Fingerprinting maps construction of *Brassica napus* and the rapeseed germplasm with super-high oil content in China

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Abstract: In this study, we selected 7 rapeseed germplasms with super-high oil content and 16 main rapeseed cultivars from the different planted regions to construct their fingerprinting maps by SSR molecular markers. Using 28 SSR primer pairs from the different linkage groups, 302 SSR bands were amplified for the all materials, 279 of which were polymorphism bands, each primer amplified 6-16 bands with 10.79 bands on average, the average of polymorphism rate reached 92.38%. By this way, we could distinguish all the materials better. UPGAM analyzing revealed that there were the large difference among the high oil content germplasms and between the high oil content germplasms and the main cultivars. At genetic distance of 0.171, the 23 *B. napus* could be divided into nine groups, in which the super- high oil content germplasms were divided into four groups, and 8 main cultivars were divided into five groups. These results showed that all materials had the most abundant genetic diversity, and there were large genetic differences between the super-high oil germplasms and the main rapeseed cultivars.

Key words: *B. napus*; high oil content; SSR molecular markers; Fingerprinting maps

Excellent germplasm resources are the base of continuous improvement for crop cultivars. China is the first enormously rapeseed producing country in the world. However, oil content of rapeseed cultivars was still lingered at around 40% for a long time(Liu et al. 2008), which was lower than 2-5 percentage points with Canada rapeseed have higher oil content in the world. According to the rapeseed planting area, average yield level and rapeseed prices of china at present, if the oil content of rapeseed increased one percentage point, which was equivalent to increase production 37.5kg/ha, there were 7 millions ha of rapeseed planting area, the yield of rapeseed would increase 262.5 thousands tons, and would squeeze out 100 thousands tons of high quality canola every year, as well as increased or created the economic benefit of 900 millions RMB. Therefore, improving the oil content of rapeseed had become an important goal of rapeseed breeding (Wang.2004), and a key method of improving rapeseed production efficiency (Li et al.2006; Suo et al.2007), which had the great development potential. At present, the yield and oil output of unit area had been introduced as an equally important quantification investigation index and a judgment standard to cultivars assessment in china, moreover, it got more and more attention in the process of cultivars releasing and application. Therefore, the study of rapeseed high oil content and cultivar breeding had been changed greatly in recent years, oil content of cultivars of passed cultivar assessment was significantly improved, the level of high oil content rapeseed germplasms were refreshed unceasingly. Oil content of rapeseed was about 50% in early stage, then Wang Hanzhong reported that oil content was 54.72% in 2007 (Liu et al. 2008),until Shaanxi Hybrid Rapeseed Research Centre created about 60% of oil content of super-high rapeseed germplasms through the working hard of 15 years, which not only showed the great potential of improving rapeseed oil content level ,but also proved us some important problems about how to effectively protect and make full use of these rapeseed germplasms to service for high oil content breeding.

In order to utilize the rapeseed germplasms, we should well know them firstly and clarify genetic relationship and fingerprint differences of germplasms at the molecular level, which not only was the basis of the further researching and breeding application, but also was of great significance to the intellectual property protection. SSR molecular markers technique had been used to construct fingerprinting maps and protect intellectual property of cultivars, and had been applied in fingerprinting maps construction and analysis of genetic diversity of maize, rice, soybean et al (Smith et al.1997). Therefore, the study used SSR molecular markers technique to construct fingerprinting maps for partial super-high oil content rapeseed germplasms and main planting rapeseed cultivars from the different planted regions, and made a discussion for methods on making full use and effective protection of various specific rapeseed germplasms.

1 Materials and methods

1.1 Material

Test materials were provided by Shaanxi Hybrid Rapeseed Research Centre, it included 7 rapeseed germplasms with super-high oil content that was bred by kinds of breeding techneques of shuttle breeding in different ecological regions, multiple genes polymerization and microspore culture, and so on, and 16 main rapeseed cultivars from the different planted regions (Table 1).

1.2 Methods

1.2.1 SSR system and the construction of fingerprinting maps

The total volume of SSR reaction system was 20μl, it included: 10× Buffer with $(NH4)_2SO_4$ 2.0μl,25mmol/L $MgCl_2$1.5μl, 10mmol/LdNTP0.4μl, 5U Taq DNA Polymerase 0.2μl, 2.5μmol/L primer1μl, 20-80ng DNA1.0μl, ddH_2O 12.9μL. Amplified products were mixed into loading buffer of a half volume, which could be separated by polyacrylamide gel electrophoresis(6%), 120 voltage prerun 30minutes, joined in 6μL sample ,electrophoresis 2.5 hours. Silver staining referenced Lu Guangyuan et al(Lu et al.2001).

Primers screened: selected primers that had the stable and clear polymorphism amplification bands as a target marker primer to construct fingerprinting maps. Bands recorded: existed bands recorded “1” and no bands recorded “0”.

1.2.2 Data statistics and analysis

According to the mathematical statistics theory (Nanjing Agricultural University.1998) and the molecular genetics theory (Zhejiang Agricultural University.1998; Xu et al.1994), appearing the same probability formula of fingerprint was $1/2^n$ to count confidence probability of 23 *B. napus* fingerprinting maps. Through UPGMA and CLUST statistics software constructed a dendrogram of 23 *B. napus*.

