

# Medicinal and Aromatic Plant Science and Biotechnology

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**Scope and target readership:** *Medicinal and Aromatic Plant Science and Biotechnology* will provide a complete analysis and understanding of all aspects of medicinal and aromatic plant science and biotechnology.

*Medicinal and Aromatic Plant Science and Biotechnology* primarily wishes to examine:

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- 2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture, bioreactor system production);
- 3) Mycorrhizal symbioses (and effects on plant physiology, productivity, reproduction and disease resistance);
- 4) Novel techniques for analysis (genetic, biochemical, biophysical);
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- 6) Pathology;
- 7) Production of secondary metabolites, organic and inorganic biochemistry, and phytochemistry; pharmacological properties;
- 8) Storage of valuable genetic material (cold-storage or cryopreservation).

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**Cover photo:** Organogenesis and somatic embryogenesis in *Bacopa monnieri*. (Left) A plantlet obtained from each embryoid further produced multiple shoots and roots. (Right) Cordate- and torpedo-shaped embryoids showing the presence of a distinct meristematic layer of cells when treated with the fluorochrome Auramine O. More details in Sharath *et al.*, pp 138-141.

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## CONTENTS

### SPECIAL SECTION: GARLIC & HEALTH

<b>R. Kamenetsky (Israel), F. Khassanov (Uzbekistan), H.D. Rabinowitch (Israel), J. Auger (France), C. Kik (The Netherlands)</b> Garlic Biodiversity and Genetic Resources	1
<b>S-J. Zheng (The Netherlands), R. Kamenetsky (Israel), L. Féréol (France), X. Barandiaran (Spain), H.D. Rabinowitch (Israel), V. Chovelon (France), C. Kik (The Netherlands)</b> Garlic Breeding System Innovations	6
<b>O. Huchette, J. Auger, I. Arnault (France), X. Barandiaran (Spain), V. Chovelon, R. Kahane (France)</b> Garlic Cultivation for High Health-Value	16
<b>M.G. Jones, H. A. Collin, A. Tregova, L. Trueman, L. Brown, R. Cosstick, J. Hughes, J. Milne, M.C. Wilkinson, A.B. Tomsett, B. Thomas (United Kingdom)</b> The Biochemical and Physiological Genesis of Alliin in Garlic	21
<b>S. Espirito Santo (The Netherlands), H-P. Keiss, K. Meyer (Germany), R. Buytenhek (The Netherlands), Th. Roos, V. Dirsch, G. Buniatian, C. Ende, J. Günther, K. Heise, D. Kellert, K. Lerche, S. Pavlica, F. Struck, E. Usbeck, J. Voigt, S. Zellmer (Germany), J.M.G. Princen (The Netherlands), A.M. Vollmar, R. Gebhardt (Germany)</b> Garlic and Cardiovascular Diseases	25
<b>M.H. Siess, A.M. Le Bon, C. Teyssier, C. Belloir, V. Singh, R. Bergès (France)</b> Garlic and Cancer	31
<hr/>	
<b>Deborah Helena Markowicz Bastos, Daniela Moura de Oliveira, Ruth Lobato Teixeira Matsumoto, Patrícia de Oliveira Carvalho, Marcelo Lima Ribeiro (Brazil)</b> <i>Yerba maté</i> : Pharmacological Properties, Research and Biotechnology	37
<b>Cheng-Jiang Ruan (China), Jaime A. Teixeira da Silva (Japan), Hua Jin, He Li, Dai-Qiong Li (China)</b> Research and Biotechnology in Sea Buckthorn ( <i>Hippophae</i> spp.)	47
<b>Damintoti Karou, Wendyame M.C. Nadembega, Lassina Ouattara, Dénise P. Ilboudo (Burkina Faso), Antonella Canini (Italy), Jean Baptiste Nikiéma, Jacques Simporé (Burkina Faso), Vittorio Colizzi (Italy), Alfred S. Traore (Burkina Faso)</b> African Ethnopharmacology and New Drug Discovery	61
<b>Fhatuwani N. Mudau, Hintsu T., Araya, Elsa S. du Toit, Puffy Soundy, Jana Olivier (South Africa)</b> Bush Tea ( <i>Athrixia phylicoides</i> DC.) as an Alternative Herbal and Medicinal Plant in Southern Africa: Opportunity for Commercialization	70
<b>Fhatuwani N. Mudau, Asanda Ngele, Phathu W. Mashela, Puffy Soundy (South Africa)</b> Seasonal Variation of Tannin Content in Wild Bush Tea	74
<b>Mogotlane I. Daniel, Fhatuwani N. Mudau, Phathu W. Mashela, Puffy Soundy (South Africa)</b> Seasonal Responses of Total Antioxidant Contents in Cultivated Bush Tea ( <i>Athrixia phylicoides</i> L.) Leaves to Fertilizer Rates	77
<b>Z. Mhinana, R.M. Coopoosamy, B. Mayekiso, M.L. Magwa (South Africa)</b> Characterization and Biological Activity of Essential Oils of Vaaltee, <i>Plecostachys serpyllifolia</i> Leaves	80
<b>Dalia B. Varghese, B.N. Sathyanarayana (South Africa)</b> Induction of Variations in Two Cultivars of <i>Bacopa monnieri</i> by Gamma Irradiation of <i>In Vitro</i> Cultures	86
<b>Donatus Ebere Okwu (Nigeria)</b> Nigerian Medicinal Plants I	90
<b>Donatus Ebere Okwu (Nigeria)</b> Nigerian Medicinal Plants II	97
<b>Donatus Ebere Okwu, Ezinne Okoro (Nigeria)</b> Phytochemical Composition of <i>Brachstegia eurycoma</i> and <i>Mucuna flagellipes</i> Seeds	103
<b>Alexander S. Lukatkin (Russia), Jaime A. Teixeira da Silva (Japan)</b> Effects of cultivation parameters of <i>Stevia rebaudiana</i> Bertoni Callus culture on Callus Proliferation	107
<b>H. Raja Naika, V. Krishna, N. Varadaraj, H.M. Kumara Swamy (India)</b> Micropropagation and Evaluation of Genetic Variability Among <i>in Vitro</i> Regenerants of <i>Naravelia zeylanica</i> (L.) DC.	112

