**Review Article**

**In vitro Propagation of Stevia rebaudiana (Bertoni): An Overview**

Manvender Singh*, Vinod Saharan, Jyotsna Dayma, Deepak Rajpurohit, Yadunandan Sen and Ajay Sharma

Department of Molecular Biology and Biotechnology, Rajasthan Collage of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

*Corresponding author

**A B S T R A C T**

Stevia rebaudiana is herbaceous perennial plant of Asteraceae family and an excellent substitute of sugar with medicinal importance. The potential uses of Stevia which produces stevioside, a non-caloric sweetener that does not metabolize in the human body, hence control blood sugar level. Conventional propagation methods are not produce adequate planting material. Plant tissue culture techniques are only technique to produce quality planting material. In present review, micro propagation methods and protocols are compile and generate the information to researchers’ for further exploration for improvement of this valuable medicinal plant.

**Keywords**

In-vitro culture, Micro propagation, Stevia, Rebaudioside.

**Article Info**

Accepted: 17 June 2017
Available Online: 10 July 2017

**Introduction**

Stevia rebaudiana (Bertoni) is a medicinal plant belongs to Asteraceae family and known as stevia, sweet leaf, honey leaf, and candy leaf. Stevia is native to Paraguay (South America) and also called sweet herb of Paraguay. The leaves of this plant has a pleasantly sweet and refreshing taste which is educe by diterpene glycosides (stevioside and rebaudiosides), a high-potency sweeteners and substitute to sugar, being 300 times sweeter than sucrose (Madan et al., 2010; Megeji et al., 2005; Singh and Rao, 2005; Soejarto et al., 1983; Soejarto DD, Kinghorn AD, 1982; Yadav et al., 2011). Stevia is a well-known therapeutic agent serve as an efficient medication for diabetes, hypertension, myocardial and antimicrobial infections, dental troubles, and tumors (Chan et al., 1998; Gregersen et al., 2004; Jayaraman et al., 2008; Jeppesen et al., 2003, 2002; Kujur et al., 2010; Marcinek and Krejpcio, 2016; Muanda et al., 2011; Philippe et al., 2014; Planas and Kucacute, 1968; Shivanna et al., 2013; Šic Žlabur et al., 2013; Singh et al., 2015). In present time people are very calorie conscious which increases use of stevia in preparation of non-calorie food stuffs and become a major sweetening agent in food products in South-east Asia (Ashwell, 2015; Durán A. et al., 2013; Marcinek and Krejpcio, 2016; Panpatil and Polasa, 2008; Salunkhe and Bhise, 2010; Savita et al., 2010). Recently FDA of United States of America issued GRAS status to stevia product. The market grows at a rate of 4% per annum and has a business of around 1.3
billion US dollar. Two international business groups, Wilmar and Olam, have started a joint venture to invest 106.2 million US dollar to globally enhance the production and consumption of Stevia. India is supposed to have suitable conditions for the cultivation of stevia. It has been found that Indian stevia plant gives a higher stevioside yield of 10–18 percent in comparison to the reported 8–12 percent from other countries (Yadav et al., 2011).

An International stevia supplier called GLG Life Tech has taken the initiative of introducing stevia to the Indian market and to facilitate its production and extraction in India. India itself is also stepping forward to compete in the stevia sweetener international market (Savita et al., 2010; Yadav et al., 2011). This will paved the path for commercial cultivation of stevia in developed as well as in developing countries, ahead to replace the cane sugar with a global production estimated to be around 40,000 million tons and for India it may be around 600 tons (Ahmed B., Hossain M., Islam R., Kumar Saha A., 2011; Gantait et al., 2015; Yadav et al., 2011). But still the cultivation and commercialization of stevia has not achieved the expected heights as the conventional propagation methods lack in providing rapid quality planting material to the farmers.

Today demand will need to be supported by high biomass yield varieties with improved agronomical traits as well as higher quantities and quality of diterpene glycosides production. This generates the need of stevia development in vitro.

The purpose of this overview is to summarize the existing literature for the in vitro culture of stevia that may help to recognize from beginning to end tissue culture technology in stevia and provide baseline for further improvement.

Conventional propagation methods in stevia

In nature, seed germination in stevia is poor and unsuccessful commonly due to infertile seed (Goettemoeller and Ching, 1999; Kumar, 2013) and small endosperm (Yadav et al., 2011). Even some plant selections produce virtually no viable seed due to their self-incompatibility (Raina et al., 2013; Ramesh et al., 2006; Yadav et al., 2011). Plant raised from seed does not allow the production of homogenous plant population resulting in great variability in important features like sweetening levels and compositions (Brandle and Telmer, 2007; Kovyljava et al., 2007).

