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Review Article

IN VITRO PROPAGATION OF GENUS DIOSCOREA – A CRITICAL REVIEW

SUPRIYA DAS*[1], M. DUTTA CHOUDHURY[1], P.B.MAZUMDER[2]

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

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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* 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. eg. *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].

Yam propagation by seeds using conventional methods is slow and not adequate for rapid multiplication. Tuber yield is drastically reduced by viral and nematode infections, through infected tubers it is transmitted to the next generation [11] and it also deteriorate the quality of the tuber [12]. *In vitro* propagation may help to overcome constraints related with availability of high quality of planting material [13].For their nutritional value yams are used as staple food. But many reasons restrict production of tuber such as lack of agronomic constraints, phytosanitary problems and lack of good healthy planting materials. Tissue culture technique provides a way to increase the rapid production of virus free plant material.

This review will summarize some of the important reports on the *in vitro* propagation of *Dioscorea* species from literature data of recent years. This review will focus the significant achievements of recent years in the field of micropropagation of yam.

Establishment of Aseptic Cultures

Explants collected from field-grown plants are usually contaminated by various microorganisms. To exclude the surface contaminants common sterilizing agents like sodium hypochlorite (1-5%), ethyl alcohol (70-90%) and mercuric chloride (0.1-1%) are used by washing in the appropriate solution for 10-30 min followed by several rinses in sterile water. Bavistin (0.3%) benomyl (1.0%) streptomycin (0.3-0.5%) and detergent are also used as surface sterilizing agent.

Basic Culture Media

The formulation described by Murashige and Skoog [14] is most commonly used for *in vitro* propagation of various species of *Dioscorea* [15-21]. A nutrient medium consists of all the essential major and minor plant nutrient elements, vitamins, plant growth regulators and as carbon source carbohydrate with other organic substances as optimal additives. For micropropagation of *Dioscorea alata* D-571culture medium was used [22] and Linsmaier and Skoog culture medium was used for in vitro culture of *D. japonica* [23].For preparing solid and semi solid culture media Agar powder is most commonly used.

Growth Substances

Success of tissue cultural work much depends on the levels and kinds of plant growth regulators included in the culture medium. Root and shoot initiation, callus formation and differentiation are closely regulated by the relative concentration of Auxins and cytokinin in the medium [24,25] .The gibberellins stimulate callus formation.

Incubation Condition

Light and temperature are two important parameters in culture incubation. Exposure to light for 12-16 h per day under 30-50µmolm^{-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 axillary buds in culture. Several factors are reported to influence the growth of *in vitro* propagated plants. Different explants are used by different worker to propagate their plant.

Mahesh *et al*.,[20] worked with *Dioscorea wightii* and propagate 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 0.15-1.75µM BA, 0.75-5.0µM 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. *cayenensis -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 medium was superior to solid medium in terms of shoot proliferation, 6.9 number of node produced in liquid medium where 2.1 node produced in solid medium in *Dioscorea japonica.* 6-Benzylaminopurine at 0.44 µM produced highest number of nodes (7), shoot (2.60) and the fresh weight 336.0mg.40 ml LS medium supplemented with 0.44µMBAand 0.44µM 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.

Table1: *In vitro* multiplication of genus *Dioscorea* by different workers

Plant Name	*Explant Source*	*References*
D. floribunda	*Nodal segment*	*Chaturvedi,[29] Lakshmi sita et al.,[30] Uduebo,[18]*
D. deltoidea	*Axillary meristem*	*Furmanowa et al.,[31] Grewal et al., [32]*
D. composita	*Nodal segment*	*Ammirato,[33]Datta et al.,[34]*
D. bulbifera	*Axillary meristem*	*Uduebo,[18]*
D. rotundata	*Nodal segment*	*Mantell et al.,[35]*
D. opposita	*tuber*	*Jin xu et al.,[36]*
D. zingiberensis	*Nodal segment*	*Chen et al., [37]*
D. oppositifolia D. pentaphylla	*Nodal segment*	*Poornima & 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*	*Heping et al.,[41]*
D. alata	*Nodal segment*	*Wheatley et al.,[42]*
D. balcanica	*tuber*	*Savikin- 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*	*Mascarenhas et al., [44]*
D. floribunda	*internode*	*Ammirato, [45]*
D. alata	*root*	*Twyford & Mantell, [46]*
D.cayenensis- D.rotundata complex & D.praehensilis	*Meristem tip*	*Malaurie et al.,. [47]*

Chen *et al*.**,** [37] developed a protocol for rapid in vitro propagation of *D.zingiberensis* 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 4.9 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. cayenensis- 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* tuber formation and sprouting in yam (*D. cayenensis- 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 strong 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 0.5-2.0mg/L BA + 0-2.0mg/L NAA and best callus formation was observed in medium with 0.5mg/L BA + 2.0mg/L 2,4-D from stem explants. Chu *et al*.,[50] cultured *Dioscorea* species in different day length, different concentration of BAP and sucrose and accumulation of soluble carbohydrate was found in leaves with the increasing concentration of BAP (0-22 µM/L)and sucrose(1.5-8%). Bazabakana *et al*.,[51] investigated the effects of applying exogenous jasmonic acid (JA) on the microtuber germination of *D. alata* and listed JA at concentration (0.1-1 µM) promoted the germination but JA at concentration 30 and 100 µM completely inhibited the germination. Lauzer *et al*.,[52]noticed that nodal segment less than 5 cm length was less suitable for *in vitro* propagation when worked with *D. abyssinica* and *D. mangenotiana*. Wheatley[42] designed a experiment to develop salt tolerant yam (*D. alata*) and observed higher level of NaCl (200 mM) show devastating effect on shoot proliferation as well as root development, at concentration 100 mM NaCl development of new node and leaves formation was noticed.

