Int.J.Curr.Microbiol.App.Sci (2020) *9*(8): 2887-2910

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 8 (2020)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2020.908.324

Advances in Tissue Culture of Cucurbits: A Review

Sangram S. Dhumal[1*], B. Veerendra Naik[1] and Mansinghraj S. Nimbalkar[2]

[1]Department of Horticulture, Rajarshee Chhatrapati College of Agriculture, Kolhapur-416 004, Maharashtra, India (Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India)
[2]Department of Botany, Shivaji University, Kolhapur-416 004, Maharashtra, India

**Corresponding author*

Keywords

Tissue culture, Cucurbits

Article Info

Accepted:
22 July 2020
Available Online:
10 August 2020

A B S T R A C T

Cucurbitaceae is the one of the elite family in the plant kingdom and have importance in its daily utilization for cuisine preparation as a source of vegetable and medicinal plant. This family consists of hundreds of edible species, qualitative and quantitative improvement plays a vital role in the processing industry and Indian medicine system of Ayurveda (AYUSH). Plant tissue culture techniques have been used extensively for propagation of cucurbitaceae by using various explants and methods from last few decades. This review aims to describe and list all the major findings related with the tissue culture of cucurbitaceae.

Introduction

Crops belongs to Cucurbitaceae are generally known as cucurbits or gourds. The family Cucurbitaceae is largest among the known vegetables comprising of 117 genera and includes 825 species in tropical parts of the world. It includes the cucumber, Squashes, Pumpkin, Luffa, Melons, Watermelon, Spine gourd, Sweet gourd, Bottle gourd, Sponge gourd, Snake gourd, Pointed gourd etc. It is widely distributed around the tropics. It is also listed in the earliest cultivated plantsof old and new world for the edible fruits and vegetables. It consists of wide range of vegetables which can be used in various purposes such as, salad (cucumber), for cooking (all type of gourds), pickling (gherkins), as a dessert food (Musk melon and watermelon) and as a candy (ash gourd). The Cucurbitaceae family consists of widely spread and genetically diverse group of plants. It occupies largest area through out the world. This genetically diversified group of plant includes traditional cultivars, landraces, edible as well as nonedible wild and cultivated forms, weedy species and related non-edible wild species.

Its use is important because of some vital minerals, calories, or vitamins. The most of the cucurbits generally contain low to moderate nutrients, however few exceptions like Pumpkin (Vit-A, 1600 IU/100g), Bitter

gourd (rich in Vit-C, 96mg/100g), Kakrol (High protein, 3.1 g/100g) are also reported. Moreover, the cucurbit seeds more valued for their protein and high oil contents. Seed proteins which are rich in methionine, are comparable with the legumes. Cucurbit crops are very important for small land holding farmers and this is cash crop for several rural families. In Tropical countries, a number of minor and major cucurbits are cultivated as a popular kitchen gardening crop and considering its crop duration it is also included in cropping system a cash crop.

The large-scale production of sex specific plants in cucurbits using the conventional propagation methods has several limitations. These limitations have forced many scientists to look forward towards tissue culture because of its immense potential in efficient clonal propagation. Improvement of plant species via biotechnological approach depends largely on plant tissue culture. Micropropagation helps to overcome the problems in conventional method of propagation in great extent and systematic improvement is boon for Horticulture, pharmaceutical industry and Ayurveda, high multiplication ratio achieved rapid multiplication of disease and pest free elite plant within short span of time and space (Ghive. 2006). The major advantage of getting unlimited planting material can be achieved using *in-vitro* propagation, irrespective of season of growing. The better genetic upgradation is possible using non-conventional approaches such as plant tissue culture. Its application mainly depends on a reliable and successful plant regeneration system. Many scientists have successfully developed micropropagation protocol for the commercial production of many crops including cucurbitaceous vegetables. The purpose of this review article is to present the recent advancements, current status and developments in micropropagation techniques in cucurbitaceous crops. It also focuses on the increasing collective interest in the search of new protocols and major findings of non-conventional techniques of propagation in cucurbitaceous vegetable crops.

Traditional methods of propagation in cucurbits

Seed

At present most of the cucurbits are propagated by seeds like water melon, cucumber, Luffa, squashes *etc.* but using of seed for propagation in most of the crop is shows the late germination and uneven germination, some may remain in soil as it is due to dormancy.

Cuttings

Using of the cutting is the only way of propagation in some species of cucurbits like pointed gourd and ivy gourd, while using cuttings major problem is the less sprouting and rooting.

Tubers

Underground storage organs like tubers also used for propagation in Spine gourd and other crops but main threat in using of tubers is low rate of multiplication and improper establishment in the field.

Strategies for tissue culture

Micropropagation

Micropropagation have been attempted by using apical bud, axillary bud and cotyledon in various crops like *Momordica dioica* (Kulkarni. 1999, Choudhary *et al.,* 2017, Ghive *et al.,* 2006[b], Jamatia. 2016, Karim and Ullah. 2011, Arekar. 2012, Mustafa *et al.,* 2012, Jadhav. 2015, Shekhawat *et al.,* 2011,

Govind *et al.*, 2012, Thiruvengadam *et al.*, 2012 and Kapadia. 2018),*Momordica sahyadrica* (Rajashekharan *et al.*, 2012), *Cucumis melo* (Venkateshwaralu. 2012, Parvin *et al.*, 2013, Huda and Sikdar. 2006, Faria *et al*., 2013, Keng and Hoong. 2005, Venkateshwaralu *et al.*, 2010, Randall *et al.*, 1989.), *Trichosanthesdioica* (Abdul–awal *et al.*, 2005, Komal. 2011[c], Malex *et al.*, 2010), *Cucumis sativus* (Mohammadi and Siveritepe. 2007, Ahamad and Anis. 2005, Kielkowska and Havey. 2011), *Cucumis sativus* by using MS + Kinetine (6µm) (Sangeetha *et al.*, 2011, Firoz Alam *et al.*, 2015), *Cucurbita maxima* (Mahzabin. 2008), Watermelon (Khalekuzzaman *et al.*, 2012, Li *et al.*, 2011, Khatun *et al.*, 2010[b], Suratman, 2009, Chaturvedi and Bhantnagar. 2001), *Trichosanthescucumerina* (Devendra *et al.*, 2008, Kawale *et al.*, 2009.), *Benincasa*hispida (Kausar *et al.*, 2013, Haque *et al*., 2008), *Cucumis hystrix* (Compton *et al.*, 2001), Momordica charantia (Verma *et al.*, 2014, Sultan. 2005, Sultana. 2003.), *Cucumis anguria* (Margareate, 2014), *Momordica balsamina* (Thakur *et al.*, 2011), *Sechiumedule* (Abdelnour *et al.*, 2002), *Citrullus colocynthis*. (Rama Krishna and Shashtri. 2014), *Luffa acutangula* (Zohura *et al.*, 2013)), *Cucurbita ficifolia* (Kim *et al.*, 2009).

Somatic embryogenesis

The many pioneer scientists are worked on cucurbits with an objective to explore the potentiality of somatic embryogenesis, *viz*., *Cucurbita pepo* (Paula. 1992), *Momordica dioica* (Hoque *et al.*, 2007 and Karim and Ahamad, 2010) with a highest percentage of callus in internodal explants, *Cucumis sativus* (Hisajima and Arai. 1989,Elmeer *et al.*, 2009 and Usman *et al.*, 2011), *Cucumis melo* (Gray *et al.*, 1993), *Cucurbita moschata* (Valdez-Melara *et al.*, 2009), *Momordica charantia* (Thiruvengadam *et al.*, 2006), *Cucurbita pepo*cv. YC60 (Paula *et al.*, 1990) and *Momordica dioica* (Raju *et al.*, 2015).

Organogenesis

Many scientists have also worked on direct and indirect organogenesis in order to produce callus in cucurbits. The organogenesis in *Momordica dioica* was studied by Nabi *et al.*, (2002[a]), Swamy *et al.*, (2015), Devendra (2009), Nabi *et al.*, (2002[b]), Karim (2013), Hoque *et al.*, (2000), Karim (2011), Patel (2015), Debnath (2013), Mustafa *et al.*, (2012) and Thiruvengadam *et al.*, (2007). Thiruvengadam *et al.*, (2012) used MS and Gamboge + NAA (3.0µm) + TDZ (1.0µm) + Putrecine (1.0µm) to induce the callus in *Momordica dioca.* Similarly organogenesis was studied in *Luffa cylindrical* by Srivastava and Roy. 2012, Han *et al.*, 2004 and in *Citrullus lunatus* by Sultana (2004) Vedat Pirinc *et al.*, 2002, Khatun *et al.*, 2010[a] and Compton and Grey (1992)who developed the triploid water melon. The scientists Krug *et al.*, 2005 used the coconut water along with media to induce the good callus in watermelon. The organogenesis was also reported in *Momordica charantia* (Saima malik. 2007), *Citrullus colocynthis* (Shasthree *et al*2014), *Trichosanthesdioica* (Sourab *et al.*, 2017), *Cocciniaabyssinica* (Guma *et al.*, 2015), *Cucumis melo* (Rahaman *et al.*, 2012), *Cucumis trigonus* (Satapathy *et al.*, 2014), *Citrullus colocynth* (Savitha *et al.*, 2010), *Luffa acutangula* (Umamaheshwari *et al.*, 2014, Vellivella. 2016,Moideen and Prabha. 2014). Moideen and Prabha (2013) concluded that best callusogenesis response in *Luffa acutangular* was observed in media treated with 2, 4–D + TDZ-2.0mg/l. The effect of commercial fruit juices on callus induction in *Cucumis sativus* was also studied by Ikram-ul Haq *et al.*, (2013). The organogenesisin *Cucumis sativus* was also reported by Selvaraj *et al.*, (2006), Jesmin and Mian (2016).

Similarly it is also reported in *Cucurbita pepo* (Pal *et al.,* 2007), *Lagenariasiceraria* (Hasbullah *et al.,* 2007), *Benincasahispida* (Thomas *et al.,* 2004), *Cucumis figarei* and *Cucumis metuliferus* (Yutaka *et al.,* 1998), *M omordica cochinchinensis* (Debnath *et al.,* 2013) and *Momordica cymbalarias* (Devi *et al.,* 2017).

Other

Thiruvengadam *et al.,* (2006), optimized a somatic embryogenesis system using embryogenic suspension culture in bitter melon. In Spine gourd, Thiruvengadam *et al.*(2013), evaluated an efficient method of somatic embryogenesis using exogenous polyamines through suspension culture. Ghive *et al.,* (2006) reported the highest survival and establishment rate in spine gourd with healthy shoots on its own root systems. Thiruvengadam *et al.,* (2013) achieved somatic embryogenesis from cell suspension cultures in *Cucumis anguria*. While Claveria *et al.,* (2005) concluded that homozygous doubled haploid lines in cucumber were helpful to breed resistant varieties. Agrobacterium mediated genetic transformation had been also carried out in several crops viz., *Cucumis melo* (Chovelon. 2008, Bezirganoglu *et al.,* 2014), *Cucumis sativus* (Nanasato *et al.,* 2013), *Citrullus colocynthis* (Dadauza *et al.,* 1997) and Sponge gourd (Singh *et al.,* 2011).

