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BRIEF COMMUNICATION

Flooding tolerance and genetic diversity in populations of *Luehea divaricata*

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Abstract

We investigated some aspects of flooding tolerance in two riparian populations (exposed and no exposed to flooding) of *Luehea divaricata* C. Martius. Plants derived from seeds collected in each population were submitted to flooding (30 and 60 d), submergence and re-aeration treatments. Plants exposed to flooding showed development of aerenchyma, hypertrophic lenticels and new adventitious roots. Interestingly, whereas the plants originated from population naturally exposed to flooding developed some of these alterations more markedly, they could not survive when totally submerged. The random amplified polymorphic DNA (RAPD) markers, showed a significant difference between populations, suggesting that seasonal flooding on riparian populations of *L. divaricata* has been selecting individuals who are more adapted to survive in these conditions.

Additional key words: adventitious roots, aerenchyma, hypertrophic lenticels, RAPD, riparian populations.

The conditions prevailing in river banks are examples of extreme environments, since the periodically water-saturated soils exclude oxygen, one of the basic requirements for plant life (Visser *et al.* 2003). The establishment of flooding gradients may force individuals into a selection resulting in intraspecific differentiation, unless phenotypic plasticity or gene flow overrides natural selection (Lessen *et al.* 2004). Intraspecific differentiation has been previously reported. Chan and Burton (1992) found that seeds of *Trifolium repens* from a site constantly challenged by flooding presented higher flood tolerance, while seeds from a site without flooding history presented lower flood tolerance. Lessen *et al.* (2004) showed that flooding induces genetic variation within *Ranunculus reptans* populations on a small spatial scale.

Luehea divaricata (C. Martius), a South American pioneer tree species, is frequently used in reforestation programs of tropical riparian forests in Brazil. This species is found from North to South of Brazil occupying areas of riparian forests on both wet and drained soil. In the present study we delimited two populations of *L. divaricata* across a soil humidity gradient in a river bank, to find if there are differences in growth ability and morphological and anatomical traits induced by flooding between these two populations.

The study area was a fragment of riparian forest located along the Tibagi River basin in the State of Paraná, Brazil (23°16' S, 50°59' W). Plants located at low elevation riverside areas are frequently exposed to flooding, whereas plants located at high elevation landside areas are not exposed to flooding. This distribution allowed us to divide a 150 × 200 m area occupied by adult individuals of *L. divaricata* in two populations: naturally exposed and unexposed to flooding. A total of 48 individuals having mature fruit, 24 from each population, were selected for study. They were marked and used for seed and leaf sampling.

An equal number of seeds extract from mature fruits of both populations were germinated in a controlled condition. The ability of seedlings to grow under flooding was tested by comparing the dry mass of individuals grown for 30 d in three conditions: drained (D), flooded (F) and submerged (S). Drained condition was obtained by watering the plants once a day in order to keep the soil humid. Flooded condition was obtained by sealing the

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Abbreviations: D - drained; F - flooded; RAPD - random amplified polymorphic DNA; RGR - relative growth rate; S - submerged.

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pots to avoid water leakage and watering them twice a day in order to keep the water level 5 cm above soil surface. For the submersion condition, pots with plants were totally submerged in a container filled with running water. The experimental design was 3 treatments × 2 populations, with 10 plants per population in each treatment. The lengths of roots and the diameter at the base of stems (submerged portion) were also measured.

To test the ability of both populations to develop morphological and anatomical alterations related to flooding tolerance, 40 individuals were kept in two conditions: drained (D30), flooded (F30). In order to observe intensified morphological alterations and survival rates in both exposed and unexposed populations, we also conducted flooding treatments for 60 d by applying the following conditions: drained (D60), flooding (F60), flooding for 30 d followed by submersion for additional 30 d (FS), and flooding for 30 d followed by soil re-aeration for 30 d (FR). The FS condition simulates an intensification of flooding along the inundation period, resulting in seedlings submergence, while FR simulates a situation where flooding is established and then it is discontinued. The re-aeration of flooded soil was accomplished by unsealing the pots and allowing the soil to return naturally to drained condition. The experimental design was 2 treatments × 2 populations for the 30 d experiments and 4 treatments × 2 populations for the 60 d experiments, with 10 plants per population in each treatment. The parameters for morphological and anatomic analysis were evaluated according to Medri *et al.* (2002).

For growth, morphological, anatomical, and survival evaluations, the analysis of variance (*ANOVA*) was carried out using the *SAS 1990* program. The averages were compared by the Tukey test ($P < 0.05$).

