Olive Tree Galega Cultivar Accessions' Identification Based on RAPD Markers

ESCOLA SUPERIOR AGRÁRIA
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PURPOSE OF THE STUDY

Questions

1) Do the 75 *Olea europaea* accessions belong to the Galega cultivar?

2) Is it possible to fingerprint the Galega cultivars with RAPDs ?

RESULTS

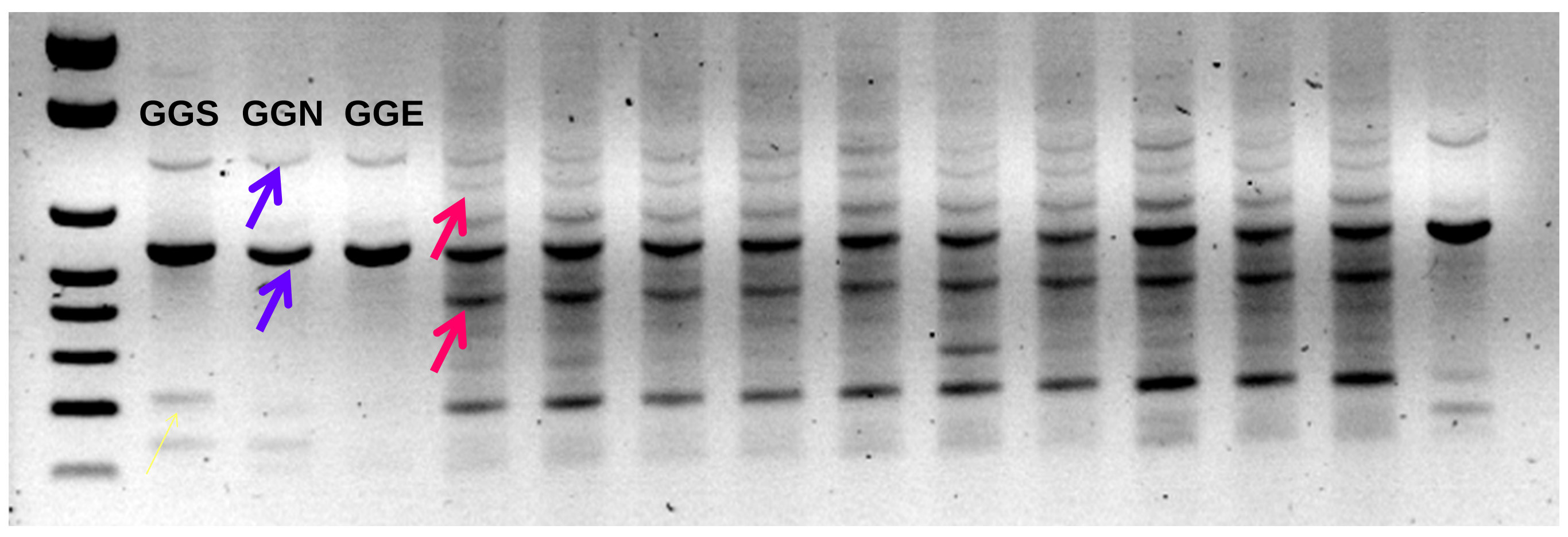


Fig. 2. Amplified products from OPC-2 RAPD marker. Polymorphic (pink arrows) and monomorphic bands (blue arrows). GGS, GGN and GGE are putative Galega cultivars.

