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ORIGINAL ARTICLE

# Genetic variation in natural populations of *Acacia visco* (Fabaceae) belonging to two sub-regions of Argentina using AFLP

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**Abstract** *Acacia visco* is a tree native to South America that grows in central and northwest region of Argentina, north Chile and Bolivia and has also been introduced to Africa and naturalized in Europe. Little is known about genetic diversity and genetic structure of this species. Therefore, we studied natural Argentinean populations of *A. visco* using the AFLP technique, by determining the genetic diversity of the species and its genetic structure, considering the proportion of the species diversity explained within and between the two sub-regions where populations were this species is present in the country. Fourteen of the 445 loci obtained appeared to be under diversifying selection. The remaining 431 neutral loci showed a mean of 60.89 % of percentage of polymorphic loci. The estimates of genetic diversity $H_E$ were generally high. The $F_{ST}$ (0.126) was highly significant, providing evidence for genetic structure among populations. Hierarchical AMOVA indicated that variation between sub-regions was 2.1 % and highly significant. The higher component of variance was found within populations (77.4 %). STRUCTURE analysis showed an optimal number of $K = 6$. This result was consistent with those obtained by UPGMA from Nei's distances and Canonical Discriminant Analysis. Since differentiation of *A. visco* populations in sub-regions was highly significant, a suitable management strategy for the use of this species in restoration programs would be focus on sampling seeds of a high number of individual trees within populations and also ensure a comprehensive coverage of the entire ecological amplitude of this species.

**Keywords** *Acacia visco* · AFLP · AMOVA · Genetic differentiation · *Senegalia visco* · STRUCTURE

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## Introduction

The genus *Acacia* Mill. includes over 1450 species distributed in tropical and subtropical regions of the Americas, Australia, Africa, and southern Asia (Guinet and Vassal 1978; Ross 1981; Luckow 2005). Additionally, in many dryland areas it is the dominant shrub or tree on which humans and animals depend (Rico-Arce 2007). In northern and central Argentina *Acacia* is represented by 21 woody species. Most of them are trees or shrubs 2–6 m high, although some species reach up to 20 m.

The circumscription of the genus *Acacia* is currently controversial, since it may be treated as a single genus or as comprising multiple genera. Considerations about this subject can be found in Orchard and Maslin (2005), Smith et al. (2006), Van Rijckevorsel (2006) and Moore et al. (2011).

Following Vassal's treatment (Vassal 1972; Polhill et al. 1981), *Acacia* is considered as a single genus with three subgenera (*Acacia*, *Aculeiferum* Vassal and *Phyllodineae* (DC.) Ser.). The native American species of *Acacia* belong to two subgenera: *Acacia* and *Aculeiferum*. However,

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recently other authors (Seigler et al. 2006) included the American species of subg. *Aculeiferum* in *Senegalia* Raf. In the present work, for the benefit of the users, the authors recognize this last classification although they will use the name *Acacia* s. l.

*Acacia visco* Lorentz ex Griseb. belongs to the subg. *Aculeiferum*. It is native to South America and it has also been introduced to Africa and naturalized in Europe. This plant is commonly known as "viscote" or "arca", and it is cultivated as ornamental plant (Pedernera et al. 2010). As most Leguminosae, their roots are able to fix atmospheric nitrogen and contribute to improve soil fertility in arid regions. Among other potential uses it was shown that extracts from leaves and bark of this species have anti-inflammatory properties (Pedernera et al. 2010).

Since half 19th century there have been many tries to summarise the Latin-American biome in a varied number of regions and biogeographic provinces (Cabrera 1976; Burkart et al. 1999). The main differences among previous proposals were the criterion for delimitation of the areas, which give variable relative weight to geographical, paleontological, floral or faunal considerations. However, different authors generally recognize implicitly that the units defined on their schemes represent historical entities. In 2001, Morrone made a revision of some previous schemes of Latin-American and the Caribean Biogeography and proposed a more natural classification that arranges hierarchically the space in regions, sub-regions and biogeographic provinces. According to Morrone (2001), native *A. visco* populations can be found in two regions, Neotropical and Andean. Within the Neotropical Region *A. visco* is found in the Chaqueña sub-region in the biogeographical provinces of Chaco and Monte, which, according to a biogeographical cladistic analysis based in floral and faunal taxa (Morrone 1993), are closely related to each other. Taken as a whole, Chaco and Monte provinces occupy southern Bolivia, western Paraguay, southern Brasil, and northern and central Argentina to the parallel 44°S to the north west of the political province of Chubut.

