In vitro propagation strategies for ex-situ conservation of Inula royleana DC., a threatened medicinal plant of Kashmir Himalaya

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ABSTRACT
Inula royleana DC is a threatened medicinal plant belonging to genus Inula, tribe Inuleae and family Asteraceae. It is a rich source of lycocotnine and anthranoyl-lycetoctine alkaloids. Sesquiterpene lactones of eudesmane type, abietane diterpenes and diterpene alkaloids are also reported from its roots. This plant is also used traditionally to cure various diseases like headache, dermatitis, allergy, intestinal problems, lowering hypertension etc. This plant has ethnoveterinary importance as well where it is used to cure wounds and inflammation of hooves. During present study efficient in vitro propagation strategies have been developed for I. royleana by using different explants (leaf, petiole, involucral bracts and seeds). MS medium was supplemented with different growth hormones both individually and in different combinations but MS medium containing BAP(5mg/l) and IAA(2mg/l) in combination proved to be most effective in inducing maximum callus and regenerating 7.6±0.49 mean number of shoots with mean length of 6.2±0.28cm from leaf explant. Regenerated shoots produced 4±0.2 mean number of roots on MS medium supplemented with IBA (0.8mg/l). These in vitro plantlets were successfully acclimatized within 4 weeks with 100% survival rate.

Keywords: Shoot regeneration, root regeneration, acclimatization

Abbreviations: MS medium - Murashige and Skoog’s medium; BAP - 6-benzylaminopurine; Kn – Kinetin; IAA – Indole 3-acetic acid; IBA – Indole 3- butyric acid; NAA – α-Naphthylene acetic acid; 2,4-D – 2,4-dichlorophenoxyacetic acid
1. INTRODUCTION

Genus Inula belongs to tribe Inuleae, family Asteraceae and comprises about 100 species (Qaiser and Abid, 2003). This genus is known for diverse biological activities like anticancer, antibacterial, hepatoprotective, cytotoxic, and anti-inflammatory (Zhao et al., 2006). So far as I. royleana (Fig. 1a) is concerned, it is one of the medicinally reputed plant species of this genus (Kaul, 1997), which is distributed throughout Western Himalaya (Stojakowska and Malarz, 2004) at an altitude of 2800-3400 m (Khuroo et al., 2007) and has become threatened due to illicit trade, overgrazing and overexploitation (Dar et al., 2002). According to the study of Edwards and Rodger (1959), this plant is rich in lycocitonine and anthranoyl-lycöctonine alkaloids which were previously named as Royline and Inuline respectively (Chatterjee and Talapatra, 1957). Sesquiterpene lactones of eudesmane type (Bohmann et al., 1978; Quirishi et al., 1980), abietane diterpenes (Edwards et al., 1962; Bhat et al., 1975) and diterpene alkaloids (Khaleque et al., 1959; Hegnauer, 1964) are also reported from its roots. It is due to the presence of these bio-chemicals that this plant is used as an insecticidal (Jennings et al., 1986), insect repellent (Ulubelen et al., 2001), antimicrobial (Yang et al., 2001), anti-inflammatory (Dirsch et al., 2000) and antiproliferative against different cancer cell lines (Lawrence et al., 2001; Konishi et al., 2002) and have neuromuscular blocking properties (Manchanda et al., 2000). Moreover, vasodepressor effect of some abietanes is reported (Kolak et al., 2001; Ulubelen et al., 2002). This plant is traditionally used to cure headache (Kala, 2006), dermatitis (Kaul, 1997), throat sores, wounds and inflammation of hooves in cattle (Khuroo et al., 2007), intestinal problems (Khan and Khatoon, 2008), in lowering hypertension (Haq and Alam, 2010) and as an anti-allergic and antiseptic (Prakash and Aggrawal, 2009). Due to such a wide array of biological activities and its threatened status, this plant was chosen for tissue culture studies for mass propagation. Rapid micropropagation protocols have been developed for I. royleana by using different explants. The selected explants viz., leaf, petiole, involucral bract, seeds and embryos responded differently, in terms of callus production and shoot regeneration, to growth hormones which were added to the MS basal medium in different concentrations and combinations.

