

***In vitro* propagation of goldenberry (*Physalis peruviana* L.): A Review**

Shivani Yadav¹, Navneet Barnwal¹, Govind Kumar Rai², Monika Bajpai¹ and Neha Prakash Rai^{1*}

Received: June 2019 / Accepted: November 2019

Abstract

Goldenberry (*Physalis peruviana* (L.)) has great economic importance due to its nutritional values as well as the medicinal properties. Due to such properties, this plant is gaining focus in pharmaceutical industries. The use of tissue culture methods might be conducive to select cultivars of desired characters and micropropagation for clonal propagation and agricultural practices of golden berry, considering the limited plant production and high consumption due to value-added natural compounds. *P. peruviana* is recalcitrant to shoot organogenesis through adventitious regeneration from leaf explants, therefore, there is a very limited number of *in vitro* regeneration studies. This review summarizes available tissue culture reports on *P. peruviana* for explants and plant growth regulators used and the technological advances made.

Keywords: *Physalis*, micropropagation, regeneration, goldenberry, *in-vitro*, solanaceae

Introduction

Goldenberry (*Physalis peruviana* (L.)) a golden berry of future, belongs to the family Solanaceae. *P. peruviana* is a small herbaceous, semi shrub, and an annual plant which also grow as weed in the crop field (Ramirez et al. 2013). This golden fruit is also known as rasbhari, cape gooseberry, physalis, goldenberry, aguaymanto, topotopo, uvilla, uchuva, physalis, giant ground cherry, pokpok, harankash, inca berry, African ground cherry, Peruvian ground cherry, Peruvian cherry, or Aztec berry (Puente et al. 2011; Ramadan et al. 2012). It is native to tropical Peru and other warm temperate and subtropical regions throughout the world (Ramirez et al. 2013). It is commercially cultivated in Ecuador, South Africa, Kenya,

Zimbabwe, Australia, New Zealand, Hawaii, India, Malaysia, Colombia, and China. The fruits are smooth berry, resembling a miniature yellow tomato, exhibits a characteristic mild tart flavour with sweetness when ripe. Its peculiar taste makes it ideal for snacks, pies, or jams. The seeds are bright yellow to orange in color.

This plant has increasing popularity due to its nutritional and medicinal values of the fruits as well as other parts of the plant (including leaves and stems). Many researchers indicated that *Physalis peruviana* are widely used medicine for anticancer, anti-mycobacterial, antipyretic, immunomodulatory properties (Pietro et al. 2000; Soares et al. 2003). It has been used for treating diabetes, asthma, malaria, dermatitis, hepatitis, ulcers, and several other diseases (Soares et al. 2003; Mayorga et al. 2002; Arun and Asha 2007).

Main content of the goldenberry fruits are protein (0.054g/100g), fat (0.16g/100g), fiber (4.9g/100g), ash (1.01g/100g), calcium (8.0mg/100g), phosphorus (55.3mg/100g), iron (1.23mg/100g), carotene (1.613mg/100g), thiamine (0.101mg/100g), riboflavin (0.032mg/100g), niacin (1.73mg/100g) and ascorbic acid (43.0mg/100g). However, the fruits also contain high levels of vitamins A, C, B-complex, compounds with anti-inflammatory and antioxidant properties (Strik, 2007). Many reports show the tremendous medicinal value of goldenberry for curing out different diseases. The ethanol extracts from *Physalis peruviana* contain well an antioxidant activity and highest (95%) antioxidant properties (Wu et al. 2005). *Physalis peruviana* showed main elements of K, Mg, Ca and Fe in its mineral composition and the lipidic fraction presented predominance of the linoleic acid (72, 42%) in its composition (Rodrigues et al. 2009). Some researchers noted that *Physalis peruviana* has several physalin compounds (Kawai et al. 1992; Sen and Pathak 1995). Some physalin compounds like physalin B and F were noted to have great potential for treating tumour (Antoun et al. 1981; Chiang et al. 1992a, b and Sunayama et al.