Choice of explant

Apical bud

Apical bub is the one of standardized explant for the *in-vitro* propagation. Several scientists have tried apical bud as an explant in cucurbitaceous crops in their investigations *viz*., *Benincasa hispida* (Haque *et al.,* 2008, Kausar *et al.,* 2013), *Citrullus lanatus* (Compton and Grey. 1992, Khalekuzzaman *et al.,* 2012, Vedat *et al.,* 2002), *Cucumis hystrix* [Compton *et al.,* 2001 got the successful plantlet using combinations of growth regulators like MS + Sucrose (30g) + myo-inositol (0.1g) + Agargelplus (5g) + IBA (1.7µM) + Kinetin (0.5µM) + GA3 (0.3µM)], *Cucumis melo* (Faria *et al.,* 2013, Huda and Sikdar 2006, Venkateshwaralu. 2012), *Cucumis sativus* (Mohammadi and Siveritepe. 2007, Sangeetha *et al.,* 2011), *Cucurbita maxima* (Mahazabin. 2008), *Cucurbita pepo* (When most of the scientists used the apical bud for the micropropagation, Paula *et al.,* (1990) reported somatic embryogenesis by apical bud), interspecific *Cucurbita* hybrid (Sarowar *et al.,* 2003), *Trichosanthesdioica* (Abdul-Awal *et al.,* 2005) and *Trichosanthes cucumerina* (Devendra *et al.,* 2008).

Axillary bud

The use of axillary bud was reported in *Citrullus lanatus* (Khatun *et al.,* 2010[b]), *Cucumis anguria* (Margareate, 2014), *Cucumis melo* (Parvin *et al.,* 2013), *Cucumis sativus* (Ahamadand Anis. 2005, Firoz Alam *et al.,* 2015), *Cucurbita maxima* (Hoque *et al.,* 2008), *Momordica balsamina* (Thakur *et al.,* 2011), *Momordica charantia* (Sultana *et al.,* 2003, Sultana *et al.,* 2005, Verma *et al.,* 2014), *Momordica cymbalarica* (Devi *et al.,* 2017), *Momordica dioica* (Choudhary *et al.,* 2017, Debnath, 2013, Ghive *et al.,* 2006[b], Govind *et al.,* 2012, Jadhav, 2015, Kapadia, 2018, Kulkarni, 1999, Mustapha *et al.,* 2012, Mustapha *et al.,* 2013, Patel and Kalpesh, 2015, Shekhawat *et al.,* 2011.), *Trichosanthes dioica* (Komal. 2011[a], Komal 2011[b], Komal 2011[c]). Venkateshawaralu *et al.,* 2010 used BAP 1 and 2 mg however Keng and Hoong 2005 used BAP 8.0 mg and found good result of plant initiation by using axillary bud in *Cucumis melo*. A good percentage of callus was obtained from the axillary buds in *Momordica cochinchinensis* in MS agar gelled + 2, 4-D (2mg) + Coconut milk (15% v/v) (Debnath *et al.,* 2013).

Leaf

Citrullus colocynth (Devendra, 2009; Guma *et al.,* 2015), *Citrullus lanatus* (Moideen and Prabha, 2013), *Cocciniaabyssinica*(Raju *et al.,* 2015 reported molecular confirmation of sex by leaf explant), *Cucumis anguria* (Saima malik *et al.,* 2007), *Cucumis melo* (Satapathy *et al.,* 2014), *Cucumis sativus* (Savitha *et al.,* 2010), *Cucumis trigonus* (Shashtree *et al.,* 2014), *Luffa acutangula* (Sourab *et al.,* 2017), *Luffa cylindrical* (Srivastava and Roy. 2012), *Momordica charantia* (Sultana *et al.,* 2004, Swamy *et al.,* 2015), *Momordica dioica* [(Thiruvengadam *et al.,* 2006,Usman *et al.,* 2011)], *Trichosanthesdioica* (Rahaman *et al.,* 2012). In *Momordica dioica,* Thiruvengadam *et al.,* 2013, found somatic embryogenesis in MS media supplemented with 2, 4-D (3.3µm) + Putrecine (0.5µm) using leaf as an explant.

Cotyledon

Beninca sahispida (Thomas *et al.,* 2004), *Citrullus colocynth*(Rama Krishna and Shashtri. 2015, found the best results for rhizogenesis by cotyledon explant), *Citrullus lanatus* [(Suratman *et al.,* 2009, Dadauza *et al.,* 1997, Khatun *et al.,* 2010a, Krug *et al.,* 2005, Li *et al.,* 2011)], *Cucumis figarei* (Yutaka *et al.,* 1998), *Cucumis melo* (Chovelon *et al.,* 2008, Grey *et al.,* 1993, Bezirganoglu *et al.,* 2014, Randall *et al.,* 1989), *Cucumis metuliferus* (Yutaka *et al.,* 1998), *Cucumis sativus* (Yutaka *et al.,* 1998, Nanasato *et al.,* 2013, Hisajima and Arai. 1989), *Cucurbita ficifolia* (Kim *et al.,* 2010), *Cucurbita moschata* (Valdez-Melara *et al.,* 2009), *Cucurbita pepo* (Paula. 1992), *Lagenaria siceraria* (Han *et al.,* 2004), *Luffa acutangula* (Umamaheshwari *et al.,* 2014), Zohura *et al.,* (2013), *Luffa cylindrical* (Singh *et al.,* 2011), *Trichosanthescucumerina* (Kawale and Choudhary, 2009), *Trichosanthes dioica* (Malex *et al.,* 2010) and in *Momordica dioica* (Hoque *et al.,* 2000, Karim, 2013, Karim and Ullah. 2011, Nabi *et al.,* 2002[a], Nabi *et al.,* 2002[b] and Karim. 2011). All the scientists used cotyledon as an explant in Spine gourd on a MS media supplemented with BAP 1.0µm +NAA0.1µmhoweverArekar (2012), used BAP (4.44 and 8.88µm). Chaturvedi and Bhantnagar, 2001, used MS + BAP (3.0µM) + 2iP (3.0µM) and showed best result in *Citrullus colocynth* using cotyledon explant.

Other explants

The other explants used by many scientists include leaf node, somatic embryo, hypocotyle etc. and got success to some extent. A recent work on cucurbits using explants other than leaf, cotyledons, apical and axillary buds is reported hereunder crop wise. Leaf node was used as an explant in *Cucumis melo*by Rahaman *et al.,* 2012. In *Cucumis sativus*, cuttings (Ikram-ul haq *et al.,* 2013), hypocotyle (Selvaraj *et al.,* 2006), parthenogenic embryo (Claveria *et al.,* 2005), somatic embryo (Elmeer *et al.,* 2009) and stem (Jesmine and Mian 2016 and Kiełkowska and Havey, 2011) were used as an explant. The use of hypocotyl as an explant was also recorded in *Cucurbita pepo* (Pal *et al.,* 2007). The stem fragments were used in *Lagenariasiceraria* by Hasbullah. 2017 while in *Luffa acutangula*, Moideen and Prabha (2014) and Vellivella *et al.,* (2016) used petiole as an explant. Similarly, the petiole was also used to get success in *Momordica charantia* by Thiruvengadam *et al.,* (2012). The encapsulated shoot tips (Thiruvengadam *et al.,* 2012), healthy shoots (Ghive *et al.,* 2006[a]), immature embryo (Hoque *et al.,* 2007), internode (Karim and Ahamad. 2010), node and leaf (Jamatia. 2016) and leaf (Thiruvengadam *et al.,* 2007) were used as an explant in *Momordica dioica*. Rajashekharan *et al.,* (2012) used seedling explants in *Momordica sahyadrica* while stem in *Sechiumedule (*Abdelnour *et al.,* 2002) and

cotyledonary nodes in *Trichosanthes cucumerina* (Kawale and Choudhary, 2009).

Effect of growth regulators

Micropropagation

Apical bud

In Spine gourd, Thiruvengadam *et al.,* (2012) developed efficient protocol for in vitro regeneration by using encapsulated shoot tip as an explant. They obtained 100 per cent conversion into plantlets from encapsulated shoot tip explants when placed on 0.5µM BAP supplemented full strength MS containing the 0.7% agar. Hardened and acclimatized plant in field reported the 90 % survival rate and grew well without considerable variation. Kausar *et al.,* (2013) in *Benincasahispida* used shoot tip and node as explant but shoot tip showed the highest rate of multiple shoots at 1.5mg/l BAP + 0.2mg/l $GA_{3,}$ wherenormal number of shoots per culture recorded was 5.55. The lower concentration of GA_3inducd multiple shoots effectively. When Kausar *et al*(2013)used only BAP and GA_3, Huda and Sikdar (2006)used not only BAP and GA3 but also in combination With IBA and found good shoot initiation and elongation. Shoot proliferation rate, shoot quality, and other parameters showed best result at the combination of MS with BAP 0.4µM. The highest rooting frequencies were observed in PGR free medium (Mohammadi and Siveritepe, 2007). In *Trichosanthes* cucumerina, after 4^{th} sub culture maximum number of shoots 12.00±0.70 were recorded at concentration of BAP 1.0mg/l in combination with lower amount of NAA 0.1mg/l. Out of different chemical combinations used 100% multiple shoot formation was noticed in BAP 1mg/l + NAA 0.2mg/l (Devendra *et al.,* 2008, Abdul-Awal *et al.,* 2005). Wherever Shoot tip is used as explant BAP is used up to 3.0mg/l concentration but in *Citrullus lanatus* combination of MS + BAP (5.0) + IAA (0.1) registered maximum frequency (73%) with better growth response. The percentage of successful hardening (72%) from regenerated plantlets was recorded with best survival in the soil condition (Khalekuzzaman *et al.,* 2012). For the induction of the multiple shoots in shoot tip explants MS augmented with IAA (0.5 mgl^{-1}) + BAP (2.0 mgl^{-1}) was proved to be best (Venkateshwaralu. 2012). Efficient cloning of *Cucumis hystrix* was also reported using 1mm shoot-tip explants. Establishment of Stage I cultures was greatest (83%) when shoot tips were cultured on (per liter) 30 g sucrose, 0.1g myo-inositol, and 5g Agargelplus, 1.7µM IBA, 0.5µM kinetin and 0.3 µM GA_3 (IKG). Among all the growth regulators tried, BAP 5µM proved best for Stage II shoot proliferation. It was also observed that plantlet height influenced acclimatization and over 72% of plantlets survived (Compton *et al.,* 2001).

Rajashekharan *et al.,* (2012) conducted investigation on conservation and in-vitro propagation of *Momordica sahyadrica* species. *In-vitro* grown seedlings were selected as explants and cultured on modified MS fortified with the BAP. Shoot and root differentiation was reported on the MS media supplemented with BAP+IBA/NAA. MS media without hormones reported the induction of multiple shoots with good number of roots. Finally 40% of the plants were survived after transplanting to the ex-vitro field condition. Most of the research scientist reported that the BAP in the concentration range of 1.0- 3.0mg gave good results with the shoot tip as an explant in *Cucumis sativus, Cucumis melo, Cucurbita maxima* (Sangeetha *et al.,* 2011,Faria *et al.,* 2013, Mahazabin. 2008) but some scientist were reported that usage of BAP in combination with NAA and IAA in the range of 0.1- 0.5mg helps in establishment of the

plant (Abdul-Awal *et al.,* 2005, Devendra *et al.,* 2008, Khalekuzzaman *et al.,* 2012, Venkateshwaralu, 2012).

