Plant Let Regeneration from Leaf Explants through Organogenesis in Bitter Melon (Momordica Charantia L.)

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Abstract

Efficient plant regeneration via organogenesis was established using leaf explant. Callus cultures from the leaf explants were tested for growth and organogenic capacity on MS medium fortified with different concentrations and combination of 2,4-D with BAP and 2,4-D with TDZ. The maximum morphogenic callus induction rate (65%) was observed from leaf explant by culturing in MS medium supplemented with 2.5 mg/L 2,4-D + 3.0 mg/L BAP when compared to 2.5 mg/l 2,4-D + 3.0 mg/l TDZ (63%). High frequency shoot regeneration (75%) from leaf derived callus was observed on MS medium supplemented with 2.5 mg/l 2,4-D and 3.0 mg/l TDZ. At the end of 3 weeks the regenerated shoots were transferred on the same medium (MS + 2.5 mg/l 2,4-D + 3.0 mg/l TDZ) for further proliferation and elongation. The regenerated shoots were rooted with high frequency (60%) in MS medium supplemented with 1.5 mg/L IBA when compared to other auxin NAA. The in vitro raised plantlets were successfully established in green house and transplanted to natural conditions with 70% survival.

Keyword: *Tables-2 Figures-0 References-24 In vitro, organogenesis, Thiadiazuron, Rhizogenesis, bitter melon.*

1. Introduction

Cucurbits belong to the family Cucurbitaceae and consist of about 118 genera and 825 species, according to the last taxonomic treatment[1] Cucurbits are among the most important plants supplying humans with edible fruits and useful seeds. Plants of this family are very similar in above ground development, but they have high genetic diversity for fruit, shape and other characteristics, resulting in a variety of uses. The most important cultivated genera bitter gourd (*Momordica charantia* L.) is the summer vegetable grown extensively throughout the country and covers an area of about 5697 ha with an annual production of about 52099 tons in the country[2] which serve as the main source of nutrition, energy, valuable vitamins and minerals. A good micropropagation protocol could reduce the cost of hybrid seed production, which can account for 30% of the total seedling cost. The commercial application of *in vitro* techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported from excised cotyledons [3,4,5,6,7and 8] leaf explants[9,10,11 and 8] The present communication describes *in vitro* multiple shoot

regeneration from Leaf explants, and the rooting and successful greenhouse establishment of bitter melon.

2. Material and Methods

The seeds of Bitter melon (*Momordica charantia.* L.) were collected from Agriculture Research Institution Karimnagar (A.P) India. These seeds were washed in running tap water for three minutes and then washed repeatedly in double distilled water Bitter melon (*Momordica charantia.* L.) Now under aseptic conditions the seeds were surface sterilized with 70% ethanol for one minute followed by a twenty minute treatment with 2% sodium hypochloride and washed with sterilized triple distilled water five times followed by 0.1% Mercuric chloride ($HgCl_2$) for five minutes and rinsed five times in sterile distilled water. The sterilized seeds were then placed on MS basal medium[12] solidified with 0.8% bacto agar for germination in 250 ml culture bottles, 20 seeds were cultured per bottle containing 30 ml of medium. This was incubated in dark at 26^oC till it germinated and then transferred to cool-white-fluorescent light room and incubated at 24$\pm$2^oC and allowed to grow. The plant after reaching a height of 6 centimeters was taken in an aseptic condition the leaf explants were excised using a sterile scalpel and cut into 6-8 mm sections.

Sterilized leaf explants were cultured on MS medium supplemented with various concentrations of 2, 4-D (1.0-4.0mg/L) with BAP (0.5-6.0mg/L) and 2,4-D(1.0-4.0mg/L) with TDZ(0.5-6.0mg/L) (Table 1). After 3 weeks, efficient callus was induced and sub-cultured into fresh media with various concentrations and combinations of 2,4-D with BAP and TDZ for developing potentially organogenic nature (Table 1). Nodular and friable calli are potentially organogenic and were sub cultured for adventitious shoot bud induction and plantlet regeneration. The regenerated shoots were sub cultured onto the same shoot induction medium after 21-28 days for shoot proliferation and elongation. *In vitro* raised micro shoots after attaining a height of 1-1.5 cm were transferred to MS medium fortified with different concentrations of IBA and IAA for root induction (Table 2).

