Biotechnological interventions in banana: current knowledge and future prospects

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ABSTRACT

Banana is an important food crop responsible for ensuring food security, nutrition, and employment for a significant portion of the world population. It has fairly broad genetic diversity and is distributed widely across the globe. Due to its socio-economic importance, there has been growing demand for healthy and improved planting materials of banana. In recent years many companies and organizations are working hard to narrow down the gap between demand and supply of quality planting materials. The other challenges include its susceptibility to adverse environmental conditions, attack of various pests/pathogens and improvement of nutritional quality of bananas. To address these issues, refinement of existing techniques and introduction of new experimental tools are required. However, the genetic improvement of bananas to a large extent is limited by using conventional methods due to polyploidy, heterozygosity, and sterility of this plant. For rapid multiplication and obtaining disease free and healthy plants, efficient in vitro propagation techniques and fine tuning of the existing protocols are being tried in many laboratories across the globe. Besides, for developing a successful protocol for propagation of different cultivars of bananas, a deeper understanding of the factors associated with various steps of its multiplication till transfer to the land is immensely critical. Similarly, developing biotic and abiotic stress tolerant banana and enhancing its commercial value through biotechnological interventions could be very useful. The key intent of this review is to highlight the research endeavor in this direction, associated challenges and future prospects.

1. Introduction

Banana (Musa spp. L.) is one of the most important cash crops and contributes immensely to global food security (Esan et al., 2022; Tripathi et al., 2020). It belongs to the family Musaceae and is distributed all around the world. With an annual estimated production of 153 million tonnes (FAOSTAT, 2019), the banana is the most popular fruit in terms of international trade (Tripathi et al., 2020). The major producers of bananas include India, China, Indonesia, Brazil, Ecuador, Philippines, Guatemala, Colombia, and Angola, where India is the largest producer with 30.808 million metric tons of production (FAO, 2020). Banana fruit is very popular especially in developing nations due to its low price and high nutritive value. It is a rich source of carbohydrates, proteins, minerals (such as potassium calcium, magnesium, and manganese), and vitamins including vitamins A, C, and B6 (Ranha et al., 2020). Besides the fruit, the other parts obtained from the banana plant such as peels, pseudo-stem, rhizome, leaves, etc. are utilized in various industries such as agro, food, textiles, etc (Adeniyi et al., 2019, 2021; Ighalo and Adeniyi, 2019; Akatwijuk et al., 2022; Mathew et al., 2021).

The genus Musa is divided into 5 sections viz. Australimusa, Callimusa, Rhodochlamys, Ingentimusa, Eumusa (Lamare et al., 2017). Section Australimusa having basic chromosome number 10 is distributed in Queensland, New Caledonia, Philippines, and Australia and is mainly used in the form of fiber, fruit, and vegetables. It includes 5–6 species (M. textilis, M. maclayi, M. lolodensis, M. peckelii, and M. fehi). Section Callimusa with chromosome number 10 is distributed in Indo-China and

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Indonesia and is mainly used for ornamental purposes. It consists of three species (M. coccinea, M. violascens, and M. gracilis). Section Eumusa having chromosome number 11 is distributed in India and is mainly used in the form of fiber, fruit, vegetables and have medicinal applications. It consists of the following species: M. acuminata, M. balbisiana, M. cheesmmani, M. flaviflora, M. itinerans, M. schizocarpa, M. sikkimensis, M. nagnsium, M. halabanensisand, M. ochracea. Section Rhodochlamis possessing chromosome number 11 mainly consists of ornamental species (M. aurantiaca, M. latertia, M. sanguinea, M. ornate, M. velutina, M. marnii, M. rosea and, M. rubra) which are distributed throughout India, Indo-China, Philippines, Thailand, and Malaysia (Debnath et al., 2019). There are more than 1000 varieties of banana cultivated all over the world, however, the Cavendish varieties of banana are considered as one of the most important commercial varieties (Tripathi et al., 2019; Thangavelu et al., 2021).

Banana cultivation largely regulates the agri-based global bio-economy. Hence, understanding the associated challenges in its production and developing appropriate strategies for addressing these concerns are of paramount importance. In recent years, there has been increasing interest to grow healthy and commercially important cultivars of bananas. However, there exists a huge gap between the demand and supply of healthy planting materials (Jacobsen et al., 2019; Nkengla-Asi et al., 2021). Further, in the last few decades, banana production is under threat due to different climatic factors and pathogenic agents such as bacteria, viruses, fungi, and nematodes (Tripathi et al., 2019). To circumvent these challenges and to produce agronomically superior disease-resistant banana crop, various traditional breeding methods have been employed such as diploid breeding (Rowe and Rosales, 1994), 3x/2x strategy (pollination of susceptible triploids with male fertile diploids that are resistant), and 4x/2x strategy which involves the production of a tetraploid parent by chromosome doubling of an ancestral diploid with good agronomic trait followed by the production of triploid hybrids through hybridization of a diploid parent with the tetraploid (Menon, 2016). However, polyploidy in Musa is the biggest challenge in banana breeding which ranges from diploid to tetraploidy (Nansamba et al., 2020). Thus, the development of a new banana cultivar is very exhaustive via breeding because the selection of desirable characters can take more than 12 years (Menon, 2016). A high level of heterozygosity and the requirement of a large population for the selection of individual clones with desirable agronomic traits, make it more cumbersome. Moreover, introgression of desired gene loci from diploid wild cultivars of banana also carries certain undesirable traits such as non-parthenocarpy and low yield (Menon, 2016). Thus, to sustain banana cultivation in the era of changing global climate, the development of new elite banana varieties and preparation of disease-free planting material for its commercial plantations using non-conventional approaches is much warranted. Biotechnology has largely provided solutions to many of these existing problems. The present review focuses on biotechnological interventions in bananas for their mass multiplications and improvement.

2. Micropropagation of banana

Banana plants are traditionally propagated through vegetative means using suckers (Nkengla-Asi et al., 2021). However, plants produced through suckers have their own limitations as it leads to disease transmission, low productivity, and poor preservation of original plant genetic material (Hussein, 2012). Moreover, there is a huge demand for quality planting materials to narrow the gap between demand and supply. In this scenario, micropropagation techniques have been used in many parts of the world to produce healthy, disease-free banana plants throughout the year that perform better under field conditions (Abdalla et al., 2022).

There are several reports available on micropropagation of different cultivars of banana (Table S1). These reports suggest that for successful micropropagation of bananas, optimization of micropropagation protocol is critical. In the present review, we have divided the protocol of micropropagation of bananas into four distinct stages such as (i) initiation of aseptic cultures (ii) shoot multiplication (iii) in vitro rooting of microshoots (iv) hardening of rooted microshoots (Figure 1) and tried to understand the factors that influence each of these stages. A better understanding of these factors will lead to the development of robust micropropagation protocols for large-scale propagation of different cultivars of bananas.