Axillary bud

In plant tissue culture technique, most of the axillary buds were used to get multiple shoots due to absence of apical dominance. Most of the pioneer investigators used the BAP in the range of 0.5, 1.0, 1.5, and 2.0 alone or in combination with the different growth regulators for nodal explants (Verma *et al.,* 2014, Ahamad and Anis. 2005,Jamatia. 2016, Choudhary *et al.,* 2017, Margareate. 2014, Thakur *et al.,* 2011, Venkateshawaralu *et al.,* 2010, Jadhav.2015, Khatun *et al.,* 2010[b], Kapadia. 2018, Firoz Alam *et al.,* 2015, Sultana *et al.,* 2005, Hoque *et al.,* 2008, Parvin *et al.,* 2013, Shekhawat *et al.,* 2011, Sultana *et al.,* 2003) but Keng and Hoong (2005)reported that multiple shoots could be induced on MS supplemented with 8.0mg/l BAP in Musk melon cv. Honey dew (*Cucumis melo*). When majority of the scientists reported to use the full strength MS medium for their research purpose, Verma *et al.,* (2014)used half strength MS with 0.5 mg/l BAP in monoecious bitter melon and reported more number of shoots (3.4) after 3[rd] sub culture with shoot length (2.7 cm). Addition of casein hydrolysate 200mg/l to the shoot induction medium (MS + BAP) significantly enhanced the number of multiple shoots in *Cucumis sativus L.* but casein hydrolysate 200mg/l + 0.9µM BAP helped in enhancing the axillary shoot proliferation in case of nodal explants of Spine gourd. Highest number of shoots i.e., 6.2 shoots per explants was recorded with the 100 % shoot regeneration frequency. Especially in case of male genotype CH helped in inducing the callus formation healthy shoots and proved inhibitory action for the shoot length and shoot differentiation (Ahamad and Anis. 2005, Govind *et al.,* 2012). Good amount of compact, green callus and organogenesis is obtained in 2.0 mg/l 2, 4-D + 1.0mg/l BAP in *Momordica dioica* (Mustafa *et al.,* 2012). In *Momordica dioica* itself MS + $AdSO_4$ (70/80) + BAP (1.0) + NAA (1.0) is used to get a maximum number of multiple shoots whereas the highest number of shoots 45.30 ± 3.83 with average length of shoot 6.52±0.89cm were differentiated on MS + BAP (0.5) + IAA (0.1) + Ascorbic acid (50)+ Adenine sulphate, Citric Acid, L-arginine (25), later regenerated plants were evaluated for genetic stability. For this, PCR techniques like RAPD and ISSR were used for the amplification of the micropropagated plants and mother plants which found to be monomorphic in nature depicting the genetic stability of the in-vitro propagated plants (Ghive *et al.,* 2006[b,] Choudhary *et al.,* 2017). In *Cucumis melo var utillisimus*highest concentration of Adenine sulphate (15mg/l) in combination with BAP were found to be best for multiple shoot induction (Venkateshawaralu *et al.,* 2010). In case of *Momordica dioica, Citrullus lunatus* and *Momordica charantia* BAP 1.0 or 2.0 mgl^{-1} in combination with NAA 0.1 or 0.2 mgl^{-1}were used for early shoot initiation, establishment and maximum shoot multiplication with significantly more height and good percentage of acclimatize and successful survival of rooted plants in *ex-vitro* condition (Jadhav. 2015, Khatun *et al.,* 2010[b], Kapadia. 2018, Sultana *et al.,* 2003).

Sultan (2005) used nodal explants of *Momordica charantia* in a media with different levels of pH and agar infused with different concentrations of sucrose. Maximum shoot induction was recorded in medium containing MS+2.0mg/l BAP+0.2mg/l NAA, with 30g/l sucrose, 7 g/l agar and 5.5-6.0 level pH (Table 1).

Table.1

Review of Literatures in table form					
Sr. No.	**Crop**	**Explant**	**Best treatments (mgl^{-1})**	**Result**	**Author**
1	*Sechiumedule*	Stem part	MS + BAP (0.1)	Full plantlet in soil	Abdelnour *et al.*, (2002)
2	*Trichosanthesdioica*	Shoot tip	MS + BAP (1.0) + NAA (0.2)	Full plantlet in soil	Abdul-Awal, *et al.*, (2005)
3	*Cucumis sativus L.*	Node	MS + BAP (1.0 µM) + Casein hydrolysate (200)	Full plantlet in soil	Ahamad and Anis (2005)
4	*Momordica dioica*	Cotyledon	MS + BAP (4.44 and 8.88µm)	Full plantlet in soil	Arekar (2012)
5	*Cucumis melo L.*	Cotyledon	Bacteria concentration of OD_{600} 0.6, inoculation for 30 min,	Genetic transformation	Bezirganogalu, *et al.*, (2014)
6	*Citrullus lanatus*	Cotyledon	MS + BAP (3.0µM) +2iP (3.0µM)	Full plantlet in soil	Chaturvedi *et al.*, (2001)
7	*Momordica dioica*	Node	MS + BAP (0.5) + IAA (0.1) + Ascorbic acid (50) +Adenine sulphate, Citric Acid, L-arginine (25)	Full plants in soil, monomorphic, genetic stability	Choudhary, *et al.*, (2017)
8	*Cucumis melo L.*	Cotyledon	MS + BAP + 2.0-iP	Agrobacterium mediated Genetic transformation	Chovelon, *et al.*, (2008)
9	*Cucumis sativus L.*	Parthenogenic embryo	500 gamma radiation, Co 60 Ý- rays source	Haploid production	Claveria, *et al.*, (2005)
10	*Citrullus lanatus*	Shoot tip	MS + BAP (1.0)	Full plantlet in soil	Compton and Grey (1992)
11	*Cucumis hystrix*	Shoot tip	MS + Sucrose (30 g) + myo-inositol (0.1 g) + Agargelplus (5g) + IBA (1.7µM) + Kinetin (0.5µM) + GA_3 (0.3µM)	Full plantlet in soil	Compton, *et al.*, (2001)
12	*Citrullus lanatus*	Cotyledon	Agrobacterium tumefaciens LBA4404 + vector pBI121 + r gene β–glucuronidase (gus) + neomycin phosphotransferase(nptII)	For transgenic	Dabauza, *et al.*, (1997)
13	*Cucurbitaceae*	Cotyledon		Reviewed somatic embryogenesis	Debeaujon and Brancherd (1993)
14	*Momordica dioica*	Node	MS + 2, 4-D (2.0) + BAP (0.5) / Coconut milk	Organogenesis	Debnath *et al.*, (2013[a])

			(15% v/v).		
15	*Momordica cochinchinensis*	Node	MS agar gelled + 2, 4-D (2.0) + Coconut milk (15% v/v)	Callus	Debnath, *et al.*, (2013[b])
16	*Momordica dioica*	Leaf	MS + 2, 4-D (1.0) + BAP (2.0)	Organogenesis	Devendra *et al.*, (2009)
17	*Trichosanthescucumerina*	Shoot tip	MS + BAP (1.0)+ NAA (0.1)	Full plantlet in soil	Devendra *et al.*, (2008)
18	*Momordica cymbalarica*	Node	MA + BAP(2.0)	Full plantlet in soil	Devi, *et al.*, (2017)
19	*Cucumis sativus L.*	Somatic embryo	Primers (OP-C10, OP-G14, OP-H05, OP-Y03 and OP-AT01)	Genetic stability by RAPD	Elmeer, *et al.*, (2009)
20	*Cucumis melo L.*	Shoot tip	MS + BAP (2.0)	Full plantlet in soil	Faria, *et al.*, (2013)
21	*Cucumis sativus L.*	Node	MS + BAP (1.5)	Full plantlet in soil	Firoz Alam, *et al.*, (2015)
22	*Momordica dioica*	Healthy shoots	MS + IBA (1.0)	Highest percent of rooting	Ghive *et al.*, (2006[a])
23	*Momordica dioica*	Node	MS + $AdSO_4$ (70/80) + BAP (1.0) + NAA (1.0)	Multiple shoots	Ghive, *et al.*, (2006[b])
24	*Momordica dioica*	Node	MS + BAP (0.6µm) + Casein hydrolysate (200)	Assessedgenetic stability by RAPD	Rai, *et al.*, (2012)
25	*Cucumis melo L.*	Cotyledon	MS + 2,4-D (5)+ TDZ (0.1)	Somatic embryogenesis	Grey, *et al.*, (1993)
26	*Cocciniaabyssinica*	Leaf	5% NaOC with 10 Minutes	Sterilization	Guma, *et al.*, (2015)
27	*Lagenariasiceraria*	Cotyledon	MS + BAP (3) +$AgNO_3$ (0.5)	$AgNO_3$ derived plants are diploid	Han, *et al.*, (2004)
28	*Benincasahispida*	Shoot tip	MS + BAP (1.5)	Full plantlet in soil	Haque, *et al.*, (2008)
29	*Lagenariasiceraria*	stem	MS + BAP (2.0) + NAA (0.5)	Full plantlet in soil	Hasbullah (2017)
30	*Cucumis sativus L.*	Cotyledon	MS+ BAP (2.5-5µm)	Multiple shoots	Hisajima and Arai (1989)
31	*Momordica dioica*	Cotyledon	MS + BAP (2.0) + NAA (0.5)	Organogenesis	Hoque. *et al.*, (2000)
32	*Momordica dioica*	Immature embryo	MS + IBA (10.8) + NAA (1.08) + GA_3 (0.54)	Full plantlet in soil	Hoque, *et al.*, (2007).
33	*Cucurbita maxima*	Node	MS + BAP (2.0)	Full plantlet in soil	Hoque, *et al.*, (2008)
34	*Cucumis melo L.*	Shoot tip	MS + BAP (1.0) + IBA (0.1) + GA_3 (0.3)	Full plantlet in soil	Huda and Sikdar(2006)
35	*Cucumis sativus L.*	Cuttings	MS + Orange juice	Callus	Ikram-ulhaq, *et al.*, (2013)
36	*Momordica dioica*	Node	MS + BAP (1.0) + NAA	Full plantlet with	Jadhav (2015)

			(0.2)	Genotypes response	
37	*Momordica dioica*	Node	MS + BAP (1.5)	Full plantlet in soil	Jamathia (2016)
38	*Cucumis sativus L.*	Stem	MS + BAP (0.5) + NAA (1.0)	Callus	Jesmine and Mian (2016)
39	*Momordica dioica*	Node	MS + BAP (1.0) + NAA (1.0)	Full plantlet in soil	Kapadia (2018)
40	*Momordica dioica*	Cotyledon	MS + BAP(1.5)	Full plantlet in soil	Karim (2011)
41	*Momordica dioica*	Cotyledon	MS + BAP (1.0)	Full plantlet in soil	Karim (2013)
42	*Momordica dioica*	Cotyledon	MS + BAP (1.0)	Plantlet regenerated from calli	Karim and Ullah (2011)
43	*Momordica dioica*	Internode	MS + BAP (0.1) + NAA (0.1) + Sucrose (30g/l w/v)	Somatic embryogenesis	Karim and Ahamad(2010)
44	*Benincasahispida*	Shoot tip	MS + BAP (1.5) + GA_3 (0.2)	Full plantlet in soil	Kausar *et al.*, (2013)
45	*Trichosanthescucumerina L.*	Cotyledonary node	Kinetin (0.1) and BAP (2.0)	Full plantlet in soil	Kawale and Choudhary (2009)
46	*Cucumis melo L.*	Node	MS + BAP (8.0)	Full plantlet in soil	Keng and Hoong (2005)
47	*Citrullus lanatus*	Shoot tip	MS + BAP (5.0)+ IAA (0.1)	Full plantlet in soil	Khalekuzzama, *et al.*(2012)
48	*Citrullus lanatus*	Cotyledon	MS + 2, 4-D (1.0)	Callus	Khatun *et al.*, (2010^a)
49	*Citrullus lanatus*	Node	MS + BAP (1.0) + NAA (0.2)	Full plantlet in soil	Khatun *et al.*, (2010^b)
50	*Cucumis sativus L.*	Stem fragments	MS + Kinetine (6.0 µm)	Flower and pollen production	Kiełkowska and Havey (2011)
51	*Cucurbita ficifolia*	Cotyledon	MS + zeatin (1.0) + IAA (0.1)	Full plantlet in soil	Kim *et al.*, (2010)
52	*Trichosanthesdioica*	Node	MA + BAP (2.0) + NAA (0.3)	Full plantlet in soil	Komal (2011^a)
53	*Trichosanthesdioica*	Node	MS + BAP (2.5)	Callus	Komal (2011^b)
54	*Trichosanthesdioica*	Node	Semi solid MS + Coconut milk (15%)	Full plantlet in soil	Komal (2011^c)
55	*Citrullus lanatus*	Cotyledon	MS + BAP (1) + coconut water (10%).	Organogenesis	Krug, *et al.*, (2005)
56	*Momordica dioica*	Node	MSHP + $AdSO_4$ (80 ppm) + BAP (10 ppm) + IBA (5ppm) + myo-inositol (100) + Agar agar (0.8%) + Sucrose (3%)	Full plantlet in soil	Kulkarni (1999)
57	*Cucumis melo L.*	Cotyledon	MS + BA (2.0) + IAA	Full plantlet in soil	Li, *et al.*, (2011)