3. Results

The leaf induced efficient callus on MS medium containing 2.5 mg/l 2,4-D + 3.0 mg/l BAP and 2.5 mg/l 2,4-D + 3.0 mg/l TDZ respectively (Plate I, Fig 1 & 2 and Table 1). Highest growth response was obtained with 2,4-D and TDZ (63%) than with 2,4-D and BAP (65%). Levels above or below this gradually decreased the frequency of callus induction. After 3 weeks, the actively growing callus were sub cultured on fresh medium of culture on the same composition of medium enhanced peripheral greening of callus inducing shoot buds (Plate I, Fig 3). Combination of 2,4-D and TDZ showed low response to callus formation compare to 2,4-D and BAP showed best results from leaf explants culture. The calli derived from leaf explants were best for regeneration and were sub cultured on MS medium with 2.5 mg/L2,4-D + 3.0 mg/L TDZ and 2.5 mg/L 2,4-D + 3.0 mg/L BAP. After 2 weeks shoot buds from green callus were regenerated to plantlets (Plate I, Fig 4).

The combination of 2.5 mg/L 2,4-D and 0.5 mg/L TDZ is effective and induced minimum percentage of (36%) in plantlet formation when compared to 2.5 mg/L 2,4-D + 1.5 mg/L BAP (55%) (Table 1). The percentage of culture response in inducing callus and regeneration from callus derived from leaf is low when the concentration of increased to BAP/TDZ up to 3.0mg/L shoots formation were increased then 3.0 to 6.0mg/L BAP/TDZ shoots formation were decreased. The regenerated micro shoots were sub cultured on the same composition of medium for further shoot proliferation and elongation.

3.1 Rooting of shoots

Micro shoots (3-4 cm) developed from leaf regenerated were excised and cultured on MS medium

supplemented with (0.5-2.0mg/L) IAA and (0.5-2.0mg/L) IBA. Profuse rhizogenesis was observed on (1.0 mg/L) IAA compared to (1.0 mg/L) of IBA alone is most potential in inducing high percentage (70%) of rooting with highest number of roots per shoot (3.2±0.38) when compared to IBA (1.0 mg/l) induced (54%) of rooting with highest number of roots (2.8± 0.87) per shoot (Table 2). Most of the shoots had produced roots within 2 weeks after placing on rooting medium.

Figure1: Plantlet induction of Leaf explants through organogenesis of *Momordica charantia.*

L a) Initiation of callus from leaf explants on MS+ 2.5mg/l 2,4-D+3.0mg/L BAP b) Greening of callus derived from leaf explants on MS+ 2.5mg/l 2,4-D+3.0mg/L TDZ c) Multiple shoot buds developed on callus culture of leaf on MS+ 2.5mg/l 2,4-D+3.0mg/L TDZ d) In direct shoots formation on MS+ 2.5mg/l 2,4-D+3.0mg/L TDZ after six weeks

3.2 Acclimatization of the plantlets

After *in vitro* rooting the regenerated plantlets were taken out and were washed carefully to remove agar and then transferred to pots containing sterile vermiculite. Each pot was enclosed in a polyethylene bag after watering and maintained in a plant growth chamber at 25±1°C under 16-h illumination with fluorescent lamps. Bags were progressively opened weekly. After 3 weeks of field. The percentage of survival was found to be 70% and the plants were morphologically identical to the acclimatization, plantlets were transferred to large pots filled with garden soil and farmyard manure (1:1) in the open parental plants. In all experiments a minimum of three plates were cultured. Each single treatment consisted of five to ten explants per plate. Data recorded at 3 weeks included the number of shoots per explants, length of shoots and rooting were statically analyzed using one way analysis of variance.

Table-1: Frequency of callus induction and shoot regeneration from Leaf explants of Bitter melon (*Momordica charantia.* L) On MS medium with different concentration of auxin and cytokinin.

Hormone cone (mg/L)	% of cultures responding	% of Shoot regeneration
2.4–D+BAP		
1.0 +0.5	28.0	16.0
1.5+1.0	35.0	20.0
2.0+2.0	45.0	25.0
2.5+3.0	65.0	36.0
3.0+4.0	48.0	23.0
3.5+5.0	32.0	15.0
4.0+6.0	20.0	12.0
2.4–D+TDZ		
1.0 +0.5	36.0	18.0
1.5+1.0	42.0	24.0
2.0+2.0	55.0	28.0
2.5+3.0	63.0	40.0
3.0+4.0	50.0	26.0
3.5+5.0	45.0	17.0
4.0+6.0	32.0	14.0

Table-2: Rooting ability of regenerated shoots from Leaf explants culture of Bitter melon (*Momordica charantia.* L) Cultured on MS medium supplemented with IAA and IBA.

