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Short-term conservationof a woody climber, *Decalepishamiltonii* Wight.andArn.through*in vitro* technology

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Abstract: *An efficient protocol was developed for short-term conservation of a woody medicinal climber, Decalepishamiltonii, using micropropagated nodal segments. Nodal segments maintained in reduced culture condition and low light intensity (2.97 $\mu m^{-2}s^{-1}$)and at 10^0 C. MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown highest survival per cent (80%), significantly high shoot length (3.24±0.11), high shoot number/explant (2.6±0.54) and significantly high number of leaves/explant (5.4±1.34) after six months of in-vitro conservation.*
Keywords *D. hamiltonii*, Nodal segments, *In vitro*, Conservation

I. Introduction

Conservation of biodiversity is considered fundamental because climate change, alien species and use of land for intensive farming and development are causing severethreat to the plant genetic diversity worldwide. *Decalepishamiltonii* Wight.&Arn.also known as swallow root, is a woody climber belonging to family Asclepiadaceae. It is widely found in moist as well as dry deciduous forest of peninsular India (1). It is found growing between altitude of 300 and 1200m. There are several vernacular names of *Decalepishamiltonii* in different languages. In Telugu, it is known as Neemamtheega (chenchu tribes), maregugaddalu, Maredukommulu and Barresugnadhi. In Kanada, it is known as Magadiberu and Makaliberu. In Tamil, it is known as Mahalikizhangu, Mavilingakilangu and Peru nannari. In Malayalam, it is known as Nannari. In Sanskrit, its known as Sariba and Svetasariva. In English, it is known as Swallow root (2). *D. hamiltonii* roots known to contain abundant 2-hydroxy-4-methoxy benzaldehyde (HMB) (3).*D. hamiltonii* used as blood purifier, wound healer, bronchial asthma, fever, intrinsic haemorrhage, kushtha, erysipelas, poisoning, paediatric rejuvinative and general vitaliser in Ayurveda, Siddha and Folk systems of medicines (4). Chewing the roots and drinking Nannari (Herbal drink prepared from roots by Yanadi tribe) considered as good digestive aid and appetizer (5). Due to the health promoting properties of its roots, pickles and juices prepared out of its roots and consumed (6). In various food and pharmaceutical applications, it is used as preservatives due to bacteriostatic properties of its volatile principle compound (7). Due to the similar aromatic properties, its roots are used as a substitute for *Hemidesmusindicus* in ayurvedic preparation of ancient Indian medicine (8). It is used to cure skin diseases, nutritious disorders (9), epilepsy and central nervous system disorders (10). The roots are used as a flavouring principle, preservative (11), demulcent, diaphoretic and diuretic. It is also used to treat diarrhoea and also used as bioinsecticide for stored food grains (12). The highly aromatic roots have been subjected to overexploitationby destructive harvesting that affects the survival ofthis plant in its wild habitat (13).Extended flowering pattern, self-incompatibility, pollinatorlimitation, absence of seed dormancy, abortion of a considerablepercentage of seedlings prior to establishment arecontributing factors for the regulation of its population size (14). Moreover, the absence of any organizedcultivation of this plant calls for immediate conservationmeasures (15).

Hence, this study aimed to optimize the culture condition for short term *in vitro* conservation of the micropropagated nodal segments of *D. hamiltonii.*

II. Materials and methods

The experiment was conducted at Division of Plant Genetic Resources, ICAR-IIHR, Bangalore. Short term conservation of *in-vitro* raised plants of *D. hamiltonii* was done for six months to slow down its growth by reducing further sub-culturing and providing limited light intensity for growth. In order to accomplish this, equal number of eight weeks old tissue cultured plants from each treatments (MS medium + Hormonal combinations) were taken and kept under low light intensity(2.97 $\mu m^{-2}s^{-1}$) in a chamber having ambient temperature maintained at 10^0C (Fig. 1). Equal number of replicates from each treatments were kept under standard culture condition in order to compare it with those which are kept under reduced culture condition. Before transferring the replicates from each treatments for *in-vitro* conservation, proper sub-culturing done in every 4 weeks. Readings of growth parameters were recorded at regular intervals (Table 1)

