SSR and AFLP fingerprinting of 89 newly bred winter rapeseed cultivars in China

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Abstract

The technique of DNA fingerprinting is very important for new cultivars identification, registration and protection. In this study, 89 candidate rapeseed (*Brassica napus* L.) cultivars for the 2004-2005 National List were fingerprinted by 15 core SSR primers and 4 AFLP primer combinations, which yielded a total of 41 and 67 polymorphic bands, respectively. Either 41 SSR markers or 67 AFLP markers were sufficient to discriminate all cultivars completely, thus constructing a primary DNA fingerprint database. The genetic cluster analysis showed that cultivars bred by the same breeder have the highest level of genetic similarity, and that the genetic diversity of newly bred cultivars is increasing compared to those released in 1980-1990 in China. Furthermore, the highest level of genetic diversity was found in three-line hybrids, following by open pollination varieties, and two-line hybrids the least.

Key words: *Brassica napus* L., Official field trail, DNA fingerprinting, SSR, AFLP

Introduction

Rapeseed is a major oil crops in china, and also a promising crop for biofuel. Due to its economics importance, the rapeseed production, breeding and extending of new varieties developed quickly in recent years. China has now become the largest rapeseed production and consumption region in the world. Several dozen of new varieties were bred and applied for official field trail each year in china.

The traditional registration and protection of rapeseed variety heavily relies on a limited number of morphological traits. However, as the number of cultivars increase rapidly, it is more difficult to distinguish them on the basis of these traits. Therefore, new descriptors such as molecular markers are needed to maintain the efficiency of registration testing.

In this study, we employed SSR and AFLP markers to fingerprint candidate rapeseed varieties for the National List, and assess their DUS characteristics, aiming at providing an effective alternative for cultivar registration and protection.

Material and Methods

Eighty nine newly bred rapeseed varieties being surveyed at 2004-2005 National Official field trial were used as plant materials (Fig.1).

For each cultivar, total DNA was isolated from a mixture of 15-30 seedlings using a modified SDS method (Lu et al., 2003). SSR and AFLP marker analysis were performed as described by Lu et al. (2003) and Vos et al. (1995), respectively.

Only polymorphic SSR and AFLP bands with strong intensity were scored, as '1' for presence and '0' for absence. Genetic clustering was performed using UPGMA method. To evaluate the genetic diversity level, Shannon-Weaver diversity index and Simpson diversity index were used, which were calculated as:

$$H' = -\sum_{i=1}^{s}(P_i \ln P_i)\,;\quad D = 1 - \sum_{i=1}^{s}(P_i^2)\,,$$

where P_i is the frequency of the i^{th} allele, and s is the total number of alleles in that locus (Nei and Li, 1979).

Results and Discussion

After extensive screening, a set of 15 core SSR primers and 4 AFLP primer combinations (PC) were selected and used for DNA fingerprinting of 89 varieties. In SSR analysis, a total of 2-5 bands were amplified from each of the primer, with an average of 3.4 bands per PC. A total of 41 polymorpchic bands were observed, with a mean polymorphic ratio of 79.4%. In AFLP analysis, a total of 67 polymorphic bands resolving by 4 PCs were generated, with an average polymorphic ratio of 27.8%. Thus, this set of SSR and AFLP markers consisted of a primarily DNA fingerprint database, in which every variety have its own unique banding profile.

The clustering result showed that, at a minimum genetic distance of 0.02 (similarity of 0.98), all varieties were completely separate into different group using either SSR or AFLP data (Fig.1), meaning that it is possible to identified each variety by DNA fingerprinting.

The clustering result also showed that: 1) Generally, variety bred by different breeder or originated from different

province have a higher level of distinctness and separated into distinct groups; 2) Variety come from the same breeder share a higher degree of genetic background and first cluster together, such as H4270、H0203 and H0201 provided by Huazhong Agricultural University; 3) The most unique variety revealed by SSR and AFLP marker were 4028 and 99-1055, respectively (Fig.1).