2. MATERIALS AND METHODS

2.1. Explant selection and sterilisation

Explants like leaf, petiole, involucral bract and seeds, selected for present study, were collected from the plants growing in wild habitat. These were thoroughly washed under running tap water in order to remove dirt and dust. This was followed by washing with detergent labolene and surfactant tween-20 that helps the detergent to spread all over the surface of the explants. After washing with double distilled water, the explants were treated with chemical sterilants like 2% sodium hypochlorite for 8-10 min in case of leaf, petiole and involucral bract and 0.1% mercuric chloride for 10-15 min in case of seeds. This was followed by washing with autoclaved double distilled water and finally inoculation on sterilised nutrient medium. The sterilisation procedure was carried out under laminar air flow hood.

2.2. Preparation of medium and maintenance of culture conditions

Murashige and Skoog’s (MS, 1962) medium, gelled with 0.8% agar containing 30 g sucrose was supplemented with different concentrations of auxins and cytokinins both individually and in combination. Auxins like 2,4-D; IAA; NAA; IBA and cytokinins like BAP and Kn were used in concentration range of 0.1-5 mg/l. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C and 15 lb. The cultures were incubated at 22±4°C and exposed to 24h photoperiod supplied by fluorescent tubes.

2.3. Hardening procedure/Acclimatization

The in vitro regenerated plantlets with well differentiated shoots and roots were taken out of culture vials. Their basal portion was thoroughly washed with double distilled water in order to remove the adhering medium. These were then planted in jiffy pots and trays containing a mixture of sand + soil in the ratio 1:1. The jiffy pots and trays were maintained in the green house under controlled conditions of temperature (20±2°C) and relative humidity (60%). These pots and trays were watered after every 2 days.

2.4. Data analysis

The experiments were repeated thrice and effects of different treatments were quantified. The data was analysed by calculating Standard Error (SE) of various treatments.

3. RESULTS

3.1. In vitro response from leaf explants

3.1.1 Callus production: The leaf explants excised from the wild plants produced callus on MS medium supplemented with different growth hormones (BAP, Kn, IAA, NAA, IBA and 2,4-D) both individually as well as in different combinations (Table 1). Among cytokinins, BAP at the concentration of 1mg/l was effective in producing nodular and green colored callus in 80% cultures within 30 days (Fig. 2a). Among auxins, 2,4-D at the concentration of 0.2mg/l was effective in producing friable and green colored callus in 50% cultures within 34 days (Fig. 2b). Among auxin-cytokinin combinations, BAP(5mg/l)+IAA(2mg/l) (Fig. 2c), BAP(2mg/l)+IAA(3mg/l) (Fig. 2d) and BAP(3mg/l)+IAA(1mg/l) (Fig. 2e) supplemented MS medium was effective in inducing callus differentiation within 32, 39 and 43 days in 100%, 40% and 40% cultures respectively. The callus produced was nodular and greenish in color. When MS medium was fortified with Kn(2mg/l)+IAA(1mg/l) nodular and cream colored callus was obtained in 30% cultures within 56 days (Fig. 2f).
3.1.2 Shoot regeneration: Callus obtained from leaf explants regenerated shoots on MS medium containing BAP and IAA in combination (Table 2). Leaf callus without sub-culturing produced 7.6±0.49 mean number of shoots with mean length of 6.2±0.28cm on MS medium containing BAP(5mg/l)+IAA(2mg/l) in 90% cultures within 38 days (Fig. 3a). After sub-culturing the callus, 5.4±0.5 mean number of shoots with mean length of 5.1±0.9cm was obtained on same hormone combination in 80% cultures within 36 days (Fig. 3b). 4.6±0.57 mean number of shoots with 4.2±0.11cm mean length were produced in 70% cultures within 36 days when MS medium was supplemented with BAP(2mg/l)+IAA(3mg/l) (Fig. 3c). When MS medium was fortified with BAP(5mg/l)+IAA(5mg/l) 3.5±0.43 mean number of shoots with 3.4±0.06cm mean shoot length were produced in 70% cultures within 43 days (Fig. 3d). In addition to BAP and IAA combinations, leaf callus regenerated 4.03±0.49 mean number of shoots with 3.5±0.08cm mean shoot length in 38 days in 70% cultures when MS medium was supplemented with NAA at the concentration of 1mg/l (Fig. 3e).

