COST Action FP1202

"Strengthening conservation: a key issue for adaptation of marginal /peripheral populations of forest trees to climate change in Europe (MaP-FGR)"

Topic

"Marginal populations of a native Mediterranean pine in the Iberian Peninsula (*Pinus halepensis*)"

STSM Reference code

COST-STSM-ECOST-STSM-FP1202-010914-044277

Grantee's name

Enrique José Hernández Tecles

REPORT ON THE SHORT TERM SCIENTIFIC MISSION

In the frame of the STSM within the COST Action FP1202 (MaP-FGR), I have visited Dr. Vendramin's lab at the IBBR-CNR of Florence (Italy) from 01/09/2014 to 31/10/2014.

The purpose of my visit was to continue working with *Pinus halepensis* Mill. natural populations in Spain, with focus on peripheral populations in the region of Murcia nearby semi-desert areas. Jointly with Dr. Vendramin and his group we tried to improve the genetic knowledge about the species, considering natural populations and surrounding plantations which could be considered as a risk in terms of genetic introgression.

Previously, we had been collecting plant material in Spain from selected pine populations in the region above mentioned. For natural populations, we obtained the geographic coordinates from each tree within a 15-20 meters distance in order to contrast possible spatial genetic structure. For planted ones, we tried to select trees very close trees with similar phenotypic characteristics assuming they came from the same seedlot.

Once in the lab, the first step comprised isolation of total DNA from collected material of 288 individuals with Qiagen-DNeasy 96 plant DNA kit. To control the quality and quantity of DNA yield we used Nanodrop spectrometry and electrophoresis gel on 1% agarose. Then we proceeded to the optimization of protocols for multiplexing of 7 nuSSRs and 2 single nuSSRs which had been amplified previously and had proved polymorphic (Table 1). In addition, we tested 5 nuSSRs recently developed through single PCR. After this, we checked for the successfully amplification of PCR products on 2% agarose gel electrophoresis system. Finally we proceeded to fragment analysis of PCR products (both for singleplex and multiplex) on capillary electrophoresis bioanalyzer (ABI-3500).

Table 1. Description of 9 nuclear microsatellites used

marker	Motif	Size	Reference
NZPR544_8	(CA)5(AC)12(TA)5	244-254	Chagné et al. 2004
epi3_FW	(TC)15	219-222	Chagné et al. 2004
FRPP94	(CT)22	143-151	Mariette et al. 2001
pEST2669	(TA)19	140-146	Steinitz et al. 2011
ITPH4516_3	(CT)27	134-174	Mariette et al. 2001
pEST8	(AT)11	147-151	Steinitz et al. 2011
PtTX3116	(TTG)7…(TTG)5	131-137	González-Martínez et al. 2004
PtTX3030	(TA)4…(GGT)10	348-357	Elsik et al. 2000
B4F08	(TTG)7…(TTG)5	177-199	Guevara et al. 2005

Once the lab tasks were finished, we started with the bioinformatics methodologies. First of all, scoring of resulted peaks from capillary biosystem analysis through GeneMarker V2.4.0 (SoftGenetics, State College, PA, USA) (Fig. 1) and continuing with the binning through R script prepared by Dr. Vendramin´s group.

