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Tissue culture of *Momordica charantia* L.: A review

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Abstract: Plant tissue culture is the technique to culture plant cells or tissues under controlled aseptic conditions on a synthetic medium .It has value in basic research like cell biology, genetic transformation studies &biochemistry for the production of medicinally valuable secondary metabolites. Besides this also has commercial application. This review work outlines the work done on the tissue culture of Momordica charantia L. *Momordica charantia L.* commonly known as bitter melon/gourd, a member of Cucarbitaceae, is a slender, tendril climbing, annual vine. Bitter melon is a common food item of the tropics and is used for the treatment of cancer, diabetes, AIDS and many ailments. It is a potent hypoglycemic agent and its hypoglycemic actions for potential benefit in diabetes mellitus are possible due to at least three different groups of constituents in bitter melon. These include alkaloids, insulin like peptides, and a mixture of steroidal sapogenins known as charantin. Clinical studies with multiple controls have confirmed the benefit of bitter melon for diabetes. Alpha and beta momarcharin are two proteins found in bitter melon, which are known to inhibit the AIDS virus.

Keywords: Tissue Culture, *Momordica charantia*, Hypoglycemic, *In Vitro* Regeneration

1. Introduction

Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (*in vitro*).It has applications in research and commerce. In commercial settings, tissue culture is primarily used for plant propagation and is often referred to as micro propagation [1]. Another way to define the plant tissue culture is the culture of plant cells or plant tissues in a synthetic culture medium under controlled aseptic conditions is called 'Tissue culture'. The controlled conditions give the culture a suitable microenvironment for the successful growth [2] Plant tissue culture now has direct commercial applications as well as basic research into cell biology, genetics and biochemistry. The techniques include culture of cells, anthers, ovules and embryos, protoplast isolation and fusion, cell selection merited and bud culture on experimental to industrial scales [3]. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation which include-

1. The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits, to produce mature plants and multiples of plants in the absence of seeds or necessary pollinators to produce seeds.

2. Regeneration of whole plants from plant cells that have been genetically modified.

3. Production of plants from seeds that have very low chances of germinating and growing i.e. Orchids and nepenthes. Other applications like germplasm maintenance, hybrid productions for incompatible species, production of haploid plants etc [4-5].

There are different types of plant tissue culture ,callus cultures, cell suspension cultures [6(BotinoPJ,1981) , protoplast culture, Explants culture, microspore culture, embryo culture, ovary culture, root cultures, shoot tip and meristem culture, anther and pollen culture[7], mass cell culture, organ culture[8], nucellus culture[9].

Modern plant tissue culture is performed under aseptic conditions under filtered air .Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting material (explants) in chemical solutions (usually alcohol or bleach) is required[10]. Mercuric chloride is seldom used as a plant sterilant today as it is dangerous to use and difficult to dispose off. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and

plant hormones .Solid media are prepared from liquid media with the addition of gelling agent, usually purified agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explants. For example, an excess of auxin (Indoleaetic acid, Naphthalene acetic acid)by stimulating cell expansion, particularly cell elongation promote the proliferation of roots, while an excess of cytokinins (Zeatin, Kinetin) may yield shoots by producing two immediate effects on undifferentiated cells: the stimulation of DNA synthesis and increased cell division[11]. A balance of both auxin and cytokinin will often produce an organized growth of cells, or callus because both cell division and cell expansion occur in actively dividing tissue ,but morphology of the outgrowth will depend on the plant species as well as medium composition .As cultures grow pieces are typically sliced off and transferred to new media (sub-cultured) to allow for growth or to alter the morphology of the culture .As shoots emerge from a culture ,they may be sliced off and rooted with auxin to produce plantlet ,when mature can be transferred to potting soil for further growth in the greenhouse as normal plants[12].

