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Review

Conservation Strategy for African Medicinal Species: *In Vitro* Biotechnological Approach

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Abstract: The use of medicinal plants for different therapeutic values is well documented in African continent. African diverse biodiversity hotspots provide a wide range of endemic species, which ensures a potential medicinal value. The feasible conservation approach and sustainable harvesting for the medicinal species remains a huge challenge. However, conservation approach through different biotechnological tools such as micropropagation, somatic embryogenesis, synthetic seed production, hairy root culture, molecular markers based study and cryopreservation of endemic African medicinal species is much crucial. In this review, an attempt has been made to provide different *in vitro* biotechnological approaches for the conservation of African medicinal species. The present review will be helpful in further technology development and deciding the priorities at decision-making levels for *in vitro* conservation and sustainable use of African medicinal species

Keywords: Biotechnological approach; conservation; medicinal plant; African traditional medicine (ATM); start codon targeted polymorphism (SCoT); somatic embryogenesis

1 Introduction

For the success of primary health care, availability and usage of appropriate drugs are one of the important requirements. Plants based traditional medicine is easily accessible and commonly used source for the treatment in the primary health care system. Medicinal plants are mostly used as herbal remedies and play an essential role in traditional healthcare in most of the developing countries. In ethno-medicine

system, several plant species are used to treat wide range of diseases. Due to this multi-usage, trade network demand for the different medicinal plant species can extend to international boundaries.

The diverse and rich plants landscape of the African continent ensures the livelihoods and primary healthcare of African population. A large proportion of the African population depends on medicinal plants based herbal medicine for their primary health care needs [1,2]. The innovative utilization of medicinal plants as a source of ailments and their rich history of African cultures has been passed down through generations [3]. However, indiscriminate harvesting, industrialization and lack of knowledge among local population lead to medicinal plant biodiversity loss in Africa. Therefore, different conservation strategies need to be promoted to ensure their conservation and long-term sustainability. In past few decades plant biotechnology has emerged as pioneer tool for the conservation of different genotypes of medicinal species. *In vitro* biotechnological approaches represent essential resources for the selection, multiplication, germination and conservation of surviving populations and their genetic resources. In this regards, *in vitro* plant regeneration via micropropagation and somatic embryogenesis holds significant potential for the production of elite, virus-free clones. While, synthetic seed technology and cryopreservation technique provide a long-term conservation of medicinal and aromatic species using alginate beads and liquid nitrogen (−196°C) respectively. Molecular markers such as RAPD, RFLP, ISSR, IRAP, and SCoT could be utilized for the identification of genetic divergence, reproduction and conservation practices for several medicinal species.

Hairy root interest in the plant biotechnology also represents a key step toward diverse objectives including *in vitro* conservation, commercialization and production of bioactive compounds for African medicinal species. In this review, we summarize different *in vitro* biotechnological approaches for the conservation of African medicinal species. We trust that present review will be useful for the reader to get an idea on current advances of the biotechnological approaches for *in vitro* conservation.

1.1 In vitro Biotechnological Application

Harvesting of various medicinal species from the nature remains the key resource for raw materials [4]. The global utilization of medical plants based medicine exposed the local plant resources to uncontrolled harvesting, which bring some important species on verge of extinction. In addition, the ever-increasing demand for the medicinal plants on the national and international trade has led to unsustainable, indiscriminate and uncontrolled harvesting [5,6].

Conservation approach on medicinal plant species remain a global challenge [2,7,8]. According to IUCN [9], huge number of plant species currently under strain (threatened, vulnerable or declining, critically endangered and endangered) in African countries. A number of biotechnological tools offer for the conservation and improvement of different plant species (Fig. 1). These biotechnological approaches are essential to get elite clones and conserve the crucial genotypes of different medicinal species.

1.2 Micropropagation

In vitro plant tissue culture involves the induction, multiplication and proliferation of plant tissues or organs in a nutrient medium with or without plant growth regulators (PGRs) under aseptic conditions [10,11]. *In vitro* regeneration also maintains the clonal uniformity that is less approachable by conventional methods [12].

Micropropagation is an important biotechnological tool for the conservation of medicinally and economically important plant species which have difficulties in regeneration using conventional methods. It allows the sustainable production of thousands of plant species in a short period of time. Attempts have been made for the conservation of different African medicinal plants via *in vitro* regeneration methods [13–18].

