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96

MINI-REVIEW

Biotechnology for propagation and secondary metabolite production in *Bacopa monnieri*

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Abstract

Bacopa monnieri (L.) Wettst. or water hyssop commonly known as "Brahmi" is a small, creeping, succulent herb from the *Plantaginaceae* family. It is popularly employed in Ayurvedic medicine as a nerve tonic to improve memory and cognition. Of late, this plant has been reported extensively for its pharmacologically active phyto-constituents. The main phytochemicals are brahmine, alkaloids, herpestine, and saponins. The saponins include bacoside A, bacoside B, and betulic acid. Investigation into the pharmacological effect of this plant has thrived lately, encouraging its neuroprotective and memory supporting capacity among others. Besides, it possesses many other therapeutic activities like antimicrobial, antioxidant, anti-inflammatory, gastroprotective properties, etc. Because of its multipurpose therapeutic potential, it is overexploited owing to the prioritization of natural remedies over conventional ones, which compels us to conserve them. *B. monnieri* is confronting the danger of extinction from its natural habitat as it is a major cultivated medico-botanical and seed propagation is restricted due to less seed availability and viability. The ever-increasing demand for the plant can be dealt with mass propagation through plant tissue culture strategy. Micropropagation utilizing axillary meristems as well as de novo organogenesis have been widely investigated in this plant which has also been explored for its conservation and production of different types of secondary metabolites. Diverse in vitro methods such as organogenesis, cell suspension, and callus cultures have been accounted for with the aim of production and/or enhancement of bacosides. Direct shoot-organogenesis was initiated in excised leaf and internodal explants without any exogenous plant growth regulator(s) (PGRs), and the induction rate was improved when exogenous cytokinins and other supplements were used. Moreover, biotechnological toolkits like *Agrobacterium*-mediated transformation and the use of mutagens have been reported. Besides, the molecular marker-based studies demonstrated the clonal fidelity among the natural and in vitro generated plantlets also elucidating the inherent diversity among the natural populations. *Agrobacterium*-mediated transformation system was mostly employed to optimize bacoside biosynthesis and heterologous expression of other genes. The present review aims at depicting the recent research outcomes of in vitro studies performed on *B. monnieri* which include root and shoot organogenesis, callus induction, somatic embryogenesis, production of secondary metabolites by in vitro propagation, acclimatization of the in vitro raised plantlets, genetic transformation, and molecular marker-based studies of clonal fidelity.

Key points

- *Critical and up to date records on in vitro propagation of Bacopa monnieri*
- *In vitro propagation and elicitation of secondary metabolites from B. monnieri*
- *Molecular markers and transgenic studies in B. monnieri*

Keywords Micropropagation · Biotechnology · Cell suspension cultures · Elicitation · Saponins · Bacosides · In vitro propagation · Pharmacological activity

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Introduction

Bacopa monnieri (L.) Wettst. from the *Plantaginaceae* family is an amphibian plant of the tropical regions that usually grow on the banks of rivers and lakes (Binita et al. 2005). It

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is found in the tropical and subtropical regions worldwide which include India, Sri Lanka, Nepal, Taiwan, China, Pakistan, and Vietnam. It is also reported from Florida, Hawaii, southern states of the USA, and the Mediterranean Basin. In India, it occurs in Assam, Delhi, Manipur, Goa, Andhra Pradesh, Gujarat, Bihar, Kerala, Karnataka, Tamil Nadu, Punjab, Andaman, Rajasthan, Orissa, and West Bengal. It is also found throughout the Western and Southern Peninsula (Lansdown et al. 2013). Figure 1 presents the global distribution of *B. monnieri* (source: https://www.gbif.org/species/3171169). Popularly referred to as Jalanimba, Brahmi, or the thinking man's herb, *B. monnieri* is a major ancient Medhya Rasayana drug in the Ayurveda (Abdul Manap et al. 2019). It is employed as a valuable component in a number of Ayurvedic preparations, such as Brahmi rasayana, Brahmivati, Brahmighrit, and Sarasvatarisht (Sharma et al. 2016). In Ayurveda, it has been used as a brain tonic to improve memory, concentration, and learning capacity and also to cure mental illness (Brimson et al. 2021; Lopresti et al. 2021). The plant improved cognitive and behavioral parameters in children as well as in adolescents (Kean et al. 2017). *B. monnieri* has been suggested in the Indian Materia Medica and Traditional Chinese Medicine for the remedy of a variety of mental medical conditions such as insomnia, poor cognition, anxiety, psychosis, a deficit of concentration, epilepsy, insanity, Alzheimer's disease, and depression (Moskwa et al. 2020; Halder et al. 2021; Anand et al. 2022). The plant was found to improve respiratory function during bronchoconstriction and also is utilized as a cardiac tonic and digestive aid in India and Pakistan (Saha et al. 2020). Additionally, the plant possesses neuroprotective, anti-neuro-inflammatory, pro-cognitive, neuropsychiatric, antinociceptive, analgesic, anticancer, antioxidant, antipyretic, and anticonvulsant properties (Nemetchek et al. 2017; Ranjan et al. 2018; Abdul Manap et al. 2019; Brimson et al. 2020; Castelli et al. 2020; Jeyasri et al. 2020; Kiani et al. 2020; Micheli et al. 2020; Cheema et al. 2021; Datta et al. 2021; Dutta et al. 2021; Paul et al. 2021; Sharma et al. 2022). The plant extract also offered protection against tacrolimus-mediated kidney toxicity and opioid induced toxicity (Shahid et al. 2016; Oyouni et al. 2019). The plant has also exhibited anti-anhedonia (Micheli et al 2020), vasodilatory (Kamkaew et al. 2019), hippocampus-strengthening (Promsuban et al. 2017), and anti-cytotoxic-genotoxic (Dogan and Emsen 2018) properties. The plant also did not exhibit any acute and chronic toxicities in a rat model (Sireeratawong et al. 2016). The active phytochemicals of *B. monnieri* include saponins, alkaloids, and sterols. The alkaloid brahmine was first reported from the plant. Eventually, several other alkaloids such as nicotine and herpestine were also reported. The plant houses other major phytochemicals viz. des-saponin glycosides-triterpenoid and saponins (bacosides A and B). It also possesses other minor constituents such as betulic acid, bacosides A1 and A3, hersaponin, monnierin, herpestin and flavonoids, glucuronyl-7-luteolin, luteolin-7-glucoside, and glucuronyl-7-apigenin. The pharmacological attributes of *B. monnieri* for enhancing memory and cognition have been credited to the presence of various triterpenoid saponins like bacosides A, B, C, and D also referred as "memory chemicals" (Dey et al. 2019; Banerjee et al. 2021; Nandy et al. 2022).

