BIOLOGIA PLANTARUM 49 (1): 125-127, 2005

BRIEF COMMUNICATION

Characterisation of Chinese elite cultivars and genetic resources of chestnut by AFLP

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Abstract

We selected a informative set of twelve amplified fragment length polymorphism (AFLP) primer pairs suitable for evaluation of Chinese chestnut (*Castanea mollissima* Blume) genotypes. Cluster analysis based on 198 polymorphic AFLP amplified by these 12 primer pairs clearly divided investigated genotypes according to their place of origin. We showed, that genetic basis of modern genotypes is narrow also in the case of this species.

Additional key words: AFLP, Chinese chestnut, *Castanea mollissima*, diversity, fingerprinting, genetic resources.

The genus *Castanea* includes about 10 species (Kubitzki *et al.* 1993). Among them chestnut *Castanea mollissima* Blume is of great economic importance and form also a natural part of the landscape in China (Liu *et al.* 1995, Bounous and Liu 1996). For the characterization of genetic resources within *Castanea* sp. several methods have been used: morphological or phenological studies, isozyme markers, or random amplified polymorphic DNA (RAPD) markers (Bassi and Marangoni 1984, Huang *et al*. 1994, Gao *et al*. 2001). Simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) analyses have been introduced and widely applied to characterise genetic resources of many plant species (Vos *et al*. 1995, Mukherjee *et al*. 2003) including genus *Castanea* (Yamamoto *et al*. 1998, Botta *et al*. 1999). However, a specialised study focused on *C. mollissima* has not been available.

In the present study we aimed to select an informative set of AFLP primer pairs and estimate the diversity within the set of 24 Chinese *C. mollissima* genotypes, which are used in productive orchards in sub-tropical China. AFLP was chosen for several criteria: *a*) AFLP has good information content (Powell *et al*. 1996), *b*) AFLP is considered to be reproducible (Jones *et al*. 1997), and *c*) AFLP can be applied to any plant species without previous knowledge of the sequence (Ashikawa *et al*. 1999).

Genomic DNAs were extracted from young leaves collected from selected chestnut (*Castanea mollissima* Blume) genotypes grown in productive orchards in Hunan province, P.R. China, using DNeasy *Plant Mini Kit* (*Quiagen*, Hilden, Germany). AFLP assay was carried out according to the *Perkin-Elmer* (Wellesley, USA) protocol (*Part No. 402083, Rev. A*, 1995). For each accession, a binary matrix reflecting specific AFLP fragments presence (1) or absence (0) was generated. Pair-wise distances between the accessions based on Jaccard similarity metrics (Jaccard 1907) were calculated. Diversity indexes (D) were calculated according to Dahleen (1997).

A total of 93 selective primer pairs were screened for their ability to produce reproducible profiles. Based on the screening a set of 12 selective primer pairs was selected, amplifying more then 7 polymorphic fragments per reaction (Table 1). Selective primer pair *Mse*I-CAT/*Eco*RI-ATC with D = 0.317 (Table 1) was shown to

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Received 27 August 2003, *accepted* 15 April 2004.
Abbreviations: AFLP - amplified fragment length polymorphism; D - diversity index; RAPD - random amplified polymorphic DNA; RS - rootstock; SSR - simple sequence repeat; UPGMA - unweighted pair group method with arithmetic averages.
Acknowledgement: The study was supported by Project No. ME 325 of Ministry of Education, Youth and Sports of the Czech Republic. We thank Irena Nováková for her previous work and Hana Udavská for the excellent technical assistance.
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be the most suitable for differentiation of analysed accessions. This selective primer pair allowed differentiate 21 accessions, 3 cultivars were not differentiated. Any combination of two selective primer pairs differentiated among all genotypes analysed. Much higher number polymorphic fragments were revealed by AFLP in our study in comparison with isozyme alleles (Zhang and Liu 1997, Lang *et al*. 1999) or RAPD analysis (Gao *et al*. 2001, Weng *et al*. 2001). An informative set of AFLP primer combinations discriminating effectively among *C. mollissima* genotypes was thus assembled.

Table 1. The number of detected AFLP polymorphic fragments per each primer pair used in the investigation across the studied chestnut cultivars. D - diversity index as a measure of the information value per each primer pair calculated according to Dahleen (1997).

