DNA markers to study genetic diversity and fingerprinting in *Arbutus unedo* L. (strawberry tree)

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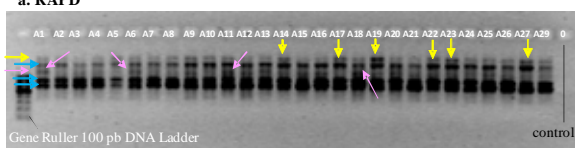
PURPOSE OF THE STUDY

Questions

What are the genetic distances and the level of diversity among 27 Portuguese *Arbutus unedo* genotypes ?

RESULTS

a: RAPD

Gene Ruller 100 pb DNA Ladder   control

b: SSR

Au 1   M₂ - CA421F   Allele's sizes 171 - 185
Au 2   Allele's sizes 149 - 203
Au 3   Allele's sizes 185 - 203
Au 4   Allele's sizes 149 - 156
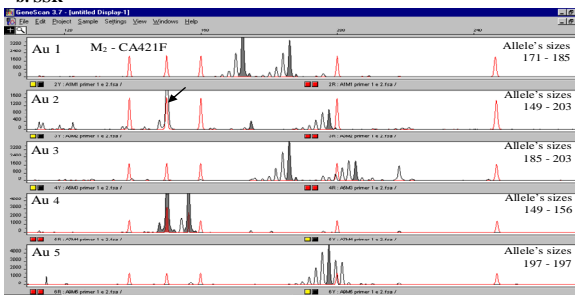Au 5   Allele's sizes 197 - 197

Fig. 1. RAPD (OPC9), 2 polymorphic (yellow and rose arrows) and 3 monomorphic bands (blue arrows) (**a**); M2 SSR automatic sequencer output. Sample number in the left upper corner. Red peak=ROX marker (**b**).
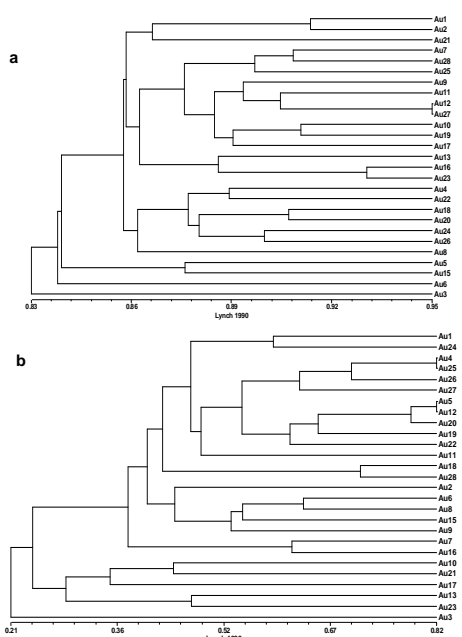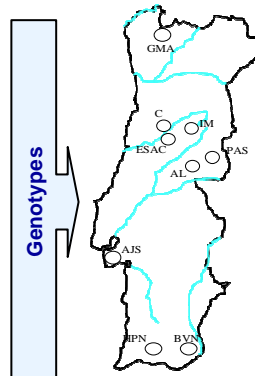

Fig. 2. UPGMAs based on a band similarity coefficient Lynch (1990): RAPD (**a**) and SSR (**b**).

MATERIAL AND METHODS

Region	Code	Local name
GMA	Au 7 / Au 10 / Au 22	Gerês Mata de Albergaria
C	Au 3 / Au 11 / Au 15	Coimbra
ESAC	Au 21 / Au 23 / Au 27	Coimbra (ESAC)
IM	Au 13 / Au 16 / Au 24	Alva e Alvoco
PAS	Au 6 / Au 9 / Au 19	Serra da Gardunha
AL	Au 2 / Au 5 / Au 17	Serra Alvélos e Muradal
AJS	Au 1 / Au 20 / Au 25	Serra da Arrábida (Portinho)
HPN	Au 4 / Au 8 / Au 28	Algarve /Silves /Herdade da Parra
BVN	Au 12 / Au 18 / Au 26	Algarve/Serra do Caldeirão/ Barranco Velho

Molecular markers: 19 RAPDs (OPC) and 5 SSRs from *Vaccinium* (Ericacea)

RAPDs

Parameter	value	Parameter	value
Nº of bands	124	Polymorfic level	57.3%
Nº of polymorfic bands	71	He	**0.27** ±0.014

SSR

Locus	Na	He	Ho	Fis	P-value	Sig.	F (null)	PIC
M2	22	0.90	0.85	0.05	0.2018	NS	0.0546	0.88
M4	9	0.79	0.78	0.02	0.0146	NS	0.0534	0.75
M7	7	0.78	0.69	0.11	0.0159	NS	0.0985	0.73
M10	6	0.57	0.56	0.03	0.004	NS	0.0868	0.51
M11	14	0.73	0.62	0.16	0.0007	*	0.0887	0.70
Mean	**11.6**	**0.75**	**0.70**	**0.07**	**0.0474**		**0.0764**	**0.71**

Tab. 1. Genetic diversity parameters estimates: Nº of alleles/*locus* (Na), expected heterozigocity (He), observed heterozigocity (Ho), fixation index (Fis), polymorphic information content (PIC). Sig. refers to the Sig. resulting from the HWE test (after Bonferroni correction), and F(null) refers to null allele frequency.

CONCLUSIONS

RAPD: some genotypes shared up to 95% of the bands. The tree topology (fig. 2a) was supported by a moderate correlation (r=0.63; P<0.001).

SSR: the UPGMA showed a degree of band similarity between genotypes up to 82% (fig. 2b). The Mantel test validated the tree topology (r=0.75; P<0.001). Finally, no correlation was found between the SSR and the RAPD pair-wise similarity matrices.

The diversity with SSRs (He=75%) was much higher than the 27% uncovered with the RAPDs, in agreement with the high microsatellites polymorphism, though monomorphic bands were used in the latter (Tab 1).

Mantel tests unfolded the lack of correlation between genetic and geographical distance matrices, for both SSRs and RAPDs. Indeed, the genotypes did not group according to their geographical origin, in both cases. Reduced gene flow, due to fragmentary distribution of the species in the country, together with the type of mating system (pollination by small insects), are plausible explanations for these results.

References : Boches, PS, Bassil, NV, Rowland, LJ, 2005. Microsatellite markers for *Vaccinium* from EST and genomic library. Mol. Ecol. Notes **5**, 657-660; Bassil, NV, Boches, P., Rowland, L., 2006. Genetic diversity in the highbush blueberry evaluated with microsatellite markers. J. Amer. Soc. Hort. Sci. 131, 674-686; Lynch M., 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol. 7(5): 478-484; Ratnaparkhe, MB, 2007. Blueberry. Genome Mapping and Molecular Breeding in Plants, vol.4. Spring.-Verlag, Berlin, Heidelberg, pp. 217-227.