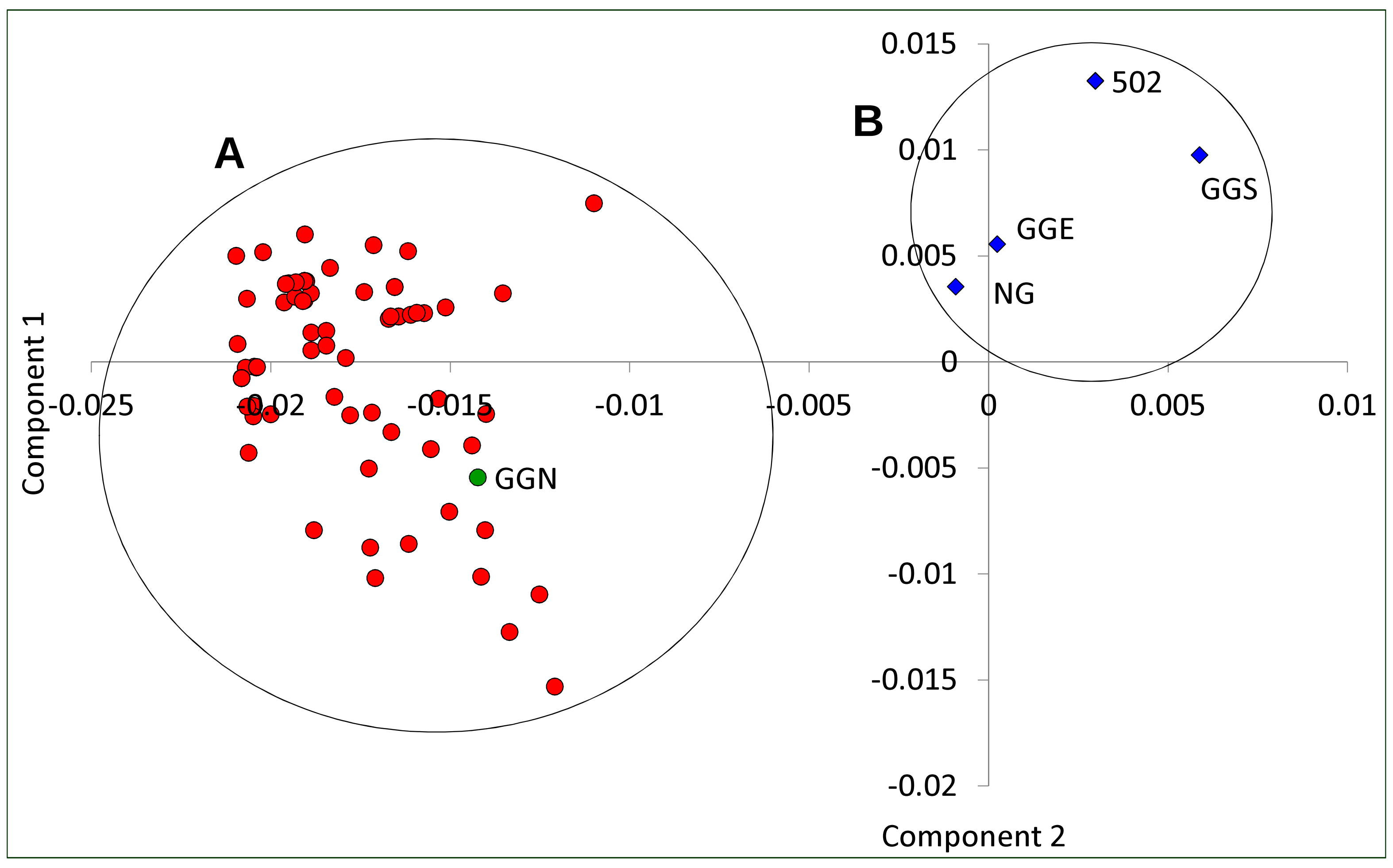
