Hybridization and conservation status of *Echium portosanctensis*: first results using ISSR markers

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INTRODUCTION

Echium L. (Boraginaceae) is represented in the Madeira Archipelago by three endemic woody taxa: *E. nervosum* W.T. Aiton (Madeira Island, Porto Santo Island and Desertas - Bugio Islet, Fig.1 and 2), *E. candicans* Lf. (Madeira Island) and *E. portosanctensis* J.A.Carvalho, Pontes, Bat.-Marques & R.Jardim (Porto Santo Island, Fig. 3).

In the island of Madeira *E. candicans* inhabits high-altitude sites in complete isolation of *E. nervosum* a characteristic species of the *Euphorbietum piscatoriae* therefore occurring in low altitude habitats. In the Porto Santo Island *E. portosanctensis* occurs in a few and scattered populations usually in sea cliffs and in the higher peaks of the island, that correspond to the *Siderito multiflorae-Echietum portosanctensis* community. The native status of *E. nervosum* is not clear in Porto Santo, even though there are some references for the existence of a native population in a restricted geographic location, but most *E. nervosum* in lower altitude and ruderal habitats seem to result from garden escapes (i.e. from plants of Madeiran origin).

Interspecific hybridization resulting from the introduction of non-indigenous con-generic plants is recognized as a threat to endemic taxa (Rhymer & Simberloff, 1996). To this hybridization process are associated several problems, especially when heterotic hybrids emerge. The loss of genetic variability in endemic taxa is verified, as former separate genetic pools are allowed to fuse, due to the disruption of reproductive barriers between species. This process can lead to the extinction of native flora and fauna, since the new hybrid taxon competes directly with the native species (Todesco *et al*., 2016; Quilodrán *et al*., 2018).

The occurrence of morphological putative hybrids (Fig. 4) seems to support an undergoing hybridization process between *E. portosanctensis* and *E. nervosum*, and for this reason *E. portosanctensis* is included in the IUCN list of threatened species as critically endangered (Rivers & Menezes de Sequeira, 2017). Aiming to evaluate existing hybridization processes between these species, a preliminary molecular study was carried out using ISSR markers.

MATERIAL AND METHODS

In this study twenty-two individuals were sampled from *E. nervosum*, *E. portosanctensis* and putative *E. nervosum* x *E. portosanctensis* hybrids (Table 1). Leaves, harvested between 2012 and 2018, were dried in silica gel and ca. 20 mg used in the extraction of total DNA by the CTAB method (Doyle & Doyle, 1990) with minor modifications. PCR reactions were executed with 20-30 ng of DNA, 0.3 µM UBC primers (British University of Columbia, Canada) and 1x of MyTaq-Plant polymerase PCR mix (Bioline, UK). The reactions were carried in an Applied Biosystems 2720 Thermal Cycler using the following program: 94°C for 2´, 35 Cycles [94°C for 30´´; 52°C (UBC 890) / 55°C UBC 888/889) for 30´´; 72°C for 2´] and a final extension for 5´ at 72°C. PCR products were visualized in 1% agarose gels in TAE 1X containing ethidium bromide. Binary matrices were built grounded on the presence/absence of unambiguous and reproducible loci. Statistical analysis was carried out using NTSYS version 2.20e (Rolf, 2005). A dendrogram for the sampled individuals using Jaccard´s similarity coefficient, and UPGMA as clustering method, was used to access genetic proximity.

RESULTS AND DISCUSSION

The amplification of DNA generated in total 56 loci, of which 53 were polymorphic. Despite the high polymorphic variation detected by primers (93% to 95%), it was not recognized a species specific locus. Figure 5 reveals high intra- and inter-population genetic variability in the Madeiran endemic taxa.

The dendrogram (Fig. 6), based on UPGMA analysis of ISSR polymorphisms using Jaccard coefficient, does not support any clear segregation of *Echium* samples according to their origin or even recognized species.

Although some aggregation of *E. portosanctensis* and *E. nervosum* from Ponta de S. Lourenço can be observed it is evident the dispersal of putative hybrids along the graphic, such dispersion could be assigned to several events of hybridization (ie. introgressive hybridization that result in complex mixtures due to transfer of parental genes mediated primarly by backcrossing), but results so far obtained do not support any clear taxonomic/geographical structure. The putative hybrid individuals might belong to distinct hybrid generations with different degrees of *E. portosanctensis* and *E. nervosum* genetic pools integration. ISSR data suggest that the putative hybrid from Pico Juliana shows more similarities to *E. portosanctensis* individuals. Putative individuals of Pico do Facho population are closest to *E. nervosum* from the Porto Santo Island while Ribeiro Cochino individual resembles *E. nervosum* from Madeira Island.

