IN VITRO PROPAGATION OF GENUS DIOSCOREA – A CRITICAL REVIEW

SUPRIYA DAS*,1, M. DUTTA CHOUDHURY1, P.B.MAZUMDER2

1Department of Life science and Bioinformatics, Tissue Culture Lab. Assam University. Silchar. 2Department of Biotechnology, Assam University. Silchar. E-mail: supriya1august@gmail.com

Received: 6 June 2013, Revised and Accepted: 20July 2013

ABSTRACT

The monocotyledonous Dioscorea genus is known as yam. Many species of Dioscorea genus are economically important crops of worldwide and many of them have been used in the pharmaceutical industry. In vitro propagation of Dioscorea species pave the way to meet the demand of this economically important plant. The protocols are designed to provide the optimal levels of mineral nutrients, environmental factors, vitamins and carbohydrates to achieve the high regeneration rate of the different species of Dioscorea in vitro. This review summarizes some of the important reports on micropropagation technique of Dioscorea from the literature data.

Keywords: Dioscorea, In vitro, micropropagation, Yam.

INTRODUCTION

The monocotyledonous Dioscorea genus is known as yam. It is named after the ancient Greek physician and Botanist Dioscorides. The genus Dioscorea includes 600 Species and is of considerable economic importance [1]. Many wild Dioscorea species are a very important source of secondary metabolites used in pharmaceutical industry and medicine. A number of Dioscorea wild species are source of compounds used in synthesis of sex hormones and corticosteroids [2] and cultivated species are the source of food in some countries [3]. Dioscorea alata L. is an important tuber crop and is a staple food for millions of peoples in tropical and subtropical countries [4]. Root and Tuber crops are the most important food crops after cereals. Tuber crops find an important place in the dietary habits of small and marginal farmers especially in the food security of tribal population. India hold a rich genetic diversity of tuber crop especially yam Dioscorea [5].

Yams are valuable source of carbohydrates, fibers and low level of fats which make them a good dietary nutrient and also processed into various staple intermediate and end product forms [6]. Species of genus Dioscorea are tuberous herbaceous perennial vine reaching a length of 2-12 meters or more. Leaves are spirally arranged mostly broad heart shaped. Flowers are unisexual and fruits capsules, few species are monoecious. Dioscorea alata is edible and used as a staple food. Powdered tubers used as a remedy for piles, gonorrhea and applied externally to sores.

The tubers of some species of Dioscorea are important source of diosgenin a chemical used for the commercial synthesis of sex hormones and corticosteroids which are widely used for anti-inflammatory, androgenic and contraceptive drugs [7]. Many species of Dioscorea genus are economically important crops worldwide, e.g. D. alata, D. Cayenensis, D. rotundata are main crops in Caribbean, Central and South America and West Africa [8]. The tuber of D. oppositifolia are used as herbal tonic which stimulate stomach and spleen and also has effect on lung and kidney. The tubers are used to treat appetite, diarrhea, asthma, cough, frequent urination, diabetes and emotional instability.

Out of six hundred species of Dioscorea 14 are used as edible tubers. Tubers have a dual agricultural function. They supply nourishment as a source of food and tubers are also act as a planting material [9]. Dioscorea species are vegetatively propagated by using tuber pieces. The production of tuber is hampered by several significant virus and fungal diseases [10].
Incubation Condition
Light and temperature are two important parameters in culture incubation. Exposure to light for 12-16 h per day under 30-50 µmol m⁻² s⁻¹ provided by cool, white fluorescent lamps is usually recommended. The temperature 25°C±1 is employed in the culture incubation room.

Micropropagation of Dioscorea

Stem as explants
Micropropagation of Dioscorea has been achieved through rapid proliferation of shoot-tips auxillary buds in culture. Several factors are reported to influence the growth of in vitro propagated plants. Different explants are used by different workers to propagate their plant.