<b>K. Padmalatha, M.N.V. Prasad (India)</b> Inter and Intrapopulation Genetic Diversity of <i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz, an Endangered Medicinal Plant, by RAPD Analysis	118
<b>B.N. Maruthi Prasad, B.N. Sathyanarayana, Gowda Balakrishna, R. Sharath (India)</b> <i>In Vitro</i> Regeneration of Drug-Yielding Tuber Crop <i>Chlorophytum borivilianum</i>	124
<b>B.N. Maruthi Prasad, B.N. Sathyanarayana (India), Jaime A. Teixeira da Silva (Japan), R. Sharath, Gowda Balakrishna (India)</b> Regeneration in <i>Chlorophytum borivilianum</i> through Somatic Embryogenesis	128
<b>N.L. Raju, M.N.V. Prasad (India)</b> Cytokinin-Induced High Frequency Shoot Multiplication in <i>Celastrus paniculatus</i> Willd., a Red Listed Medicinal Plant	133
<b>R. Sharath, V. Krishna, B.N. Sathyanarayana, B.N. Maruthi Prasad, B.G. Harish (India)</b> High Frequency Regeneration through Somatic Embryogenesis in <i>Bacopa monnieri</i> (L.) Wettst., an Important Medicinal Plant	138
<b>Neeta Shrivastava, Tejas Patel (India)</b> <i>Clerodendrum</i> and Healthcare: An Overview	142
<b>Anshu Srivastava, Avani Kothari, M. Rajani, Harish Padh, Neeta Shrivastava (India)</b> Sustainable Production of a Therapeutically Important Tree ( <i>Holarrhena antidysenterica</i> ) of India	151

<b>K. Padmalatha, M.N.V. Prasad (India)</b> Inter and Intrapopulation Genetic Diversity of <i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz, an Endangered Medicinal Plant, by RAPD Analysis	118
<b>B.N. Maruthi Prasad, B.N. Sathyanarayana, Gowda Balakrishna, R. Sharath (India)</b> <i>In Vitro</i> Regeneration of Drug-Yielding Tuber Crop <i>Chlorophytum borivilianum</i>	124
<b>B.N. Maruthi Prasad, B.N. Sathyanarayana (India), Jaime A. Teixeira da Silva (Japan), R. Sharath, Gowda Balakrishna (India)</b> Regeneration in <i>Chlorophytum borivilianum</i> through Somatic Embryogenesis	128
<b>N.L. Raju, M.N.V. Prasad (India)</b> Cytokinin-Induced High Frequency Shoot Multiplication in <i>Celastrus paniculatus</i> Willd., a Red Listed Medicinal Plant	133
<b>R. Sharath, V. Krishna, B.N. Sathyanarayana, B.N. Maruthi Prasad, B.G. Harish (India)</b> High Frequency Regeneration through Somatic Embryogenesis in <i>Bacopa monnieri</i> (L.) Wettst., an Important Medicinal Plant	138
<b>Neeta Shrivastava, Tejas Patel (India)</b> <i>Clerodendrum</i> and Healthcare: An Overview	142
<b>Anshu Srivastava, Avani Kothari, M. Rajani, Harish Padh, Neeta Shrivastava (India)</b> Sustainable Production of a Therapeutically Important Tree ( <i>Holarrhena antidysenterica</i> ) of India	151

**SPECIAL SECTION: GARLIC**

**R. Kamenetsky (Israel), F. Khassanov (Uzbekistan), H.D. Rabinowitch (Israel), J. Auger (France), C. Kik (The Netherlands)** Garlic Biodiversity and Genetic Resources (pp 1-5)

**ABSTRACT**

**Invited Mini-Review:** Garlic clones exhibit a wide variation in vegetative traits, flavor and pungency; bolting capacity, and fertility. Cultivar characteristics differ considerably with the location of cultivation, and climate has a significant impact on garlic bulbing, florogenesis and flavor. All cultivated garlic clones are sterile, thus an increase in genetic variation is possible only via random or induced mutations, somaclonal variation and introduction of new genetic variation using modern molecular techniques. Conservation of garlic germplasm began about 30 years ago. Since then, field genebanks were established in Germany, Israel, Poland, Czech Republic, Russia, and the USA. Yet, only limited effort has been invested in systematic collection and preservation of wild *Allium* species of potential economic value. Isozyme and molecular analyses show considerable genetic diversity within the garlic species complex, and AFLP technology facilitates the evaluation of genetic diversity in garlic collections. Biochemical and molecular studies suggest that the highest level of heterogeneity occurs within the Central Asian gene pool, which may contain genes of interest for future use in genetic studies, as well as for plant improvement programs. Until recently, however, this genepool did not attract much attention by researchers from outside the region. An international effort aimed at immediate collection and preservation of this heritage is imperative. It should be followed by an intensive evaluation effort and accompanied by substantial preservation projects, to halt the rapid and irreversible erosion of the Central Asia genepool of garlic landraces and wild populations.

**S.-J. Zheng (The Netherlands), R. Kamenetsky (Israel), L. Féréol (France), X. Barandiaran (Spain), H.D. Rabinowitch (Israel), V. Chovelon (France), C. Kik (The Netherlands)** Garlic Breeding System Innovations (pp 6-15)

**ABSTRACT**

**Invited Review:** This review outlines innovative methods for garlic breeding improvement and discusses the techniques used to increase variation like mutagenesis and *in vitro* techniques, as well as the current developments in florogenesis, sexual hybridization, genetic transformation and mass propagation. Sexual sterility of garlic reduces its potential for improvement of desired traits. Restoring fertility in this crop, which has been vegetatively propagated for millenia, provides new genetic possibilities for breeding purposes and/or genetic studies. In this context the recent developments on the manipulation of garlic florogenesis are discussed and it is shown that specific environmental conditions might allow for fertility restoration and seed production in bolting garlic. Furthermore the introduction of *Agrobacterium*-mediated and biolistic gene transfer systems in garlic, a species known for its recalcitrant behaviour in *in vitro* culture, are reviewed. Attention is paid to the development of a high quality callus year-round production method for transformation. Also the first garlic transgenics resistant to beet armyworm (*Spodoptera exigua*) and herbicides will be introduced. Garlic friable embryogenic calluses are of pivotal importance for the establishment of cell suspension cultures needed for rapid multiplication of elite garlic genotypes. The development of these suspension cultures are discussed in this review. It is shown that a large number of somatic embryos (potentially  $8 \times 10^9$  to  $10^{11}$ ) can be produced annually, from a single clove, for each current variety and that the conversion into plantlets amounts approximately 50%.