			(0.2)		
58	*Cucurbita maxima*	Shoot tip	MS + BAP (3.0)	Full plantlet in soil	Mahazabin (2008)
59	*Trichosanthesdioica*	Cotyledon	MS + BAP (1.0)	Full plantlet in soil	Malex, *et al.*, (2010)
60	*Cucumis angurea*	Node	MS + BAP (1) +NAA (0.2)+L - glutamine (20)	Full plantlet in soil	Margareate (2014)
61	*Cucumis sativus L.*	Shoot tip	MS + BAP (0.4µm)	Full plantlet in soil	Mohammadi and Siveritepe (2007)
62	*Luffa acutangula*	Leaf	2, 4 – D + TDZ –(2.0)	Callusogenesis	Moideen and Prabha (2013)
63	*Luffa acutangula*	Petiole	MS + 2, 4-D + TDZ(1.5)	Callus	Moideen and Prabha (2014)
64	*Momordica dioica*	Node	MS + 2, 4-D (2.0) + BAP (1.0)	Organogenesis	Mustapha, *et al.*, (2012)
65	*Momordica dioica*	Node	MS + BAP (2.0) + L-glutamic (2.0)	Callus and shoot buds	Mustapha, *et al.*, (2013)
66	*Momordica dioica*	Cotyledon	MS + BAP (1.0) + NAA (0.1)	Multiple shoots	Nabi, *et al.*, (2002[a])
67	*Momordica dioica*	Cotyledon	MS + BAP (1.0) + NAA (0.1)	Organogenesis	Nabi *et al.*, (2002[b])
68	*Cucumis sativus L.*	Cotyledon	Kanamycin resistance and green fluorescent protein (GFP) fluorescence,	Genetic transformation	Nanasato, *et al.*, (2013)
69	*Cucurbita pepo*	Hypocotyle	MS + Thidiazuron (0.5)	Full plantlet in soil	Pal, *et al.*, (2007)
70	*Cucumis melo L.*	Node	MS + BAP (2.0)	Full plantlet in soil	Parvin *et al.*, (2013)
71	*Momordica dioica*	Node	MS + NB6 + BAP (0.5+0.5)	Shoot multiplication from callus	Patel and Kalpesh (2015)
72	*Cucurbita pepo*	Cotyledon	2.,4,5-T (4.7µm)+ BAP (4.0 µm) + Kinetine (0.5µm)	Somatic embryos	Paula (1992)
73	*Cucurbita pepo*	Shoot tip	MS + 2,4,5-T (1.2) + BAP (0. 8) + Kinetin (0.I)	Somatic embryogenesis	Paula, *et al.*, (1990)
74	*Cucumis melo L.*	Leaf node	MS + BAP (1.0)	Full plantlet in soil	Rahaman, *et al.*, (2012)
75	*Momordica sahyadrica*	Seedlings	MS + BAP	Full plantlet in soil and conservation	Rajashekharan, *et al.*, (2012)
76	*Momordica dioica*	Leaf	MS + 2, 4-D (2.0) + BAP (2.0)	Molecular confirmation of sex	Raju *et al.*, (2015)
77	*Citrullus colocynth*	Cotyledon	MS + IAA (2.0) + IBA (1.5)	Rhizogenesis	Ram and Shashtri (2015)

78	*Cucumis melo L.*	Cotyledon	MS + IBA (5.0 µM)+ BAP (5.0 µM)+ 25-29°C + light intensity (5-30µmolm^{-2}s^{-2})	Factors of influence	Randall, *et al.*, (1989)
79	*Momordica charantia*	Leaf	MS + BAP (1.5)	Callusogenesis	Saima malik, *et al.*, (2007)
80	*Cucumis sativus L.*	Shoot tip	MS + BAP (1.0)	Full plantlet in soil	Sangeetha, *et al.*, (2011)
81	*Interspecific Cucurbita hybrid*	Shoot tip	MS + BAP (3.0)	Full plantlet in soil	Sarowar, *et al.*, (2003)
82	*Cucumis trigonus*	Leaf	MS + BA (1.0) + IAA (0.25)	Full plantlet in soil	Satapathy *et al.*, (2014)
83	*Citrullus colocynth*	Leaf	MS + 2,4-D (1.5) + BAP (1.0)	Callus	Savitha, *et al.*, (2010)
84	*Cucumis sativus L.*	Hypocotyle	MS + Sucrose (87.64µM) + agar (0.8%) + 2,4-D (3.62µM) + BAP (2.22µM)	Organogenesis	Selvaraj, *et al.*, (2006)
85	*Citrullus colocynth*	Leaf	MS + Kn (2.0) + TDZ (1.0)	Callusogenesis	Shashtree, *et al.*, (2014)
86	*Momordica dioica*	Node	MS + BAP (2.0) + IAA (0.1)	Full plantlet in soil	Shekhawat, *et al.*, (2011)
87	*Luffa cylindrica*	Cotyledon	MS salts + B5 + BAP (10µM)	Resistant GUS (β-Glucuronidase)	Singh, *et al.*, (2011)
88	*Trichosanthesdioica*	Leaf	MS + BAP (0.5) + 2,4-D (0.5)	Callus	Sourab, *et al.*, (2017)
89	*Luffa cylindrica*	Leaf	MS + BAP (1.5) + NAA (1.0)	Callus	Srivastava and Roy (2012)
90	*Momordica charantia*	Node	MS + BAP (2.0) + NAA (0.2)	Full plantlet in soil	Sultana, *et al.*, (2003)
91	*Citrullus lanatus*	Leaf	MS + 2, 4-D (2.5)	Organogenesis callus	Sultana, *et al.*, (2004)
92	*Momordica charantia*	Node	MS + BAP (2) + NAA (0.2) + Sucrose 30 gl^{-1} + Agar 7.0 gl^{-1}+ pH (5.5-6.0)	Effects of sucrose, agar pH	Sultana, *et al.*, (2005)
93	*Citrullus lanatus*	Cotyledon	MS + BAP (20.0 µM)	Full plantlet in soil	Suratman, *et al.*, (2009)
94	*Momordica dioica*	Leaf	MS + BAP(3.0) + NAA (0.5)	Organogenesis	Swamy, *et al.*, (2015)
95	*Momordica balsamina*	Node	MS + BAP (1.0)	Full plantlet in soil	Thakur, *et al.*, (2011)
96	*Momordica charantia*	Leaf	MS + 2,4-D(1.0)	Embryogenesis	Thiruvengadam, *et al.*, (2006)
97	*Momordica dioica*	Petiole	MS + 2,4-D (2.2µm) +	Somatic emryoids	Thiruvengadam,

			L- glutamine (0.5µm)		*et al.*, (2007)
98	*Momordica dioica*	Encapsulated Shoot tip	MS (0.7% agar solidified) + BAP (0.5µm)	Full plant let in soil without variation	Thiruvengadam, *et al.*, (2012[a])
99	*Momordica charantia*	Petiole	MS and Gamboge + NAA (3.0µm) + TDZ (1.0 µm) + Putrecine (1.0µm)	Plantlet from organogenesis	Thiruvengadam, *et al.*, (2012[b])
100	*Momordica dioica*	Leaf	MS + 2, 4-D (3.3µm) + Putrescine(0.5µm)	Somatic embryogenesis	Thiruvengadam, *et al.*, (2013)
101	*Benincasahispida*	Cotyledon	MS + BAP (1–6µM) + NAA, 0.2 and 0.5µM	Full plantlet in soil	Thomas, *et al.*, (2004)
102	*Luffa acutangula*	Cotyledon	MS + BAP (1.0) + Zeatin (0.2) + NAA (0.2) + 2,4-D (0.6) + Picloram (0.1) +AdS (20).	Full plantlet in soil	Umamaheshwari, *et al.*(2014)
103	*Cucumis sativus L.*	Leaf	MS + 2,4-D (5) + TDZ (0.1)	Somatic embryogenesis	Usman, *et al.*, (2011)
104	*Cucurbita moschata*	Cotyledon	Callus induction medium (CIM) + 2,4-D (0.5 or 3.5)	Somatic embryogenesis	Valdez-Melara, *et al.*, (2009)
105	*Citrullus lanatus*	Shoot tip	MS + BAP (0.5)	Full plantlet in soil	Vedat, *et al.*, (2002)
106	*Luffa acutangula*	Petiole	MS + BAP (2.0) + NAA (0.2)	genetic stability by IISR	Vellivella, *et al.*, (2016)
107	*Cucumis melo var utillisimus*	Node	MS + BAP (1.0) + Adenine sulphate (15)	Multiple shoots	Venkateshawaralu, *et al.*(2010)
108	*Cucumis melo L.*	Shoot tip	MS + IAA (0.5) + BAP (2.0)	Multiple shoots	Venkateshwaralu (2012)
119	*Momordica charantia*	Node	½ MS + BAP (0.5)	Full plantlet in soil	Verma, *et al.*, (2014)
110	*Cucumis figarei*	Cotyledon	MS + BAP (1.0) + ABA (1.0 or 2.0)	Full plantlet in soil	Yutaka, *et al.*, (1998)
111	*Cucumis metuliferus*	Cotyledon	MS +BAP (1.0) + IAA. (0.2)	Full plantlet in soil	Yutaka *et al.*, (1998)
112	*Luffa acutangula*	Cotyledon	MS + BAP(1.5) + NAA (1.0)	Organogenesis	Zohura, *et al.*, (2013)

The use of 30g/l sucrose gave 100% shoot proliferation with 5.1±0.8 shoots having length of 5.6 ± 0.4cm.MS medium having 7g/l agar showed 100% frequency in shoot proliferation. Highest frequency of multiple shoot was regenerated on MS medium containing 1.0 mgl^{-1}BAP + 0.2mgl^{-1}NAA + L - glutamine (20mgl^{-1}) and elongation of shoots were achieved by adding GA_3 (0.5mg/l) in *Cucumis anguria* (Margareate 2014). In female plants of *Momordica dioica*bud breaking occurrence of nodal explants was noticed in very low concentration of IAA (0.1mg) with BAP (2.0mg) (Shekhawat *et al.*, 2011). Kulkarni (1999) conducted micropropagation studies in Kartoli by using nodal segment as an explant

and developed a proper method of *in-vitro* regeneration and multiplication. MSHP + 80ppm $AdSO_4$ + 10ppm BA + 5ppm IBA +100mg myo-inositol + 0.8 %Agar agar + 3% sucrose gave good results (75 %). The same medium gave the maximum multiple shoots per culture (81±1.28) at the end of 4th subculture. It was found that the nodal segment cultures of spine gourd initiated maximum rooting response (86.66%) to the medium, MS basal+ 3ppm NAA + 0.8% Agar agar + 3% sucrose + 0.2% activated charcoal. Among the different potting mixture compositions tried for hardening of the *in-vitro* developed plantlets vermiculite alone gave maximum (77.33%) survival and the lowest survival was observed in potting mixture with FYM (20 %) alone. A minimal medium and protocol has been formulated to reduce the cost and time period of micropropagated raised plants of *Trichosanthesdioica* Roxb. Semi solid MS with 15% coconut water showed the highest percentage of plantlet regeneration (99%) and rhizogenesis was observed when explants were cultured on this medium within 5-6 days, followed by shoot formation in 8-10 days (Komal 2011^{c}).

