar cultivars namely, HG 2.20, HVG 2.30, FS 277 and wild species showed maximum regeneration response of 100, 74.73, and 93.33 per cent, respectively on $Z_1$ (Zeatin 1.0mg/l) medium where as guar cultivar HG 563 showed maximum regeneration response, 83.50 per cent was observed $Z_2$ (Zeatin 3.0mg/l) on medium. Similarly which had low concentration of KB$_1$ (Kinetin 1mg/l + BAP 0.5 mg/l) medium showed best response of regeneration in guar cultivars and wild species (Table 5).

Recently, Virender (2008) reported direct shoot morphogenesis from cotyledonal node in guar using low concentration of TDZ in combination with NAA.

The best response to direct shoot morphogenesis was observed in BAP (0.5 mg/l) and combination with kinetin (1.0 mg/l) in both the species. Our success in achieving direct shoot regeneration in present study in cotyledonal node explants is not surprising, various workers have observed similar results by using cotyledonal node explants in various legumes. Mundhara et al. (2006) reported regeneration from cotyledonal nodes at low concentration of TDZ (10 μM) in Vignaradiata and it was also observed that TDZ was more effective than BAP (10 μM) for getting direct shoot regeneration.

Regeneration of shoots from cotyledonary node explants of chickpea was reported on MS medium supplemented with low concentration of TDZ, 2-ip and kinetin. Surekha and Arundhati (2007) also observed regeneration from cotyledonary node in peanut cultured on BAP and in combination with kinetin. Hoque et al., (2007) while working on vignaradiata observed shoot morphogenesis from cotyledonal explants on high BAP (0.5 mg/l). But
high concentration of kinetin/cytokinin resulted in condensed and vitrified shoots.

In the present investigation, it was found that cotyledonary node explants of guar genotypes and wild species were more responsive for direct shoot regeneration on Z₁ medium [MS+ Zeatin (1.0 mg/l)] and K₁ medium [MS+ kinetin(1.0 mg/l) + BAP 0.5] with an average number of 2 shoots per explants. Regeneration was observed on cotyledon explants via somatic embryogenesis in other legumes. But in the present study, our attempts to get direct shoot regeneration were unsuccessful from cotyledon, mature embryo and hypocotyl explants. Only callus induction was observed on all the media used in C. Serrata and four cultivar of C. tetragonoloba.

Tivareker and Eupen (2001) in Vignaradina reported shoot formation from immature cotyledon on the medium containing BAP and IAA.

ROOTING

Six rooting media, ½ MS basal media supplemented with IBA (0.5 mg/l), IBA (1 mg/l), IBA (2 mg/l), IBA (0.5 mg/l) + Charcoal (3.0 g/l), IBA (1 mg/l) + Charcoal (3.0 g/l), IBA (2 mg/l) + Charcoal (3.0 g/l), were used for rooting in all cultivars in regenerated shoots from cotyledonary nodes explants. No rooting response was observed. Rooting was only observed in regenerated shoots obtained from callus of mature embryo explants (somatic embryogenesis) medium containing IBA (0.5 mg/l) + Charcoal (3.0 g/l), out of 4 shoot cultured three showed rooting.

In the present investigation, the best rooting response was observed in half strength MS medium supplemented with IBA 1mg/l and GA₃ 0.1mg/l. Similar results were obtained by Zambreeet al., 1998 in Phaseolus vulgaris and Prem et al. (2005) in cluster bean. Prem et al. (2003) observed rooting response from regenerated shoots obtained from cotyledonary node explants in guar on MS medium supplemented with IBA (4.9 M). Our attempts of root regeneration from cotyledonary explants, however, were unsuccessful. In most of the examples cited for rooting, media subsequently used is supplemented with an auxin source.

CONCLUSION

In the present study on Cyamopsis species best media composition for callus induction i.e. Cl₂ (2,4-D 2.5 mg/l + BAP 0.5 mg/l), and Cl₃ (2,4-D 2 mg/l + BAP 2 mg/l) were found in the guar cultivars and the wild species. Somatic embryogenesis was observed in FS 277 (20%) and HVG 2-30 (14%) in medium containing NAA (1 mg/l) and BAP (0.5 mg/l) along with charcoal.

Direct regenerations were observed in case of cotyledonary node explants where as other explants were failed to respond. Direct shoot morphogenesis was observed in all guar cultivars and the wild species of cotyledonary node explants upon culturing on MS medium supplemented with Zeatin alone and kinetin and BAP combinations. Maximum response was observed in medium with zeatin (1.0 mg/l) and Kinetin 1.0 + BAP 0.5 mg/l. Maximum of 2-3 shoots per explants were observed in both guar cultivars and the wild species. Genotypic differences in terms of statistical significance were found on all the responding media both for callus induction and plant regeneration.

Rooting was observed surgically separated regenerated shoots from embryo derived shoots on half-strength MS medium containing IBA (1 mg/l) with charcoal (3mg/l).

REFERENCES


Edited by: Sethi, S. K., Chhabra, A. K., Raj, K., Yadav, B. S. and Singh, D.