Within the Andean Region, *A. visco* can be found in the Puna biogeographical province of the Puneña sub-region. This province occupies Bolivia, northern Argentina and Chile, and southern Perú. In this work we choose the more conservative classification of sub-regions (Puneña and Chaqueña) (Fig. 1). The most important climate and ecological characteristics of the two sub-regions can be summarised as follows. The climate in Chaqueña sub-region is continental, warm-subtropical, with areas presenting the maximum absolute temperatures of the continent. The mean annual temperature varies from north to south, from 23 to 18 °C. Precipitation varies from 500 to 700 mm per year and it occurs mainly during the summer, decreasing towards the south–west to the limit with the Puneña sub-region. Characteristic vegetation is xerophilous woods. Also savannas and grazing lands can be found.

In the Puneña sub-region the climate is cold and dry, presents high daily thermal oscillation that can reach 30 °C, annual media lower than 8 °C and winter minima lower than −20 °C. Precipitation is in summer and averages 100–200 mm per year, increasing to the north. The predominant vegetation is shrubby steppe, which contains species which grow as scattered groups of shrubs.

Aronson (1992) advanced the hypothesis that geographical separation in species of *Acacia* may be associated

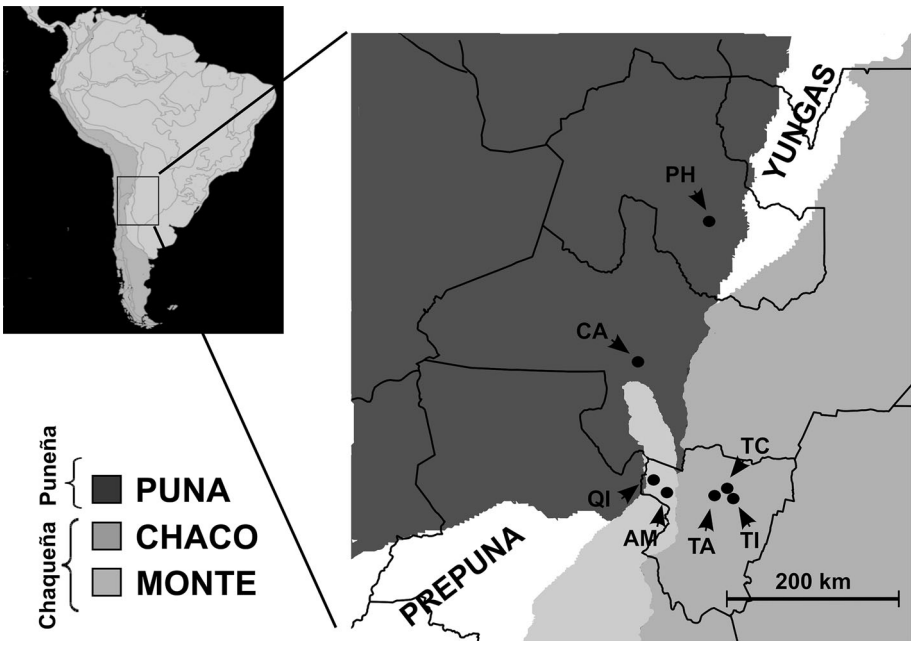


**Fig. 1** Map of South-America showing the two regions present: *dark grey* Neotropical Region; *light grey* Andean Region, and sampled area of Argentina showing the two sub-regions and the populations where the *Acacia visco* specimens were sampled from. *AM* Amaicha, *CA* Cachi, *PH* Posta de los Hornillos, *QI* Quilmes, *TI* Ruta a Ticucho, *TA* Tapia, *TC* Ticucho

with ecological differentiation among regions, but in the case of *A. visco* little is known about genetic diversity and differentiation among natural populations.

Genetic diversity is critical for adaptation to environmental changes for long-term survival of a species. Knowledge of genetic diversity and its causes can provide insights into their ecological and evolutionary histories; meanwhile, such information also may help conservation and restoration (Hamrick and Godt 1996; Avise 2006).

DNA-based markers provide accurate and useful information to study genetic diversity in many plant species (Villalobos-Barrantes et al. 2015). The amplified fragment length polymorphism (AFLP) technique is very useful and powerful compared to other DNA markers, because (1) it is applicable to all organisms without previous sequence information, (2) it provides the capability to detect various polymorphisms in different genomic regions, simultaneously allowing the separation of closely related species and (3) it provides highly reproducible and robust data in comparison to other dominant multilocus marker systems, as it generates a larger number of amplified products in a single reaction (Campbell et al. 2003; Savelkoul et al. 1999; Vuylsteke et al. 2007).