2. Plant Profile

2.1. Botanical Description

Momordica Charantia L. commonly known as bitter melon, balsam pear, bitter cucumber, or bitter gourd, karela (India), fukwa (China), and ampalaya (Philippines) . It is a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne on the leaf axils native to eastern India and southern China and cultivated widely throughout the world. The textured leaves look as though a bite has been taken from them giving the plant its Latin name Momordica which means to bite. The orange fruits are soft when ripe and inside black seeds have a red covering. These warty, cucumber-shaped fruit, which varies in length from 12 to 30 centimeters, harvested while immature, before the seeds harden. The young fruit is emerald green, turning to orange-yellow when ripe. At maturity, the fruit splits into three irregular valves that curl backwards and release numerous reddish-brown or white seeds encased in scarlet arils. Momordica charantia is used as a food, bitter flavoring, and medicine. The plant grows well in a variety of soils, and begins flowering about one month after planting.

2.2. Habitat

Found throughout the tropics. Bitter melon grows in tropical areas, including parts of the Amazon, East Africa, India, Asia, and the Caribbean.

2.3. Biological Name

Momordica charantia L.

2.4. Family

Cucurbitaceae

2.5. Common Name

Bitter Melon, Balasam Pear

2.6. Part Used

2.6.1. Fruit

2.6.1.1. Main Preparation Method

Fruit Juice

2.6.1.2. Active Components

Mixture of steroidal sapononins known as charantin, insulin like peptides and alkaloids. These three different groups of constituents have been reported to have hypoglycemic or other actions of potential benefit in diabetes mellitus. Two proteins, known as alpha and beta momarcharin, inhibit the AIDS virus [13-20].

2.6.1.3. Medical Potentialities

Bitter melon is reported to help in the treatment of Diabetes, HIV support, Psoriasis, High cholesterol and triglyceride levels, high pylori ulcers, Bitter digestive aid for intestinal gas blotting, stomachache and sluggish digestion, Intestinal parasites.

2.6.1.4. Properties/Actions Documented By Research

Hypoglycemic, hypocholesterolemic (lowers cholesterol), antibacterial, carminative (expels gas), bitter, Abortive, contraceptive, antimicrobial

2.6.2. Leaf/Stem

2.6.2.1. Main Preparation Method

Leaf decotation, Capsules

2.6.2.2. Main Actions

Ant cancerous, antiviral, antibacterial, digestive stimulant, hypoglycemic

2.6.2.3. Medical Potentialities

Helps in the treatment of Cancer, Viral infections (HIV, herpes, Epstein Barr, hepatitis, influenza and measles), Bacterial infections (Staphylococcus, Streptococcus and Salmonella), Bitter digestive aid (dyspepsia and sluggish digestion), Diabetes.

2.6.2.4. Properties Actions Documented by Research

Antibacterial, anti-inflammatory, antimalarial, antiparasitic, antiseptic, bitter carminative, digestive stimulant, febrifuge (reduces fever), hyposensitive (lowers blood pressure) lacto ague (promotes milk flow), menstrual stimulator, purgative, vermifuge (expels worms), wound healer.

2.6.3. Other Uses

Fruits are eaten as vegetables and are a good source of Vit C, VitA, phosphorus and iron.

3. Plant Tissue Culture of *Momordica Charantia L.*

3.1. Establishment of in Vitro Regeneration System of Bitter Melon (Momordica Charantia L.)

Manye et al (2004) [21] reported Production of three types of callus from M.charantia seedling: green, yellow green and fragile yellow. They showed that in the process of callus differentiating adventitious bud, the kind, proportion and quantity of phytohormone and the type of callus made different result. The adventitious buds were induced successfully on MS medium + 6-BA (N_6-benzyladenine) 4.0 mg l_{-1} + KT(kinetin) 2.0 mg l_{-1}) by yellow green callus. The frequency was about 66.7 %. Yellow callus did not differentiated adventitious bud. The frequency of green callus differentiation was very low (< 15.0 %) even under the most suitable conditions. The MS + ZT (zeatin) 5.0 mgl_{-1} + KT 0. 5 mgl_{-1} medium was suitable for the proliferation of bud and three weeks later, the coefficient of proliferation was about 5-6. The ½ MS + ZT 0.02 mgl_{-1} or ½ MS media were suitable for in vitro rooting of shoot, the shoot on them could produce 6 - 7 new roots in three weeks. After plantation test, the survival rate of tube plantlets was about 70% and their characteristics were the same as those from seed by field test.