Cotyledon

In micropropagation cotyledons play a vital role in giving the successful plantlets. In thisregard, most of the scientist used the BAP alone or in combination with the other growth hormone for regeneration of plant. As per the opinions of most of the investigators BAP in the range 1.0, 1.5, 2.0 showed the best results for optimum plant regeneration (Karim and Ullah 2011, Malex *et al.,* 2010, Zohura *et al.,* 2013, Li *et al.,* 2011). In *Luffaacutangula,* by using cotyledon as explant, organogenesis was found best on media supplemented with BAP 1.5mg/l and NAA 1.0mg/l (Zohura *et al.,* 2013). When watermelon is treated with the lower concentrations of BAP (20µM) the highest mean number of shoots obtained was (9.83±0.81), whereas another scientist used cotyledons excised from 7-day-old aseptic seedlings the Sugar baby variety of *Citrullus lunatus* Thumb. Matsum and Nakai, the maximum number of shoots were recorded on MS + BAP 3.0µM +2iP 3.0µM and MS + BA 3.0µM + IAA 3.0µM. Finally, 55% plants showed success in field (Suratman. 2009, Chaturvedi and Bhantnagar. 2001). Arekar (2012) used the decoated seeds of *Momordica dioica* for shoot regeneration and got maximum number of shoots in 7-8 weeks on 4.44µM and 8.88µM BAP. The rooting was recorded within 45 days when supplemented with 0.049mM IBA. Indole-3 Acetic acid is used by the several scientists in *Cucurbita ficifolia, Citrullus lanatus, Citrullus colocynth* for getting plantlet but use of MS + IAA (2.0) + IBA (1.5) in *Citrullus colocynth*reported rhizogenesis (Rama Krishna and Shashtri 2015). In addition to this, MS + IAA (0.1) + zeatin (1.0) was found to be efficient for shoot regeneration in *Cucurbita ficifolia* (Kim *et al.,* 2009).

Randall *et al* (1989) used cotyledonary explants of *Cucumis melo* in MS medium fortified with 5µM IBA and 5µM BAP and incubated at 25-29°C under low light intensity of 5-30µmol$m^{-2}s^{-2}$. They observed that the presence of ABA significantly enhanced the number of explants producing shoot buds. It was also observed that seedling age, genotype, temperature and light intensity affected bud initiation. The addition of various phytohormones like thidiazuron, gibberellic acid or silver nitrate to regeneration medium was not noticed in improving, either bud initiation or shoot regeneration.

Somatic embryogenesis

Plant regeneration via somatic embryogenesis follows the initiation of embryonic culture, proliferation of embryonic culture, prematuration of somatic embryo, maturation of somatic embryo and plant development on nonspecific media. So many interested scientists were worked on plant regeneration by using somatic embryogenesis. High frequency somatic embryogenesis(3.3 somatic embryos) was noticed in *Cucumis melo* on 2, 4-D at 5 mg/l and TDZ at 0.1mg/l while 3% sucrose was found to be highly significant in embryo

induction and development (Gray *et al.,* 1993). RAPD markers viz; OP-G14, OP-C10, OP-Y03, OP-H05 and OP-AT01were used to evaluate the genetic stability of regenerants of *Cucumis sativus* plants obtained through somatic embryogenesis and found that there are no significant visual differences between the somatic embryo plants and F1 hybrids (Elmeer *et al.,* 2009). Immature embryo and immature cotyledon of *Momordica dioica*were used to get higher percentage of somatic regeneration but immature embryo showed best response than immature cotyledon for shoot proliferation onMS + IBA (10.8) + NAA (1.08) + GA3 (0.54) (Hoque *et al.,* 2007). 2, 4-dichloro phenoxy acetic acid is one of the major auxin in inducing somatic embryos as reported by many persons who worked on this one and in all the cases 2, 4-D 1.0 mgl^{-1} and 2.0 mgl^{-1} in single or in combination with other growth regulators like BAP, TDZ were also used to get somatic embryos (Thiruvengadam *et al.,* 2006, Raju *et al.,* 2015). Hisajima and Arai (1989) reported that BAP (2.5-5µm) in *Cucumis sativus* by using cotyledons as explant gave multiple shoots. Raju *et al.,* (2005) worked on molecular sex confirmation in *Momordica dioica*by the plant regenerated from leaf callus of somatic embryogenesis at MS + 2, 4-D (2.0) + BAP (2.0).Maximum amount of callus (94.16%) was inducedon leaf disc explants of cucumber on MS medium fortified with 2, 4-D @ 2.0 mg/1. Callus induced from leaf disc explants at higher level of 2, 4-D (5 mgl^{-1}) yielded the highest percentage of embryo formation i.e. 23% (Usman *et al.,* 2011).Plants were regenerated from the callus of shoot tip, petiole, leaf explant, internode and nodal explants. The highest callus induction was noticed in internodal explant of teasle gourd on semi-solid MS media containing basal salts and growth regulators fortified with 1.0 mg^{-1} BAP, 30 g/l (w/v) and 0.1mgl^{-1} NAA sucrose (Karim and Ahamad. 2010). Somatic embryogenesis was successfully achieved in *Cucurbita pepo* by using shoot tip and cotyledon at various combination and concentration of 2,4,5-T (1.2) + BAP (0. 8) + Kinetin (0.1) and 2.,4,5-T (4.7µm)+ BAP (4µm) + kinetine (0.5µm), respectively where best callus was noticed in cotyledon derived explant (Paula *et al.,* 1990 and Paula 1992).

Good amount of friable embryogenic callus was recorded on callus induction medium fortified with 0.5mgl^{-1} or 3.5 mgl^{-1} 2, 4-D from zygotic embryos(53-56%) and cotyledonary seedlings (70%) derived from *Cucurbita moschata* cv. Sello*de* Oro. Among the 75 per cent of the evaluated pure lines of *Cucurbita moschata*, embryogenic calli induction with the frequency range from 5% to 34% were registered. Regenerated plants from micropropagation looks morphologically normal and sets the flower, fruit and seed which could germinate normally (Valdez-Melara *et al.,* 2009). Various main cucurbits such as cucumber, watermelon, squash, and melons were studied to build a protocol for somatic embryogenesis. Out of several explants used, cotyledons and hypocotyls gave the best results. In somatic embryogenesis, genetic constitution of mother plants playeda vital role. Somatic embryos showed the abnormalities during the growth phase of plants, if they were raised from the protoplast derived cultures (Debeaujon and Branchard, 1993).

Organogenesis

Organogenesis is defined as the development of adventitious organs of plant part or primordia from the mass of undifferentiated cells which is called as callus, by the process of differentiation. The regeneration of plant or plant organs only taken place by the expression of cellular totipotency of the callus tissue. In the process of organogenesis, a good quality of callus initiation plays a vital role for the further regeneration. In most of the findings researchers had used cotyledon as an explant in various cucurbit crops. They used BAP as good callus inducing growth hormone either single or in combination with various kind of growth regulators. In allthe best callusing reports, BAP range is 1.0 1.5, 2.0 and 3.0mg/l (Krug *et al.,* 2005, Compton and Grey. 1992, Karim, 2013, Yutaka *et al.,* 1998, Nabi *et al.,* 2002[b], Hoque *et*

al., 2000, Hasbullah, 2017, Han *et al.,* 2004, Karim, 2011). The potentiality of watermelon callus induction and its successive regeneration from cotyledon and internode was studied by Khatun *et al.,* (2010[a]). Greenish compact callus was achieved from cotyledon on MS fortified with 1mg/l 2, 4-D within one week of inoculation. When most of the scientists in their research used the BAP more than 1.0mg/l, Vedat Pirinc *et al.,* (2002) used the BAP at lower concentration i.e. 0.5mg/l and got 50% more number of shoots which were higher than Kinetin @ 1.0 mg/l in *Citrullus lanatus.*

Organogenesis in water melon was studied and the best result was obtained in cotyledon segments which were taken from the proximal region. The explants were inoculated on MS medium supplemented with 1.0 mgl^{-1}BAP and 10% coconut water. It was revealed from the histological study that the organogenesis occurs directly without any callus formation on epidermal layer and sub-epidermal layers of the explants (Krug *et al.,* 2005). In the process of organogenesis, BAP supplemented with the other growth hormones were found to be best for callusing. In this regards, many persons (Nabi *et al.,* 2002[b], Hoque *et al.,* 2000, Hasbullah, 2017)used the BAP @ 1.0-2.0mg/l supplemented with the lower concentrations of NAA 0.1 to higher concentration of 0.5 mg/l in *Momordica dioica* and *Lagenaria siceraria*. The explants used in the study were cotyledon and stem respectively. By using cotyledon explantsin *Luffa acutangula* L. Roxb., multiple shoots were induced via indirect organogenesis on MS media fortified with BAP (1.0) + Zeatin (0.2) + NAA (0.2) + 2, 4-D (0.6) + Picloram (0.1) + $AdSo_4$ (20). The average shoots per explants produced were 10.3 in 78.34 per cent of the cotyledon derived callus (Umamaheshwari *et al.,* 2014). Maximum shoot regeneration was observed in proximal parts of cotyledons derived from 4-day-old seedlings of bottle gourd when cultured on MS medium with 3mg/l BAP and 0.5mg/l $AgNO_3$ under a 16hr photoperiod. The diploid cultured were recorded in the most of the $AgNO_3$ supplementation. This observation was reported by flow cytometric analysis (Han *et al.,* 2004). The effect of commercial fruit juices was also tested for callus induction, its proliferation and plant regeneration in cucumber. The orange, apple, strawberry and red grapes were used in the place of 3% sucrose. Out of these, MS medium supplemented with Orange juice was found to be the best source of callusing as reported by Ikram-ul Haq *et al.,* (2013). In *In-vitro* organogenesis from hypocotyle explant of *Cucumis melo* var Poinsette, calli were induced on MS + Sucrose (87.64µM) + agar (0.8%) + 2,4-D (3.62µM) + BAP (2.22µM) and regeneration of adventitious shoot from these calli (25 shoots per explant) were achieved on MS + 8.88µM BAP + 2.5µM zeatin + 10% coconut water (Selvaraj *et al.,* 2006). The 2, 4-dichloro phenoxy acetic acid at 2.5mg/1 gave best callusing percentage in hypocotyle explants of *Cucurbita pepo* and the highest percentage of shoot regeneration (85%) was obtained at 0.5mg/l TDZ, About, 70% of regenerated plantlets survived under *ex-vitro* conditions (Pal *et al.,* 2007). MS + BAP (1–6µM) + NAA, 0.2 and 0.5µM was observed best response on cotyledon explant of *Benincasahispida* (Thomas *et al.,* 2004).