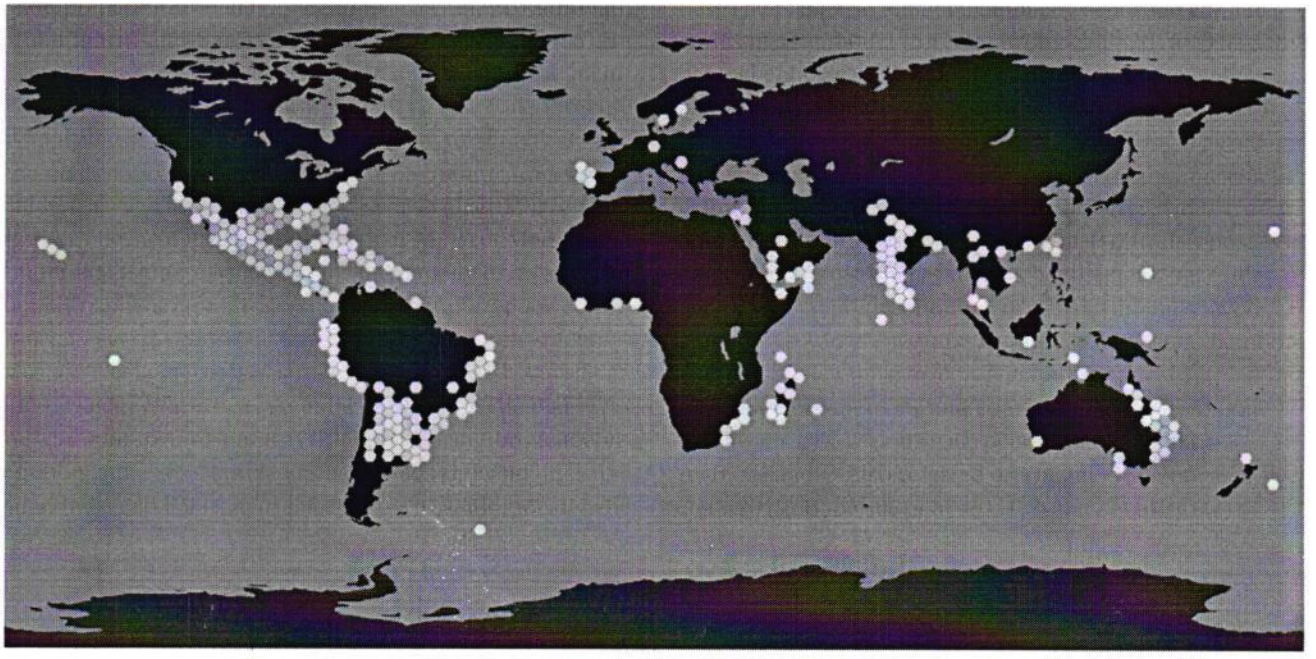

Fig. 1 Worldwide distribution of *B. monnieri* (source: https://www.gbif.org/species/3171169)

The seeds of *B. monnieri* are considered as poor propagules because of their short viability (two months), and the seedlings often die at the two-leaved stage, making the growth difficult from seeds. Vegetative propagation is slow which is also hampered by particular habitat conditions and inferior performance of the propagules. Further, rising demand for the plant materials due to over-exploitation put pressure on the supply of this medicinal species causing adulteration of plant materials (Tiwari et al. 2001). An effective and most suitable alternative is the development of in vitro techniques for the conservation and sustainable yield of medico-botanicals and their phytochemicals. True-to-type, infection-free and compatible plants for identification, characterization, and quantification of phytochemicals can be provided by the in vitro propagation. In the past two decades, in vitro technology has been progressively applied for rapid clonal propagation and conservation of threatened and valuable plant germplasm. Therefore, the implementation of in vitro techniques might be a promising alternative for *B. monnieri* multiplication and conservation.

Taxonomic description

B. monnieri also known as *Herpestis monniera* or water hyssop, locally called Jalanimba or Brahmi in India, is a much used Ayurvedic herb belonging to the *Plantaginaceae* family.

Taxonomic classification

Kingdom: *Plantae*
Division: *Angiosperms*
Class: Eudicots
Order: Lamiales
Family: Plantaginaceae
Genus: *Bacopa*
Species: *monnieri*

B. monnieri is a small, annual, succulent, creeping, multi-branched herb with numerous prostrate branches and roots arising from the nodes. The plant grows to a height of about 2–3 feet (60–90 cm), and the branches are 10–35 cm long. Roots developing from the nodes are small, thin, wiry, much branched, and creamish-yellow in color. The stem is soft, green or purplish green, thin measuring about 1–2 mm in thickness, and slightly bitter in taste. The nodes and internodes are prominent and glabrous. Each flower is small, solitary, and axillary in position having four to five petals. The shades of the flower range from white, purple, pink to pale violet. The pedicels are 6–30 mm long and bracteoles shorter than pedicels. The leaves of *B. monnieri* are succulent or fleshy and relatively thick. They are oblanceolate in shape, sessile, stalkless measuring about 0.6–2.5 cm long and 3–8 mm broad, and oppositely arranged on the stem (Rameshwari et al. 2013). The fruits are ovoid, two celled and two valved capsules, acute apex, and tipped with style base. It is slightly bitter in taste with no distinct odor. Figure 2A represents the habit of *B. monnieri* (source: Wikimedia commons; Creative Commons Attribution 3.0 Unported license; Attribution: Forest & Kim Starr), and Fig. 2B represents the flower of *B. monnieri* (source: Wikimedia commons; Creative Commons Attribution 2.0 Generic license). Species, as well as family names, are verified from www.theplantlist.com.

Phytochemistry

Phytochemicals are classified into two categories viz., primary and secondary components. The primary components in *B. monnieri* are proteins, amino acids, sugar, and chlorophyll. *B. monnieri* contains alkaloids, saponins, and sterols (secondary components). The alkaloids, "brahmine" was isolated at first, and other alkaloids such as nicotine and herpestine were also isolated later. Subsequently, saponins like *D*-mannitol, hersaponin, and sterols like β-sitosterol and stigmasterol were obtained. Besides, monnierin, wogonin, betulic acids, and oxindin were also reported (Al-Snafi 2013). The major compounds were recorded as tritetrapenoid saponins such as bacosides and bacopasaponins. Besides, two saponins were found as aglycones, viz. jujubogenin and pseudojujubogenin (Dey et al. 2019) which mainly differ from each other in the nature of sugar units, because the position of the glycosidic chain and the olefinic side chain which is different in the aglycone (Rajan et al. 2015). Few other major active components recorded were bacopaside I, bacopaside II, bacopaside X, bacoside A3, bacopaside N2, and bacopasaponin C. Some minor active components were bacopaside III, bacopaside IV, bacopaside V, bacopasaponin E, and bacopasaponin F (Murthy et al. 2006). The most common and major phytochemical, bacoside A, was found to be levorotatory which is the mixture of four triglycosidic saponins like bacoside A3, bacopasaponin C, bacopaside II, and jujubogenin; this jujubogenin is an isomer of bacopasaponin C (Dey et al. 2020). Besides, bacogenins A1, A2, A3, and A4 were identified from bacoside A, with the help of hydrolysis process. Another common phytochemical, bacoside B, which is the mixture of four diglycosidic saponins like bacopaside N1, bacopaside N2, and bacopasides IV and V (Dey et al 2020). The dammarane-type triterpenoid saponins viz. bacopasaponins A, B, and C, which were isolated with different names, such as 3-*O*-a-L-arabinopyranosyl-20-*O*-a-Larabinopyranosyl-jujubogenin, 3-*O*-[a-L-arabinofuranosyl (I → 2) a-Larabinopyranosyl] pseudojujubogenin and 3-*O*-[β-D-glucopyranosyl (1 → 3)