2.1. Factors that influence micropropagation of banana

2.1.1. Stage I: initiation of aseptic cultures

Initiation of aseptic culture is one of the most important steps for the development of a robust micropropagation protocol (Kaur et al., 2022).

2.1.1.1. Choice of explants. Shoot cultures of bananas can be initiated conventionally from mother sword suckers (Hasan et al., 2020) (Figure 2a). However, explant material from mature individuals with known responses to environmental factors and with proclaimed quality traits is preferred. Moreover, larger explants are advantageous because they contain a shoot apex with more lateral buds that develop into shoots, but they are more susceptible to contamination and blackening (Strosse et al., 2014; Kannahi and Buvaneswari, 2019). Among different plant parts of banana, suckers are considered the most suitable explant for micropropagation as they have a vascular connection with the mother plant, are easy to isolate and culture, and produce true to mother type plantlets (Heslop-Harrison and Schwarzacher, 2007).

2.1.1.2. Sterilization. Surface sterilization of explants is a process in which explants are immersed into the appropriate concentration of chemical sterilant to establish contamination-free cultures (Bello et al., 2018). The type of sterilant used primarily depends on the size and type of explants along with the procedure of disinfection (Strosse et al., 2004; Mihaljevic et al., 2013). Sodium hypochlorite is the most commonly used agent for surface sterilization. It is very effective against all types of bacteria, viruses, fungi, and nematodes (Tripathi et al., 2019).

![Figure 1. Different steps involved in the in vitro propagation of banana.](image-url)
microbial contaminations. It causes biosynthetic alterations in cellular metabolism through phospholipids destruction, fatty acid degradation, formation of chloramines that interfere in cellular metabolism, oxidative damage, and irreversible enzymatic inactivation in bacteria (Estrela et al., 2002). Mercuric chloride (HgCl$_2$) has antimicrobial activity against both fungi and bacteria. It may be used at 0.05–0.1% for 1–5 min according to the type of explant of different plants. It has been observed that a longer duration of 0.1% concentration of mercuric chloride treatment is effective to decrease bacterial contamination in cultures. However, higher concentrations of mercuric chloride can be toxic to
plant cells and tissues (Kang et al., 2020; Kapadia and Patel, 2021). Besides, bavistin (0.1%) and 70% (v/v) ethanol also act as a powerful sterilizing agent for banana explants (Bhutani et al., 2021; Prakash et al., 2019; Yadav et al., 2021). In addition, some antibiotics such as penicillin, ampicillin, and ticarcillin have also been used to curb bacterial contaminants in banana tissue cultures (El-Banna et al., 2021). Liquid cultures containing rifampicin for up to 30 days were able to inhibit the growth of Gram-positive bacteria in banana shoot tips with no effect on plant growth (Van Den Houwe and Swennen, 1999).

2.1.1.3. Browning of the medium. Browning of the medium occurs due to the oxidation of phenolic compounds produced by banana explants resulting in reduced cell division and plant growth (Rodrigues et al., 2022). These exudates form a barrier around the tissue which prevents nutrient uptake (Tabiyeh et al., 2005; Krishna et al., 2008). They further leach into the culture medium and result in the browning of the medium. It is for this reason that fresh shoots need to be transferred to new media every now and then (Ahmad et al., 2013). It has been observed that when banana explants were treated with citric acid and ascorbic acid for 30 min, the browning ceased (Ko et al., 2009; De Anicezio, 2012). Cessation of media browning could be attributed to the ascorbic acid’s activity to scavenge oxygen radicals produced when the plant is wounded which prevents the cells from further damage. Washing of explants with antioxidant solution (0.125% potassium citrate: citrate (K:C:C) in a ratio of 4:1 w/w) was useful to eliminate browning in Musa spp. cv. Kanthali (Titov et al., 2006). A similar response was observed when explants of banana were pre-soaked/pre-treated with 0.1–0.5 mg/ml of potassium citrate and citrate (K:C:C) (Onouha et al., 2011). Pre-soaking of explants in 1.2 g/l of ascorbic acid solution prevented lethal browning in local Musa spp. cv. Mzuzu (Ngomuo et al., 2014). For genotypes like Musa spp. ABB and BB groups, which produce compact proliferating masses of buds, the addition of activated charcoal to the medium minimized the phenol ABB and BB groups, which produce compact proliferating masses of buds, and shoot multiplication, while auxins such as indole acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) promote root development (Gupta et al., 2020; Kaur et al., 2022). BAP is the most preferred cytokinin to enhance shoot multiplication in Musa spp. due to its high cytokinin activity, accessibility, and low cost (Shirani et al., 2009; Singh et al., 2017). It has been reported to be the optimum cytokinin for shoot multiplication in bananas either alone or in combination with different auxins such as IAA and NAA (Huq et al., 2012; Shirani et al., 2009; Ahmed et al., 2014; Mahdi et al., 2014; Shankar et al., 2014; Fersdous et al., 2015; Qamar et al., 2015; Suman and Kumar 2015; Uzairabara et al., 2015; Hossain et al., 2016; Devi et al., 2017; Khatun et al., 2017; Safarpour et al., 2017; Khabab et al., 2017; Hoque et al., 2018; Rajoriya et al., 2018; Selvakumar and Parasurama, 2020) (Table S1). MS medium fortified with BAP (20.0 μM) in combination with NAA (1.0 μM) was reported to give the best proliferation for banana cultivar Grand Nain (Safarpour et al., 2017). Moreover, a higher multiplication rate in Dwarf Cavendish bananas on media supplemented with BAP (13.31 μM) and IAA (2.28 μM) was observed in the five subcultures which then declined after the 5th cycle (Dagniew et al., 2012). However, the proliferation of shoots was lesser at lower concentrations of BAP (10 μM) compared to a higher concentration of BAP (30.0 μM). For the banana cultivars ‘SH3362’, ‘‘Basrai’, ‘William’, ‘GN60A’ and ‘High gate’, MS + BAP (10.0 μM) + IAA (5.0 μM) + 40 mg/l cytokine HCl + 4% sucrose was used for initiation of cultures, and MS + BAP (20.0 μM) + 40 mg/l cytokine HCl + 4% sucrose for the shoot proliferation (Khatri et al., 1997). In addition, other cytokinins like TDZ and KN have been used for in vitro shoot proliferation in bananas (Gubkub and Pekmezci, 2009; Farahani et al., 2008; Gubkub, and Pekmezci 2009; Roy et al., 2010; Hrahsel et al., 2014). TDZ at a lower concentration (2.0 μM) promoted shoot proliferation (Shirani et al., 2009; Manjula et al., 2015), however, a higher concentration of TDZ (5.0 μM) was reported to cause a high abnormality index of shoots in bananas (Shirani et al., 2009). Besides, a new compound with cytokinin-like activity, named meta-Topolin (N6-(3-hydroxy-benzyladenine) (mT) has been used in place of BAP for the micropropagation of certain banana genotypes (Aremu et al., 2012; Escaño et al., 2003; Bairu et al., 2008).