III. Results and discussion

Nodal segments grown in MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shownhighest survival per cent (80%), significantly high shoot length (3.24±0.11), high shoot number/ explant (2.6±0.54) and significantly high number of leaves/explant (5.4±1.34) (Table 1). A comparison was made between *in-vitro* conserved plantlets and equal number of tissue cultured plantlets regenerated in standard culture conditions for survival per cent, shoot length, number of shoots and number of leaves (Table 2). Plantlets kept in standard culture condition were sub cultured in every 4 weeks. MS medium supplemented with BA (0.886mg/l)+2ip(0.24mg/l) shown highest survival per cent in both *in-vitro* conserved plantlets(80%) and plantlets under normal conditions (100%). The same hormonal combination shown significantly high shoot length in both *in-vitro* conserved plantlets (3.24±0.11) and plantlets kept in normal conditions (5.08±0.29).MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown highest number of shoots both *in-vitro* conserved plantlets (2.6 ±0.54) and plantlets kept in normal conditions (3.4 ±0.54).As far as number of leaves are concerned, MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown significantly high number of leaves both in *in-vitro* conserved plantlets (5.4 ± 1.34) and plantlets kept under normal conditions (6.6±0.54).

The objective to go for *in-vitro* conservation by providing limited nutrients, low light intensity (2.97 $\mu m^{-2}s^{-1}$) and maintaining a temperature of 10^0 C was to reduce the growth and other growth related traits in order to conserve it for six months and analysing the survival per cent, shoot length, number of shoots and number of leaves after six months of conservation. Krishnan *et al*. (2011) studied the status of medicinal plants of Western Ghats of India and concluded that not only *in-situ* methods but also *ex-situ* methods through biotechnological tools are required to conserve those important medicinal plant species.MS medium supplemented with BA (0.886 mg/l)+2ip(0.24 mg/l) shown highest survival per cent which indicates that, this particular concentration level of the combination of cytokinins is optimum for the cell division to occur leading to slow shoot growth for six months.The same combination i.e., BA (0.886mg/l)+2ip(0.24mg/l) shown significantly high shoot length, number of shoots/explant and number of leaves when compared to other treatments indicating the occurrence of constant but slow cell division up to six months. Hence, MS medium supplemented with the hormonal combination of BA(0.886mg/l)+2ip(0.24mg/l) may be used for short term *in-vitro* conservation of *D. hamiltonii*.It was found that MS medium supplemented with BA (0.886 mg/l)+2ip(0.24 mg/l) shown highest survival per cent, shoot length, number of shoots and number of leaves in both *in-vitro* conserved plantlets and tissue cultured plantlets regenerated in normal ambient conditions. This shown that, this particular hormonal combination and concentration may be used for short term conservation as well as normal growth of tissue cultured nodal segment.

IV. Conclusion

In-vitro conservation was achieved by providing limited nutrients, reduced light intensity (2.97 $\mu m^{-2}s^{-1}$) and maintaining a temperature of 10^0 C to reduce the growth and other growth related traits in order to conserve it for six months. 8 weeks old nodal segment as explant cultured on MS medium supplemented with various combinations of hormones were kept under above mentioned ambient condition. Among various combinations, satisfactory results obtained in terms of survival per cent, shoot length, number of shoots and number of leaves by using a combination of BA (0.886 mg/l) +2ip (0.24 mg/l).

Table 1.Estimates of growth after six months of *in-vitro* conservation.

Sl. No.	MS Media + Growth regulators (mg/l)	Survival %	Shoot length (Mean ± SD)*	No. of shoots/explant (Mean ± SD)*	No. of leaves/explant (Mean ± SD)*
1	BA(0.443)	40	3.04 ± 0.11	1.4 ± 0.54	4.2 ± 0.8
2	BA(0.443)+2ip(0.24)	60	3.0 ± 0.15	2.6 ± 0.54	4 ± 1
3	BA(0.443)+NAA(0.27)	20	3.08 ± 0.08	1.6 ± 0.89	4.8 ± 0.83
4	BA(0.886)	40	3.0 ± 0.12	1.6 ± 0.54	3.8 ± 0.44
5	BA(0.886)+2ip(0.24)	80	3.24 ± 0.11	2.6 ± 0.54	5.4 ± 1.34
6	BA(0.886)+NAA(0.27)	60	3.08 ± 0.08	1.8 ± 0.44	5.2 ± 0.83
7	BA(2.22)	40	2.98 ± 0.08	1.6 ± 0.89	4.2 ± 0.83
8	BA(2.22)+2ip(0.24)	60	3.1 ± 0.07	2.2 ± 0.44	5.2 ± 0.83
9	BA(2.22)+NAA(0.27)	80	3.04 ± 0.11	1.6 ± 0.89	4 ± 0.70
10	BA(5.37)	60	3.0 ± 0.15	1.6 ± 0.89	5 ± 0.70
11	BA(5.37)+2ip(0.24)	60	2.88 ± 0.17	2 ± 0.70	4.4 ± 1.14
12	BA(5.37)+NAA(0.27)	60	2.98 ± 0.13	1.4 ± 0.54	5 ± 1.22
13	BA(10.74)	40	3.0 ± 0.12	2 ± 0.70	3.4 ± 0.54
14	BA(10.74)+2ip(0.24)	60	3.02 ± 0.08	2 ± 1	4 ± 1
15	BA(10.74)+NAA(0.27)	40	2.92 ± 0.23	1.6 ± 0.54	4 ± 1.41

