A Review on *In Vitro* Regeneration of Ethnomedicinal Plant Turkey Berry (*Solanum torvum* Swartz)

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**ABSTRACT**

In this review, we report on the different plant growth regulators (PGRs) affecting the callus induction, callus-mediated regeneration, organogenesis, somatic embryogenesis from different explants and micropropagation through shoot and nodal cultures in Turkey Berry (*Solanum torvum*SW). The fruits of this plant guard against liver and kidney damage, stop certain cancers, and regulate blood sugar levels. They benefit digestion, the management of gout and menstruation, the treatment of anaemia and diabetes, the prevention of intestinal worms, cardiovascular disorders, and strokes. The protocol developed for the induction of callus can be utilized to isolate pharmaceutically important secondary metabolites in *S. torvum*, and the regeneration protocols optimized in this study can be used for genetic transformation and conservation of *S. torvum*, not only as a medicinal plant but also as a model system.

Keywords: Callus induction, Organogenesis, Plant growth regulators, *Solanum torvum*, Somatic embryogenesis

I. INTRODUCTION

*In vitro* plant cell, tissue, and organ culture has emerged as a key tool in the conservation of endangered/threatened and medicinally important taxa. It has since evolved into an important component of plant biotechnology; we have now progressed to the point where we can manipulate to isolate pharmaceutically potential biomolecules. Plant tissue culture is important in the development of pathogen-free plants, synthetic seeds, somatic hybrids, and cybrids, as well as the basic requirement for genetic transformation to develop transgenics.

The technology of plant tissue culture has a significant impact on the propagation and preservation of therapeutic, threatened, and endangered plants. This innovative method not only shortens the time required for micropropagation and the selection of desirable features, but it also makes it possible to raise many plants in a small amount of time and space.

There has been a lot of research into developing agronomically important transgenic plants using transformation technology. Many laboratories, particularly in European and Western countries, have been conducting extensive research to develop plants (Pharma) that produce edible vaccines to cure a variety of diseases such as diabetes, hepatitis-B, diarrhoea, cholera toxin-B, tooth decay, and so on. As a result, these pharma plants show promise as low-cost vaccine production systems. Many immunotherapeutic proteins are being expressed at high levels in plants in order to use them as modern bioreactors (Sharma et al., 1999). Research in these areas frequently used to enhance the yield of important plant derived compounds. Thus, tissue culture technology is used in all of these potential areas of modern research.

It has become more and more important to realise *in vitro* multiplication of numerous clonal plants with better features in order to satisfy the constantly expanding commercial requirements. Thus, *in vitro* micropropagation can produce a significant number of clonal plants for ongoing plant establishment as well as being crucial for the preservation of germplasm.

It is undeniable that medicinal plant biotechnology grown from *in vitro* cell culture technology. Plant cell and tissue culture, genetic engineering and bioprocessing technologies have provided mankind with a tremendous opportunity to exploit medicinal plants under *in vitro* conditions (Narula et al., 2004). As a result of all of these advances in plant biotechnology, the present review has concentrated on the *in vitro* regeneration and conservation of an ethnomedicinally important plant *Solanum torvum* SW.

A. Introduction to Species

The species *Solanum torvum* Swartz, often known as the Turkey Berry or Pea Eggplant, is a prickly, tomentose, erect little shrub that grows 1.5 to 3.0 metres tall. The fruits of this plant are frequently sold in markets and are consumed as...
whereas saponins were discovered to be a significant source of antibacterial compounds (Cowan 1999), and nutritional value (Mackeen 2001; Gilbert and Mc Bain, 2003), so it is possible that these plants will yield drugs that will improve the treatment of microorganism infections.

Turkeyberry contains a number of potentially pharmacologically active chemicals, including chlorogenin, a sapogenin steroid. Turkey berry aqueous extracts kill mice by reducing the number of erythrocytes, leukocytes and platelets in their blood (Ghan Singh et al., 2021a). Plant extracts have been reported to be effective in the treatment of pimpls, skin diseases, leprosy, hyperactivity, colds, and cough (Ghan Singh et al., 2021a). Turkey berry is high in vitamin A and carotenoids, and it improves eyesight, skin texture, and prevents chronic diseases. Fruits are high in iron and thus beneficial in the treatment of anaemia, digestion, indigestion, stomachaches, and diarrhoea. Prevents intestinal worms, treats diabetes, and prevents and heals colds and flu, as well as protecting against cancer.

The aqueous extract of S. torvum induced a dose-dependent analgesic effect against the writhing syndrome, indicating a peripheral effect (Atta and Alkofahi, 1998). Prostaglandins and kinines appear to play an important role in the pain process in peripheral tissues (Hajare et al., 2000), and writhing induced by chemical substances injected intraperitoneally is thought to be the result of prostaglandin sensitization of chemosensitive nociceptors (Maria Elena et al., 1997).

The crude extracts (showed glykoalkaloids) isolated from S. torvum demonstrated anticancer properties (Lee et al., 2004; Friedman et al., 2005). The glycoalkaloid solasodine found in its leaves and fruits are used in India for the production of steroidal sex hormones for oral contraception (Silva et al., 2005). The aqueous and methanol extracts of S. torvum leaves have anti-ulcerogenic characters, which may be due to a cytoprotective mechanism. These findings support the plant’s ethnomedical uses in the treatment of gastric ulcers.

In view of its medicinal value, use as a vegetable, and overexploitation, an attempt has been made to review the regeneration potential of an ethnomedicinal species S. torvum in order to conserve it.

II. CALLUS INDUCTION

Plant regeneration can occur directly or indirectly through suspension cultures. Most plant regeneration occurs through the formation of adventitious shoot buds from explants or through the de novo organization of shoot and root meristems in the callus (Vasil, 1984). The potential number of plants regenerated from callus cultures can thus be very high. A variety of plants have been studied for later plant regeneration.
regeneration via adventitious shoot and root formation (Collonnier et al., 2001; Rama Swamy et al., 2004; Rama Swamy et al., 2005). Adventitious shoots proliferation is the most commonly used technique in micropropagation of commercially important medicinal plants for large-scale in vitro multiplication.

