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Restoration over time and sustainability of *Schinus terebinthifolius* Raddi

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ABSTRACT. The success of recovery programs on degraded areas is dependent on the genetic material to be used, which should present heterozygosity and genetic diversity in native and recovered populations. This study was carried out to evaluate the model efficiency to enable the recovery of a degraded area of the Lower São Francisco, Sergipe,

Brazil. The target species for this study was *Schinus terebinthifolius* Raddi. Three populations were analyzed, the recovered area, seed-tree source population, and native tree population border established to the recovered area. The random amplified polymorphic DNA (RAPD) markers were used for diversity analysis. Genetic structure was estimated to evaluate the level of genetic variability existent in each population. There was no correlation between the spatial distribution and the genetic distances for all trees of the recovered area. The heterozygosity present in the recovered population was higher than the native tree population. The seed-tree source population presents genetic bottlenecks. Three clusters were suggested ($\Delta K = 3$) with non-genetic structure. High intra-population genetic variability and inter-population differentiation are present. However, gene flow may also introduce potentially adaptive alleles in the populations of the recovered area, and the native population is necessary to ensure the sustainability and maintenance of the populations by allelic exchange.

Key words: Recovery programs; Inbreeding; Gene flow; Pink pepper; Seed collection

INTRODUCTION

In Brazil, all the major economic cycles, such as wood, sugarcane, gold mining exploration, followed by coffee and industrialization, were based on the logic of higher gain in the short term, resulting in strong changes in natural environments. These events were intense along the coastland, where the first colonization centers were settled, and which was originally occupied by the Atlantic Forest (Lino, 2013). The State of Sergipe has an area of approximately 21,918.4 km², which is approximately the same area of Israel (20,770 km²), El Salvador (21,041 km²), and Haiti (27,750 km²). The state is the smallest of the Brazilian confederation with some particularities, such as eight river basins, the mouth of the São Francisco River, and the Atlantic Forest and Caatinga Biomes. Sergipe is one of the 17 Brazilian states with Atlantic Forest remnants (Brasil, 2006); these forest areas are small and highly fragmented due to the large human impact (RBMA, 2008).

As a result of the fragmentation process to which ecosystems are exposed, their restoration can be an instrument for the establishment of ecological corridors, and the gene flow continuity, which is necessary for the maintenance of species and populations viability. Therefore, the analysis of population genetic structure is necessary for both the knowledge in the maintenance of the existing populations and for the population that will compose the recovery source of a degraded area. Thus, for the success of recovery programs, the genotypes should present the highest heterozygosity possible, since the succession of the area will depend on the genetic produced locally in further generations (Wang, 2005). And for these activities, seeds must be collected from populations that have reasonable levels of genetic variability and with a minimum of inbreeding (Sebbenn et al., 2003).

With the recovery models of degraded areas, genetic connectivity must be continually achieved among and within populations to manage the species and to evidence if the historical and current connectivity can be distinguished (Frankham et al., 2014). Substantial increase in

the effective size (N_E) can usually be carried out in isolated populations of a given species, since it had previously been connected by gene flow, and can become reconnected using artificial gene flow, or habitat corridors (Frankham et al., 2010).

However, to establish artificial corridors, the seeds should be spread or collected for seedling production and planting, which is a procedure that not always samples all the genotypic diversity of seed source populations, mainly due to the low number of individuals in fragmented native areas. As a result, most of the time, the few genotypes represented in seed sampling lots allow the production, in the nursery, of half-sib seedlings from the same individual. Thus, the objectives of the present study were to evaluate the maintenance of the genetic diversity of the *Schinus terebinthifolius* Raddi in the recovery area and to point out the perspective for further tree populations.