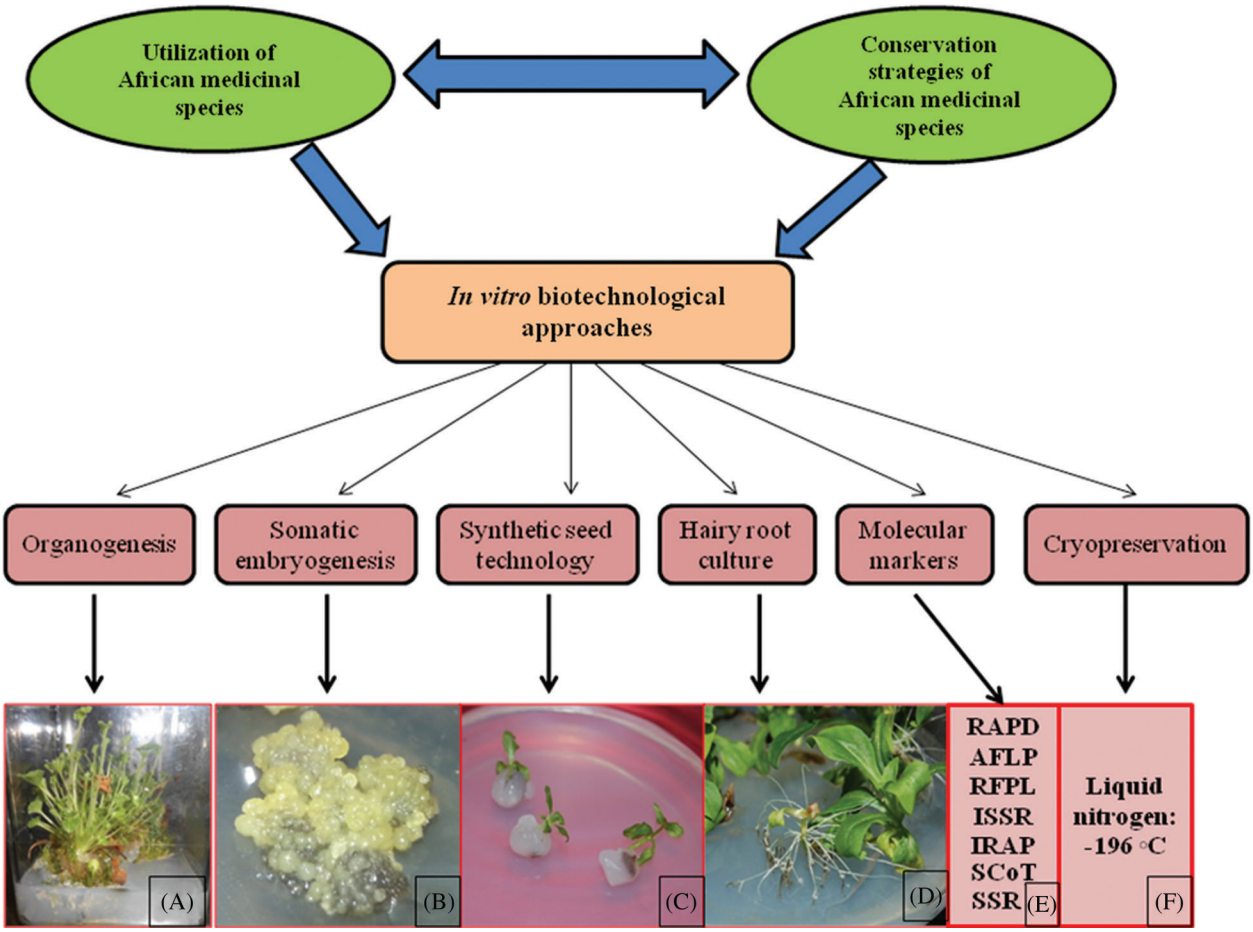

Figure 1: Showing different *in vitro* biotechnological strategies for the conservation of medicinal species. [A] *In vitro* organogenesis; [B] *In vitro* somatic embryogenesis; [C] Synthetic seed production and germination; [D] Hairy root induction by using *Agrobacterium rhizogenes*; [E] Assessment of genetic variation based on different molecular markers; [F] Cryopreservation technique with liquid nitrogen at −196°C

In vitro propagation through organogenesis and conservation strategies for *Cyranthus mackneii* have been reported [16]. MS [19] medium supplemented with increased amount of sucrose (40 g L^{-1}), 10 µM picloram, 20 µM N^6 benzyladenine (BA) and 20 µM glutamine produced a maximum number of shoots in a short period of time.