Table1 Name and their sources of the tested material

NO.	Name	Source	Oil content(%)	Category of varieties
1	M1	Shan'xi	57.69%	Breed resource
2	M2	Shan'xi	59.10%	Breed resource
3	M3	Shan'xi	58.06%	Breed resource
4	M4	Shan'xi	59.29%	Breed resource
5	M5	Shan'xi	61.40%	Breed resource
6	M6	Shan'xi	57.17%	Breed resource
7	M7	Shan'xi	61.30%	Breed resource
8	Zhongyou821	Hubei	38.48%	Variety
9	Rongyou No.4	Sichuan	40.00%	Hybrid
10	Deyou No.4	Sichuan	37.85%	Hybrid
11	Deyou No.5	Sichuan	39.49%	Hybrid
12	Youyan No.10	Guizhou	46.00%	Hybrid
13	Qinyou No.10	Shan'xi	42.76%	Hybrid
14	Ningza No.10	Jiangsu	43.34%	Hybrid
15	Fengyou701	Hubei	41.67%	Hybrid
16	ZhongshuangNo.1	Hubei	49.00%	Hybrid
17	Ronghuayou No.2	Shan'xi	40.29-45.42%	Hybrid
18	Huayouza No.10	Hubei	39.7-41.12%	Hybrid
19	Zheshaung No.8	Zhejiang	45.86%	Variety
20	Chuyou No.1	Anhui	40.28%	Variety
21	Xinyouza No.6	Xinjiang	45.00%	Hybrid
22	Zheshaung No.6	Zhejiang	39.78%	Variety
23	Zheshaung No.3	Zhejiang	42.90%	Variety

2 Results and analysis

2.1 SSR polymorphism primer screening

The study obtained more than 50 polymorphism good primers from over 300 pairs of primers, and selected 28 pairs of primers of plentiful polymorphism. Finally, clear and stable bands from different linkage groups were used to construct fingerprinting maps by comparing and screening again. Total 302 SSR bands were amplified by28 SSR primer pairs, 279 of which were polymorphism bands, each primer pair amplified 6-16 bands with 10.79 bands on average, and the average of polymorphism rate reached 92.38%. S598 amplified 16 bands, which was a primer pair with the most bands amplified, but S648 and S705 only were amplified 6 bands, which were primers with the least bands amplified.

2.2 Fingerprinting maps construction and confidence probability analysis

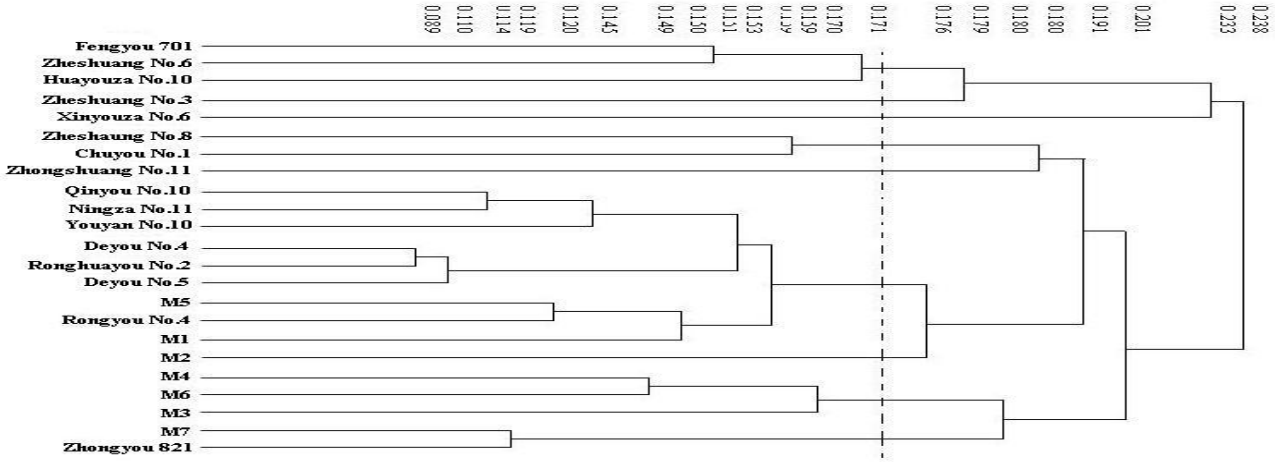
Using 28 SSR primer pairs, DNA amplification analysis of 7 rapeseed germplasms with super-high oil content and 16 main rapeseed cultivars were carried out, the fingerprinting maps of cultivars were recorded “0”or “1” as a cultivar digital map, and combining each cultivar digital map of each primer to construct the digital combinational maps of each cultivar. For example, amplified result of S648 and S705 primers for M1 were the digital map of 001010 and 010001 respectively, combining together both S648 and S705 amplified result the digital combinational map of 001010010001 was got. Combining 28 SSR primers amplified result got 302 digital maps with composed of "0”and “1”, and these digital combinational maps constructed the database of the digital combinational maps of 23 materials or cultivars.

