International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(2): 209-212 April 2016
DOI Number: 10.5958/2230-732X.2016.00028.0

MICROBIOLOGY

Putative endophytic fungi from taro (*Colocasia Esculenta*), greater yam (*Dioscorea Alata*) and elephant foot yam (*Amorphophallus Paeoniifolius*)

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Paper No. 430 Received: 17-1-2016 Accepted: 8-4-2016

Abstract

Endophytic microorganisms which remain asymptomatically inside plants have the potential to be used widely in agricultural field and valuable for agriculture as a tool to improve crop performance. Tuber crops are the second most important group of crop plants and among them tropical root and tuber crops which include cassava, sweet potato, yams and aroids, are essential as staple food and are utmost important for world food security. Research on these crops has been neglected and regarding endophytic colonisation, some of these tuber crops have been examined. Healthy asymptomatic leaves of three tropical tuber crops viz. taro (*Colocasia esculenta*), greater yam (*Dioscorea alata*) and elephant foot yam (*Amorphophallus paeoniifolius*) were inoculated on potato dextrose agar media to detect endophytic colonisation. Total six different putative endophytic fungi were isolated from these crops which were found to be non-sporulating when observed under microscope. Further works are being carried out for the identification and utilisation of these organisms in tuber crops improvement. This communication is to report the presence of putative endophytic fungi in taro, yam and elephant foot yam for the first time.

Highlights

- Tropical root and tuber crops are most important for world food security
- Endophytic microorganisms have the potential to boost agriculture
- Incidence of endophytic colonisation was observed first time in taro (*Colocasia esculenta*), greater yam (*Dioscorea alata*) and elephant foot yam (*Amorphophallus paeoniifolius*)

Keywords: Root crops, endophytes, greater yam, taro, elephant foot yam

Endophytes are defined as organisms (both bacteria and fungi) that asymptomatically infect the internal tissues of plants during at least part of their life cycle. Exploitation of beneficial properties of endophytes is of great relevance at an applied level, either to increase production yields of agricultural crops, control of plants diseases or pests, adapt plant to suitable growth conditions or in reforestation activities (Azevedo *et al*. 2000, Momota *et al*. 2012). Endophytic microorganisms are excellent sources of bioactive natural products and the capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop performance. The symbiotic technology may be useful in mitigating impacts of climate change on crops and expanding agricultural production onto

marginal lands (Redman *et al*. 2011).Tuber crops (with 5.4% energy) are the second most important group of crop plants providing food energy to humans after cereals (49%). More importantly, tropical root and tuber crops which include cassava, sweet potato, yams and aroids, are essential as staple food in fragile ecosystems and going to play significant role for world food security.The tropical tuber crops (TTCs) contribute 3.9% of human energy for an average consumption of 28.6 kg capita^{-1} day^{-1}(76 kcals capita^{-1} day^{-1}). Three of the TTCs like cassava, sweet potato and yam - rank among the top 15 crop plants of the world in area under cultivation (Nayar, 2014). Despite of such importance in food security, tuber crops have not been subjected to intense research. Further, the studies regarding the association of endophytes with these crops have been sparsely studied. Presence of some fungal and bacterial endophytes in cassava (*Manihot esculenta* Crantz ssp. *esculenta*), sweet potato (*Ipomoea batatas* (L.) Lam), yam (*Dioscorea* species) and yam bean (*Pachyrhizus erosus*L. Urban) was reported (Rivera *et al*. 1992, CIAT 1992, De Melo *et al*. 2009, Chauhan *et al*. 2013, Puranwati and Nirwanto 2013, Teixeira *et al*. 2007, Adachi *et al*. 2002, Khan and Doty 2009, Hoipal 2012, Maggiwar *et al*. 2013, Ravi *et al*. 1996, Zhang *et al*. 2010, Omoregie et al. 1999, Stamford et al. 2001, Stamford *et al*. 2007). However reports regarding the endophytic presence in taro (*Colocasia esculenta*), greater yam (*Dioscorea alata*) and elephant foot yam (*Amorphophallus paeoniifolius*) etc. are lacking in the current database. Hence in the current investigation we have tried to isolate some putative entophytic fungi associated with three tuber crops viz. taro (*Colocasia esculenta*), greater yam (*Dioscorea alata*) and elephant foot yam (*Amorphophallus paeoniifolius*).

Materials and methods

Fresh leaves having no sign of any disease and insect attack were collected both from exotic and indigenous genotypes of taro viz. BL/SM/158, BL/SM/132 and CE/IND/10.One each of greater yam genotype (BBSR-1) and elephant foot yam genotype (NDA-4) were also used. The leaves were first washed with sterile water to remove dust and dirt and then dried. The leaves were then wiped with tissue paper wet with ethanol to remove waxy materials from leaf surface to make surface sterilization better. Leaves were then cut into small pieces of 2cm length and kept in 70% ethanol for 30seconds. Leaf bits were then transferred to 0.1% $HgCl_2$ solution and kept for 30 seconds which were then transferred to sterilized water and washed thoroughly. The leaf pieces were blot dried and placed on PDA media (Potato Dextrose Agar: extract of 250g peeled potato, dextrose-20g and agar 10g per litre). Twenty leaf beats for each plant variety was plated. The PDA plates were then incubated at ambient temperature for the appearance of fungal growth. The fungal colonies were isolated and maintained on PDA media. Fungal colony features and microscopic characters were observed on 12days after inoculation in all replicated cultivars. All the processes were carried out under laminar air flow chamber.

