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ENDOPHYTIC FUNGAL COMMUNITY OF ALIEN *Nicotiana glauca* IN TENERIFE, CANARY ISLANDS

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Abstract: *Nicotiana glauca* it is naturally distributed throughout South America. It was introduced in North America, Europe, Africa, Asia and Oceania and arrived to the Canary Islands as an ornamental plant, where has been invading altered soils. Endophytic fungi are known to produce active compounds that provide protection to their host against diseases and attack of herbivores, these microorganisms being an interesting source of novel molecules. So far, the endophytic fungal communities of *Nicotiana* have not been studied in the Canary Islands. The goal of this study is to evaluate the diversity of the fungal endophytes community associated with *Nicotiana glauca*. A total of 36 fungal species were isolated from roots, stems and leaves of plants collected in three locations from Tenerife Island - San Miguel de Abona, Fasnia and Puerto de la Cruz. The highest species richness values were found in leaves and stems (Margalef index = 3.33 and 3.36, respectively) versus roots (Margalef index = 2.52). Simpson's index complements the results of the Margalef index, indicating a fungal community with a high dominance value in roots (D = 0.65) due to the presence of multiple *Fusarium* species. Fungal community in Fasnia had the highest value of species richness (Margalef index = 3.69 versus 2.17 and 2.27 for San Miguel de Abona and Puerto de la Cruz, respectively). Results indicate fungal specificity to organ and location with 13 genera isolated from a single location and organ, among which rare species like *Collariella* and *Gelasinospora*. In this study was detected and isolated for the first time in Canary Islands the fungal species *Collariella hilkhuijsenii* which is of importance for the ecology of this genus scarcely known. Special attention should be offered to the presence of *Fusarium*, which possibly relates to the alkaloid production ability of both, the plant and the fungal strains.

***Key words**: fungal communities, alien plant, diversity, Volcanic Island.*

INTRODUCTION

Nicotiana glauca it is a sempervirens shrub (Sanz-Elorza et al., 2005; Silva et al., 2008) with a natural distribution throughout South America. However, it was introduced in North America, Europe, Africa, Asia and Oceania and arrived to the Canary Islands as an ornamental plant. It has ruderal behaviour, therefore invading all altered places. It is common on borders of roads and highways, as well as in the bed of ravines. It also grows near crops and in house gardens (Silva et al., 2008). In the Canary Islands it was introduced as ornamental in the 1930s. The consequences of invasive species on ecosystems are accentuated when it comes to endemic flora. Invasive species are considered a real issue in different ecosystems as they compete and interfere with endemic flora, which determines changes in the plant communities and functions of ecosystems. Endophytic fungi are microorganisms that live intracellularly in all plant organs without causing disease (Strobel & Long, 1998) for which multiple definitions have been proposed (Cosoveanu & Cabrera, 2019)

Studies show the high abilities of endophytic fungi to produce bioactive compounds and protect their host against pathogens and pest, being a source of new molecules (Strobel & Daisy, 2003). *Nicotiana* is a plant genus that possess antibiotic, anti-inflammatory, antifungal and insecticidal properties. Chemical profiles of *N. glauca* have revealed alkaloides like nicotine, isinicotine, and anabasine, highly used as insecticides. So far, the endophytic fungal community of *Nicotiana* has not been studied in the Canary Islands.

The objective of this study was to evaluate the diversity of the fungal endophytes community associated with *Nicotiana glauca*.

MATERIAL AND METHODS

Plants Sampling. Plants samples were collected in three locations from Tenerife Island, Canary Islands -San Miguel de Abona, Fasnia and Puerto de la Cruz. Only plants without disease symptoms or insects attack were sampled. For each plant, stems, leaves and roots were cut, labelled and maintained in zip-lock bags at 4-5°C, until processed, within 24 hours from the recollection.