Mahesh et al. [20] worked with Dioscorea wightii and propagated the plant using nodal segment as explants. BA and kinetin was used for the multiplication of nodal segment. Callus initiation was observed in MS medium supplemented with 0.15-1.75 µM BA, 0.75-5.0 µM kinetin, 0.15-0.30 µM 2iP and shoot formation was observed in all growth regulators tested in BA, Kinetin and 2iP. Ovono et al. [26] reported that presence of kinetin (2mg/L) reduce the shoot length, root length, node numbers but root length was increase when sucrose concentration increased 3%-5% in case of D. cayennensis-D. rotundata complex. It is observed that cytokinin required in optimal quantity for shoot proliferation in many genotypes but addition of low concentration of auxins along with cytokinins triggered the shoot proliferation [27]. Kadota and Niimi [23] reported that liquid culture using nodal segment as explants. BA and kinetin was used for plant using nodal segment as explants. BA and kinetin was used for

Plant Name | Explant Source | References
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D. floribunda | Nodal segment | Chaturvedi,[29] Lakshmi sita et al.,[30] Uduebo,[18]
D. composita | Nodal segment | Grewal et al.,[32]
D. bulbifera | Axillary meristem | Uduebo,[18] Datta et al.,[34]
D. rotundata | Nodal segment | Manet al.,[35]
D. opposita | tuber | Jin xu et al.,[36]
D. zingiberensis | Nodal segment | Chen et al.,[37]
D. oppositifolia | Nodal segment | Poornima &
D. pentaphylla | Nodal segment | Ravishankar,[38]
D. oppositifolia | Nodal segment | Behera et al.,[25]
D. alata | Nodal segment | Borges et al.,[22]
D. wightii | Nodal segment | Mahesh et al.,[20]
D. zingiberensis | Stem, leaves, petioles | Shu et al.,[39]
D. opposita | Stem segment | Nagasawa and Finer[40]
D. zingiberensis | tuber | Hepping et al.,[41]
D. alata | Nodal segment | Wheatley et al.,[42]
D. balcanica | tuber | Savkin-Fodulovic et al.,[43]
D. zingiberensis | inflorescence | Huang et al.,[15]
D. bulbifera | Nodal segment | Narula et al.,[21]
D. alata | Nodal segment | Jova et al.,[19]
D. deltoidea | Nodal segment | Annirata et al.,[45]
D. floribunda | internode | Mascarenhas et al.,[44]
D. alata | root | Tuyford & Mantell,[46]
D. cayennensis | Meristem tip | Malaurie et al.,[47]
D. rotundata | | complex & D. praehensilis

Chen et al., [37] developed a protocol for rapid in vitro propagation of Dioscorea rotundata using stem as explants. Medium supplemented with 4.4µM BAP +1.1 µM NAA produced shoots on nodal segments within 20 days. Callus formed on MS +8.9 µM BA + 5.4 µM NAA in 30 days, 22.2 µM BAP and 1.1 µM NAA regenerated shoot from callus and for rooting 9.4 µM IBA was used. Poornima and Ravishankar [21] used nodal segments to propagate D. Oppositifolia and D. pentaphylla and reported multiple shoots produced on MS medium with 8.8 µM BAP and 0.3% activated charcoal, rooting was observed in MS medium with 2.67 µM IBA and developed tuber on MS medium with 8.8µM BAP. Behera et al.,[25] carried out a work to regenerate plantlet of D. Oppositifolia where nodal segment was used as explants and culture it on MS medium supplemented with BAP and NAA. They reported best shoot proliferation was observed in MS medium + 2mg/L kinetin + 1.0 mg/L BAP + 0.5 mg/L NAA + 100 mg/L ascorbic acid where 90% explants showed proliferation and half strength MS supplemented with 2.0mg/L NAA found to be best for root formation. Mantell [48] studied the association of microbes with tissue and cell cultures of tropical Dioscorea yams for that nodal segment were used as explants. Ovono et al., [49] gave an account on effect of polyamines on in vitro tuber formation and development in D. cayennensis-D. rotundata complex and found that in presence of polyamines in culture medium accelerated tuber formation. Ovono et al., [26] investigated the effect of reducing sugar on in vitro shoot formation and sprouting of D. rotundata complex and observed for earlier tuber formation 1% sucrose needed but it decrease the length and weight of tuber, tuber obtained 3% sucrose sprouted rapidly.

