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BRIEF COMMUNICATION

Random amplified DNA polymorphism of *Nicotiana tabacum* L. cultivars

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Abstract

The polymorphism, similarities and relationships among *Nicotiana tabacum* L. cultivars were assessed with RAPD analyses. One hundred and forty-nine bands were detected, of which 94 were polymorphic (63.1 %). A primer distinguishing all of the tested cultivars was found. High similarity between cultivars was revealed, and cultivar relationships were estimated through cluster analysis (UPGMA) based on RAPD data.

Additional key words: flue-cured tobacco, genetic diversity, RAPD, UPGMA.

Flue-cured tobacco (*Nicotiana tabacum* L.) is one of the most important commercial types for tobacco production in the world. Undoubtedly the study of the genetic diversity of flue-cured tobacco cultivars would be important not only for germplasm conservation but also for parental choice for breeding purposes. Diversity among and within breeding populations and elite germplasm is key to successful breeding programs (Renganayaki 2001).

Random amplified polymorhic DNA (RAPD) is the simplest and fastest of DNA-based techniques in genetic similarity studies (Gwanama *et al*. 2000). A number of scientists have used RAPD markers to study polymorphism in various plants (Ortiz *et al*. 1997, Ranade *et al*. 2002, Rout *et al*. 2002, Samal *et al*. 2003).

In this preliminary report the RAPD procedure was used to assess the level of polymorphism, the similarities and relationships among flue-cured tobacco cultivars from major production countries China, USA and Brazil.

Twenty-four accessions of *Nicotiana tabacum* L. with desirable agronomic characteristics, such as large leaf size, high leaf yield, low nicotine content, or resistance to various diseases or insects (Rei *et al*. 1997), were selected for evaluation in this study (Table 1).

Seeds were planted in pots and grown in greenhouse at temperature 28 to 32 °C. Twenty days after germination, shoots were harvested from 40 seedlings of each accession. DNA was extracted from shoots by the CTAB method (De Riek *et al*. 2001). Amplification was performed in volumes of 0.02 cm^3 containing 0.002 cm^3 of the 10x buffer, and 100 mM each of dNTPs, 0.4 mM primer, 25 ng genomic DNA, and 1 unit of polymerase (*Sangon*, Shanghai, China). The reaction mixture was overlaid with 0.04 cm^3 mineral oil. Amplifications were carried out using a *2400 Perkin-Elmer* (USA) thermal cycler programmed for 40 cycles as follows: 30 s at 94 °C, 30 s at 36 °C, 1.5 min at 72 °C, with an initial melting of 6 min at 94 °C, and a final extension of 6 min at 72 °C. Amplification products were analyzed by electrophoresis in a 1.5 % agarose gel with 1 × TAE buffer (0.004 M Tris-acetate and 0.002 M EDTA). Individual RAPD fragments for each primer-genotype combination were scored as 1 (presence) or 0 (absence), and a note of their sizes was made. The set of fragments co-migrating across the 24 cultivars was referred to as a band. Pairwise similarity matrices were generated using Nei's coefficient of similarity (Nei and Li 1979), and UPGMA clustering was then used to produce a dendrogram. The above procedures were performed using *STATICA-pc*.

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Abbreviations: RAPD - random amplified DNA polymorphism; UPGMA - unweighted pair group method.
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Table 1. Flue-cured tobacco cultivars used in RAPD analysis.

No.	Cultivar	Origin	No.	Cultivar	Origin
1	HB39P	Brazil	13	Reams44	USA
2	RGH51	Brazil	14	Coker213	USA
3	PVH08	Brazil	15	Cu236	USA
4	PVH01	Brazil	16	RG8	USA
5	14×L8	Brazil	17	G-23	USA
6	PVH09	Brazil	18	K326	USA
7	RGH04	Brazil	19	P3	USA
8	PVH05	Brazil	20	Baofong	China
9	PVH03	Brazil	21	311	China
10	PVH02	Brazil	22	Yanyan97	China
11	RGH12	Brazil	23	Gaozhao74	China
12	Oxford1	USA	24	Guanghuang55	China

Of the two hundred primers used in this study, 61 (30.5 %) produced amplification products that were too faint to score or could not be consistently reproduced, and 122 (61 %) produced monomorphic banding patterns. Thus only 17 (8.5 %) out of 200 primers were scored. A total of 149 bands were scored from the comparison of amplifications with 17 primers of DNAs from 24 flue-cured tobacco cultivars, with an average of 8.8 bands scored per primer. Three to fifteen bands generated by a single primer of variable lengths were detected.