Using S648 and S705 primers combination, the constructed digital combinational maps of constructed M1 and M2 *B. napus* materials were 001010010001 and 011111010001 and were corresponded to DNA - SSR fingerprinting maps of two materials respectively. Two materials had three differences from a digital combinational maps, therefore, two materials identical probability was $(1/2)^3$ or 0.125. SSR primers combination could get 9.96 polymorphism loci on average, and two primers combination could average get about 20 polymorphism loci in this study, so the average probability of the same fingerprinting maps amplified for two materials was about$(1/2)^{20}$ or 9×10^{-6}. However, different primer showed difference, probability of the same amplified results among materials should be very low. 23 materials were identified by 28 primer pairs and got 279 polymorphism bands, the same probability of the fingerprinting maps was $(1/2)^{279}$. It was distinguished greatly among material through the fingerprinting maps, so these specific fingerprinting maps provided the effective guarantee for 7 rapeseed germplasms with super-high oil content and 16 main rapeseed cultivars of participating in the construction.

2. 3 UPGMA clustering analysis

Using CLUST statistics software and UPGMA, a dendrogram of 7 rapeseed germplasms with super-high oil content and 16 main rapeseed cultivars was constructed (Fig1). The genetic differences of high oil materials and between high oil materials and main cultivars were significance in fig 1. At genetic distance of 0.171, the 23 *B. napus* could be divided into nine groups, 7 super- high oil content germplasms were divided into four groups. M1 and M5 with super- high oil content germplasms and 7 main cultivars were clustered in one group, M3, M4 and M6 were clustered in one group, M7 and Zhongyou 821 were clustered in one group, and M2 was alone one group, while the rest 8 cultivars were clustered in other 5 groups respectively. All materials had more abundant genetic diversity, especially the large genetic differences were found between the super-high oil germplasms and the main rapeseed cultivars. The clustering results embodied genetic relationship of 23 materials at the DNA level in a certain extent, and provided the background reference for pedigree analysis and matching combination among materials.

Fig 1 Dendrogram of genetic relationship of 7 rapeseed germplasms with super-high oil contentand 16 main rapeseed cultivars detected by SSR in *B. napus*



3 Discussion

Traditional crops and cultivars were identified and distinguished by a lot of phenotypic information, but these information was various and complex, and most of which were fuzzy and similarity, moreover, the difficulty of distinguished or some traits of crops maybe be changed because of the influence and change of environmental conditions. That result in windage that was not avoided, especially when lots of sample would be identified, which showed more troubles and longer cycle. DNA molecular markers based on DNA polymorphisms to reflect difference and performance and abundant polymorphism of the DNA level, the result often showed dominant or codominant. DNA bands form reflected which were intuitive, stable and reliable. This technique overcame various drawbacks in the process of the quantitative traits investigation, and it could analyze lots of cultivars by combining with computer technology through the establishment of the database to discern. This technique had the short cycle and high efficiency, and wasn't influenced by environment, so results were correct and reliable, which had become preferred technique in the process of fingerprint analysis (Fang et al.2000).Wen Yancheng et al had constructed cultivars fingerprinting maps with SRAP and SSR markers in *B.napus*, and they thought SSR markers had a lot of advantages with good stability, high repetition, easy operation and clear bands, and easy to identify and do statistics, and so on, so primer combinational method was suitable to construct fingerprinting maps of cultivars (Wen et al.2006).

In this study, using SSR molecular markers constructed fingerprinting maps for 7 rapeseed germplasms with super-high oil content and 16 main rapeseed cultivars by28 SSR primer pairs. The constructed fingerprinting maps with SSR technique had the suited-number, clear and stabilized bands, abundant fingerprint differences and high Polymorphism ratio. Therefore, SSR technique was the ideal methods of constructing rapeseed fingerprinting maps. The fingerprinting maps were not only effectively method to identify among cultivars or germplasms, but also to analyze the genetic relationship of all materials. The genetic distance relation got through the DNA level direct information, which was more accurate and reliable than the field traits and general genealogical relationship. Because of Chinese *B. napus,* resources were from abroad, including Europe and Japan etc (Wang et al.2009), and resources were frequent exchanged and crossed transduction in the process of breeding in recent years, as well as the directional or multiple selection methods were used for the different breeding goals, therefore, Chinese materials were not far apart with that from abroad with complex performance. It was hard to judge the genetic distance among the materials through external traits, especially to the high generation resources and cultivars releasing, in which the genetic background was more polymerized for the same genetic background of many excellent traits, and made the genetic relationship of the materials near and complicated, the corresponding extent between the traits and the genetic relationship also became less and less (Wang et al.2009). Using morphological characteristics and general methods assess relationship to be difficult implement. For example, correlation was near or far for the same super-high oil content germplasms, and the genetic distance of each main cultivars was also not different. Therefore, DNA fingerprint technique function was fully used, which not only had the important significance on identifying and protecting of many precious germplasms and cultivars, but also had the irreplaceable value on the genetic relationship analysis of materials, the matching and selecting cross combination and heterosis using .

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