Numbers of reports are available on successful propagation of Stevia via stem cuttings (Ramesh et al., 2006; Shock, 1982; Smitha and Umesha, 2011). But the direct planting of stem cuttings in the field has limited success due to poor rooting (Khalil et al., 2014; Pande and Gupta, 2013; Smitha and Umesha, 2011). Further, location from where cuttings are taken, the pair of leaves, length of the cuttings as well as the season effect rooting percentage and growth (Brandle et al., 1998; Carneiro et al., 1997; Ceunen and Geuns, 2013; Khalil et al., 2014; Rajasekaran et al., 2007). Published work indicated the necessity of higher quantities of starch, carbohydrates, sugar and phenolic compounds for root initiation (Brandle et al., 1998; Ibrahim et al., 2008).

Rooting of cuttings can sometimes be stimulated by the use of growth regulators (Brandle et al., 1998; Ibrahim et al., 2008). Moreover, a huge number of stem cuttings are required for mass propagation of plants which is an obstacle as number of mother plant need to be vanish. Although, vegetative propagation has been found next considerable option for the mass propagation of stevia. But ample supply of planting material is still not adequate for farmers because vegetative
propagation is limited by production of less number of individuals multiply from a single explant (Gantait et al., 2015; Pande and Gupta, 2013; Philippe et al., 2014; Yadav et al., 2011).

**In vitro tissue culture of stevia**

Plant regeneration from *in vitro* culture can be achieved by either organogenesis or embryogenesis. Administration of different phytohormone enhances and accelerates the production of *in vitro* plants with good agronomical traits and steviosides content in leaves (Brandle et al., 1998; Durán A. et al., 2013; Jain et al., 2014). However, there are some fundamental factors like growth condition of the source material, medium composition, culture conditions and genotypes of donor plants whose role cannot be avoided for the successful *in vitro* culture plants (Gantait et al., 2015; Ibrahim et al., 2008; Pande and Gupta, 2013; Philippe et al., 2014).

**Establishment of in vitro culture of stevia through nodal explant**

Nodal part is the choicest explant for *in vitro* culture of stevia for researchers. Various reports on *in vitro* use of nodal explant had been well documented (Ahmed et al., 2007; Atalay et al., 2011; Debnath, 2008; Laribi et al., 2012; Mitra and Pal, 2007; Nepovim and Vanek, 1998; Sivaram and Mukundan, 2003; Sung, 2006; Tamura et al., 1984; Thiyagarajan and Venkatachalam, 2012). Initial work on nodal explant was stated by Yang and his co-workers with the achievement of highest axillary shoot proliferation. They concluded that the type of cytokinin was the most important factor affecting shoot multiplication (Table 1). Also in our lab we have obtain similar results, using TDZ for shoot induction and kin for shoot multiplication (Fig 1). Increasing BA concentration promoted shoot multiplication. Similar results were obtained by Sivram and Mukundan with shoot apex and leaf explants (Sivaram and Mukundan, 2003). However, medium supplemented with kin resulted in elongated shoots. For root induction, different concentrations of IBA and NAA were assayed. IBA showed to be more significant and effective for rooting than NAA in all concentrations used. The maximum root induction (100%) was observed on medium supplemented with 1.0 or 2.0 IBA (mg/l) and in our lab we observed root induction on MS basal medium without PGR/low concentration of IBA i.e. 0.5 mg/l (Fig 2). Similar result was obtained by medium fortified with low concentration of IAA (0.1 mg/l) and it was found that root induction gradually decreased with increasing concentration of auxin (Ahmed et al., 2007; Atalay et al., 2011). Venkatachalam also studied the effect of different concentrations of BAP in combinations with various auxins on multiple shoot bud regeneration. Of the three auxins combinations tested (IAA/IBA/NAA), BAP with IAA combination was found to be superior for induction of highest percent (92%) of multiple shoot bud development, followed by BAP and NAA (83%) and BAP along with IBA (75%) combinations. However, both last combinations (BAP with NAA and BAP with IBA) produced more callus with low percent (50%) of multiple shoot bud regenerations (Thiyagarajan and Venkatachalam, 2012). The lower percent of multiple shoot bud regenerations may be due to the profuse callusing at the basal part of differentiated shoot buds.

**Establishment of in vitro culture of stevia through stem tip explant**

*In vitro* clonal propagation by stem-tips culture with a few leaf primordia was reported by Tamura and his group (Tamura et al., 1984). They found that neither roots nor
callus developed when stem-tips were grown on a medium supplemented with cytokinin only whereas, the similar dose of kinetin proved effective in vegetative propagation, yielding 50-100 shoots from a single stem-tip in 80 days. Addition of auxin (NAA) along with kin could not enhance shoot formation, but did induce callus formation. The study suggested that the ability to form multiple shoots is dependent on the size of excised stem tip and/or the number of leaf primordia. Similar studies on shoot proliferation had been performed (Akita et al., 1994; Ferreira and Handro, 1988; Sivaram and Mukundan, 2003) (Table 1).

They also reported that plant hormone is necessary for shooting, elongation and rooting. Recently similar results with MS medium in place of previously used LS medium nourished with kin were obtained by Das and coworkers (Das et al., 2011). For root induction, they found that MS media without growth regulators worked dynamically whereas when it was supplemented with auxins (IAA and BA), it had an adverse effect on root induction. They also performed peroxidase assay along with Inter-simple sequence repeat (ISSR) fingerprinting to confirm the genetic fidelity of in vitro generated propagules.