2.1.1.4. Status of the medium. Besides media composition and plant growth regulators, the type of gelling agent used in a tissue culture medium is also critical (Thorpe et al., 2008). Gelling agents such as agar and phytagel are commonly used in plant tissue culture because of their higher gelling capabilities. Agar is routinely used because of its inertness, stability, and clarity (Palanuyandy et al., 2020). However, due to the high cost of tissue-culture-grade agar, the use of alternative cost-effective gelling agents including phytagel, gelrite, isabagol, etc is being explored (Ayeweh et al., 2021; Dhawale et al., 2021). During the in vitro
propagation of banana Grand Nain, when the gelrite in the medium was replaced with a mixture of gelrite/starch, the micropropagation rates were found to be relatively equal (Kodym and Zapata-Arias, 2001). When isabgol was used as an alternate gelling agent to phytagel and agar for in vitro propagation of banana cv. Karpura Chakkarakeli (AAB; Mysore subgroup), there was no significant difference in the number of shoots produced, although the survival rate of the shoots was higher with a slower multiplication rate in isabgol media than in phytagel and agar supplemented media. This could be ascribed to the low availability of water and a hence slower rate of absorption of nutrients from the isabgol matrix than that of other media tested (Agrawal et al., 2010). Use of phytagel in micropropagation of banana cultivar Dwarf Cavendish resulted in a higher shoot number and shoot weight than agar-agar, agargel, and plant agar (Kacar et al., 2010). Besides, a higher shoot multiplication rate and fresh weight were also observed in medium solidified with 0.9 g/l gelrite in comparison to medium gelled with 2.6 g/l gellan gum and 4–8 g/l agar in Shima Banana (AAA) (Bhush et al., 1999). In addition, when saog + Isabgol were used, the maximum number of shoots was observed in ‘Udhayam’ and ‘Ranthali’ cultivars compared to the control (Saraswathi et al., 2016). Nevertheless, till now, there are only a few reports on the use of low-cost gelling agents for banana micropropagation and thus more research is necessary to optimize their use in banana in vitro propagation.

Total elimination of gelling agents (liquid medium) has also been tried for banana micropropagation by different researchers (Alvard et al., 1993). It has been observed that the rate of shoot multiplication and dry weight of shoots was higher on liquid media when compared with gelled media (Alvard et al., 1993). However, these experiments required specialized culture vessels for highly controlled intermittent submergence of cultures in the medium. A simple polypropylene container with specialized culture vessels for highly controlled intermittent submergence of cultures in the medium. A simple polypropylene container with cotton fiber support was found more effective than that of an agar-gelled medium for micropropagation in banana (Musa acuminata) cv. Grand Nain (Prabhuling and Sathyaranayana, 2017). The plantlets produced were also sturdier and were of better quality in comparison with the agar-gelled medium. Liquid medium not only reduces the cost of propagation but also facilitates better availability of nutrients and plant growth regulators leading to higher shoot multiplication. Moreover, the liquid medium promotes proper aeration of cultures and dilutes any exude from the explant which might inhibit the growth of the culture (Ziv and Halevy, 1983; Abdulmalik et al., 2020). However, in general, many in vitro propagated plants respond poorly to liquid culture medium due to hyperhydricity that is a result of prolonged contact between the explants and liquid culture (Ziv, 2005; Snyman et al., 2011). To circumvent this challenge, a partial immersion system is utilized to make sure explants are fully aerated. Materials such as rockwool, coconut coir, filter paper, luffa sponge, cotton fiber, glass wool, polystyrene foam, nylon cloth, polyester screen raft, and polypropylene membrane raft are utilized to ensure there is contact between the lower portion of the explant and the culture medium (Gupta and Prasad, 2006). Filter sterilized air can also be bubbled through the medium for micropropagation (Preil, 1991). In bananas, four different liquid medium systems (solid medium (A), liquid medium with the immersion of plants (B), liquid medium with cotton culture support (C), and liquid medium aerated by bubbling (D) were compared for micropropagation. In this study, maximum shoot number in a temporary immersion bioreactor was observed in banana cultivar Musa cv. Dwarf Cavendish and hyperhydricity were observed in shoots that were cultured in a continuously aerated liquid media setup (Preil, 2011). Thus, temporary immersion bioreactors can help to overcome the hyperhydricity deformities through better aeration, and intermittent or partial submergence of the cultures in the medium (Sajid and Parvaiz, 2008; Farahani and Majid, 2012).

2.1.2.5. Physical factors. Temperature plays a critical role in the shoot multiplication of bananas. For banana shoot proliferation, a temperature in the range of 24 °C–26 °C has been reported by various workers to be optimum (Safarpour et al., 2017; Bello-Bello et al., 2019). However, there are few reports where a temperature up to 28 ± 2 °C is recommended for banana micropropagation (Alvard et al., 1993; Gebeeyehu 2015; Bohra et al., 2016). Along with temperature, light intensity and photoperiod also depict an important role in plant tissue culture (Kaur et al., 2021). In bananas, most workers have reportedly used a light intensity in the range of 30 and 100 μmol/m²/s (El-Mahdy and Youssef 2019; Mekonen et al., 2021; Subrahmanyawari and Gantait, 2022). However, optimum banana shoot proliferation was found at 40 μmol/m²/s (Wilken et al., 2014). Exposure to higher light intensity during later stages led to the improved survival rate of banana plantlets upon subsequent transfer to soil (Suman, 2017). Fluorescent lamps are currently the most common light for photosynthesis; however they are expensive and produce unnecessary wavelengths and radiations (Yeh et al., 2009; Sonthisut et al., 2022). Thus, the use of light-emitting diodes (LEDs) (combining LEDs emitting in far red, red, and blue colors) is encouraged these days for better plant growth under in vitro conditions (Nhut and Nam, 2010). It was reported that blue and red LED lights (B:R = 1:1) are more suitable for higher fresh and dry weight in bananas (Duong et al., 2009). Moreover, a maximum number of shoots was observed under the LED lamps as compared to the leaves under the white fluorescent lamps (Bhaya and Al-RazzaqSalim, 2019). Besides, high chlorophyll content was also observed in the case of two ornamental banana varieties using LED illumination (Vendrame et al., 2022).