In this context, we used the AFLP technique to study natural populations of *A. visco* from the two sub-regions where the species occurs in Argentina. The main objective was to determine the proportion of genetic variability represented at different hierarchical levels (sub-regions, populations, and individuals), as well as differentiating random demographic processes from adaptive processes. This information is key to a conservation and management program for *A. visco* and the results might be extrapolated to other *Acacia* species.

## Materials and methods

### Study species

*Acacia visco* is a leguminous tree, without spines, prickles or thorny stipules; it can grow up to 20 and 50 cm of diameter at breast height (dbh). On its specific form, it has a globose top and semi-perennial foliage. Its flowers are white, not scented and its fruit is a typical dehiscent legume, of 10–17 cm long and 1.5–2.5 cm wide. Flowers bloom from October to December and the fruits ripen between February and April (Cialdella 1984). Chromosome numbers for this species have been reported as $2n = 26$ (Zanín et al. 1998), although Covas and Schnack (1946) also recorded diploid and tetraploid karyotypes ($2n = 26$ and $2n = 52$). Pollen is shed in polyads consisting of 16 grains (Cialdella 1984). Seeds are found in numbers of 8–12 per fruit (Cialdella 1984). *A. visco* is found in Chile, Bolivia and Argentina, being these countries the native range of the species. In Argentina *A. visco* grows in the provinces of Salta, Jujuy, Tucumán, Catamarca, La Rioja, San Juan and San Luis.

### Study sites and sample collection

Seven populations of *A. visco* were collected in Northwest Argentina (Table 1; Fig. 1). The total number of sampled individuals was 362. Sampling methodology was that of Vilardi et al. (1988) and Saidman and Vilardi (1993). Approximately 50 seed pods were collected from 5 to 12 mother trees per population that were separated from each other by more than 50 m.

Representative vouchers of each population are deposited at the herbarium SI, Instituto de Botánica Darwinion, San Isidro, Buenos Aires, Argentina.

### AFLP methods and data analysis

#### *DNA extraction*

Cotiledons were ground to a fine powder in liquid nitrogen and then placed in a microtube. The DNeasy Plant kit (QIAGEN Inc., Valencia, CA, USA) was used for DNA extraction following the manufacturer's instructions. DNA was stored at −20 °C.

The AFLP assay was performed as described by Vos et al. (1995), but with a slight modification. Three selective

**Table 1** Populations of *Acacia visco* sampled in this study

| Population | Population code | Latitude (°S) | Longitude (°W) | Altitude (m o.s.l.) | Province (state) | Region | Sub-region | Bio-geographic province |
|---|---|---|---|---|---|---|---|---|
| Amaicha | AM | 26°35′36.00″ | 65°51′58.02″ | 2200 | Tucumán | Neotropical | Chaqueña | Monte |
| Cachi | CA | 25°10′0.8″ | 66°10′59.3″ | 2350 | Salta | Andina | Puneña | Puna |
| Posta de los Hornillos | PH | 23°39′15″ | 65°25′52″ | 2400 | Jujuy | Andina | Puneña | Puna |
| Quilmes | QI | 26°27′55.02″ | 66° 2′11.40″ | 1850 | Tucumán | Neotropical | Chaqueña | Monte |
| Ruta a Ticucho | TI | 26°36′29.88″ | 65°10′17.76″ | 703 | Tucumán | Neotropical | Chaqueña | Chaco |
| Tapia | TA | 26°36′31.6″ | 65°20′35.4″ | 983 | Tucumán | Neotropical | Chaqueña | Chaco |
| Ticucho | TC | 26°34′15.00″ | 65°16′28.20″ | 769 | Tucumán | Neotropical | Chaqueña | Chaco |

primers were combined as follows: E + ACA/M + CTT (C1), E + AGG/M + CAG (C3) and E + AAC/M + CAA (C4). In all cases, primers M + 3 were labelled with a fluorescent dye 6-FAM. A complete technical replicate was run for a subset of 30 randomly selected individuals (about 10 % of the total) from the restriction-ligation stage. Negative controls were carried out to test for systematic contamination and dye blobs. PCR products were electrophoresed in an ABI313XL (HITACHI) automated DNA sequencer and automatically sized with the size standard GS500 LIZ using GENEMAPPER ver 3.7 (Applied Biosystems). The size of AFLP bands (bins) scored ranged from 50 to 400 bp. All bins were set to a width of two bases pair, and those fragments with peak heights below 50 relative fluorescence units (RFU) were assumed to represent instrument noise and were not scored. To ensure that bin positions were assigned accurately, all bins were then checked manually. Any bins possessing fragments that overlapped with adjacent bins were removed. As well, we adjusted bins assigned off-centre of any peak distributions. Finally, all AFLP profiles were checked manually to ensure successful amplification and were either re-run or removed from analysis if the fingerprint failed to amplify or appeared to possess many unique fragments.