Young leaves from 48 individuals, 24 of each population (exposed and unexposed to flooding), were used for DNA extraction according to Doyle and Doyle (1987). Seventeen ten-mer primers (*Operon Technologies*, Alameda, USA) that revealed sufficient number of polymorphic, reproducible and scored bands were selected for DNA amplification. PCR reactions were performed as described in Silveira *et al.* (2003). Amplified products were scored as discrete characters (present/absent). Since *L. divaricata* is an outbreeding species, genotype information could be missing due to the dominance of RAPDs, consequently causing a biased estimation of null-allele frequency when the null homozygotes are rare within populations (Lynch and Milligan 1994). To minimize this problem, two groups of data were submitted to analysis: *a*) the total number of markers and *b*) the markers with frequencies lower than 1 - [3/N], where N represents the number of sampled plants, as suggested by Lynch and Milligan (1994). The analysis of molecular variance (*AMOVA*) defined for RAPDs was applied to estimate the distribution of genetic variation within and between populations using the software *Arlequin v. 2.0* (Schneider *et al.* 2000).

Abiotic stresses can produce corresponding patterns of differentiation between adjacent populations or generations (Linhart and Grant 1996). The present study showed that some morphological parameters between populations had significant *F* values for interaction treatment × population (Table 1) in *L. divaricata*. The seedlings originated from the population naturally exposed to flooding developed a higher number of hypertrophied lenticels, and a higher diameter of cortical parenchyma cells, in comparison to seedlings from the population that do not experience flooding (Table 1). Likely, this morphological differentiation reflects, at least in part, the genetic variation observed between populations (Table 2), suggesting a higher efficiency of the population exposed to flooding in developing structures related to flooding tolerance.

RAPD markers were used to evaluate the genetic differentiation between populations exposed and unexposed to flooding. The percentage of genetic variation estimated using the total RAPD markers and those markers scored according to Lynch and Milligan (1994) were similar (Table 2), suggesting that the number of generated markers was sufficient to discriminate the populations and to reduce the bias caused by the dominant nature of RAPDs. The hierarchical analysis by *AMOVA* revealed that the majority of the variation (89.61 %) was within populations, while the genetic variation between populations was 10.39 % (Table 2). The pattern observed in the distribution of genetic variation within and between populations of *L. divaricata* was similar to that found for other pioneer, perennial and cross pollinated tree species that have pollen and seed dispersion by the wind (Nybom and Bartish 2000, Sun and Wong 2001). In most situations, genetic differentiation between co-specific populations could have resulted from either an adaptation to environmental variation or balances between gene flow and genetic drift (Linhart and Grant 1996, Latta and Mitton 1997). The high percentage of genetic variation within populations suggests that the observed differences may have derived from pressure of natural selection, instead of genetic drift. These variations are reflected in the several morphological and anatomical responses developed by plants under flooding. For instance, increment of root porosity, adventitious roots, development of sprouts associated with the hypertrophied lenticels at the base of flooded stems, and increase in root diameter (Table 1). Similar response to flooding was observed in other tropical tree species from the Tibagi river basin (Medri *et al.* 2002).

Flooding is an important factor determining differences in species growth and survival along flooding gradients (Vervuen *et al.* 2003, Sairam *et al.* 2008). Flooding stress acted as a selecting pressure in determining genetic differentiation along small-scale flooding gradients in the semi-aquatic *Ranunculus reptans* (Fischer *et al.* 2000, Prati and Schmid 2000, Lessen *et al.* 2004). Possibly, flooding may also result in genetic differentiation between populations in plant species of riparian forest fragments, such as *L. divaricata*, where only the plants at the river banks are exposed to

Table 1. Morphological and anatomical parameters tested in two populations of *Luehea divaricata*, naturally exposed and unexposed to flooding treatments, under different conditions of soil aeration. D30 and F30 are drained and flooding soils for 30 d. D60 and F60 are drained and flooding soils for 60 d. FR is flooding for 30 d following by re-aeration for 30 d. FS is flooding for 30 d following by submersion for 30 d. Means with the different superscripted letters are significant according to Tukey test ($P \leq 0.05$). Uppercase letters compare the means of the 30-d treatments and lowercase letters compare the means of 60-d treatments.