Wang et al(2008)[22] established in vitro plant regeneration system from cotyledon node of *Momordica charantia* L. with different genotype including Dading, Yingyin and Lubaoshi as tested materials, and showed the effect of different seedling age, combinations of different hormones and their concentration ratio, as well as different genotypes on the generation of morphogenesis in vitro of *M.charantia* . When the *M.charantia* explants was used for inducing directly the generation of adventitious bud, the influence of seedling age was the biggest. The seedlings of *M.charantia* cultured for about 10 d were optimal for inducement of multiple shoot.6-BA, ZT and NAA showed significant induction effect on clustered shoots, and the good induction effect could be attained only at the low concentration ratio, with better induction effect in MS+6-BA 0.5 mg/L+NAA 0.2 mg/L and MS+ZT 2.0 mg/L+NAA 0.1 mg/L. Although there was some difference in clustered shoots induction of *M.charantia* with different genotype, the difference was less, and effective seedling rate of Lvubaoshi was the highest. The judgment of explants seedling age by integration of cultivated days and cotyledon color can be helpful to select appropriate explant for high-effective clustered shoots, induction of *M.charantia*.

3.2. In vitro Plant Production through Apical Meristem Culture of Bitter Gourd (Momordica Charantia L.)

Huda et al(2006)[23] observed growth of meristem on semisolid MS medium [24]supplemented with 0.05 mg/l Kn + 0.1 mg/l GA_3. After three weeks, meristems were transferred to MS medium supplemented [28] with BA, Kn, IBA, NAA and IAA singly or in combination for shoot elongation and root initiation. Among different treatments for shoot initiation with elongation were obtained in MS supplemented with 1.0 mg/l BA + 0.1 mg/l IBA + 0.3 mg/l GA_3. On the other hand good rooting was observed when 0.5 mg/l IBA and 0.1 mg/l NAA were used to fortify MS semisolid medium. Ten weeks old in vitro plantlets were successfully planted in soil through gradual acclimatization.

3.3. In vitro Plant Regeneration from Direct and Indirect Organogenesis of Momordica Charantia L.

3.3.1. In Vitro Regeneration

Malik et al(2007)[25] analyzed the effect of plant growth regulators on callogenesis and direct and indirect organogenesis of *Momordica charantia* and reported Callus formation from leaf, stem and cotyledonary explants of *Momordica charantia*, at different auxin and cytokinin concentrations and combinations in MS medium. The best callogenic response was observed from all three explants (leaf, stem and cotyledon) on MS medium supplemented with 1.0 and 1.5 mg L^{-1} BAP with 1.5 mg L^{-1} NAA and 1.0 mg L^{-1} 2,4-D, respectively. 2,4-D, a sole PGR for callus induction produced maximum callus from all three explants (leaf, stem and cotyledons) but failed to produce callus at low level (0.1 and 1.0 mg L^{-1}). However, leaf explants showed maximum callus percentage and callogenic response then other two explants; stem and cotyledons, when tested on MS medium supplemented with BAP, NAA and Kin. The callus produced at these PGRs concentrations including 2,4-D, were of different texture and morphology depending upon nature of PGR. At 2,4-D all concentrations the calluses were brownish to yellowish green but compact and hard. These totipotent cells were failed to give rise shooting response when transferred to same or different growth regulator containing medium as second subculture. Indirect organogenesis response was very low or absent due to hardening of callus and habituation. Momordica callus did not produce any shoot at any concentration/combination tested. At few combinations (BAP with TDZ or NAA), only leaves were formed that were lush green in color. At all concentrations tested the callus turned hard and embryogenic but failed to produce shoot. Cotyledonary node explants showed comparatively low response on same hormonal combinations giving maximum regeneration frequency 50.0% that was 100.0% in case of shoot tip explant.

Shoot tip explant showed best shooting response at BAP with NAA (1.0+0.2 mg L^{-1}) supplemented in MS medium where average number of shoots per flask were 2.75±0.71 in which shoots attained maximum length of 1.74±0.69 cm. BAP with TDZ also exhibited good shooting response with average number of shoots 1.50±1.69 and length 0.800±0.899 cm. However at BAP alone shooting response was low. Best shooting was observed at 1.0 mg L^{-1} BAP + 0.1 TDZ and 1.5 mg L^{-1} BAP + 0.2 mg L^{-1} NAA from shoot tip and cotyledonary node explants.

