High frequency regeneration of plantlets from leaf derived callus cultures of *Tylophora indica* Burmf. An important medicinal plant

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Abstract — *In vitro* clonal propagation of medicinal plants enables large scale production of therapeutically high value for commercialization. An efficient protocol is developed for the rapid *in vitro* multiplication of an endangered medicinal plant *Tylophora indica* (Burm.f) via callus from leaf explants collected from one year old plant. Low concentration of BAP +2, 4-D (1.0mg/l each) favoured green callus induction after 16 days of culture. When the green callus was cultured on MS medium supplemented with 1.0mg/l BAP + 2.0 mg/l L-glutamic acid and 3.5% sucrose, developed shoot buds after 4 weeks. The same cultures were allowed for 2nd passage to develop large number of plantlets after three weeks. Such plantlets were separated and allowed for rooting on half strength MS medium supplemented with 4.0mg/l IBA.

Index terms — Organogenesis, Passages, Regeneration, Standardization, Subculture, *Tylophora indica*.

1 INTRODUCTION

Tylophora indica (Burm.f) Merrill, previously called as *Tylophora asthmatica* a member of Asclepiadaceae, now merged in Apocynaceae is an important indigenous medicinal plant found is restricted locations in Indian subcontinent. The roots have a sweetish taste. The plant is used as folk remedy in certain regions of India for treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis [1]. The powdered leaves and roots contain the alkaloid tylophorine [2] and Tylophorinine. The roots also contain a potential anticancer alkaloid Tylophorinidine [3]. Due to non availability of sufficient quality planting material, commercial plantation of this important aromatic and medicinal species have not been widely attempted and presently only the wild population is exploited for extraction purpose. The lack of cultivation produces and the indiscriminate way in which this plant is collected from its natural habitat pose a serious threat to its existence in the wild. The propagation in its natural habitat is a rare phenomenon evidenced by close field observation. Propagation either by seeds or vegetative cutting is rather difficult. Stem cuttings failed to produce proper root when treated with different growth regulators [4]. Plant Tissue culture is alternative for its propagation to shorten the long sexual cycle and other problems like limited seed availability and problem of seed physiology. It is difficult to collect the seed as they are dispersed by wind on attaining maturity. Reports on Regeneration are limited [5], [10], [11].So here we have developed an efficient and high frequency regeneration plantlets from the Leaf derived callus and direct regeneration from leaf explant. This is highly useful for commercial exploitation of this medicinally important plant.

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2 MATERIAL AND METHODS

Healthy leaves of *T .indica* were collected from one year old plant in the University campus. The leaves were cut into small pieces and washed under running tap water for 10 minutes and surface sterilized with 0.1% $Hgcl_2$ solution for 6minutes and finally rinsed 4-5 times with sterile distilled water. Leaf explants were inoculated MS medium fortified with varied concentration of Auxins, cytokinins and L-Glutamic acid and for the induction of callus, callus growth and regeneration of plantlets and direct regeneration of plantlets. All the cultures were incubated under 2000lux of light for a photoperiod of 12 hrs for callus induction and16 hrs for regeneration of plantlets at 25 ± 2^0c.temperature.

3 RESULTS AND DISCUSSION

In the present investigation the pieces of leaf explants were inoculated on MS medium fortified with various concentrational combinations of 1 mg/l 2, 4-D + 1.0 mg/l BAP, for the induction of callus was observed after 20 days of culture, the callus growth was promoted on the same medium after I passage of cultures on the same medium. Lower concentrations of both the hormones did not promote callus induction, however by increasing the concentration of both the phytohormones promoted in the induction of green compact callus favoured the result (Table-1).

Table – (1) Effect of 2, 4-D and BAP combination for the induction of callus from leaf explant of *Tylophora indica* Roxb.

S.No	MS medium + 2,4-D + BAP (mg/l)	Callus morphology
1	0.5 + 0.5	No response
2	1.0 + 0.5	Scanty friable callus
3	1.5 + 0.5	White friable callus
4	2.0 + 0.5	Light green compact callus
5	2.5 + 0.5	White friable and nodular callus
6	3.0 + 0.5	White nodular callus
7	0.5 + 1.0	White compact callus
8	1.0 + 1.0	Green compact callus
9	1.5 + 1.0	Green compact callus
10	2.0 + 1.0	White compact callus
11	2.5 + 1.0	White friable callus
12	3.0 + 1.0	White friable callus

*Data was collected after five weeks of culture.

The obtained callus was green and compact, optimum for organogenesis and the growth of the callus was optimized on the same medium after I subcultured on MS medium supplemented 1.0 mg/l 2, 4-D + 1.0 mg/l BAP 3.0% sucrose after 28 days of subculture. The callus culture turned to green and compact in the centre and light, green in the periphery. When the green compact callus was cultured on MS medium fortified with different concentrational combinations of BAP and L-glutamic acid started to initiate regeneration. The lower concentration of L-glutamic acid promoted less number of shoot bud differentiation as concentration of L-glutamic acid was increased from 0.5mg/l to 2.0mg/l significantly enhanced the regeneration of plantlets (Table - 2).