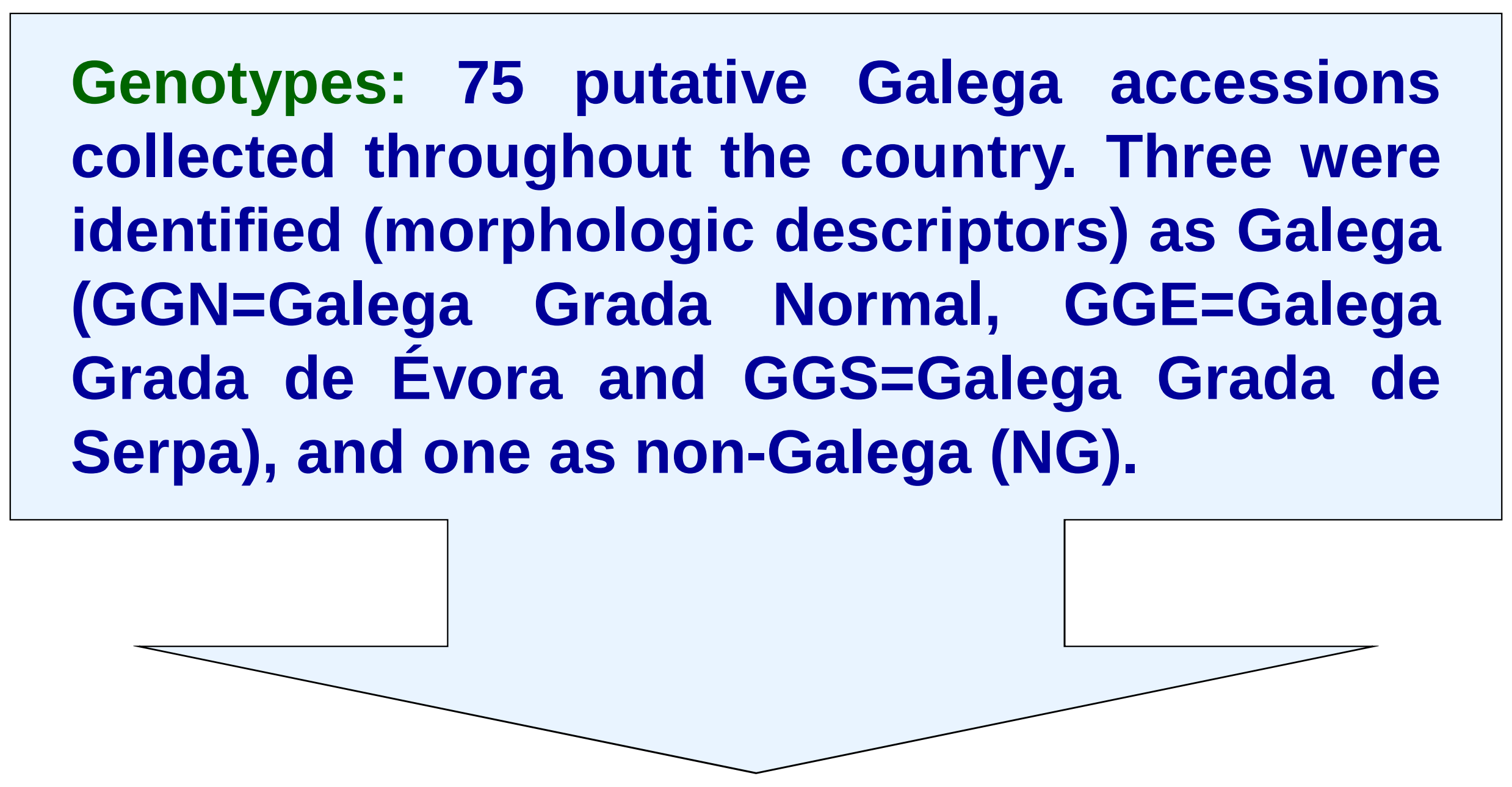