While root formation was achieved when generated shoots were transferred to MS medium both full and half strength supplemented with different auxin concentrations.

Best rooting response from generated shoots was observed

at half strength of MS medium supplemented with 0.5 mg L^{-1} NAA with 2.63±1.30 average number of roots giving 87.5% response and half strength MS without any growth regulator that gave 100% rooting response.

3.3.2 Callogenesis

Thiruvengadam *et al*. (2006)[26] stated that MS medium containing 1.0 mg L^{-1} 2,4-D approximately 90% of leaf explants of *Momordica charantia* L. gave rise to a well organized friable calli. At different concentrations of BAP and Kn green, compact and hard calluses produced. These calluses turned to be embryogenic under the stress of PGR. Berg *et al*. (1997)[27] reported BAP as sole plant growth regulator, successfully preferred for good texture callus development. Nabi *et al*. (2002) [28]found that for teasel gourd (*Momordica dioica* Roxb) callogenesis combination of 1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA was most suitable that produced soft, light green and friable calli.

3.3.3. Direct and Indirect Shooting Response

Shooting response is dependent upon concentration of cytokinin supplemented in the medium. Cytokinin work as signaling molecules that activate totipotent cells of callus for shoot organogenesis where as in the case of direct organogenesis, these molecules activate preexisting machinery in the case of somatic cells (leaf, stem, cotyledon etc.) while in the case of shoot apex they stimulate the growth due to presence of meristemic cells at the tip of explant. These cytokinins may also turn the explant to produce multiple shooting response.

Pierik (1987)[29] stated that cytokinins are often used to stimulate growth and development, Kn and BAP being in common use. They usually promote cell division especially if added together with an auxin. At higher concentrations they can induce adventitious shoot formation by decreasing apical dominance and they retard aging. Cotyledonary node explants showed comparatively low response on same hormonal combinations giving maximum regeneration frequency 50.0% that was 100.0% in case of shoot tip explant.

Agarwal and Kamal (2004)[30] observed shoot differentiation in *Momordica charantia* when alone BAP was used. They also obtained good results on NAA in combination with IBA. Sultana and Bari (2003)[31] obtained similar results as that of present study. According to them best response towards multiple shoot regeneration was obtained from the nodal segments of *Momordica charantia* L. on MS medium supplemented with BAP and NAA. Hoque *et al*. (1995)[32] also found that BAP and NAA as the best combination for adventitious multiple shoot formation in teasle gourd.

Agarwal and Kamal (2004)[30] and Sultana and Bari (2003)[31] showed that full strength MS medium 0.1 mg L^{-1} NAA gave maximum rooting response giving 1.38±1.06 average roots per flask. Acclimatization percentage was 62.5%.

The endogenous and exogenous level of growth regulators is important for callogenesis and for differentiation. But habituation property of callus hinders the organogenic response in *Momordica charantia*.

3.4. High-Frequency Shoot Regeneration from Leaf Explants through Organogenesis in Bitter Melon (Momordica Charantia L.)

Thiruvenagadam et al (2010) [33] gave an efficient protocol for in vitro organogenesis was achieved from callus-derived immature and mature leaf explants of *Momordica charantia*, a very important vegetable and medicinal plant. Calluses were induced from immature leaf explants excised from in vitro (15-day-old seedlings) mature leaf explants of vivo plants (45 days old). The explants were grown on Murashige and Skoog (MS) medium [24]with Gamborg (B5)[34] vitamins containing 30 g l^{-1} sucrose, 2.2 g l^{-1} Gelrite, and 7.7 μM naphthalene acetic acid (NAA) with 2.2 μM thidiazuron (TDZ). Regeneration of adventitious shoots from callus (30–40 shoots per explant) was achieved on MS medium containing 5.5 μM TDZ, 2.2 μM NAA, and 3.3 μM silver nitrate ($AgNO_3$). The shoots (1.0 cm length) were excised from callus and elongated in MS medium fortified with 3.5 μM gibberellic acid (GA_3). The elongated shoots were rooted in MS medium supplemented with 4.0 μM indole 3-butyric acid (IBA). Rooted plants were acclimatized in the greenhouse and subsequently established in soil with a survival rate of 90%. This protocol yielded an average of 40 plants per leaf explant with a culture period of 98 days.