**Establishment of in vitro culture of stevia through leaf explant**

It is well established that a precise level of cytokinin would play a critical role in shoot organogenesis in various plants. Sreedhar and co-workers had tested various combinations of growth regulators and found that BA and kin could successfully induced adventitious bud from the midrib of leaf explant.

It was the first report of direct shoot induction from leaf explant. Initially, the shoot buds appeared as white knob-like structures which later turned green leading to the formation of a pair of green leaves (Sreedhar et al., 2008). In an earlier study, kin in combination with NAA or IAA failed to induce shoot formation on leaf explants of stevia whereas BA was found to be more effective (Sivaram and Mukundan, 2003). Contrarily, Tamura et al., showed the formation of shoots only from the margins of stevia leaves in shoots cultured on a very high concentration of kin for 40 days (Tamura et al., 1984).

This was probably because of the accumulation of high amount of cytokinin that is known to induce adventitious shoot formation. Effect of copper on in vitro culture of stevia leaf explants was also studied. It was found that an optimum level of copper helps to achieve maximum shoot bud induction and elongation along with BAP and IAA PGRs. This has also gave a positive effect on chlorophyll and biomass production (Jain et al., 2009). A promising method of micropropagation of stevia has been developed with an aim to increase the biomass, survivability of the plantlets and stevioside production, using chlorocholine chloride, a plant growth retardant. The application of chlorocholine chloride along with IBA on in vitro generated microshoots from cotyledonary leaves explants found to be effective for inducing certain beneficial changes like desirable reduction in stem, elongation, profuse rooting, bigger leaf size, increase fresh weight of the plantlets, longer chlorophyll retaining capacity and higher stevioside production (Dey et al., 2013).

Recently, Ramírez-Mosqueda and Iglesias-Andreu, 2016 made a recipe for in vitro plantlet regeneration through thin cell layer method. Different concentration and combination of exogenous growth regulators (BA and 2, 4-D) were tested with transverse thin cell layers and 2, 4-D showed best organogenesis.
Table 1 In vitro culture of stevia

| Type of explants                  | Shooting media                      | Rooting media                   | References                        |
|-----------------------------------|-------------------------------------|---------------------------------|-----------------------------------|
| Leaflets                          | 2-10 mg/l BA                        |                                 | Chen and Li, 1993                |
| Shoot primordial                  | 1.0 mg/l BA                         | 0.1 mg/l NAA                    | Akita et al., 1994               |
| Shoot apex, nodal, and leaf explants | 2.0 mg/l BA and 1.0 mg/l IAA        | 1.0 mg/l IBA                    | Sivaram and Mukundan, 2003       |
| Nodal                             | 2.0 mg/l IAA and 0.5 mg/l kin       | 2.0 mg/l IBA                    | Hwang, 2006                      |
| Nodal                             | 1.5 mg/l BA + 0.5 mg/l kin          | 0.1 mg/l IAA                    | Ahmed et al., 2007               |
| Nodal                             | 2.0 mg/l BA and 1.13 mg/l IAA       | 2.0 mg/l IBA                    | Debnath, 2007                    |
| Midrib                            | 2.0 mg/l BA and 1.0 mg/l kin        | 1.0 mg/l IBA                    | Sreedhar, 2008                   |
| Leaf and Nodal                    | 0.5mg/l BA and 0.5 mg/l IAA         |                                 | Jain et al., 2009                 |
| Nodal                             | 0.5 mg/l BA + 0.5 mg/l kin          | 1.0 or 2.0 mg/l IBA             | Alhady, 2011                     |
| Nodal                             | 1.0 mg/l BA                         | 0.4 mg/l NAA                    | Venkatachalam, and Thiagarajan, 2012 |
| Nodal                             | 1.0 mg/l BA                         | 0.5 mg/l IBA or IAA             | Labiri, 2012                     |
| Stem-tips with a few leaf primordial | 10 mg/l kin                       | 0.1 mg/l NAA                    | Labiri, 2012                     |
| Nodal segment                     | MS + 0.5 BAP mg/l + 2.0 Kin mg/l    | MS +1.0 IBA mg/l                | Mehta et al., 2012               |
| Nodal segment                     | MS + 0.5 BAP mg/l + 2.0 Kin mg/l    | MS +0.1 IBA mg/l +100 ppm Charcoal | Modi et al., 2012               |
| Shoot tip, nodal segment          | MS + 1 BAP mg/l + 2 Kin mg/l        | MS +0.5 IBA mg/l                | El-Motaleb et al., 2013          |
| Shoot tip                         | MS + 1.5 BAP mg/l + 10 Spermine mg/l| MS + 1.5 IAA mg/l               | Guruchandran and Sasikumar, 2013 |
| Shoot tip, nodal segment          | MS + 2.0 BAP mg/l                   | MS+0.5 IBA mg/l                 | Hassanen and Khalil, 2013        |
| Shoot tip                         | MS + 1.0 BAP mg/l                   | MS + 0.4 IBA mg/l               | Javad et al., 2013               |
| Nodal segment MultSht             | MS + 1.0 I M TDZ                     | ½ MS                           | Lata et al., 2013                |
| Nodal explant                     | ½ MS + 0.01 TDZ mg/l                | ½ MS + 1.0 IBA mg/l             | Singh and Dwivedi, 2013          |
| Nodal segment                     | MS + 1.0 BAP mg/l + 0.05 NAA mg/l   | MS+0.5 IAA mg/l                 | Soliman et al., 2013             |
| Shoot tip                         | MS + 1.0 IAA mg/l +1.0 BA mg/l      |                                 | Taleie et al., 2013              |
| Nodal segment                     | MS + 1.0 IAA mg/l                   | MS + 0.2 IAA mg/l               | Nower, 2014                      |
| Nodal segment MultSht             | MS + 0.5 Kin + 1.0 IBA              |                                 | Singh et al., (2014)             |
| Thin cell layer                   | 6.78 µM 2, 4-D                      | Without PGR                    | Ramirez-Mosqueda and Iglesias-Andreu, 2016 |
Table.2 Somatic embryogenesis studies in stevia plants