The individual effects of sucrose, plant growth regulators and basal salt medium formulations on microtuber induction and development were investigated by Alizadeh et al., [17] and reported BA at 1.25 and 2.5 µM strongly inhibitory effects on microtuber induction while promotive effect was shown by NAA and IBA at 5.0 µM.

In vitro regeneration and multiplication of Dioscorea alata was studied by Borges et al.,[22] and high rates (100%) of explants regeneration was observed in D-571 medium with 1.5% manitol +1mg/L BAP + 2g/L activated charcoal. An improved method of in vitro propagation of D. bulbifera was established by Forsyth and Staden [24] for this they cultured nodal segment on MS medium and reported that with the increasing concentration kinetin shoot formation per node was increased. Primary callus was induced by Shu et al.,[39] culturing stems, leaves, petioles on MS medium supplemented with 0.44µMBA and 0.4gM NAA with no gelling agents was optimum for shoot proliferation. Jova et al.,[28] investigated the effect of temporary immersion system on formation of micro tuber in Dioscorea alata and reported TIS show a positive effect on shoot growth.

### Table 1: In vitro multiplication of genus Dioscorea by different workers

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Explant Source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. floribunda</td>
<td>Nodal segment</td>
<td>Chaturvedi,[29] Lakshmi sita et al.,[30] Uduebo,[18]</td>
</tr>
<tr>
<td>D. composita</td>
<td>Nodal segment</td>
<td>Grewal et al.,[32]</td>
</tr>
<tr>
<td>D. bulbifera</td>
<td>Axillary meristem</td>
<td>Uduebo,[18] Datta et al.,[34]</td>
</tr>
<tr>
<td>D. rotundata</td>
<td>Nodal segment</td>
<td>Manet al.,[35]</td>
</tr>
<tr>
<td>D. opposita</td>
<td>tuber</td>
<td>Jin xu et al.,[36]</td>
</tr>
<tr>
<td>D. zingiberensis</td>
<td>Nodal segment</td>
<td>Chen et al.,[37]</td>
</tr>
<tr>
<td>D. oppositifolia</td>
<td>Nodal segment</td>
<td>Poornima &amp;</td>
</tr>
<tr>
<td>D. pentaphylla</td>
<td>Nodal segment</td>
<td>Ravishankar,[38]</td>
</tr>
<tr>
<td>D. oppositifolia</td>
<td>Nodal segment</td>
<td>Behera et al.,[25]</td>
</tr>
<tr>
<td>D. alata</td>
<td>Nodal segment</td>
<td>Borges et al.,[22]</td>
</tr>
<tr>
<td>D. wightii</td>
<td>Nodal segment</td>
<td>Mahesh et al.,[20]</td>
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<td>D. zingiberensis</td>
<td>Stem, leaves, petioles</td>
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<td>D. zingiberensis</td>
<td>tuber</td>
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<td>D. balcanica</td>
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<tr>
<td>D. zingiberensis</td>
<td>inflorescence</td>
<td>Huang et al.,[15]</td>
</tr>
<tr>
<td>D. bulbifera</td>
<td>Nodal segment</td>
<td>Narula et al.,[21]</td>
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<td>D. alata</td>
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<td>D. deltoidea</td>
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<td>D. floribunda</td>
<td>internode</td>
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<tr>
<td>D. alata</td>
<td>root</td>
<td>Tuyford &amp; Mantell,[46]</td>
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<td>D. cayennensis</td>
<td>Meristem tip</td>
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<td></td>
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</table>
proliferation. Nodal culture without growth regulators has been shown to be an efficient way for multiplication of several species of *Dioscorea* [54].