2.1.3. Stage III: rooting of microshoots

For an efficient micropropagation protocol, rooting of microshoots is necessary for the successful transfer of in vitro propagated shoots to the field (Waman et al., 2015) (Figure 2e). MS basal medium was found to be the best suitable medium for inducing rooting from shoot tips in Musa spp. (cv. Sirimalai (Mahadev et al., 2011)). 1/2 MS, MS50 media alone or supplemented with activated charcoal and other additives have also been reported to promote rooting of in vitro banana microshoots (Mahadev et al., 2011; Hrashel et al., 2014; Thanakronpaisan et al., 2019; Selvakumar and Pararumana, 2020). Moreover, rooting in micropropagated shoots was successfully induced in the banana cultivar Elaki using 1/2 MS medium augmented with 20 mg/l adenine sulfate + 200 mg/l activated charcoal (AC) + 3% sucrose (Selvakumar and Pararumana, 2020). Further, MS medium supplemented with chitosan (20 mg l⁻¹) showed maximum rooting in shoots obtained from shoot tips (Thanakronpaisan et al., 2019).

MS media augmented with different auxins such as IBA, IAA and NAA had also been used for in vitro rooting of banana microshoots (Table S1). Auxins stimulate lateral root initiation and primordium growth by stimulating cell division, differentiation, and expansion (Kaur et al., 2021). Among different auxins, IBA has been reported by most workers as the most ideal auxin for rooting of in vitro developed banana shoots (Suman et al., 2013; Safarpour et al., 2017; Kavitha et al., 2021; Quiñonez et al., 2021). MS medium with 3% sucrose supplemented with IBA (1.47 μM) + 1 g/l activated charcoal was able to elicit rooting in vitro propagated shoots of Musa (AAB) Curare (Quiñonez et al., 2021). It was also observed that MS medium fortified with BAP (8.87 μM) + IAA (11.42 μM) + 0.1% activated charcoal is suitable for root induction in shoot tip culture (Vani et al., 1999). Further, MS media supplemented with NAA (2.65 μM) + 0.2% activated charcoal successfully induced rooting in bananas (Shashikumar et al., 2017). Besides, IAA (2.8 μM) in combination with BAP (22.19 μM) has also been widely used by different groups for in vitro rooting of banana shoots (Khan et al., 2021; Quiñonez et al., 2021).

2.1.4. Stage IV: hardening of rooted microshoots

Successful hardening of in vitro propagated plantlets is a prerequisite for an efficient lab-to-land transfer protocol (Khatik and Joshi, 2016) (Figures 2f, 2g). It is dependent on factors such as the composition of potting mixture, genotype of the plant, temperature, and humidity, etc.
(Twaij et al., 2020; Waman et al., 2015). Soil mixtures containing different ratios of vermiculite, peat moss, perlite, sand, and vermicompost have been used for the successful hardening of banana cultivars (Robinson and Sauco, 2009; Safarpour et al., 2017; Chamling et al., 2021). Besides combinations of bio-fertilizers (including VAM), peat fortified with nitrogen fixing and phosphate solubilizing microbes (each 1 g/plantlet) has been used for hardening in banana plantlets (Vasane et al., 2008). For hardening of banana cultivar Grand Nain (cocopeat: red soil: sand (1:1:1) was found to be the optimum potting mix for vigorous growth with a 96.5% survival rate (Selvakumar and Parasaruma, 2020).

In a similar study for Grand Nain banana, cocopeat (temperature 25 ± 2 °C) was used for hardening in the green house and further acclimatization was carried out using soil and vermicompost (1:1) (Manokari et al., 2022). Sand and farmyard manure (FYM) have also been used for acclimatization and hardening of banana cv. Malbhog and B. B. Battisa, with a survival rate of 95 % and 80%, respectively (Suman and Kumar, 2015).

3. Somatic embryogenesis system

The establishment of a high-frequency regeneration protocol is an important prerequisite for direct regeneration and genetic transformation (Bajput et al., 2022). It relies on the utilization of PGs to induce tissue differentiation thus forming embryogenic callus (EC). Embryogenic callus has been found to have a high competence for embryogenesis (Rustagi et al., 2019). Moreover, the embryogenic callus also provides the starting material for the formation of embryogenic cell suspensions (ECS). From these cell suspensions, somatic embryos are produced and plants are regenerated. The first successful protocol on somatic embryogenesis in bananas was reported by Cronauer-Mitra and Krikorian (1988) followed by Escalant Teision (1989). However, despite their high regeneration potential, they are not widely used for propagation because of the frequent occurrence of somaclonal variations (Kavitha et al., 2021). Banana regeneration has been successfully achieved using different types of explants as discussed in the following sections (Table S2).

3.1. Embryogenesis from leaf sheath and rhizome

Modified Schenk and Hildebrandt medium fortified with TDZ (5.0 μM) and 20.0 μM Dicamba (3,6-dichloro-2- methoxy benzoic acid) was used for inducing callus from rhizome slices and leaf bases of cooking banana (ABB) and dessert banana (AAA). Later, somatic embryos were obtained from the induced callus in cell suspension after one month in media augmented with zeatin (5.0 μM) in dessert (AA and AAA) and cooking (ABB) bananas (Musa spp.) (Novak et al., 1989). Leaf sheath disks of ‘Nanico’ banana (Musa sp., AAA group, Cavendish subgroup) were used to induce embryogenic callus on MS medium supplemented with activated charcoal (0.2 %), MES (2 [N-morpholino]ethanesulfonic acid) (15.3 mM), Picloram (414.0 μM), 2-iP (492.0 μM) and arginine (300 mM) (DA- Silva et al., 1998). Besides, MS medium supplemented with picloram (16.56 μM) induced embryogenic callus in Musa acuminata cv. Njaliapoon (AB). Further, subculturing of the callus in dark on MS basal media resulted in the development of shoots and roots (Smitha and Nair, 2011).

3.2. Embryogenesis from zygotic embryos

Somatic embryogenesis was induced in ornamental banana (Musa ornata Roxb.) using zygotic embryos propagated on semi-solid MS medium supplemented with 2,4-D (2.25 μM, 4.52 μM, 9.04 μM) + coconut water (5%) + 3% sucrose (Cronauer and Krikorian, 1988). Embryo germination and growth were achieved through the elimination of 2,4-D and subsequent transfer of the somatic embryos to Schenk and Hildebrandt media. Similarly, somatic embryos were obtained in Musa balbisiana (BB) and Musa acuminata (AA) from zygotic embryos using MS medium containing picloram (7.5 μM) or NAA (5.5 μM). Plant regeneration was achieved on MS media augmented with NAA (5.3 μM) (Echalant and Teision, 1989). Immature zygotic embryos of Musa acuminata ssp. burmannicoides and Musa acuminata ssp. malaccensis were used to induce embryogenic calli on MS medium containing picloram (7.5 μM). These calli were used to prepare embryogenic cell suspensions (ECS) in a liquid MS medium. ECS produced somatic embryos that germinated on an MS medium supplemented with BAP (0.22 μM) + IAA (1.14 μM) (Morroquin et al., 1993). However, regeneration of plantlets from immature embryos was higher than mature embryos (Uma et al., 2021).