*Data scoring and analysis*

Data were scored as band presence (1) or absence (0). Each AFLP band was considered as a single bi-allelic locus with an amplifiable (dominant) and a null (recessive) allele.

Population structure analysis is based on the correlation of random gametes within subdivisions relative to the total population (quantified by the $F_{ST}$ coefficient), which assumes that the differentiation among populations results from interaction between drift and migration (Wright 1978). Therefore, genetic structure parameter estimates should be based on neutral loci. Besides, pairwise genetic distances between populations are assumed to be correlated with divergence time if based on neutral loci (Nei 1975).

In order to distinguish parts of the genome subjected to natural selection from neutral loci we applied a population genomics approach (Foll and Gaggiotti 2008). This method is based on checking outlier $F_{ST}$ loci. The basic rationale is that loci influenced by directional (also called adaptive or positive) selection will show a larger genetic differentiation ($F_{ST}$) than neutral loci, and loci that have been subject to balancing (also called negative or purifying) selection will show a lower genetic differentiation.

In order to check for outlier-$F_{ST}$ loci, BayeScan v2.1 program (Foll and Gaggiotti 2008) was used with a burn-in period of 50,000, a thinning interval of 10, a number of iterations of 100,000, a number of pilot runs of 20 and a length of each pilot run of 5000. The dataset was divided in two subgroups, "neutral" and "selective" (outlier) AFLP loci as suggested by Luikart et al. (2003).

For neutral loci, allele frequencies were estimated using the Bayesian method with non-uniform prior distribution of allele frequencies, as described by Zhivotovsky (1999) by means of the software AFLP-SURV (Vekemans 2002), following Lynch and Milligan (1994) approach. The distribution of whole genetic diversity at different hierarchical sub-regional levels was estimated by analysis of molecular variance (AMOVA) considering sub-regions, populations, and individuals as nested levels. The decomposition of variance by AMOVA was conducted following Excoffier et al. (1992), using the matrix approximations from Dyer et al. (2004) with the software GeneticStudio (Dyer 2008). Non hierarchical Wright (1978) $F_{ST}$, variability measures, Nei (1973) genetic diversity $H_E$ and pairwise Nei (1978) genetic distances between populations were also estimated using the software AFLP-SURV (Vekemans 2002). Band richness ($B_R$, Coart et al. 2005) expected at each AFLP locus was computed using the rarefaction approach, based on a sample size of 18 using AFLPDIV 1.0 (http://www.pierroton.inra.fr/genetics/labo/Software/). Number of private bands (PB) and number of private fixed bands (PFB) per population and per sub-region were calculated with LibreOffice Calc (ver. 4.2.8.2) (The Document Foundation 2000–2014).

A neighbor-joining unrooted tree using the AFLP matrix of all individuals as input file was obtained using the package *ape* (Paradis et al. 2004) of the program R (R Core Team 2015). The bootstrap support of branches was obtained using the command *boot.phylo* of the same package using 1000 replicates.

A matrix of pairwise Nei's genetic distance among populations was obtained using the software AFLP-SURV (Vekemans 2002) as indicated before, and used to obtain a phenogram by the UPGMA clustering method (Sneath and Sokal 1973) with the software Statistica 5.5 (StatSoft Inc. 2000). Support for branching pattern was determined using 1000 bootstrap permutations of the data set using the software Phylip ver. 3.695 (Felsenstein 2005, http://evolution.genetics.washington.edu/phylip/getme.html).

To identify spatial structure in the populations of *A. visco*, a Bayesian model-based cluster analysis was performed using the STRUCTURE program version 2.3.4 (Pritchard et al. 2009). The burn-in period and the number of MCMC repetitions were set respectively to 50,000 and 100,000. An admixture model was used, with correlated allele frequencies. *K* was set at 2–8, and the highest *K* value was identified as the run with the highest likelihood value, following the recommendations of Pritchard et al. (2000). In addition, *K* values were averaged across ten iterations. The results of STRUCTURE were edited with software

CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and Distruct 1.1 (Rosenberg 2004) to obtain the plot.

Geographic distances between sample sites were estimated using Google Earth 4.3 measuring tool (2008) (http://earth.google.es). A Mantel test was performed using the routine ISOLDE of the program GENEPOP (Raymond and Rousset 1995), to asses isolation-by-distance by testing the relationship between pairwise $F_{ST}$ and geographic distances.

Canonical discriminant analysis was applied to AFLP data to summarise variation between predefined classes (populations) for classification variables (band presence/absence). This analysis was carried out with the software Statistica 5.5 (StatSoft Inc. 2000).