In vitro regeneration of *H. hemerocallidea* was established via corm explants [13]. The maximum number of adventitious and transplantable shoots was recorded on MS medium fortified with 10 µM *m*T. Interestingly, total of 89% of produced adventitious shoots were transplantable.

In another study by Moyo et al. [15], *Pelargonium sidoides* plants were cloned using *in vitro* tissue culture method for the conservation strategy. Different cytokinins (CKs) including *m*T and its derivatives were used to evaluate *in vitro* plant growth and found that increased number of shoots was produced from CKs treated medium. Moyo et al. [15] demonstrated that this technique had the capacity to produce thousands of virus-free *P. sidoides* clones throughout the year. Witbooi et al. [17] developed a micropropagation technique for *Agathosma betulina*, a highly valued medicinal plant in South Africa. A significantly increased number of shoots was recorded in MS medium supplemented with 2.2 µM of

2,4-D. Recently, Zhang and co-workers [18] developed an efficient protocol for *in vitro* regeneration of an important medicinal plant in Africa. *In vitro* regeneration of *Warburgia ugandensis* was established from shoot tips and petiole bases. High density of buds induced on MS medium with 0.2 mg L^{-1} Indole-3-butyric acid (IBA) and 0.8 mg L^{-1} BA [18]. Although, *in vitro* micropropagation system of few African medicinal plants has listed in Tab. 1. These findings supply the increasing research knowledge on the importance of *in vitro* regeneration via micropropagation and the role of different PGRs as reliable alternatives of conservation strategy. In addition, these findings also provide plant materials for the extraction of bioactive compounds and will help for the recovery of these important African species. In future, more advanced biotechnological approach of *in vitro* regeneration including scale-up biomass using bioreactor would be helpful for the conservation of medicinally important flora of African region as well as for the pharmaceutical industries.

Table 1: *In vitro* plant tissue culture approaches for the conservation of some African medicinal plant species used in African traditional medicine

Plant Species	Family	*In vitro* conservation method	Explant type	References
Ansellia africana	Orchidaceae	*In vitro* regeneration using encapsulated protocorm-like bodies	Nodal segment of seedlings	[20]
Agathosma betulina	Rutaceae	*In vitro* micropropagation	Seed	[17]
Albuca bracteata	Hycinthaceae	*In vitro* micropropagation	Bulblets	[21]
A. nelsonii	Hycinthaceae	*In vitro* micropropagation	Bulblets	[21]
Aloe polyphylla	Asphodelaceae	*In vitro* micropropagation	Shoot tip	[22]
Barleria greenii	Acanthaceae	*In vitro* micropropagation	Shoot tip	[23]
Brunsvigia undulata	Amaryllidaceae	*In vitro* bulblet propagation	Bulb	[24]
Crinum macowanii	Amaryllidaceae	*In vitro* micropropagation	Bulb, Floral stem	[25,26]
Cotyledon orbiculata	Crassulaceae	*In vitro* propagation via organogenesis	Leaf	[27]
Cyranthus mackneii	Amaryllidaceae	*In vitro* propagation through organogenesis and somatic embryogenesis	Leaf	[16]
Dioscorea bulbifera	Dioscoreaceae	*In vitro* tuberous induction	Tuberous root	[28]
D. hirtiflora	Dioscoreaceae	*In vitro* tuberous induction	Tuber root	[28]
Drimia robusta	Hyacinthaceae	*In vitro* regeneration via direct and indirect somatic embryogenesis	Leaf	[29]
Dierama erectum	Iridaceae	*In vitro* regeneration	Hypocotyl	[30]
Eucomis zambesiaca	Hyacinthaceae	*In vitro* micropropagation	Leaf	[31]
E. poleevansii	Hyacinthaceae	*In vitro* micropropagation	Leaf	[32]

Table 1 (continued).