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Explant</th>
<th>Growth Regulators</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. japonica</em></td>
<td>Shoot tips</td>
<td>LS + 0.44 µM BA</td>
<td>Kadota &amp; Nii [23]</td>
</tr>
<tr>
<td><em>D. zingiberensis</em></td>
<td>Nodal segment</td>
<td>MS + 4.4 µM BAP + 1.1 µM NAA</td>
<td>Chen et al. [37]</td>
</tr>
<tr>
<td><em>D. oppositifolia</em></td>
<td>Nodal segment</td>
<td>MS + 8.8 µM BAP + 0.3% charcoal</td>
<td>Poornima &amp; Ravishankar [20]</td>
</tr>
<tr>
<td><em>D. pentaphylla</em></td>
<td>Nodal segment</td>
<td>MS + 2mg/L KN + 1.0mg/L BAP + 0.5mg/L NAA + 100mg/L ascorbic acid</td>
<td>Behera et al. [25]</td>
</tr>
<tr>
<td><em>D. opposita</em></td>
<td>Nodal segment</td>
<td>MS + 1.0mg/L NAA + 0.5-1.0mg/L BA</td>
<td>Shin et al. [55]</td>
</tr>
<tr>
<td><em>D. hispida</em></td>
<td>Nodal segment</td>
<td>MS + 2.0mg/L BAP + 0.5mg/L NAA + 100mg/L ascorbic acid</td>
<td>Behera et al. [56]</td>
</tr>
<tr>
<td><em>D. bulbifera</em></td>
<td>Nodal segment</td>
<td>MS + 0.5 µM/L NAA + 5mg/L KN</td>
<td>Narula et al. [21]</td>
</tr>
</tbody>
</table>

### Table 2: Growth regulators used for shoot formation by different workers

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Explant</th>
<th>Growth Regulators</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. oppositifolia</em></td>
<td>Nodal segment</td>
<td>MS + 30g/L sucrose + 2.0mg/L KN + 1.0mg/L BA + 0.5mg/L NAA</td>
<td>Behera et al. [25]</td>
</tr>
<tr>
<td><em>D. bulbifera</em></td>
<td>Nodal segment</td>
<td>MS + 5% sucrose + 0.1% BAP + 0.4mg/L KN</td>
<td>Kohmura et al. [57]</td>
</tr>
<tr>
<td><em>D. composita</em></td>
<td>Nodal segment</td>
<td>MS + 2.5 µM KN</td>
<td>Forsyth &amp; van Staden [58]</td>
</tr>
</tbody>
</table>

### Seed as Explant

Heping et al. [41] worked with *D. Zingiberensis* and for callus induction seeds were cultured on MS medium + 1.0mg/L BAP + 0.5mg/L KN. Plantlets regenerated on solid MS medium with 0.2mg/L BAP and half MS medium with 0.5 mg/L NAA favoured root formation in regenerated shoots.

Effect of sodium nitroprusside on callus induction and plant regeneration was investigated by Xu et al. [36] in *D. opposita* and noticed supplementation with 40 µM SNP markedly promotes callus induction frequency, higher number of shoots produced in SNP supplemented medium than the medium without SNP. Callus was induced from seed (Savikin-Fudlovic et al., 1998) on MS medium supplemented with 5mg/L 2,4-D + 0.5mg/L BAP for 5 days and later they lowered the concentration at 0.66mg/L. Heping et al. [41] produced tetraploid plants of *D. Zingiberensis* using seed by colchicines.

### Inflorescence as Explant

Inflorescence induction and morphogenesis of regenerated flowers was investigated in *D. zingiberensis* [15] and for that experiment male inflorescence was used as explants. According to the observation MS + 2mg/L BA + 0.5mg/L BAP was favorable for highest inflorescence induction where GA showed reverse effect when kinetin combined with 0.4mg/L NAA explants developed inflorescence.

