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## Developing clonal propagation technique for conservation of (Endangered medicinal plant) *Dioscorea deltoidea* Wall

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**Abstract**

The present study was conducted to standardize the propagation protocol for *Dioscorea deltoidea* Wall during the year 2008 in polybags under shade net in Completely Randomized Design (CRD) with seven treatments and three replications. Rhizome cuttings of 2-3cm length were treated with six (6) concentrations of NAA & IBA (i.e; NAA 20ppm, NAA 30ppm, IBA 20ppm, IBA 30ppm, NAA 10ppm+IBA 10ppm, NAA 15ppm+IBA 15ppm) and control (distilled water) for 12 hours. In general, IBA at 30ppm resulted in early sprouting (14.43 days), maximum sprouting percentage (93.33%), early root emergence (12.37 days), maximum rooting percentage (89.33%), maximum number of secondary roots (40.60), longest root (34.07 cm), maximum root diameter (1.27 mm) and root propensity (4.00), while as, control recorded delayed sprouting & rooting.

**Keywords:** NAA, IBA, *Dioscorea deltoidea*, rhizome, sprouting, rooting

**Introduction**

Medicinal and Aromatic plants are the emerging crops of the future because of the resurgence of interest in their curative and aromatic properties. Even today 80% of the world population is directly or indirectly dependent on plant based drugs for their health care. In India, nearly 5-8 thousand plant species are known for their medicinal value and a large number of them are used in different indigenous medicine system (Kirtikar and Basu, 2001) [4]. Demand for medicinal plants are increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor (Verma, 2008) [10].

As pointed by Threatened Plant Species Committee of the Survival (TPSCS) of IUCN indicate that one in ten species of vascular plants on earth is endangered or threatened due to commercial exploitation and international trade. It has been pointed out that 60000 plant species may be in danger of extinction leading to gene erosion during the next 30-40 years (Sahu and Sahu, 2001) [6]. Thus there is an urgent need to domesticate such species so that they can be introduced in regular farming system. The present investigation has accordingly been undertaken to standardize the vegetative propagation in *Dioscorea deltoidea* Wall.

*Dioscorea deltoidea* Wall ex Kunth commonly known as medicinal yam belongs to family Dioscoreaceae (Fig.1). It is a twining herb, which grows at high altitudes. The glabrous and left twining stem bears alternate petiolate leaves. The flowers are born on axillary spikes, male spike 8-40cm long and stamen 6cm. Female spikes are 15cm long with 4-6 seeded. Seeds are winged all round. Rhizome is lodged (In soil) superficial, horizontal, tuberous, digitate and chestnut brown in color. Its tubers contain purest available source of diosgenin. The major uses of steroids are for production of corticosteroids, sex hormones, and anti-fertility compounds.

**Material and Methods**

The experiment was carried out during 2008 at 1730m above mean sea level at the experiment farm of the division of the Floriculture, Medicinal and Aromatic plants, SKUAST-K, Shalimar campus. The experiment was laid under Completely Randomised Design (CRD) with 25 polybags per treatment. Each treatment was replicated 3 times. The treatments consisted of six (6) concentrations of growth regulators (viz.; NAA 20ppm, NAA 30ppm, IBA 20ppm, IBA 30ppm, NAA 10ppm+IBA 10ppm, NAA 15ppm+IBA 15ppm) and control (distilled water). Rhizome cuttings of *Dioscorea deltoidea* were dipped in different concentrations for 12 hours prior to planting. Dipping of rhizome cuttings in distilled water served as control. There were

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thus 7 treatments of 3 replications with a sample size of 25 cuttings. Planting was done in polybags filled with uniform composition of Soil+Sand+FYM under 35% shade net. Data was recorded up to the period of 45 days after planting. Data recorded on various parameters of shoot/root growth were subjected to statistical analysis under Completely Randomised Design (CRD) following the standard methodology (Gomez and Gomez, 1984) [2].

### Result and Discussion

Data reveal variation in the response of rhizome cuttings to applied growth regulators in terms of days taken to sprout (Table- I). Growth regulator IBA at 30ppm took minimum number of days (14.43 days) to sprout, followed by NAA 15ppm+IBA 15ppm and NAA 10ppm+IBA10ppm. NAA treatments at both concentrations i.e., 20ppm and 30ppm delayed sprouting than IBA but were at par with IBA at 20ppm. Control took significantly maximum days to sprout (24.77days) (Table-I).