MATERIAL AND METHODS

Three populations were used for analysis: recovered population (R), seed source population (P), and native population (N) located at the border of the recovered area. The recovered area (R) is located at the Mãe Natureza Farm (10°15'24"S/36°38'31"W) in the municipality of Santana do São Francisco-SE (10°18'11"S/36°35'56"W). The recovered population was established in June 2003, and is compose of 12 Brazilian native species (Table 1), being 17 individuals from seeds of *S. terebinthifolius* from two regions: São Cristóvão-SE (P), and the National Forest of Ibura, located in the municipality of Socorro-SE (10°50'32"S/37°8'24"W). Fourteen individuals were sampled in the seed source natural population (P) located on the campus of the Federal University of Sergipe, in São Cristóvão-SE (10°55'35"S/37°6'9"W). The native population (N) is located in Santana do São Francisco-SE, approximately 2 km far from the recovered area, on the banks of São Francisco River. This area is inserted in a region with intense community extractive activity. The same number of individuals was used for all populations, based on the minimum number of specimens found.

For RAPD analysis, DNA extraction was carried out using 2 g young leaves macerated with liquid nitrogen and 2% cetyltrimethylammonium bromide (CTAB) 2X buffer (100 mM Tris, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl and 1% PVP) and 20 μL β-mercaptoethanol, following the protocol described by Nienhuis et al. (1995), with modifications.

In the amplification reactions, 3.0 μL DNA, 2.92 μL ultrapure H_2O, 1 μL $MgCl_2$, 1.04 μL dNTPs, 1.04 μL BSA, 1.0 μL PCR 10X buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl) 0.2 μL *Taq* Polymerase, and 2.5 μL decamer oligonucleotides were used (Williams et al., 1990).

Amplifications were carried out in the Biometrics Tpersonal thermocycler, programmed for an initial cycle at 94°C for 5 min, followed by 45 cycles at 94°C for 60 s, 36°C for 2 min, and 72°C for 60 s.

Amplification products for 20 oligonucleotides were separated by electrophoresis on 0.8% agarose gel in TBE 0.5X buffer (Tris-borate, Tris base, boric acid, EDTA, and water) stained with 25 μL/500 mL ethidium bromide of distilled water, and visualized under ultraviolet light.

For the evaluation of the results obtained in RAPD, each fragment present on the gel was assigned with one (1), and each fragment absent on the gel was assigned with 0 (zero). A binary matrix was constructed and used to estimative the genetic similarities using the Jaccard coefficient by the statistical package NTSYS-pc version 2.1. Similarity clusters were obtained and represented in a dendrogram and performed using unweighted pair group method with

arithmetic mean (Rohlf, 2001). Correlation analysis between spatial and genetic distances (Mantel test) was obtained by the NTSYS-pc version 2.1 software.

Genetic structure of populations and the principal coordinates analysis (PCoA) were obtained using the GenAlEx software (Peakall and Smouse, 2006) that estimated: number of observed alleles (N_A), number of effective alleles (N_E), genetic diversity of Nei ($\hat{H}_E$), percentage of polymorphic loci (P%), and the Shannon index (I) (Smith, 2002).

Genetic structure of population was carried out on the Bayesian clustering model, using the STRUCTURE v.2.3.3 software (Hubisz et al., 2009). The set of parameters assumed the "admixture" model, with correlated allele frequencies. Simulations were performed in burn-in of 100,000 replications, with values of k ranging from 2 to 8. The number of clusters (ΔK) was estimated according to the STRUCTURE HARVESTER of Evanno method (Earl and VonHoldt, 2012).

RESULTS

The seed source population (P) presented genetic similarity ranging from 33 to 68% and the average value was 43.63%. There was low genetic similarity (41%) among the individuals of this population, even when they presented low geographical proximity (St$_6$ and St$_{14}$ - 30 m). The most geographically distant individuals, St$_1$ and St$_8$ (1.125 m) presented 46% of genetic similarity. The correlation matrix between the genetic diversity and spatial distance was negative and significant (r = -0.364 and P = 0.0097).