Different biotechnological investigations require the induction and development of callus. Callus induction is dependent not only on the presence of endogenous plant growth regulators (PGRs) in the explant (Sinnot, 1960), but also on the exogenous supply of PGRs, namely auxins and auxin-cytokinin combinations, for inducing callus from cut ends of any explant. The influence of various factors on callus induction from different explants was recently published in medicinal plants (Rama Swamy et al., 2004; Rama Swamy et al., 2005).

Callus is classified into two types: (i) embryogenic callus, which is smooth and friable and is either white/creamy/yellowish in colour, and (ii) non-embryogenic callus, which is composed of tubular cells with a compact, rough, crystalline texture and a yellow to brown colour. The genotype, age of the explants, and even the season can affect the induction and development of callus. The callus texture, shapes, and colouring, is reliant on PGRs.

The in vitro culture system is a viable alternative to the traditional method for isolating secondary metabolites (Sarin, 2005). Callus production requires only a small portion of the plant as an explant rather than using leaves, stem parts, or/and the entire plant, which can eventually contribute to the species’ extinction/endangerment. As a result, in order to preserve medicinal plants, we must employ the modern method of callus induction. Many factors influence successful callus induction, including genotype, explants, culture media, PGRs, and culture conditions (Sarin, 2005). It has previously been demonstrated for many Solanaceae species for callusing from roots, leaves, hypocotyls, nodal segments, and petiole explants of S. viarum (Mahadev et al., 2014), S. nigrum (Zou et al., 2017), S. melongena (Alim et al., 2014) and S. surattense (Rama Swamy et al., 2004).

Callus plays an important role for the induction of shoot buds/shoot buds+ roots/rhizogenesis, somatic embryogenesis, somaclonal variations with agronomically significant traits, and the isolation of secondary metabolites of medicinal significance using cell suspension culture methods.

Chandan Kumar Singh et al. (2018) investigated the influence of PGRs on callus induction from S. torvum leaf explants. Leaves of various ages (1st to 5th, from shoot apex to base) were chosen and cultured on MS medium augmented with various concentrations (1.0-5.0 mg/L) of NAA/2,4-D/KIN/NAA+KIN and 2,4-D+KIN. They have observed a lack of callusing ability.

Ghan Singh et al. (2023) have studied the effect of PGRs on callus induction from different types of explants in S. torvum, including cotyledon, hypocotyl, and leaf. S. torvum cotyledons, hypocotyls, and leaf explants were cultured on MS medium fortified with 1.0-6.0 mg/L 2,4-D/ NAA/IAA. In cotyledon explants, the percentage of responding cultures increased from low concentration to 4.0 mg/L NAA/2,4-D/IAA but then decreased at all other concentrations. At 4.0 mg/L NAA/2,4-D/IAA, the maximum percentage of callusing response was 88%, 82%, and 88%, found respectively. At 2.0-4.0 mg/L NAA, 4.0-5.0 mg/L 2,4-D, and 3.0-5.0 mg/L IAA, a high level of callusing ability was observed. At 1.0–3.0 mg/L 2,4-D, white-compact callus was observed, followed by creamy-compact callus at 4.0–6.0 mg/L 2,4-D. In contrast, friable callus was discovered in all of the investigated NAA concentrations, but friable callus only formed at 2.0-3.0 mg/L IAA.

High percentage of callusing response was observed at 4.0-5.0 mg/L 2,4-D including high amount of callus development at the same concentration of 2,4-D in hypocotyl explants. At 4.0 mg/L NAA/IAA, there was a higher percentage of responding cultures as well as the induction of a greater amount of callus. At 3.0-4.0 mg/L NAA and 3.0 mg/L IAA, friable callus formed.

Ghan Singh et al. (2023) have reported the induction of callus from leaf explants on MS medium fortified with different concentrations of 2,4-D/NAA/IAA in S. torvum. The maximum percentage of callusing response was found at 3.0-4.0 mg/L 2,4-D/NAA/IAA. At 4.0 mg/L 2,4-D and 3.0-4.0 mg/L NAA/IAA, a significant amount of callus was formed. At 2.0 mg/L NAA and 1.0 mg/L/4.0-5.0 mg/L IAA, friable callusing was observed. The embryogenic callus was formed at 1.0 mg/L NAA.

Ya-Long Qin et al. (2017) used MS medium fortified with BA (0.5-3.0 mg/L)+NAA (0.1-0.6 mg/L) to induce callus from stem segments and leaf explants. At 1.0 mg/L BA+0.5 mg/L NAA, a high amount of callus was induced from both the explants.

S. torvum leaf explants had the highest percentage of culture response and callusing ability, followed by cotyledon and hypocotyl explants (Ghan Singh et al. 2023). S. torvum callus produced from various explants varied in texture, form and coloration. In all S. torvum explants tested, the growth regulator NAA induced the greatest amount of callus, followed by IAA and 2,4-D (Ghan Singh et al., 2023). The same response was observed in S. surattense (Rama Swamy et al., 2004; Rama Swamy et al., 2005). Similarly, the effect of NAA on callus induction in several Solanaceae species has been reported (Soloukiet et al., 2011; Govindarajan and Chinnachamy, 2014; Ewais et al., 2015). Callus tissues have recently been shown to be superior for the synthesis of secondary metabolites/biomolecules (Solouki et al., 2011; Govindarajan and Chinnachamy, 2014; Ewais et al., 2015).

The optimal concentration of these PGRs may be affected by variety of factors, including the mother plant's genotype, explant origin, and explant source. In S. torvum, 2,4-D induced less callus proliferation than other auxins tested in all the explants studied (Ghan Singh et al., 2023). The maximum callusing ability in Solanum viarum (Tejavathi and Bhuvana, 1998) was found to be at 2.0 mg/L NAA, as observed in S. torvum by Ghan Singh et al. (2023). Callus induction is dependent not only on the presence of endogenous PGRs in the explants, but also on the exogenous supply of PGRs, namely, auxins and auxin-cytokinin combinations, which are important for inducing and promoting callus formation from cut ends of any explant. However, depending on the species and type of explant, callusing ability can be improved by combining a low concentration of Cytokinins with a high concentration of auxins in the medium. At certain
concentrations, auxins and cytokinins work synergistically to promote callus induction.