¹Institute of Applied Medicines and Research, Duhai, Ghaziabad 201206, UP

²Biosafety Support Unit, Regional Centre for Biotechnology, 1st Floor, National Productivity Council Building, Lodhi Road, New Delhi-110 003

*Corresponding author; E-mail: nehaprai21@yahoo.in

1993). Wu et al. (2009) reported that supercritical carbon dioxide extracts of *P. peruviana* leaves induced cell cycle arrest and apoptosis in human lung cancer cells. Similarly, Çakir et al. (2014) reported that phenolic content of ethanolic leaf extracts (100 µg ml⁻¹) possessed high cytotoxic effects on HeLa cell line. Yen et al. (2010) reported that goldenberry-derived 4-HWE is a potential DNA-damaging and chemotherapeutic agent against lung cancer. Some medicinal plants may exhibit a certain type of toxicity on humans; however, the toxicity of goldenberry was demonstrated just at high concentrations in males only (Perk et al. 2013). Leaf and shoot extracts possess a cytotoxic effect on HeLa cells when applied as 100 µg ml⁻¹ concentration and can alter anti-apoptotic genes (Çakir et al. 2014). Furthermore, *P. peruviana* extracts were reported to have anti-hepatoma activity due to the induction of apoptosis in a human hepatocellular carcinoma (Hep G2) cell line (Wu et al. 2004). Due to such medicinal properties, this plant is gaining focus in pharmaceutical industries.

Goldenberry plant is conventionally propagated through seed. However, there are many problems associated with seed propagation such as inadequate availability of the propagation material, poor seed germination rate, variability in vigour, seedling growth, genetic variation occurs in seed from cross pollinated plants because they are heterozygous. Plant tissue culture techniques may play an important role in the clonal propagation of elite clones and germplasm conservation of this plant species. The use of tissue culture methods for the selection of the best cultivars for agricultural practices and clonal propagation might be conducive for micropropagation, especially when considering high consumption due to value-added natural compounds, limited plant production, and requirements of manpower, transport and storage (Rodrigues et al. 2013a). Enhanced production of secondary metabolites can also be possible through *in vitro* plant cell culture (Barz and Ellis 1981; Deus and Zenk, 1982).

Regeneration studies

In vitro propagation has the potential to circumvent such problems for cultivation as well as could be an effective approach to conserve the germplasm and genetic improvement (Boulay 1987; Rai et al. 2012a). The capability for regenerating and propagates plants from cultured cells and tissues is one of the most useful aspects of *in vitro* cell and tissue culture (Rai et al. 2012b). Therefore, it is important to develop an efficient micropropagation and regeneration technique for *P. peruviana* to rapidly disseminate superior clones once

they are identified. Numerous studies and industrial applications of *in vitro* propagation of different plant species have shown that this technique effectively does rapid propagation of target plant species. Used in conjunction with classical breeding methods, an efficient *in vitro* shoot proliferation and regeneration system could accelerate cultivar development programs. *In vitro* techniques are important tools for modern plant improvement programs introducing new traits into selected plants, to multiply elite selections and to develop suitable cultivars in the short time also (Rai et al. 2012a, 2012b; Taji et al. 2002).

P. peruviana is recalcitrant to shoot organogenesis through adventitious regeneration from leaf explants (Torres, 1991). There is a very limited number of *in vitro* regeneration studies concerning the micropropagation of *P. peruviana* (Rodrigues et al., 2013b), these reports do not provide information about the synergistic effects of plant growth regulator (PGR) combinations on growth parameters. Methods on propagation and conservation of plant genetic resources remain a major challenge. Several studies on *in vitro* propagation of other *Physalis* species, including *P. minima*, *P. ixocarpa*, and *P. pruinosa*, were also reported (Bapat and Rao, 1977; George and Rao, 1979; Gupta, 1986; Ramirez-Malagón and Ochoa-Alejo, 1991; Rao et al., 2004). Synthetic seed production in *Physalis* through encapsulation technology might also provide year-round productivity because most plants produce seeds only during certain months of the year. However, since goldenberry is recalcitrant to adventitious regeneration, synthetic seed production has not been exploited much, but it could be a promising approach.