3.2. In vitro response from petiole explants

3.2.1 Callus production: The petiole explants produced callus on MS medium supplemented with both auxins (IAA, NAA, IBA, 2,4-D) and cytokinins (BAP, Kn) individually as well as in different combinations. Among cytokinins, BAP at the concentration of 1mg/l was effective in differentiation of friable and cream colored callus in 60% cultures within 49 days (Fig. 4a) while as Kn at the concentration of 0.5 mg/l was effective in producing nodular and green colored callus in 40% cultures within 62 days (Fig. 4b). Among auxins, IAA at the concentration of 1mg/l was effective in inducing callus differentiation in 60% cultures within 39 days (Fig. 4c). The callus produced was friable and creamish in colour. Among auxin-cytokinlin combinations, only BAP+IAA in different concentrations were effective in inducing callus differentiation. When MS medium was supplemented with BAP (5mg/l)+IAA(3mg/l) and BAP(5mg/l)+IAA(2mg/l), nodulear and cream colored callus was obtained in 35 and 38 days in 90% and 70% cultures respectively (Fig. 4d, 4e). Nodular and green colored callus was obtained when petiole explants were inoculated on BAP(3mg/l)+IAA(1mg/l) containing MS medium (Fig. 4f) in 50% cultures within 55 days (Table 3).

3.2.2 Shoot regeneration: The shoots were regenerated from the callus initiated on MS medium containing BAP(3mg/l)+IAA(1mg/l) (Fig. 5). Mean number of shoots obtained was 4.5±0.56 with 2.5±0.05cm mean shoot length in 70% cultures within 31 days (Table 2).
4. DISCUSSION

The present work has been carried out to develop rapid in vitro propagation strategies for conserving *Inula royleana* DC. using different explants. The selected explants viz., leaf, petiole, involucral bract, seeds and embryos responded differently, in terms of callus production and shoot regeneration, to growth hormones which were added to the MS basal medium in different concentrations and combinations. For Leaf explants MS medium supplemented with BAP(5mg/l)+IAA(2mg/l) was most effective in producing maximum callus in 100% cultures within 32 days. However, callus was also obtained when MS medium was fortified with BAP(1mg/l); 2,4-D(0.2mg/l); BAP(2mg/l)+IAA(3mg/l); BAP(3mg/l)+IAA(1mg/l) and Kn(2mg/l)+IAA(1mg/l), but amount of callus and percent culture response were less (Fig. 15). Similar results were obtained on callus induction studies of *Onobrychis sativa* from its leaf explants by Mohajer et al. (2012), but they used NAA as the source of auxin instead of IAA. They achieved maximum callus production on MS medium supplemented with BAP(2.5mg/l) and NAA(0.5mg/l). Wani et al. (2010) also obtained callus from leaf explants of *Tridax procumbens* by using BAP(0.5mg/l) in combination with 2,4-D(0.5mg/l).