According to Ya-Long Qin et al. (2017), the combination of 1.0 mg/L BA+0.5 mg/L NAA in the medium was the best for callus induction from *S. torvum* stem segments and leaf explants. When a single PGR was added to the medium, *S. torvum* showed a higher percentage and quantity of callusing ability (Ghan Singh et al., 2023). This difference in callusing ability suggests that the tissues contain varying levels of endogenous growth regulators. Shoot bud formation was suppressed in *S. torvum* on MS medium with all of the auxins tested, but callusing was promoted, as seen in medicinal plants such as *S. surattense* (Rama Swamy et al., 2004; Rama Swamy et al., 2005) and *S. nigrum* (Zou et al., 2017).

Induction of friable callus plays a vital role in the formation of adventitious shoots and roots in a variety of medicinal plants. The most commonly used technique in micropropagation for large-scale plant multiplication *in vitro* is adventitious shoots proliferation. From the foregoing, it is clear that among the auxins, NAA was found to be the most potent for callus induction, proliferation in *S. torvum*, followed by IAA and 2,4-D (Ghan Singh et al., 2023). On MS+NAA, callus proliferation was faster, and a very high yield of callus mass was produced in 4 weeks, followed by IAA and 2,4-D. The highest amount of callus development was observed on MS medium augmented with 3.0 and 4.0 mg/L NAA, with the highest percentage of responding cultures in all explants tested. Thus, among the explants tested, leaf explants are extremely effective at inducing callus production in *S. torvum* (Ghan Singh et al., 2023). The callus produced by 2,4-D and IAA was white and brownish/gray in nature, whereas the callus produced by NAA was friable/embryogenic in almost all concentrations and explants used, indicating the capability for regeneration and suspension culture. Thus, the genotype influences the texture, morphology, and coloration of the callus, as well as the age of the explants. The season also influences the induction and development of the callus.

*In vitro* cell culture technology can produce a variety of valuable secondary metabolites using cell suspension culture technology. The benefit of this method is that it can eventually provide a consistent, dependable source of natural products. Furthermore, because of the lack of significant amounts of pigments, compounds from tissue culture are easily purified, lowering production costs. These secondary metabolites include alkaloids, glycosides (steroids and phenolics), terpenoids, and a variety of flavours, fragrances, perfumes, agrochemicals, commercial insecticides, shikonin, and naphthaquinone (used as dye and pharmaceutical) (Ghan Singh et al., 2022a). The basic technologies for producing high yield of specific secondary metabolites from large-scale cultures are still under development.

Thus, the callus induction in *S. torvum* plays an important role for isolation of secondary metabolites. The success of callus induction protocol developed for *S. torvum* can be used to isolate glykoalkaloids/solasodine which is antinecancerous and also used as oral contraceptives. All these pharmacologically important compounds can be isolated through cell suspension culture technology.

### III. PLANT REGENERATION/ORGANOGENESIS

*In vitro* plant regeneration/organogenesis has pivotal role in multiplication and conservation of an endangered, medicinal plants and also in production of transgenic plants. *In vitro* plant regeneration can be achieved by three ways: (i) through callus mediated regeneration/organogenesis (ii) direct shoot buds proliferation/regeneration/organogenesis, and (iii) somatic embryogenesis. These three pathways depend upon the concentration and combinations of auxin/cytokinin/cytokinin alone present in the nutrient medium. Regenerative capacity is also dependent upon the genotype of explants; age and physiological activity of the donor plant.

Nutrient medium supplemented with PGRs viz., auxins or auxin-cytokinin combination and concentration and also the culture conditions during the incubation period play an important role in plant regeneration (Rama Swamy et al., 2004; Rama Swamy et al., 2005). The effect of these factors was reported on *in vitro* regeneration in *Solanum lycopersicum* (Praveen and Rama Swamy, 2011), *S. surattense* (Rama Swamy et al., 2004; Rama Swamy et al., 2005), *S. nigrum* (Zou et al., 2017) and *S. torvum* (Ghan Singh et al., 2022a, 2022b).

The current review attempts to report the protocol developed for *In vitro* plant regeneration and multiplication of *S. torvum* via callus mediated and direct regeneration.

**A. Indirect Regeneration/Organogenesis/Callus Mediated Regeneration**

Callus mediated regeneration is influenced by various factors. In general, the shoot organogenesis/regeneration from callus can be induced by decreasing the auxin concentration and raising the cytokinin concentration, or by the presence of solely cytokinin in the nutritional medium. Despite being undifferentiated, the cells contain all of the genetic material found in the parent plant. From callus cultures, it is feasible to promote the formation of organogenesis, i.e., roots, shoots, and the entire plant, by appropriately manipulating PGRs and medium components.

Callus mediated regeneration is a more efficient method of plant multiplication than organogenesis for producing many number of plants in a short duration of time. The callus-mediated regeneration of *S. torvum* is described in this review.

Ya-Long Qin et al. (2017) described a callus-mediated regeneration system in *S. torvum* using stem segments and leaf explants. MS+0.3 mg/L NAA+2.0 mg/L BA+1.0 mg/L 2,4-D was used to culture calli from stem segments and leaf explants. For plant regeneration, the proliferated callus from both explants was cultured on MS medium augmented with BA (0.5-3.0 mg/L)+2,4-D (0.5-3.0 mg/L). The medium containing 0.5 mg/L BA+1.0 mg/L 2,4-D had the highest regeneration frequency.

1) **Regeneration from Cotyledon Derived Callus**

Ghan Singh et al. (2022a) used callus induced at 3.0-4.0 mg/L NAA from cotyledon explants cultured on MS medium fortified with various concentrations of BAP/KIN for callus mediated regeneration. In all concentrations of BAP/KIN used as sole PGR, adventitious shoots were induced. In comparison to KIN alone, 2.5 mg/L BAP produced the
highest percentage of response (90%) and the highest number of shoots formation (27.0±0.19) per explant. To determine the effect of auxin on multiple shoot formation, 0.5 mg/L IAA was added to MS medium along with various concentrations of cytokinin (BAP/KIN). Shoot formation per explant was increased in all concentrations of BAP/KIN and IAA. The maximum number of shoots per explant (35.2±0.25) was observed at 0.5 mg/L IAA+2.5 mg/L BAP followed by 2.0 mg/L BAP.