Similar findings with IBA, NAA & IAA have been reported in *Leptaedenia reticulata* (Shivanna *et al.*, 2006) [7] and in *Dendrocalamus hemiltonii* (Singh *et al.*, 2001) [9]. Auxins are known to change the cell wall structure thus allowing increase in the volume of the individual cells. This increased cell volume might be responsible for early sprouting of auxin treated propagules. IBA and NAA are known to form conjugates with other chemicals inside the plant system. However, IBA is more freely available for a longer time and hence is more effective in decreasing number of days to sprout than NAA & IBA/NAA combinations.

Data in Table-I also reveal significantly improved sprouting percentage with the application of auxins. Maximum percentage sprouting (93.33%) was recorded under IBA at 30ppm followed by NAA 15ppm+IBA 15ppm. Control recorded significantly lowest percentage sprouting. Here quicker sprouting due to auxin might be the reason behind improved sprouting percentage under auxin application.

The rhizome cutting took as many as 19.76 days for root emergence under control conditions which was reduced by use of growth regulators with minimum of 12.37 days under IBA 30ppm followed by NAA 15ppm+IBA 15ppm and IBA 20ppm. Rhizome cuttings treated with NAA at 20ppm & 30ppm took more number of days to root emergence. The effectiveness of IBA over NAA in inducing rhizogenesis is quite in agreement with the observation of Chandra-Gowda *et al.* (2006) [1] in *Thymus vulgaris*, Okezie (1985) [5] in *Dioscorea rotundata* and Joshi *et al.* (2004) [3] in *Heracleum*

*candicans*. Auxins in general decreased the number of days to root emergence. Auxins are known to promote differentiation of xylem elements, a process considered precursor to root initiation.

IBA at 30ppm also recorded maximum percentage of rooting (89.33%) but was at par with NAA 15ppm+IBA 15ppm. Control recorded lowest percentage of rooting (57.33%). Earlier root emergence with IBA has had a positive cascading effect on the percent rooting of the *Dioscorea* propagules.

Number of secondary roots per propagule ranged from 21.27 (control) to 40.60 (IBA 30ppm), which shows a clear cut influence of different growth regulators

(Table- II). IBA significantly increased the number of secondary roots per propagule. Auxins are known to initiate cambial cell division which might be the reason for significantly more number of secondary roots. These results are in conformity with the findings of Okezie (1985) [5] in *Dioscorea rotundata* and Joshi *et al.* (2004) [3] in *Heracleum candicans*.

As is evident from Table-2, length of longest root (34.07cm) was recorded at 30ppm IBA which was at par with NAA 15ppm+IBA 15ppm. However, control recorded shortest root length of 14.65cm. NAA increased root length but was significantly lower than IBA and in combination with IBA. This might be due to the early start given by IBA in terms of root emergence and secondary root development. These findings get support from Okezie (1985) [5] in *Dioscorea rotundata*. IBA showed more efficient, since it is more stable. The success of IBA may be due to its slow activity and its slow degradation by auxin destroying enzyme (IAA-oxidase). Root diameter was also recorded higher (1.27mm) at 30ppm IBA treatment but was significantly higher than control (0.88mm), while as NAA at 20ppm increased root diameter than control (Table- II). These results are in conformity with the findings of Joshi *et al.* (2004) [3] in *Heracleum candicans*. As early root establishment ensures early commencement of growth above ground. This in turn results in early unfolding of leaves and photosynthesis. Hence, photosynthates are translocated to under ground rhizomes including roots in auxin treated propagules. This can be the possible explanation for significantly increased root diameter with IBA treated plants. Same trend is reflected on evaluation of root propensity under different auxin treatments with the highest of 4.00 recorded with NAA 15ppm+IBA 15ppm and IBA 20ppm. Control recorded lowest root propensity of 2.00. These findings are in conformity with the findings of Siddique *et al.* (2009) [8] in *Picrorhizia kurrooa*.

