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Genetically and functionally diverse symbiotic and non-symbiotic native bacteria colonized root nodules of *Erythrina brucei* growing in different land use types in Ethiopia

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ABSTRACT

Erythrina brucei is a woody legume tree used as mulch, green manure and cover crop in conventional agroforestry practices. This paper explores genetic diversity and plant growth promoting traits of bacteria associated with nodules of *E. brucei* by trap culture method using 15 field soils. Only 67% of soil samples induced nodulation and 27 bacteria were isolated, in which 15 possessed *nifH*, 9 phosphate solubilizers and IAA producers and showed growth at 1.0–3.5% of NaCl. The ARDRA analysis revealed 12 clusters at 75% similarity level and distributed into 6 genera and 9 species. Species *Bradyrhizobium cajani* and *Bradyrhizobium cytisi* were observed for the first time in the nodules *E. brucei.* Genetically, functionally and phylogenetically diverse bacteria occur in the nodules of *E. brucei.* Isolate BU1 could be a potential bioinoculant candidate which can be used for the promotion of the host plant.

Erythrina brucei (Papilionoideae) is an endemic leguminous tree widely distributed in different land-use types in Ethiopia. It is used by local farmers as a source of mulch, green manure and cover crop. *E. brucei* has desirable agro-forestry attributes, such as rapid establishment, tolerance to sunlight, possession of spreading canopy, high rate of litter production, rapid litter decomposition, vigorous regrowth after cutting and coppicing, as well as rapid recovery through profuse foliage production after a period of sustained drought (Powell and Westley, 1993).

Leguminous plants establish symbiosis with rhizobia and the symbiosis fix atmospheric nitrogen. The symbiotic nitrogen fixation supplies a larger proportion of nitrogen for food production and agricultural sustainability (Suleiman and Tran, 2013). Rhizobia were considered the only inhabitants of root nodules for several decades. However, non-rhizobial bacteria were isolated from nodules of several leguminous plants (Martínez-Hidalgo and Hirsch, 2017). In the past, non-symbiotic nodule endophytes and their contributions to plant growth promotion, nodule development, and biological nitrogen fixation remained ignored (Lu et al., 2017).

Recent studies revealed that the non-symbiotic nodule bacteria are involved in biological nitrogen fixation through inducing nitrogen fixing nodules on roots of host legumes (Lu et al., 2017) and assist in nodulation ability and improved nodulation and nitrogen fixation through synergistic interaction with rhizobia (Andrews et al., 2010). They benefit the legume host by improving plant growth through producing phytohormones, fixing nitrogen, and solubilizing mineral nutrients (Peix et al., 2015). The contribution of bacterial nodule endophytes in plant growth promotion (Santoyo et al., 2016) and alleviation of abiotic and biotic stresses (Zhang et al., 2019) and also salinity (Etesami and Adl, 2020) has been reported. Like other legume plants, *E. brucei* establishes a significant ecosystem for the symbiotic interaction with different groups of nitrogen fixers (Wolde-meskel et al., 2004; Aserse et al., 2013). Previous reports showed that the nodules of *E. brucei* dominantly harbored *Bradyrhizobium* spp. .

Much research efforts have been focused towards maximizing rhizobia efficiency and yield of food legume commodities, however, less attention was given to efficient nitrogen fixation by leguminous woody and cover legume plants. Studies on woody legume nodule bacteria biodiversity are important to find more efficient nitrogen fixer and eco-physiological stress tolerant strains as resilience to respond to future

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