2) Regeneration from Hypocotyl Derived Callus

Hypocotyl derived callus was cultured on MS medium fortified with various concentrations (0.5-4.0 mg/L) of BAP/KIN, as well as 0.5 mg/L IAA (Ghan Singh et al., 2022a). Ghan Singh et al. (2022a) have observed that 2.5 mg/L BAP produced more multiple shoots per explant (18.2±1.25), as well as the highest percentage (85%) of responding cultures. BAP was found to have a higher percentage of response and the development of multiple shoots/explant than KIN. The percentage of responding cultures and the number of shoots per explant were found to be higher in calli cultured on BAP/KIN as the sole growth regulator. To determine the effect of 0.5 mg/L IAA on the induction of multiple shoots in S.torvum, the MS medium was fortified with BAP/KIN.

The highest frequency of shoot buds induction (23.4±0.11) per explant was observed at 0.5 mg/L IAA+3.0 mg/L BAP than at any other BAP/KIN concentration used. It is also interesting to note that adventitious shoots formation along with the rooting was observed at 3.0-4.0 mg/L BAP in combination with 0.5 mg/L IAA (Ghan Singh et al., 2022a).

3) Regeneration from Leaf Derived Callus

Leaf derived callus at 2.0 mg/L NAA was cultured on MS medium supplemented with 0.5-4.0 mg/L BAP/KIN alone and in combination with 0.5 mg/L IAA/NAA. Ghan Singh et al., (2022a) have reported that 2.5 mg/L BAP produced the maximum frequency number of shoots (36.4±0.13) per explant than all other concentrations of KIN and BAP alone in the medium. In comparison to BAP/KIN alone in the medium, all concentrations of BAP/KIN with IAA (0.5 mg/L) produced more shoots per explant. In comparison to all other BAP/KIN concentrations tested, the highest frequency number of shoots (38.0±0.55) per explant was observed at 0.5 mg/L IAA+2.5 mg/L BAP. To find out the efficacy of auxin on the induction of multiple shoots, 0.5 mg/L NAA was also added to the medium along with BAP/KIN (0.5-4.0 mg/L). When leaf derived calli cultured in all concentrations of BAP/KIN along with 0.5 mg/L NAA when compared to IAA used in S.torvum, the number of shoots per explant was found to be increased. In S.torvum, the highest frequency of shoots (43.0±1.50) per explants was found at 0.5 mg/L NAA+2.5 mg/L BAP, followed by 2.0 mg/L BAP and 3.0 mg/L KIN (Ghan Singh et al., 2022a).

Adventitious multiple shoots were induced in all the concentrations and combination of PGRs used (Ghan Singh et al., 2022a). In comparison to KIN as the sole growth regulator, 2.5 mg/L BAP induced the greatest number of shoots per explant. Similarly, Oceania et al. (2015) and Papry et al. (2016) obtained the highest shoot multiplication in Solanum lycopersicum callus cultures supplemented with 3.0 mg/L and 2.0 mg/L BAP, respectively. While the addition of low levels of auxin (0.5 mg/L IAA) to cytokinin (BAP/KIN) improved the development of shoots per explant from all cytokinin concentrations tested. However, among all other PGRs combinations and concentrations tested, the proliferation of shoot buds was found to be greatest at 0.5 mg/L IAA+2.5 mg/L BAP.

In leaf cultures of S.torvum, the 0.5 mg/L NAA+2.5 mg/L BAP combination produced the highest frequency number of shoots per explant (Ghan Singh et al., 2022a). Shahzad et al.,(1999) also reported the efficacy of NAA+BAP in plant regeneration from S. nigrum leaf derived callus cultures. Kurnlay and Erisci, (2015) also observed the highest number of shoots per explant in S. tuberosum in combination with BAP+GA. In Solanum trilobatum, Sreenu et al. (2019) found the highest mean number of shoots differentiated de novo augmented with TDZ+NAA. Similarly, Ya-Long Qin et al. (2017) observed the maximum regeneration frequency at 0.5 mg/L BA+1.0 mg/L 2,4-D from S. torvum leaf and stem segments derived callus, as it was observed by Ghan Singh et al. (2022a) showing the synergistic effect of both auxin+cytokinin combinations.

In vitro micropropagation via callus is an important technique for rapid multiplying and propagating a species. However, plantlets regenerated through callus cultures may exhibit genetic variability in a variety of morphological abnormalities. However, Ghan Singh et al. (2022a) and Ya-Long Qin et al. (2017) found that all plants regenerated through callus cultures showed normal flowering and fruiting in S. torvum.

Based on our review, the leaf derived callus was found to be more potential than all other explants tested in terms of producing a high frequency number of shoots per explant. Cytokinin BAP in combination with IAA/NAA/2,4-D was found to be more effective in inducing enhancement of shoot organogenesis in all the explants of S. torvum studied. However, the combination of 2.5/3.0 mg/L BAP with 0.5 mg/L IAA/NAA/2,4-D induced the greatest number of shoots per explant. As a result, the current regeneration protocol developed through callus mediated can be used as a model system for mass-scale production of the species Turkey berry as well as genetic transformation studies to introduce novel traits for enhancement of secondary metabolites production.

B. Direct Regeneration/Organogenesis

Organogenesis is the morphogenetic event that occurs directly from any type of explant without the callus phase. This technique reduces the use of in vitro culture and results in the formation of new shoots directly from the explants. Longitudinal sections of leaf explants during the culture formed numerous meristematic zones within the tissue, which subsequently converted into shoot buds (Mukherjee et al., 1991). The appearance of shoot apex with developing leaf primordia characterised of shoot buds (Sarketet et al., 2006). The genotype is important in the organogenesis of shoots directly from explants. Different varieties and species, including Solanum aethiopicum, S. macrocarpon and S. melongena, demonstrated varying potential in direct plant regeneration, yielding 70-100% explants with an average of two to seven shoots per explant (Sarker et al., 2006; Gisbert et al., 2006; Zayova et al., 2012; Robinson and Saranya, 2013; Bhat et al., 2013). The potential for direct regeneration varied depending on the tissue system.
cultured in a well-defined medium. Different explants responded differently to regeneration on different cytokinin, auxin and auxin-cytokinin containing media combinations (Taha and Tizan, 2002; Sarker et al., 2006; Gisbert et al., 2006; Kanna and Jayabal, 2010; Kaur et al., 2011; 2013; 2020).