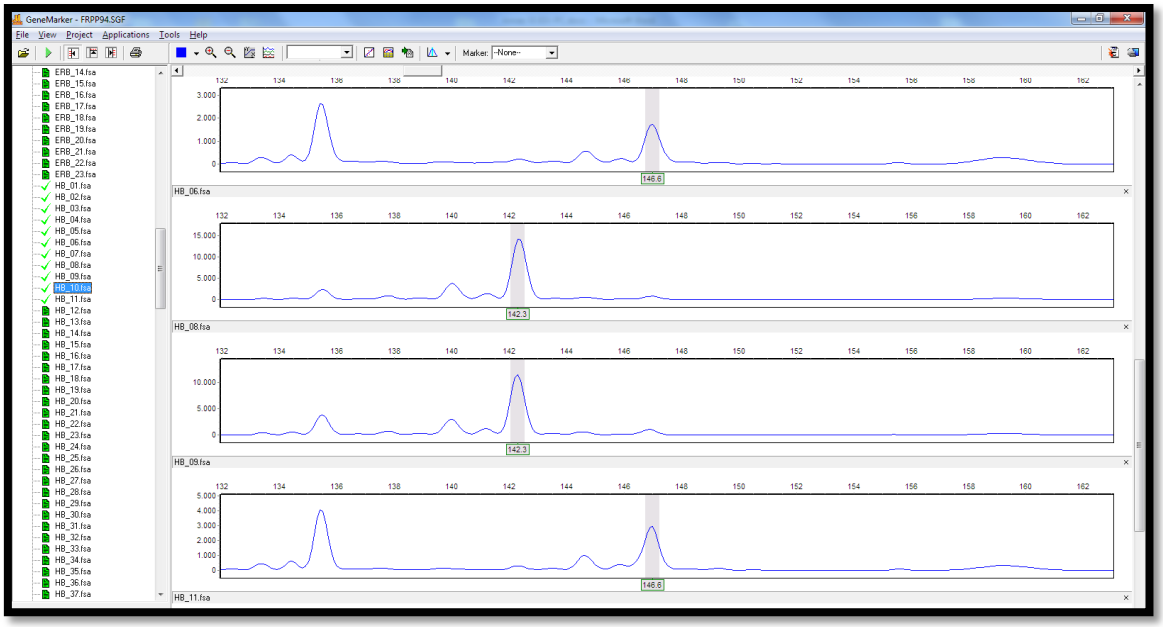

Figure 2. Example of scoring FOR marker FRPP94 using Genemarker software

We started the genetic analysis of refined data from all the markers using GenAlex v.6.5 (Peakall & Smouse, 2012) were we obtained principal parameters for population showing low levels of differentiation between natural and planted populations (Table 2).

Natural 1	Natural 2	Natural 3	Planted 1	Planted 2	Planted 3	Planted 4	Planted 5	Planted 6	
-------									Natural 1
0,013	-------								Natural 1
0,020	0,014	-------							Natural 1
0,020	0,019	0,011	-------						Planted 1
0,027	0,023	0,012	0,022	-------					Planted 2
0,015	0,022	0,017	0,015	0,031	-------				Planted 3
0,021	0,018	0,013	0,010	0,025	0,026	-------			Planted 4
0,014	0,018	0,019	0,018	0,019	0,025	0,018	-------		Planted 5
0,021	0,017	0,022	0,025	0,037	0,022	0,024	0,025	-------	Planted 6

Table 2. Pairwise population Fst values

We continued the analysis searching for presence of genetic structure among populations, with the help of specific software: Structure v.2.3 (Hubisz et al., 2009) and Tess v.2.3 (Durand et al., 2009). Although weak, as confirmed by low values of model from DIC (Fig. 2), both clustering approached revealed a genetic structure between natural populations (Fig. 3). Finally we studied carefully autocorrelogram for genetic and spatial structure in order to contrast the signal presented through previous software. E.g.: in the first natural population (Natural 1) there is some significant spatial genetic structure in very near trees which disappears through distance (Fig. 4).

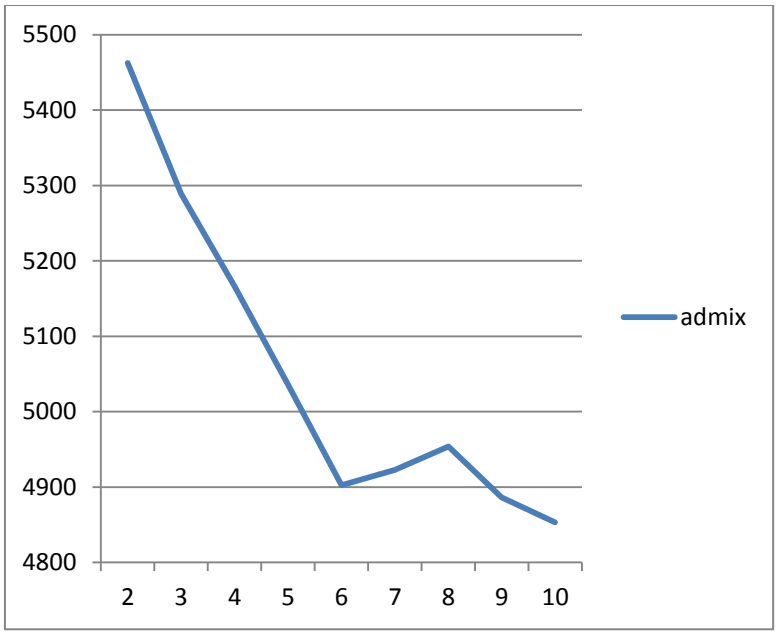

Figure 2. Clustering DIC values with admixture model from TESS software

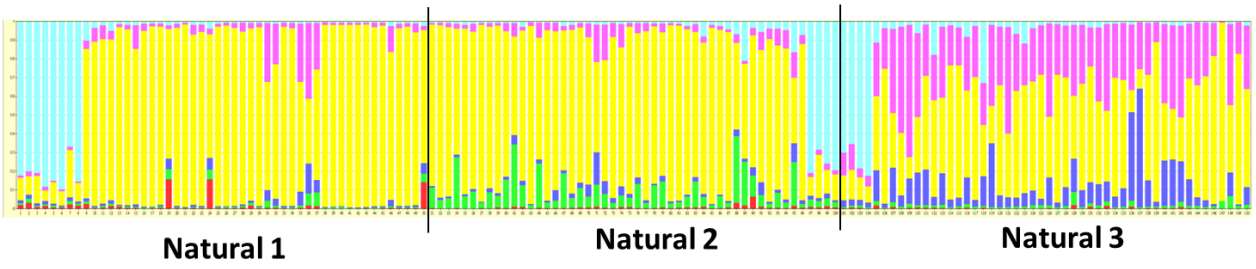

Figure 3. Genetic structure of natural populations by Tess software (K=6)