### Tuber Development

Ovono et al. [49] worked with *D. cayenensis* - *Drotundata* complex and reported jasmonic acid (JA 10µM) increase the tuber formation in absence of kinetin. In vitro production of micro tubers has been reported in a number of species [59-63]. Jasik and Mantell [64] reported media supplemented with 20g/L sucrose produce higher micro tuber number and greater micro tuber size than 40g/L sucrose. A decrease in the percentage of micro tuber number and greater micro tuber size than 40g/L sucrose was observed when sucrose concentration was 30g/L [28]. Alizadeh et al. [17] reported NAA and IBA at 5.0 µM showed promotive effect on micro tuber formation and growth on in vitro culture of *D. composita*. They observed no tuber induced on medium containing 20g/L sucrose whereas tuber developed on medium containing 80 and 100g/L sucrose. In TIS maximum micro tuber number per plant, fresh weight, and diameter were obtained [28] in comparison with culture medium. MS medium has been reported to be inhibitory in case of tuber formation [66]. Highest number (2.2±0.14) of micro tubers were obtained on MS + 30g/L sucrose + 0.2mg/L KN + 1.0mg/L BAP + 0.5mg/L NAA [25].

Kohmura et al. [57] studied the effect of sucrose concentrations (3 and 6%) in D. opposita with 8.0 µM BAP and they reported 6% sucrose was found to be more effective in tuber formation. Chen et al. [67] indicated sucrose provide a carbon source and energy for induction of shoot and micro tuber. Chu et al. [50] observed micro tubers in culture of D. delacata only after 10 month under 8% photoperiod and *D. bulbifera* developed bulbs under short days after 8 month. Micro tuber germination was promoted when jasmonic acid present in the medium at concentration 0.1 or 1 µM but jasmonic acid at concentration 30 & 100 µM inhibited germination [51].

Lauzer et al. [52] reported that under 8h day length in both species *D. abyssinica* and *D. mangenotiana* microtuber was induced on nodal segment. In *D. abyssinica* microtubers induced when sucrose was present in the medium at concentration 20, 40, 60 and 80 g/L whereas in *D. mangenotiana* tuberization favored only at concentration 40 and 60g/L.

### Root Development

Behera et al. [56] used NAA and IBA to induce rooting from in vitro raised shootlets of *D. hispida*, and they observe highest rooting in half strength MS basal medium + 2mg/L NAA + 2g/L Ac and 2mg/L IBA + 2g/L Ac in half strength MS basal medium induce second highest rooting. For rooting Behera et al. [25] in vitro micro shootlets

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
of Doppasiitofila inoculated on half MS medium supplemented with 2mg/L NAA and profuse rooting was observed on this medium. 2mg/L NAA in combination with 0.2 and 0.5 mg/L BA produced root in D. esculenta [68]. Poornima and Ravishankar [28] reported that efficient rooting was observed on MS medium +2.67µM NAA after 30 days.

Sucrose concentration when raised 3% to 8% an increase in root number was observed [59], sucrose concentration when increased 3% to 5% root length also increased. Rooting frequency was higher in the solid medium but number of roots produced by each shoots was greater in liquid medium and the roots produced in gelan gum medium was longest[23]. In hormone free medium within 10 days all the shoots produced root, when medium was supplemented with 4.9µM or 9.8µM IBA induced fastest rooting with higher number of roots per plant was observed [37].

**Acclimatization and field establishment**

Kadota and Niimi [23] reported that when micropropagated plants of D. japonica were transferred to pots containing 1:1:vermiculite and soil (v/v/v) mixture under green house condition about 80% of the plants survived. Micropropagated plants were transferred to the pots containing mixture of soil + sand + manure in 1:1:1 ratio [25] and 90% plants survived. Rooted plantlets were transferred to the pots containing sand, compost and mould mixture (1:1:2) after 8 month acclimatized plants produced tuber [51]. Rooted plantlets were transferred to soil rite (equal proportion of decomposed compost and peat moss) for acclimatation[26].

**REFERENCES**