3.5. Additives Promote Adventitious Buds Induction from Stem Segments of Bitter Melon (Momordica Charantia L.)

Tang et al(2011)[35] investigated the effects of thidiazuron (TDZ), silver nitrate ($AgNO_3$) and triacontanol on adventitious buds induction from stems of balsam pear (*Momordica charantia* L.)and found that TDZ was necessary for bud development and the higher concentration of it could induce adventitious buds efficiently, while 0.1 mg/L was the best concentration to induce adventitious buds considered effect and cost. Bud formation was significantly affected by $AgNO_3$ and triacontanol. Best results were obtained at concentration of 2.0 mg/L separately

3.6. In vitro Regeneration from Internodal Explants of Bitter Melon (Momordica Charantia L.) Via Indirect Organogenesis

3.6.1. In vitro Regeneration

Triruvenagadam et al (2012)[36]reported induction of organogenic callus and high frequency shoot regeneration internodal explants of bitter melon. About 97.5% of internodal explants derived from 30 day old *in vivo* grown plants produced green, compact nodular organogenic callus in Murashige and Skoog (MS)[24] plus Gamborg et al. (1968) [34](B_5) medium containing 5.0 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 μM thidiazuron (TDZ) after two successive transfers at 11 days interval. Adventitious shoots were produced from organogenic callus when it was transferred to MS medium supplemented with

4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine with shoot induction frequency of 96.5% and regeneration of adventitious shoots from callus (48 shoots per explant). In this investigation, L-glutamine is the best for induction of shoots as compared to L-asparagine. Their was requirement of high auxin : low cytokinin ratio for callus induction and low auxin : high cytokinin ratio for shoot induction from callus. Shoot proliferation occurred when callus with emerging shoots was transferred in the same medium at an interval of 15 days. The regenerated shoots were elongated on the same medium. The elongated shoots were rooted in MS medium supplemented with 3.0 µM indole 3-butyric acid (IBA). Rooted plants were acclimatized in green-house and subsequently established in soil with a survival rate of 95%. This protocol yielded an average of 48 shoots per internodal explant after 80 days of culture.

3.6.2. Callus Induction

Nabi et al. (2002)[28], Devendra et al. (2009)[37] and Selvaraj et al. (2006)[38] found that the combination of BAP with NAA or 2,4-D produced organogenic callus in *Momordica dioica* and *Cucumis sativus*. The combination of 7.7 µM NAA with 2.2 µM TDZ produced greenish compact callus from leaf explants of *M. charantia*. Handley and Chambliss (1979)[39] reported that NAA and Kn combination in MS medium produced nodular compact callus in cucumber. Selvaraj et al. (2006)[38] obtained nodular, greenish compact and organogenic callus in the presence of 2,4-D and BAP for hypocotyl explants of cucumber. Punja et al. (1990)[40], Seo et al. (2000)[41] and Selvaraj et al. (2007)[42] reported callus formation in cucumber cultivars in the combination of NAA and BAP for petiole, leaf and cotyledon explants, respectively.

3.6.3. Adventitious Shoot Formation

Savitha et al. (2010)[43] reported that MS medium with 2.5 mg/l 2,4-D and 0.5 mg/l TDZ produced high frequency shoot regeneration from leaf derived callus of *Citrullus colosynthis*.

TDZ is more effective in shoot regeneration as compared to BAP. The effectiveness of TDZ over other cytokinins has also been reported in other cucurbits such as *Cucurbita pepo* (Pal et al., 2007)[44], *C. colosynthis* (Savitha et al., 2010)[43] and *Melothria maderaspatana* (Baskaran et al., 2009)[45]. MS medium containing 4.0 µM TDZ and 1.5 µM 2,4-D and various concentrations of L-asparagine and L-glutamine significantly increased adventitious shoots from internode derived callus (Table 3). Selvaraj et al,2007[42] reported NAA (1.34 µM), BAP (8.88 µM), zeatin (0.91 µM) together with L-glutamine (136.85 µM) produced large number of shoots in cucumber . Addition of L-glutamine in the adventitious shoot regeneration medium greatly enhanced the production of shoots from callus [45-46]).