In the recovered area (R), the mean genetic similarity was superior compared with population P, with values of 55.62 and 43.64%, respectively. Population P has fixation index (*f*) of 0.30. Due to the geographical distance (10.52 km) between the regions of the National Forest of Ibura and the region of São Cristóvão, the mixture of seed subsamples of these two areas could contribute to increasing the genetic diversity in the recovered area (R) (Figure 1).
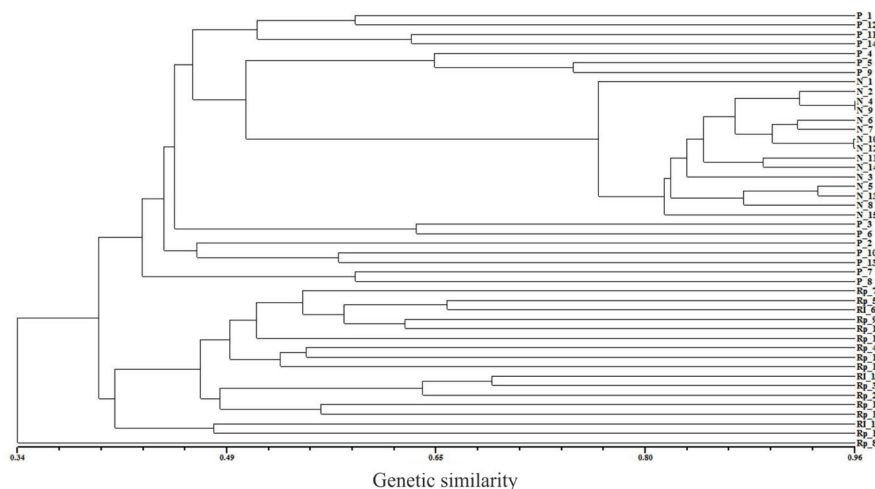

Figure 1. Genetic similarity of populations of *Schinus terebinthifolius* in the State of Sergipe. P: Population used for seed collection - São Cristóvão/SE; Rp: individuals originated from seeds of population P; RI: individuals originated from the National Forest Ibura, Socorro/SE; N: individuals belonging to natural population located next to the recovered area, in the municipality of Santana do São Francisco/SE.

The native population (N) presents high genetic similarity (70 to 95.91%) low value of heterozygosity (0.218) and the Shannon index (0.318) for this population when compared with the others (Table 1).

Table 1. Genetic parameters of individuals of *Schinus terebinthifolius* located in the State of Sergipe.

	N	N_E	H_E	I
Population 1 (P1)	14	1.579	0.345	0.519
Recovered area (R2)	17	1.535	0.315	0.478
Natural population (N3)	15	1.385	0.218	0.318

P1: donor population used for seed collection - São Cristóvão/SE; R2: population originated from seeds of the P1 population - Santana do São Francisco/SE. N = number of individuals sampled; N_E = effective number of alleles per locus; H_E = genetic diversity; I = Shannon index.

For the PCoA (Figure 2) three clusters were obtained, and each sampled population was represented by a cluster, consistent with the high genetic differentiation (F_{ST}) (0.39).
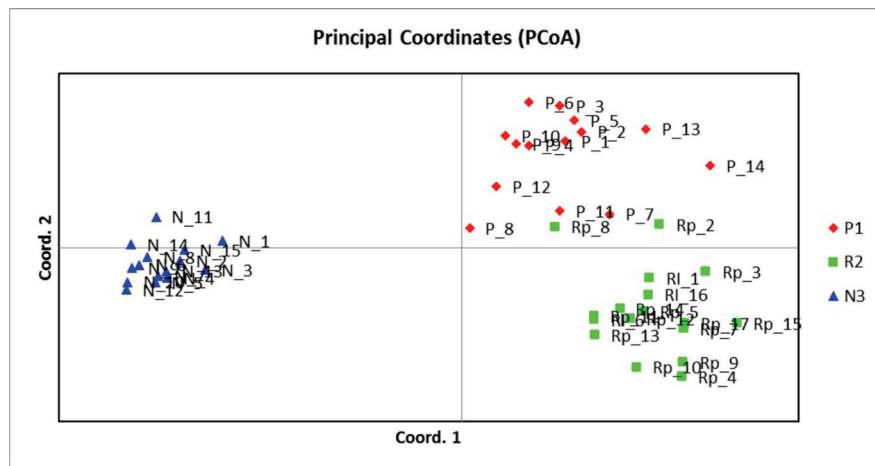

Figure 2. Principal coordinate analysis of populations of *Schinus terebinthifolius* located in the State of Sergipe. P1: Population used for seed collection - São Cristóvão/SE; R2: population originated from seeds of the P1 population - Santana do São Francisco/SE.