The polymorphic bands were 94 (63.1 %), and in average the band polymorphism per genotype was 7.1 %. Of course, the genetic loci which were invariant are also important for their potential to detect polymorphism in other flue-cured tobacco genotypes. In this study, the average number of bands per primer detected among all

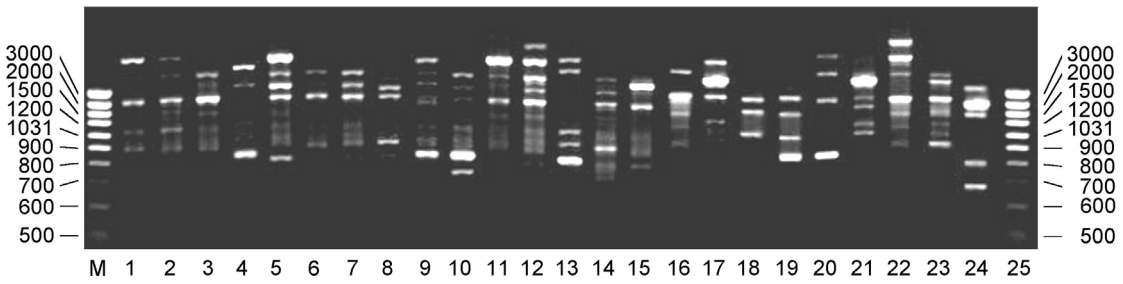

Fig. 1. RAPD fingerprints of 24 flue-cured tobacco cultivars (the primer was A-7). The number representing cultivar code was shown in the Table 1, and M was 100 bp ladder.

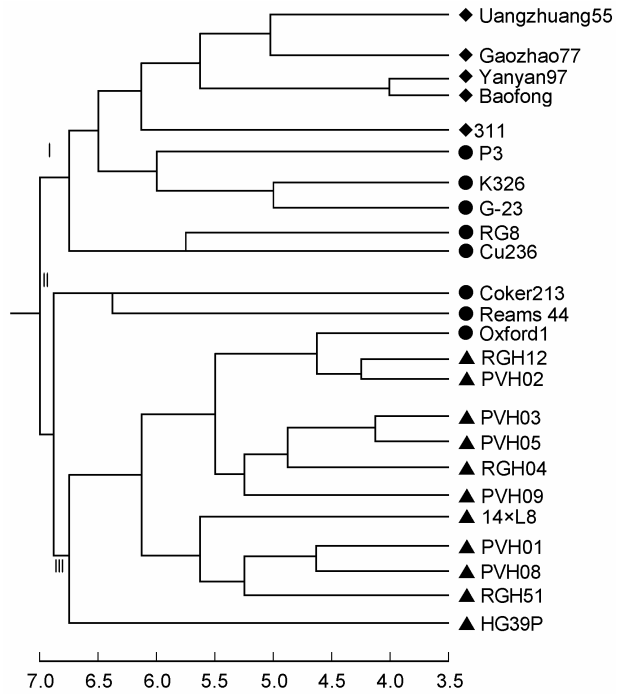

Fig. 2. Dendrogram of the 24 flue-cured tobacco cultivars based on cluster analysis (UPGMA) of the genetic distances calculated from 94 polymorphic RAPD fragments. Symbols indicate country of origin: *rhombs* - China, *circles* - USA, and *triangles* - Brazil.

the 200 primers tested was 0.47 polymorphic and 0.745 total (invariant plus polymorphic). These suggested the existence of limited genetic variation in flue-cured tobacco cultivars.

The use of primers selected for faithful reproduction of higher polymorphism in the target group of genotypes could further increase the efficiency and the applications of the RAPD approach. One example of more polymorphic primers (A-7) distinguishing all of the tested cultivars is in Fig. l, which shows the 24 cultivar profiles, each consisting of a number of amplified fragments.

Characterization and quantification of genetic diversity has long been a major goal in breeding. In plant breeding programs, information on the genetic diversity within and among closely related crop species is essential for a rational use of genetic resources. The dendrogram (Fig. 2), produced by cluster analysis revealed three clusters. The entire collection of Brazilian accessions in the cluster III, together with one US cultivar Oxford 1. All cultivars from Chine grouped together with 5 cultivars of USA. The dendrogram indicated a clear pattern of division among the flue-cured tobacco accessions based on geographic origin, as seen in some other crops (Paul *et al*. 1997, Spooner *et al*. 1996). Some American cultivars clustered together with some Chinese or Brazilian cultivars, maybe they shared the same ancestors; a lot of Chinese and Brazilian cultivars were bred with American cultivars (Wuang *et al*. 1995). The

genetic diversity within US cultivars is larger than that within Brazilian and Chinese accessions. In addition, it seems that genetic diversity of flue-cured tobacco is larger among different countries than within a specific country. Divergent genotypes may have good breeding value, and genotypes in the same cluster may represent members of one heteromorphic group. For example, Reams 44 and Coker 213 clustered together in the cluster II. They are both the offspring of Coker 319. There was also a close relationship being detected between the cultivars Baofong 44 and Yanyan 97 (pairwise similarities coefficient, Ps = 0.89) as well as between PVH03 and PVH05 (Ps = 0.86). These two pairs of cultivars are both similar in morphological traits, and both originated from the same parents too (Rei *et al*. 1997). The result showed that RAPD assay could discriminate those flue-cure tobacco cultivars with similar genotypes. It seems that RAPD is an effective tool for flue-cured tobacco germplasm management, cultivar protection, and cultivar improvement.

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