Table – (2) Regeneration of plantlets from leaf derived callus of *Tylophora indica* on MS medium with BAP + L-glutamic acid combination (mg/l)

MS medium with growth regulators (mg/l) BAP + L-glutamic acid	% of cultures showing response	Number of shoots/explant (Mean±SE)	Length of shoot (cm) (Mean±SE)
0.5 + 1.0	5	5.5 ± 0.4	5.04 ± 0.24
1.0 + 1.0	5	6.1 ± 0.6	4.56 ± 0.16
1.5 + 1.0	60	9.0 ± 0.5	4.46 ± 0.15
2.0 + 1.0	70	10.1 ± 0.3	5.38 ± 0.23
2.5 + 1.0	60	8.2 ± 0.5	5.24 ± 0.19
3.0 + 1.0	50	5.2 ± 0.5	5.14 ± 0.20
0.5 + 2.0	65	8.0 ± 0.4	5.11 ± 0.22
1.0 + 2.0	60	10.2 ± 0.7	4.92 ± 0.23
1.5 + 2.0	70	16.1 ± 0.4	4.32 ± 0.22
2.0 + 2.0	90	18.0 ± 0.4	4.44 ± 0.20
2.5 + 2.0	80	15.0 ± 0.7	4.50 ± 0.31
3.0 + 2.0	60	12.2 ± 0.6	4.01 ± 0.22

*Data was collected after 4 weeks of culture

MS + 2.0 mg/l BAP + 2.0 mg/l L- glutamic acid was proved the best for high frequency of regeneration in the present investigation (fig. A-F). Among all the tested concentrations of sucrose, 35% of sucrose is suitable for the enhancement of shoot buds. These buds later transformed in to healthy shoots with a maximum of 14.24± 0.87 shoots per culture, which is much higher than the previous reports [15].The advantageous role of BAP alone for satisfactory. Shoot bud differentiation was reported by Failsal and Anis 2005 in

Tylophora indica, in the higher concentration of BAP, the number of shoots were decreased [16] in *Tylophora indica* recorded that the higher concentration of BAP had an inhibitory effect on shoot bud formation. The synergistic effect of BAP and Auxins has been demonstrated in many medicinal plants of Asclepiadaceae family such has *Gymnema Sylvestre* [6], *Holestemma annulare* [8], *Hemidesmus indicus* [7] and *Ceropegia candelabrum* [9]. In accordance with these reports the present investigation has been show the low concentration of BAP in Combination with L- glutamic acid on shoot bud differention and BAP L-glutamic acid showed synergistic influence in high frequency regeneration (Table-2; fig. C, D)

In our results a novel method is used to make the rapid regeneration of plantlets by using aminoacid like L-glutamic acid. The previous reports related to the aminoacid like L-glutamic acid, urea and alanine served as reliable substitutes for the induction of somatic embryogenesis [19]. Aminoacid like proline is known to enhance somatic embryogenesis in maize [14] and pollens somatic embryogenesis in cereals [17], while tryptophan favoured somatic embryogenesis in some cultures of rice [18]. MS medium containing BAP (2.0mM), NAA (1.5mM), PVP (25mM) and Glycine (25mM) also induced number of shoots from cotyledon (15.4/ explant) and embryonal axis (12.2/ explant) but the response was lower than other aminoacid studied in the present investigation [20]. IAA or IAA- Glycine, IAA- Phenylalanine, IAA- amine, IAA-aspartic acid at concentration of 0.5Mm induced on shoot but regeneration leaf disc of peanut [13].

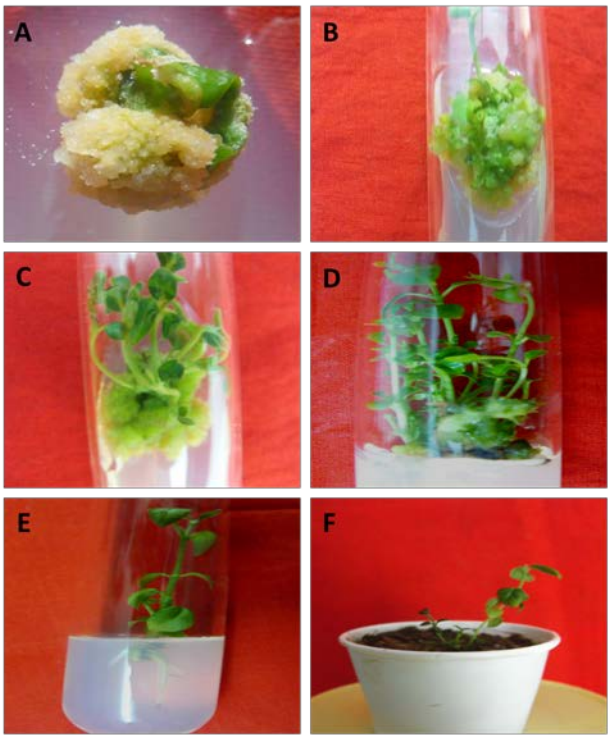

Fig. Regeneration of plantlets from leaf explants of *Tylophora indica*.

A. Induction white friable callus on MS + 1.5 mg/l 2, 4-D + 0.5 mg/l BAP.
B. Induction Green compact callus on MS +1.5 mg/l 2, 4-D +1.0 mg/l BAP.
C. Regeneration of plantlets on MS + 1.0 mg/l BAP + 1.5 mg/l L-glutamic acid.
D. High frequency regeneration of plantlets on MS + 1.0 mg/l BAP +2.0 mg/l L-glutamic acid.
E. Rooting of plantlets on ½ streghth + 4.0 mg/l IBA.
F. Hardening of plantlets of *Tylophora indica*

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