Except for Ghan Singh et al. (2021b), we found no reports on direct regeneration from S. torvum explants in our literature search. As a result, we reviewed the work of Ghan Singh et al. (2021b) on the direct organogenesis of S. torvum from various explants. They cultured cotyledon, hypocotyl, and leaf explants for direct regeneration on MS medium fortified with various concentrations of BAP/KIN/TDZ and also combination with 0.5/1.0 mg/L IAA.

1) Regeneration from Hypocotyl Explants

In comparison to all other concentrations of BAP/KIN alone and IAA+KIN, a high percentage (92%) of responding cultures were found at 0.5 mg/L IAA+2.5 mg/L BAP. The formation of more adventitious shoots was also observed in all concentrations of BAP alone and in combination with IAA. At 0.5 mg/L IAA + 2.5 mg/L BAP, the maximum frequency number of shoots (31±0.17) was induced, followed by 1.0 mg/L IAA + 3.0 mg/L BAP (24±0.15) (Ghan Singh et al., 2021b).

2) Regeneration from Cotyledon Explants

The highest percentage of response (96%) was observed at 0.5 mg/L IAA+2.5 mg/L BAP, followed by 2.0 mg/L BAP (92%). The percentage of response and average number of shoots per explant were found to be higher in all BAP concentrations than in KIN concentrations. Cotyledon explants were also cultured on MS medium supplemented with 0.5/1.0 mg/L IAA and 0.5-4.0 mg/L BAP/KIN. However, the auxin-cytokinin combination IAA+BAP induced more shoots than IAA+KIN. By demonstrating its superiority, the maximum number of shoots formation (55.0±0.15) was recorded at 0.5 mg/L IAA+2.5 mg/L BAP (Ghan Singh et al., 2021b).

3) Regeneration from Leaf Explants

The highest percentage of response was found at 0.5 mg/L IAA+2.5 mg/L BAP, as well as the highest frequency of shoot formation when compared to KIN alone. MS medium fortified with 0.5-1.0 mg/L IAA+BAP/KIN, the number of shoots formed was found to be increased. The percentage of response and average number of shoots formed per explant were also higher in all BAP concentrations than in KIN in combination with IAA. After 6 weeks of first subculture, shoots developed on MS+2.5/3.0 mg/L BAP in combination with 0.5/1.0 mg/L IAA showed profuse rhizogenesis (Ghan Singh et al., 2021b).

In this review, the role of different PGRs on direct regeneration from various S. torvum explants (cotyledon, hypocotyl, and leaf) was reported, as well as the efficacy of cytokinins and auxin-cytokinin combinations in S. torvum (Ghan Singh et al., 2021b). 0.5/1.0 mg/L IAA+2.5 mg/L BAP/3.0 mg/L KIN, on the other hand, only induced shoots and rhizogenesis in leaf explants. When 0.5/1.0 mg/L IAA was added to MS medium containing BAP/KIN, all explants showed increased shoot buds proliferation. On MS medium supplemented with 0.5 mg/L IAA+2.5-3.0 mg/L BAP, a high frequency number of shoots was induced. Leaf explants produced the most shoots per explant, followed by cotyledon and hypocotyl explants. As a result of the preceding, it is clear that BAP outperformed KIN in inducing direct organogenesis from all explants used in S. torvum. Similarly, in Solanum lycopersicum (Praveen and Rama Swamy, 2011), and S. surattense (Rama Swamy et al., 2004; Rama Swamy et al., 2005), the combination of IAA+BAP was found to be more efficient in inducing the greatest number of shoots from different types of explants. The highest number of shoots per explant was developed in Solanum sisymbriifolium leaf explants on MS medium augmented with 0.5 mg/L IAA+2.5 mg/L BAP (Rao et al., 1997) compared to all other concentrations of BAP alone and in combination with 1.0 mg/L IAA. Similar to the current findings in S. torvum, increasing the IAA concentration to 1.0 mg/L reduced the efficiency of shoot buds induction in S. sisymbriifolium. Tejavathi and Bhuvana (1998) discovered that IAA+BAP/2-ip resulted in the greatest shoot regeneration and that BAP over KIN in S. viarum as observed in S. torvum (Ghan Singh et al., 2021b).

In S. aethiopium, explant age also influenced regeneration, with younger leaves showing better organogenesis than mature ones (Zhang, 1999). The presence of any cytokinin in the medium resulted in the formation of shoot organogenesis from leaf explants (Ghan Singh et al., 2021b). However, auxin and cytokinin combinations and concentrations should be optimised for maximum number of regenerated shoots in eggplant (Magioliet al., 1998; Zang, 1999; Picoli et al., 2000; Sarkeret al., 2006). The gelling agents had also an effect on the shoot regeneration process, and agar was found to be superior to gerlite (Perrone et al., 1992). Peptone had no effect on S. melongena and S. integrifolium hyperhydric shoots. Culture vessels with gas-permeability through a membrane filter have a lower percentage of hyperhydric shoots and a higher survival rate than sealed vessels (Takamura et al., 2006).

According to the preceding discussion, leaf explants have a higher potential for forming a high frequency number of shoots per explant than all other explants tested in S. torvum (Ghan Singh et al., 2021b). Cytokinins BAP/KIN, either alone or in combination with IAA, were found to be effective in inducing direct shoot regeneration in all S. torvum explants. Different explants cultured on the same concentration of cytokinins and auxin-cytokinin combinations produce varying results in the same species, either through callus mediated organogenesis or direct regeneration. Many factors influence explant morphogenesis, including the balance of exo- and endogenous auxin-cytokinin levels (Rama Swamy et al., 2004). Thus, morphogenesis in a specific direction is manifested as a result of the cumulative effect of many factors, including growth substances, nutrient medium, temperature, humidity, photoperiod, and so on. These are important morphogenetic tools for controlling the cell's internal environment (Rama Swamy et al., 2004; Rama Swamy et al., 2005). Thus, plants grown in vitro via direct organogenesis may have greater genetic stability than those grown from callus.

The current review on regeneration protocol developed can suggest for mass-scale propagation of the species as well as genetic transformation studies to introduce agronomically
important traits to enhance secondary metabolites production and resistance against pests/insects. Based on this, the plant *S. torvum*, like tobacco and cress plants, can be used as a model system (organism).