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	2.3 ± 0.37
1.0	-	70	3.2 ± 0.38
2.0	-	60	2.6 ± 0.38
-	0.5	44	1.3 ± 0.36
-	1.0	54	2.8 ± 0.87
	2.0	50	2.0 ± 0.36

* Mean ± Standard Error

4. Discussion

In our study, high rate of callus growth was induced on MS medium containing 2-4-D and BAP than 2,4-D and TDZ. 2,4-D is widely used for *in vitro* callus induction in a wide range of plant species. Combination of 2.5 mg/L 2,4-D + 3.0 mg/L BAP induced potentially organogenic callus from leaf explant. Levels above or below this gradually decreased the frequency of callus induction. Similar results were reported previously in *Cucurbit pepo* L.13 Moreover, there is a report of induction of organogenic calli using a combination of BAP and NAA in *Citrullus vulgaris*14. Friable and creamy calli derived from leaf explants were sub cultured on (2.5 mg/L) 2,4-D + (3.0 mg/L) TDZ are suitable for acquiring green granular organogenic callus with subsequent shoot bud induction after 3 weeks. In *Cucumis metuliferus* and *Cucumis figrei*, highest regeneration frequency (92.5%) was achieved under the combination of (1.0 mg/L BAP and 0.2 mg/L TDZ) 15 and 16.

Regeneration of plantlets was observed on the same medium after four weeks of subculture. Organogenic response in Cucurbitaceae is highly genotype dependent. An expressive organogenic response in cotyledon explant from *Momordica charantia.* L using only BA as growth regulator was

reported 17. Also, organogenesis depends on the endogenous concentration of plant growth regulator. An adverse effect of prolonged *in vitro* cultures reduce shoot organogenesis was reported 18 and 19. Shoot bud proliferation is satisfactory by cytokinin BAP (1.0 mg/l) alone in *Momordica charantia.* L 20. Among different concentration it was concluded that (2.5 mg/L 2,4-D) with (3.0 mg/L) TDZ was impressive and best suitable phytohormones for shoot regeneration from leaf explants of *Momordica charantia.* L*.* Further an increase or decrease of this hormone level showed a negative trend in multiple shoot formation. In our studies, TDZ at low concentration (0.5 mg/L) increased shoot differentiation and marked effect on the quality of regenerated plants when compared to BAP. At higher level of TDZ lead to undifferentiated hard green callus development. In the present investigation rooting occurred in all concentrations, but with different rooting percentages.

Highest numbers of roots were produced at (2.0 mg/L) IBA and (1.5 mg/L) IAA. When exposed to high concentration above (3.0 mg/L) IBA/ IAA shoots become necrotic, lost leaves and the shoot tips died gradually. While at lower below (1.0 mg/L concentration of IBA and IAA low frequency number of roots was induced. The present study reveals that auxin, IBA is better than IAA in inducing rooting ability. Among all plants growth regulators, IBA is widely used for root induction in Cucurbits 21, while IAA is also used22. Efficient rooting was achieved in *Trichosanthes dioica* at different concentration if IBA (0.5 mg/L) and NAA (2.0 mg/l) 23. Variation in rooting response may be a result of genotype or culture conditions. Subsequently, the rooted plantlets were removed from agar medium, washed thoroughly and placed in soil pots after 2 weeks for acclimatization and initial hardening under culture room conditions. Almost 70% of these regenerants survived and developed new branches and were ready for planting in the field for further growth.

The importance of *Momordica charantia.* L*.* as a medicinal cucurbit is growing up substantially with increasing and stronger reports in support of its multifarious therapeutic uses. Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Greater demand for these plants especially for the purpose of food and medicine is one of their rapid depletion from primary habits 24. In the present study, shoot induction and plantlet regeneration from leaf explant is the best and first report to our knowledge on large scale multiplication in a short period of time for conservation of medicinally important species *Momordica charantia.* L*.*

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