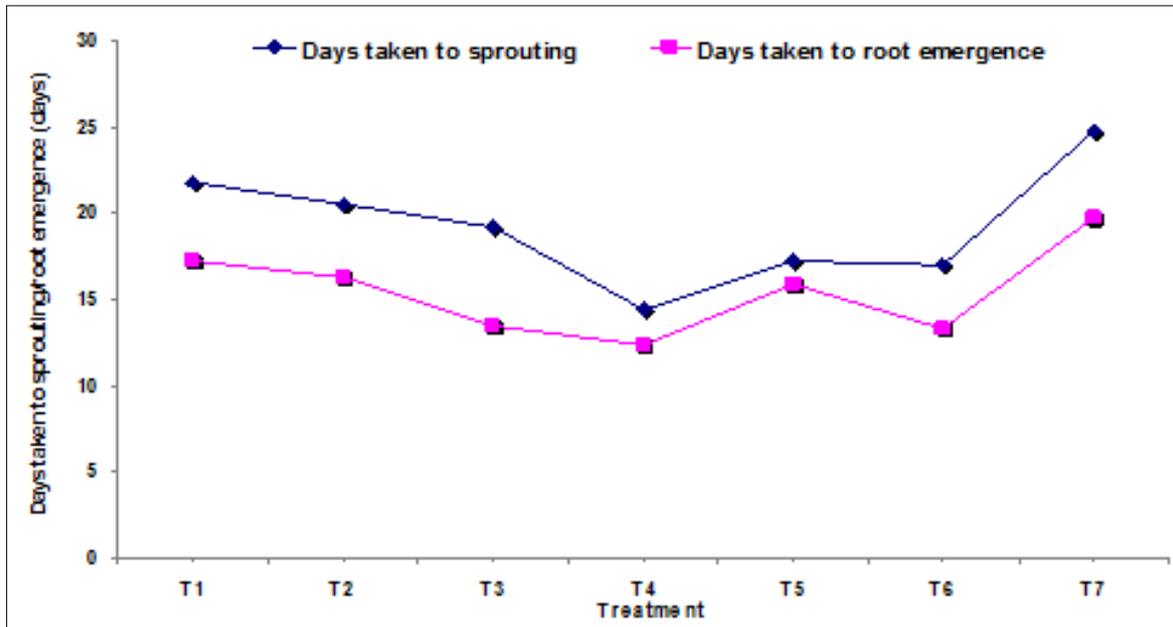
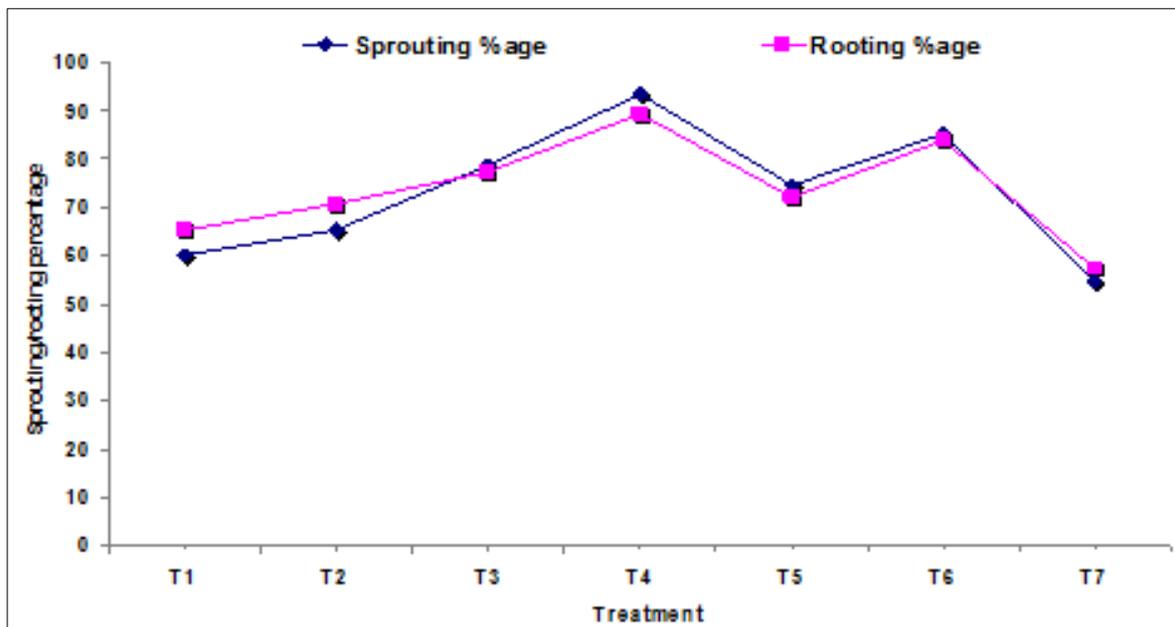
**Table I:** Effect of NAA and IBA on sprouting and rooting of *Dioscorea deltoidea* Wall

