

Use of endophytic bacterium (*Pseudomonas* sp., MTCC9476) in propagation and conservation of *Ginkgo biloba* L.: A living fossil

Ginkgo biloba L. (English name: maiden hair, Hindi name: balkuanri, family: Ginkgoaceae), often referred to as living fossil, is recognized for its sturdiness, resistance to diseases and remarkable longevity. The leaves are bi-lobed, without midrib and irregularly crenate, and the tree is dioecious where male and female sporangia are borne on separate trees. The species has been well recognized for medicinally important active ingredients. Traditionally, it has been used on an extensive basis, either as food or medicinal component. While the *Ginkgo* leaves are still used in form of tea, leaf extracts in the form of various bioformulations are commercially available in Europe and America. The leaf extracts of *Ginkgo* contains flavonoides, glycosides and ginkgolides that improve blood flow, act as antioxidants and are mainly used as memory enhancers and anti-vertigo agents¹. Besides rich antioxidant activity, the leaf extracts have also been reported for antimicrobial activity²⁻⁴.

The species is also interesting from the taxonomic and evolutionary point of view, as it is the only surviving genus and species of Ginkgoales that reached its height in the Jurassic period. While the natural habitat of *Ginkgo* is in China, Japan and Korea, some established trees have been reported from the hilly areas of Indian Himalayan Region (IHR); maximum being in the State of Uttarakhand⁵. The Botanical Survey of India

has declared this as a rare species. In view of the existence of limited number of established trees of *Ginkgo* in India and its evolutionary and medicinal importance, need of conscious efforts for propagation and conservation of the species has been realized⁶⁻⁸. *Ginkgo* is an extremely slow-growing tree and its regeneration through seeds is very poor. Propagation through cuttings is the only possible option for regeneration of this species.

Aiming successful propagation and conservation of the species, a detailed study on rhizosphere microflora associated with *Ginkgo* trees growing under temperate climatic conditions of IHR has been conducted⁸. Besides colonization of free-living microorganisms and arbuscular mycorrhizal (AM) fungi, the occurrence of endophytic microorganisms (bacteria and fungi) was among the significant findings of this study. An endophytic plant growth promoting bacterium, isolated from the mycorrhizae-infected cortical root cells of *Ginkgo*, was demonstrated for its plant growth promoting and biocontrol properties. The psychrotolerant bacterium, based on 16S rRNA analysis, was identified as *Pseudomonas* sp. The bacterium possesses potential for propagation of *Ginkgo* under temperate climatic conditions. The bacterium and the nucleotide gene sequence have been deposited in the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Tech-

nology, Chandigarh, India (accession number = MTCC9476) and NCBI (accession number = EU702439) respectively⁹. The bacterium, in liquid formulation, has been successfully used for healthy propagation of *Ginkgo* through stem cuttings.

The bacterium raised in suspension culture was tested for its growth promotion abilities through plant-based assays in the Institute's net-house. Simultaneously, the suspension culture was inoculated in the rooted stem cuttings of *Ginkgo* in polybags. One-year-old plants from these bags (Figure 1 a) were transferred to earthen pots. Growth monitoring was performed by measuring plant biomass, biochemical constituents and the rhizosphere colonization efficiency of the inoculated bacteria. 3-4-year-old plants were then transferred to various locations in Uttarakhand.

The bacterial inoculation of *Ginkgo* plants showed positive influence in terms of plant biomass, colony-forming units of beneficial plant growth promoting microorganisms, colonization by mycorrhizae and endophytes, and biochemical constituents. More than 200 plants (treated with the bioformulation as well as non-treated) have been transferred to various locations in Uttarakhand (courtesy: Silviculture, Nainital district; Foundation for Contemplation of Nature, Majkhali, Almora district; 35 Battalion, Indo-Tibetan Border Police Force, Almora district). Kalika (Figure 1 b) and



Figure 1. Propagation of *Ginkgo biloba*. *a*, One-year-old stem cuttings under Institute's net-house. *b*, Six-year-old plants growing after successful transfer at Kalika nursery, Ranikhet, Almora district, Uttarakhand.

Sariatal Forest Nurseries (Almora and Nainital districts respectively) in January 2011 showed 90% survival of *Ginkgo* (inoculated) compared to 42% (uninoculated), after one year of transfer. Besides, plants have also been transferred to the Arboretum and other locations in the Institute from conservation viewpoint.

The outcome of the research work, specifically isolation and identification of an endophytic bacterium and its bioformulation resulting in successful establishment of cutting-raised *Ginkgo*, needs sharing with the local communities, such as Forest Department officials, Central Armed Police Force, etc. (engaged with forest/plantation management). Right from the beginning of work on *Ginkgo*, the research group has involved foresters in activities such as soil sampling, site selection and transfer of treated plants. Further success of this research will be achieved through organization of location-specific training workshops with a view to (i) explain the importance of this medicinal plant, and (ii) demonstrate the use of 'bioformulation' technology for successful plant establishment. Prepara-

tion and distribution of information leaflets explaining the methodology of propagation and the growth of *G. biloba*, in local/simplified languages, will be helpful to reach out to the local community.

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ANITA PANDEY*
PRIYANKA SATI
MUKESH K. MALVIYA
SHIPRA SINGH
AJAY KUMAR

*Biotechnological Applications,
G.B. Pant Institute of Himalayan
Environment and Development,
Kosi-Katarmal,
Almora 263 643, India
*For correspondence.
e-mail: anita@gbpihed.nic.in*