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OUR ECOSYSTEM

Zoological Diversity
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Indigenous Bioresources of NER

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The Northeastern Region (NER) of India comprising of eight states (Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim) is blessed with smoky mountains, rivers, waterfalls, evergreen forests, valuable natural flora and fauna that should be protected, explored and used sustainably. NER shares both, Himalaya and Indo-Burma biodiversity hotspots that are the natural habitats of several endemic species, which are unexplored, untouched, and extremely beneficial. Its scenic beauty and exotic biodiversity have attracted scientists, policymakers and various stakeholders to work together as one coherent unit for overall wellness of the people of NER.

Landlocked by international borders, NER states are innately connected with nature and have a rich socio-economic and cultural heritage. The mighty Brahmaputra River and several affluents enrich soil fertility in an adjacent valley and support agriculture growth and allied sectors. Even though there is immense potential in agriculture, the majority of the tribal community from NER practices jhum/shifting cultivation, which accelerates habitat destruction, deforestation and environmental pollution. To call self-sufficiency in agriculture, the policy makers are aiming for doubling of farmers' income by the introduction of high-yielding varieties and modern scientific farming strategies that improve productivity. Recent studies show that extensive urbanisation, wild harvests of natural flora and changing environmental conditions become an extreme threat to NER. As a result, many medicinally and commercially important plant species are on the verge of extinction. In such a scenario, in-vitro plant tissue culture techniques are a highly reliable approach for germplasm conservation, eco-restoration and phytopharmaceutical production, especially for several medicinally and commercially important plant species.

Qualitative Improvement of Genetic Resources of Indigenous Tree Species Camellia sp.

Tea plant (*Camellia* sp.) is an evergreen socio-economic crop species and belongs to the family 'Theaceae'. The

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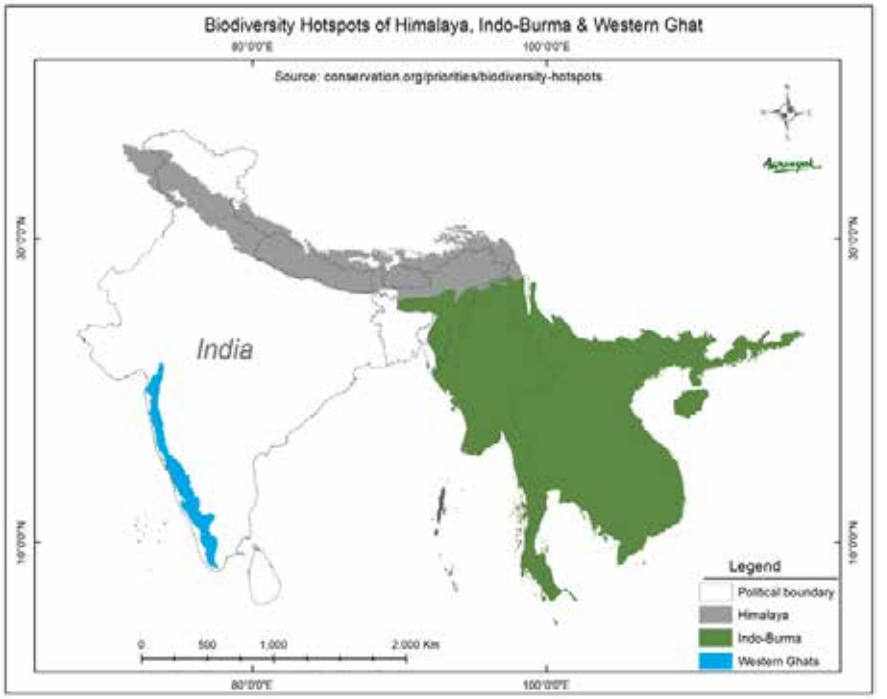

indigenous tea cultivator of Assam is named *Camellia assamica* ssp. *assamica* (TV21) having broad leaves and high content of catechins, dominates in black tea production as compared to China type tea (*Camellia sinensis*). The extreme cross-pollinating nature of tea plant results in high heterozygosity (high variability), which leads to inconsistent quantity and quality of phytochemicals. Conventional methods of plant propagation through seeds do not produce genetically uniform clones/identical plants and, on the other hand, stem-cutting and grafting have a poor survival rate and require adequate care to best suit the changing environmental conditions. Being a woody perennial, tea plant requires a longer time period to attain reproductive maturity, which causes less success rate of the development of superior clones/plants from existing parent plants following conventional farming strategies. Furthermore, improvement of genetic constituents and a further selection of superior varieties require several years in conventional breeding practices. In such scenario, in-vitro tissue culture method not only acts as a potential way of qualitative development of superior plants but can also produce large number of true-to-type (identical) plants in comparatively shorter duration. The in-vitro developed plants could serve as a potential source for the development of pure breeding plants and would also facilitate the consistent production of medicinally important bioactive metabolites, independent of seasonal variation.