Plant Species	Family	*In vitro* conservation method	Explant type	References
Harpagophytum procumbens	Pedaliaceae	*In vitro* micropropagation	Nodal	[33]; [34]
Hoslundia opposita	Lamiaceae	*In vitro* micropropagation	Node	[35]
Huernia hystrix	Asclepiadaceae	*In vitro* micropropagation	Stem	[36]
Hypoxis rooperi	Hypoxidaceae	*In vitro* micropropagation	Corm	[37]
H. colchifolia	Hypoxidaceae	*In vitro* micropropagation	Corm	[38]
H. hemerocallidea	Hypoxidaceae	Organogenesis and callus culture	Corm	[13]
H. hemerocallidea	Hypoxidaceae	*In vitro* somatic embryogenesis	Corm	[39]
Kniphofia pauciflora	Asphodelaceae	*In vitro* micropropagation	Leaf peduncle	[40]
K. leucocephala	Asphodelaceae	*In vitro* micropropagation	Meristem from lateral bud	[41]
Ledebouria revoluta	Asparagaceae	*In vitro* somatic embryogenesis and artificial seed production	Bulb	[42]
L. ovatifolia	Asparagaceae	*In vitro* micropropagation and somatic embryogenesis	Leaf	[43]
Maesa lanceolata	Primulaceae	Cryopreservation of hairy root culture	Root tip	[44]
Merwilla plumbea	Hyacinthaceae	*In vitro* somatic embryogenesis	Leaf	[45]
Mondia whitei	Apocynaceae	*In vitro* somatic embryogenesis and synthetic seed production	Leaf	[46]
Pelargonium sidoides	Geraniaceae	*In vitro* tissue culture	Shoot tip	[14]
Pelargonium sidoides	Geraniaceae	*In vitro* somatic embryogenesis	Leaf	[47]
P. sidoides	Gerniaceae	*In vitro somatic embryogenesis*	Petiole and inflorescence shoot	[48]
Podocarpus henkelii	Podocarpaceae	*In vitro* micropropagation	Axillary bud	[49]
P. elongatus	Podocarpaceae	*In vitro* micropropagation	Axillary bud	[49]
Salvia africana	Lamiaceae	*In vitro* cultivation via shoot induction	Hypocotyl	[50]
S. stenophylla	Lamiaceae	*In vitro* micropropagation	Seedlings	[51]
Searsia dentate	Anacardiaceae	*In vitro* micropropagation	Shoot tip, Node	[52]

(Continued)

Table 1 (continued).

Plant Species	Family	*In vitro* conservation method	Explant type	References
Scilla nervosa	Hyacinthaceae	*In vitro* micropropagation	Leaf	[53]
S. natalensis	Hyacinthaceae	*In vitro* micropropagation	Bulb	[54]
Sutherlandia frutescens	Fabaceae	*In vitro* micropropagation	Node	[55]
Tulbaghia simmleri	Alliaceae	*In vitro* micropropagation	Leaf, Peduncle	[56]
T. simmleri	Alliaceae	*In vitro* organogenesis and somatic embryogenesis	*In vitro* seedlings	[57]
Veltheimia bracteata	Hyacinthaceae	*In vitro* micropropagation	Leaf, Bulb	[58]
Warburgaria ugandensis	Cancellaceae	*In vitro* regeneration via callus culture	Petiole bases and shoot tip	[18]
Zygophyllum potaninii	Zygophyllaceae	Micropropagation via somatic embryogenesis	Hypocotyl or Cotyledon segment	[59]

1.3 Somatic Embryogenesis

Somatic embryogenesis (SE) is a unique developmental pathway which describes the cell differentiation process with a bipolar structure and has a potential interest for plant biotechnology [60]. The SE process provides an alternative approach for the large-scale production of plants [61,62], genetic manipulation opportunity [63], production of synthetic or artificial seeds [46,64], germplasm cryopreservation [65], and production of biological active compounds in a less period of time [66]. It is a well-documented biotechnological tool for clonal propagation and has been extensively explored in a wide range of African species [6,29,39,45,47,67–69].

In vitro regeneration system via somatic embryogenesis has been established in a number of medicinally important African plant species by us [39,47]. In *Pelargonium sidoides*, highest frequency of somatic embryos was developed from leaf in six weeks. A combination of picloram, thidiazuron (TDZ) and glutamine induced large amounts of somatic embryos [47]. Similarly, an efficient reproduction system for *P. sidoides* via somatic embryogenesis has been established from inflorescence shoots and petioles [48]. An efficient *in vitro* regeneration via somatic embryogenesis system was established in highly valuable African medicinal plant *Hypoxis hemerocallidea* from corm explants [39]. A combination of 2,4-Dicholorophenoxyacetic acid (2,4-D) and BA produced a large number of somatic embryos. The germination frequency of somatic embryos was reached up to 89%.