Nonetheless the dispersal of putative hybrids is coherent with the fully naturalization of *E. nervosum* in Porto Santo Island, probably due to its introduction as an ornamental garden plant. In fact the references to *E. nervosum* as native of Porto Santo are all possibly related to *E. portosanctensis* and need further study. Moreover, results suggest that *E. portosanctensis* is genetically closer to the *E. nervosum* populations in the east side of the Madeiran Island (Ponta de São Lourenço), the geographical site on the Island closest to Porto Santo. But similarity with one individual of *E. nervosum* from Garajau, is hard to explain (Garajau population showed the highest genetic variability).

In conclusion, ISSR data obtained so far do not reveal a clear evidence of the hybridization processes between *E. nervosum* and *E. portosanctensis*. Thus, further studies, with an increased number of populations and individuals (both in Madeira and Porto Santo), in addition to a different set of primers are needed, in order to clarify the putative ongoing hybridization process and *E. portosanctensis* conservation status.

Fig. 1. *Echium nervosum*, Prazeres, Madeira Island.

Fig. 2. *Echium nervosum*, Ponta de S. Lourenço, Madeira Island.

Fig. 3. *Echium portosanctensis*, Pico Branco, Porto Santo Island.

Fig. 4. *Echium* putative hybrid, Pico Juliana, Porto Santo Island.

Table 1. List of samples included in this study.

Taxa	Identification code of population	Number of Samples	Harvest location	Madeira Archipelago
E. nervosum	En_M_PSL	3	Pta. São Lourenço	Madeira Island
	En_M_PN	3	Porto Novo	
	En_M_PP	2	Ponta do Pargo	
	En_M_G	3	Garajau	
	En_PS_C	3	Calheta	Porto Santo Island
	En_PS_Z	1	Zimbralinho	
E. putative hybrid	Eph_PS_PF	2	Pico do Facho	
	Eph_PS_PJ	1	Pico Juliana	
	Eph_PS_RC	1	Ribeiro Cochino	
E. portosanctensis	Ep_PS_PB	3	Pico Branco	

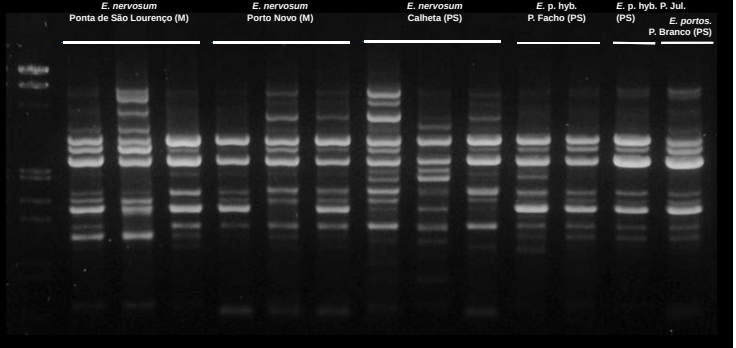

Figure 5: ISSR profile obtained for primer UBC 890. From left to right: 1, Lambda DNA/ *EcoRI+HindIII* Marker; 2, En_M_PSL_1; 3, En_M_PSL_2; 4, En_M_PSL_3; 5, En_M_PN_1; 6, En_M_PN_2; 7, En_M_PN_3; 8, En_PS_C_1; 9, En_PS_C_2; 10, En_PS_C_3; 11, Eph_PS_PF_1; 12, Eph_PS_PF_2; 13, Eph_PS_PJ; 14, Ep_PS_PB_1.

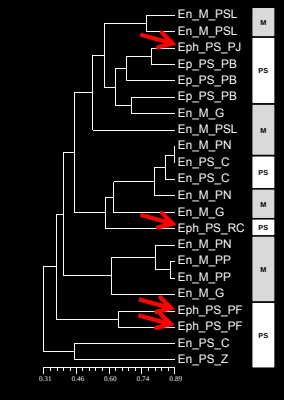

Figure 6. Dendrogram of 22 individuals of *Echium* based on UPGMA analysis of ISSR polymorphisms using Jaccard similarity coefficient. En – *E. nervosum*, Eph – E. putative hybrid, Ep – E. *portosanctensis*; PS – Porto Santo; M - Madeira

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