In order to evaluate if the patterns of genetic differentiation differs between selective and neutral loci, the degree of genetic differentiation for selective loci was also estimated by AMOVA at different levels: between sub-regions, among populations within sub-regions, and within populations.

## Results

The three primer pair combinations used for AFLP analysis generated a total of 669 bands in the interval of 50–400 bp. The great number of bands gave the opportunity of selecting those with 100 % reproducibility. After checking error rates by replicating a blind sample of 30 individuals, the original matrix was pruned and only loci with 0 % error rate (445 loci) were included in further analysis. The number of bands retained was adequate for the purpose of this work, according to the recommendations of Bonin et al. (2007).

Each of the 362 individuals analysed showed a distinctive banding pattern. Analysis of presence of outlier-$F_{ST}$ AFLP loci with a chosen $q$ value threshold of 10 %, showed that about 3 % of loci (14 out of 445) appeared to be under diversifying selection. According to these results the 14 outlier loci were removed and further analysis (with the exception of AMOVA were both data sets were used) were based on the 431 loci assumed as neutral.

The percentage of polymorphic loci (PPL) ranged from 43.4 % in TA population to 74.2 % in TC with a mean of 60.89 % over the seven populations. Estimations of genetic diversity varied from $H_E = 0.16$ (TA) to $H_E = 0.26$ (TC) with a mean of $H_E = 0.20$. Band richness ranged from $B_R = 1.48$ in AM population to $B_R = 1.78$ in TC (Table 2). Moreover, only CA, PH, TC and TI populations showed private bands (PB) (10, 3, 4 and 1, respectively) (Table 2). At the level of sub-region, 13 private bands were found in Chaqueña and 24 in Puneña sub-region. In no case the private bands were fixed in the corresponding population.

The analysis of population structure performed with the software AFLP-SURV indicated that the component of variability within populations ($H_w = 0.20$) was higher than among populations ($H_b = 0.03$). The non hierarchical $F_{ST}$ estimate (0.126) was highly significant ($P = 0.000$), providing evidence for genetic structure among populations. However, Mantel test between pairwise $F_{ST}$ and geographical distances was not significant ($P = 0.08$).

**Table 2** Summary of genetic diversity based on 431 neutral AFLP loci

| Population | *N* | PB | PPL | $H_E$ | SE ($H_E$) | $B_R$ |
|---|---|---|---|---|---|---|
| AM | 46 | 0 | 58.0 | 0.17 | 0.01 | 1.48 |
| CA | 93 | 10 | 71.7 | 0.25 | 0.01 | 1.75 |
| PH | 44 | 3 | 56.6 | 0.17 | 0.01 | 1.55 |
| QI | 42 | 0 | 61.0 | 0.21 | 0.01 | 1.60 |
| TI | 30 | 1 | 61.3 | 0.19 | 0.01 | 1.57 |
| TA | 89 | 0 | 43.4 | 0.16 | 0.01 | 1.49 |
| TC | 18 | 4 | 74.2 | 0.26 | 0.01 | 1.78 |

*N* sample size, *PPL* percentage of polymorphic loci, $H_E$ expected heterozygosity under panmixia, *SE* ($H_E$) standard error of $H_E$, $B_R$ band richness values overall neutral AFLP loci in each population, *PB* private bands per population

The results from hierarchical AMOVA with neutral loci indicated that of the total genetic diversity, 77.4 % resided within populations, 20.5 % resided among populations and that the variation between sub-regions was low (2.1 %) but highly significant (Table 3). By contrast, hierarchical AMOVA with selective loci did not show evidence of significant variation between sub-regions (Table 4).

Analysis of data using STRUCTURE revealed that $K = 6$ had the highest mean probability of density [Ln P(D) = −46,303.52], after which this value reached a plateau, which suggested that the optimal number of $K$ was 6. In this analysis individuals from close populations of AM and QI were genetically similar constituting a group. In the same way individuals corresponding to TI and TC populations joined together in another group. The remaining populations of *A. visco* were clearly differentiated from each other, as they were constituted by individuals from different groups and they were not associated by sub-region. Moreover, the STRUCTURE results detected admixture individuals in all populations (Fig. 2).

The neighbor-joining tree obtained from the original matrix of all individuals showed that most of them belonging to the same population were clustered and four populations from the Chaqueña sub-region (AM, QI, TI, and TC) appeared grouped in the tree. In the same way, individuals of TA and PH populations grouped together, but not those individuals from CA population, where a large proportion of individuals are found together but a little group remains apart (Fig. 1a, b, Online resource 1). This situation is similar to that observed in the results of STRUCTURE (Fig. 2).