Effect of growth hormones on callus production from petiole explant is also clear from the present study. It has been found that petiole explants produced maximum callus in 90% cultures when MS medium was supplemented with BAP(5mg/l)+IAA(3mg/l). The time taken was 35 days. Callus differentiation in petiole explants was also initiated on MS medium containing BAP(5mg/l)+IAA(1mg/l); IAA(1mg/l); BAP(1mg/l); BAP(3mg/l)+IAA(1mg/l) and Kn(0.5mg/l), but the amount of callus produced and percent cultures responding were less in these cases. Further, time taken for callus differentiation on these concentrations and combinations was comparatively more (Fig. 16). Faisal et al. (2005) also have obtained callus from petiole explants of *Tylaphora indica* on MS medium containing auxins and cytokinins in combination. However, they used TDZ instead of BAP and 2,4-D instead of IAA. They achieved maximum callus production on MS medium containing 2,4-D(2.2mg/l)+TDZ(0.5mg/l). Similarly, Jianbin et al. (2008) obtained maximum petiole callus in case of *Amorphophallus albus* Liu & Wei on MS medium supplemented with BAP(0.9mg/l)+NAA(0.9mg/l).

There are no reports of callus production from involucral bracts in any medicinal plant. However, during present study callus was also obtained from involucral bracts by adding growth hormones (BAP, Kn, IAA, NAA, 2,4-D) in combinations to MS medium. Maximum amount of callus was produced in 60% cultures within 48 days when MS medium was supplemented with BAP(2mg/l)+NAA(1mg/l). MS medium containing Kn(2mg/l)+IAA(1mg/l) and BAP(2mg/l)+2,4-D(1mg/l) was also effective in inducing callus differentiation but time taken was comparatively more and amount of callus produced along with percent cultures responding was less (Fig. 17).

Callus production was also induced in in vitro raised seedlings from seeds. These seedlings differentiated maximum callus when inoculated on MS medium containing BAP(3mg/l)+NAA(5mg/l) in 60% cultures within 55 days. Callus was also obtained when MS medium was fortified with BAP(2mg/l)+IAA(3mg/l) but the response was observed in 40% cultures after an extended time period of 63 days (Fig. 18). Similar results were obtained by Prakash et al. (1999) in *Hybanthus enneaspermus* L. Muell. on MS medium containing BAP(0.49mg/l)+NAA(0.48mg/l) but callus was derived from seeds instead of in vitro seedlings.

Shoot regeneration was achieved in callus produced from all explants viz., leaf, petiole, involucral bract and in vitro seedlings. Callus produced from leaf explants regenerated shoots on the same medium. A maximum mean number of shoots 7.6±0.49 with 6.2±0.28cm mean shoot length were produced in 90% cultures within 38 days. Shoots were also regenerated when leaf callus was sub-cultured on MS medium containing BAP(5mg/l)+IAA(2mg/l); BAP(2mg/l)+IAA(3mg/l); NAA(1mg/l) and BAP(2mg/l)+IAA(5mg/l). But number of regenerated shoots (5.4±0.5; 4.6±0.57; 4.03±0.49 and 2.8±0.43 with 5.1±0.09cm; 4.2±0.11cm; 3.5±0.08cm and 3.4±0.06cm mean shoot length) and percent cultures responding (80%, 70%, 70%, 70%) were less (Fig. 19a). Petiole callus initiated on MS medium containing BAP(3mg/l)+IAA(1mg/l) also regenerated shoots that too in only 31 days but mean number of shoots (4.5±0.56 with 2.5±0.05cm mean shoot length) and percent cultures responding (70%) were less as compared to leaf regenerated callus. Involucral bracts regenerated 4.4±0.9 mean number of shoots with 1.7±0.04cm mean shoot length from callus initiated on MS medium supplemented with BAP(2mg/l)+NAA(1mg/l). The time taken was 39 days and percent cultures responding was 60%. In case of in vitro seedling, callus initiated on MS medium containing BAP(2mg/l)+IAA(3mg/l) regenerated 2.6±0.4 mean number of shoots with 2.3±0.05cm mean shoot length in 32 days (Fig. 19b). These results reveal that for the regeneration of shoots auxins and cytokinins were required in combinations. Similar results were obtained by Stojakowska and Malarz (2004) from nodal explants of *I. royleana* wherein they obtained 5.2±1.7 mean number of shoots on MS medium containing Kn(2.25mg/l)+NAA(0.04mg/l). Further, according to Winand et al. (1986); Akita et al. (1994); Laparra et al. (1997); Stojakowska and Kisiel (1997); Wildi et al. (1998) and Liu et al. (1998), BAP in combination with NAA is preferable for multiple shoot induction and maintenance in family Asteraceae.