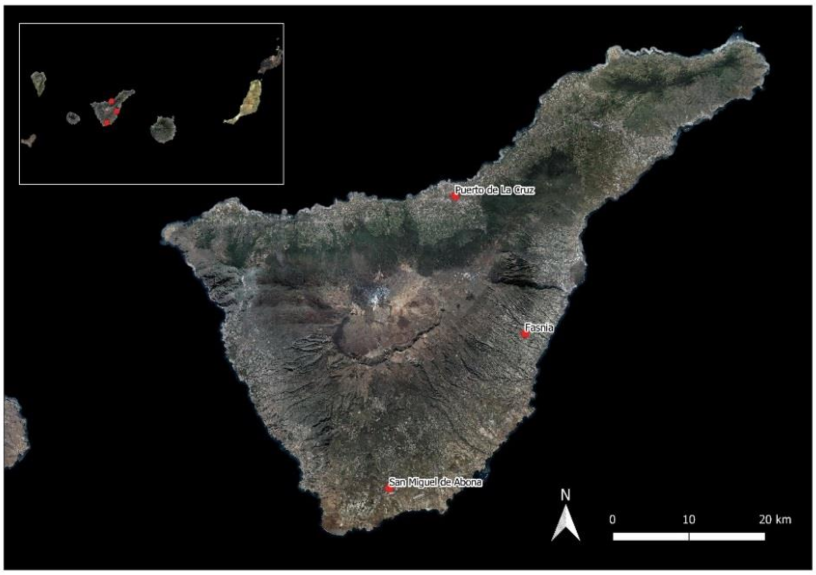

Figure 1. Map with the recollection points of the *N. glauca* plant samples in Tenerife

Fungal Endophytes Isolation. Plant fragments were superficially sterilized to avoid growth of epiphytic. Standard classical procedure for fungal endophytes was employed (Cosoveanu et al., 2018). Briefly, plates incubated with the plant segments were incubated at 25°C in the dark for 5-10 days and observed daily for fungal growth. When fungal outgrowth from the plant tissues occurred, observations on emerged fungi were made. Only the fungi with different morphological characteristics were subcultured. Eventually, when an endophyte was acquired in pure culture it was preserved in Czapek medium, at 5 °C and in glycerol 20% in deionized H_2O at -32°C and silica gel at 5°C.

Morphological Identification To analyse the fungal diversity, each replicate of the distinct stem fragments was noted. Prior to taxonomic identification, a preliminary classification was made to avoid the selection of identical strains arising from the same plant individual, separating isolates into morphotypes (Cosoveanu & Cabrera, 2018). Observations targeted characteristics related to the colony and medium as: colony shape, texture and colour; exudates, medium colour and growth rate. For the microscopic observations mounting slides with tween 20 + ethanol + sodium hydroxide were used. Molecular identification of the fungal strains was performed using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer pair to amplify the 5.8S rDNA and the two internal transcribed spacers ITS1 and ITS2 (Rottstock et al., 2014)

Endophytic fungal communities characterization For the diversity of endophytic fungi, the Margalef index and Simpson's dominance index were used. Margalef index measures species richness while Shannon index measures evenness of the community sometimes called index of dominance. The Margalef index was calculated using formula d = (S-1)/ln N, where S is the number of species (OTUs) and N is the number of individuals in the sample. Simpson's index of diversity was calculated according to the formula D=1-∑[ni(ni-1)/N(N-1)], where ni is the number of individuals belonging to i species and N is the total number of individuals. For the diversity indices, PAST software version 3.15 was used (copyright Hammer & Harper, Natural History Museum, University of Oslo, Norway)

RESULTS AND DISCUSSION

A total of 36 fungal species were isolated (Table 1).

Table 1. Endophytic fungal species isolated from *N. glauca* within three locations and organs.