3.3. Embryogenesis from immature male/female flowers

Immature floral tissues have been reported to have excellent embryogenic potential and are thus widely used in in vitro cultures (Anmimrita, 1983). MS medium supplemented with 2,4-D (1.0 μM) + NAA (5.7 μM) + IAA (5.4 μM) + 1 mg/l biotin + 100 mg/l glutamine + 100 mg/l malt extract + 3% sucrose and gelled with 2.6 g/l phytagel was used for induction of embryogenic callus and regeneration of Musa acuminata cv. Mas (AA) using immature male flowers as the starting material (Jalil et al., 2003). Callus was obtained in MS medium supplemented with 2,4-D (4.52 μM). However, embryo development occurred on MS medium supplemented with 2,4-D (0.23 μM). For embryo-to-plant retrieval, MS medium with BAP (9.76 μM) was used. 2,4-D has been used in many studies to induce somatic embryos from immature male flower buds of different cultivars of banana (Wei et al., 2005; Kulkarni et al., 2006; Sidha et al., 2006; Ali et al., 2013). Besides, embryogenic calli of Musa acuminata cv. Matti was obtained through inoculation of broth explants on MS medium supplemented with TDZ (0.45 μM) + 3% sucrose (Divakaran and Nair, 2011). Embryo development was attained on MS + 8.18 μM biotin. Similar results were obtained in Musa acuminata cv. Njaliapoon using MS medium with TDZ (4.5 μM) + 3% sucrose for initiation of embryogenic callus and MS medium supplemented with 16.37 μM biotin for embryo development. In addition, embryogenic callus in Musa sp. Rasthali (AAB) was established using shoot tips as explants on MS medium augmented with 2, 4-D (9.05 μM) + zeatin (1.0 μM) and 1 mg/l D-biotin and MS medium supplemented with 2,4-D (4.5 μM) + 1 mg/l D-biotin + 100 mg/l glutamine + 100 mg/l malt extract for induction of embryogenic cell suspension. Embryo development was finally achieved on ½ MS medium supplemented with Zeatin (10.0 μM) + 3% sucrose (Ganapathi et al., 2001). In a recent study, somatic embryos were induced in three ornamental bananas using immature male flower buds as the starting material. In this study, it was observed that embryogenic calli desiccated up to 2 h at 25 ± 1 °C resulted in higher frequencies of embryo induction and maturation in comparison with non-desiccated embryos (Natarajan et al., 2020).

3.4. Embryogenesis from scaps

Liquid ½ MS medium supplemented with zeatin (1.0 μM) and 2, 4-D (5.0 μM) was used to induce embryogenic cell suspensions of cooking banana cv. ‘Bluggo’ (Musa spp. ABB group) using scaps as the explant. Embryo maturation was achieved in MS basal medium and further plant regeneration was induced in ½ MS medium supplemented with BAP (10.0 μM) (Dheeda et al., 1991). Shoot tips of Musa sp. cavendish were inoculated in ½ MS medium supplemented with BAP (22.19 μM) and IAA (1.14 μM). Explants were subsequently transferred to a medium composed of ½ MS salts augmented with BAP (1.02 μM) and NAA (1.08 μM). The explants were further transferred to MS medium supplemented with BAP (0.88 μM) + 2, 4-D (9.04 μM) + 1 mg/l biotin, whereby somatic embryos were observed after two weeks. Moreover, somatic embryos of hybrid banana FHIA-18 were obtained using the liquid medium in a bioreactor (Kosky et al., 2002).

3.5. Embryogenesis from shoot tips obtained from in vitro cultures

Direct somatic embryos in banana (Musa acuminata AAA cv. Grand Nain) were induced from split shoot tips obtained from 4 weeks old in
vitro multiple shoot cultures on MS medium supplemented with picloram (4.14 μM) and BAP (0.22 μM). These somatic embryos germinated into plantlets on MS medium supplemented with NAA (0.53–2.68 μM) together with BAP (2.22–44.39 μM), or thidiazuron (4.54 μM) plus glutamine (200 mg/l) (Remakanthan et al., 2014).

3.6. Plant regeneration from protoplasts

The isolation of protoplasts in a banana for the very first time was reported in 1984 from inflorescence-derived callus of Cavendish (Musa AAA) (Bakry, 1984). However, no regeneration was obtained from the protoplasts. Later, successful plant regeneration from protoplasts in wild banana Long Tavoy (AA) was obtained using immature seeds (Megia et al., 1993). Embryogenic cell suspensions were established from the upper meristematic part of proliferating shoot-tips in cv. 'Bluggoe' (Musa spp., ABB subgroup). Protoplasts were isolated from ECS and they directly formed somatic embryos without undergoing any callus phase on ½ MS salt solution, mannitol (5%), 2,4-D (5.0 μM), and agarose (0.8%) or Gelrite (0.2%) (Panis et al., 1993). Moreover, protoplast regeneration through somatic embryogenesis using immature male flowers of seven banana cultivars viz. Dominico and curare Enano (subgroup plantain AAB), IRFA 903, SF 265 and Col 49 (AA); Grand Nain and Gros Michel (subgroup Cavendish AAA) were achieved (Assani et al., 2001). The conversion rate of protoplast into somatic embryos was 2% on MS medium supplemented with BAP (2.2 μM) and IAA (11.4 μM), vitamins of Morel + 3% sucrose, and solidified with 0.75% agarose. Of all the embryos, 43% were able to germinate and develop into plantlets (Assani et al., 2001).

4. Genetic transformation of banana

In the current scenario of global climate change and threatened food security, genetic improvement of bananas using biotechnological tools has fascinated the researchers (Ganapath et al., 2021; Subrahmanyaswari and Gantait, 2022). There are several methods available for the genetic transformation of bananas including electroproporation of protoplasts, Agrobacterium-mediated transformation, and particle bombardment using embryogenic cells (Ganapathi et al., 2021; Tripathi et al., 2015). Nevertheless, Agrobacterium-mediated transformation is the most sought-after approach for the genetic improvement of bananas with the advent of biotechnology. This method has an edge over other techniques as it relies on the utilization of differentiated tissues that can be regenerated into the complete plant using routine protocols (Tripathi et al., 2015). Besides, it offers integration of the transgene in low copy number and can transform larger stretches of DNA as compared to other direct methods of transformation (Tripathi et al., 2015). In recent decades, banana has been successfully transformed using several genes that have resulted in improved stress endurance, introduction of novel attributes, and value addition into the Musa spp. as discussed below:

4.1. Genetic transformation for production of transgenic banana resistant to biotic and abiotic stresses

Banana plants experience different biotic and abiotic stresses through their production cycle that significantly hampers their overall productivity (Li et al., 2021; Wang et al., 2021). Among biotic factors, the major diseases that are causing serious concerns and losses to banana yield currently include black Sigatoka, Fusarium wilt tropical race 4 (TR4), banana Xanthomonas wilt (BXW), and banana bunchy top disease (De souza-pollo and de Goes., 2020). Among these, Fusarium wilt (Panama disease) caused by Fusarium oxysporum f. sp. cubense (Foc) was the first major calamity of banana (Gros Michel). There are four different races of Foc fungus, and all of these except race 3 are pathogenic (Ganapathi et al., 2021). Fusarium wilt tropical race-4 (TR-4) imposes the sternest threat on the cultivation of bananas as it can destroy the banana plantations and result in global pandemics (Rocha et al., 2021). As control of Foc using different physical, chemical, or biological methods is practically impossible, thus, in the past few decades, sufficient attention has been given to the development of Foc-resistant genetically engineered banana varieties (Wang et al., 2021). Foc-resistant banana varieties have been generated by overexpressing the genes encoding for antifungal proteins like ferredoxin-like proteins, defensins, chitinase, lysozyme, anti-apoptosis proteins, etc from other organisms (Table S3). Another yield-limiting fungal pathogen that affects banana production is Mycosphaerella fijiensis which results in black Sigatoka disease. This disease can be managed by the use of certain chemicals, however, the arbitrarily use of such chemicals imposes a serious hazard on mankind and the environment (Sowmya et al., 2016; Soares et al., 2021). Thus, the development of Sigatoka-resistant banana varieties by employing genetic engineering approach has provided a most suitable option to protect the plants from this harmful fungus (Sowmya et al., 2016). Sigatoka-resistant banana has been developed by overexpressing antifungal proteins including chitinase, glucanase, RCC2, RCC3, etc (Table S3) (Kosky et al., 2010; Vishnevetsky et al., 2011; Kovaic et al., 2013). Apart from fungal pathogens, the transgenics approach has been effectively used for the management of the bacterial disease Xanthomonas wilt which can devastate banana production (Ocicati et al., 2020). The transgenic banana plants developed using sweet pepper Ppflor, Hrp genes showed high resistance to Xanthomonas campestris pv. Musa orienum that causes Xanthomonas wilt disease (Namukwya et al., 2012; Tripathi et al., 2010; 2014a). Similarly, transgenic banana plants expressing the rice H21 gene showed increased resistance towards the Xanthomonas wilt pathogen (Tripathi et al., 2014b).

Apart from bacterial and fungal pathogens, infestation by nematodes and weevils is another persistent challenge for banana cultivation. Nematodes account for almost 20% loss in global banana production that may rise to even 40% in tropical storms-prone areas (Roderick et al., 2016). Nematodes are conventionally controlled with the use of harmful pesticides that contaminate the environment (Tripathi et al., 2019). Thus, to circumvent this problem, nematode-resistant transgenic banana cultivars were developed using cysteine proteinase inhibitors (cystatins) and nicotinic acetylcholine receptors (naCHR) inhibiting peptides encoding genes. Cystatins inhibit the dietary protein's intestinal digestion, whereas naCHR inhibiting peptides interfere with the nervous system of pests (Roderick et al., 2016) (Table S3). Rice cystatin (OcIJD86) transformed banana plants have been developed that show almost 69–70% resistance towards Radopholus similis (Atkinson et al., 2004). In another attempt, genetic transformation of banana using maize naCHR inhibiting peptide or cystatin or both of these used together conferred resistance against Helicoverpenchus multicinctus, and Radopholus similis under field trials conducted in a confined area in Uganda (Roderick et al., 2012). Moreover, transgenic banana 'Sukali Ndzi' (ABB) has been developed using the cystatin (CpCY5-Mut89) gene from papaya for their potential resistance against common pests that infest banana. However, a precise evaluation of such plants for resistance against nematodes and weevils is yet to be conducted (Namuddu et al., 2013).

Besides different biotic agents, abiotic stresses including salinity, sub-optimum temperatures, and drought also act as a major impediment to banana cultivation (Ganapathi et al., 2021). Thus, researchers have undertaken profound efforts to improve the abiotic stress tolerance window of banana plants using a transgenic approach. Genetically modified banana plants with enhanced tolerance against salinity and drought stress were generated by overexpression of genes that belong to different families including WRKY transcription factors, late embryogenesis abundant (LEA) proteins, aquaporin coding genes, pathogenesis-related (PR) proteins, MYB transcription factors, NAC transcription factors, and several other stress-associated proteins, etc (Table S4) (Sreedharan et al., 2012, 2013, 2015; Shekhawat and Ganapathi 2013; Rustagi et al., 2015; Dou et al., 2016). However, limited attention has been given to the generation of genetically engineered banana cultivars that can resist changes in their optimum growth temperature.

Although, appreciable efforts have been undertaken by the researchers to develop stress-tolerant genetically modified banana
cultivars, yet there is no report so far on the commercialization of such varieties. A major portion of such studies are greenhouse restricted and are not extended to field conditions. However, recent field trials conducted for over 3 years using the RGA2 gene (resistance gene analogue 2) expressing TR4-resistant Cavendish transgenic plants showed promising results of resistance towards TR4 Foc strain (Dale et al., 2017). Similarly, field trials are underway for the transgenic banana plants resistant to Xanthomonas wilt disease and nematodes. Moreover, researchers have identified several candidate genes that are associated with the stress-induced senescence and decreased productivity of banana plants. The manipulation of such genes using modern genomics approaches can enhance the stress endurance and production of banana plants in the coming years (Lira et al., 2017; Tak et al., 2018; Ma et al., 2018).