| Treatment               | Days taken to sprouting(days) | Sprouting percentage (%) | Days taken to root emergence(days) | Rooting percentage (%) |
|-------------------------|-------------------------------|--------------------------|------------------------------------|------------------------|
| NAA 20 ppm              | 21.84                         | 60.00 (50.78)            | 17.28                              | 65.33 (53.94)          |
| NAA 30 ppm              | 20.59                         | 65.33 (53.94)            | 16.32                              | 70.67 (57.26)          |
| IBA 20 ppm              | 19.25                         | 78.67 (62.64)            | 13.49                              | 77.33 (61.59)          |
| IBA 30 ppm              | 14.43                         | 93.33 (75.20)            | 12.37                              | 89.33 (71.01)          |
| NAA 10 ppm + IBA 10 ppm | 17.25                         | 74.67 (59.80)            | 15.87                              | 72.00 (58.09)          |
| NAA 15 ppm + IBA 15 ppm | 16.99                         | 85.33 (67.81)            | 13.33                              | 84.00 (66.53)          |
| Control                 | 24.77                         | 54.67 (47.69)            | 19.76                              | 57.33 (49.22)          |
| LSD(p=0.05)             | 4.37                          | 7.94                     | 2.51                               | 5.72                   |

Figures in parentheses are arc sin transformed values

**Table II:** Effect of NAA and IBA on various rooting traits of *Dioscorea deltoidea* Wall

| Treatment               | Average No. of secondary roots | Length of longest root (cm) | Root diameter (mm) | Root propensity |
|-------------------------|--------------------------------|-----------------------------|--------------------|-----------------|
| NAA 20 ppm              | 25.33                          | 18.14                       | 0.98               | 2.33            |
| NAA 30 ppm              | 27.73                          | 22.47                       | 1.07               | 2.67            |
| IBA 20 ppm              | 36.53                          | 27.12                       | 1.19               | 3.67            |
| IBA 30 ppm              | 40.60                          | 34.07                       | 1.27               | 4.00            |
| NAA 10 ppm + IBA 10 ppm | 33.40                          | 24.72                       | 1.15               | 3.00            |
| NAA 15 ppm + IBA 15 ppm | 38.87                          | 29.17                       | 1.21               | 4.00            |
| Control                 | 21.27                          | 14.65                       | 0.88               | 2.00            |
| LSD <sub>(p=0.05)</sub> | 4.61                           | 5.19                        | 0.23               | 0.67            |

**Fig 1:** Effect of NAA and IBA on days taken to sprouting and root emergence of *Dioscorea deltoidea* Wall**Fig 2:** Effect of NAA and IBA on sprouting and rooting percentage of *Dioscorea deltoidea* Wall

T1 = NAA 20 ppm; T2 = NAA 30 ppm; T3 = IBA 20 ppm; T4 = IBA 30 ppm;  
 T5 = NAA 10 ppm + IBA 10 ppm; T6 = NAA 15 ppm + IBA 15 ppm; T7 = Control



*Dioscorea deltoidea*



Rhizome of *Dioscorea deltoidea*



Plate 1: *Dioscorea deltoidea* in polybags



Plate 2: Effect of different concentrations of PGRs on the rhizogenesis of *Dioscorea deltoidea*

T<sub>1</sub> = NAA 20 ppm; T<sub>2</sub> = NAA 30 ppm; T<sub>3</sub> = IBA 20 ppm; T<sub>4</sub> = IBA 30 ppm;  
T<sub>5</sub> = NAA 10 ppm + IBA 10 ppm; T<sub>6</sub> = NAA 15 ppm + IBA 15 ppm; T<sub>7</sub> = Control

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