Yan e*t al*.,[53] studied the effect of temporary immersion system on growth and quality of *D. fordii* and *D.alata* and results reported by them indicated that TIS improved the growth and quality of the plantlets in terms of proliferation rate ,shoot length, fresh weight, dry weight of shoot and biomass. Nodal segments were used as explants by them. Highest rate of shoot proliferation was observed on MS medium with 2.0mg/L Kn+1.0mg/L BAP+ 0.5 mg/L [25] Chen *et al*.,[37] used MS +8.8 µM/L BA+4.65 µM/L NAA for callus induction and MS + 4.44 µM/L BA + 2.32 µM/L NAA callus

proliferation. Nodal culture without growth regulators has been shown to be an efficient way for multiplication of several species of *Dioscorea* [54].

Table 2: Growth Regulators used for shoot formation by different workers

Plant Name	Explant	Growth Regulators	References
D. japonica	Shoot tips	LS + 0.44µM BA	Kadota & Niimi,[23]
D. zingiberensis	Nodal segment	MS + 4.4 µM BAP +1.1 µM NAA	Chen *et al*.,[37]
D.oppositifolia *D. pentaphylla*	Nodal segment	MS + 8.8 µM BAP+ 0.3% charcoal	Poornima & Ravishankar [28]
D.oppositifolia	Nodal segment	MS+2mg/LKN+1.0mg/LBAP+0.5mg/LNAA+100mg/L ascorbic acid	Behera *et al*.,[25]
D. opposita	Nodal segment	MS+ 1.0mg/L NAA+0.5-1.0mg/L BA	Shin *et al*.,[55]
D.hispida	Nodal segment	MS+2.0mg/LBAP + 0.5mg/LNAA + 100mg/L ascorbic acid	Behera *et al*.,[56]
D.bulbifera	Nodal segment	MS+0.5 µM/LNAA+5mg/L KN	Narula *et al*.,[21]

Table3: Growth regulators used for tuber formation by different workers

Plant Name	Explant	Growth Regulators	Reference
D.opposita	Nodal segment	MS+30gm/Lsucrose+2.0mg/LKN+1.0mg/LBAP +0.5mg/LNAA	Behera *et al.,* [25]
D.opposita	Nodal segment	MS+3%sucrose+8.9 µMBAP	Kohmura *et al*.,[57]
D.bulbifera	Nodal segment	MS+2-8%sucrose+23.2-46.4 µM KN	Forsyth & van staden,[58]
D.composita	Nodal segment	MS+2.5 µM KN	Alizadeh *et al*.,[17]

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 IAA. 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-Fodulovic *et al*.,1998) on MS medium supplemented with 5mg/L 2,4-D + 0.5mg/L BAP for 5days 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 BA 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.*,(worked with *D. cayenensis* - *D.rotundata* 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 microtuberization with 8 and 10% sucrose and 2.5 µM kinetin in *D.rotundata* was reported by Ng [60]. Higher level of kinetin (23.2-46.4 µM) raised the microtuber formation frequency was reported by Forsyth and Van Staden [24].Influence photoperiod on *in vitro* tuber formation was examined by Jean & Cappadocia[16] and indicated 16 and 24 hour photoperiod was favorable to produce highest number of microtubers whereas 8 hour photoperiod was effective larger micro tubers. Mantell et al.,[35] observed 2% sucrose produce maximum number of micro tuber in nodal culture of *D. opposita* and *D. alata*. Ovono *et al.*,[49] studied the effect of polyamines in tuber formation of *D. cayenensis – D. rotundata* complex and reported low concentration of putrescine (10^{-5},10^{-6}M) produce tuber earlier. The effect of reducing sugar level on tuber formation, development and sprouting in *D. cayenensis* - *D. rotundata* complex was investigated[26] and reported that lower concentration of sucrose delayed the micro tuber formation and also decrease tuber weight as well as tuber length, micro tuber sprouted later which were obtained on reduced sucrose level, only 29% of the explants showed tuber formation after 3 weeks in presence of 1% sucrose. For tuber growth increasing amount sugar is required in the medium [65].

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 0n 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 +2.0mg/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 opposite 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.delicata* only after 10 month under 8h photoperiod and *D. bulbifera* developed bulbils 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 on 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

of *D.oppositifolia* 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 gellan 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 1:1vermiculite and soil (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 coir and peat moss) for acclimatization[28].

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