IV. SOMATIC EMBRYOGENESIS

Somatic embryogenesis is an effective method for plant micropropagation and the production of a large number of elite and transgenic plants (Ammirato, 1983). Somatic embryogenesis is an alternative method of plant propagation to organogenesis/regeneration. Plants regenerated through somatic embryogenesis are of single cell origin, true-to-type, and produced in large numbers in a short period of time (Ammirato, 1983).

Somatic embryogenesis can be induced directly from a variety of explants or indirectly through *In vitro* manipulation of non-embryogenic callus. Cell totipotency is best demonstrated in the formation of somatic embryos from single cells, as well as their growth and development to form a complete plantlet (Ammirato, 1983; Rama Swamy *et al.*, 2005; Sharada *et al.*, 2019; Ghan Singh *et al.*, 2021a). Because somatic embryos are thought to be formed from a single cell, plants derived from them are genetically identical. As a result, it has significant role in tissue culture technology. For starters, because somatic embryos have preformed root and shoot meristems, several labor-intensive steps involved in subculture, separation, and rooting of individual shoots are eliminated. Second, single cells could be prodigiously induced to form embryoids. There is already an experimental system for encapsulating somatic embryos with various hydrogels, resulting in artificial seeds (Ghan Singh *et al.*, 2021a), which play an important role in germ plasm conservation of threatened and endangered species, as well as medicinal and commercially important species.

Somatic embryogenesis is a preferred method for rapid *In vitro* plant multiplication by producing artificial/synthetic seeds, as well as for *Agrobacterium tumefaciens*-mediated genetic transformation and transgenic plant regeneration (Rama Swamy *et al.*, 2006).

Somatic embryogenesis is dependent on many factors such as genotype, explant, the combination of PGRs and their concentrations, and also culture conditions in *Solanum* (Kantharajah and Golegaonkar, 2004). Genotype is the most important factor influencing somatic embryogenesis, with significant quantitative differences in their ability to form emboids in *S. melongena, S. nigrum* and *S. torvum* (Huda *et al.*, 2007; Mir *et al.*, 2008; Mir *et al.*, 2011; Sidhu *et al.*, 2014; Sharada *et al.*, 2019; Ghan Singh *et al.*, 2021a).

The morphogenic event occurs from any explant in the form of callus mediated (Indirect) or direct somatic embryogenesis, depending on a variety of factors. We have now reviewed direct somatic embryogenesis in *S. torvum*. Only the work of Ghan Singh *et al.* (2021a) on somatic embryogenesis in *S. torvum* was found in our review of the literature.

A. Somatic Embryogenesis from Cotyledon Explants

Somatic embryogenesis was induced directly from cotyledon explants cultured on all NAA (1.0-5.0 mg/L) + 0.5 mg/L BAP concentrations except 6.0 mg/L NAA. At 0.5 mg/L BAP+2.5 mg/L NAA, the highest percentage of somatic embryogenesis was observed. The highest frequency of somatic embryo formation was also discovered at the same NAA concentration. On all NAA concentrations, globular embryos were converted into bipolar embryos. The level of NAA was discovered to be a factor in embryo conversion. At 2.5 mg/L NAA, the highest percentage of bipolar/torpedo-shaped embryos were formed (Ghan Singh *et al.*, 2021a).

B. Somatic Embryogenesis from Leaf Explants

In all concentrations of NAA except the highest (6.0 mg/L), somatic embryogenesis was initiated directly from the explant. At 0.5 mg/L BAP+2.5 mg/L NAA, the highest percentage of somatic embryogenesis was observed, followed by 0.5 mg/L BAP+2.0 mg/L NAA. The maximum number of somatic embryos per explant was observed at 0.5 mg/L BAP+2.5 mg/L NAA.

Except at 6.0 mg/L NAA, all concentrations of NAA tested resulted in the conversion of somatic embryos from globular to torpedo-shaped. At 0.5 mg/L BAP+2.5 mg/L NAA, the highest percentage of bipolar embryos was observed. Bipolar somatic embryos did not mature further after a second subculture on the same fresh medium. However, the number of somatic embryos per explant was increased. After 4-6 weeks of culture, individual embryos developed into distinct bipolar structures and went through all of the typical developmental stages (globular, heart, torpedo/bipolar). Asynchronous development of somatic embryos as a result, different stages of embryo development were visible in the same cluster of embryos derived from the explant. It is also interesting to note that a cluster of torpedo-shaped embryoids development was observed (Ghan Singh *et al.*, 2021a).

C. Somatic Embryo Germination and Plantlet Formation

According to Ghan Singh *et al.* (2021a) somatic embryos did not germinate on ½ strength MSO and also on MSO medium. In *S. torvum*, the highest frequency of embryo germination (75%) was observed on MS medium supplemented with 0.5 mg/L IAA+2.0 mg/L BAP. The conversion of cotyledonary stage embryos in a group was also recorded. When the embryos were shifted to a medium fortified with 0.5 mg/L IAA+2.0 mg/L BAP, they turned green with folded cotyledons and eventually developed into whole plantlets (Ghan Singh *et al.*, 2021a).

Auxins such as NAA, as well as the cytokinin BAP, are required for inducing somatic embryogenesis in *S. torvum*, according to Ghan Singh *et al.*(2021a). The nature of growth regulators and their combinations used in the culture medium are critical for somatic embryogenesis. The type of auxin or auxin combined with cytokinin used in the induction medium can have a significant impact on the frequency of somatic embryos in *S. torvum*.