4.2. Genetic transformation of banana for crop improvement

The improvement of banana cultivars for better agronomic traits to enhance their growth, yield and nutritional value are one of the prime agendas of researchers for long time (Sipen et al., 2011; Ganapathi et al., 2021; Wang et al., 2021). A Global Programme for Musa Improvement named ‘PROMUSA’ has been initiated by the World Bank and INIBAP since 1997. The major aim of this program is to develop novel banana varieties using conventional breeding approaches in conjunction with genetic engineering to address the needs and challenges faced by banana farmers in different regions of the world (Frison et al., 1998; Eksoy, 2018). It has been realized that semi-dwarf banana varieties with thick/sturdy stems and root system that shows better hydrotropism are ideal for increasing the production of banana (Wang et al., 2021). However, limited genetic engineering efforts have been made to impart these traits in banana varieties. In one such investigation, the overexpression of the NAC domain-containing MaVND1, MaVND2, and MaVND3 genes in bananas has been observed to modulate the secondary wall deposition that may influence the stem thickness (Negi et al., 2015, 2016). In addition, the bio-fortification of bananas is one of the key components of the global mission of banana improvement, especially in the African continent (Paul et al., 2018). Banana is one of the staple foods for African populations; however, it has a lower content of iron, vitamin A, and protein (Paul et al., 2018). As people in African nations especially younger women and children are mostly deficient in micronutrients; thus reasonable efforts have been undertaken for the biofortification of bananas to enhance the content of iron and vitamin A (Amah et al., 2019; Ganapathi et al., 2021; Yadav et al., 2017). Iron biofortification of bananas has been done using IRT1, FRO2, SFER, NAS1, NAS2, FER1, and YSL2 genes from different sources (Kumar et al., 2011; Moses et al., 2016; Yadav et al., 2017). Similarly, PSY2a, PSY1, and CRTL genes have been used to generate pro-vitamin A-rich transgenic banana plants (Paul et al., 2017). Moreover, a banana project named Banana-21 has been initiated by the National Banana Research Program of the National Agricultural Research Organisation (NARO) of Uganda in collaboration with the Centre for Tropical Crops and Biocommodities at Queensland University of Technology (QUT) in Australia since 2005 for the development of vitamin A-rich transgenic banana by stacking and overexpressing various genes that participate in the pro-vitamin A synthesis (Paul et al., 2017, 2018).

Along with biofortification, the genetic transformation of banana plants for ‘molecular farming’ has come into the limelight in recent years (Tak et al., 2016). Molecular farming involves the production of pharmaceutically important products including antibodies, hormones, enzymes, and vaccines using plants (Schillberg and Finnern, 2021). Bananas can serve as the ideal system for molecular farming owing to their year-round availability, large-scale cultivation, easy digestibility especially by infants, and availability of an efficient gene transfer protocol, etc (Tak et al., 2016). Earlier, embryogenic cells of bananas have been successfully transformed with hepatitis B surface antigen (HBsAg) for the generation of edible banana vaccines against hepatitis B (Kumar et al., 2005). Thus, in the future banana can provide a suitable platform for the large-scale production of a range of pharmaceuticals.

5. Genome editing system

Genome editing is the latest approach that is in limelight for improving the different traits in crop plants (Alvarez et al., 2021; Hüdig et al., 2022; Kaur et al., 2019, 2022). Although, there are not many reports on exploring the potential of genome editing for the genetic improvement of bananas, yet, in the forthcoming era, genome editing may act as a saviour for sustaining banana cultivation. The initial experiments of genome editing in bananas were conducted on the Phytotoxin desaturase (PDS) gene. The PDS enzyme participates in the carotene biosynthetic pathway and its silencing results in the albino phenotype (Tripathi et al., 2019). The mutations induced in this gene using single gRNA resulted in the silencing of the PDS gene with 59% efficiency in the “Rusthali” cultivar (Kaur et al., 2018). Later, the same gene was mutated in the “Cavendish Williams” banana cultivar with the advent of polycistronic gRNAs (Naim et al., 2018). In the year 2020, the PDS gene was silenced in “Sukali Ndizi” and “Gonja Manjaya” cultivars by employing multiple gRNAs with an efficiency of almost 100% (Ntui et al., 2020). All these efforts paved the way for the successful generation of genome-edited banana plants with specific elite traits. In 2019, endogenous banana streak virus (eBSV) that integrates into the B genome of Musa balbisiana was mutated using CRISPR/Cas. eBSV can reactivate upon exposure to stress conditions and due to this reason, the banana cultivars that comprise of atleast one B genome are not selected as a parent for genetic improvement of banana. Upon disruption of eBSV sequence in the B genome of banana using multiple gRNAs, approximately 75% of the mutated plants were observed to be asymptomatic in response to water stress thus affirming the successful deactivation of eBSV (Tripathi et al., 2019). Moreover, the transgenic banana plants that carried the small interfering RNA (siRNA) designed to target the Fusa- rium transcription factor 1 (ftf1) and the Foc velvet genes showed increased resistance towards Foc Race 1 (Ghag et al., 2014). Similarly, silencing of ERG6 and ERG11 genes involved in ergosterol biosynthesis genes enhanced the resistance of banana plants towards Foc Race 4 (Dou et al., 2020). The introduction of RNAi targets designed against replicase-associated protein (Rep) gene of viral origin was found to successfully resist BBTV infection in banana plants (Table S3) (Shekhawat et al., 2012; Elayabalan et al., 2013). In another report, semi-dwarf plants of “Gros Michel” have been developed by mutating the gibberellin 20ox2 (MaGA2ox2) gene involved in gibberellin biosynthesis (Shao et al., 2020). Besides, the shelf life of banana fruits has been improved through the modulation of the MaRCO1 gene that plays a key role in the ripening of banana fruits using CRISPR/Cas (Hu et al., 2021). In a recent report, the role of the carotenoid cleavage dioxygenase4 (CCD4) gene in the regulation of carotenoid accumulation was unravelled using CRISPR/-Cas. In this investigation, transgene-free editing of the banana genome was successfully done by transforming the embryogenic cells and protoplasts of the banana (Awasthi et al., 2022).

Till now, in the majority of the reports, the agrobacterium-mediated transformation method has been used for transforming the genome editing cassette to the different banana cultivars. But, as the banana is a sterile plant, the elimination of foreign DNA sequences derived from plasmids including the selection marker is a major hurdle (Tripathi et al., 2021). To overcome this issue, researchers have suggested that preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) may be designed with specific gene targets (Awasthi et al., 2022; Ntui et al., 2020). These RNPs could be coated on gold particles and can be transferred to either embryonic cells or protoplast through particle bombardment, etc. thus escaping the GM legislation and to further easing the commercialization of genome-edited bananas (Awasthi et al., 2022; Ntui et al., 2020).