Genus	Species	Organ	Location
Alternaria	*Alternaria alstroemeriae*	Stem	Fasnia
Aspergillus	*Aspergillus aflatoxiformans*	Stem	Pto de la Cruz
Aspergillus	*Aspergillus micronesiensis*	Roots	San Miguel de Abona
Aspergillus	*Aspergillus ochraceus*	Roots	San Miguel de Abona
Aspergillus	*Aspergillus tabacinus*	Leaves	Pto de la Cruz
Aspergillus	*Aspergillus pulvericola*	Leaves	Pto de la Cruz
Botryotrichum	*Botryotrichum verrucosum*	Leaves	Fasnia
Canariomyces	*Canariomyces notabilis*	Leaves	Fasnia
Chaetomium	*Chaetomium subglobosum*	Leaves	Fasnia
Chaetomium	*Chaetomium subglobosum*	Stem	San Miguel de Abona
Chaetomium	*Chaetomium hexagonosporum*	Leaves	Pto de la Cruz
Cladosporium	*Cladosporium endophyticum*	Leaves	Pto de la Cruz
Collariella	*Collariella hilkhuijsenii*	Leaves	Fasnia
Cunninghamella	*Cunninghamella gigacellularis*	Roots	San Miguel de Abona
Curvularia	*Curvularia coatesiae*	Roots	San Miguel de Abona
Dothiorella	*Dothiorella iberica*	Stem	Fasnia
Dydimella	*Didymella keratinophila*	Roots	San Miguel de Abona
Fusarium	*Fusarium falsiforme*	Roots	San Miguel de Abona
Fusarium	*Fusarium foetens*	Roots	San Miguel de Abona
Fusarium	*Fusarium foetens*	Roots	San Miguel de Abona
Fusarium	*Fusarium foetens*	Stem	Fasnia
Fusarium	*Fusarium hainanense*	Roots	San Miguel de Abona
Fusarium	*Fusarium longifundum*	Stem	San Miguel de Abona
Fusarium	*Fusarium oxysporum*	Roots	Fasnia
Fusarium	*Fusarium oxysporum*	Roots	Fasnia
Fusarium	*Fusarium tonkinense*	Roots	San Miguel de Abona
Fusarium	*Fusarium tonkinense*	Roots	Fasnia
Gelasinospora	*Gelasinospora saitoi*	Stem	Fasnia
Kabatiella	*Kabatiella bupleuri*	Stem	Fasnia
Paraconiothyrium	*Paraconiothyrium estuarinum*	Leaves	Pto de la Cruz
Penicillium	*Penicillium crustosum*	Stem	San Miguel de Abona
Penicillium	*Penicillium murcianum*	Roots	Fasnia
Penicillium	*Penicillium olsonii*	Leaves	Pto de la Cruz
Stagonosporopsis	*Stagonosporopsis lupini*	Roots	San Miguel de Abona
Stemphylium	*Stemphylium botryosum*	Leaves	Pto de la Cruz
Stemphylium	*Stemphylium botryosum*	Leaves	Fasnia

The highest species richness values were found in leaves and stems (Margalef index = 3.33 and 3.36, respectively) versus roots (Margalef index = 2.52) (Figure 1). Fungal community in Fasnia had the highest value of species richness (Margalef index = 3.69 versus 2.17 and 2.27 for San Miguel de Abona and Puerto de la Cruz, respectively). There is a major difference between the ecosystems of the selected locations, with Fasnia being at the highest elevation (489 m) compared to Abona and Puerto de la Cruz which are below 200 m. It was previously observed (Rottstock et al., 2014; Li et al., 2022) that a higher diversity of plants in the area may influence in a higher diversity of the fungi inside one of the plant species. All plants were indeed collected from the borders of roads but a distinction should be made between the areas. In Fasnia, the collection site is in a rural area with multiple local and endemic plant species both perennial and annual co-habiting with human civilization whereas Puerto de la Cruz is a dense urban area surrounded by banana crops and Abona is the most arid site in an industrial area, with abandoned fields.

