Cucurbitaceae is one of the elite families 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.

Keywords
Tissue culture, Cucurbits

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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., 2006b, 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), Trichosanthes dioica (Abdul–awal et al., 2005, Komal. 2011c, Malex et al., 2010), Cucumis sativus (Mohammadi and Siveritepe. 2014), Elmeer et al., 1993, Citrullus colocynthis, Sechium edule (Thiruvengadam et al., 2011), Cucurbita maxima (Mahzabin, 2008), Watermelon (Khalekuzzaman et al., 2012, Li et al., 2011, Khatun et al., 2010b, Suratman, 2009, Chaturvedi and Bhanthnagar, 2001), Trichosanthes cucumerina (Devendra et al., 2008, Kawale et al., 2009.), Benincasahispida (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).

**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., (2002a), Swamy et al., (2015), Devendra (2009), Nabi et al., (2002b), Karim (2013), Hoque et al., (2000), Karim (2011), Pateli (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 dioica. Similarly organogenesis was studied in Luffa cylindrica by Srivastava and Roy. 2012, Han et al., 2004 and in Citrullus luminus by Sultana (2004) Vedat Pirinc et al., 2002, Khatun et al., 2010b 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), Trichosanthes dioica (Sourab et al., 2017), Coccinia abyssinica (Guma et al., 2015), Cucumis melo (Rahaman et al., 2012), Cucumis trigonus (Satapathy et al., 2014), Citrullus colocynthis (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 acutangula 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), *Cucumisfigarei* and *Cucumismetuliferus* (Yutaka et al., 1998), *Momordica 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., *Cucumismelo* (Chovelon, 2008, Bezirganoglu et al., 2014), *Cucumissativus* (Nanasato et al., 2013), *Citrulluscolocynthis* (Dadauza et al., 1997) and Sponge gourd (Singh et al., 2011).

**Choice of explant**

**Apical bud**

Apical bud 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), *Citrulluslanatus* (Compton and Grey, 1992, Khalekuzzaman et al., 2012, Vedat et al., 2002), *Cucumishystrix* (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)), *Cucumismelo* (Faria et al., 2013, Huda and Sikdar 2006, Venkateshwaralu. 2012), *Cucumissativus* (Mohammadi and Siveritepe. 2007, Sangeetha et al., 2011), *Cucurbita maxima* (Mahazabin. 2008), *Cucurbitapepo* (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 *Citrulluslanatus* (Khatun et al., 2010), *Cucumisanguria* (Margareate, 2014), *Cucumismelo* (Parvin et al., 2013), *Cucumissativus* (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 cymbalara* (Devi et al., 2017), *Momordica dioica* (Choudhary et al., 2017, Debnath, 2013, Ghive et al., 2006b, Govind et al., 2012, Jadhav, 2015, Kapadia, 2018, Kulkarni, 1999, Mustapha et al., 2012, Mustapha et al., 2013, Patel and Kalpesh, 2015, Shekhwat et al., 2011.), *Trichosanthesdioica* (Komal. 2011a, Komal 2011b, Komal 2011c). Venkateshwaralu 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 *Cucumismelo*. 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 colocynthis* (Devendra, 2009; Guma et al., 2015), *Citrullus lanatus* (Moideen and Prabha, 2013), *Coccinia abyssinica* (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* (Shahshteari 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)], *Trichosanthes dioica* (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

*Beninc a sahis pida* (Thomas et al., 2004), *Citrullus colocynthis* (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), *Trichosanthes cucumerina* (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., 2002a, Nabi et al., 2002b and Karim. 2011). All the scientists used cotyledon as an explant in Spine gourd on a MS media supplemented with BAP 1.0µm +NAA0.1µm howeverArekar (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 colocynthis* 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 melob* Rahaman et al., 2012. In *Cucumis sativus*, cuttings (Ikram-ul haq et al., 2013), hypocotyle (Selvaraj et al., 2006), parthenogenenic embryo (Claveria et al., 2005), somatic embryo (Elmeer et al., 2009) and stem (Jesmine and Mian 2016 and Kielkowska 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., 2006a), 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 *Benincasa hispida* 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 GA3, where normal number of shoots per culture recorded was 5.55. The lower concentration of GA3 induced multiple shoots effectively. When Kausar et al.(2013) used only BAP and GA3, 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 4th subculture 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 mg l⁻¹) + BAP (2.0 mg l⁻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 GA3 (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, Venkateshwaralu et al., 2010, Jadhav, 2015, Khatun et al., 2010b, 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 3rd 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 + AdSO₄ (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., 2006b, Choudhary et al., 2017). In *Cucumis melo var utilisimus* highest concentration of Adenine sulphate (15mg/l) in combination with BAP were found to be best for multiple shoot induction (Venkateshwaralu et al., 2010). In case of *Momordica dioica, Citrullus lunatus* and *Momordica charantia* BAP 1.0 or 2.0 mg/l in combination with NAA 0.1 or 0.2 mg/l 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., 2010b, 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