Heterozygosities (H_E) observed for populations were 0.34 (P), 0.31 (R), and 0.21 (N) (Table 1), and the genetic structure by the Bayesian analysis presented three genetic clusters (ΔK = 3) (Figure 3).

DISCUSSION

Studies on *S. terebinthifolius* populations are important due to exploitation of pink pepper fruits for subsequent use in agroindustries, which has led local communities to extract large amounts of the fruit in the Lower São Francisco (Jesus and Gomes, 2013), despite the constant dissemination of the impacts that the irrational exploitation of individuals causes breaking branches or even trees suppression. Thus, the recovery of these areas should become a public policy for vegetation restoration.
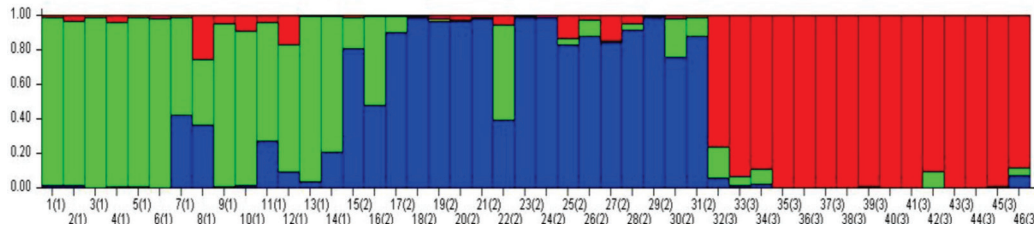
Figure 3. Genetic structure of populations of *Schinus terebinthifolius*: 1-14: population P (seed trees) (São Cristóvão); 15-31: population R (individuals originated from seeds of population P (São Cristóvão), and the National Park of Ibura (Socorro), located and recovered in the municipality of Santana do São Francisco); 32-46: population N (natural population located in the municipality of Santana do São Francisco), obtained by Bayesian analysis. The y-axis indicates the rainfall percentage in each of the estimated K groups.

The main object for the establishment of recovery areas, seeds should be collected at a recommended minimum distance of 50 to 100 m (Kageyama and Gandara, 1999). However, when working in extremely fragmented areas, or with few matrices per area, the recommendation distance is unfeasible to be used as standardization. Although some seed trees of population P present spatial distances shortest than 50 m, there is low genetic proximity (Gaia et al., 2004).

In a study of genetic diversity in natural populations of *Enterolobium contortisiliquum*, with eight individuals, in the Lower São Francisco - Sergipe, low genetic similarity (46%) was observed, despite being located at approximately 40 m spatial distance. Genetic similarity between the most distant pair of individuals (110 m) presents the highest value (67%), although the spatial distance between them is above the recommended distance (Santana et al., 2008). For the authors, by making proper management in seed collection for seedling production, it is possible to use these matrices as seed trees. Besides, studies on *Ziziphus joazeiro* Mart. in population located in the municipality of Canhoba (Sergipe), with collection carried out in the recommended minimum spacing between supplying seeds of 50 m, the mean genetic similarity was 55.0%, and similarity ranged from 32 to 68% (Gois et al., 2014), which are values similar to those found in population P of *S. terebinthifolius*.

In the population P, it is evident the absence of a spatial distribution pattern with the genetic distances, reinforcing that stochastic effects can prevail over environmental patterns (Hubbell, 2001). Given the results, obtaining genetic diversity is important for a low number of individuals, even if they present geographic proximity, i.e., less than 50 m. For seed collection programs, the most relevant factor for the population sustainability is to maintain heterozygosity.