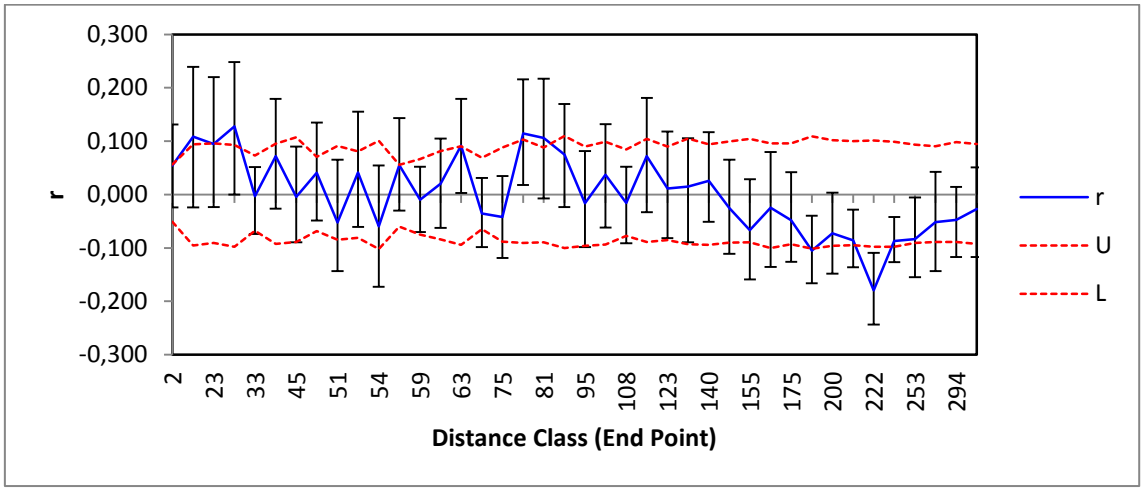

Figure 4. Results of spatial structure analysis in Natural 1 population

Considering these preliminary results we can conclude that seed used for plantations in the area must have been local, from old natural nearby populations, and there is no present risk of genetic introgression from undesirable provenances of Aleppo pine in the area. This is a clear evidence of ecological restoration principles were local seed sources should be always considered (MCPFE 2007).

There is a clear evidence of the potential of the new markers developed and tested with this experiment at Dr. Vendramin´s lab. The species presents low levels of differentiation at neutral markers (Soto et al., 2010), therefore new advances would help to find a higher resolution for *P. halepensis* future studies. This species is essential in context of climate change and special attention should be paid to marginal/peripheral populations, such as nearby semi-desert areas presented.

All molecular analyses are shared as a common success for future preservation and protection of mentioned species. Results of this STSM are part of candidates' postdoctoral research and in the near future it would be submitted a manuscript to peer-reviewed international scientific journal.

We are looking forward to continuing future collaboration with Dr. Vendramin's group and it would be highly recommendable to support upcoming STSM project proposals of the candidate.

Bibliography

Elsik CG, Minihan VT, Hall SE, Scarpa AM, Williams CG (2000). Low-copy microsatellite markers for *Pinus taeda* L. Genome 43:550–555.

Chagné D, Chaumeil P, Ramboer A, Collada C, Guevara A, Cervera MT et al. (2004). Cross-species transferability and mapping of genomic and cDNA SSRs in pines. Theor Appl Genet 109: 1204–1214.

Durand E, Jay F, Gaggiotti OE, François O (2009). Spatial inference of admixture proportions and secondary contact zones. Mol Biol Evol 26:1963-1973.

González-Martínez SC, Robledo-Arnuncio JJ, Collada C, Díaz A, Williams CG, Alía R, Cervera MT. 2004. Cross-amplification and sequence variation of microsatellite loci in Eurasian hard pines. Theor Appl Genet 109: 103-111.

Guevara MA, Soto A, Collada C, Plomion C, Savolainen O, Neale DB, González-Martínez SC, Cervera MT. 2005. Genomics applied to the study of adaptation in pine species. Invest. Agr.: Sist. Recur. For. 14: 292-306.

Mariette S, Chagné D, Lézier C, Pastuszka P, Raffin A, Plomion C, Kremer A. (2001). Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. J Hered 86: 469-79.

MCPFE. 2007. Pan-European Recommendations for Afforestation and Reforestation in the context of the UNFCCC. MCPFE, Varsow.

Peakall R and Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinfor 28, 2537-2539.

Soto A, Robledo JJ, González-Martínez SC, Smouse PE, Alía R (2010). Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. Mol Ecol 19: 1396–1409.

Steinitz O, Troupin D, Vendramin GG, Nathan R (2011). Genetic evidence for a Janzen-Connell recruitment pattern in reproductive offspring of Pinus halepensis trees. Mol Ecol 204: 152–4164.

Murcia, 10th of November 2014

Enrique José Hernández Tecles