Adding nontoxic glutamine (Gamborg et al., 1968)[34] to the medium maintains a high growth rate of cells for a longer period. Locy and Wehner (1982)[47] demonstrated that L-asparagine was the best nitrogen source for the growth of cucumber shoots. The highest number of shoots (34.5 shoots) was produced on MS medium containing 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-asparagine . Malepszy and Nadolska-Orezyk (1983)[48], Bergervoet et al. (1989)[49] and Trulson and Shahin (1986)[50] advocated repeated subcultures of callus to obtain high frequency shoot regeneration in cucumber. Cucumber regeneration via organogenesis showed the production of 36 shoots per explant [42].

3.6.4. Rooting and Acclimatization

The effectiveness of IBA in rooting has been reported in *M. dioica* [51] [28], *Benincasa hispida* [52] and *M. maderaspatana* [45]. In contrast, NAA have been used for *in vitro* rooting in *Cucurbita pepo* [53]. Thiruvengadam et al. (2006)[30] and Han et al. (2004)[54] reported that IAA is the best for root induction in *M. dioica* and *Lagenaria siceraria*.

3.7. In vitro Induction of Multiple Buds from Cotyledonary Nodes of Balsam Pear (Momordica Charantia L.)

3.7.1. In vitro Induction of Multiple Buds

Chou et al(2012)[55]studied the effects of different genotypes of Balsam pear, hormone combinations, seedling ages, dark period and $AgNO_3$ concentrations on the bud regeneration from cotyledonary nodes of Changbai, Dabai and Youlv Balsam pear (*Momordica charantiaL.)*

The results showed that eight-day old seedling was the best for multiple buds regeneration. There were significant differences in the induction rate of the different pear seedling ages. Shoot regeneration frequency in eight-day old seedlings is higher than in six- and 10-day old seedlings. The induction rate is 78.63%. In addition, the induction rate of multiple buds is high and the regenerated seedlings are vigorous when the seedling age is eight-day-old.

The combination of 6-benzylaminopurine (6-BA) and indole-3-butyric acid (IBA) had the best effect on the induction of multiple buds. The optimum medium for the induction of multiple buds in Balsam pear was Murashige and Skoog (MS) medium [24]supplemented with 2.5 mgL^{-1} of 6-BA and 0.1 mgL^{-1} of IBA. The cotyledonary nodes of Balsam pear are cultured on a medium and they begin to turn green and swell after two to three days. Callus appears at the lower edges of the cotyledonary nodes and multiple buds also appear after a week. Multiple buds can be induced on all kinds of media. Auxins and cytokinins play a great role in promoting the induction of multiple buds, particularly the effect of 6-BA.

The average of multiple buds was highest (up to 4.42) when explants were cultured on MS medium supplemented with 2.0 mgL^{-1} 6-BA and 0.1 mg L^{-1} NAA, but the buds were too weak and yellowish; it was difficult to elongate them. The multiple buds were not only large in number, but also vigorous when explants were cultured on MS medium supplemented with 2.5mg L^{-1} 6-BA and 0.1 mg L^{-1} IBA . In addition, the induction rate of these different hormone types and proportions of MS medium reached as high as 90.26%. So these different hormone types and proportions of MS medium were the optimum medium in the regeneration of

Balsam pear. The phenomenon of crisped-leaf, expanded stem, and vitrifycation occurred commonly when the MS medium was supplemented with high concentration of 6-BA. However, a low concentration of 6-BA (1.0 mgL^{-1}) was good for the elongation of buds when they were moved to the MS medium supplemented with it. Different hormone types and proportions had a great influence on the induction of multiple buds in this experiment. Inducing frequency varied with the genotypes, of which Changbai showed highest percentage of induction. They concluded that there were significant differences in the induction rate of the three Balsam pear cultivars in the experiment conducted. The induction rate of Youlv Balsam pear is highest (up to 80.65%), followed by the induction rate of Changbai Balsam pear (78.79%). The induction rate of Dabai Balsam pear was found to be the lowest (7.74%). However, the average multiple buds of Changbai were greater than that of the other Balsam pear cultivars. We also found that buds of "Changbai" Balsam pear are vigorous and easy to grow and it is the optimum genotype in the three varieties of Balsam pear.