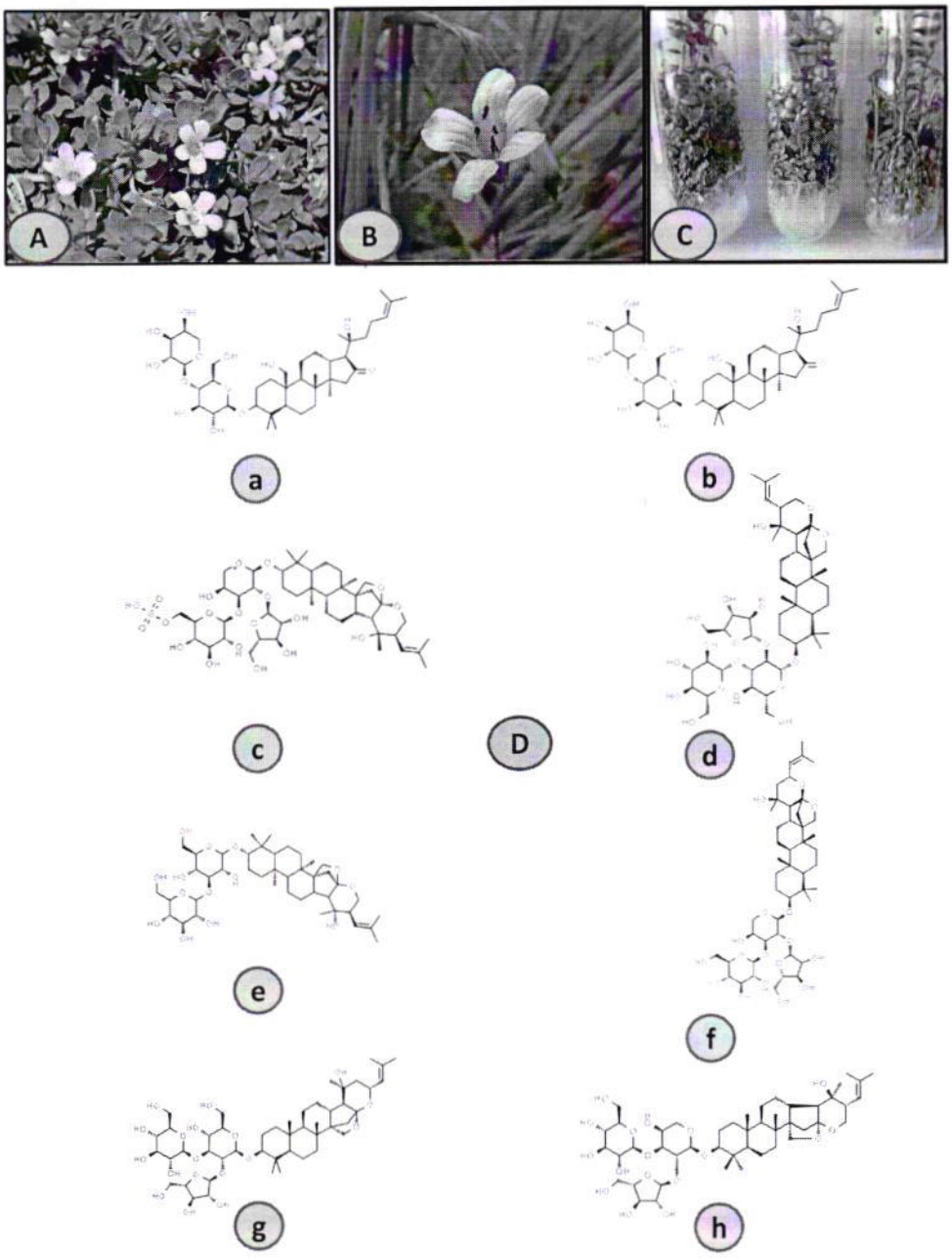

Fig. 2 (**A**) Habit of *B. monnieri* (source: Wikimedia commons; Creative Commons Attribution 3.0 Unported license; Attribution: Forest & Kim Starr). (**B**) Flower of *B. monnieri* (source: Wikimedia commons; Creative Commons Attribution 2.0 Generic license). (**C**) Microprpagation in *B. monnieri* (unpublished photograph of Dr. Dey). (**D**) 2D structures of the major secondary metabolites found in *B. monnieri* (a: bacoside A, b: bacoside B, c: bacopaside I, d: bacopaside II, e: bacopaside N2, f: bacopaside X, g: bacoside A3, h: bacopasaponin C) (structure source: http://www.chemspider.com/)

{a-L-arabinofuranosyl (1 → 2)}a-Larabinopyranosyl]. Bacopasaponin D is also isolated as 3-*O*-[α-Larabinofuranosyl (I → 2) β-D-glucopiranosyl] pseudojujubogenin by the spectroscopic and chemical transformation method. The saponin, bacoside A1, and triterpenoid saponin A3 were also identified (Dey et al. 2020). A minor saponin, bacoside A1 which is known as 3-O-[a-L-arabinofuranoyl (1 → 3)-β-L-arabinopyranosyl] jujubogenin and triterpenoid saponin, bacoside A3 were also isolated by chemical and spectral analyses and known as 3-b-[*O*-b-Dglucopyranosyl (1–3)-*O*-[aL-arabinofuranosy (1 → 2)]-*O*b-D-glucopyranosyl)oxy] jujubogenin. Along with this bacogenin A4 was characterized as ebelin lactone pseudojujubogenin. Le et al. (2015) recognized novel saponins like bacopasides I–XII. Bacopasides N1-N2 were also recorded from the plant. (3alpha)-3-Hydroxylup-20(29)-en-27-oic acid which is triterpene bacosine was isolated and identified from the aerial parts of *B. monnieri* (Kishore et al. 2017; Ghosh et al. 2011). From the aerial four cucurbitacins, bacobitacins A-D (1–4) and cucurbitacin E(5), a known cytotoxin along with other three known phenylethanoid glycosides, monnieraside I, III, and plantioside B, were also isolated (Bhandari et al., 2007). Besides, stalks and leaves of *B. monnieri* were found to contain 88.4% moisture. Carbohydrates (5.9 g), fat (0.6 g), protein (2.1 g), crude fiber (1.05 g), ash material (1.9 g), phosphorus (16.0 mg), calcium (202 mg), iron (7.8 mg),

nicotinic acids (0.3 mg), ascorbic acids (63 mg), and some amount of energy (38 cal) were recorded along with this moisture (Devendra et al. 2018). Figure 2D represents the 2D structures of the major secondary metabolites found in *B. monnieri* (a: bacoside A, b: bacoside B, c: bacopaside I, d: bacopaside II, e: bacopaside N2, f: bacopaside X, g: bacoside A3, h: bacopasaponin C) (structure source: http://www.ChemSpider.com).

Biotechnological aspects of *B. monnieri*

Source of explants

The factors on which the regeneration potential of an explant depends are genotype, size, age, source of explant, physiological and developmental stage, presence of meristematic region, proper sterilization, etc. (Dey et al. 2020; Tandon et al. 2021). Almost all healthy plant parts can be employed as the explant-source like apical or nodal meristem, root, shoot, leaf, bud, seed, etc. Leaf, internodes, and nodes are mostly used as explants for plant regeneration, especially for shoot formation. Direct somatic embryos were also found using the leaf explants derived from the microshoots (Khilwani et al. 2016). The use of microshoots, leaf, or internodal explants excised from the basal region of the plant *B. monnieri* was found to be very effective for direct organogenesis of shoots (Sarkar and Jha 2017). Position, type, and orientation of explants in the medium were recorded to influence the direct and indirect organogenesis of shoots (Saha et al. 2020). For successful synthetic seed production in *B. monnieri*, shoot tips were used as explants, cultured in the MS medium, and supplemented with 75 mM calcium chloride and 2.5% sodium alginate (Pramanik et al. 2021). Nodal segments and leaves, as explants, were also reported for bacoside production (Praveen et al. 2009). The uses of axillary bud, younger nodes, shoot tips, and young leaves excised from the young shoots have been used for the establishment of callus cultures (Bhusari et al. 2013; Showkat et al. 2010).

Culture conditions

For in vitro propagation, plants are cultured in a suitable culture vessel containing various nutrient media under aseptic and controlled parameters. Culture conditions like temperature, light intensity, pH, CO_2 concentration, etc. are needed to be optimized for the best growth and morphogenic response of the plant materials in vitro. The light intensity used was 50 µmol/m2/s PPFD (photosynthetic photon flux density) by Dey et al. 2019 which has been reported as 80–100 µmol/m^2/s PPFD by Banerjee and Srhivastava (2007). In another study, a fluorescent lamp of 40 W was used as a light source at night (Naik et al. 2017). The temperature was reported to be 23 ± 2 °C (Samanta et al. 2019). The relative humidity provided for the incubation was found to be 55–60% (Ranjan et al. 2018). Some sources have shown to adjust the pH at 5.8 of the media with 1 N HCl or 1 N NaOH solutions, and then, the culture media were autoclaved for 15–20 min at 121 °C temperature and 15 lb pressure (Binita et al. 2005). In some experiments, 4.5 pH has also been provided for the accumulation of biomass and production of bacoside (Naik et al. 2010). If culture vessels were used, after autoclaving, they were transferred to the media room under controlled aseptic conditions for further experiments. After this, all the subcultures were generally conducted in intervals of four weeks (Srivastava et al. 2017).