**Table 3** Analysis of molecular variance (AMOVA) based on 431 neutral AFLP loci in two sub-regions

| Source of variation | *df* | SSD | MSD | Variance (%) | $\Phi$ | *p* |
|---|---|---|---|---|---|---|
| Between sub-regions | 1 | 435.50 | 435.50 | 2.1 | 0.021 | 0.001 |
| Among pop. within sub-regions | 5 | 1173.25 | 234.65 | 20.5 | 0.210 | 0.001 |
| Individuals within populations | 355 | 6384.80 | 17.98 | 77.4 | | |

$\Phi$ fixation index

**Table 4** Analysis of molecular variance (AMOVA) based on 14 AFLP loci candidate to diversifying selection in two sub-regions

| Source of variation | *df* | SSD | MSD | Variance (%) | $\Phi$ | *p* |
|---|---|---|---|---|---|---|
| Between sub-regions | 1 | 74.24 | 74.24 | 0.0 | −0.07 | 1.000 |
| Among pop. within sub-regions | 5 | 319.57 | 63.91 | 29.7 | 0.70 | 0.001 |
| Individuals within populations | 355 | 209.62 | 0.59 | 70.3 | | |

$\Phi$ fixation index

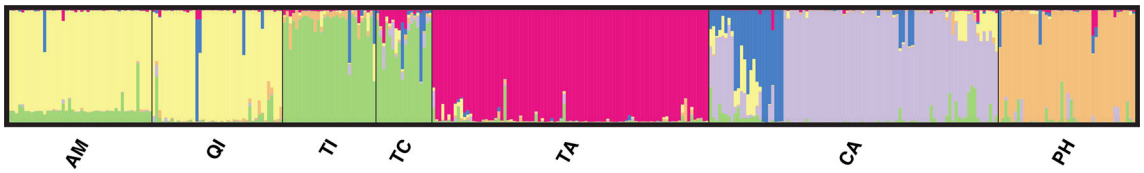


**Fig. 2** Clustering of individuals made by STRUCTURE for $K = 6$. Each individual is represented by a *vertical coloured line*. *Same colour in different individuals* indicates that they are belonging to the same cluster or group. Population codes are the same used in Fig. 1

Due to the high number of individuals studied in this work, the degree of differentiation among populations was more clearly visible when a phenogram was obtained for population level rather than individual data. The cluster analysis of populations using UPGMA showed a main group of four populations from the Chaqueña sub-region (AM, QI, TI, and TC), consistent with the results obtained in the tree based on individuals. The bootstrap support of this group was very high (96 %). This cluster could be separated in two subgroups: one formed by AM and QI populations and another by TI and TC populations, and in both cases the support was higher than 50 %. The other populations were separated from these two groups and from each other (Fig. 3).

Canonical discriminant analysis based on allele frequencies succeeded to differentiate all the populations studied. Ninety-nine of 431 neutral loci were sufficient to differentiate populations. The correct classification of individuals into their respective populations based on AFLP profiles was 100 % in all cases. The first, second and third canonical roots accounted for 40, 22.5 and 16.1 % of variation respectively, explaining a cumulative 78.6 % of molecular variation. The plot showed clearly four groups of populations consistent with the phenogram in Fig. 3, two of them corresponding to populations from Puneña sub-region: CA and PH; the third group was represented by individuals from TA and the fourth group was heterogeneous, including four populations

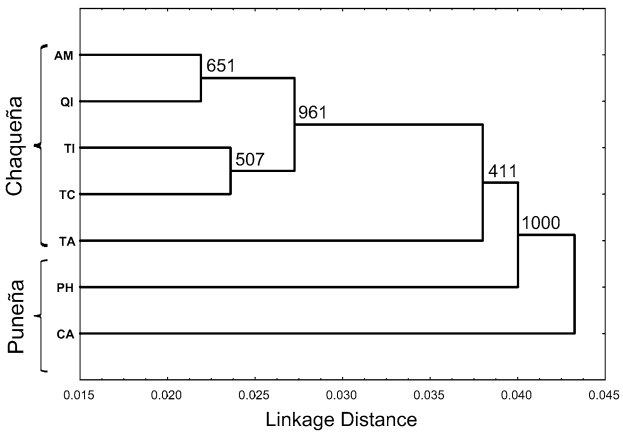


**Fig. 3** UPGMA phenogram showing the genetic relationships based on Nei's genetic distances among the seven populations of *Acacia visco* using AFLP dataset with bootstraping values based on 1000 bootstraps

belonging to Chaqueña sub-region (AM, QI, TI, and TC) (Fig. 4).