**O. Huchette, J. Auger, I. Arnault (France), X. Barandiaran (Spain), V. Chovelon, R. Kahane (France)** Garlic Cultivation for High Health-Value (pp 16-20)

**ABSTRACT**

**Invited Mini-Review:** Garlic (*Allium sativum* L.) is well-known for presenting numerous health benefits linked to the high amount of organo-sulfur compounds, especially aliin, produced in the bulbs. The accumulation of aliin and its precursors in garlic is dependent on both genetic factors and environmental conditions in which the plants are cultivated. Indeed, different organo-sulfur compounds profiles were obtained for several accessions originating from Central Asia when grown in natural conditions in two different climatic contexts. Field trials carried out on three commercial varieties grown under two different

climatic conditions of Western Europe confirmed this observation and suggested an important role of the cropping temperature, soil status and water stress conditions. Experiments performed under fully controlled conditions, *in vitro* and in the greenhouse, showed that sulfur fertilisation as well as light conditions could also have an impact on the organo-sulfur composition of garlic bulbs. However, the interaction with the genotype has to be considered as spring-varieties and winter-varieties did not react the same way to variations in fertilising and environmental conditions. In the mean time, the effect of increasing mineral sulfur should be considered in relation to other mineral fertilising components, like nitrogen and selenium, as well as to other sulfur sources, from the soil and from the atmosphere, as garlic seems to be able to use atmospheric sulfur. Multiple factors affect alliin accumulation in garlic, so its quality for human health. These factors should be considered when growing garlic for flavour or therapeutic value.

**M.G. Jones, H. A. Collin, A. Tregova, L. Trueman, L. Brown, R. Cosstick, J. Hughes, J. Milne, M.C. Wilkinson, A.B. Tomsett, B. Thomas (United Kingdom)** The Biochemical and Physiological Genesis of Alliin in Garlic (pp 21-24)

#### ABSTRACT

**Invited Mini-Review:** The health-giving properties of garlic are thought to be primarily derived from the presence and subsequent breakdown of the alk(en)ylcysteine sulphoxide (CSO), alliin and its subsequent breakdown to allicin. Two biosynthetic pathways have been proposed for CSOs, one proceeds from alkylation of glutathione through  $\gamma$ -glutamyl peptides to yield S-alkyl cysteine sulphoxides while the alternative is direct thioalkylation of serine followed by oxidation to the sulphoxide. Addition of allyl thiol to differentiating garlic tissue cultures resulted in the appearance of detectable levels of both S-allyl cysteine and alliin and also demonstrated that S-allyl-cysteine was oxidised stereospecifically to (+)-alliin by garlic tissue cultures, indicating the presence of a specific oxidase in the cells. Although these reports provide good evidence that S-allyl cysteine can be converted to alliin by garlic tissue cultures, it does not indicate whether  $\gamma$ -glutamyl-S-allyl cysteine or S-allyl cysteine is the substrate for oxidation *in vivo*. Garlic contains several cysteine synthases and at least one has the capacity to synthesise alliin. Studies on alliin distribution during bulb development are consistent with a process within which CSOs are synthesised primarily in leaves and translocated to garlic clove tissues during bulb development. Alliinase is the enzyme that initiates the conversion of alliin to allicin and its derivatives. Although multiple sequences can be identified within a single variety, the expression of the dominant isoform in both clove and leaf tissue does not vary significantly with stage of development, consistent with alliinase genes being constitutively expressed.

**S. Espirito Santo (The Netherlands), H-P. Keiss, K. Meyer (Germany), R. Buytenhek (The Netherlands), Th. Roos, V. Dirsch, G. Buniatian, C. Ende, J. Günther, K. Heise, D. Kellert, K. Lerche, S. Pavlica, F. Struck, E. Usbeck, J. Voigt, S. Zellmer (Germany), J. M. G. Princen (The Netherlands), A.M. Vollmar, R. Gebhardt (Germany)** Garlic and Cardiovascular Diseases (pp 25-30)

#### ABSTRACT

**Invited Mini-Review:** Based on extensive use in traditional medicine garlic has been claimed to prevent or mitigate cardiovascular disease. Lipid-lowering with particular emphasis on cholesterol and anti-inflammatory effects were considered as major mechanisms. This view, however, was challenged by an increasing number of clinical studies denying significant influence of various garlic preparations on these functions. Therefore, one of the aims of the European Garlic & Health project was the brought evaluation of the influence of well-defined garlic powders or of garlic-derived organosulphur compounds on surrogate markers of cardiovascular disease by *in vitro* assays, animal studies and a human intervention study. The results are briefly reviewed herein together with work published in parallel by other investigators. In conclusion, the investigations within the Garlic & Health project as well as the majority of independent studies provided compelling evidence against a beneficial influence of garlic powders or garlic constituents on risk factors and pathological aspects of cardiovascular disease in animals and humans.

**M.H. Siess, A.M. Le Bon, C. Teyssier, C. Belloir, V. Singh, R. Bergès (France)** Garlic and Cancer (pp 31-36)

#### ABSTRACT

**Invited Mini-Review:** This review summarizes current knowledge on the anticarcinogenic properties of garlic. Collectively epidemiologic studies – mostly case-control studies – provide strong evidence that garlic consumption reduces the risk of cancer especially, the risk of gastric and colon cancer. Furthermore many experimental studies demonstrate that organosulfur compounds (OSCs) and garlic extracts can prevent or slow down the carcinogenic process induced by a variety of chemical

carcinogens in animals. Garlic and OSCs have been shown to be active during all the stages of carcinogenesis. Several mechanisms have been proposed to explain the cancer-preventive effects of garlic and related OSCs. These include inhibition of the carcinogen formation, antioxidant action, inhibition of genotoxicity and/or mutagenicity of carcinogenic agents, modulation of the carcinogen metabolizing enzymes, effect on cell proliferation and apoptosis, and inhibition of angiogenesis. While research on garlic is promising, the outcome can not be directly translated in specific recommendations for garlic consumption. However this outcome must sustain the general recommendation of consuming daily a variety of vegetables and fruits.