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Azadirachta Indica

Azadirachta indica, commonly known as Neem plant, is native to the Indian subcontinent and Southeast Asia. All parts of the Neem tree, the leaves, stems, roots, flowers, fruits, and seeds contain medicinal metabolites and are used for household remedies against various diseases. Additionally, this magnificent tree is also renowned for its eco-friendly, insect-eliminating properties, thus regularly used in agriculture. The extreme cross-pollinating nature of Neem plant causes high variability in plants which leads to inconsistent quantity and quality of phytochemicals. To satisfy the growing demand and the vast need for pharmaceutical industries, attaining enhanced production of metabolite compounds should be our utmost priority. Neem tree is conventionally propagated through seeds. However, the low seed viability and seed-borne variability limit uniform and consistent metabolite production. In-vitro tissue culture methods would be the most suitable alternative strategy for the production of homozygous clones (pure breed plants) containing high amounts of metabolites compared to seed-borne trees. In the aseptic condition of the laboratory, pollen grains (male gametes) present in the male reproductive parts were successfully allowed to induce the haploid plants in suitable nutrient media. Haploid plants (sporophytes) are the product of meiosis and are the natural recombinant that carry the variable amount of metabolites in haploid plant lines. Haploids possess a single set of chromosome (n) so they will not form the seeds but otherwise grow normally as a tall tree. Successful production of seeds requires an even number of chromosomes (2n) in parental lines. Therefore, the genome of these haploid plants was doubled to obtain seed-bearing doubled haploids plants that can be utilised as a pure breed variety. Screening and quantification of secondary metabolites (azadirachtin, salannin and nimbin) were analysed from these improved plants of the Neem plants. It has been observed that the newly developed haploid/doubled haploid plants contain higher azadirachtin, salannin and nimbin contents as compared to naturally grown parental diploid Neem plants. Despite these, overall methods have expedited the growth cycle with

added advantages of the production of genetically improved plant lines that are impossible to produce following conventional methods of breeding. Lab-to-field transfer of plants requires 6-8 weeks and lakhs of plants are multiplied around the year. Now, this genetically improved variety is up for commercialisation and industry tie-up.

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Micropropagation and Biomass Resources Utilisation for High Value Metabolites Production

Lantana Camara

Lantana camara L. is an aromatic, evergreen shrub belonging to the family Verbenaceae. Each and every plant part is a natural reservoir of terpenoids, glycosides and flavonoids. Continued production of high-value metabolites at a commercial scale requires stable in-vitro elite cell lines. In this context, in-vitro culture from leaf explants was established in the laboratory to get a constant source of medicinally important compounds, in higher amounts, all year round. Betulinic acid, oleanolic acid and ursolic acid are three pharmacologically active pentacyclic triterpenes that have been identified and quantified by various analytical methods. Moreover, the in-vitro derived cell extract exhibited cytotoxic activity on cancerous HeLa cells.

Spilanthes Paniculata

Spilanthes paniculata Wall. ex DC. is a perennial herb belonging to Asteraceae family. The plant is commonly available in Northeastern India. It is a natural source of various important anti-malarial medicinal metabolites like N-alkylamides. Leaves and flowers are major edible parts of the plant, especially in Tripura and Arunachal Pradesh. The plant is known to possess anti-pyretic, anti-inflammatory, local anaesthetic, and anti-malarial properties. The primary active component is spilanthol, an isobutylamide mainly isolated from different parts of the plant, such as the flower and leaf. The high medicinal importance and increasing demand for plant-derived medicines lead to overharvesting of plants from natural habitats. In-vitro micropropagation is an excellent alternative to producing the plant at a large scale. It provides a rapid and constant supply of raw materials to produce important metabolites without seasonal and regional restrictions. Apart from micropropagation, adventitious root in suspension culture is also an important method of in-vitro biomass generation. This method can easily be scaled up from the flask to the bioreactor level for large-scale production of biomass as well as metabolites. In laboratory, we explore the alternate in-vitro approaches to biomass generation at a small reactor level that can be further scaled up to a commercial level.

Stevia Rebaudiana

Stevia rebaudiana belonging to the family Asteraceae, is a medicinally important plant containing low-calorie sweeteners (steviol glycosides) in leaves. Health-conscious consumers around the world are recently looking for low-calorie artificial sweeteners as a substitution for sugar. Excess consumption of sucrose in the diet is associated with diabetes and cardiovascular diseases. Vegetative propagation of *Stevia rebaudiana* is limited by unfavourable climatic condition, and seeds of the stevia plant show low viability in the field. However, in-vitro propagation is a rapid reliable system for the production of a large number of genetically uniform disease-free plantlets, irrespective of the seasonal variations. In this context, the accelerated in-vitro micropropagation protocol is established in the laboratory by nodal segments culture, followed by screening and quantification of steviol glycosides (stevioside and rebaudiosides) from various plant parts.