## Discussion

Since genetic diversity is considered the result of long-term evolution and represents the evolutionary potential of a species, to survive in a harsh environment, a species has to

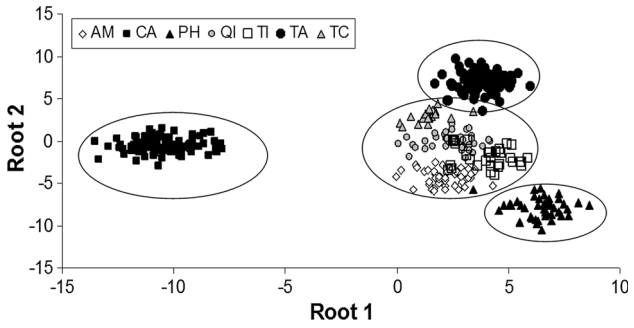


**Fig. 4** Plot of canonical discriminant functions 1 and 2 of *Acacia visco* populations from AFLP data

change in some aspects and accumulate more genetic variation in order to adapt itself to various ambient pressures (Li et al. 1999). The ability to detect of high levels of polymorphism makes AFLP analysis a powerful tool for assessing genetic diversity in many species (Mueller and Wolfenbarger 1999; Bensch and Akesson 2005). In this study, we used AFLP markers to investigate genetic diversity and structure in *A. visco.* The analysis showed that each individual here studied was genetically unique based on its band pattern, indicating that this level of resolution was suitable to distinguish all multilocus genotypes.

Since loci under selection will often behave different from neutral ones revealing "outlier" patterns of variation is extremely important to reliably infer population-demography history and to detect selected loci (Luikart et al. 2003). In this work, only 14 of the 445 loci produced by the AFLP technique appear to be under diversifying selection, so they were treated as "selective" ones.

Within the neutral loci, the number of private bands (PB) per population was low (ranging from 0 to 2 % of the total number of loci analysed) and even at the sub-region level the number of private bands is only about 5 % of the loci. Moreover, the private bands are mostly in low frequencies as to be useful for diagnosing populations or regions. For selective loci no private bands were observed whatsoever.

With the 431 neutral loci the mean heterozygosity ($H_E = 0.20$) and the mean percentage of polymorphic loci (PPL = 60.89 %) coefficents indicated that the genetic variation in *A. visco* is relatively high. Indeed, the estimated $H_E$ is 40 % of the theoretically maximum expected for a bi-allelic system (0.5). Unfortunately, percentage of polymorphism is difficult to compare across studies since the investigated loci have usually been preselected for high levels of polymorphism. For comparisons across studies, estimates of heterozygosity still appear to be the most suitable and commonly reported parameters, although these are also influenced by choice of bands and loci (Nybom 2004). Keeping in mind this consideration, comparison of our results was made only with previous studies using markers of the same nature (dominant ones). Similar results were found in other widely distributed American acacias like *A. farnesiana* (average $H_E = 0.12$, PPL = 26.03 %) and *A. curvifructa* ($H_E = 0.21$, PPL = 51.2 %) (Pometti et al. 2015), and in an African species, *A. senegal* ($H_E = 0.255$) (Chiveu et al. 2008).

The estimates of band richness were similar to those obtained by AFLP for the South American species *Schinus molle*, $B_R$ between 1.404 and 1.855 (Lemos et al. 2015).

The genetic structure of *A. visco* was assessed by several approaches in this work.

A significant amount of genetic differentiation among populations was observed using both the Wright's approach ($F_{ST} = 0.126$) and AMOVA ($\Phi_{ST} = 0.23$) and a large proportion of genetic variation (about 77.4 %) existed within populations, analyzing the neutral loci data set.

The analysis of molecular variance showed that the variance between sub-regions was relatively low (2.1 %), but highly significant.

Although among populations diversity estimates yielded similar results for AFLP and RAPD, ISSR-derived estimates have sometimes been higher (Nybom 2004). Therefore, this consideration was taken into account for comparisons, analyzing only the general trend of the results. Relatively high levels of variability within populations were also detected by AMOVA in other African species of *Acacia*: 91 % in populations of *A. senegal* var *kerensis* (Omondi et al. 2010), and 86 % in populations of *A. senegal* analysed as a whole (Chiveu et al. 2008). Moreover, high levels of variation within populations were detected in American species of *Acacia*: 68.5 % in populations of *A. caven* (Pometti et al. 2012) and 77.6 % in populations of *A. farnesiana* (Pometti et al. 2015). However, little or no evidence of genetic structure was found in *A. senegal* and *A. albida* by means of Wright's $F_{ST}$ index (Omondi et al. 2010; Joly et al. 1992).