Surface sterilization

Different plant parts/organs collected from the field or greenhouse are surface sterilized before setting up the in vitro cultures. Surface disinfection of the explants is a significant step prior to the establishment of in vitro culture since microorganisms develop quicker in tissue culture medium than the explants do (Kim et al. 2017). Contamination with microorganisms such as viruses, bacteria, yeast, fungi, etc. is counted to be one of the most important hindrances during in vitro culture of plants. These microbes compete unfavorably with plant tissues for nutrients and elevate the culture mortality rate. They can also result in tissue necrosis, invariable growth, decreased shoot proliferation, and reduced rooting. The very first step in surface sterilization of the cut explant of *B. monnieri* was performed by a thorough washing of the explant under running tap water for 20 min to 2 h to remove the superficial mud and dust particles adhering to the surface (Banerjee and Modi 2010; Sharma et al. 2016). Labolene (5% v/v for 15 min) (Banerjee and Modi 2010), teepol (5% for 30 min) (Mohapatra and Rath 2005), Cetavlon (1—2% for 10 min) (Sharma et al. 2010), etc. have also been used as mild liquid detergents for initial microorganism elimination from the surface of the explants. Kalita et al. (2018) in their experiment used 70% ethanol for 30 s to disinfect nodal segments of *B. monnieri* followed by a solution with 25% sodium hypochlorite: 0.01% Tween-20 for about 25 min. Afterwards, the explants were washed thrice with sterile distilled water. Few researchers have also recommended the use of a systemic fungicide called Bavistin®. Ceasar et al. (2010) soaked excised shoot buds in 0.1% (w/v) Bavistin® containing carbendazim for 20 min and then washed thrice with sterile distilled water. As reported by most of the researchers, the final step of washing was performed with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 3 to 10 min followed by washing with sterile distilled water to remove traces of $HgCl_2$ (Haque et al. 2017; Parale et al. 2010; Sharma et al. 2010). The final wash was

performed carefully several times (4–5) with autoclaved distilled water prior to inoculation of the explants in the culture medium. In place of $HgCl_2$, 40% diluted H_2O_2 (v/v) for 10 min was used as a surface sterilant by Karataş and Aasim (2014). Then, they were washed with double distilled water for 5 min by continuous stirring. Some researchers also used 70% alcohol for surface decontamination (Ceasar et al. 2010; Kalita et al. 2018).

Media and plant growth regulators (PGRs)

Plant tissue culture medium is most important for plant growth in vitro and basal medium, in the same way, is fortified with some necessary nutrients like carbohydrates, vitamins, minerals, and many additives for the proper growth of the plant. Murashige and Skoog (MS) medium, B5 (Greenway et al. 2012), Linsamaier and Skoog (LS), and Schenk & Hildebrandt (SH) are the most used media for in vitro culture of the plants. For *B. monnieri*, MS medium of full strength has been proven as the most suitable culture medium but several works like shoot multiplication and bacoside production have been observed in Gamborg's B5 medium (Koul and Mallubhotla 2020). Plant growth regulators (PGRs) are some chemical compounds that regulate plant's growth and development in many ways by promoting or inhibiting them. In plant tissue cultures, many PGRs are supplemented to the medium for seed germination, promoting elongation or differentiation of cells etc. MS medium fortified with different concentrations of PGRs like auxins, cytokinins, etc. in different combinations has shown to produce multiple shoots and buds. Using nodes of *B. monnieri* as explants, Sanputawong et al. (2021) conducted a study where MS medium was supplemented with 0–1 mg/L napthalene acetic acid (NAA) and 0–2 mg/L 6-benzyladenine (BA). These combinations of PGRs have produced good callus growth and shoot amplification. BA and thidiazuron (TDZ) (0.5 mg/L and 0.25 mg/L, respectively) have also been shown to enhance the bacoside production in the cell suspension cultures (Kharde et al. 2018). MS basal medium of half strength, supplemented with 0.5 mg/L benzyl amino purine (BAP) and 1 mg/L indole-3-butyric acid (IBA), was found to provide better results in terms of induction of longer shoots and roots, respectively, in greater numbers (Ceasar et al. 2010). For micropropagation of *B. monnieri*, MS medium supplemented with gibberellin A_3 was found as one of the most suited PGRs with different combinations of NAA, 2–4-D, kinetin (kin), BAP, etc. for callus induction, multiple shoot formation, and root growth (Murthy et al. 2019). In addition, 100 ml/L of banana extract and 100 ml/L of coconut water were added as the sources of PGRs in the MS basal medium which showed maximum numbers of rooting from the regenerated shoots (Soundararajan and Karrunakaran 2011). In another study, *Gracilaria salicornia* extracts were used as a source of PGR for *B. monnieri* in vitro propagation as 25% of this extract had shown 82.2% root induction and 60% of this extract had shown 85.9% shoot induction after induction to the medium (Rency et al. 2017). Figure 2C presents the micropropagation in *B. monnieri* (unpublished photograph of Dr. Dey).

Carbon source

Carbohydrates are one of the main sources of carbon for plants as it controls the developmental patterns, absorption of energy, etc. (Dey et al. 2020). Sucrose is usually found to be the best carbon source in the culture media among all the carbohydrates which can be substituted by galactose, lactose, mannose, melibiose, and cellobiose in the culture (Srivastava et al. 2017). However, sucrose is the main carbohydrate to help in the translocation of phloem sap (Fink et al. 2018). Even 2% sucrose supplementation in the MS medium increased the shoot-biomass and enhanced bacoside A content in the regenerated shoots (Naik et al. 2010). Besides, 20 g/L sucrose with 7 g/L agar, supplemented to MS medium, was found to be best suited for induction of roots (Ranjan et al. 2018). Supplementation of 250 mM sucrose enhanced the somatic embryogenesis up to 70% probably via supplying the necessary energy needed to form somatic embryos (Saha et al. 2020). Glucose as well increased the biomass as well as bacoside A accumulation in hairy roots (Bansal et al. 2015). Sucrose, sorbitol, maltose, fructose, and glucose in different combinations in different media were also supplemented for shoot regeneration, bacoside, and biomass production. In one study, it was suggested to check every sugar combination for their suability in obtaining the best results in the propagation of plants in vitro (Naik et al. 2017).

Nitrogen source

Nitrogen has a plethora of roles in plant growth and development in in vitro conditions. Nitrogen has been reported to influence cell differentiation and to enhance the totipotency of the cell. A nitrogen source is not always required to be added exogenously to the culture medium but for some special advantage, it may be applied (Kovalchuk et al. 2018). Double strength of NH_4NO_3 added to the MS medium produced maximum and efficient yield of bacoside A. Even this supplemented media produced biomass and shoots in greater numbers (Naik et al. 2011). Potassium nitrate (KNO_3) was also found to be effective as a nitrogen source for bacoside A production and for obtaining increased biomass (Bansal et al. 2015). Nitrogen is the main component of amino acids, and sometimes amino acids like L-asparagine, L-glutamate, adenine, etc. are added to the culture media as organic nitrogen sources. In an experiment on biomass production in *B.*

monnieri, they added 0.1 g/L l-tryptophan; 0.5 g/L and 0.25 g/L serine were added in the MS medium for better response (Muszyńska et al. 2016).

Additives

The beneficial nutritive chemicals, those act as chelators or pH controlling buffer systems to improve the production rate, micro-salts availability to the plant part used as explant by providing sufficient nutrients, are known as additives (Dey et al. 2020). Most of the time, agar is used as an additive to solidify the medium for better rooting and shoot regeneration (Showkat et al. 2010). In many studies, casein hydrolysate was used to supplement the liquid MS medium for suspension culture to produce maximum bacoside (Kharde et al. 2018). Methyl jasmonate was also found to be effective in bacoside A production, as an additive in in vitro raised shoots of *B. monnieri*. In addition, salicylic acid (SA) and pyruvic acid (PA) were applied as additives for bacoside production (Koul and Mallbhotla 2020; Parale et al. 2010). Zinc oxide nano-particles were also added in the suspension cultures for better plant growth (Bhardwaj et al. 2018). Magnesium sulfate, zinc hydroaspartate, and anthranilic acid have produced increased biomass following being supplemented in the media for *B. monnieri* shoot cultures (Lojewski et al. 2014).

In vitro *propagation*

In vitro propagation or micropropagation is a technique used for the vegetative growth and multiplication of plants from tissues or seeds in aseptic and controlled conditions on artificial growth media. Micropropagation is usually executed in two ways: direct and indirect. The indirect process involves callus development from explant followed by shoot and root formation, while the direct process involves shooting and rooting on the explant. With its rapidity and limitless potential, plant tissue culture offers novel opportunities to deal with various shortcomings in the areas of medicinal plant cultivation, conservation, and exploitation. Some of the exciting applications are exploiting genetic engineering and somaclonal variation for crop improvement, fast micropropagation to produce quality plants, in vitro conservation and germplasm exchange and production of secondary metabolites, in vitro selection for resistance to biotic and abiotic stress, etc. A great deal of medicinal plants are not the producer of seeds, or the seeds are too minute to be germinated in soil. Thus, mass-scale propagation of disease-free plants is a common problem. In this situation, the plant tissue culture technique presents a remarkable potential for fast and true-to-type mass scale propagation of the plants in disease-free conditions.