For the root regeneration the *in vitro* raised shoots were inoculated on both full salt strength and half salt strength MS medium containing auxins like IAA, NAA, IBA and 2,4-D individually at various concentrations and also in combinations with cytokinins like BAP and Kn. Various MS medium supplemented with growth hormones could not affect root induction. However, full salt strength MS medium supplemented with IBA(0.8mg/l) was effective in inducing *in vitro* regeneration of roots (4±0.2) in 43 days. The present results are in agreement with the study of Stojakowska and Malarz (2004) who obtained root regeneration in *I. royleana* by using IBA at the concentration of 0.02mg/l and Yong-Mei et al. (2008) who obtained rooting in case of *Inula japonica* on NAA supplemented MS medium.

5. CONCLUSION

During present study many in vitro protocols were developed in order to propagate *Inula royleana* at a very large scale that will ultimately lead to its conservation. Four different explants viz., leaf, petiole, involucral bract and in vitro seedlings were used and MS medium was supplemented with different growth hormones both individually and in different combinations. It was found that among all the explants leaf
expant was most responsive in producing maximum amount of callus and maximum number of shoots on MS medium containing BAP in combination with IAA. The shoots obtained were successfully rooted on IBA containing MS medium.

**SUMMARY OF RESEARCH**

1. In Kashmir Himalaya, sub-alpine and alpine medicinal plants are facing threat to extinction mostly because of overgrazing and overexploitation for medicinal purposes, so there arises a need for their large scale propagation for conservation.

2. Plant tissue culture techniques, an important tool for ex-situ conservation of plants species by producing large number of plants in minimum time and space, can be extended to these plant species as well.

3. *Inula royleana* DC., a threatened medicinal plant species growing in Kashmir Himalaya can be rescued from extinction by subjecting its various explants to in vitro regeneration, thus producing a large number of plants.

4. Growth regulators viz., auxins and cytokinins were found to be responsible for inducing dedifferentiation and redifferentiation in mature and differentiated tissues of leaf, petiole, involucral bracts, seeds and embryos which were used as explants.

5. The most responsive explant for callus production and subsequent shoot regeneration was found to be leaf explant. However, for direct shoot and root regeneration embryos gave best result.

**FUTURE ISSUES**

The biggest challenge to plant tissue culture technique according to me is whether the in vitro raised plants have genetic stability with the naturally growing plants or not. If yes, then these plants will have secondary metabolite content equal to the natural ones and it will conserve the mentioned plant species in real terms. So, there is a scope of research in this field.

**DISCLOSURE STATEMENT**

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**Figure 1**
*Inula royleana* DC.
(a) Population (b) Individual plant

**Figure 2**
Callus production from leaf explant on MS medium containing
a) BAP(1mg/l)  b) 2,4-D(0.2mg/l)  c) BAP(5mg/l)+IAA(2mg/l)  d) BAP(2mg/l)+IAA(3mg/l)  e) BAP(3mg/l)+IAA(1mg/l)  
f) Kn(2mg/l)+IAA(1mg/l)
Figure 3
Shoot regeneration from leaf callus on MS medium containing
a) BAP(5mg/l)+IAA(2mg/l)  b) BAP(5mg/l)+IAA(2mg/l)
c) BAP(2mg/l)+IAA(3mg/l)  d) BAP(5mg/l)+IAA(5mg/l)  e) NAA(1mg/l)
Figure 4
Callus production from petiole explant on MS medium containing
a) BAP(1mg/l)  b) Kn(0.5mg/l)  c) IAA(1mg/l)  d) BAP(5mg/l)+IAA(3mg/l)
e) BAP(5mg/l)+IAA(2mg/l)  f) BAP(3mg/l)+IAA(1mg/l)