| Sr. No. | Crop               | Explant | Best treatments (mg/l) | Result                                      | Author                          |
|---------|--------------------|---------|------------------------|---------------------------------------------|---------------------------------|
| 1       | *Sechium edule*    | Stem part | MS + BAP (0.1)         | Full plantlet in soil                       | Abdelnour *et al.*, (2002)      |
| 2       | *Trichosanthes dioica* | 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 OD₆₀₀ 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 Y- 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) + GA₃ (0.3µM) | Full plantlet in soil              | Compton, *et al.*, (2001)       |
| 12      | *Citrullus lanatus* | Cotyledon | Agrobacterium tumefaciens LBA4404 + vector pBI121 + β glucuronidae (gus) + neomycin phosphotransferase(nptII) | For transgenic                     | Dabauza, *et al.*, (1997)       |
| 13      | Cucurbitaceae       | Cotyledon | Reviewed somatic embryogenesis |                                      | Debeaujon and Brancheard (1993) |
| 14      | *Momordica dioica*  | Node     | MS + 2, 4-D (2.0) + BAP (0.5) / Coconut milk | Organogenesis                           | Debnath *et al.*, (2013ª)       |
| No. | Species/Species Name | Type | Media/Conditions | Response | Ref. |
|-----|----------------------|------|------------------|----------|------|
| 15  | Momordica cochinensis| Node | MS agar gelled + 2, 4-D (2.0) + Coconut milk (15% v/v) | Callus | Debnath, et al., (2013) |
| 16  | Momordica dioica     | Leaf | MS + 2, 4-D (1.0 ) + BAP (2.0) | Organogenesis | Devendra et al., (2008) |
| 17  | Trichosanthes cucumerina | Shoot tip | MS + BAP (1.0)+ NAA (0.1) | Full plantlet in soil | Devendra et al., (2009) |
| 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) |
| 23  | Momordica dioica     | Node | MS + AdSO₄ (70/80) + BAP (1.0) + NAA (1.0) | Multiple shoots | Ghive, et al., (2006) |
| 24  | Momordica dioica     | Node | MS + BAP (0.6µm) + Casein hydrolysate (200) | Assessed genetic 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  | Coccinia abyssinica  | Leaf | 5% NaOC with 10 Minutes | Sterilization | Guma, et al., (2015) |
| 27  | Lagenaria siceraria  | Cotyledon | MS + BAP (3) +AgNO₃ (0.5) | AgNO₃ derived plants are diploid | Han, et al., (2004) |
| 28  | Benincasa hispida    | Shoot tip | MS + BAP (1.5) | Full plantlet in soil | Haque, et al., (2008) |
| 29  | Lagenaria siceraria  | 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) + GA₃ (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) + GA₃ (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) |
|   | Plant Name                  | Organ | Medium Components | Response                  | Reference       |
|---|-----------------------------|-------|-------------------|---------------------------|-----------------|
| 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) + GA₃ (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) |
| 49 | Citrullus lanatus           | Node  | MS + BAP (1.0) + NAA (0.2) | Full plantlet in soil     | Khatun et al., (2010) |
| 50 | Cucumis sativus L.          | Stem fragments | MS + Kinetine (6.0 µm) | Flower and pollen production | Kielkowska 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 (2011a)   |
| 53 | Trichosanthesdioica        | Node  | MS + BAP (2.5)    | Callus                    | Komal (2011b)   |
| 54 | Trichosanthesdioica        | Node  | Semi solid MS + Coconut milk (15%) | Full plantlet in soil     | Komal (2011c)   |
| 55 | Citrullus lanatus           | Cotyledon | MS + BAP (1) + coconut water (10%). | Organogenesis               | Krug, et al., (2005) |
| 56 | Momordica dioica            | Node  | MSHP + AdSO₄ (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) |
|   | Species                        | Morphology   | Medium                                      | Response                          | References                      |
|---|--------------------------------|--------------|---------------------------------------------|-----------------------------------|---------------------------------|
| 58| *Cucurbita maxima*              | Shoot tip    | MS + BAP (3.0)                              | Full plantlet in soil             | Mahazabin (2008)                |
| 59| *Trichosanthes dioica*          | 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., (2002a)           |
| 67| *Momordica dioica*             | Cotyledon    | MS + BAP (1.0) + NAA (0.1)                  | Organogenesis                     | Nabi, et al., (2002b)           |
| 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 (0.1) | 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 colocynthis*        | Cotyledon    | MS + IAA (2.0) + IBA (1.5)                  | Rhizogenesis                      | Ram and Shashtri (2015)         |
|   | Species                | Plant Part | Media Composition                                                                 | Factor of Influence               | Reference                          |
|---|-----------------------|------------|-----------------------------------------------------------------------------------|-----------------------------------|-----------------------------------|
|78 | *Cucumis melo L.*     | Cotyledon  | MS + IBA (5.0 µM) + BAP (5.0 µM) + light intensity (5-30µmolm⁻²s⁻³)               |                                   | Randall, *et al.*, (1989)         |
|79 | *Momordica charantia* | Leaf       | MS + BAP (1.5)                                                                     | Callusogenesis                    | Saima *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 | *Trichosanthes dioica* | 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                     | Sultana, *et al.*, (2004)         |
|92 | *Momordica charantia* | Node       | MS + BAP (2) + NAA (0.2) + Sucrose 30 g l⁻¹ + Agar 7.0 g l⁻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, *et al.*, (2006)   |
| No. | Species                      | Tissue Type | Medium Composition                                                                 | Regeneration Type                              | Authors, Year |
|-----|------------------------------|-------------|-----------------------------------------------------------------------------------|------------------------------------------------|---------------|
| 98  | *Momordica dioica*           | Shoot tip   | MS (0.7% agar solidified) + BAP (0.5µm)                                          | Full plantlet in soil without variation         | Thiruvengadam, et al., (2007)                      |
| 99  | *Momordica charantia*        | Petiole     | MS and Gamboge + NAA (3.0µm) + TDZ (1.0 µm) + Putrecine (1.0µm)                  | Plantlet from organogenesis                     | Thiruvengadam, et al., (2012)                      |
| 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 utilissimus* | Node        | MS + BAP (1.0) + Adenine sulphate (15)                                           | Multiple shoots                                 | Venkateshwaralu, et al.(2010)                     |
| 108 | *Cucumis melo L.*            | Shoot tip   | MS + IAA (0.5) + BAP (2.0)                                                       | Multiple shoots                                 | Venkateshwaralu (2012)                            |
| 109 | *Momordica charantia*        | Node        | 1/2 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 mg/l 1BAP + 0.2mg/l NAA + L - glutamine (20mg/l) and elongation of shoots were achieved by adding GA₃ (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 AdSO₄ + 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 *Trichosanthes dioica* 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).}