Nodal explants have been the explant of choice for tissue culture of Goldenberry plants. Otrushy et al. (2013) used leaf and nodal explants tissue culture of goldenberry and reported maximum regeneration at a high concentration of benzyl amino purine (BAP) in combination with Kinetin; maximum callus induction (100%) was observed from the nodal segment at the lower concentration of cytokinin and presence of IBA. Similarly, Singh et al. (2016) also cultured nodal segments (1-2cm) having at least one axillary bud on Murashige and Skoog (MS; 1962) medium supplemented with BAP and IBA. They reported that nodal explants were found superior to internode explants for organogenesis. However, Ramar et al. (2014) observed efficient *in vitro* regeneration from the node, internode as well as leaf explants of *P. peruviana* on MS medium supplemented with B5 vitamins and different concentrations and combinations of BAP, GA3 and 2,4-D.

Use of BAP at the concentration of 2.5 mg l⁻¹ along with IBA at the concentration of 0.05 mg l⁻¹ was found to be optimum for shoot proliferation based on sprouting percentage, several shoots, shoots length and number of leaves (Singh *et al.* 2016). Gunay *et al.* (2016) also observed differed effects of various levels of BAP with IBA and NAA combinations based on shoot numbers and length. They reported higher shoot number and length on the medium supplemented with BAP and IBA combinations than BAP with NAA. The highest shoot numbers (6.00) were obtained from 2 mg l⁻¹ BAP with 0.4 mg l⁻¹ IBA combinations and longest mean shoot length (3.30 cm) obtained in 2 mg l⁻¹ BAP with 0.2 mg l⁻¹ IBA combinations (Gunay *et al.* 2016). As for the effects of BAP and NAA combinations; the longest shoot length (3.33 cm) were obtained from 2 mg l⁻¹ BAP without NAA combinations while the lowest one was in 3 mg l⁻¹ BAP with 0.4 mg l⁻¹ NAA combinations (Gunay *et al.* 2016). In contrast, Ramar *et al.* (2014) reported maximum numbers of multiple shoots were achieved from nodal and internodal explants on the combination of 2.0 mg l⁻¹ BAP, 1.0 mg l⁻¹ GA3 and 1.0 mg l⁻¹ 2,4-D. The high frequency of shoot multiplication observed from leaf explants on 3.0 mg l⁻¹ BAP, 1.0 mg l⁻¹ GA3 and 1.0 mg l⁻¹ 2,4-D. Celikli *et al.* (2017) investigated *in vitro* salinity (0-100 mM NaCl) of goldenberry shoot apex cultured on MS medium supplemented with 1 mg l⁻¹ indole acetic acid (IAA), 3% sucrose and 0.7% agar containing NaCl (0, 25, 50, 75 or 100 mM). The explants were incubated at 25±2°C for 4 weeks and related parameters, such as shoot, leaf and root formation, were measured. Experimental results revealed that different level of salinity treatments on *in vitro* culture had a notable effect on above stated growth parameters. These parameters decreased significantly by increasing salinity level for excluding shoot diameters.

For root induction, full-strength semi-solid MS medium with 1.0 mg l⁻¹ IBA exhibited the best *in vitro* rooting in finding of Otrosky *et al.* (2013) while Singh *et al.* (2016) reported MS medium supplemented with a much lower concentration of IBA (0.05 mg l⁻¹) is sufficient superior root initiation, number of roots and root length. Addition of activated charcoal at 450 mg l⁻¹ resulted in 100 per cent browning free culture (Singh *et al.* 2016). Ramar *et al.* (2014) transferred shoots regenerated on 3.0 mg l⁻¹ BAP, 1.0 mg l⁻¹ GA3 and 1.0 mg l⁻¹ 2,4-D on to half strength MS medium fortified with IBA for root induction, the rooted plantlets were successfully acclimatized. On the contrary, Gunay *et al.* (2016) obtained satisfactory rooting on medium containing NAA compare to the IBA, the highest root numbers were obtained from NAA application (1-2 mg l⁻¹). Yücesan *et al.* (2015) develop a new regeneration system for