The ability of rapid multiplication into true-to-type plants and efficiency in *B. monnieri* transplantation can be employed in conservation strategies and mass propagation of the plant for commercial use. *B. monnieri* has an upright market demand owing to the medicinal attributes it possesses. In India, the estimated consumption of this plant is approximately 1000 tons/year (Kharde et al. 2017). The annual demand of *B. monnieri* during the years 2004 to 2005 was 6621.8 tons with an annual growth rate of 7% according to the National Medicinal Plants Board (NMPB). This demand is growing rapidly with the growing popularity of drugs consist *B. monnieri* (Sharma et al. 2010). Recently, NMPB and Technology Information Forecasting and Assessment Council (TIFAC) have identified this plant among the seven prime medico-botanicals suggested for prompt recognition to be provided and are indexed in the list of highly endangered Indian medicinal plants (Tripathi et al. 2012). Micropropagated plants grow rapidly and mature early compared to the progenies propagated via seeds. Plants generated from tissue culture can multiply through the increased production of axillary and/or adventitious shoots either by direct or indirect organogenesis followed by generation of roots and also by somatic embryogenesis.

Callus induction

A callus is an undifferentiated and unorganized mass of cells produced from plant tissue (explant) cultured on an appropriate medium supplemented with PGRs. Callus cultures are a source of tissues for plant regeneration, chromosomal variation (somaclonal variation), secondary metabolite production, and cell suspension culture. Apical shoot tip, leaf, embryo, stem, nucellus, germinating grains, stamen, root, basal plate meristem, etc. can be used as explants for the inception of callus cultures. The cells of explants divide continuously to give rise to a soft, irregular shaped callus. In most cases, the explant produces callus within 3 to 8 days of incubation (Jat et al. 2016). Callus formation and its subsequent regeneration are the main steps in *in vitro* propagation of plants. From the shoot tip explant of *B. monnieri*, callus was induced in MS media fortified individually with various auxins (NAA, IAA, and 2,4-D). Callus development was recorded the highest in media with 2,4-D (2 mg/L) and moderate in media treated with IAA and NAA (Talukdar 2014). Soft, yellowish green to brownish callus tissues were obtained from the leaf explants on MS media supplemented with 0.5 mg/L 2,4-D (Showkat et al. 2010). MS media composed of various concentrations and combinations of PGRs were used for callus formation of which combination of 6 BA (2 ppm) and IAA (1 ppm) were the most useful for the formation of soft, yellowish-green calli in 15 days (Ahmed et al. 2014). In callus initiation from leaf explant, the best result was obtained when 0.5 mg/L NAA and 0.5 mg/LBAP

were added to the MS basal media resulting in 75% callus formation. In addition, for the nodal segment as explants, 0.5 mg/L IAA and 4.0 mg/L BAP added to MS media supported 85% callus formation (Ranjan et al. 2018). Hegazi et al. (2017) recorded that 9 μM 2,4-D and 2.3 μM kin added to MS medium produced the best callus initiation in *B. monnieri*. In addition, it generated an increased percentage of fresh weight and yellowish, white friable callus. Ali et al. (2021) obtained maximum callus generation from the leaf explants with NAA (2.5 mg − 1) showing 94.22% generation rate accompanied via 2,4-D (2.5 mg − 1) showing 82.43% generation rate; in the case of nodal explants, the highest callus formation was detected with 2,4-D (2.5 mg − 1) showing 71.14% generation rate followed by NAA (2.5 mg − 1) showing 62.15%. In intermodal explants, most of the calli formation was noted in the presence of 2,4-D (2.5 mg − 1) showing 65.21% generation rate followed by NAA (2.5 mg − 1) showing 52.14% generation rate. Samanta et al. (2019) in their experiment observed that when BAP (5 mg/L) was supplemented in MS media following 60 days of culture, callus formation and potent growth were achieved. Hence, they concluded that BAP (5 mg/L) needed more time to initiate callus formation. During in vitro propagation, leaf segments were chosen mostly over the internodal segments as explant sources for callus formation because of the presence of soft tissue, no woody structure, and broad surface area (Dey et al. 2020). High concentrations of phenolic compounds are often correlated with the superior antioxidant capacity of the plant (Aras et al. 2018; Silinsin and Bursal 2018). Many phenolics such as quinic acid, p-coumaric acid, and malic acid were found to be present in the plant extracts (Bursal et al. 2019). Owing to the presence of high phenolics in *B. monnieri*, the callus culture often became brown and to prevent this browning; different PGRs (2,4-D, NAA, IBA, and BAP) at different concentrations were used by Meenashree et al. (2017), out of which NAA produced healthy callus without any browning. Lowering the amount of nitrate source and incorporation of ascorbic acid (100 mg/L) in media also helped in attenuating the browning of callus. Dogan (2020) reported that the percentage of callus induction and intensities of callus growth from nodal explants reduced with an increment in NaCl concentrations (salt stress). In addition, browning, yellowing, or even deaths of the callus were observed due to salt toxicity.

Shoot organogenesis

Shoot multiplication from a single explant is one of the prime highlights for micropropagation, germplasm conservation, and biomass production. Many researchers recommended the potency of nodal explant to produce multiple shoots (Dey et al. 2020). Shoot organogenesis can directly take place on the isolated explant such as leaf and stem via direct organogenesis or can be found only following callus generation by indirect organogenesis. The concentration of cytokinins and auxins in the culture medium is a significant factor affecting the degree of multiplication. The cytokinin signaling pathway exhibits a potential target for controlling de novo shoot organogenesis and in vitro plant regeneration. The two fundamental types of cytokinins utilized in plant tissue culture are BAP and kin. Binita et al. (2005) reported solid media with more potential for bud proliferation from the leaf whereas the liquid medium was suggested to be more effective for bud proliferation from axillary nodes and internodes. Saha et al. (2020) reported enhanced direct shoot organogenesis from the leaf and internodal explants without the supply of exogenous PGRs, and the induction rate was increased when exogenous cytokinins and some additives were used. Direct shoot organogenesis was obtained in a culture medium containing a combination of BAP (17.80 μM) and IAA (2.28 μM) producing maximum shoot initiation (85.2) with larger shoot production (Mahender et al. 2012). MS medium fortified with 0.25 mg/L BA + 0.25 mg/L NAA showed the maximum number of shoots per explant compared to the medium with other combinations of BA + NAA. Shoot regeneration is inhibited with a higher concentration of NAA in combination with all concentrations of BA. The shoots developed from the leaf explants were comparatively longer than those from the other explants (Karatas et al. 2013). When the swollen nodes were sub-cultured either on MS medium or MS media supplemented with 1.0 mg L-1 GA3, the highest shoot proliferation (114.2 shoots/ node) with an average shoot length of 6.4 cm was noted. Chauhan and Shirkot (2020) in their experiment observed that MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L kin showed the best in vitro shoot multiplication. The shoots produced from MS medium containing 0.5 mg/L BAP were reported long and healthy and on increasing the BAP concentration to 2.0 mg/L, the rate of shoot multiplication declined. Various concentrations of different carbon sources (glucose, sucrose, and mannitol) were evaluated to find out the best response in regeneration events, among which 5% sucrose in MS media was recorded to be the most useful for shoot generation (22.6 shoots/explant), and in case of leaf explant, 3% sucrose was found more effective (20.6 shoots/explant) (Srivastava et al. 2017). Similarly, it was noted that the medium with 2% sucrose and 4.5 pH demonstrated increased shoot biomass up to 150.50 shoots/explant, fresh wt. 6.31 g, and dry wt. 250 mg (Naik et al. 2010). In a study on the effect of different concentrations of NaCl on *B. monnieri*, it was observed that in shoot, the Na^+ content was enhanced with a rise in NaCl level in the medium, and both K^+ and Ca^{2+} levels decreased in the shoot, and as a result, a remarkable reduction was recorded in shoot number/culture, fresh and dry weights, shoot length, and water content in the tissues (Ahire et al. 2013).