Parameters	D30	F30	D60	F60	FR	FS
Length of stem [cm]	61.30^{A}	43.40^{B}	78.40^{a}	59.20^{b}	58.80^{b}	38.30^{c}
Length of main root [cm]	39.90^{A}	34.30^{A}	43.00^{a}	37.00^{b}	37.30^{b}	31.50^{b}
Basal stem region [cm]	0.80^{B}	1.00^{A}	1.10^{b}	1.50^{a}	1.10^{b}	0.50^{c}
Number of hypertrophied lenticels	0.00^{B}	120.40^{A}	0.00^{c}	95.80^{a}	60.50^{b}	0.00^{c}
Height of hypertrophied lenticels [cm]	0.00^{B}	0.30^{A}	0.00^{c}	0.60^{a}	0.20^{b}	0.00^{c}
Number of sprout in base of stems	0.60^{B}	4.20^{A}	0.00^{c}	5.00^{b}	4.20^{b}	0.00^{c}
Number of adventitious roots	0.00^{B}	0.10^{A}	0.00^{b}	1.20^{a}	0.05^{b}	0.00^{b}
Number of superficial roots	0.00^{B}	4.30^{A}	0.0^{c}	5.30^{a}	1.50^{b}	0.00^{c}
Root relative growth [mg g^{-1}(d.m.) d^{-1}]	0.04^{A}	0.02^{B}	-	-	-	0.02^{C}
Stem relative growth [mg g^{-1}(d.m.) d^{-1}]	0.04^{A}	0.02^{B}	-	-	-	0.02^{C}
Leaf relative growth [mg g^{-1}(d.m.) d^{-1}]	0.03^{A}	0.02^{B}	-	-	-	0.03^{C}
Total relative growth [mg g^{-1}(d.m.) d^{-1}]	0.06^{A}	0.02^{B}	-	-	-	0.03^{C}
New leaves	9.90^{A}	5.55^{B}	-	-	-	0.00^{C}
Foliar abscission	0.65^{B}	0.95^{B}	-	-	-	10.30^{A}
Number of cell layers in stem	13.15^{A}	11.64^{A}	-	-	-	-
Stem porosity [%]	10.00^{B}	30.00^{A}	-	-	-	-
Diameter of the root section [μm]	513.70^{B}	734.20^{A}	-	-	-	-
Diameter of root central cylinder [μm]	286.00^{A}	251.00^{B}	-	-	-	-
Thickness of root cortical tissue [μm]	234.20^{B}	471.00^{A}	-	-	-	-
Root porosity [%]	5.05^{B}	7.28^{A}	-	-	-	-
Diameter of root cortical cell [μm]	16.29^{B}	30.36^{A}	-	-	-	-

periodic flooding. The oxygen stress induced by flooding may favour those genotypes that grow and develop more effectively, inducing the increase in the frequency of those alleles related to flooding tolerance in the riverside population. Recent studies report the correlation between genetic control and flooding tolerance traits in rice cultivars that differ in flooding or submergence tolerance (Siangliw *et al.* 2003, Toojinda *et al.* 2003).

Possibly, the genetic differentiation between populations of adult individuals in the riparian forest is not reproduced in seeds after one generation of out-crossing, given the open occurrence of genetic flow between them. Hence, we could suppose that gene flow between populations prevails over selection pressure imposed by flooding. Barrett *et al.* (1993) also reported that genetic differentiation along gradients with temporal variation in environmental conditions is rare, possibly because such selection pressures can be overridden by gene flow.

Furthermore, seed dispersal by transport with the rising in water level may represent an important factor contributing to the introduction of genetic variation in the populations at river banks. This assumption is supported by our results where the percentage of polymorphic loci is 6.21 % higher in the population that experience flooding when compared to the value obtained for the population that do not experience flooding. This suggests that selection pressure resulted from the seasonal alterations between flooding and well-drained soil conditions do not reduce genetic variation, instead, this stress might be responsible for maintaining within-population genetic variation as reported by Lessen *et al.* (2004).

It seems that periodic flooding influences genetic differentiation between populations along a small-scale flooding gradient in *L. divaricata*. However, the lack of a

Table 2. Analysis of molecular variance (*AMOVA*) within and between the two populations of *L. divaricata* (naturally exposed and unexposed to flooding). The analysis is based on the total number of markers and on those markers that followed the Lynch and Milligan (1994) criterion. *** - $P < 0.001$(levels of significance are based on 1023 permutations).

Analysis	Source of variation	d.f.	Sum of squares	C.V.	Variance [%]
Total markers	between populations	1	73.31	2.25	10.39***
	within population	46	891.46	19.38	89.61
Markers analyzed by L & M criterion	between populations	1	44.25	1.09	10.48***
	within population	46	852.86	20.31	89.52

strong differentiation in flood tolerance between seedlings can be attributed to high levels of gene flow, seed dispersal and to the cyclic patterns of the stress. In addition, the use of molecular markers to study the pattern of genetic variation between populations could be interesting to investigate the pattern of expression of genes implicated in the control of flooding response in this important tree species.

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