**Deborah Helena Markowicz Bastos, Daniela Moura de Oliveira, Ruth Lobato Teixeira Matsumoto, Patrícia de Oliveira Carvalho, Marcelo Lima Ribeiro (Brazil)** *Yerba maté: Pharmacological Properties, Research and Biotechnology* (pp 37-46)

#### ABSTRACT

**Invited Review:** *Maté* (*Ilex paraguariensis* St. Hilaire) is a plant originary from the subtropical region of South America, and present in the South of Brazil, North of Argentina, Paraguay and Uruguay. *Maté* beverages have been widely consumed for hundreds of years as infusions popularly known as *chimarrão*, *tererê* (both from green dried mate leaves) and *maté* tea (roasted mate leaves). The popular medicine and the herbalists recommend it for arthritis, migraines, constipation, rheumatism, hemorrhoids, obesity, fatigue, retention of liquid, hypertension, and for stomach and liver diseases. Recently published research has proved scientifically the actions of *maté* which may explain many of the cited pharmacologic effects such as its chemopreventive activity, choleric effect and intestinal propulsion, vasodilatation effect, inhibition of the glycation and as a free radical scavenger. *Maté* beverages are rich in many bioactive compounds such as caffeine, phenolic compounds (mainly phenolic acids) and saponins. This review discusses the latest scientific data on *maté* physiological properties and their correlation with the bioactive compounds present in the *maté* leaves and aqueous infusions.

**Cheng-Jiang Ruan (China), Jaime A. Teixeira da Silva (Japan), Hua Jin, He Li, Dai-Qiong Li (China)** *Research and Biotechnology in Sea Buckthorn (*Hippophae* spp.)* (pp 47-60)

#### ABSTRACT

**Invited Review:** Sea buckthorn (*Hippophae* spp.), which holds environmental importance and is a new commercial berry crop, is naturally distributed from Asia to Europe. Its berries are rich in phytonutrients and bioactive substances, products often used for medical and pharmaceutical purposes. This review summarizes recent research and biotechnology in this genus, including the improvement of ecological environments, chemical composition and nutritional values, medicinal uses and sea buckthorn products, selection and breeding for improving yield and quality, tissue culture, genetic relationship and diversity at the molecular level (isozymes, RAPD, AFLP, ISSR, cpDNA and ITS). Good adaptability, fast-growth, protection against wind and preventing sand from drifting, use in soil and water conservation and improvement of soil by efficient nitrogen fixation make sea buckthorn to be planted at a large scale in China and other countries. Sea buckthorn contains different kinds of nutrients and bioactive substances such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids, elemental components, and others. These components vary substantially among populations, origins or subspecies, and their presence is more important for the health of individual. The clinical trials and scientific studies during the 20<sup>th</sup> century confirm the medicinal and nutritional value of sea buckthorn. The present review describes some areas of research that have been important points, for example in cancer therapy, cardiovascular diseases, treatment of gastrointestinal ulcers, skin disorder and as a liver protective agent. Mutation, crossing and conventional breeding were widely used in its selection and in breeding for improving yield and quality. Based on the differences in agronomic traits among two different subspecies, crosses between *H. rhamnoides* ssp. *sinensis* (good adaptability, more thorns and small fruits) and *H. rhamnoides* ssp. *mongolica* (few thorns, big fruits and a long fruit stalk) yielded several fine hybrids. The genetic relationship and diversity based on molecular approaches in sea buckthorn provides a potential for improving the breeding strategy. Dried-shrink disease (*Plowvigneia hippophaeos*) and drought are the main problems restricting the sustainable development of sea buckthorn, and molecular biotechnology may be able to provide for a solution to overcome these problems and limitations. Plants have been successfully regenerated using different organs on different media, and regularly clonal propagation using hardwood and softwood cuttings in the greenhouse and field has been used. Currently, we are cloning genes related to resistance to dried-shrink disease, and expecting to get fine resistant lines by genetic transformation and marker-assisted selection.

**Damintoti Karou, Wendyame M. C. Nadembega, Lassina Ouattara, Dénise P. Ilboudo (Burkina Faso), Antonella Canini (Italy), Jean Baptiste Nikiéma, Jacques Simpore (Burkina Faso), Vittorio Colizzi (Italy), Alfred S. Traore (Burkina Faso)** African Ethnopharmacology and New Drug Discovery (pp 61-69)

#### ABSTRACT

**Invited Review:** This review covers the general area of disease and problems such as malaria, bacterial and fungal infections, free radical damage and the decline in the immune system. After a brief history of ethnopharmacology, we discuss the scientific approaches that have been used in the screening of medicinal plants and identify some African medicinal plants that are used successfully in the treatment of these diseases. It is evident that African medicinal plants are continuously being screened for their pharmacological properties and many interesting results with crude extracts have occasionally been obtained through the isolation and identification of the active principles. However, as a source of new drugs, African medicinal plants are understudied, considering the high percentage of plants not yet screened for their biochemical composition or for their pharmacological properties.

**Fhatuwani N. Mudau, Hintsu T., Araya, Elsa S. du Toit, Puffy Soundy, Jana Olivier (South Africa)** Bush Tea (*Athrixia phylicoides* DC.) as an Alternative Herbal and Medicinal Plant in Southern Africa: Opportunity for Commercialization (pp 70-73)

#### ABSTRACT

**Invited Mini-Review:** Bush tea (*Athrixia phylicoides* DC.) is a plant indigenous to South Africa and is commonly known as bushman's tea (English); Boesmanstee (Afrikaans); Icholocholo, itshelo, umthsanelo (Zulu). It is a herbaceous plant that belongs to the Asteraceae family. People of South Africa have predominantly used it throughout history as a medicinal tea, for cleansing or purifying the blood, treating boils, headaches, infested wounds, cuts and the solution may also be used as a foam bath. The foam bath brew can also be used as lotion dabbed on to the boil, skin eruption or cut. The tea is also excellent for coughs and colds and as a gargle for throat infections and loss of voice. It is also believed to have aphrodisiac properties in some parts of southern Africa. The leaves contain 5-hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol as a new flavonol which is a recently discovered flavonoid. Today, herbal tea cultivation is a big business in many parts of the world. South Africa is well known for its indigenous herbal tea production such as honey bush, rooibos and bush tea. There are increasing demands for such products, especially in the light of growing health consciousness worldwide. This necessitated the establishment and revival of bush tea as a healthy herbal beverage alternative to caffeine-containing beverages. Current research suggests that there is a great need to standardize processing methods and production protocols for consistent quality.