Recently, Rama Swamy *et al.* (2005) reported that both auxin-cytokinin combinations are required for inducing somatic embryogenesis in *S. surattense*, a medicinal plant. Direct somatic embryogenesis was induced on medium containing BAP in *S. nigrum*, and the number of embryos was increased further by enriching the medium with NAA (Sharada *et al.*, 2019). In comparison to cotyledon explants, leaf explants produced the highest frequency number of somatic embryos and converted the most into bipolar.
embryos at 5.0 mg/L BAP + 2.5 mg/L NAA (Ghan Singh et al., 2021a). *S. surattense* exhibited the same response (Rama Swamy et al., 2005). Somatic embryo maturation is a critical step in somatic embryogenesis that leads to the formation of all plantlets. Heart/torpedo shaped/bipolar embryos in *S. torvum* required a combination of 0.5 mg/L IAA + 1.0-4.0 mg/L BAP for germination (Ghan Singh et al., 2021a). This is most likely due to some of the heart-shaped embryos being converted to torpedo or cotyledonal stage embryos and then germinating in the presence of IAA+BAP. Thus, it appears that a combination of auxin and cytokinin is required for the maturation and germination of bipolar somatic embryos in *S. torvum* (Ghan Singh et al., 2021a). The requirement of auxin-cytokinin combination for germination of torpedo stage embryos was also reported in *S. surattense* (Rama Swamy et al., 2005), as it was in *S. torvum*.

According to Zimmerman (1993), new gene products are required for progression from the globular to the heart-stage, and these new products can only be synthesized when an exogenous auxin is removed. However, Ghan Singh et al. (2021a) state that an auxin-cytokinin combination is required for the induction of somatic embryos in *S. torvum*. At higher auxin concentrations, the population of embryogenic cells likely decreases due to disruption and elongation, and the cultures’ embryogenic potential is lost (Bhojwani and Razdan, 1996). Similarly, embryogenesis was inhibited in *S. torvum* at 6.0 mg/L NAA + 0.5 mg/L BAP (Ghan Singh et al., 2021a).

Thus, the type of auxin/cytokinin/auxin+ cytokinin and their concentrations in the medium are always important in somatic embryogenesis. The type of PGR and its concentration also differ between genotypes. Somatic embryogenesis is preferred because it allows for plant production without somaclonal variation and can be used for genetic transformation studies. These induced somatic embryos in ethnomedicinally important plant, *S. torvum* can also be used to develop synseeds for germplasm storage, conservation, and exchange.

V. MICROPROPAGATION

Micropropagation/Clonal propagation techniques are important for mass-scale multiplication and conservation of an endangered or threatened species that is medicinally important in a short period of time and limited space. Because the explants consist of an organised meristem, the plants produced by this method are true-to-type and fungal/pathogen-free.

Shoot tip/shoot meristem cultivation is essential in mericlone technology for in vitro shoot regeneration. This technology is mainly used for elimination of virus and other pathogens that are present in the species. Nodal culture is also another important in vitro technique for multiplication and conservation of medicinal plants. The axillary / nodal buds induction is an efficient method of plant micropropagation. Nodal segments have been found to be the most suitable for clonal propagation in several medicinal plants.

Shoot tip and nodal explants of *S. torvum* were cultured on MSO and MS media augmented with different concentrations (1.0-5.0 mg/L) of BAP/KIN alone as well as in combination with 0.1/0.5 mg/L IAA. According to Ghan Singh et al., (2022b). shoot tips and nodal segments responded with 8% and 10%, respectively, when cultivated on MSO medium, and formed a single shoot. The axillary buds began to function within a week of inoculation, and after 2-3 weeks, new shoots with leaves and internodes had emerged in all concentrations and combinations of PGRs used.

A. Effect of BAP and IAA+BAP

After one week of culture without the callusing phase, Ghan Singh et al. (2022b) observed direct numerous shoot buds proliferation from the shoot apices of *S. torvum* in all concentrations of BAP used alone and in combination with IAA. The highest percentage of responsive cultures was observed at 4.0 mg/L BAP, followed by 5.0 mg/L BAP alone. At 4.0 mg/L BAP, a high frequency number (29.0±0.56) of shoots per explant was also observed. At high BAP concentrations, the shoot number was found to be reduced with a short shoot length. Additionally, shoot tip explants were also grown on MS media containing 0.1/0.5 mg/L IAA and 4.0/5.0 mg/L BAP. The combination of 0.5 mg/L IAA + 4.0 mg/L BAP resulted in the highest frequency number of multiple shoots (42.0±0.23) development. Multiple shoot formation was improved at 4.0/5.0 mg/L BAP in combination with auxin IAA when compared to using BAP/KIN alone in the medium. Multiple shoot development and rhizogenesis were observed at 0.1/0.5 mg/L IAA + 4.0 mg/L BAP.

Ghan Singh et al. (2022b) observed single shoot only induction of nodal segments in MS medium with 1.0 mg/L BAP supplementation. The highest number of shoots proliferation (5.0 ±0.62) per explant was observed at a concentration of 3.0 mg/L BAP alone. The percentage of reaction was also higher at 3.0 mg/L BAP. When BAP concentration reached 3.0 mg/L, it was gradually observed that the multiplication of shoot buds increased. It was discovered that as BAP concentrations increased, both the number and length of the shoots decreased. MS media supplemented with 0.5 mg/L IAA+4.0 mg/L BAP caused the highest frequency of multiple shoots (11.2±0.44), when compared to all other BAP/KIN concentrations and also in combination with 0.1/0.5 mg/L IAA. The addition of IAA (0.1/0.5 mg/L) to BAP (4.0/5.0 mg/L) increased shoot bud proliferation and response percentage. Rhizogenesis was also observed at 0.1/0.5 mg/L IAA + 4.0 mg/L BAP.

B. Effect of KIN and IAA+KIN

According to Ghan Singh et al. (2022b), multiple shoots induction from *S. torvum* shoot tips was observed on MS medium enriched with various concentrations of KIN alone and in combination with IAA. The number of numerous shoot buds that formed per explant increased as KIN concentration increased. While the percentage of response and the average number of shoots per explant decreased at high concentrations (5.0 mg/L KIN). Longer shoots resulted in the development of a greater number of multiple shoots per explant at 4.0 mg/L KIN alone.