One week dark treatment could increase the rate of shoot regeneration from 76.91 to 80.91%. The addition of $AgNO_3$ which varied from 1 to 6 mg L^{-1} in the medium could not help to stimulated shoot regeneration.

When the regenerated buds reached a height of 2 to 3 cm, the individual buds were carefully excised and transferred to different rooting media for proper rooting. In this experiment, IBA was effectively used as a root inducing hormone. The best root development was obtained in the rooting medium containing a half strength of MS medium with 0.4 mg/L IBA (Table 6) and the rooting rate reached 80 to 90% . About two weeks was required to get healthy root formation

3.7.2. Effect of Seedling Age on Induction of Multiple Buds

Seedling age is reported to be an important factor for obtaining high frequency adventitious shoot regeneration and the research results are diverse [31][22][56-57]. The seedling stage determines the physiological state of explants. Different physiological states of explants obviously, affect shoots regeneration capacity of explants [58-59]. Therefore, the seedling stage is a key success factor of *in vitro* Balsam pear regeneration.

3.7.3. Effect of Genotype on Induction of Multiple Buds

Many experimental results demonstrate the effect of genotype in the regeneration capacity of other plants in the Cucurbitaceae family [57] [60]. In Balsam pear tissue culture, genotype also has a considerable impact on the organogenic response [22][61].

3.7.4. Effect of Hormone Types and Proportion on Induction of Multiple Buds

The dedifferentiation and redifferentiation of plant cells is highly affected by the different plant hormone types and proportion, especially the proportion of auxins and cytokinins. Although some progress in regeneration improvement of Balsam pear has been made, the studies on the various hormones and proportion are diverse [22] [30] [31] [61] [62].

3.7.5. Rooting

Rooting of regenerated buds is important for establishing tissue culture derived from plants. It is generally accepted that the high rate of auxin / cytokinin helped to regenerate roots. On the contrary, the low rate of auxin / cytokinin helped to regenerate buds. IBA was found to be effective in the induction of root in different plants like *Momordica charantia* L. [30],) and *Limonium altaica* L.[63].

3.8. In Vitro Flowering of Bitter Melon

Wang et al (2001)[64]observed that flowers were formed from shoot tips of bitter melon (*Momordica charantia* L.) cultured on Murashige and Skoog[24] medium supplemented with 90 m*M* sucrose, 0.05 m*M* Fe^{2+} and 4 µ*M* N^6-benzyladenine (BA). The addition of 0.05 m*M* Fe^{2+} to the medium prevented chlorosis of the explant and promoted normal flowering. Increasing the ratio of carbon to nitrogen promoted male flower formation but intensively inhibited vegetative growth. The influence of cytokinin on the morphogenesis of the explants was highly notable. Flowers could be formed after a 15- to 20-day exposure to kinetin (Kin) or BA. Kin and BA had opposite effects with regard to the development of the explants. Kin promoted flower formation, especially female, but inhibited branch bud formation. Conversely, BA promoted branch bud formation and also promoted male flower formation when present at a concentration of 1–2 µ*M*, but completely inhibited flower formation at 4–8 µ*M*. Fluoresce in di-acetate staining and in vitro germination showed that in vitro pollen were of a fairly high viability.

3.9. Micro Propagation of Momordica Charantia L.

Mishra et al(2012)[65]elucidated the potential of *Momordica charantia* L. for *in vitro* propagation studies .They established the in vitro development of *Momordica charantia* on MS medium (Murashige and Skoog's)[28]which gives(100%) germination of the seeds with normal hypocotyals using different concentrations of auxins and cytokinins. maximum seed germination was observed in basal MS media and multiplication of shoot, callus formation and rooting of shoot was observed in the presence of MS+BAP+IBA in *Momordica charantia* L. Shoot apex (1.5cm-2.0cm)long were inoculated on MS medium augmented with different concentration of BAP(1mg/l,2mg/l,4mg/l)showed good number of shoots. *In vitro* shoot segments were inoculated on MS medium with different concentrations of IBA(0.1mg/land 1mg/l)showed massive rooting with slight callusing in two weeks.