For the production of callus, TDZ is one of the major source for callusogenesis in single or in combination. Most of the research workers used combination form of TDZ with 2,4-D and Kinetine, Best callusogenic response was observed in 2, 4–D + TDZ-2.0mg/l and MS + Kn (2.0) + TDZ (1.0) in *Luffa acutangula* and *Citrullus colocynth* respectively by using leaf as explant (Moideen and Prabha,2013 and Shashtree *et al.,* 2014). Guma *et al.,* (2015) conducted study to develop efficient protocol for sterilization and callus induction in *Cocciniaabyssinica*. Maximum number of clean explants with better survival rate (82.5±0.5) was obtained when they were treated with 5% NaOCl for 10 minutes sterilization period and maximum amount of callus induction i.e., 80±2.0 was achieved from the combination of 5.0 µm BAP+2, 4-D. During the course of organogenesis, callusing is the first step to induce a good quality callus. Majority of the

research workers, preferred to use 2,4-D either single or in combination with various growth hormones. In some investigations, best callusing range of 2,4-D reported was 0.5, 1.0, 1.5 and 2.5mg/l with BAP 0.5, 1.0 and 2.0mg/l in leaf explants of *Momordica dioica, Citrullus lanatus, Citrullus colocynth, Trichosanthesdioica* (Devendra, 2009, Sultana *et al.,* 2004, Savitha *et al.,* 2010,Sourab *et al.,* 2017). Some of the scientists had used either BAP alone or BAP with IAA to get the callus followed by organogenesis. The BAP @ 1.0 and 1.5mg/l concentrations was used in alone. When used with IAA then MS + BA (1.0) + IAA (0.25) was found best in *Cucumis trigonus, Cucumis melo L. Momordica charantia.* The explants used were leaf explants (Satapathy *et al.,* 2014, Rahaman *et al.,* 2012 and Saima malik *et al.,* 2007). In case of *Momordica dioica* and *Luffa cylindrica*, BAP@ 1.0, 1.5 and 3.0mg/l in combination with the NAA @ 0.1, 0.5 and 1.0mg/l was found to be the best source of callusing and organogenesis. In some cases, use of BAP 1.0 +NAA 1.0mg/l was reported for multiple shoot regeneration from callus leaf explants (Nabi *et al.,* 2002[a], Srivastava and Roy. 2012, Swamy *et al.,* 2015).

Coconut milk is the one of the very good organic source of growth hormones mainly cytokinins. In this regard Debnath *et al.,* (2015) by using nodal explants of *Momordica dioica* and *Momordica cochinchinensis* media supplemented with coconut milk (15% v/v) and 2, 4-D (2.0 mg/l)had reported highest percentage of callusing and organogenesis, in both the species. Further they added 05. mg/l BAP with agar gelled MS as a basal medium for *Momordica dioica*while in case of *Momordica cochinchinensis*, did not usethe BAP with agar gelled MS as basal medium. In another treatment, for *Momordica dioica*, coconut milk was avoided and good amount of compact and green callus was obtained on 2, 4-D (2.0) + BAP (1.0) supplemented medium (Debnath. 2013, Debnath *et al.,* 2013, Mustapha *et al.,* 2012). Direct organogenesis was reported in *Momordica cymbalarias* on BAP2.0 mg/l for shoot regeneration (Devi *et al.,* 2017). In case of *Momordica dioica*, for inducing the callus and multiplication of shoot, first timeNB_6 phytohormones was used. NB_6 + BAP (0.5 + 0.5 mgl^{-1}) recorded the highest percentage of shoot multiplication within 15 days of inoculation with shoot length 5.2 ± 0.37cm and shoot numbers 10 ± 1.4 (Patel and Kalpesh, 2015).

Addition of polyamines in culture media has enhanced the percentage of callus induction in organogenesis of bitter melon by using petiole as explant. The medium supplemented with 3.0µM NAA, 1.0µM TDZ and 1.0 µMputrecine induced 95.0% callus induction while regeneration of adventitious shoots from callus was achieved i.e., 53 shoots per explant on medium with 3.0µM TDZ with 1.0µM NAA and 1.0µM Spermidine (Thiruvengadam *et al.,* 2012). Callus induction and multiplication was tried on *Luffa acutangula* by using node, leaf and petiole explant. Out of all explants used for experimentation, the petiole showed the best callusing percentage in 2, 4-D + TDZ – 1.5mg/l. (Moideen and Prabha. 2014).The IISR marker techniques were used to find out the clonal fidelity from the callus derived regenerated plant of *Luffa acutangular*. In this, 2mg/l BAP and 0.2mg/l NAA were used for highest callus (Vellivella. 2016). The highest percentage of callus was obtained from stem explant (89.0 ± 0.75%) followed by leave (79.05 ± 3.28) in NAA and BAP but addition of 2, 4-D on growth medium had promoted the slow growth and low quality callus in cucumber (Jesmin and Mian. 2016).

Other

Homozygous doubled haploid lines (DHLs) from cucumber could be helpful to breed resistant varieties. Parthenogenic embryos are induced by irradiated pollen with Co_{60}gamma-rays at 500 gamma. The SSR markers were used to discriminate the undesirable zygotes. Chromosome doubling of haploid was done by Colchicine 500µM. Selfing was done between the colchicine treated haploid plants and those plants were allowed for the perpetuation by

seed of homozygous lines. Percentage of seed set was 90%, and it was concluded that DHLs are ideal resources for genomic analyzer (Claveria *et al.,* 2005). Rooting studies on spine gourd conducted reported that plants of healthy shoots with their own root system were able to survive and became complete plantlet. The highest percentage of rooting was obtained on IBA (1.0) (Ghive *et al.,* 2006a).

In cell suspension culture, single or small aggregates of cells multiply while suspended in agitated liquid medium. Thiruvengadamis one of the pioneer research scientist who worked more and more on the cell suspension culture of some cucurbits to achieve somatic embryogenesis. In all his findings, he used the 2, 4-D either single or in combination with the other plant hormones. The range of 2, 4-D at 2.2µm and 2.0µM with L- glutamine (0.5µm) was found to be the best for somatic embryogenesis by using petiole and leaf explants in *Momordica dioica* and *Cucumis anguria* respectively. For the leaf explant of *Momordica dioica*, addition of putrecine (0.5µm) rather than L- glutamine was found to be the best for somatic embryogenesis (Thiruvengadam *et al.,* 2006, Thiruvengadam *et al.,* 2007, Thiruvengadam *et al.,* 2013, Thiruvengadam *et al.,* 2013).

From last few decades, tremendous advancement has been made in cucurbitaceae family through tissue culture technique. Micropropagation techniques are already standardized in various species of cucurbitaceae family. It is assessed that several millions of plants are now propagated from various explant sources each year. Despite these advancements, much focused research is needed in various areas such as somatic embryogenesis, rooting studies, genetic engineering etc. To increase the percentage of success rate in recalcitrant genotypes there is need to choose the explant source judiciously, coupled with some improvements in media composition. Improvement in regeneration ability and acclimatization in *ex-vitro* condition are crucial for extreme exploitation of this family. When more importance was given to these research areas the future commercial *in-vitro* micropropagation of cucurbitaceae family will be revolutionized.

Declaration of conflicting interests:

The authors declare that there is no conflict of interest.

References

Abdelnour, A., Ramirez, C. and Engelmann, F. (2002). Micropropagacin of Chayote (*Sechium Edule* Jacq. Sw.) Apartir De Brotes Vegetativos. *Agronom ameso americana*,13(2): 147-151.

Abdul-Awal, S. M., Alam, J. Md., Ali, R. Md., Hassan, N. Md., Basunia, S. R., and Rehaman, S. M. M. (2005). *In-vitro* propagation of Pointed gourd (*Trichosanthes dioica*) from shoot tips. *Biotech.,* 4(3): 221-224.

Ahmad, N. and Anis, M. (2005). *In-Vitro* Mass Propagation of *Cucumis Sativus*. *Turk J. Bot.*, 29: 237-240.

Arekar, A. R., Janhavi, A., Arekar, S. S., Barve and Paratkar, G. T. 2012. *In- vitro* regeneration of *Momordica dioica* (Roxb.). *J. of Applied and Natural Sci*., 4 (2): 297-303.

Bezirganoglu, I., Hwang, S. Y., Shaw, J. F., and Fang, T. J. (2013). Efficient production of transgenic melon via Agrobacterium-mediated transformation. Genetics and Molecular Research, 13(2): 3218-3227.

Chaturvedi, R., and Bhatnagar, S. P. (2001). High-Frequency Shoot Regeneration From Cotyledon Explants Of Watermelon Cv. Sugar Baby. *In-Vitro Cell. Dev. Biol. Plant*.37: 255-258.

Choudhary, S. K., Patel. A. K., Harish, Shekhawat, S., Narpat, S., Shekhawat. 2017. An improved micropropagation system, *ex-vitro* rooting and validation of genetic homogeneity in wild female *Momordica dioica*: an underutilized nutraceutical vegetable crop. *Physiol. Mol. Biol. Plants*., 23(3): 713–722.

Chovelon, V., Restier, V., Dogimont, C. and

Aarrouf, J. (2008). Histological study of shoot organogenesis in melon (*Cucumis melo* L.) after genetic transformation. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008.*

Claveria, E., Garcia-mas, J. and Dolcet-Sanjuon, R. (2005). Optimization of cucumber Doubled Haploid Line production using *in-vitro* rescue of *In-vivo* Induced Parthenogenic embryos. *J. Amer. Soc. Hort. Sci.*, 130(4): 555-560.

Compton, M. E., Brenda, L., Pierson and Staub, J. E. (2001). Micropropagation for recovery of *Cucumis hystrix*. *Plant Cell, Tissue and Organ Culture*, 64: 63–67.

Compton, M. E. and Gray, D. G.(1992). Micropropagation as a means of rapidly propagating Triploid and tetraploid watermelon. *Proc. Fla. state Hort. soc*, 105:352-354.

Dabauza, M., Bordas, M., Salvador, A., and Roig, L. A. (1997). Plant regeneration and Agrobacterium-mediated transformation of cotyledon explants of *Citrullus colocynthis* L. Schrad. *Plant Cell Reports*, 16: 888–892.

Debeaujon, I. and Branchard, M. (1993). Somatic embryogenesis in Cucurbitaceae. *Plant Cell, Tissue and Organ Culture*, 34: 91-100.

Debnath, B., Sinha, S. and Sinha, R. K. (2013). *In-Vitro* Differentiation And Regeneration of *Momordica Cochinchinensis* (Lour.) Spreng . *Ind. J. of Plant Sci.*, 2(2): 2319-3824.

Debnath, B., Sinha, S. and Sinha, R. K. 2013. Rapid in vitro differentiation and regeneration of *Momordica dioica*Roxb. Ind. J. of Plant Sci., ISSN: 2319-3824, 2 (3): 43-47.

Devendra, N. K., Subhash, B. and Seetharam, Y. N. 2009. Callus growth and plant regeneration in *Momordica dioica* (Roxb.) Wild. Cucurbitaceae. *American-Eurasian J. of Sustainable Agric.*, 3(4): 743-748, ISSN 1995-0748 © 2009.

Devendra, N. K., Rajanna, L., Sheetal, C. and Seetharam, Y. N. (2008). *In-vitro* Clonal Propagation of *Trichosanthes cucumerina* L. var. cucumerina. Plant Tissue Cult. and Biotech. 18(2): 103-111.

Devi, T., Rajasree, V., Premalakshmi, V. and Hemapra, K. (2017). *In-vitro* Protocol for Direct Organogenesis in *Momordica cymbalaria*. Fenzl. *Int. J. Curr. Microbiol. App. Sci.*, 6(4): 2392-2402.

Elmeer, K. M. S., Thomas, F., Gallagher, and Hennerty, M. J. (2009). RAPD-based detection of genomic instability in cucumber plants derived from somatic embryogenesis. *African J. of Biotech.*8(14): 3219-3222.

Faria, A. L., Tanziman, A., Karim, R., Islam, R., and Hossain, M. (2013). Rapid *in-vitro* clonal propagation of two hybrid muskmelon cultivars and their field evaluation in agro climatic condition of Bangladesh. *J. of Genetic and Envi. Resou. Conservation*, 1(3):247-253.

Firoz Alam M., Ruhul, A., Ekhlas, U. M. and Sudhan. (2015). Regeneration of Shoot from Nodal explants of *Cucumis sativus* considering different Hormonal concentration. *Inter. Research J. of Biol. Sci.*, 4(7): 48-52.