In comparison to KIN alone, addition of IAA 0.1/0.5 mg/L in the medium to 4.0/5.0 mg/L KIN increased the maximum frequency number of shoots per explant. The combination of 0.5 mg/L IAA + 4.0 mg/L KIN had the highest response rate (96%) and the most multiple shoots per explant than any other combination. Shoots grown from shoot
tip cultures containing 0.1/0.5 mg/L IAA + 4.0 mg/L KIN also showed abundant rooting. According to Ghan Singh et al. (2022b), nodal segments were grown on MSO and MS media supplemented with various concentrations of KIN (0.1-5.0 mg/L) as well as in conjunction with IAA (0.1/0.5 mg/L). On MSO, there were no shoot formations. A solitary shoot was inducible from the axillary bud on MS medium with 1.0 mg/L KIN. Several shoots grew on all of the measured KIN concentrations, especially when combined with IAA. After 10 days of culture, axillary bud break was observed. The highest percentage of responsive cultures and the more number of shoots per explant were discovered at 4.0 mg/L KIN. There were fewer shoots per explant at a high concentration of KIN (5.0 mg/L). The combination of auxin IAA (0.5 mg/L) and cytokinin KIN (4.0 mg/L) produced the maximum number of shoots (8.4±0.17) per explant and a high proportion of responsive cultures. The possibility of multiple shoots being introduced from *S. torvum* nodal explants was enhanced when auxin and cytokinin were combined.

According to Ghan Singh et al. (2022b) both auxins and cytokinins had the highest frequency of shoots per explant. It's also interesting to note that BAP outperformed KIN at causing many shoots in *S. torvum*, either on its own or in combination with IAA. The similar superiority of BAP in shoot meristem cultures was shown in other Solanaceous medicinal plants as well, including *S. surattense* (Rama Swamy et al., 2005) and *S. nigrum* (Sharada et al., 2019). Merclone technology is a terrific way to speed up *in vitro* multiplication and cultivate virus-free and pathogen-free plants. This meristem cultivation has been utilised successfully with a number of medicinal plants (Sudershan et al., 2000; Geetha and Shetty, 2000; Rama Swamy et al., 2005).

Ghan Singh et al. (2022b), found that BAP superiority over KIN in stimulating the proliferation of numerous shoots per node, either alone or in conjunction with IAA. Despite the fact that many researchers have noted the importance of both auxins and cytokinins in the induction of shoots (Das and Mitra, 1990; Roy et al., 1993), cytokinins alone, such as BAP/KIN, were sufficient for the development of adventitious shoot buds in *S. torvum* nodal cultures.

Our review on the micropropagation of *S. torvum*, an important medicinal plant, using shoot meristem/nodal cultures demonstrated the importance of large-scale multiplication. Because this technique only uses ordered meristems, progeny that are genetically stable and true-type can be recovered. This type of clonal propagation is useful for producing hundreds of plantlets from a single individual in a short period of time and in a small amount of space. As a review, on the micropropagation of *S. torvum*, an essential medicinal plant, employing mericlone technology demonstrates the great value for large-scale propagation.

VI. IN VITRO ROOTING AND PLANTLET ESTABLISHMENT

*In vitro* rooting is an important phase in regenerating whole plant using *in vitro* culture technology. After successful development of regeneration protocol from any plant species, *in vitro* rooting is prerequisite for conservation and multiplication of the species. For induction of *in vitro* rooting, different types of auxins and their concentrations show variability. This phenomenon it varies from species to species. Even it depends upon the type of explant from which the micro-shoots were developed for *in vitro* rooting.

Ya-Long Qin et al. (2017) investigated *in vitro* rooting from micro-shoots formed by callus mediated regeneration of stem segments and leaves. They inoculated the micro-shoots on 1/2 strength MS medium supplemented with 0.1 mg/L IAA and discovered profuse rhizogenesis at 0.1 mg/L IAA.

Ghan Singh et al. (2021b, 2022a) cultured the micro-shoots developed from different explants derived callus cultures and from direct organogenesis were transferred on MS medium augmented with 0.25-2.0 mg/L NAA/IAA. *In vitro* rooting was observed in all NAA/IAA concentrations. In comparison to all other NAA/IAA concentrations tested, 1.0 mg/L IAA produced the highest percentage of response (90%) and the maximum frequency number (18.2±0.03) of roots.

According to Ghan Singh et al. (2022b), *in vitro* rooting was established from shoots developed from shoot tip and nodal cultures. Micro-shoots developed from shoot tip, and nodal cultures were transferred to 1/2 strength MS and MS media without growth regulators, as well as MS medium supplemented with various concentrations (0.25-2.0 mg/L) of NAA/IAA/IBA. *S. torvum* showed no *in vitro* rooting on half-strength MSO medium without PGRs. On MSO medium with 4-6 weak roots, poor and moderate rooting was observed with a very low (10%) percentage. Roots developed without a callus phase in all IAA and IBA concentrations tested. As NAA concentration increased, the percentage of reaction increased to 0.5 mg/L NAA. Furthermore, abundant rhizogenesis and shoot development were observed at 0.5 mg/L NAA in comparison to other NAA concentrations used. When the concentration of NAA increased above 0.75 mg/L, root growth and callus induction improved gradually. The highest proportion of reaction was discovered at 1.0 mg/L IBA, followed by 0.75 mg/L IBA. The greatest number of roots, including long roots in *S. torvum*, were also induced at the same IBA concentration. 1.0 mg/L IAA produced the highest percentage of response and the maximum frequency number of roots per shoot than the other concentrations of IAA/IBA/NAA used.

In a greenhouse, all the *in vitro* rooted plantlets were hardened. Before being placed in the research field, these plantlets were housed in a darkened environment for a month. The healthy, normal morphological traits, flowering, and fruiting of the regenerated plants were found to be identical to those of the donor plant.

VII. CONCLUSION

We reviewed the different types of *in vitro* regeneration protocols i.e., callus induction, regeneration (indirect/direct), somatic embryogenesis, micropropagation (using shoot meristem/nodes) and rooting and plantlet establishment by using different types of explants for improvement of *S. torvum*. Since it has importance in Ayurveda, the biotechnological techniques developed in this review can be used all year to produce secondary metabolites (bioactive molecules) such as glycoalkaloids, which are important in the pharmaceutical industry. The plant can also be used as a plant factory for the production of plant-made pharmaceuticals as...
a model system for introducing various types of novel genes using these standardized regeneration protocols in the field of molecular farming.

VIII. ACKNOWLEDGEMENTS

GSM is thankful to the University Grants Commission, New Delhi, India for providing the financial assistance under Rajiv Gandhi National Fellowship as JRF/SRF (Ref. No. F.14−2/2006 (SA-II)) to MGS.

IX. CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in the present review.

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DOI: http://dx.doi.org/10.24018/ejbio.2023.4.3.443 Vol 4 | Issue 3 | July 2023