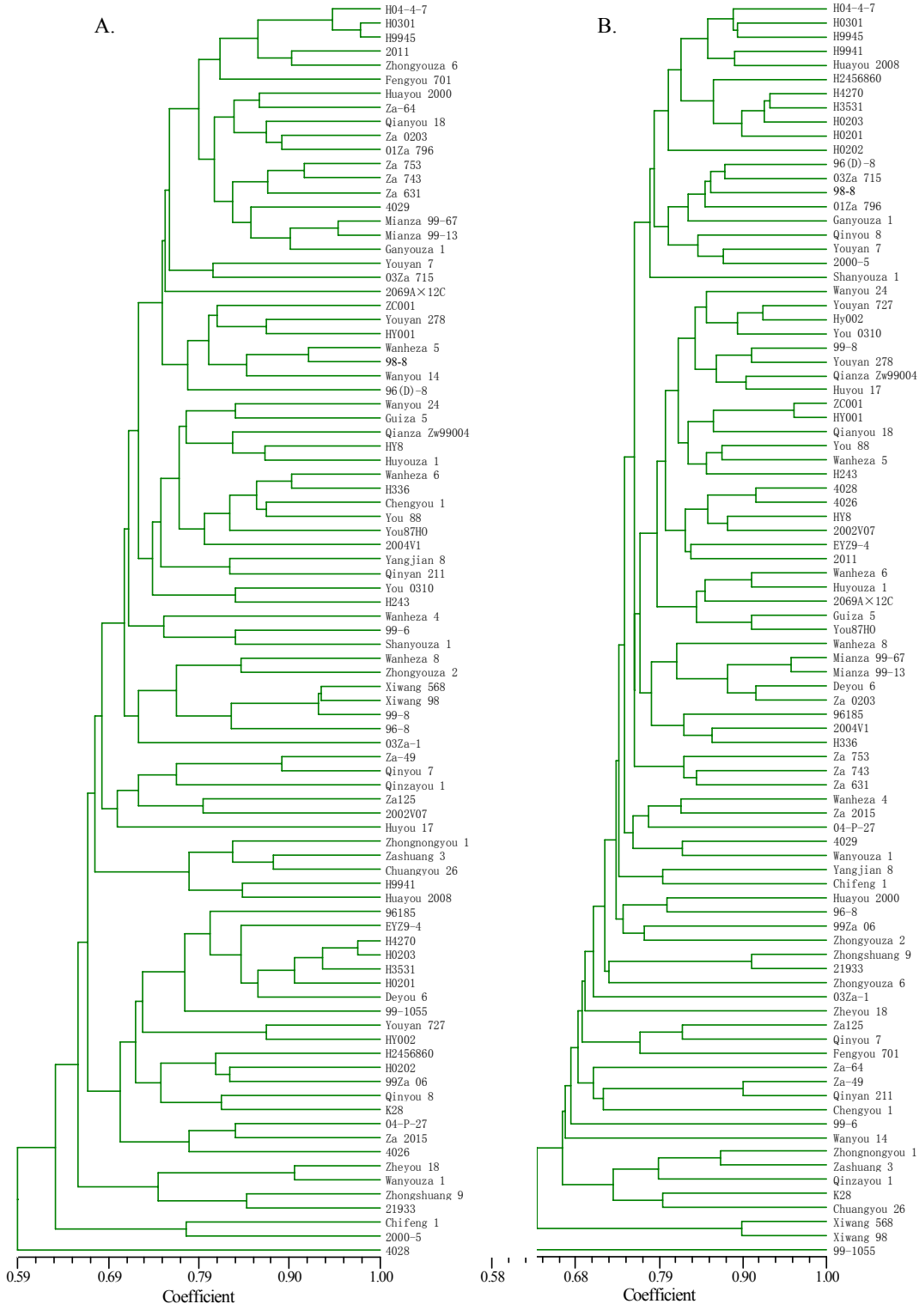

Fig. Dendrograms of 89 rapeseed cultivars based on 41 SSR markers (A) and 67 AFLP markers (B)

The tested 89 varieties can be classified into three distinct types, i.e. three line hybrid (THL), two line hybrid (TWL) and

Open pollination variety (OP). Shannon-Weaver index (H) and Simpson diversity index (D) were used to assess the genetic diversity of different varieties types. Taken 89 varieties together, the respective value of H and D were 0.4424 and 0.2884, which were higher than those of the varieties released in 1980-1990 in china (data not shown). Within the three types, the highest diversity level was observed in THL, OP second to it, and TWL was the lowest (Table 1). This result showed that the genetic diversity level of newly bred variety in china was growing, and the richness of genetic diversity can greatly reduce the potential risk of genetic fragile.

Table1 Genetic diversity of variety type resolved by SSR and AFLP marker

Variety Type	No. access.	Shannon index		Simpson index	
		SSR	AFLP	SSR	AFLP
Three-line hybrid	67	0.4424	0.4065	0.2884	0.2597
Two-line hybrid	15	0.4449	0.3248	0.2898	0.2083
Open pollination	7	0.3697	0.3637	0.2450	0.2460
total	89	0.3946	0.4061	0.2688	0.2586

Conclusion

89 candidate rapeseed varieties in the National list were fingerprinted by SSR AND AFLP markers. Our data showed that both marker system are suitable for DNA fingerprinting and distinctness, uniformity and stability (DUS) assessment. In order to counterbalance advantages and disadvantages of each marker system, AFLPs and SSRs could be used in a complementary way to unambiguously distinguish varieties. For the first stage, SSR is applied to separate most varieties; then, at the second stage, AFLP is further applied to characterize the most similar ones due to a higher discrimination power.

For a practical application in real case, however, an optimized set of core SSR and AFLP primers, a standard procedure, and a suitable criteria to declare DUS must first to be established.

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