| Explants            | media                                      | References                               |
|---------------------|--------------------------------------------|------------------------------------------|
| Cell suspensions    | BA (0.5 mg/l)+2,4D (0.5 mg/l)              | Ferreira and Handro, 1988                |
| Leaf                | NAA, 0.5 mg/l+ BAP, 0.5 mg/l              | Swanson et al., 1992                    |
| Anther              | BAP, 0.1 to 1.0 mg/l                      | Flachsland et al., 1996                 |
| Floret              | 4.0 mg/l 2,4-D 0.5 mg/l kin               | Bespalhok-Filho and Hattori, 1997        |
| Leaf                | 2,4-D (2.0 mg/l)+ kin (0.2 mg/l)           | Das et al., 2006                        |
| nodal, leaf and root| NAA (1.0 mg/l)                             | Gupta et al., 2010                      |
|                     | NAA (2.0 mg/l)                             |                                          |
|                     | IBA (0.5 mg/l)                             |                                          |
| Leaf disc           | MS + 1.5 NAA mg/l or 5.0 Cyanobacterial media | Banerjee and Sarkar, 2008              |
| Nodal and leaf segment | MS + 1.0 2,4-D mg/l + 0.2 BAP mg/l + 0.2 TDZ mg/l | Banerjee and Sarkar, 2009               |

Fig.1 Shoot multiplication through nodal explants
Establishment of callus culture

Callus culture in stevia has been studied for the production of organs or somatic embryos. Regeneration from callus derived from different explants, including cell suspension (Ferreira and Handro, 1988), leaves (Gupta et al., 2010; Swanson et al., 1992), nodal (Gupta et al., 2010), flower (Ahmed et al., 2007), root (Gupta et al., 2010), anthers (Flachsland et al., 1996) has been achieved (Table 2). Callus is important for rapid mass multiplication, generation of variability, cell suspension culture, preservation of cell line culture and production of secondary metabolites. Uddin and workers reported the establishment of callus culture in stevia through Leaf, nodal and inter-nodal segments. They observed that inter-nodal segments initiated callus earlier than node and leaf. It was also reported that the highest amount of callus was found in MS medium with 2, 4-D at 3.0 mg/l but poorest callus was resulted with the increase in 2, 4-D concentration (Uddin et al., 2006). In a similar study 100% callusing was obtained from leaf explants cultured on combination of NAA and 2,4-D (Gupta et al., 2010). Studies suggested that leaf explants could serve as a best explant for callus production. The calli obtained from leaf
and root explants were shiny green while with nodal explants it was hard and brown. Though the role of 2, 4-D was well established in callus production but the study of Das and group reported that 2, 4-D in combination with kin is best for callus induction whereas, NAA and BAP are superior for callus maintenance (Das et al., 2014). Recently, it is found that leaf explants of stevia when subjected to varying concentrations of sodium azide and colchicine (0- 0.250%) solution for varying period (12- 24 h), this influence the callus induction and growth but same will be delay when the concentration of mutagen increases (Pande and Khetmalas, 2012).

Somatic embryogenesis in stevia

Bespalhok-Filho and coworkers reported the somatic embryogenesis in stevia from leaf explant to investigate the influence of growth regulators on the induction of somatic embryogenesis. They concluded that, combination of 10 or 25 mM 2, 4-D and 1.0 mM BA were found to be effective for somatic embryogenesis (Bespalhok-Filho et al., 1993). In another experiment they used floret as explants and employed 2, 4-D and kin and observed a light green or light yellow color embryogenic callus, which was characterized by compact structure and presence of globular somatic embryos on its surface (Bespalhok-Filho and Hattori, 1997).