**Cotyledon**

In micropropagation cotyledons play a vital role in giving the successful plantlets. In this regard, 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 *Luffia acutangula*, 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 lanatus* 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⁻¹. 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-AT01 were 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 on MS + IBA (10.8) + NAA (1.08) + GA3 (0.54) (Hogue 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 mg/l and 2.0 mg/l 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 induced by leaf disc explants of cucumber on MS medium fortified with 2, 4-D @ 2.0 mg/l. Callus induced from leaf disc explants at higher level of 2, 4-D (5 mg/l) 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/l BAP, 30 g/l (w/v) and 0.1 mg/l 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 0.5mg/l or 3.5 mg/l 2, 4-D from zygotic embryos(53-56%) and cotyledonary seedlings (70%) derived from Cucurbita moschata cv. Sellode 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 played a 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 all the 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., 2002b, Hoque et
Organogenesis in watermelon 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 mg/l 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., 2002b, 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 explants in 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) + AdSo4 (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 AgNO3 under a 16hr photoperiod. The diploid cultured were recorded in the most of the AgNO3 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/l 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 colocynthis 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 Coccinia abyssinica. 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 2.5 mg/l with BAP 0.5, 1.0 and 2.0 mg/l in leaf explants of *Momordica dioica*, *Citrullus lanatus*, *Citrullus colocynthis*, *Trichosanthes dioica* (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 1.5 mg/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 3.0 mg/l in combination with the NAA @ 0.1, 0.5 and 1.0 mg/l was found to be the best source of callusing and organogenesis. In some cases, use of BAP 1.0 +NAA 1.0 mg/l was reported for multiple shoot regeneration from callus leaf explants (Nabi et al., 2002a, 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 use the 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 time NB6 phytohormones was used. NB6 + BAP (0.5 + 0.5 mg/l) recorded the highest percentage of shoot multiplication within 15 days of inoculation with shoot length 5.2 ± 0.37 cm 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 μM putrecine 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 (Thiruvenkadham et al., 2012). Callus induction and multiplication was tried on *Luffia acutangular* 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 *Luffia 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.

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