Figure 5
Shoot regeneration from petiole callus on MS medium containing BAP(3mg/l)+IAA(1mg/l)
Figure 6
Callus production from involucral bract on MS medium containing
a) BAP(2mg/l)+NAA(1mg/l)  b) Kn(2mg/l)+IAA(1mg/l)
c) BAP(2mg/l)+2,4-D(1mg/l)

Figure 7
Shoot regeneration from bract callus on MS containing BAP(2mg/l)+NAA(1mg/l)
Figure 8
(a) Seed germination on basal MS medium
(b) Callus production from in vitro raised seedlings on MS medium containing BAP(3mg/l)+NAA(5mg/l)
(c) Callus production from in vitro raised seedlings on MS containing BAP(2mg/l)+IAA(3mg/l)

Figure 9
Shoot regeneration from in vitro seedling callus on MS medium containing BAP(2mg/l)+IAA(3mg/l)

Figure 10
Rooting of regenerated shoots on MS medium containing IBA (0.8mg/l)
Figure 11
Acclimatization/Hardening in greenhouse in a) Jiffy pots b) Trays

Table 1
Effect of different hormones on callus production from leaf explants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of days taken for callus production</th>
<th>Amount of callus produced</th>
<th>Texture and color of callus</th>
<th>% Culture response</th>
</tr>
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<tbody>
<tr>
<td>MS basal</td>
<td>No response</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>MS+BAP(1mg/l)</td>
<td>30</td>
<td>Moderate</td>
<td>Nodular, Cream colored</td>
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<td>High</td>
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<td>MS+2,4-D (0.2mg/l)</td>
<td>34</td>
<td>High</td>
<td>Friable, Green colored</td>
<td>50</td>
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<tr>
<td>MS+BAP(2mg/l)+IAA(3mg/l)</td>
<td>39</td>
<td>Moderate</td>
<td>Nodular, Green colored</td>
<td>40</td>
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<tr>
<td>MS+BAP(3mg/l)+IAA(1mg/l)</td>
<td>43</td>
<td>Moderate</td>
<td>Nodular, Green colored</td>
<td>40</td>
</tr>
<tr>
<td>MS+Kn(2mg/l)+IAA(1mg/l)</td>
<td>56</td>
<td>High</td>
<td>Nodular, Cream colored</td>
<td>30</td>
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(30 replicates per treatment)
### Table 2
Effect of different hormones on callus production from petiole explants

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<tr>
<th>Treatments</th>
<th>No. of days taken for callus production</th>
<th>Amount of callus produced</th>
<th>Texture and color of callus</th>
<th>% Culture response</th>
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<tr>
<td>MS basal</td>
<td>No response</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS + BAP(5mg/l) + IAA(3mg/l)</td>
<td>35</td>
<td>High</td>
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<td>38</td>
<td>High</td>
<td>Nodular, Cream colored</td>
<td>70</td>
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<tr>
<td>MS + IAA(1mg/l)</td>
<td>39</td>
<td>Little</td>
<td>Friable, Cream colored</td>
<td>60</td>
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<td>MS + BAP(1mg/l)</td>
<td>49</td>
<td>High</td>
<td>Friable, Cream colored</td>
<td>60</td>
</tr>
<tr>
<td>MS + BAP(3mg/l) + IAA(1mg/l)</td>
<td>55</td>
<td>High</td>
<td>Nodular, Green colored</td>
<td>50</td>
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<tr>
<td>MS + Kn(0.5mg/l)</td>
<td>62</td>
<td>Little</td>
<td>Nodular, Green colored</td>
<td>40</td>
</tr>
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</table>

(30 replicates per treatment)