Baskaran et al. [29] and [45] developed somatic embryogenesis protocols for *Merwilla plumbea* and *Drimia robusta* respectively. Embryonic cell suspension cultures were used for the enhance production of somatic embryos. Recently, rapid *in vitro* propagation system via somatic embryogenesis was developed in *Mondia whitei*, an important medicinal plant extensively used in African traditional medicine (ATM) [69]. In their study, they found that a combination of 1-napthalene-acetic acid (NAA) and *meta* topolin (*m*T) significantly increases the embryogenesis and liquid media produces a higher number of somatic embryos.

A somatic embryogenesis system in a cardioprotective African medicinal plant *Ledobouria revoluta* has been developed [42]. MS medium supplemented with 3.0 mg L^{-1} TDZ, 0.75 mg/L^{-1} NAA and 1.75 mM spermidine produced high amounts of somatic embryos. However, list of *in vitro* somatic embryogenesis system of few African medicinal plants has listed in Tab. 1.

Finally, we can conclude that SE system is an innovative tool for germplasm conservation, large scale propagation of plants and provides a system for genetic transformational studies and production of bioactive compounds. In future, novel strategies in plant embryogenesis and designing new techniques at molecular levels for plant productivity and conservation will be crucial.

1.4 Cryopreservation

Plant germplasm preservation plays a crucial role in the avoidance of genetic erosion and the maintenance of biodiversity. Cryopreservation is an important biotechnological tool for long-18term maintenance and conservation of plant germplasm with low risk of loss of preserved materials as well as low cost and labour [70,71]. Plant germplasm cryopreservation (In liquid nitrogen, −196°C) has clear benefits over *in vitro* in terms of cost-effective, long term storage, improved phytosanitation and space saving [72–76]. *In vitro* cryoconservation comprises several manipulations such as freezing, thawing, recovery, culture initiation and embryo rescue, osmotic pretreatments and regeneration [77]. Successful cryopreservation is judged by ability to regenerate into complete plantlets and upon survival rate. Although, this may result in somaclonal variation or genetic change [78]. Cryopreservation technique has been successfully documented in wide range of medicinal plant species [79–85]. Cryopreservation of other plant species in Africa is extensively studied [77] however, there are occasional instances of *in vitro* cryoconservation are reported in medicinal species. Kioko et al. [86–88] discussed the use of cryopreservation for the conservation of *Warburgia salutaris*. In *Dioscorea rotundata* cryoconservation of shoot-tips was achieved by Quain et al. [80]. In *Haworthia koelmaniorum* and *limifolia*, globular somatic embryos have been cryopreserved with 50% of survival rate [89]. Although, the ability of the somatic embryos to survive cryostorage did not improve after exposure to a cryoprotectant solution such as sucrose and glycerol [89]. However, cryopreservation technique for germplasm preservation of plant diversity studied in Africa but not practised on a large-scale. In future, main effort could be directed at the cryopreservation of endangered African medicinal species that cannot be stored conventionally or do not produce seeds.

1.5 Synthetic Seed Technology

Many medicinal species occur recalcitrant seeds or desiccation–sensitive which confine the storage of these species up to months or years. An increasing interest has shown for the potential usage of synthetic seed production for the conservation of high valuable medicinal species. Synthetic or artificial seed technology is a modern application in plant biotechnology which offers an outstanding potential for the conservation of several medicinal plant species.

Synthetic seed is defined as artificially encapsulated plant propagule such as somatic embryos, shoot tips, protocorm-like bodies, nodal segments/axillary buds, hairy roots, calli or any other tissue and that possesses the ability to germinate into complete plantlets under *in vitro* or *ex vitro* conditions [20,64,90,91]. This technology delivers an easy handling, large-scale plant production, storage shipping, cost-effective and exchange of germplasm between laboratories and pharmaceutical industries without losing the viability of the plants [91–94]. Synthetic seed production has been extensively studied by several workers for a wide range of plant species including medicinal plants, vegetables, fruits, cereals, orchids, ornamental plants and other forest trees [18,64,95–98]. However, a few numbers of researches have been done on the synthetic seed production of African medicinal species. Recently, Bhattacharyya et al. [20] have established *in vitro* encapsulation based storage of *Ansellia africina* using protocorm-like

