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PLANT TISSUE CULTURE

Management of microbial contaminants in the *In Vitro* Gene Bank: a case study of taro [*Colocasia esculenta* (L.) Schott]

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Abstract

Ex situ conservation of vegetatively propagated crops by tissue culture is sometimes hampered by covert endophytic bacteria which become visible after repeated subculture over prolonged periods. In the present study, we identified bacterial contaminants and devised a strategy for their elimination from 20 *in vitro* conserved accessions of taro [*Colocasia esculenta* (L.) Schott] held in the *In Vitro* Gene Bank (IVGB) at ICAR-National Bureau of Plant Genetic Resources (NBPGR). Visually, the cultures were exhibiting white to dark brown exudation in tissue culture growth medium and undergoing slow degeneration as recorded by wilting and necrosis of leaves and shoots. On culturing the macerated pieces of shoots, corms, and leaf petioles from the contaminated taro cultures on bacterial indexing medium, six distinct bacterial colonies were observed. The identity of these bacteria was established through 16S rDNA sequencing as gram-negative strains (2) of *Ralstonia* spp. and gram-positive strains (4) of *Paenibacillus* spp. Thereafter, two strategies were adopted for elimination of contaminants from the cultures: (i) use of antibiotic supplemented media and (ii) hardening of micro-corms in field and re-establishment under *in vitro* conditions. Antibiotic supplemented media was not effective in eliminating contamination as reappearance of bacterial colonies was observed after subculturing in antibiotic-free growth medium. In contrast, re-establishment *in vitro* from hardened infected plants resulted in an average of 6.03% bacteria-free cultures in 14 accessions. The findings indicate that endophytic bacteria associated with the host (taro) plants under tissue culture conditions may efficiently be mitigated through periodic transfer of the tissue culture derived corms to *ex vitro* conditions and re-introduction of the bacteria-free shoot tip explants *in vitro*.

Keywords Bacterial endophytes · Culture revival · *In vitro* conservation · *Paenibacillus* spp. · *Ralstonia* spp.

Introduction

Plant genetic resources are the backbone of crop improvement programs and vital for sustainable agriculture and human survival. Tropical root and tuber crops provide food and nutritional security to a large number of small-hold farmers, especially in developing countries. Taro [*Colocasia esculenta* (L.) Schott], one of the minor root crops, remains a neglected and orphan species with less development of improved varieties as compared to cassava, sweet potato, and yams (Matthews *et al.* 2017). However, its potential to strengthen food security has been recognized. It was grown in tropical Asia for more than 10,000 y (Fullagar *et al.* 2006) and now its cultivation spreads throughout the wet tropics (Matthews *et al.* 2017). Taro is one of the important members of the Araceae family which is consumed as a staple food crop in many countries in the humid tropics and subtropics (Matthews *et al.* 2017). Before the start of the global trade and transport of agricultural

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