*Mean value of growth parameters of 5 plantlets per treatment.

Fig. 1: Micropropagated*D. hamiltonii* plantlets kept for *in-vitro* conservation

Table 2.Comparisons of growth for tissue cultured plantlets kept under standard culture conditions and reduced culture condition after six months.

Sl. No.	MS Media + Growth regulators (mg/l)	Survival %		Shoot length (Mean ± SD)*		No. of shoots/explant (Mean ± SD)*		No. of leaves/explant (Mean ± SD)*	
		a	B	a	b	a	b	a	b
1	BA(0.443)	40	80	3.04±0.11	3.78 ± 0.19	1.4 ±0.54	2.4 ±0.54	4.2 ± 0.8	4.6 ±0.54
2	BA(0.443)+2ip(0.24)	60	80	3.0 ± 0.15	3.72 ±0.27	2.6 ±0.54	2.6 ±0.54	4 ± 1	4.4 ±0.54
3	BA(0.443)+NAA(0.27)	20	60	3.08±0.08	3.56 ±0.25	1.6 ±0.89	2 ±0.70	4.8 ± 0.83	5.2 ±0.83
4	BA(0.886)	40	80	3.0 ± 0.12	3.74 ±0.24	1.6 ±0.54	2.4 ±1.14	3.8 ± 0.44	5 ±0.70
5	BA(0.886)+2ip(0.24)	80	100	3.24±0.11	5.08 ±0.29	2.6 ±0.54	3.4 ±0.54	5.4 ± 1.34	6.6 ±0.54
6	BA(0.886)+NAA(0.27)	60	100	3.08±0.08	4.6 ±0.38	1.8 ±0.44	2.2 ±0.83	5.2 ± 0.83	5.2 ±0.44
7	BA(2.22)	40	60	2.98±0.08	3.92 ±0.22	1.6 ±0.89	1.8 ±0.83	4.2 ± 0.83	4.6 ±0.89
8	BA(2.22)+2ip(0.24)	60	80	3.1 ± 0.07	4.78 ±0.19	2.2 ±0.44	3 ±0.70	5.2 ± 0.83	5.4 ±0.54
9	BA(2.22)+NAA(0.27)	80	80	3.04±0.11	4.46 ±0.19	1.6 ±0.89	1.6 ±0.54	4 ± 0.70	4.6 ±0.89
10	BA(5.37)	60	100	3.0 ± 0.15	3.84 ±0.18	1.6 ±0.89	1.6 ±0.89	5 ± 0.70	4.4 ±0.54
11	BA(5.37)+2ip(0.24)	60	100	2.88±0.17	4.06 ±0.15	2 ± 0.70	1.6 ±0.54	4.4 ± 1.14	4.8 ±0.83
12	BA(5.37)+NAA(0.27)	60	80	2.98±0.13	4.04 ±0.15	1.4 ±0.54	2 ±1	5 ± 1.22	5 ±1.4
13	BA(10.74)	40	80	3.0 ± 0.12	3.94 ±0.16	2 ± 0.70	1.8 ±0.83	3.4 ± 0.54	5.4 ±0.54
14	BA(10.74)+2ip(0.24)	60	60	3.02±0.08	4.12 ±0.19	2 ± 1	1.8 ±0.83	4 ± 1	4.8 ±0.83
15	BA(10.74)+NAA(0.27)	40	80	2.92±0.23	3.92 ±0.14	1.6 ±0.54	1.6 ±0.54	4 ± 1.41	4.8 ±1.0

* Mean value of growth parameters of 5 plantlets per treatment.

a: plantlets kept in reduced culture condition

b: tissue cultured plantlets kept under standard culture conditions.

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