In conclusion, *Stevia rebaudiana* is a new emerging alternative source of calorie free sweetener gaining popularity worldwide. Lack of quality planting material is a bottle neck in large-scale cultivation of stevia. Tissue culture technique proved to be boon for the production of high quantity and quality planting material for farmers. At present, direct regeneration of plantlets via adventitious shoot bud induction from nodal explants is considered as preferred method for stevia plant regeneration. Future research emphasised on the development of protocols for direct regeneration of shoot buds from leaf explants, protocols for regeneration through somatic embryo-genesis need to be developed as it can help in producing true to type and homozygous plants with improved quality and development of improved genotypes with a high content of rebaudioside-A, higher biomass production, wider adaptability, better germination, viable seed production. Presently, the research is going on isolation, selection and multiplication of variants with high stevioside content to sustainably meet the worldwide demand of Stevia to the food processing and pharmaceutical industry.

Acknowledgement

The study was supported by the Department of Science and Technology, Government of Rajasthan and Rajiv Gandhi National Fellowship by University Grant Commission, Government of India.

References

Ahmed, M.B., Salahin, M., Karim, R., Razvy, M. A., Hannan, M.M., Sultana, R., Hossain, M., and Islam, R. 2007. An Efficient Method for in vitro Clonal Propagation of a Newly Introduced Sweetener Plant (*Stevia rebaudiana* Bertoni.) in Bangladesh. American-Eurasian Journal of Scientific Research. 2 (2): 121–125.

Ahmed, B., Hossain, M., Islam, R., Kumar, S.A., and Mandal, A. 2011. A review on natural sweetener plant - stevia having medicinal and commercial importance. Agronomski Glansnik. 1(2): 75–92.

Akita, M., Shigeoka, T., Koizumi, Y., and Kawamura, M. 1994. Mass propagation of shoots of *Stevia rebaudiana* using a large scale bioreactor. Plant Cell Reports. 13: 180–183.

Ashwell, M. 2015. Stevia, Nature’s Zero-
Calorie Sustainable Sweetener: A New Player in the Fight Against Obesity. Nutrition today.50: 129–134.

Atalay, E., Erisen, S., Yorgancilar, M., and Tanur, M. 2011. Micropropagation of Stevia rebaudiana Bertoni. Current Opinion in Biotechnology. 22S: S15–S152.

Banerjee, M., and Sarkar, P. 2008. In vitro callusing in Stevia rebaudiana Bertoni using cyanobacterial media- a novel approach to tissue culture. International Journal of Integrative Biology. 3: 163–168.

Banerjee, M., and Sarkar, P. 2009. Somatic embryogenesis in Stevia rebaudiana Bertoni using different concentration of growth hormones. International Journal of Plant Science. 5: 284–289.

Bespalhok-Filho, J. C. and Hattori, K. 1997. Embryogenic callus formation and histological studies from Stevia rebaudiana (Bert.) Bertoni floret explants. Revista Brasileira de Fisiologia Vegetal. 9: 185-188.

Bespalhok-Filho, J. C., Hashimoto, J. M. and Vieira, L. G. E. 1993. Induction of somatic embryogenesis from leaf explants of Stevia rebaudiana. Revista Brasileira de Fisiologia Vegetal. 5: 51-53.

Brandle, J.E., Starratt, A.N., and Gijzen, M. 1998. Stevia rebaudiana: Its agricultural, biological, and chemical properties. Canadian Journal of Plant Science. 78: 527–536.

Brandle, J.E., Telmer, P.G., 2007. Steviol glycoside biosynthesis. Phytochemistry. 68: 1855–1863.

Carneiro, J.W.P., Muniz, A.S., and Guedes, T.A. 1997. Greenhouse bedding plant production of Stevia rebaudiana (Bert) Bertoni. Canadian Journal of Plant Science. 77: 473-474.

Ceunen, S., and Geuns, J.M.C. 2013. Influence of photoperiodism on the spatio-temporal accumulation of steviol glycosides in Stevia rebaudiana (Bertoni). Plant Science. 198:72–82.

Chan, P., Xu, D.Y., Liu, J.C., Chen, Y.J., Tomlinson, B., Huang, W.P., and Cheng, J.T. 1998. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. Life sciences. 63: 1679–84.

Chen, S.Y. and Li, Q.R. 1993. Effect of growth substances on the stevioside content of Stevia rebaudiana. Plant Physiol. 29: 265-267.

Das, A., Gantait, S., and Mandal, N. 2011. Micropropagation of an elite medicinal plant: Stevia rebaudiana bert. Internation Journal of Agricultural Research. 6(1): 40-48.

Das, K., Dang, R., and Rajasekharan, P.E. 2014. Establishment and maintenance of callus of Stevia rebaudiana bertoni under aseptic environment. Indian Journal of Natural Products and Resources. 5: 373–376.

Debnath, M. 2008. Clonal propagation and antimicrobial activity of an endemic medicinal plant Stevia rebaudiana. Journal of Medicinal Plants Research. 2(2): 045-051.