**Fhatuwani N. Mudau, Asanda Ngele, Phathu W. Mashela, Puffy Soundy (South Africa)** Seasonal Variation of Tannin Content in Wild Bush Tea (pp 74-76)

#### ABSTRACT

**Short Communication:** The objective of the study was to determine seasonal variation in the concentration of tannins in wild bush tea (*Athrixia phylicoides* L.) leaves. The  $\text{BuOH-HCl-Fe}^{\text{III}}$  reagent was used to analyze condensed tannins, whereas hydrolysable tannin was analyzed using potassium iodate. The absorbance was measured using a spectrophotometer. Significant seasonal variations in both condensed and hydrolysable tannins in leaf tissue occurred. The highest concentrations of condensed tannins were found in autumn (4.82%) compared with winter (2.44%), spring (2.66%) and summer (3.04%). The hydrolysable tannins were lowest during summer (0.10%) compared with autumn and winter (0.14%) and spring (0.13%). The data obtained from this suggest that winter and autumn are the best times to harvest bush tea to maximize tannin contents of bush tea leaves.

**Mogotlane I. Daniel, Fhatuwani N. Mudau, Phatu W. Mashela, Puffy Soundy (South Africa)** Seasonal Responses of Total Antioxidant Contents in Cultivated Bush Tea (*Athrixia phylicoides* L.) Leaves to Fertilizer Rates (pp 77-79)

#### ABSTRACT

**Short Communication:** The objective of this study was to determine the seasonal effects of nitrogen (N), phosphorus (P) and potassium (K) fertilizer on total antioxidant content of cultivated bush tea (*Athrixia phylicoides* L.) leaves. Three independent trials of N, P and K were conducted per season i.e. autumn, winter, spring and summer. Treatments consisted of 0, 100, 200, 300, 400 or 500 kg/ha N, P or K replicated four times in a randomized complete block design. At harvest, leaves were freeze dried and ground for total antioxidant using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Results of this study demonstrate

that regardless of season, the application of N, P and K fertilizers quadratically increased total antioxidant content, with most of the increase occurring at 0-300 N, 300 P and 100 K kg/ha. Therefore, for improved total antioxidant contents in cultivated bush tea leaves, 300 N, 300 P and 100 K kg/ha N is recommended.

**Z. Mhinana, R.M. Cooposamy, B. Mayekiso, M.L Magwa (South Africa)** Characterization and Biological Activity of Essential Oils of Vaaltee, *Plecostachys serpyllifolia* Leaves (pp 80-85)

#### ABSTRACT

**Original Research Paper:** *Plecostachys serpyllifolia* (Berg.) Hilliard is commonly termed Hottentot tea, trailing licorice or *Vaaltea*. It has been used as a remedy for cold and pains associated with the chest. The yield of essential oil extracted from 500 g of leaves of the plant by steam distillation was approximately 1.3% (v/w). The oil was analysed by GC-MS analysis. According to the analysis  $\beta$ -phellandrene was the dominant component followed by  $\alpha$ -thujone. Extracts were positive against most gram positive bacteria tested. Extracts showed a range of inhibitory activity against fungal species tested. The methanol extract provided 100% inhibition against *Thamnidium elegans*, whereas the acetone extract showed 100% inhibition on *Rhizopus stolonifer*. This ability of inhibition of both bacterial and fungal species concurs with the broad spectrum antimicrobial activity of this plant species and could be attributed as to why *P. serpyllifolia* is often used in treatment of various diseases as a herbal or traditional remedy.

**Dalia B. Varghese, B.N. Sathyanarayana (South Africa)** Induction of Variations in Two Cultivars of *Bacopa monnieri* by Gamma Irradiation of *In Vitro* Cultures (pp 86-89)

#### ABSTRACT

**Original Research Paper:** *In vitro* cultures of nodal segments and calli of two cultivars of *Bacopa monnieri* viz., 'Pragyashakthi' and 'Calcutta Local' were subjected to gamma irradiation (30 Gy to 100 Gy for nodal segments and 30 Gy to 80 Gy for calli) with a view to induce variations and to assess the sensitivity of the cultivars to the mutagen treatment. The mutagenic treatments generated substantial variability in the morphological characters of the regenerated plantlets. Variation in leaf morphology induced by the mutagen was observed in plantlets of both the cultivars, while distinctly dwarf plantlets were observed only in case of 'Calcutta Local' with a high gamma ray dose of 100 Gy. The results indicate that both cultivars respond differently towards mutagenic treatments, visible from the frequency of mutations induced. Nodal segments of 'Calcutta Local' showed a higher mutation frequency while, in the case of calli, 'Pragyashakthi' was more sensitive to gamma irradiation. Such variation between cultivars depending on the type of explant used has previously never been reported. Variation was also observed with regard to the bacoside (active principle) content of the plantlets derived from the gamma-irradiated explants. Variants with high content of bacoside (to a maximum of 3.03%) could be isolated from the fourth subculture in the case of both the cultivars. The encouraging results obtained from the present investigation emphasize the efficiency of *in vitro* mutation induction for the improvement of this important medicinal plant.

**Donatus Ebere Okwu (Nigeria)** Nigerian Medicinal Plants I (pp 90-96)

#### ABSTRACT

**Invited Mini-Review:** Nigerian medicinal plants (*Aspilia africana*, *Bryophyllum pinnatum*, *Garcinia kola*, *Spondias mombim* and *Uvaria chamea*) are used in herbal medicine to cure diseases, protect and regenerate liver cells, prevent alcoholic liver damage, viral liver damage and toxic liver damage. The plants contain proteins, carbohydrates, vitamins, minerals and phytochemicals. The phytochemicals comprise flavonoids, phenolic compounds, alkaloids, tannins and saponins. The flavonoids and phenolic compounds present in the plants exhibit antioxidant, anti-inflammatory, anti-tumor, anti-hepatotoxic, anti-microbial and anti-ulcer properties. Several epidemiological studies have suggested that micro-chemicals present in plants could be the most desirable agents for the prevention and /or intervention of human cancer. These plants have been extensively used to prevent colic disorders, suppress cough, heal injuries and used in the treatment of cirrhosis and hepatitis. There is a need to pool and conserve the genetic wealth available in these plants at a national and international level. The conservation of the genetic diversity of these plants is very essential and vital for their future use. Emphasis was placed on these plants in view of their popular use in herbal medicine. The importance of the chemical constituents available in these plants is discussed with respect to their role in herbal medicine in Nigeria.