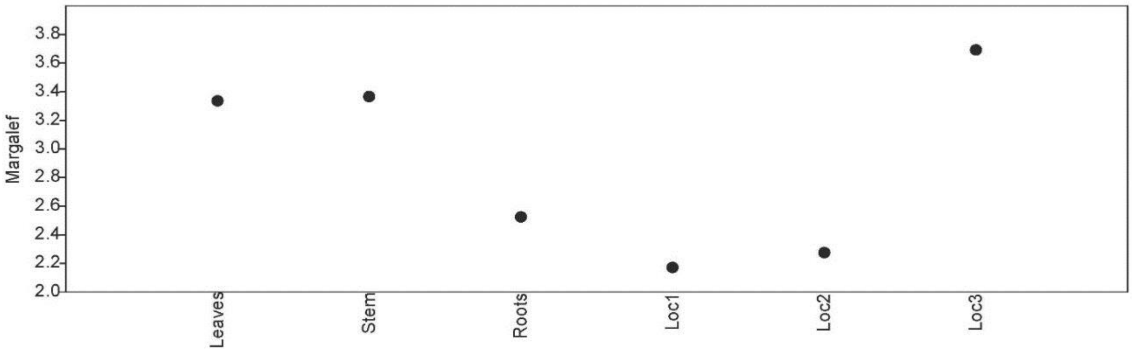

Figure. 1 Margalef index data for endophytic fungi species isolated from *Nicotiana glauca* roots, stem and leaves in all three locations (Loc 1 -San Miguel de Abona, Loc 2 - Puerto de la Cruz, 3 - Fasnia).

Simpson's index complements the results of the Margalef index, indicating a fungal community with a high dominance value in roots (D = 0.65) due to the presence of multiple *Fusarium* species (Figure 2).

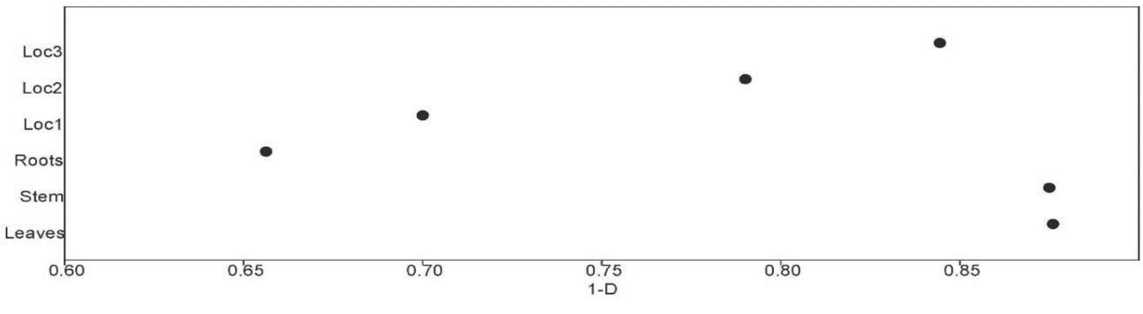

Figure 2. Plotted values of Simpson index of diversity representing endophytic fungi species isolated from *Nicotiana glauca* roots, stem and leaves in all three locations (Loc 1 - San Miguel de Abona, Loc 2 - Puerto de la Cruz, 3 - Fasnia).

Of 18 fungal genera isolated, only three were obtained from all organs, namely *Fusarium*, *Aspergillus* and *Penicillium* (Figure 3). It is noteworthy to mention that 13 genera were present in a single location and organ. Majorly, fungal genera were restricted to one or two locations with 70 and 26%, respectively. Only *Fusarium, Aspergillus, Penicillium,* *Chaetomium* and *Stemphyllium* were isolated from two locations. In Fasnia, the most arid location of the sampled ones, were found most of the genera.

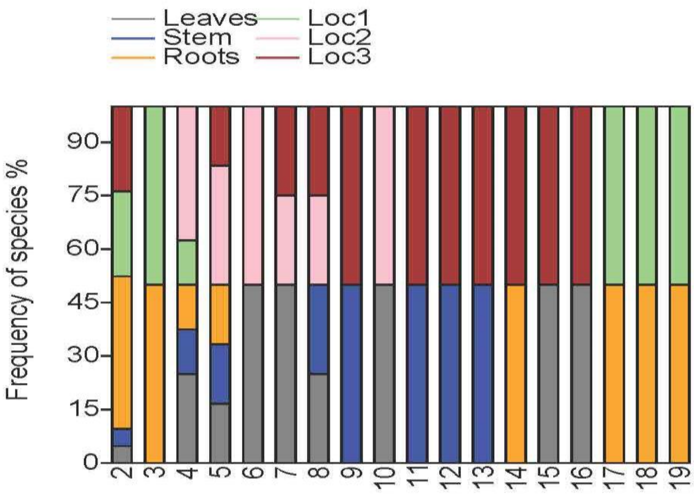

Figure 3. Frequency of endophytic 18 fungal genera (different species) isolated from *N. glauca* expressed in percentage across three locations (Loc 1 – San Miguel de Abona, Loc 2 -Puerto de la Cruz, 3 - Fasnia) and organs. Endophytic fungi genera: 2 -Fusarium, 3 -*Didymella*, 4 -*Aspergillus*, 5 -*Penicillium*, 6 -*Cladosporium*, 7 -*Chaetomium*, 8 -*Stemphylium*, *9 -Alternaria, 10 -Paraconiothyrium, 11 -Gelasinospora, 12 -Dothiorella, 13 -Kabatiella, 14 -Collariella, 15 -Canariomyces, 16 -Botryotrichum, 17-Curvularia, 18 -Stagonosporopsis, 19- Cunninghamella*.