Heterozygosity or genetic diversity of Nei (H_E) in the population (P = 0.34, R = 0.31 and N = 0.21) is of the standard, which is usually found for tree species. Populations of *Trichilia pallida* Swartz located in the Santa Geneva Reserve (Campinas) in the Ecological Station of Caetetus, in Gália (São Paulo), presented mean H_E of 0.33 (Zimback et al., 2004). *Schizolobium parahyba* populations found in conservation units, forest edges, pastures, banana crops, and in populations inserted in an urban area (Paraty) presented H_E of 0.36 (Freire et al., 2007). Studies on native populations of *Ziziphus joazeiro* Mart., located in different municipalities of the Lower São Francisco - Sergipe, observed H_E of 0.25 (Santana do São Francisco/15 individuals), 0.30 (Canhoba/19 individuals), and 0.24 (Canindé de São Francisco/20 individuals) (Gois et al., 2014).

Although the values of genetic diversity may be different, since they depend on intrinsic factors, such as the crossing system and the effective population size, as well as extrinsic factors, such as the number of sampled individuals, fragmentation, and isolation of the sampled areas, among others (Kramer et al., 2008), higher H_E values in both populations (P and R) present moderate heterozygosity levels. However, population N deserves special attention due to its low heterozygosity.

Despite the decrease of heterozygosity in population R, probably due to inbred crosses (f = 0.30) in population P, heterozygosity is still above values found for some native populations using a sample and different types of markers.

A study carried out with *S. terebinthifolius* in native populations located next to the recovered area described that the native population presents H_E of 0.20 (Carvalho et al, 2013). Also, more recent studies comparing native fragments in three different regions of the State of Sergipe (Caatinga/30 individuals; Ecotone/47 individuals; Atlantic Forest/85 individuals) also presented lower genetic diversity (H_E = 0.20) in each studied region (Álvares-Carvalho et al., 2015). Another study using simple sequence repeats and 30 sampled individuals revealed H_E of 0.28 (Álvares-Carvalho et al., 2016), lower heterozygosity than that of the recovered area (R = 0.31).

Although the first seed collection strategy in two distant regions (population P and Ibura) failure to ensure the increment of genetic diversity when compared with populations P and R, when associated with other parameters. Moderate genetic differentiation was observed between population P and population R (F_{ST} = 0.23), the mean values usually found for tree species, since several studies indicate that F_{ST} values between 0.22 and 0.36 (0 05 > P > 0.01) are usually reported for cross-breeding system species or primary successional stage (Nybom, 2004). Thus, the value of genetic differentiation between populations clearly distinguishes individuals of populations P and R, evidencing that although the percentage of the genetic similarity between populations of São Cristóvão and Ibura, the introgression of individuals in two areas could ensure the success of the recovery area; this is evidenced especially by comparing the genetic diversity of Nei of population R (H_E = 0.31) with that of population N (H_E = 0.21). The low genetic diversity of Nei in population N confirms the need for action aimed at immediate genetic enrichment for the maintenance of the genetic diversity of the species over time in the recovered area on the banks of the São Francisco River.

By the PCoA, a greater genetic distance was observed among individuals from population N and the others, which are formed by populations P and R. However, both populations (P and R) have subdivision trend (Figure 2), which is consistent with the moderate levels of genetic similarity among individuals.

The Bayesian analysis inferred the formation of three clusters (ΔK = 3), and each cluster was represented by a population. The low genetic structure of the three populations analyzed confirms the high levels of genetic differentiation found between populations (F_{ST} = 0.39). Probably this low structuring of population R is due to inbred crosses (f = 0.30) present in P, resulted in seeds with a genetic background very similar to the seedlings from the National Forest of Ibura, and make up the recovered population (R). This fact consequently led to moderate genetic differentiation between populations P and R (F_{ST} = 0.23), and to the low genetic structure in the recovered population. Although individuals of the population R present unstructured population by the Bayesian analysis, this population shows significant levels of H_E (Table 1). However, the sustainability of this population is only ensured with the genetic exchange between individuals belonging to the native population of the surroundings, due to the presence of different genetic sets.

By comparing the recovered area (R) with the natural population located in the surroundings (N) using the Bayesian analysis (Figure 3), each population showed predominance of a genetic cluster, which leads to the inference that crosses between the two populations would favor the increase of diversity to subsequent generations, and this will promote the structuring of the population in relation to the clusters analyzed. The gene flow between populations can ensure the sustainability and maintenance of populations if management practices are used to ensure the *in situ* establishment of subsequent generations.

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