**Donatus Ebere Okwu (Nigeria)** Nigerian Medicinal Plants II (pp 97-102)

#### ABSTRACT

**Invited Mini-Review:** *Aframomum meleguata*, *Dennettia tripetala*, *Monodora myristica*, *Piper guineense*, *Tetrapleura tetraptera* and *Xylopia aethiopica* are some of the Nigerian medicinal spices. These plants are rich sources of phytochemicals, vitamins, carbohydrates and minerals and contain phenolic compounds, flavonoids, alkaloids, saponins and tannins. Many flavonoids present in the plants exhibit antioxidant, antimicrobial and oxytocic properties. These plants are used in herbal medicine to regulate irregular menstruation or dysmenorrhea (menstrual pain) and help- to normalize menstrual cycle. Other medicinal uses include inhibition of tumor growth, anti-inflammatory, antispasmodic and antiallergic action. The plants showed uterine contraction activity when administered to animals. These medicinal plants could therefore have side effects of inducing abortion when used during pregnancy.

**Donatus Ebere Okwu, Ezinne Okoro (Nigeria)** Phytochemical Composition of *Brachystegia eurycoma* and *Mucuna flagellipes* Seeds (pp 103-106)

#### ABSTRACT

**Original Research Paper:** *Brachystegia eurycoma* and *Mucuna flagellipes* seeds, which are commonly used as soup condiment and flavoring agents in South Eastern Nigeria were analyzed for their chemical composition. Phytochemical studies revealed the presence of bioactive compounds comprising flavonoids (2.24-6.23 mg.100 g<sup>-1</sup>), alkaloids (0.50-0.77 mg.100 g<sup>-1</sup>), phenolic compounds (0.05-0.07 mg.100 g<sup>-1</sup>), saponins (0.46-0.79 mg.100 g<sup>-1</sup>) and tannins (0.13-0.16 mg.100 g<sup>-1</sup>). The protein, carbohydrate, lipid and fiber content were 7.0-10.50%, 71.74-72.02%, 4.20-8.34% and 3.76-6.60%, respectively. The seeds are a good source of water-soluble vitamins, ascorbic acid (4.84-10.12 mg.100 g<sup>-1</sup>), thiamine (0.05-0.13 mg.100 g<sup>-1</sup>), riboflavin (0.13-0.22 mg.100 g<sup>-1</sup>) and niacin (0.19-0.36 mg.100 g<sup>-1</sup>). Both plant samples are good sources of minerals such as Ca, P, K, Mg, Na, Zn, Fe and Cu, while Pb and Co were not detected.

**Alexander S. Lukatkin (Russia), Jaime A. Teixeira da Silva (Japan)** Effects of cultivation parameters of *Stevia rebaudiana* Bertoni Callus culture on Callus Proliferation (pp 107-111)

#### ABSTRACT

**Original Research Paper:** Callus culture of *Stevia rebaudiana* Bertoni induced from apical buds of sterile plants was the focus of this investigation. The most intensive growth of *Stevia* callus (from 17 to 19 mg/week) occurred on modified Murashige and Skoog (MS) medium supplemented with 1-1.5 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.1 mg/l 6-benzylaminopurine (BAP). The effectiveness of callus growth is equal in both the light and dark. Adventitious roots could be obtained from callus masses on MS medium supplemented with 1 mg/l  $\alpha$ -naphthylene acetic acid, while shoots formed from callus on MS medium supplemented with 0.1 mg/l 2,4-D + 0.5 mg/l BAP. Glycosides, which are the important medicinally-important secondary metabolites from *Stevia*, were organoleptically detected in root- and shoot-regenerating calluses. Organogenesis may be an indispensable requirement for glycoside synthesis.

**H. Raja Naika, V. Krishna, N. Varadaraj, H. M. Kumara Swamy (India)** Micropropagation and Evaluation of Genetic Variability Among *in Vitro* Regenerants of *Naravelia zeylanica* (L.) DC. (pp 112-117)

#### ABSTRACT

**Original Research Paper:** An efficient protocol was developed for the regeneration of plantlets via direct and indirect organogenesis from stem nodal segments of *Naravelia zeylanica* (L.) DC. (Ranunculaceae). The interaction of 6-benzyl aminopurine (BAP) with  $\alpha$ -naphthalene acetic acid (NAA) in Murashige and Skoog (1962) medium induced a varied morphological response. A maximum of 17.6  $\pm$  1.5 shoots per explant were obtained in the presence of 4.0 mg l<sup>-1</sup> BAP and 0.6 mg l<sup>-1</sup> NAA on MS medium. A higher concentration of BAP (5.0- 8.0 mg l<sup>-1</sup>) and NAA (1.0-2.0 mg l<sup>-1</sup>) favoured callogenesis while a lower range (2.0-3.0 mg l<sup>-1</sup> BAP and 0.25-5.0 mg l<sup>-1</sup> NAA) induced adventitious shoot buds and 11.7  $\pm$  1.3 plantlets were obtained from stem calli. The excised microshoots rooted well on MS basal medium without plant growth regulators. Morphologically direct organogenesis regenerants were similar to *in vivo* plants while the callus regenerants exhibited abnormal development after acclimatization. The survival rate of the plantlets derived from direct organogenesis was 96% whereas in callus regenerants it declined to 72%. Random Amplified Polymorphic DNA (RAPD) markers were employed to analyze the level of genetic variation among the plantlets of direct and indirect organogenesis. Five groups of Operon primers – B 11-20, C

01-20, D 01-10, E 01-10, and F 11-20 – were screened, from which six were selected, among which OPF-17 and OPF-19 gave clear and distinguishable bands. A dendrogram was constructed using Euclidean distances by Ward's method. RAPD marker fingerprinting allowed a rapid assessment of the level of genetic variation among the regenerants.