Primer pairs	Total number of detected fragments	Number and % of polymorphic fragments		D
CTC/AgT-FAM	18	10	(55.6 %)	0.343
CTC/ATA-JOE	37	22	(59.5 %)	0.297
CTC/ATC-TAMRA	33	16	(48.5 %)	0.266
CTA/ AgT-FAM	27	7	(25.9 %)	0.219
CTA/ ATA-JOE	44	19	(43.2 %)	0.241
CTA/ ATC-TAMRA	36	22	(61.0 %)	0.260
CAT/ACA-FAM	36	15	(41.7 %)	0.299
CAT/ ATA-JOE	25	7	(28.0 %)	0.241
CAT/ ATC-TAMRA	39	17	(43.6 %)	0.317
CTg/ AgT-FAM	20	14	(70.0 %)	0.252
CTg/ ATA-JOE	35	24	(68.6 %)	0.297
CTg/ ATC-TAMRA	31	25	(80.6 %)	0.312
Total	381	198	(52.0 %)	-

A total of 381 different fragments, out of them 198 (52 %) polymorphic, were amplified across 24 cultivars. Based on the polymorphic AFLP fragments presence or absence consensus UPGMA dendrogram showing associations among genotypes was drawn (Fig. 1). The values on the branches were the result of 100 bootstrap replications (Felsenstein 1985). Bootstrap values above 50 % or at least 50 % are indicating statistical support for the topology at a node.

Accessions were clustered into two main groups. Local adopted genotypes represented by rootstocks (RS) formed one group, genotypes represented by cultivars used in productive orchads formed the second group. Genetic similarities ranged from 0.011 (Tuoli *versus* An 1, Tuoli *versus* Tanqiao) to 0.664 (Shao 18 versus RS-2). Special subgroups were observed within the group of productive cultivars reflecting place of their origin. For example, cvs. Huafeng and Huaguang (bootstrap value 100) with similarity value 0.043 were improved in the same Institute in Shandong province, cvs. An 1, Tuoli, Tanquiao and Xin tian dissimilarities of which exhibited the lowest values, were developed in Hunan province. The highly productive elite cultivars from Hebei province, cvs. Daye, Tie li tou and Xin li Zhuang, were also grouped together (bootstrap value 88). On the other hand, high degree of dissimilarity was found within the rootstocks. Their genetic basis significantly differed from each other and from genetic basis of productive cultivars, too.

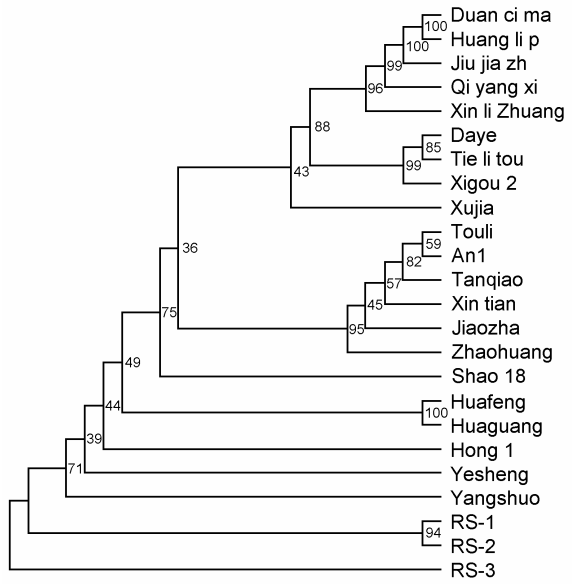

Fig. 1. Associations among chestnut (*Castanea mollissima*) genotypes as revealed by UPGMA analysis based on 198 AFLP polymorphic fragments. Numbers on the branches represent bootstrap values.

Several authors (Zhang and Liu 1998, Lang and Huang 1999, Liu 1992) reported richness and high diversity of *C. mollissima* resources in several Chinese provinces, which are considered places of species origin. Mostly natural populations were investigated. On the contrary our collection consisted of genotypes resulting from deliberated breeding. It is generally noted that breeding brings on decrease of genetic variability in comparison with wild growing species (Spillane and Gepts 2001). Already Zhang and Liu (1998) pointed out a possible effect of breeding on the variability of *C. mollissima*. Machon *et al*. (1996) also found a decrease in variability in other *Castanea* species – European chestnuts (*C. sativa* Mill.) and suggested that human interference had dramatically reduced the number of alleles per locus. Such reduction is probably due to the process of genetic drift as successively smaller numbers of genotypes have been sampled in the course of propagation of this species. Genotypes descending in other regions (Shandong, Guangdong and Zhejiang provinces, and Beijing) posses other combination of polymorphic fragments in comparison with the first two

sub-groups. Blocks of identical fragments shared by genotypes from Hunan, Hubei and Jiangsu provinces were not found for the other materials. It indicates that genetic basis of cultivars can be typical for different regions. However, more comprehensive study should be done to investigate the richness of the gene-pool in entire China. AFLP represents a suitable tool for such study.

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