Tinospora Cordifolia

Tinospora cordifolia or Giloy is a multipurpose woody liana that generally grows in tropical climates and is readily available in Northeast India. It is widely known for its immunomodulatory, hepatoprotective, anti-hypertensive, and antioxidant properties, hence called a rejuvenating herb. The nutraceutical features are also mainly due to the presence of high protein, carbohydrates, calcium, phosphorus, potassium, and iron. Recent studies show

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Tinospora Cordifolia

that consumption of *T. cordifolia* has increased the recovery rate in dengue and SARS-CoV-2 patients. Therefore, the Government of India has included this plant in the 32 prioritised plant list for conservation and research. Giloy is mainly propagated through stem-cuttings in the late spring, summer and autumn seasons. Another best-suited method for its propagation is using plant tissue culture technology, which uses micro-cuttings and grows them in controlled conditions. The quality control of *T. cordifolia* depends on its phytoconstituents content, which varies with geographical location. Chemical analyses of in-vitro regenerated plants showed higher alkaloid (berberine) content as compared to the stem and leaves of the mother plant. Additionally, inorganic nanoparticles were synthesised by utilising in-vitro cell cultures and further evaluated for biological applications.

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In-vitro Conservation and Nutraceuticals Production of Food Crops

Musa Balbisiana and Musa Paradisiaca

The *Musa sp*. commonly known as banana, belongs to the family Musaceae, and is one of the most widely distributed and consumed fruits with high content of minerals, vitamins, carbohydrates, flavonoids, and phenolic compounds. In Assam, farmers are growing bananas commercially due to high profitability. There are 15-20 different varieties of bananas available to Assam. In a seeded variety of bananas, non-viable seeds and long growth cycles limit plant propagation by the conventional method. Understanding this scenario, the laboratory is involved in micropropagation of large number of disease-free banana (*Musa* spp.) plants within a short period. Rutin, a flavonoid, naturally present in banana leaves has antioxidant properties and is beneficial to health. Extraction and quantification of rutin content were analysed in the laboratory from three different varieties of *Musa* sp., named Malbhog, Bhimko and Chinichampa. The results indicated that banana leaves, a food industry by-product, and agricultural waste, have the potential for use as an inexpensive and new source of bioactive metabolites.

Oryza Sativa

In the Northeastern region of India, different black rice (*Oryza sativa*) varieties are grown in both the wetland and upland of Manipur and Tripura. The black rice varieties have got increasing attention due to their high nutritional, antioxidant and nutraceutical properties. The dark purple pigmentation is due to the presence of high anthocyanins (Cyanidin 3-O-glucoside) in the pericarp, which act as antioxidants and have vast applications in industries as a colorant, food supplements and nutraceuticals. Moreover, black rice is propagated through seeds and the availability of seeds is limited that restrict the farm growers to meet the high demand in the market. Thus, improved high-yielding black rice will encourage the farmers to cultivate them in field. Therefore, the laboratory has implemented in-vitro tissue culture techniques involving micropropagation and further analysis of bioactive metabolites from the cultured rice plants and cells. This process is serving a dual purpose, i.e., conservation of elite plants and to study their respective commercial values.

Conclusion

Biodiversity plays a pivotal role in maintaining the ecological balance in nature. Northeastern Region (NER) sharing Himalayas and Indo-Burma biodiversity hotspots, is one of the mega biodiversity centres in India and serves as the native habitat for valuable natural flora and fauna. Nowadays, indigenous bio-resources of NER have experienced a number of challenges, such as habitat destruction due to the ever-increasing human population, illegal mining, landslide, and overutilisation and illegal trading of medicinal plants. Considering the above alarming situations, ex-situ conservation and sustainable utilisation of indigenous bioresources of NER should be given top priorities. In this current scenario, in-vitro plant tissue culture techniques have added advantages in plant, propagation, conservation, and improvement of medicinal and commercial plant species. The most important advantage of in-vitro technique is that it can further be used to produce bioactive medicinal metabolites in bulk, irrespective of geographical variations, seasonal variations, and also environmental factors. It offers a defined production system, continuous supply of products with uniform quality and yield. Novel compounds, which are not generally found in the parent plants, can be produced in the in-vitro grown plants through plant tissue culture. In addition, stereo- and regio-specific biotransformation of the plant cells can be performed for enhanced production of bioactive compounds from economical precursors. It is also independent of any political interference. Efficient downstream recovery of products and rapidity of production are its added advantages. Moreover, adoption of plant tissue culture techniques not only prevents extinction of germplasms but also makes NER self-reliant in modern agricultural practices. ❑