3.10. Development of an Embryogenic Suspension Culture of Bitter Melon (Momordica Charantia L.)

Thiruvengadam et al (2006)[66]optimized a system for the somatic embryogenic suspension culture of bitter melon (Momordica charantia L.).Friable calli could be induced in 30-day-old leaves on semi-solid MS medium (Murashige & Skoog, 1962) supplemented with1.0mg/l2,4-D.Large number

of globular embryos(24.6%)were noticed when the calli was sub-cultured in liquid medium containing 1.5mg/l 2,4-Dthe complete removal of 2,4-D in the later stages of culture, stimulated their further development to heart and torpedo stages. Microscopic examination revealed the ontogeny of somatic cell development via the formation of cell clusters, which then enlarged to pro-embryos, and give rise to heart and torpedo stages within a period of 2 weeks, somatic embryos successfully germinated on agarified MS medium with no additional growth hormones. An effect of media, other components and stimulating factors such as carbohydrates, amino acid was also evaluated for somatic embryogenesis. The full strength MS medium containing 50mg/l PVP and 40mg/l glutamine was effective to achieve a high frequency of somatic embryo induction, maturation and further development .An average of 6.2% young plants was achieved from friable callus, and was phenotypically normal. This will be a step further to facilitate genetic transformation.

3.11. Establishment of Momordica Charantia Hairy Root Cultures for the Production of Phenolic Compounds and Determination of Their Biological Activities

Thiruvengadam et al (2014)[67]established a protocol for the production of secondary metabolites from hairy root cultures of Indian *Momordica charantia* (IMC) and Korean *M. charantia* (KMC) along with their biological activities (antioxidant and antimicrobial activities) . The explants (leaves, cotyledons, hypocotyls, roots, and nodes) from in vitro seedlings were inoculated with *Agrobacterium-rhizogenes* strains (KCTC 2703 and KCTC 2704) for the induction of hairy roots. Established transgenic clones of hairy roots were confirmed by polymerase chain reaction (PCR), reverse transcription-PCR and sequencing using *rolC* specific primers. Hairy roots cultured in MS liquid medium supplemented with 3 % sucrose showed the highest accumulation of biomass [95.11 g/l fresh mass (FM) and 10.61 g/l dry mass (DM) in IMC and 93.58 g/l FM and 10.12 g/l DM in KMC]. The flask cultures of IMC and KMC increased in their biomass up to 9.6- and 9.4-fold after 20 days of culture. MS basal liquid medium supplemented with 3 % sucrose was superior for the growth of hairy roots compared with other culture media evaluated (B5, NN and N6), for biomass and phenolic compounds production. Phenolic compounds increased in hairy roots than in untransformed control roots. The antioxidant and antimicrobial activities were increased with hairy roots compare to untransformed roots. In terms of biomass, phenolic compounds and biological activities the hairy roots of IMC were superior to KMC was observed. This is the first report describing the production of phenolic compounds and biological activities from hairy root cultures of *M. charantia*.

4. Conclusion & Future Prospects

Medicinal plants and its products continue to be an important therapeutic aid for alleviating the ailments of humankind M.charantia has been used as dietary supplements and ethanomedicine throughout centuries for relieving symptoms and conditions related to diabetes. To date, M.charantia has been extensively studied worldwide for its medicinal properties to treat a number of diseases. It is described as a versatile plant worthy of treating almost any disease inflicted on mankind. This may be due to the fact that the plant possesses over 225 different medicinal constituents. These different compounds may act either separately or together to exert their medicinal effects.

Advances in biotechniques, particularly methods for culturing plant cell cultures should provide new means for the commercial processing of even rare plants and the chemicals they provide .The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products .The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions. The use of in vitro plant cell culture for the production of chemicals and pharmaceuticals has made great strides building an advances in plant science. The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolites will provide the basis for the production of commercially acceptable levels of product. The increased level of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvest has renewed interest in large-scale plant culture technology. Knowledge of biosynthetic pathways of desired phytochemicals in plants as well as in culture is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. These results show that in vitro plant cell cultures have potential for commercial production of secondary metabolites. The introduction of newer techniques of molecular biology, so as to produce transgenic cultures and to effect the expression and regulation of biosynthetic pathways, is also likely to be a significant step towards making cell cultures more generally applicable to that commercial production of secondary metabolites.

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