micropropagation and synthetic seed production using nodal segments excised from 4-week-old germinated seedlings, direct plant regeneration, without additional rooting stage, was achieved on MS medium containing 0.5 mg l⁻¹ 6- BAP, kinetin, thidiazuron (TDZ), or gibberellic acid (GA3), alone or in combination with 0.25 mg l⁻¹ indole-3-acetic acid (IAA) or IBA, after 2 weeks of incubation. The highest mean numbers of shoots and well-developed roots were obtained on MS medium containing solely 0.5 mg l⁻¹ TDZ, producing 5.3 shoots and 3.3 roots per explant after 2 weeks of incubation. Direct shoot and root formation were also recorded on MS medium containing no plant growth regulators.

Artificial seeds

Methods on propagation and conservation of plant genetic resources remain a major challenge. In recent years, encapsulation technology has provided a new understanding because of its wide use in germplasm conservation and delivery of tissue cultured plants for commercial and research purposes. Since goldenberry tissues are recalcitrant to adventitious regeneration, synthetic seed production concerning germplasm preservation, propagation, storage, and transportation issues might be promising not only for this fruit plant, but also for a wide range of solanaceous species. Synthetic seed production of goldenberry plants might also provide year-round productivity, because most plants produce seeds only during certain months of the year. Calcium alginate hollow beads are used frequently for plant cells, shoot tips, nodal segments, or somatic embryos for conservation purposes, and especially for synthetic seed production (Patel *et al.* 2000; Rai *et al.* 2009; Benelli *et al.* 2013; Kocak *et al.* 2014). Yücesan *et al.* (2015) investigated synthetic seed production using the sodium alginate encapsulation technique. Four different matrix compositions, including sodium alginate with or without MS medium containing 3% (w/v) sucrose alone or in combination with 0.5 mg l⁻¹ abscisic acid (ABA) as a growth retardant were tested for the regrowth performance of synthetic seeds after storage at 4°C up to 70 days. The highest regrowth (100%) was observed at 28 days of storage for all matrix compositions. All plantlets were acclimatized to the soil and then progressively transferred to the field. The fruits were harvested after 5 months.

Conclusion

Overall, by going through all the available literature it may be concluded that sufficient regeneration studies are not available for this plant species and there is need of extensive regeneration study for an efficient and

reproducible *in vitro* propagation method of goldenberry.

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गोल्डेन बेरी (फ़ैसलिस पेरुवियाना एल.) में पौष्टिक गुणों के साथ-साथ औषधीय गुणों का आर्थिक महत्व ज्यादा है। इन्ही गुणों के कारण यह पौधा भेषज उद्योगों में आकर्षण का केन्द्र है। ऊतक संवर्धन विधि को अपनाकर अच्छी किस्मों वांछित गुणों के लिये चयन, सूक्ष्म प्रवर्धित प्रसारण एवं गोल्डेन बेरी की कृषि उत्पादन पद्धतियों पर ध्यान दे कर कम पौध उत्पादन एवं अधिक उपभोग हेतु मूल्य संवर्धित प्राकृतिक पदार्थों की कमी को पूरा किया जा सकता है। गोल्डेन बेरी रिकैल्सीट्रेंट होने के कारण ऊतक पुर्नजनन के माध्यम से वृद्धि की जा सकती है। इसके पुर्नजनन पर आधारित अध्ययनों के परिणाम बहुत सीमित है यह शोध समीक्षा उपलब्ध ऊतक संवर्धन अनुसंधान प्रयोगों के विवरणों में से प्रयुक्त ऊतक पौध नियामकों एवं तकनीकी प्रगति को साराक्षित किया गया है।

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