### Table 3
Effect of different hormones on callus production from involucral bract explants

<table>
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<tr>
<th>Treatments</th>
<th>No. of days taken for callus production</th>
<th>Amount of callus produced</th>
<th>Texture and color of callus</th>
<th>% Culture response</th>
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</thead>
<tbody>
<tr>
<td>MS basal</td>
<td>No response</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS + BAP(2mg/l) + NAA(1mg/l)</td>
<td>48</td>
<td>High</td>
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<td>60</td>
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<tr>
<td>MS + Kn(2mg/l) + IAA(1mg/l)</td>
<td>52</td>
<td>High</td>
<td>Friable, Cream colored</td>
<td>40</td>
</tr>
<tr>
<td>MS + BAP(2mg/l) + 2,4-D(1mg/l)</td>
<td>60</td>
<td>Little</td>
<td>Friable, Cream colored</td>
<td>40</td>
</tr>
</tbody>
</table>

(30 replicates per treatment)
### Table 4
Effect of different hormones on callus production from *in vitro* raised seedlings

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of days taken for callus production</th>
<th>Amount of callus produced</th>
<th>Texture and color of callus</th>
<th>% Culture response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS basal</td>
<td>No response</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS+BAP(3mg/l)+NAA(5mg/l)</td>
<td>55</td>
<td>Moderate</td>
<td>Nodular, Green colored</td>
<td>60</td>
</tr>
<tr>
<td>MS+BAP(2mg/l)+IAA(3mg/l)</td>
<td>63</td>
<td>Little</td>
<td>Nodular, Green colored</td>
<td>40</td>
</tr>
</tbody>
</table>

(30 replicates per treatment)

### Table 5
Effect of different hormones on shoot regeneration from callus obtained from selected explants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Explant</th>
<th>Mean no. of shoots ± SE</th>
<th>Mean length of shoots (cm) ± SE</th>
<th>No. of days taken for shoot regeneration</th>
<th>% Culture response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS+BAP(5mg/l)+IAA(2mg/l)</td>
<td>Leaf callus</td>
<td>7.6±0.49</td>
<td>6.2±0.28</td>
<td>38</td>
<td>90</td>
</tr>
<tr>
<td>MS+BAP(5mg/l)+IAA(2mg/l)</td>
<td>-do-</td>
<td>5.4±0.5</td>
<td>5.1±0.09</td>
<td>36</td>
<td>80</td>
</tr>
<tr>
<td>MS+BAP(2mg/l)+IAA(3mg/l)</td>
<td>-do-</td>
<td>4.6±0.57</td>
<td>4.2±0.11</td>
<td>36</td>
<td>70</td>
</tr>
<tr>
<td>MS+NAA(1mg/l)</td>
<td>-do-</td>
<td>4.03±0.49</td>
<td>3.5±0.08</td>
<td>38</td>
<td>70</td>
</tr>
<tr>
<td>MS+BAP(5mg/l)+IAA(5mg/l)</td>
<td>-do-</td>
<td>2.8±0.43</td>
<td>3.4±0.06</td>
<td>43</td>
<td>70</td>
</tr>
<tr>
<td>MS+BAP(3mg/l)+IAA(1mg/l)</td>
<td>Petiole callus</td>
<td>4.5±0.56</td>
<td>2.5±0.05</td>
<td>31</td>
<td>70</td>
</tr>
<tr>
<td>MS+BAP(2mg/l)+NAA(1mg/l)</td>
<td>Involucral bract callus</td>
<td>4.4±0.9</td>
<td>1.7±0.04</td>
<td>39</td>
<td>60</td>
</tr>
<tr>
<td>MS+BAP(2mg/l)+IAA(3mg/l)</td>
<td>In vitro seedling callus</td>
<td>2.6±0.4</td>
<td>2.3±0.05</td>
<td>32</td>
<td>60</td>
</tr>
</tbody>
</table>

(30 replicates per treatment)