bodies (PLBs) as source of explants. In their study, synthetic seeds were maintained upto 90 days with 88.21% of survival rate. Similarly, *Ledebouria revoluta* synthetic seeds using somatic embryos were showed 57.8% germination rate after storage of 180 days [42]. In *Mondia whitei*, synthetic seed germination frequency was up to 51.6% after 50 days of storage time at 4°C [46]. A list of few African medicinal plant species for the production of synthetic seed has listed in Tab. 2. Synthetic or artificial seed technology has shown a significant potential in past few decades because of its wide range of biotechnological application in plant germplasm conservation. Therefore, synthetic seed production for the medicinal plant species would facilitate the low cost biotechnological application by the end users. However, few major issues still need to be solved for its proper commercialization. Production of high quality viable micropropagules in a cost-effective manner is remains a main problem [94,95]. During bulk alginate encapsulation reduced shoot, root growth and matrix shrinkage due to high sodium alginate concentration is also a main issue [99]. In future research, these issues need to be solved for long-term conservation practices and proper utilization of this biotechnological technique.

Table 2: Different *in vitro* biotechnological approaches for the conservation of few African medicinal plant species

Plant species	Family	Explant type	*In vitro* conservation method	Reference
Ansellia africana	Orchidaceae	Protocorm-like bodies	Synthetic seed technology; 3% sodium alginate and 100 mM Calcium chloride solution	[20]
Mondia whitei	Apocynaceae	Somatic embryo	Synthetic seed technology; 3% sodium alginate and 100 mM Calcium chloride solution	[69]; [46]
Ledebouria revoluta	Asparagaceae	Somatic embryo	Synthetic seed technology; 3% sodium alginate and 150 mM Calcium chloride solution	[42]
Khaya senegalensis	Meliaceae	Shoot tip	Synthetic seed technology; 3% sodium alginate and 100 mM Calcium chloride solution	[100]
Warburgia salutaris	Canellaceae	Seed	Cryopreservation	[88]
Dioscorea rotundata	Dioscoreaceae	Shoot tip	Cryopreservation	[80]
Pelargonium sidoides	Geraniaceae	Seedlings: hypocotyls and cotyledons	Hairy root induction (*A. rhizogenes* strain: A4T)	[101]
Gentiana scabra	Gentianaceae	*In vitro* leaf	Hairy root induction (*A. rhizogenes* strain: ATCC15834)	[102]
Nepeta pogonosperma	Lamiaceae	*In vitro* leaf and stem	Hairy root induction (*A. rhizogenes* strains: LBA9402, ATCC15834, A4, A13 and MSU440)	[103]

Table 2 (continued).

Plant species	Family	Explant type	*In vitro* conservation method	Reference
Ansellia africana	Orchidaceae	Protocorm like bodies	Assessment of genetic homogeneity by using molecular markers based study (IRAP and SCoT)	[20]
Costus pictus	Costaceae	Leaf	Assessment of genetic diversity based on ISSR and RAPD markers	[104]

1.6 Molecular Markers

In plant tissue culture, for commercial micropropagation, genetic clonal fidelity is a main concern and attained through a wide range of molecular markers. Development of molecular marker for the detection and exploitation of DNA polymorphism has been well documented in the field of plant biotechnology. Molecular markers have a potential role in the conservation of medicinal plant species, as they provide information from distribution of genetic diversity, population structure, allele frequencies and diversity at nucleotide level [105]. These are highly useful for the identification of genetic divergence as they are not manipulated by growth stage or environmental factors [91].