**K. Padmalatha, M.N.V. Prasad (India)** Inter and Intrapopulation Genetic Diversity of *Rauvolfia serpentina* (L.) Benth. ex Kurz, an Endangered Medicinal Plant, by RAPD Analysis (pp 118-123)

#### ABSTRACT

**Original Research Paper:** The present study is the first report of inter and intrapopulation diversity analysis using RAPD markers in *Rauvolfia serpentina*, an endangered, commercially and medicinally very important plant, collected from six locations of Andhra Pradesh (AP), India. In the inter-population analysis screening with forty primers revealed 263 scorable polymorphic markers out of the 379 total markers. A high proportion of polymorphism i.e., about 70% was found with 23 unique markers. Cluster analysis based on the Dice coefficient showed two major groups indicating that in cross-pollinated plants, a high level of differentiation occurs among existing accessions. The grouping of these accessions was independent of the geographical distance. The significant variation in the accession collected from Sukumamidi when compared to other accessions needs to be further investigated. Intrapopulation diversity in *R. serpentina* collected from Dulapally mostly exhibited monomorphism, which proved the maintenance of homogeneity. Hence the results of the present study can be seen as a starting point for future research on the population and evolutionary genetics of this species.

**B.N. Maruthi Prasad, B.N. Sathyanarayana, Gowda Balakrishna, R. Sharath (India)** *In Vitro* Regeneration of Drug-Yielding Tuber Crop *Chlorophytum borivillianum* (pp 124-127)

#### ABSTRACT

**Original Research Paper:** *Chlorophytum borivillianum* Sant et Fernard., commonly known as safed musli is an important medicinal herb of commercial importance. Tuberos roots of *C. borivillianum* are used as a health tonic, vitalizer, and aphrodisiac and are used as a herbal substitute for chemical-based sildenafil citrate. The natural regeneration of this herb is through tuberos roots that have become scarce in nature because of indiscriminate collection of wild material. Further, poor seed set and germination associated with this crop has added to the problems of propagation. A shoot bud with a part of the stem disc of *C. borivillianum* were cultured on MS (Murashige and Skoog 1962) medium with different concentrations of BAP (6-benzyl amino purine) at 4.54, 5.11 and 5.68 mg l<sup>-1</sup>, NAA (α-naphthalene acetic acid) at 0.93, 1.86 and 2.79 mg l<sup>-1</sup> and hormone-free medium (control). Shoot proliferation was maximum (17.82) with 5.11 mg l<sup>-1</sup> BAP alone when compared with other treatments. Among the nutrient media screened viz., MS, Gamborg's (B5), Schenk and Hilderbrandt (SH), and White's (Wh), when supplemented with 5.11 mg l<sup>-1</sup> BAP, MS medium was found to be best followed by SH for mean number and length of shoots. In separate trials, direct regeneration of shoots from the basal leaf sheath following application of thidiazuron at 0.5 mg l<sup>-1</sup> was obtained, the first report of its kind. In medium with both half and full strength of both macro and micronutrients the mean number of roots per shoot was quite high (34.2 in half strength and 38 in full strength respectively). Reducing the mineral concentration to half the normal, strength of MS media supplemented with 2 mg l<sup>-1</sup> IBA produced a maximum number (29.2) and length (3.4 cm) of roots. Encouraging results were not obtained when different concentrations of NAA and IAA were used for root induction. However, NAA at 3 mg l<sup>-1</sup> induced a few roots showing reduced root length (0.5 cm); these roots on prolonged incubation in the dark for 75 days resulted in *in vitro* tuberization. Plantlets obtained *in vitro* were best hardened on a vermicompost: sand (1:1) mixture with 70% survival.

**B.N. Maruthi Prasad, B.N. Sathyanarayana (India), Jaime A. Teixeira da Silva (Japan), R. Sharath, Gowda Balakrishna (India)** Regeneration in *Chlorophytum borivillianum* through Somatic Embryogenesis (pp 128-132)

#### ABSTRACT

**Original Research Paper:** *Chlorophytum borivillianum*, an important medicinal plant belonging to the Liliaceae family is valued for its dried fasciculated roots, which have aphrodisiac properties and also form an important ingredient of various herbal medicines. A high frequency regeneration protocol for rapid multiplication of *C. borivillianum* through the induction of somatic embryos was attempted. Microshoots along with a part of the stem disc were cultured on Murashige and Skoog (MS) medium supplemented with a low concentration range (0.25, 0.5, 0.75 and 1.0 mg l<sup>-1</sup>) of 2,4-Dichlorophenoxyacetic acid (2,4-D) alone, and in combination with different concentrations of cytokinins like 6-benzylaminopurine (BAP) and kinetin (Kn). One hundred per cent callus induction and embryogenic response was noticed at all low concentrations of 2,4-D. The number of days

required for callusing (29.8), the number of embryos (67.2), the diameter of embryogenic callus (2.28 cm) was more with  $1.0 \text{ mg l}^{-1}$  2,4-D, the average fresh (1.073 g) and dry (0.100 g) weights of callus were more when  $1.0 \text{ mg l}^{-1}$  2,4-D was used. The callus resulting from these treatments was friable, glossy and creamish-yellow in appearance. After 60 days of culture in induction media, 100% maturation was achieved on plain MS and on MS medium with  $0.1 \text{ mg l}^{-1}$  abscisic acid (ABA). Embryos derived from treatments with 2,4-D did not show 100% embryo maturation, which could, however be achieved if these were added to cytokinin-supplemented medium. Sixty two per cent of matured embryos formed shoots on media devoid of plant growth regulators but these took nine days to germinate unlike the precocious germination (5 days) demonstrated by matured embryos treated with  $0.5 \text{ mg l}^{-1}$  gibberellic acid ( $\text{GA}_3$ ). Shoot induction formed in 60% of embryos treated with  $0.5 \text{ mg l}^{-1}$  TDZ and  $0.5 \text{ mg l}^{-1}$  Kn but most shoots showed malformation during growth. Stereo and fluorescence microscopy study confirmed all the different developmental stages of embryos, including the suspensor.