Ghive, D. V., Raut, N. W. And Ghorade, R.B. (2006). Tissue Culture Studies in Spine Gourd (*Momordica dioica*Roxb.). *Inter. J. of Plant Sci*., 1(2): 266-268

Ghive, D. V., Ghorade, R. B., Khedekar, R. P., Jeughale, G. S. and Raut, N. W. 2006. In Vitro Rooting Studies in Spine Gourd (*Momordica dioica*Roxb.). *Asian J. of Biol. Sci*., 1(2): 146-148.

Govind, R. K., Singh, M., Rai, N. P., Bhardwaj, D. R. and Kumar, S. 2012. *In-Vitro* propagation of spine gourd (*Momordiacadioica*Roxb.) and assessment of genetic fidelity of micropropagated plants using RAPD analysis. *Physiol. Mol. Biol. Plants.*, 18(3): 273-280.

Gray, D. J., McColley. D. W., and Michel, E. 1993. High Freequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivar. *J. Amer. soc. Sci.*, 118(3): 425-432.

Guma, T. B., Kahia, J., Justus, O., Peter, N. K. 2015. Standardization of *In-Vitro* Sterilization and Callus Induction Protocol for Leaf Explants of Anchote: *Coccinia abyssinica.* Int. J. of Research and

Development in Pharmacy and Life Sci. 4(2); 1427-1433.

Han, J. S., Oh, D. G., Mok, I. G., Park, H. G. and Kim, C. K. (2004). Efficient plant regeneration from cotyledon explants of bottle gourd (*Lagenaria siceraria* Standl.). *Plant Cell Rep.*23:291–296.

Hasbullah, N. A., Mohammad, M., Lassim, Muhammad, A., Mazlan, Siti, Z., Lood, Muhamad, A. and Mohamed, A. 2017. Mass Propagation of *Lagenariasiceraria* through *in-vitro* Culture. J. of Advanced Agric. Technologies 4(1).

Hisajima, S. And Yujl, A. 1989. Micropropagation of Cucumber Plant through Reproductive Organ Culture and Semi-aquaculture of Regenerated Plants. *Japan. J. Trop. Agr*. 33(1): 1-5.

Haque, M. E., Sarkar, M. A. R., Mahmud, M. A., Rezwana, D., and Sikdar, B. (2008). *In-Vitro* Propagation Of Pumpkin and Ash gourd through nodal segments. *J. bio-sci*, 16: 67-71.

Hoque, M., Hossain, S., Alam, S., Arima and Islam, R. 2007. Adventitious Shoot Regeneration from Immature Embryo Explant Obtained from Female × Female *Momordica dioica*. *Plant Tissue Cult. and Biotech*., 17(1): 29-36.

Hoque, A.,Islam, R.,Arima, S. 2000. High frequency plantlet regeneration from cotyledon-derived callus of *Momordica dioica* (Roxb.) Wild. *Phytomorphology*, 50 (3/4): 267-272 ref.19.

Huda, A. K. M. N. and Sikdar, B. (2006). *In-vitro* Plant Production through Apical Meristem Culture of Bitter Gourd (*Momordica charantia* L.). *Plant Tissue Cult. and Biotech.* 16(1): 31-36.

Ikram-ul Haq, Arshad, Z. and Taseer. (2013). Establishment of Plant Regeneration Protocol in Cucumber: Fruit Juices Used as Carbon Source. *Inter. J. of Sci. and Research*, 6(4), 81-84.

Jadhav, S., Parmar, L. D. and Chauhan, R. M., 2015. Response of spine gourd genotypes (*Momordica dioica* Roxb.) to micropropagation. *4th International Conference on Agriculture and Horticulture*, 4:2 http://dx.doi.org/10.4172/2168-9881.S1.016.

Jamatia, D. 2016. Assessment of Variability and Standardization of Protocol for Micropropagation in Spine gourd (*Momordica dioica*Roxb.). A thesis submitted to *Maharana Pratap University of Agricultural andTechnology*.

Jasmine, R. and Mian, M. A. K. (2016). Callus induction and efficient plant regeneration in Cucumber (Cucumis sativus L.). *J. Biosci. Agric. Res. 09(02): 796-803* .

Kapadia, C., Patel, N., Patel, N., Ahmad, T. 2018. Optimization of Culture Medium for Higher Multiplication and Efficient Micropropagation of Spine Gourd (*Momordica dioica* Roxb.). J. of Experimental Biol. and Agric. Sci., 6(3): 599– 605.

Karim, M. A. And Ullah, M. A. (2011). *In-Vitro* Regeneration of Teasle Gourd. 2011 2nd International Conference on Biotechnology and Food Science IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

Karim, M. A. and Ullah, M. A. 2011. *In-vitro* regeneration of teasle gourd. Paper presented in 2nd *Int. Conference on Biotech. And Food Sci., IPCBEE*.7:144-148.

Karim, M. A. 2013. High Frequency Shoots Regeneration from Cotyledon Explants of Teasle Gourd via Organogenesis. *Journal of Life Scie. and Technologies*, 1(1): doi: 10.12720/jolst.1.1.79-83.

Karim, M. A. and Ahmed, S. U. 2010. Somatic Embryogenesis and Micropropagation in Teasle Gourd. *International J. of Environmental Sci. and Development*, 1(1): 10-14. ISSN: 2010-0264.

Kausar, M., Parvin, S., Haque, M. E., Khalekuzzaman, M., Sikdar, B. and Islam, M. A. (2013). Efficient Direct Organogenesis From Shoot Tips and Nodal Segments of Ash Gourd (*Benincasa Hispida* L.). *J. life Earth Scie.*,8: 17-20.

Kawale, M. V. and Choudhary, A. D. (2009). *In-Vitro* Multiple Shoot Induction In *Trichosanthes cucumerina* L. *Ind. J. Plant Physiol.*, 14(2): 116-123.

Keng, C. L. and Hoong, L. K. (2005). *In-vitro* Plantlet Regeneration From Nodal Segment of Musk melon (*Cucurbita pepo* L.). *Biotechnology*. 4(4): 354-357.

Khalekuzzaman, M., Khatun, M. M. Rashid, M.

H. Sheikh, M. I., Sharmin, S. A. and Alam, I. (2012). Micropropagation of an Elite F1 Watermelon (*Citrullus lanatus*) Hybrid from the Shoot Tip of Field Grown Plants. *Brazilian Archives Of Biol. And Tech.*, 55(3): 335-340.

Khatun, M. M., Hossain, M. S., Khalekuzzaman, M., Rownaq, A. And Rahman, M. 2010. *In-Vitro* Plant Regeneration from Cotyledon and Internodes Derived Callus in Watermelon (*Citrullus lanatus* Thumb.). Int. J. Sustain. Crop Prod. 5(4):25-29.

Khatun, M. M., Hossain, M. S., Haque, M. A. and Khalekuzzaman, M. (2010). *In- vitro* propagation of *Citrullus lanatus* Thumb. from nodal explants culture. *J. Bangladesh Agril. Univ*,8(2): 203–206.

Kielkowska, A. and Michael, J., Havey. (2011). *In-vitro* flowering and production of viable pollen of cucumber. *Plant Cell Tiss. Organ. Cult*., doi:DOI 10.1007/s11240-011-0075-8 al, C.-M. e. (n.d.).

Kim, K., Chang Kil Kim, and Jeung-Sul Han. (2010). *In-vitro* regeneration from cotyledon explants in fig leaf gourd (*Cucurbita ficifolia* Bouche.) a rootstock for Cucurbitaceae. *Plant Biotechnol. Rep.*, 4:101–107.

Komal, R. (2011). Effect of BAP and IAA on callus formation and plant regeneration in pointed gourd. *Research Article, Biotechnol. Bioinf. Bioeng*, 1(1):59-62.

Komal, R. (2011). *In-vitro* Plant Regenera of *Trichosanthes dioica*. *Ind. J. Agric. Res.*, 45(2): 140-145.

Komal, R. (2011). One step method of plantlet regeneration in *Trichosanthes dioica* Roxb. An approach towards cost effective and shorter protocol . *African J. of Biotech.* 10(1): 9-12.

Krug, M. G. Z., Stipp, L. C. L., Rodriguez, A. P. M. and Mendes, B. M. J. (2005). *In- vitro* organogenesis in watermelon cotyledons. *Pesq. Agropec. Bras., Brasília*, 40(9): 861-865.

Kulkarni, G. R. 1999. Micropropagation studies in Kartoli (*Momordica dioic*aRoxb.). A thesis submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722.

Li, J., Li, X. M., Qin, Y. G., Tang, Y., Wang, Ma, C. and Li, H. X. (2011). Optimized system for plant regeneration of watermelon (*Citrullus lanatus* Thumb.). *African J. of Biotech.* 10(48:9760-9765.

Mahazabin, F., Parvez, S., and Alam, M. F. (2008). Micropropagation Of *Cucurbita Maxima* Duch. Through Shoot Tip Culture . *J. bio-scie.*16: 59-65.

Malex, M. A., Khanam, D., Khatun, M., Molla, M. H. and Mannan, M. A. (2010). *In- Vitro* Culture Of Pointed Gourd (*Trichosanthes dioica* Roxb.). *Bangladesh J. Agril. Res.*, 35(1): 135-142.

Margaret, S., Maheswari, U., Ambethkar, Vasudevan, Sivanandhan and Selvaraj. (2014). Direct regeneration of multiple shoots from nodal explants of West Indian Gherkin (*Cucumis anguria* L.). *Inter. J. of Innovative Research in Sci, Engineering and Technology*, (6): 13876-13881.

Mohammadi, J. and Sivritepe, N. 2007. *In-vitro* Clonal Propagation of *Cucumis sativus* L. by Shoot tip culture. *J. Biol. Scie*, 7(4): 653-657.

Moideen, R. S., Prabha, L. A. 2013. *In-Vitro* Plant Regeneration of *Luffa acutangula*Roxb. Var Amara Lin.: An Important Medicinal Plant. Int. J. of Sci. and Research (IJSR), 2319-7064.

Moideen, R. S. And Prabha, L. A. 2014. *In-vitro* Studies on *Luffa acutangula* Lin. Var. Amara. Roxb - An Important Medicinal Plant. Int. J. Pharm Bio. Sci. 5(1): 925 – 933.

Mustafa, Md., Swamy, T. N., Raju, S. and Peer, S. N. 2013. Multiple shoot inductionfrom the nodal cultures of teasle gourd (*Momordica dioica*Roxb.). *Int. J. of Biosciences*, doi: *http://dx.doi.org/10.12692/ijb/3.2.8-12*. 3(2): 8-12.

Mustafa, Md., Swamy, T. N., Raju, S., Mohammad, S. K. and Suresh, V. 2012. Regeneration of Plantlets from Nodal Cultures of *Momordica Dioica*Roxb. *Int. J. Pharm Bio. Sci.* 3(4): 92 – 96.

Nabi, S. A., Rashid, M. M., Al-Amin, M. and Rasul, M. G. 2002[a]. Organogenesis in Teasle Gourd (*Momordiacadioica* Roxb.). *Plant Tissue. Cult*., 12(2): 173 - 183.

Nabi, S. A., Rasul, M. G., Al-Amin, M., Rasheed, M. M., Ozaki, Y. and Okubo, H. 2002[b]. *In-vitro* multiplication of kakrol (*Momordica dioica* Roxb.). *J. of the Faculty of Agric*., 46

(2): 303-309.

Nanasato, Y., Konagaya, K., Okuzaki, A., Tsuda, M. and Tabei, Y. (2013). Improvement of Agrobacterium-mediated transformation of cucumber (*Cucumis sativus* L.) by combination of vacuum infiltration and co-cultivation on filter paper wicks. *Plant Biotechnol Rep*, 7:267–276.