In *in vitro* raised culture somaclonal variation can have a major issue in determining the quality of individual cloned. Several factors such as long term culture maintenance in *in vitro* conditions, exposure to high concentration of PGRs (plant growth regulators) could be potential inducers for somaclonal variation [106,107]. A range of molecular markers such as RAPD, AFLP, ISSR, SSR and microsatellite have been generally used for the molecular stability of the *in vitro* derived plantlets [108–110]. Initiating a trend away from common DNA markers, a novel molecular marker called Start Codon Targeted Polymorphism (SCoT) has been reported which targets on short ATG start codon in plant genes [111]. It has several advantages over several random DNA markers, as it is reproducible, more stable and can be used to assess genetic diversity, DNA fingerprinting, population studies and genetic mapping in different plants. SCoT markers are directly involved in gene function and can be used in polymorphism and genotyping [112,113]. These markers have been successfully utilized for finger-printing and diversity analysis at molecular level in number of medicinal plant species [20,104–109]

Recently, Bhattacharyya et al. [20] has assessed the genetic homogeneity using IRAP and SCoT markers and revealed a high level of genetic homogeneity amongst *in vitro* raised *A. Africana*. Naik et al. [104] reported the genetic diversity in *Costus pictus* based on ISSR and RAPD markers. The ISSR analysis generated 177 loci, of which 77 were polymorphic with an average of 3.85 loci per primer, whereas, RAPD primers produced 343 loci, of which 124 were polymorphic with an average of 4.96 loci per primer. These findings conclude that these molecular markers could be utilized for the reproducing and conservation practices for wide range of African medicinal plants.

1.7 Hairy Root Culture

The term “hairy root” was first mentioned by Stewart et al. [120] and cited by Hildebrandt [121]. In past two decades, hairy root culture is emerged as an important biotechnological tool for the large-scale production of phytochemicals and has remarkable interest in the area of plant biology. Hairy roots are developed by *Agrobacterium rhizogenes*, which transfers the T-DNA of the Ri plasmid into the plant genome by infecting leaf or stem tissue of the plant [122–123]. Hairy roots are characterized by high branching, fast growth rate, high proliferation in PGR-free medium, biochemical and genetic stability and production of bioactive compounds over a long period of time [122–124]. In addition, hairy roots also

have been used for metabolic engineering and serve as a source material for regenerating transgenic plants [123–125].

For example, Colling et al. [101] established highly regenerative hairy roots from transformed *Pelargonium sidoides* using *A. rhizogenes*, to enhance the desired biologically active compounds. The authors produced high concentrations of bioactive compounds including flavonoids, coumarins and phenolic acids and concluded that the method has potential for *in vitro* conservation for this high valuable South African medicinal plant. Huang et al. [102] also established an efficient hairy root culture system in the important African medicinal plant *Gentiana scaraba*. *A. rhizogenes* mediated transformation induced hairy roots up to 21% from leaf explants. The authors concluded that hairy root culture is an important alternative biotechnological tool to produce pharmaceutically important compounds and *in vitro* conservation of this species. Similarly, in *Nepeta pogonosperna* an efficient hairy root culture system was established [103]. A significantly increase in hairy root transformation (91%) was recorded. In future, more detailed and extensive study is needed for the application of hairy root culture in wide range of African medicinal plant species. Hairy root interest in the industry will inject new avenues to scale up the diverse objectives including commercialization and *in vitro* conservation practices for African medical plant species.

2 Conclusions and Future Perspectives

Plant biotechnology is playing a critical role in the conservation and production of medicinal plant-based resources globally. Being a continent with diverse and rich plant biodiversity resources, Africa has a great opportunity to develop competent and potential plant biotechnology sectors. African medicinal plants biodiversity hotspots are unique with a potential medicinal value in ATM. Several endemic species offer various benefits as significant sources of both phytopharmaceuticals and novel chemical entities. However, conservation approach through different biotechnological tools of high value African medicinal species is much crucial. For the successful conservation and commercialization of African medicinal species any proposed detailed research must be considered in a wider context which includes sustainable supply of raw plants and conservation practices. Different biotechnological tools such as plant tissue culture, synthetic seed technology, hairy root culture, bioreactors and cryopreservation are requiring for the conservation and commercialization of endemic African species. However, very few researches have been done to document the feasibility and implementation of synthetic seed production on African medicinal plant species. Further detailed studies are required for long-term conservation practices and proper utilization of synthetic seed technology in several medicinally important African species. Hairy root culture can play a potential role in plant metabolic engineering and this strategy could also serve as *in vitro* conservation of several medicinally important African plant species. In future, novel biotechnological advancements in the application and development of *in vitro* plant tissue culture-based methods will require solving the issues such as shoot-tip necrosis and hyperhydricity. In addition, a better elucidation and understanding of the underlying molecular mechanisms regulating signaling pathways may provide solutions to several challenges encountered during plant tissue culture system.

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