Fig. 3. PCA based on the pairwise genetic matrix (Dice coefficient) and two groups were defined: A and B. Group A includes all the putative Galega accessions (red circles), including GGN (green circle), but not 502. Group B includes NG, GGE, GGS and 502 (blue diamonds). The 1st and the 2nd component explain 81.1 % and 6.9 % of the total variation, RESPECTIVELY

Tab. 2. AMOVA of the whole data set clustered in the two groups (A and B) according to the PCA (Fig. 3). SS=sum of squared deviation, df=degrees of freedom, P=level of probability of obtaining a more extreme component estimate by chance alone.

Source of variation	df	SS	Variance comp.	% of total variance	P
A vs. B (Φ_{ST} = 0.88)					
Among groups	1	35.703	4.62	87.68	< 0.0001
Within groups	77	49.930	0.64	12.32	
Total	78	1963.35	5.26		

MATERIAL AND METHODS



Genotypes: 75 putative Galega accessions collected throughout the country. Three were identified (morphologic descriptors) as Galega (GGN=Galega Grada Normal, GGE=Galega Grada de Évora and GGS=Galega Grada de Serpa), and one as non-Galega (NG).

DNA extraction

Fig. 1. Plant material for DNA extraction. (a) Leaves selection. (b) Conservation at -80ºC. (c) Crushing the leaves with liquid nitrogen

***Molecular markers*: 20 RAPDs (OPC)**

Tab. 1. RAPD primers used, OPC 1-20. Pb=number of polymorphic bands, Nb=number of bands, and P(%)=% polymorphic bands.

Primer	Sequence 5'-3'	Pb	Nb	P (%)
OPC-1	TTCGAGCCAG	4	12	33.3
OPC-2	GTGAGGCGTC	6	9	66.6
OPC-3	GGGGGTCTTT	1	5	20.0
OPC-4	CCGCATCTAC	3	7	42.8
OPC-5	GATGACCGCC	3	5	60.0
OPC-6	GAACGGACTC	2	11	18.2
OPC-7	GTCCCGACGA	6	12	50.0
OPC-8	TGGACCGGTG	1	8	12.5
OPC-9	CTCACCGTCC	3	7	42.8
OPC-10	TGTCTGGGTG	4	7	57.1
OPC-11	AAAGCTGCGG	0	2	0.0
OPC-12	TGTCATCCCC	2	7	28.6
OPC-13	AAGCCTCGTC	4	10	40.0
OPC-14	TGCGTGCTTG	1	6	16.7
OPC-15	GACGGATCAG	0	3	0.0
OPC-16	CACACTCCAG	2	5	40.0
OPC-17	TTCCCCCCAG	0	6	0.0
OPC-18	TGAGTGGGTG	0	3	0.0
OPC-19	GTTGCCAGCC	1	7	14.3
OPC-20	ACTTCGCCAC	3	12	25.0
Sum		46	144	

CONCLUSIONS

The RAPD markers revealed 144 bands, and 32% were polymorphic (Tab. 1). The expected heterozygosity was low (He=0.10±0.01; Lynch & Milligan method), as expected for clonal propagated plants.

The PCA using the Dice coefficient matrix distinguished 2 groups (Fig. 3). The AMOVA confirmed that group A and B are significantly different (P<0.0001), Φ_{ST}=88%, but a weak, yet prevalent variation, was found within groups, 12%, considering that the trees are putative clones (Tab. 2).

Apparently, the entire group A individuals are related among them and with GGN. Assuming GGN as Galega cultivar, we might suppose that trees within the A group are Galega cultivar accessions. Nevertheless, some variability exists within groups, may be due to the accumulation of somatic mutations and/or a putative origin from different but related genotypes.

Trees from group B have certainly a very different genetic origin compared to group A genotypes, already reflected in the different names attributed to putative Galega cultivars, even though some of them might correspond to the Galega cultivar morphologic descriptors. This confirms their lack of precision.

A future study, using a broader sampling and high discriminant molecular markers, such as microsatellites, would help to unambiguously fingerprinting the Galega cultivar genotypes, for certification purposes.

References : Besnard G. 2001. Cultivar identification in Olive based on RAPD markers. J. Amer. Soc. Hort. Sci. 126: 668-675; Cordeiro A et al. 2008. Genetic diversity assessment in Portugal accessions of *Olea europaea* by RAPD markers. Biol. Plant. 52 : 642-647; Gemas et al. 2004. Genetic diversity in the olive tree (*Olea europaea* L. subsp. *europaea*) cultivated in Portugal revealed by RAPD and ISSR markers. Genet. Resour. Crop Evol. 51:501-511; Lynch M and Milligan BG (1994) Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91-99.

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