Root organogenesis

In plant tissue culture, root organogenesis is primarily influenced by different types and concentrations of sucrose, minerals, PGRs, etc. in the media and other factors in a culture like pH, temperature, etc. Rooting is especially essential and is considered as the ultimate achievement for micropropagation (Chen et al. 2014). Best root induction (almost 80%), reported by Ranjan et al. (2018), was achieved on the MS media enriched with 7 g/L agar and 20 g/L sucrose. Combinations of 20 g/L sucrose, 30 g/L fructose, and 40 g/L glucose (all as carbohydrate sources), supplemented in liquid and solid MS medium, using a stem of *B. monnieri* with three nodes as an explant have also been recorded to produce a maximum number (23) of roots using a horizontal culture method (Ahmed et al. 2014). In addition to this, selective shoot rooting medium (SSRM, MS medium fortified with 4.9 μM IBA + 25 mg/L hygromycin) induced rooting from the shoots, resistant to hygromycin (Mahender et al. 2012). In another study, 0.5 mg/L phloroglucinol on MS medium of half strength with 1 mg/L IBA gave the best outcome for root organogenesis, in terms of length (8.7 cm) and numbers (16.5) (Ceasar et al. 2010). In vitro regeneration of *B. monnieri* has also shown the best combination of 1 mg/L of BAP and 3 mg/L of IAA for root induction (Gurnani et al. 2012). Some experiments have shown better rooting of regenerated shoots from the 1/2 MS media with 0.5 mg/L NAA, 1% sucrose, and 2% jiggery (Bhusari et al. 2013). An evaluation on the impact of some polyamines (PAs) in *in vitro* propagation of *B. monnieri* was conducted where the MS media with 1 mg/L IBA and 1 mM spermine has produced the highest number of roots from the regenerated shoots (Dey et al. 2019). Croom et al. (2016) found transverse thin cell layers (tTcl) of leaf and stem as explants producing 100% roots in the MS medium fortified with 5 μM IBA, using a liquid lab rocker (LLR) box. An investigation on the impact of abiotic stress using NaCl and polyethylene glycol (PEG) in in vitro raised *B. monnieri* has shown a good response in root formation on the solid/liquid MS medium supplemented with 0.8 mg/L NaCl whereas 26 g/L PEG and enhanced concentrations of NaCl have shown to decrease the development of roots (Hussien et al. 2017). In another study, 60 ml extracts of Cyanobacteria, *Aulosira fertillissima*, with the MS liquid medium of 40 ml have produced superior rooting response (Banerjee and Modi 2010).

Somatic embryogenesis

Somatic embryogenesis is a tool of regeneration or developmental pathway which forms the non-zygotic cell devoid of vascular connection with the original tissue. These non-zygotic embryos are formed from a single or grouped somatic cell. The rate of germination in somatic embryogenesis is very high (80%-85%). This process goes through different stages which are globular stage (small globose or spherical structure), heart shape stage (three-lobed structure with pale yellow color), and torpedo stage (elongated heart shape with pale yellow color) (Samanta et al. 2019). Many researchers reported various types of culture media for growing somatic embryos of *B. monnieri*. Other researchers recorded that the somatic embryos were developed in high frequency in B5 medium fortified with 2,4-D (0.25 and 0.5 mg/L) alone or combined with BAP (0.5 mg/L). The concentration of 2,4-D (1.0 mg/L) in the B5 medium was found to be important for the growth of somatic embryos. When 2,4-D was present at low concentration in B5 medium, the embryos grew in high frequency, and absence of 2,4-D in medium, no embryos were formed. However, somatic embryos failed to germinate in the specific media where only 2,4-D was present. BAP (0.5 mg/L) was found to be essential for the maturation of somatic embryos. PGR-fortified MS medium was reported ineffective for embryo growth (Jain et al. 2010). Parale and Sangle (2020) reported that with the decreasing concentration of 2,4-D or kin in the media, the number of embryos also decreased. BA and 2,4-D influenced somatic embryogenesis from leaf explants of *B. monnieri*. The developmental frequency of somatic embryos depended on the concentration of PGRs. MS media supplemented with BA (12.5 μM) and 2,4-D (1.0 μM) produced somatic embryos in a maximum frequency of 47.1%. When the 2,4-D concentration was increased in the medium, the frequency of explant producing somatic embryo decreased. Somatic embryogenesis was also influenced by the concentration of sucrose. Sucrose (250 mM) containing media helped to produce somatic embryos in the highest frequency (77%) as observed by Khilwani et al. (2016). Parale and Sangle (2020) cultured calli on the full strength of MS medium with 1.5%, 3%, or 4% sucrose and half strength of MS media with 1.5%, 3%, or 4% sucrose without PGRs. The full strength of MS media with a low concentration of sucrose produced the embryo, but when the concentration of sucrose was high (4%), the somatic embryos were not found. Ali et al. (2021) stated that a mixture of 2,4-D (2.0 mg/L) and kin (1.5 mg/L) was useful for somatic embryogenesis in maximum frequency (84%). Embryoid differentiation took place in MS media with 0.5 mg/L 2,4-D. MS media with or without BA were also used for further development of these embryoids (Saha et al. 2020).

Hardening and acclimatization

In vitro raised plantlets when pass through the process of acclimatization (hardening) show a higher rate of survival and vigorous growth when transferred to the soil

(Castañeda-Méndez et al. 2017). The in vitro generated plants are directly hardened in the greenhouse stage. Initially, water was needed to be sprayed five times a day at four-hour intervals to maintain high humid conditions (Ranjan et al. 2018). After root development from plantlets (3 cm in length) in culture vessels, the roots were washed properly, and a sticky semi-solid agar medium was removed carefully from roots under running tap water (Mehta 2017). Most of the reports indicated that the plantlets were treated with 0.1% Bavistin® for 10 min during hardening to protect the fungal attack. In addition, it was recommended to transfer these treated plantlets directly to the plastic pot which contained different mixtures of sterilized soil with various combinations such as soil mixed with either vascular arbuscular mycorrhizae (VAM), or farmyard manure, or fly ash, or vermicompost, or agropeat (Sharma et al. 2018). Various kinds of soil mixtures with different ratios were found to be used such as soil and organic manure (2:1); soil and vermicompost (3:1) (Haque and Ghosh 2013); soil and vermiculite (50:50) (Chaudhry et al. 2019); soil and soilrite (1:1) (Showkat et al. 2010); sand, soil, and farmyard manure (1:1:1) (Binita et al. 2005); and sand, soil, coco peat, and farmyard manure (1:1:1:1) (Ranjan et al. 2018). In a few studies, the plantlets were kept for 2 weeks (or 10–15 days) in the culture room following transformation from in vitro to plastic pots (Sharma et al. 2010). In a few reports, plantlets were shifted to 25 °C–30 °C temperature and 80%-90% relative humidity and under a photoperiod of 16 h for acclimatization (Sharma et al. 2010). According to an experiment by Chauhan and Shirkot (2020), the survival rate was found to be 80% when the plantlets were kept in coco peat following six weeks of hardening. In sand and soil, the survival rate was recorded to be 42% or 68% after six weeks of plantlets transfer. Moreover, 48% survival rate of the plantlets was noted in the mixture of coco peat and sand after six weeks of transfer to this mixture. The hardened plantlets were also transferred to a mixture of soil, sand, and farm yard manure (FYM) in 1:1:1 ratio, in a small pot covered with a glass jar for one week. After removing this glass jar, the pot was transferred to the glass house, and the growth rate of plantlets was observed for 2, 4, 6, and 8 weeks. The height of the hardened plantlets after the second week was recorded to be 2.96 cm with an average growth of the leaves that was recorded as 19.50 per plant. Following the 4th week, the plantlets reached a height of 4.22 cm, and the number of leaves were recorded as 25.50 per plant; the height of the plantlets increased to 5.18 cm as well as the number of leaves was increased to 31.00 per plant after the 6th week; after 8th week, the survived plantlets reached to 6.08 cm of height with 39.00 leaves per plant (Chauhan and Shirkot 2020). Finally, the plantlets were successfully transferred to the net house (under shade) following acclimatization for further growth (Srivastava et al. 2017). After acclimatization, 93% of *B. monnieri* plantlets survived in the natural environment (Hegazi 2016). Sharma et al. (2017a) reported 90% survival rate whereas Mehta (2017) recorded a 100% survival rate for the *B. monnieri* plantlets.