Results and discussion

In the current investigation, six fungal cultures isolated from three important tropical tuber crops were observed to be putative endophytes (E1 to E6; Figure 1). Three fungal endophytes E1, E2 and E3 were isolated from leaf parts of three taro genotypes viz. BL/SM/158, BL/SM/132 and CE/IND/10 respectively. Isolate E4 and E5 were from greater yam genotypes BBSR-1 whereas E6 was from elephant foot yam genotypes NDA-4.

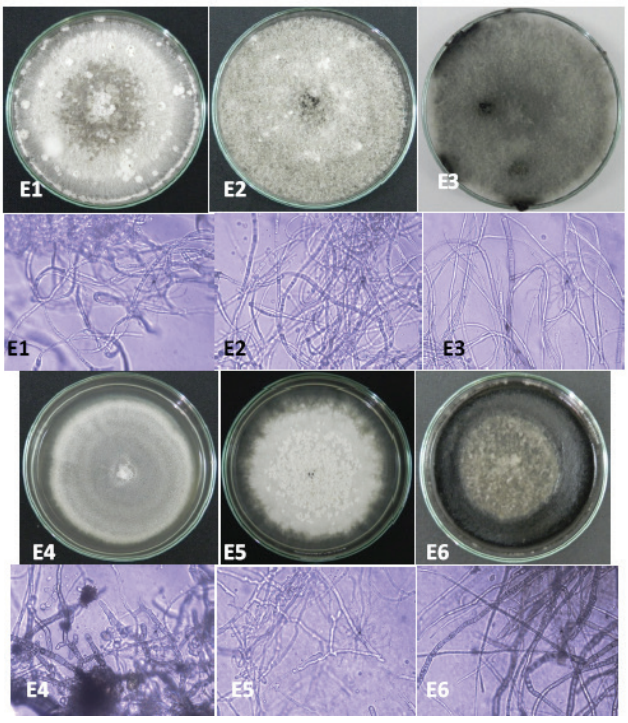

Fig. 1: Colony characters and mycelial structures of endophytes isolated from taro, greater yam and elephant foot yam.

E1 isolate observed to produce white profuse mycelia with synnemma like structures. E2 produced light grey coloured mycelia where as dark coloured mycelia were formed by E3. Mycelia growth of E4 was slow and relatively adherent to media. The mycelia of E5 were observed to be bright white in colour, sticking to media with numerous round structures at the centre. Dark grey coloured mycelia were observed in E6 and the peripheral portion extremely adhered to the media. Dark black pigmentation was also observed. Sporulation was not observed in any of the endophytic isolates. Extensive mycelial structure was observed under microscope. Dark septate mycelia were observed in case of E4 and E6.

Endophytic association in tuber crops have been earlier reported in case of cassava, sweet potato, some species of yams and yam bean. Many fungal species were found to be associated with cassava (Rivera et al. 1992), CIAT 1992). Many endobacteria were recorded to have profound antagonistic effect against many devastating plant pathogens. Some cassava cultivars reported to have strong association with bacterial endophyte *Hyphomicrobium,* even in explants when introduced to in vitro conditions from the greenhouse and field (Chauhan et al. 2013). Among these endophytes, *Pseudomonas*, *Rahnella* and *Enterobacter* produced higher amount of Indole acetic acid (IAA) which proved to be enhancing plant growth when inoculated. *Rahnella sp.* also resilient to stresses like cold shock, UV irradiation and antibiotics (Khan and Doty 2009). Plant growth promoting (PGP) activity was also observed in case of fungal endophytes of sweet potato (Hoipol 2012). The diazotrophic nature of some bacterial endophytes also has been verified by growth in nitrogen free media and with the presence of *nifH* sequences. Bacterial strains belonging to *Erwinia* and *Bacillus* species e.g. *Erwinia pyrifoliae* and *Erwini-pantoea* complex reported to have isolated from yam rhizomes or tubers. Some actinomycetes were reported to occur endophytically with yam bean tubers. These actinomycetes were found to be sources of industrially important thermostable amylolytic enzymes.

Conclusion

The three tuber crops species like taro (*Colocasia esculenta*), greater yam (*Dioscorea alata*) and elephant foot yam (*Amorphophallus paeoniifolius*) included in the current study have not been earlier investigated for the presence of endophytic associations. The six endophytes isolated during the study may possess high potential to be utilised for crop improvement. Further investigations are being continued for the identification, establishment and utilisation process of the endophytes in these import tropical food crops.

Acknowledgements

The authors are thankful to Dr. S.K. Chakrabarti, Director, ICAR-CTCRI for his constant guidance and the infrastructure facilities for the study.Financial supports of Protection of Plant Varieties & Farmers' Rights Authority (PPV&FRA), Govt. of India, New Delhi and EU aided International Network for Edible Aroids (*INEA*),Coordinated through SPC, Fiji are sincerely acknowledged.

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