6. Integration of omics studies for banana improvement

An in-depth understanding of the structure and evolution of the banana genome is critical in overcoming the challenges faced by the banana breeders (Sampangi and Ravishanker, 2016). Further, the precise
identification and elucidation of the mode of action of key genes regulating different features of the banana plant are much warranted for the implementation of modern genomics approaches including genetic engineering and genome editing for banana crop improvement (Tripathi et al., 2019). In this scenario, different omics-based methods involving metabolomics, proteomics, transcriptomics, and genomics have opened new avenues to improve banana varieties for ensuring their sustainable and eco-friendly cultivation (Mohanty et al., 2017; Kissel and Carpenteret, 2016). The size of the banana haploid genome was estimated to be 600 Mbp in *M. acuminata* and 550 Mbp in *M. balbisiana* using flow cytometry (Dolezel et al., 1994). The genome sequence of bananas was decoded by researchers working in association with the Global *Musa* Genomics Consortium in 2012 (D’Hont et al., 2012). This unravelling of the blueprint of the banana genome provided the necessary impetus to the research efforts focused on the genetic improvement of bananas. The draft sequence of the banana genome suggests that there are almost 36000 protein-coding regions, 37 Micro RNA (MiR) families and around half of the genome is comprised of transposable elements (Sampangi and Ravishankar, 2016). Besides, transcriptomics studies conducted using different parts of the plant including the flower, rhizome root, leaf, etc. aided in the precise functional interpretation of the critical elements of the banana genome (Ibrahim and Thangjam, 2015; Singh et al., 2021). The comparative transcriptome analysis of different banana varieties assisted in the identification of key genes involved in the regulation of vital processes of the banana life cycle including growth, development, and disease resistance, etc (Hu et al., 2017; Li et al., 2013; Sun et al., 2019; Dong et al., 2020; Kaushal et al., 2021; Yumbya et al., 2021). Moreover, transcriptomics studies generated useful resources to identify the critical genes that were not annotated in the banana draft genome sequence (Li et al., 2013). The deep sequencing of the transcriptome of Foc 1 and Foc race 4 infected banana plants using Illumina unravelled almost 842 genes that were not annotated in the banana draft genome sequence (Li et al., 2013). In addition to transcriptomics, several studies have been conducted to study the proteome of banana plants for the characterization and analysis of genetic variations observed in various banana varieties. Proteome analysis provided insights into the molecular dynamics of key proteins and their modulations at the post-transcriptional and post-translational levels during the vital processes of Foc resistance, drought tolerance, cold stress resistance, fruit ripening, identification of banana allergens, etc (Vanhove et al., 2012; Li et al., 2015; Nikolic et al., 2018; Dong et al., 2019; Bhuyian et al., 2020). Thus, understanding banana genetics through an integrative approach that involves comparative genomics in conjunction with transcriptomics and proteomics may provide better access to the genes and their cis-regulatory elements for the domestication of banana ‘new generations’ through breeding and for the development of genetically modified banana with elite traits.

Apart from the analysis of DNA, RNA, and proteins, the investigation of plant metabolomics has emerged as a powerful technology in recent years (Price et al., 2020; Li et al., 2021). The metabolic profiling of bananas holds promise for the precise interpretation of biochemical diversity that exists within banana germplasm (Drapal et al., 2016). Researchers have generated a database of metabolic fingerprinting of 20 different accessions of bananas using their vegetative parts. The varieties included in this collection comprised both diploid and triploid along with *Musa acuminata*, *Musa balbisiana*, and distant wild species (*Musa ornata*). These varieties were then categorized based on their differential metabolite and genotypes (Drapal et al., 2016). As banana is a nutritionally rich plant, metabolic profiling in combination with other omics approaches can help the researchers to choose the banana varieties as per the consumer demands and elite agronomic traits for future crop improvement programs (Price et al., 2020).

### 7. Nano-biotechnology for banana

The use of nano-biotechnology in the field plant science is emerging rapidly (Arab et al., 2014). Lately, the exploration of the potential of nanoparticles (NPs) in improving the different traits of plants has grasped the attention of researchers (Sanzari et al., 2019; Zahedi et al., 2020). For *in vitro* propagation and regeneration of bananas, the addition of NPs in the routine media has given promising preliminary results. The zinc (Zn) and zinc oxide (ZnO) NPs were effective in eliminating the 9 different types of bacterial and four types of fungal contaminants from *in vitro* grown cultures of bananas. They further positively influenced the rate of regeneration and rooting of plantlets (Helaly et al., 2014). Moreover, augmentation of Ag NPs promoted the shoot proliferation rate in bananas. They also increased the shoot length, the number of roots per explant, root length, fresh weight, and the number of leaves (Do et al., 2018). In addition, Ag NPs promoted callus induction, shoot regeneration, rooting, and secondary hardening during *in vitro* propagation of bananas (Huong et al., 2021). Besides, the treatment of AgNPs was also effective in combating the spread of banana bunchy top virus (BBTV) in the field-grown Grand Nain banana. The treated plants showed an increase in leaf area, and dry weight compared to the control plants (Mahfouze et al., 2020). The foliar application of chitosan nanoparticles (CH-NPs) increased the fresh weight and dry weight of hardened *Musa acuminata* var. Baxi. Further, the application of CH-NPs resulted in a decrease in reactive oxygen species (ROS) levels and malondialdehyde (MDA) content in response to cold stress (Wang et al., 2021). Silicon oxide (SiO2-NPs) promoted shoot growth and proliferation, and also improved the photosynthesis in banana plants exposed to drought stress conditions. Moreover, a significant enhancement in phenolic secretion and a reduction in lipid peroxidation rate were observed at low concentrations of SiO2 (50 mg/l) (Subrahmanyeswari and Gantait 2022). Further, silicon NPs were helpful in the mitigation of the salinity and drought stress-induced oxidative stress in banana plants. The treated plants also exhibited improved chlorophyll levels, and Na+/K+ homeostasis under stress conditions (Mahmoud et al., 2020).

### 8. Conclusion and future prospects

Banana is one of the most loved food crops across the globe owing to its taste, easy digestibility, and nutritional value. The use of biotechnology has provided ample opportunities to solve the different problems associated with the conventional cultivation of banana (Figure 3). Traditionally, banana is cultivated using suckers as the planting material. However, these suckers often act as the reservoirs for different pests/pathogens that limit the productivity of the crop. Moreover, the age, size, and uneven maturity of suckers extend the crop duration. The establishment of various protocols for *in vitro* propagation of bananas has assured the availability of disease-free planting material throughout the year. However, still, an efficient and cost-effective protocol to propagate various nutritionally rich, recalcitrant banana varieties that can thrive in the changing global climate needs to be developed. In addition to micropropagation, the genetic transformations, biofortification and use of the banana in the production of edible vaccines have resulted in value addition to the crop. Although, many success stories have emerged in the past where biotechnology has served as a saviour for banana cultivation, still many hurdles limit the use of this approach to its full potential in banana improvement. The most important among these are the strict guidelines that restrict the field trials and commercialization of genetically modified banana plants. Moreover, the lack of public acceptance of genetically modified crops is another censorious issue. In this scenario, the recent breakthrough biotechnological tool CRISPR/Cas seems to be the samaritan option that can help in sustaining banana cultivation in near future. Researchers have identified several key candidate genes that regulate the agronomic and stress ameliorative traits in bananas. The exploitation of such genes using genome editing can assist in designing next-generation banana varieties that will be more palatable to the regulatory authorities and the public. Besides, considering the public’s concern about genetically modified crops, the development of transgene-free or DNA-free genetics transformation and genome editing technology like the use of RNPs should be considered in the future.
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