In vitro *production of secondary metabolites*

B. monnieri houses its prime secondary metabolites as the dammarane type triterpenoid saponins which showed anti-oxidant, anti-amnestic, and nootropic effects (Dey et al. 2020). Among these, the most profound saponin is bacoside, and the other saponins recorded are bacopaside I-XII, including bacopasaponin F, bacopasaponin D, bacopasaponin C, bacopaside V, bacopaside II, etc. (Majumdar et al. 2011). Besides saponins, alkaloids like brahmin, herpestine, and nicotine (Lala. 2020) and flavonoids like luteolin, luteolin-7-glucoside, glucoronyl-7-lutoelin, glucoronyl-7-epigenin, etc. have also been recorded from the plant (Jat et al., 2016). Glucose (5.67%), KNO_3 (0.313%), and KH_2PO_4 (0.29%) with 0.66% inoculum density in cells cultured in MS medium demonstrated an enhanced rate of bacoside A production (Bansal et al. 2017). Another in vitro study assessed the bacoside A concentration in the shoots of *B. monnieri* cultured in MS medium supplemented with 2 mg/L kin. An addition of cytokinin resulted in a higher accumulation of bacoside A in the regenerated shoots in the liquid medium (Praveen et al. 2009). In another study, regenerated adventitious shoots produced the maximum amount of bacoside A in the medium fortified with 0.20 mM copper (Cu) and 2% sucrose at 4.5 pH (Naik et al. 2010, 2015). In addition, organic supplements like SA and PA added to the shoot cultures have shown an increase in the bacoside A accumulation in the shoots (Saha et al. 2020). Methyl jasmonate (MJ) has also been noted to promote the production of bacoside A in the shoot cultures of *B. monnieri* (Sharma et al. 2013). MJ (50 μM) combined with SA (50 μM) enhanced the accumulation of bacoside A besides producing bacoside A_3, bacopaside II, and bacopasaponon C (Largia et al. 2015). Colchicine (0.1%) treatment only for 2 h also enhanced the bacoside concentration in the regenerated plants (Kharde et al. 2017). In an *Agrobacterium rhizogenes*-transformed *B. monnieri* plant, the regenerated hairy shoots produced a higher amount of bacoside A (Largia et al. 2016). Combinations of some rhizospheric microorganisms like *Glomus intraradices*, *Trichoderma harzianum*, and *Bacillus megaterium*, using a method called Fourier Transform Near-Infrared (FTNIR), have shown to enhance the production of bacoside A in *B. monnieri* (Gupta et al. 2015). The bacoside A content was recorded higher during February to May under stress conditions which was implicated in the in vitro production of the compound under stress. Balloon type bubble bioreactor and glass bottle bioreactor have also been employed, and the former was found to be more

preferred for bacoside production (Sharma et al. 2019). Dey et al. (2020) recorded High Performance Thin Layer Chromatography (HPTLC) as a potent technique to assess bacoside A content and also compared the compound among in vitro and nature grown plantlets.

Use of molecular markers

High survival rate and genetic stability were found using molecular markers (random amplified polymorphic DNA (RAPD) (OPC-15) and inter simple sequence repeat (ISSR) (UBC-808)) on *B. monnieri* shoots conserved for 12 months (Sharma et al. 2016). RAPD studies also revealed no notable reproducible variation among the control sets and the in vitro-cryopreserved *B. monnieri* plants (Sharma et al. 2017b). In another study, 20 ISSR primers generated 130 clear and reproducible amplicons, with 125 bands showing monomorphism. In addition, 25 RAPD markers showed 115 bands with 94% of these being monomorphic following 6 months of storage (Muthiah et al. 2013). Clonal fidelity of the regenerated *B. monnieri* was investigated using ISSR and RAPD markers that also revealed high monomorphism (90%) between the mother plant and in vitro regenerated plantlets (Dey et al. 2019).

In order to determine the diversity in the wild populations, about 35% variations were found using RAPD and ISSR. However, ISSR markers showed higher variation (44.9%) than the RAPD markers (23%) (Bansal et al. 2014a). RAPD and ISSR analysis also revealed genetic diversity in 15 *B. monnieri* accessions from central India which was reported necessary to be used for their conservation as well as for breeding (Tripathi et al. 2012).

Table 1 In vitro shoot and root formation in *B. monnieri* via direct organogenesis

Explants	Culture conditions	Basal medium	Additives for root induction	Additives for shoot formation	Reference
Stem with three nodes	-	Liquid and solid MS	20 g/L sucrose, 30 g/L fructose, 40 g/L glucose	20 g/L glucose	Ahmed et al. (2016)
Nodal and leaf	-	MS	20 g/L sucrose and 7 g/L agar	1.0 mg/L BAP and 0.5 mg/L NAA	Ranjan et al. (2017)
Leaf	-	MS	-	3% sucrose, 4 mg/L BAP, 0.1 mg/L NAA, and 4 g/L Gelrite®	Thi et al. (2012)
Leaf and internode	-	½ MS	1.0 mg/L IBA and 0.5 mg/L phloroglucinol	-	Ceasar et al. (2010)
Axillary node	-	Liquid MS	60 ml *Aulosira* extracts, 1 mg/L kin (15 roots of 4 cm)	60 ml *Aulosira* extracts, 1 mg/L kin	Banerjee and Modi (2011)
Shoot tips	-	MS	1.5 mg/L IAA, 0.5 mg/L IBA	1 mg/L meta-topolin (mT)	Pramanik et al. (2021)
Leaf	-	MS	17.80 µM BAP and 2.28 µM IAA	25 mg/L hygromycin and 4.9 µM IBA	Mahender et al. (2012)
Axillary nodes and internodes	-	Liquid MS	-	0.2 µM IAA, 1.1 µM BA	Binita et al. (2005)
Microshoots/leaf or internode	50–60% humidity; 25 ± 1 °C; 16 h PP; PFD 40 µmol/S/m^2	MS	3% sucrose, 0.75% agar	-	Sarkar and Jha (2017)
Axillary nodes, young leaves, shoot tips	-	50% MS	1% sucrose, 2% jaggery, 0.5 mg/L NAA	20 g/L jaggery, 5 g/L agar, 10 g/L sucrose, 3 mg/L BAP, and 0.5 mg/L IBA	Bhusari et al. (2013)
Leaf	-	MS	-	500 µg/mL of carbenicillin or cefotaxime	Aggarwal et al. (2013)
Young shoots	-	MS	3% sucrose, 0.8% agar, 1 mg/L IBA, 1 mM spermine	2 mg/L BAP, 0.5 mg/L TDZ, 0.5 mg/L kin, 0.5 and 1 mM spermine, spermidine, putrescine	Dey et al. (2019)

Table 2 In vitro callus induction and somatic embryogenesis in *B. monnieri*

Explants	Basal medium	Additives	Response	Reference
Shoot tip	MS	2 mg/L 2,4-D	Callus development in higher percentage	Talukdar (2014)
Leaf from microshoots	MS	12.5 µM BA, 1 µM 2,4-D	Formation of somatic embryos	Khilwani et al. (2016)
Leaf	MS	2 ppm BA, 1 ppm IAA	Successful induction of soft yellowish-green callus	Ahmed et al. (2014)
Leaf petiole	MS	0.5 mg/L kin and 0.25 mg/L 2,4-D; 0.25 mg/L 2,4-D; and 0.1 mg/L BAP	Best callus induction	Mehta et al. (2012)
Leaf and stem	MS	2 µM BA, 0.2% Gelrite®, 30 g/L sucrose, 0.7% agar, 6 and 8 µM NAA	Callus induction	Shrivasatava and Rajani (1999)
Apical or axillary bud, internode, and leaves	MS	1 mg/L 2,4-D; 0.2 mg/L kinetin	Embryo maturation and production of plantlet from it	Samanta et al. (2019)
Leaf	MS	16×10^3 of silver nanoparticles	Medium callus growth	Priya et al. (2014)
Internode and leaf	MS	3% sucrose; 0.65% agar; 0.25, 0.50, and 1 mg/L of BA and NAA	Callus induction	Karatas et al. (2013)
Leaf	B5 medium	0.8% agar; 0.25 and 0.5 mg/L 2,4-D; and 0.5 mg/L BAP	Somatic embryogenesis at high frequency	Jain et al. (2010)
Leaf	½ or full strength MS	20 µM 2,4-D and 20 µM kin, 3% sucrose, 0.8% agar	Somatic embryo induction	Parale and Sangle (2020)
Young nodes	MS	1 mg/L IBA and 1 mg/L IAA, 3% sucrose, 0.65% agar	Callus formation	Showkat et al. (2010)
Nodes and leaves	MS	2 mg/L 2,4-D; 1.5 mg/L kin	Maximum (84%) induction and formation of somatic embryogenic calli	Ali et al. (2021)