**N.L. Raju, M.N.V. Prasad (India)** Cytokinin-Induced High Frequency Shoot Multiplication in *Celastrus paniculatus* Willd., a Red Listed Medicinal Plant (pp 133-137)

#### ABSTRACT

**Original Research Paper:** A protocol for rapid propagation of *Celastrus paniculatus* Willd. has been developed. A range of cytokinins and gibberellic acid were tested on Murashige and Skoog (MS) medium for multiple shoot induction and high frequency shoot multiplication with axillary nodes of seedlings. While the seeds were subjected to three different treatments (room temp, cold ( $4^\circ\text{C}$ ) and water soaking treatments), cold treatment of seeds when cultured for 3-4 weeks on MS medium with a combination of 6-benzylaminopurine (BA) and kinetin (KN) was sufficient to induce maximal shoot response (81.2%) and gave the highest number of shoots per explant (4.4). All shoots regenerated directly without a callus phase and individual hormones BA, KN and  $\text{GA}_3$  were less effective. The excision of the node and shoot tip from *in vitro* grown seedlings and their subsequent culture on MS medium supplemented with  $2.2 \text{ }\mu\text{M}$  BA and a combination of MS+  $4.4 \text{ }\mu\text{M}$  BA +  $1.1 \text{ }\mu\text{M}$  KN facilitated enhanced axillary bud proliferation (66.6%). Best rooting was observed on MS medium fortified with  $10.7 \text{ }\mu\text{M}$   $\alpha$ -naphthaleneacetic acid. The new plantlets were weaned and subsequently acclimatized in a glasshouse and a 80-90% survival rate was achieved in the field. This study has significant implications in achieving considerable progress in *ex situ* conservation initiatives for *C. paniculatus*.

**R. Sharath, V. Krishna, B.N. Sathyanarayana, B.N. Maruthi Prasad, B.G. Harish (India)** High Frequency Regeneration through Somatic Embryogenesis in *Bacopa monnieri* (L.) Wettst, an Important Medicinal Plant (pp 138-141)

#### ABSTRACT

**Original Research Paper:** *Bacopa monnieri* is well known in the Indian system of medicine or Ayurveda as brahmi or water hyssop and has been using as a brain tonic and to cure mental disorders. An efficient *in vitro* protocol for high frequency regeneration has been developed via somatic embryo-genesis from the leaf explants of two months old *Bacopa monnieri* (L.) Wettst. plants grown under greenhouse conditions. A high frequency of embryoids formed after the transfer of fleshy, nodular, leaf-derived callus onto Murashige and Skoog (MS) medium supplemented with  $0.5 \text{ mg l}^{-1}$  2,4-D (2,4-dichloro-phenoxy acetic acid). A mean of  $42.20 \pm 2.20$  embryoids developed per callus mass mean of  $1.09 \pm 0.06$ . An increase in the concentration above  $1.5 \text{ mg l}^{-1}$  of 2,4-D reduced the embryonic potentiality of the callus. Fluorescent staining of the embryogenic callus with auramine O stain revealed the occurrence of the embryogenic callus and facilitated the observation of the occurrence of globular, cordate and torpedo embryos and the subsequent differentiation of vasculature in the embryoids. The isolated cotyledonary embryoids matured and induced multiple shoots and roots on both MS basal medium and MS medium fortified with 1 to  $1.5 \text{ mg l}^{-1}$  6-benzylaminopurine, i.e., BAP. Each plantlet obtained from an embryoid, could further produce multiple shoots on MS basal medium from which a mean of  $28.6 \pm 1.37$  plantlets were harvested. Most regenerants (98%) were successfully hardened-off and transferred to the field and maintained for the evaluation of variation. The present system of somatic embryogenesis of *B. monnieri* could be a viable system for the improvement of this medicinal crop.

**Neeta Shrivastava, Tejas Patel (India)** *Clerodendrum* and Healthcare: An Overview (pp 142-150)

#### ABSTRACT

**Invited Review:** The genus *Clerodendrum* L. (Family: Lamiaceae) is very widely distributed in tropical and subtropical regions of the world. More than five hundred species of the genus are identified till now, which includes small trees, shrubs and herbs. Ethno-medical importance of various species of *Clerodendrum* genus has been reported in various indigenous systems of

medicines and as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life-threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Few species of the genus like *Clerodendrum inerme*, *C. thomsonae*, *C. indicum*, and *C. speciosum* are ornamental and being cultivated for aesthetic purposes. The powder/paste form and the various extracts of root, stem and leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria, and diseases of blood, skin and lung. To prove these ethno-medical claims, some of these species are being extensively studied for their biological activities using various animal models. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. The major chemical components reported from the genus are phenolics, steroids, di- and triterpenes, flavonoids, volatile oils, etc. This review mainly covers the extent of work done on biological activities of various *Clerodendrum* species such as *C. trichotomum*, *C. bungei*, *C. chinense*, *C. colebrookianum*, *C. inerme*, *C. phlomidis*, *C. petasites*, *C. grayi*, *C. indicum*, *C. serratum*, *C. campbellii*, *C. calamitosum* and *C. cyrtophyllum* that can be used both in conventional therapy or as replacement therapies for the treatment of various diseases.

**Anshu Srivastava, Avani Kothari, M. Rajani, Harish Padh, Neeta Shrivastava (India)** Sustainable Production of a Therapeutically Important Tree (*Holarrhena antidysenterica*) of India (pp 151-154)

#### ABSTRACT

**Original Research Paper:** *Holarrhena antidysenterica* (Linn.) Wall is an important medicinal plant of the Indian System of Medicine, the Ayurveda. The stem bark of the tree is used for antidiarrhoeal activity, owing to the presence of the bioactive compound conessine, a steroidal alkaloid. Due to the difference in environmental conditions or in genotype, the plant shows much variation in its therapeutic efficacy, hence there is a need to have more plants with an optimum and uniform therapeutic efficiency. In this study, we established a protocol for clonal multiplication of this important medicinal tree. The superior mother plant was selected on the basis of a higher concentration of the bioactive constituent, conessine, by phytochemical analysis. The maximum multiplication rate of axillary buds was observed in 8  $\mu$ M 6-benzyladenine on Murashige and Skoog (MS) medium. Multiplied shoots of 7-9 cm were transferred to rooting medium (MS medium with 0.5  $\mu$ M indole-3-acetic acid). The rooted plantlets were then transferred for primary and secondary hardening and finally transferred to the field. A 70-80% field survival was observed in regenerated plants. The established protocol offers a sustainable method for consistent production of this elite, medicinally-important tree.