The high diversity within populations and relatively lower differentiation among populations is consistent with the evidence indicating that most *Acacia* species are predominantly outcrossers, sometimes presenting self-incompatibility systems (Bernhardt et al. 1984; Kenrick and Knox 1985; Kenrick et al. 1986; Moran et al. 1989; Sedgley et al. 1992; Casiva et al. 2004; Pometti et al. 2011). In a previous study of the mating system of three populations of *A. visco* (Pometti et al. 2013) the estimate for the multilocus outcrossing rate ($t_m$) was high ($\geq$0.971) in all populations, indicating that this species is predominantly an outcrosser. The results obtained here showed that a high proportion of the variability was contained within populations, and this high genetic variability is consistent with the previous results obtained that indicated that *A. visco* is predominantly outcrosser (Pometti et al. 2013).

The analysis with STRUCTURE showed that the optimal number of *K* populations was 6, joining together the populations of AM and QI; and TI and TC. Moreover, these results were consistent with the UPGMA representation and the canonical discriminant analysis where the same four populations were grouped. All these results indicated that populations belonging to Puneña sub-region were more differentiated from the rest in comparison with those belonging to the Chaqueña sub-region. This trend was also reflected in the AMOVA.

A possible hypothesis that would support these results could be that the populations of the Puneña sub-region would be less connected to each other by gene flow than those of the Chaqueña sub-region, since PH was not grouped with CA in none of all the analyses. A possible explanation could be that the geographic distances between AM and QI, and TI and TC were smaller than that between PH and CA, however, genetic and geographical distances among populations were not significantly correlated. Moreover, the differences found between Puneña and Chaqueña sub-regions could be partially attributed to that the populations were found at different altitudes ranging from about 700 (for TI, TA, and TC) to 2400 m o.s.l. for PH. For this reason, the distribution of *A. visco* is fragmented by natural barriers represented by high hills as well as narrow ingression of the Yungas forest on the eastern slope of the Eastern Mountain Chain ("Cordillera Oriental") and Sub Andean Hills in Tucuman and Salta Provinces that separate CA and PH from the rest of populations. The situation of the TA population was rather unexpected as it was not clustered with its neighbour populations of TI and TC. The cause might be attributed to the fact that this population is sited close to the locality of Tapia, an area highly degraded by agricultural and livestock activities (Pometti et al. 2013).

In studies of other *Acacia* species, geographic patterns have been similar. For example: in the six varieties of *A. caven* (Pometti et al. 2012), in the tropical *A. mangium* (Butcher et al. 1998), in the tropical species *A. auriculiformis* (Wickneswari and Norwati 1993) and *A. tumida* (McDonald et al. 2003).

All outlier loci were identified as candidate to diversifying selection. These loci showed relatively higher levels of variation among populations and lower within populations than neutral ones. This noticeable difference between both set of data is consistent with the possibility that the selective loci could be linked to morphological or physiological traits that are able to respond to environmental challenges with allelic frequency changes rather than plasticity. AMOVA applied to these loci detected no differentiation between sub-regions; this result suggests that local differences among populations within sub-regions were the most important factor promoting genetic differences on selective traits.

## Conclusions

The knowledge of the levels and patterns of genetic diversity is important for designing conservation and management strategies since the overall genetic diversity of a taxon has great implications for its long-term survival and evolution. All rangelands in Argentina are currently experiencing some form of deterioration or desertification because the wild ecosystems of Argentina have proven to be fragile and easily damaged by unsustainable use (Fernández and Russo 1997). Conservation and management programs are needed for this and other *Acacia* species to mitigate the effect of the livestock industry which is currently based on grazing of natural vegetation without consideration of potential environmental impacts or ecosystem management techniques. A plausible strategy to be applied in woody species adapted to arid and semiarid zones could be the promotion of agro-sylvo-pastoril systems and protection of subsistence economies in areas of low productivity for traditional agriculture. The present study, together with the results of earlier investigation (Pometti et al. 2013), could be a start point to design a management program for planting projects for *A. visco*. Since differentiation of *A. visco* populations studied here in sub-regions (2.1 % with neutral loci), was highly significant, a suitable management strategy for the use of this species in restoration programs would be focus on sampling seeds of a high number of individual trees within populations and also ensure a comprehensive coverage of the entire ecological amplitude of this species.

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**Compliance with ethical standards**

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Information on Electronic Supplementary Material**

**Online Resource 1. Fig. 1** a) Neighbor-joining tree of 362 individuals of *A. visco* obtained using the AFLP of all individuals as input file; b) Neighbor-joining tree with individuals bootstrap values on each branch.

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