Dey, A., Kundu, S., Bandyopadhyay, A., and Bhattacharjee, A. 2013. Efficient micropropagation and chlorocholine chloride induced stevioside production of Stevia rebaudiana Bertoni. Comptes Rendus-Biologies. 336: 17–28.

Durán A.S., Rodríguez, N.M.P., Cordón, A.K., and Record, C.J. 2013. Stevia (Stevia rebaudiana), non-caloric natural sweetener. Estevia (Stevia rebaudiana), edulcorante natural y no calórico. 39: 203–206.

El-Motaleb, M.A., El-Hady, M.A.S., El-Kholy, M.A., and Badr, A. 2013. In vitro propagation of Stevia rebaudiana Bertoni in Egypt. Journal of Applied
Ferreira, C.M., and Handro, W. 1988. Production, maintenance and plant regeneration from cell suspension cultures of Stevia rebaudiana (Bert.) Bertoni. Plant Cell Repotrs. 7: 123–126.

Flachsland, E., Mrogrinski, L., and Daviña, J. 1996. Regeneration of plants from anthers of Stevia rebaudiana Bertoni (Compositae) cultivated in vitro. Biocell. 20: 87–90.

Gantait, S., Das, A., and Mandal, N. 2015. Stevia: A Comprehensive Review on Ethnopharmacological Properties and In Vitro Regeneration. Sugar Tech. 17: 95–106.

Goettemoeller, J., and Ching, A. 1999. Seed Germination in Stevia rebaudiana. Ptores New Crops and New Uses. 510–511.

Gregersen, S., Jeppesen, P.B., Holst, J.J., and Hermansen, K. 2004. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism: Clinical and Experimental. 53: 73–76.

Gupta, P., Sharma, S., and Saxena, S. 2010. Micropropagation of Stevia rebaudiana (natural sweetener) using kinetin for Steviol glycoside production. Research Journal of Biotechnology. 5: 63–67.

Guruchandran, V., and Sasikumar, C. 2013. Effect of polyamines on in vitro organogenesis using shoot tip explants of Stevia rebaudiana Bert. International Journal Current Biotechnology. 1: 16–18.

Hassanen, S.A., and Khalil, R.M.A. 2013. Biotechnological studies for improving of Stevia (Stevia rebaudiana Bertoni) in vitro plantlets. Middle-East Journal of Scientific Research. 14: 93–106.

Ibrahim, I.A., Nasr, M.I., Mohammed, B.R., and El-Zefzafi, M.M. 2008. Plant growth regulators affecting in vitro cultivation of Stevia rebaudiana. Sugar Tech. 10: 254–259.

Jain, P., Kachhwaha, S., and Kothari, S.L. 2014. Biotechnology and metabolic engineering of Stevia rebaudiana (Bert.) Bertoni: Perspective and Possibilities. International Journal of Life Sciences Biotechnology and Pharma Research. 3: 25.

Jain, P., Kachhwaha, S., and Kothari, S.L. 2009. Improved micropropagation protocol and enhancement in biomass and chlorophyll content in Stevia rebaudiana (Bert.) Bertoni by using high copper levels in the culture medium. Scientia Horticulturae.119: 315–319.

Javad, S., Naz, S., Ilyas, S., and Mateen, B. 2013. Establishment of the honey crop (Stevia rebaudiana) in hot semiarid climate. The Journal of Animal and Plant Sciences. 23: 108–113.

Jayaraman, S., Manoharan, M.S., and Illanchezian, S. 2008. In-vitro Antimicrobial and Antitumor Activities of Stevia rebaudiana (Asteraceae) Leaf Extracts. Tropical Journal of Pharmaceutical Research. 7: 1143–1149.

Jeppesen, P.B., Gregersen, S., Alstrup, K.K., and Hermansen, K. 2002. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine : international journal of phytotherapy and phytopharmacology. 9: 9–14.

Jeppesen, P.B., Gregersen, S., Rolfsen, S.E.D., Jepsen, M., Colombo, M., Agger, A., Xiao, J., Kruhofer, M., Ørntoft, T., and Hermansen, K. 2003. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism: Clinical and Experimental. 52: 372–378.

Khalil, S.A., Zamir, R., and Ahmad, N. 2014.
Selection of suitable propagation method for consistent plantlets production in *Stevia rebaudiana* (Bertoni). Saudi Journal of Biological Sciences. 21: 566–573.

Kovylyaeva, G.I., Bakaleinik, G.A., Strobykina, I.Y., Gubskaya, V.I., Sharipova, R.R., Al’Fonsov, V.A., Kataev, V.E., and Tolstikov, A.G. 2007. Glycosides from *Stevia rebaudiana*. Chemistry of Natural Compounds. 43: 81–85.

Kujur, R.S., Singh, V., Ram, M., Yadava, H.N., Singh, K.K., Kumari, S., and Roy, B.K. 2010. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. Pharmacognosy research. 2: 258–263.