Of the seven endophytic fungal genera isolated from Fasnia only, six of them are considered relatively rare. For instance, *Gelasinospora*, a pyrophytic fungus, closely related to *Neurospora* and *Sordaria*, has been previously isolated from macro-charcoal in Gran Canaria (Ravazzi et al., 2021), animal dung (Piasai & Sudsanguan, 2018), bird dung (Dowding, 1933), trees like *Ulex* and *Chamecyparis*, *Quercus ilex* (Collado et al., 1999) or *Styphnolobium japonicum* (Yu et al., 2021) and dwarf shrub in the polar semi-desert area (Fisher et al., 1995). *Collariella hilkhuissenii* closely related to *Chaetomium* (same family Chaetomiaceae) was first isolated from dutch soil (Wei Wang, 2017) and other closely related species were found in fresh water samples (Nguyen et al., 2019), crops of shrubs like *Rosa rosburghii* (Zhang et al., 2021), insects (Aghyl et al., 2020) and fennel seeds in Rajasthan (Dwivedi et. al., 2008). Closely related to the latter but not so scarcely present in the fungal studies, *Botryotrichum* was isolated from cucurbit plants (Huang et al., 2020), *Rhizophora mangrove* trees (Zhou & Xu, 2018), lianas (Bagchi & Banerjee, 2014), orchids like Cattleya species (Lizarazo-Medina et al., 2014), wheat rhizosphere in Iraq (Minati & Mohammed-Ameen, 2020), *Eucalyptus* spp. in Uruguay (Simeto et al., 2005) and cacao (Schmidt, 2012). In the same family, *Chaetomiaceae,* transferred recently (Wang et al., 2019) *Canariomyces* is closely related to thielavia-like species mostly found in arid and saline zones. *Canariomyces notabilis* was first isolated from litter of *Phoenix canariensis* in the Canary Islands as the name shows (Von Arx et al, 1984; Zhang et al., 2021). Species of the genus were found to determine rot in mangos (Rottstock et al., 2014; Sudrama et al., 2020) and others are medically important infective species (Wang, 2022). Yet the innocuous state has been documented in dessert soil (Wang et al., 2019), water habitats in Iraq (Abdullah & Dossary, 2014) and *Cedrus atlantica* in Algeria (Cherak et al., 2021).

More and more studies document the hypothesis of host-specificity, even in the same environments (Yu et al, 2021), naming among proposed genera *Gelasinospora*, *Sordaria* and *Thielavia*. Host species-specificity has been questioned and proposed and documented in endemic *Artemisia thuscula* (Cosoveanu & Cabrera, 2018), Mediterranean oaks (Moricca et al., 2012), tomato cultivars (Dastogeer et al., 2018) and grassland forbs) (Wearn et al., 2012) and also a pattern of community similarity associated with host genotypes of Australian *Nicotiana* species (Dastogeer et al., 2018).

By far only one study regarded endophytic fungal communities of *Nicotiana glauca* in Egipt (El-Metwally et al., 2021) and fungal identities are majorly different than in our study with only *Alternaria* and *Chaetomium* in common. In our study was detected and isolated for the first time in Canary Islands the fungal species *Collariella hilkhuijsenii* which is of importance for the ecology of this genus scarcely known.

CONCLUSIONS

These preliminary results indicate the need to further study the diversity of endophytic fungi of *Nicotiana glauca*, with special attention to the presence of *Fusarium*, which possibly relates to the alkaloid production ability of both, the plant and the fungal strains. Nonetheless, special attention should be offered to rare species like *Collariella* and *Gelasinospora.*

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