Application of transgenics

Earlier, *Agrobacterium tumefaciens* strain EHA105 harboring the binary vector pBE2113 with the genes for GUS and neomycin phosphotransferase was used to transform *B. monnieri* plants. It showed 60% transformation frequency and took two months for regeneration of the transgenics from the leaf explants (Nisha et al. 2003). In another study, *A. tumefaciens* LBA4404 harboring pCAMBIA1301 containing ß-glucuronidase (*uidA*) and hygromycin phosphotransferase (*hpt*) genes demonstrated fast and efficacious shoot organogenesis in vitro and transgenic plant production in *B. monnieri* (Mahender et al. 2012). *B. monnieri* when transformed using three strains of *A. tumefaciens* such as LBA4404, EHA105, and GV3101 containing expression vector pCAMBIA2301 with β-glucuronidase (GUS), no remarkable variation in the transformation efficiency among the three strains was noted (Yadav et al. 2014). Effective shoot regeneration (87.5%) and genetic transformation (82.5%) of *B. monnieri* were achieved by *A. tumefaciens*-mediated transformation confirmed via GUS assay and PCR mediated detection of *hpt*II gene (Kumari et al. 2015). In another report, factors influencing genetic-transformation and shoot-organogenesis in *B. monnieri* were also standardized (Aggarwal et al. 2013). In another study, a quick regenerating *A. tumefaciens*-mediated transformation method for *B. monnieri* (L.) was achieved as a heterologous expression model of *Catharanthus roseus* derived terpenoid indole alkaloid producing genes (tryptophan decarboxylase and strictosidine synthase) normally absent in *B. monnieri* (Sharma et al. 2017c). *B. monnieri* transformed with *Agrobacterium rhizogenes* strains LBA 9402 and A4 stimulated bacopa saponins synthesis in transformed calli and as well as in plants which was attributed to the endogenous elicitation mediated by *A. rhizogenes* Ri T-DNA (Majumdar et al 2011). Further, Ri *crypt*-transformed (encoding proteinaceous elicitor cryptogein) *B. monnieri* showed a notably higher accumulation of bacoside A_3 (Majumdar et al. 2012). The crypt-transformed *B. monnieri* kept long-term, exhibited notably higher bacoside content in vitro (1.66- to 2.05-fold higher than the non-transformed ones) (Paul et al. 2015). *B. monnieri*, genetically transformed with using different *A. rhizogenes* strains (viz. A4, R1000, SA79, MTCC 532, and MTCC 2364), displayed higher production of hairy root biomass and higher accumulation of bacoside A (except A4 strain) compared to the non-transformed lines (Bansal et al. 2014b). *A. rhizogenes* (A4 and MTCC 532 strains)-derived hairy roots exhibited the highest regrowth frequency. Moreover, a high biomass producing

Table 3 In vitro production and enhancement of bacosides using elicitors in *B. monnieri*

Strategies	Explants	Media	Elicitors	Reference
Cell suspension culture	Shoot	MS + 2.5 µM BA	Glucose (5.67%), KNO_3 (0.313%), and KH_2PO_4 (0.29%)	Bansal et al. (2017)
In vitro regeneration	Leaf	Semi-solid and liquid MS	2 mg/L kin	Praveen et al. (2009)
Plant cell or organ culture	Leaf	Liquid MS + 5 µM BA	100 µM pyruvic acid	Parale et al. (2010)
In vitro propagation	Stem (internodes)	MS + 0.1 mg/L TDZ	150 mg/L chitosan, 2 mg/mL yeast extracts	Kamonwannasit et al. (2008)
In vitro propagation	Leaf	MS + 2 mg/L kin	0.20 mM copper, 2% sucrose	Naik et al. (2015)
Hairy root cultures	Leaf	Liquid MS + 1 mg/L BAP, 0.1 mg/L NAA, 3% sucrose, and 0.8% agar	10 mg/L chitosan	Largia et al. (2016)
In vitro shoot cultures	Shoot	MS	45 mg/L $CuSO_4$	Roy et al. (2017); Sharma et al. (2015)
Suspension cultures	Leaf	B5 + 1 mg/L 2,4-D	1 mg/L salicylic acid	Koul and Mallubhotla (2020)
In vitro liquid shoot cultures	Shoot with 5–6 nodes	Liquid MS + 4.44 µM BAP, 0.54 µM NAA	50 µM methyl jasmonate, 50 µM salicylic acid	Largia et al. (2015)
In vitro propagation	Leaf	MS + 3% sucrose, 1.1 µM BA, 0.30 µM IBA	1% colchicine	Kharde et al. (2017)
Cell suspension culture	Leaf	Liquid MS + 0.4 mg/L 2,4-D	0.5 mg/L BA and kin, 0.25 mg/L TDZ	Kharde et al. (2018)
In vitro propagation	Stem with leaves	Liquid MS + myo- inositol, nicotinic acid, 4 mL/L vitamin B1, 1 mg/L BAP, 0.2 mg/L NAA	0.5 mg/L anthranilic acid	Lojewski et al. (2014)
In vitro propagation	Leaf and internode	MS basal salt + 3% sucrose	NaCl, $CuSO_4$	Roy et al. (2017)
In vitro propagation	Leaf	MS + 9 µM 2,4-D; 2.3 µM kin	100 mg/L chitosan and 10 mM mevanolic acid	Hegazi et al. (2017)
In vitro propagation	Stem segments and leaf	Liquid MS + 5 µM BA	750 mg/L *Saccharomyces cerevisiae* or *Mucor* sp. derived biotic elicitors	Prakash and Dayaram (2009)
Cell suspension culture	Leaf, node, internode	MS + 1 mg/L different auxins	0.75 and 1 ppm zinc oxide nanoparticles	Bharadwaj et al. (2018)
In vitro shoot regeneration	Adventitious shoots	MS + 2 mg/L kin	$2 \times NH_4NO_3$	Naik et al. (2011)

line, upon elicitation, produced 5.83% of bacoside A five and three-times more than the untransformed and transformed non-elicited control sets, respectively (Largia et al. 2016). In addition, the overexpressing *Sorghum bicolor*–derived vacuolar proton pyrophosphatase gene (*SbVPPase*) attenuated salt stress in transgenic *B. monnieri* transgenics produced by *A. tumefaciens*-mediated transformation (Ahire et al. 2018). In another report, insertion of *rol* genes modulated the morphogenic potential in transgenic *B. monnieri* derived from *A. tumefaciens*-mediated transformation (Sarkar and Jha 2021).

Conclusions

B. monnieri is considered as a potent medicinal plant containing an array of phytochemicals viz. alkaloids, flavonoids, saponins, and glycosides. The plant possesses several pharmacological activities attributed to its bioactive compounds especially bacoside A. *B. monnieri* is commercially important due to its presence in various herbal formulations used against neurological disorders. This plant has always been a subject of interest to a myriad of researchers. A substantial number of studies have been conducted on this plant focusing on its tissue culture and biotechnology to propagate the plant in vitro and also for sustainable and stable production of its phyto-constituents. The present review provides a comprehensive account of its in vitro propagation studies such as callus induction, root and shoot organogenesis, somatic embryogenesis, and secondary metabolite production (Tables 1, 2, and 3). Besides, insights in the molecular marker-based studies revealed the clonal fidelity among the natural and in vitro generated plantlets. *Agrobacterium*-mediated transformation system was mostly used to optimize bacoside production, biomass yield, and heterologous expression of secondary metabolite producing genes. The regenerated plants can be used as a continual provision for

uniform raw materials for commercial production of secondary metabolites which in turn will minimize the pressure on the natural populations and hence can be indirectly useful for conservation.

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Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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