Kumar, R. 2013. Seed Germination of *Stevia rebaudiana* Influenced by Various Potting Media. Octa Journal Biosciences. 1: 143–146.

Lata, H., Chandra, S., Wang, Y.H., Raman, V., and Khan, I.A. 2013. TDZ-induced high frequency plant regeneration through direct shoot organogenesis in *Stevia rebaudiana* Bertoni: an important medicinal plant and a natural sweetener. American Journal of Plant Sciences. 4: 117–128.

Laribi, B., Rouatbi, N., Kouki, K., and Bettaieb, T. 2012. In vitro propagation of *Stevia rebaudiana* (Bert.) -A non caloric sweetener and antidiabetic medicinal plant. International Journal of Medicinal and Aromatic Plants. 2: 2249–4340.

Madan, S., Ahmad, S., Singh, G.N., Kohli, K., Kumar, Y., Singh, R., and Garg, M. 2010. *Stevia rebaudiana* (Bert.) Bertoni - A Review. Indian Journal of Natural Products and Resources. 1: 267–286.

Marcinek, K., and Krejpcio, Z. 2016. *Stevia rebaudiana* Bertoni: health promoting properties and therapeutic applications.

Journal für Verbraucherschutz und Lebensmittelsicherheit. 11: 3–8.

Megeji, N.W., Kumar, J.K., Singh, V., Kaul, V.K., and Ahuja, P.S. 2005. Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. Current Science. 88: 801–804.

Mehta, J., Sain, M., Sharma, D.R., Gehlot, P., Sharma, P., and Dhaker, J.K. 2012. Micro propagation of an antidiabetic plant-*Stevia rebaudiana* Bertoni, (Natural Sweetener) in Hadoti region of south-east Rajasthan, India. ISCA Journal of Biological Science. 1: 37–42.

Mitra, A., and Pal, A. 2007. In vitro regeneration of *Stevia rebaudiana* (Bert) from the nodal explant. Journal of Plant Biochemistry and Biotechnology. 16: 59–62.

Modi, A.R., Patil, G., Kumar, N., Singh, A.S., and Subhash, N. 2012. A simple and efficient in vitro mass multiplication procedure for *Stevia rebaudiana* Bertoni and analysis of genetic fidelity of in vitro raised plants through RAPD. Sugar Tech. 14: 391–397.

Muanda, F.N., Soulimani, R., Diop, B., and Dicko, A. 2011. Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana* Bertoni leaves. LWT - Food Science and Technology. 44: 1865–1872.

Nepovim, A., and Vanek, T. 1998. In vitro propagation of Stevia rebaudina plants using multiple shoot culture. Planta medica. 64: 775-776.

Nower, A.A. 2014. In vitro propagation and synthetic seeds production: An efficient method for *Stevia rebaudiana* Bertoni. Sugar Tech. 16: 100–108.