Pal, S. P., Alam, I., Anisuzzaman, M., Sarker, K. K., Sharmin, S. A. and Alam, M. F., (2007). Indirect Organogenesis in Summer Squash (*Cucurbita Pepo*). *Turk J. Agric., 31: 63-70.*

Parvin, S., Kausar, M., Haque, E., Khalekuzzaman, Sikdar, B. and Islam, M. A. (2013). *In-vitro* propagation of muskmelon (*Cucumis melo* L.) from nodal segments, shoot tips and cotyledonary nodes. *Rajshahi University J. of life and earth and Agric. scie., 41: 71-77.*

Patel, M. G. and Kalpesh, B. I. 2015. *Momordica dioica* Roxb. (Spine Gourd): Multiple shoot induction from nodal cultures and its antidiabetic activity. *J. of Medicinal Plants Studies*,3(6): 82-88

Rahman, H., Shahinozzaman, M., Karim, M. R., Hoque, A., Hossain, M. M. and Rafiul, A. K. M. (2012). Rapid *in-vitro* clonal propagation of a hybrid muskmelon (*Cucumis melo* L.) cultivar from seedling explants. *Int. J. Agr. and Agri. R.*, 2(1): 47-52.

Rajasekharan, P. E., Bhaskaran, Sunitha, John, K., Joseph, Koshy, Eapen, P., Antony, V. T. 2012. *In-Vitro* Multiplication and Conservation of Wild *Momordica sahyadrica*. IUP J. of Biotech., 7: 50-56.

Raju, S., Chithkari, R., Bylla, P. and Mustafa, Md. 2015. Molecular Confirmation of Sex in Regenerated Plantlets of Spine gourd (*Momordica dioica*Roxb. Ex. WILD) by using RAPD analysis. *J. of Experimental Biol. and Agric.Sci.*, 3(5): 407-414.

Rama krishna, D, and Shasthri, T. (2015). Adventitious Rooting and Proliferation from Different Explants of *Citrullus colocynthis* L. Schrad an Endangered Medicinally Important Cucurbit. *Asian J. of Biotech.*, 7(2): 88-95.

Randall, P., Niedz, L., Smith, S. S., Kerry, B., Dunbar, Christine, T. Haeey. H. (1989). Factors influencing shoot regeneration from cotyledonary explants of Cucumis melo. *Plant Cell, Tissue and Organ Culture,* 18: 313-319.

Saima malik, Zia, M., Riaz-ur-Rehaman, and Choudhary, M. F. (2007). *In-vitro* plant regeneration from direct and indirect organogenesis of *Momordica charantia*. *Pakistan J. of Biol. Sci.*, 10(22), 4118-4122.

Sangeetha, P. and Venkatachalau, P. (2011). Induction of multiple shoots from shoot tip explants of cucumber (Cucumis sativus l.). Plant Cell Biotechnology and Molecular Biol., 12(1-4):

Sarowar, S., Oh, H. Y., Hyung, N. I., Min, B. W., Harn, C. H., Yang, S. K., Ok, S. H. Shin, and J.S. 2003. *In-vitro* micropropagation of a Cucurbita interspecific hybrid cultivar – a root stock plant. *Plant Cell, Tissue and Organ Culture,* 75: 179–182.

Satapathy, G. and Thirunavoukkarasu, M. 2014. Cytokinins effect on direct shoot bud regeneration from leaf segments of bitter cucumber (*Cucumis trigonus* Roxb.). *Int. J. Pure App. Biosci.*, 2(1): 139-146.

Savitha, R., Shasthree, T., Sudhakar, Mallaiah, B. 2010. High Frequency of Plantlet Regeneration and Multiple Shoot Induction from Leaf and Stem Explant of *Citrullus colosynthis* (l.) schrad, an endangered medicinal cucurbit. Int. J. of Pharma and Bio Sci., 1(2).

Selvaraj, N., Vasudevan, A., Manickavasagam, M. and Ganapathi. A. 2006. *In-Vitro* Organogenesis and Plant Formation in Cucumber. *Biologia Plantarum,* 50 (1): 123-126.

Shasthree, T., Ramakrishna, D., Imran, M. A. and Chandrashekar, Ch. (2014). Adventitious Shoot Organogenesis and Plant Regeneration from Leaf and Cotyledon Explants of *Citrullus colocynthis*. *J. of Herbs, Spices and Medicinal Plants,* 20:235–244.

Shekhawat, M. S., Shekhawat, N. S., Harish, Kheta, R., Phulwaria, M. and Gupta, A. K. 2011. High frequency Plantlet Regeneration from nodal segment culture of Female *Momordiacadioica* (Roxb.). *J. crop Sci. Biotech.*, 14(2): 133-137.

Singh, N., Raj, A., Sharma, A. and Kuma, P. (2011). Efficient *in-vitro* regeneration

system using cotyledon explants in Indian cultivar of sponge gourd (*Luffa Cylindrica* Roem.) compatible to *Agrobacterium tumifaciens* mediated transformation. *Reviewed Proceedings of National Seminar on Internet: Applications in Research*, 36-40, ISBN: 978-81-920945-1-9.

Saurabh, S., Prasada, D., Ambarish, S. and Vidyarthi (2017). *In-vitro* Propagation of *Trichosanthes dioica* Roxb. for Nutritional Security. *J. Crop Sci. Biotech.*, 20(2): 81-87.

Srivastava, A. and Roy, S. (2012). Callus Multiplication of a Medicinally Important Vegetable *Luffa cylindrica*. *Int. J. Pharm. Bio. Sci.*,3(3): 526-531

Sultana, R. S., and Miha, B. M. A. (2003). *In-vitro* Propagation of Karalla (*Momordica charantia* Linn.) from Nodal Segment and Shoot Tip. *J. Biol. Scie.* 3(2): 1134-1139.

Sultana. R. S., Miha, B. M. A., Rahaman, M. M. and Mollah, M. U. (2005). Asceptic Multiplication and Maintanance of Bitter gourd (*Momordica charantia*) as affected by sucrose, Agar and pH. *J. Biol. Scie.*, 5(6): 781-785.

Suratman, F., Huyop, F. and Parveez, G. k. A. (2009). In-vitro shoot regeneration of *Citrullus vulgaris*Schrad (Watermelon). *Biotechnology*,8(4): 393-404.

Swamy, T. N., Bylla, P., Suresh, V. and Mustafa, Md. 2015. *In-vitro* regeneration of *Momordica dioica*Roxb. Through leaf derived callus. *Sci. Res. Reporter*., 5(2): 177- 180.

Thakur, G. S., Sharma, R., Sanodiya, Pandey, M., Baghel, R., Gupta, A., Bisen, P. S. (2011). High frequency *in-vitro* shoot regeneration of *Momordica balsamina*, an important medicinal and nutritional plant. *African J. of Biotech*, 10(70): 15808-15812.

Thiruvengadam, M., Mohamed, S. V., Yanf, C. H. and Jayabalan, N. (2006). development of embryogenoic susupension culture of bitter melon (*Momordica dioica* L.). *Scietia Horticultureae*, 109: 123-129.

Thiruvengadam, M., Rekha, S. K., Yang, C. H. (2007). Somatic embryogenesis and plant regeneration from petiole derived callus of spine gourd (Momordica*dioica*Roxb. Ex Wild). *Functional plant sci. and Bio technology*,1(1): 200-206.

Thiruvengadam, M., Chung M-III, and Se-chun Chun. (2012). Influence of polyamines on *in-vitro* organogenesis in bitter melon (*Momordica charantia* L.) . *J. of Medicinal Plants Research*, 6(19: 3579-3585.

Thiruvengadam, M., Jeyakumar, J. J., Kamaraj, M., Lee, Y. J. and Chung, I. M. (2013). Plant regeneration through somatic embryogenesis from suspension cultures of Gherkin (*Cucumis anguria* L.). *Austrelian J. of crop scie.*,7(7): 969-977.

Thiruvengadam, M., Rekha, K. T., Jayabalan, N., Praveen, N., Kim, E. H. and Chung, I. M. 2013. Effect of exogenous polyamines enhances somatic embryogenesis via suspension cultures of spine gourd (*Momordicadioica*Roxb. Ex. WILD). *Australian J. of CropSci.*, 7(3): 449-453.

Thiruvengadam, M., Nagella, P., Ill-Min Chung. 2012. Plant regeneration from alginate-encapsulated shoot tips of *Momordica dioica* for short term storage and germplasm exchange and distribution. *Plant Omics J*. 5(3): 266-270.

Thomas, T. D. and Sreejesh, K. R. (2004). Callus induction and plant regeneration from cotyledonary explants of ash gourd (*Benincasa hispida* L.). *Scientia Horticulturae*, 100: 359–367.

Umamaheswari, C., Ambethkar, A., Margaret, F. S. and Selvaraj, N. (2014). *In-Vitro* Multiple Shoot Regeneration From Cotyledon Explants Of *Luffa acutangula* (L.) Roxb. *Inter. J. of Current Biotech.* 2(7):7-13.

Usman, Hussain, Z. and Fatima, B. (2011). Somatic Embryogenesis And Shoot Regeneration Induced in Cucumber Leaves . *Pak. J. Bot.*, 43(2): 1283-1293.

Valdez-Melara, M., García, A., Delgado, M., Andres, M., Gatica-Arias. and Ramírez-Fonseca, P. 2009. *In-vitro* plant regeneration system for tropical butternut squash genotypes (*Cucurbita moschata*). *Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744)*, 57(Suppl. 1): 119-127.

Vedat, P., Onay, A., Yildirim, H., Adiyaman, F., Ifiikalan, C. and Bafiaran, D. 2002. Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Diploid Diyarbakr Watermelon (*Citrullus lanatus* cv. Surme). *Turk J. Biol.* 27(2003): 101-105.

Velivela, Y., Narra, M., Ellendula, R., Kota, S., Abbagani, S. 2016. Establishment of *in-vitro* regeneration from petiole explants and assessment of clonal fidelity by ISSR markers in *Luffa acutangula* L. Roxb. J. of Applied Biol. and Biotech. 4(03): 041-045.

Venkateshwaralu, M. 2010. Cytokinin induced multiple shoot induction from node explants of *Cucumis melo* var. utilissimus – A potentially important medicinal plant. *Int. J. of Plant Protection* , 3(1): 107-110.

Venkateshawaralu, M. (2012). Direct multiple shoots proliferation of Muskmelon (*Cucumis melo* L.) from shoot tip explants. *Int. J. of Pharma and Bio Sci*., 3.

Verma, A. K., Kumar, M., Tarafdar, S., Singh, R., and Takur, S. (2014). Development of Protocol for Micropropagation of Gynoecious Bitter Gourd (*Momordica Charantia* L). *Inter. J. of Plant, Animal and Environmental Sci.*, 4: 275-280.

Yutaka, T., Tomohiro, Y., Toshikazu, M., and Takeshi, O. (1998). Plant Regeneration via Shoot Organogenesis from Cotyledons in Two Wild Cucumis Species, *C. Figarei and C. metuliferus*. *JARQ*, 32: 281-286.

Zohura, F.T., Haque, M. E., Islam, M. A., Khalekuzzaman, M., Sikdar, B. (2013). Establishment of an efficient *In-Vitro* regeneration system of Ridge Gourd (*Luffa acutangula* L. Roxb) from immature embryo and cotyledon explants. Int. J. of Scientific and Technology Research, 2(9).

How to cite this article:

Sangram S. Dhumal, B. Veerendra Naik and Mansinghraj S. Nimbalkar. 2020. Advances in Tissue Culture of Cucurbits: A Review. *Int.J.Curr.Microbiol.App.Sci.* 9(08): 2887-2910.
doi: https://doi.org/10.20546/ijcmas.2020.908.324