Pande, S., and Khetmalas, M. 2012. Biological Effect of Sodium Azide and Colchicine on Seed Germination and Callus Induction in *Stevia rebaudiana*. Asian Journal of Experimental
Biological sciences. 3: 93–98.
Pande, S.S., and Gupta, P. 2013. Plant tissue culture of *Stevia rebaudiana* (Bertoni): A review. Journal of Pharmacognosy and Phytotherapy. 5: 26–33.
Panpatil, V.V., and Polasa, K. 2008. Assessment of stevia (*Stevia rebaudiana*)-natural sweetener: A review. Journal of Food Science and Technology. 6: 467-473.
Philippe, R.N., De Mey, M., Anderson, J., and Ajikumar, P.K. 2014. Biotechnological production of natural zero-calorie sweeteners. Current Opinion in Biotechnology. 26: 155-161.
Planas, G., and Kucacute, J. 1968. Contraceptive Properties of *Stevia rebaudiana*. Science. 162: 1007–1009.
Raina, R., Bhandari, S.K., Chand, R., and Sharma, Y. 2013. Strategies to improve poor seed germination in *Stevia rebaudiana*, a low calorie sweetener. Journal of Medicinal Plants Research. 7: 1793–1799.
Rajasekaran, T., Giridhar, P., and Gokare, R. 2007. Production of stevioside in ex vivo and in vivo grown *Stevia rebaudiana* Bertoni. Journal of the Science of Food and Agriculture. 87: 420–424.
Ramesh, K., Singh, V., and Megeji, N.W. 2006. Cultivation of Stevia [*Stevia rebaudiana* (Bert.) Bertoni]: A Comprehensive Review. Advances in Agronomy. 89: 137-177.
Ramírez-Mosqueda, M.A., and Iglesias-Andreu, L.G. 2016. Direct Organogenesis of *Stevia rebaudiana* Bertoni Using Thin Cell Layer (TCL) Method. Sugar Tech. 18: 424–428.
Salunkhe, V.R., and Bhise, S.B. 2010. *Stevia rebaudiana*: An alternative to synthetic sweeteners. Indian Drugs. 47: 5-13.
Savita, S.M., Sheela, K., Sunanda, S., Shankar, A.G., and Ramakrishna, P. 2010. *Stevia rebaudiana* - A functional component for food industry. J.Hum.Ecol. 15: 261–264.
Shivanna, N., Naika, M., Khanum, F., and Kaul, V.K. 2013. Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. Journal of Diabetes and its Complications. 27: 103–113.
Shock, C.C., 1982. Rebaud’s stevia: natural noncaloric. Shock 4–5.
Šic Žlabur, J., Voća, S., Dobričević, N., Ježek, D., Bosiljikov, T., and Brnčić, M. 2013. *Stevia rebaudiana* Bertoni- A review of nutritional and biochemical properties of natural sweetener. Agriculturae Conspectus Scientificus. 78: 25–30.
Singh, A., Singh, K., Singh, P., and Singh, M.P. 2015. Medicinal prospective and floral biology of candy leaf (*Stevia rebaudiana* Bertoni). International Journal of Advanced Research. 3: 628–636.
Singh, P., and Dwivedi, P. 2013. Two-stage culture procedure using thidiazuron for efficient micropropagation of *Stevia rebaudiana*, an anti-diabetic medicinal herb. 3 Biotech 4: 431–437.
Singh, P., Dwivedi, P., and Atri, N. 2014. In vitro shoot multiplication of Stevia and assessment of stevioside content and genetic fidelity of the regenerants. Sugar Tech 16: 430–439.
Singh, S.D., and Rao, G.P. 2005. Stevia: The herbal sugar of 21st century. Sugar Tech. 7: 17–24.
Sivaram, L., and Mukundan, U. 2003. In vitro culture studies on *Stevia rebaudiana*. In Vitro Cellular and Developmental Biology - Plant. 39: 520–523.
Smitha, G.R., and Umesh, K. 2011. Vegetative propagation of stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] through stem cuttings.Journal of Tropical Agriculture. 50: 72–75.
Soejarto, D.D., Compadre, C.M., Medon, P.J.,
Kamath, S.K., and Kinghorn, A.D. 1983. Potential sweetening agents of plant origin. II. field search for sweet-tasting Stevia species. Economic Botany. 37: 71–79.

Soejarto, D.D., Kinghorn, A.D., and Farnsworth, N.R. 1982. Potential sweetening agents of plant origin. III. Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. Journal of Natural Products. 45: 590–99.

Soliman, H.I.A., Metwali, E.M.R., and Almaghrabi, O.A. 2013. Micropropagation of Stevia rebaudiana Bettoni and assessment of genetic stability of in vitro regenerated plants using inter simple sequence repeat (ISSR) marker. Archives Des Sciences. 66: 343–359.

Sreedhar, R. V., Venkatachalam, L., Thimmaraaju, R., Bhagyalakshmi, N., Narayan, M.S., and Ravishankar, G.A. 2008. Direct organogenesis from leaf explants of Stevia rebaudiana and cultivation in bioreactor. Biologia Plantarum. 52: 355–360.

Sung, J.H. 2006. Rapid in vitro propagation and enhanced stevioside accumulation in Stevia rebaudiana bert. Journal of Plant Biology. 49: 267–270.

Swanson, S.M., Mahady, G.B., and Beecher, C.W.W. 1992. Stevioside biosynthesis by callus, root, shoot and rooted-shoot cultures in vitro. Plant Cell, Tissue and Organ Culture. 28: 151-157.

Taleie, N., Hamidoghli, S., and Hamidoghli, Y. 2012. In vitro plantlet propagation of Stevia rebaudiana Bertoni. South West Journal Horticulture Biology and Environment 3: 99–108.

Tamura, Y., Nakamura, S., Fukui, H. and Tabata, M. 1984. Comparison of Stevia plants grown from seeds, cuttings and stem-tip cultures for growth and sweet diterpene glucosides. Plant Cell Reports. 3: 180–182.

Thiyagarajan, M., and Venkatachalam, P. 2012. Large scale in vitro propagation of Stevia rebaudiana (bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb. Industrial Crops and Products. 37: 111–117.

Uddin, M.S., Chowdhury, M.S.H., Khan, M.M.M.H., Uddin, M.B., Ahmed, R., and Baten, M.A. 2006. In vitro propagation of Stevia rebaudiana Bert in Bangladesh. African Journal of Biotechnology. 5: 1238–1240.

Yadav, A.K., Singh, S., Dhyani, D., and Ahuja, P.S. 2011. A review on the improvement of stevia [Stevia rebaudiana (Bertoni)]. Canadian Journal of Plant Science. 91(1): 1–27.

How to cite this article:

Manvender Singh, Vinod Saharan, Jyotsna Dayma, Deepak Raipurohit, Yadunandan Sen and Ajay Sharma. 2017. In vitro Propagation of Stevia rebaudiana (Bertoni): An overview. Int.J.Curr.Microbiol.App.Sci. 6(7): 1010-1022. doi: https://doi.org/10.20546/ijcmas.2017.607.122