IDENTIFICATION OF HOP VARIETIES WITH AFLP TECHNIQUE

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UDC/UDK 633.791:631.526 (045)
original scientific article/izvirni znanstveni članek
received/prispelo: 22. 09. 2006
accepted/sprejeto: 22. 11. 2006

ABSTRACT

Hop is essential raw material for brewing industry. Different hop varieties impart different bitter and different aroma to beer. It's an interesting topic for brewing industry to identify accurately hop variety. For this purpose, five EcoRI primer and eight MseI primer combinations were selected for the hop varieties identification. Among these primer combinations, five primer combinations give clearly visible polymorphism result with the AFLP method. The genetic similarity of the analyzed hop cultivars is discussed. It is possible to identify some hop varieties with several primer combinations based on this study.

Key words: hops, variety identification, AFLP

DOLOČEVANJE SORT HMELJA S TEHNIKO AFLP

IZVLEČEK

Hmelj je ena izmed osnovnih surovin za pivovarsko industrijo. Različne sorte hmelja doprinesejo pivu različno grenčico in aromo. Za pivovarsko industrijo je zato določitev sort hmelja velikega pomena. V ta namen je bilo uporabljenih 5 EcoRI in 8 MseI začetnih oligonukleotidov. Med vsemi AFLP kombinacijami začetnih oligonukleotidov je bil pri 5 kombinacijah opažen jasno razločljiv polimorfizem fragmentov. Prispevek se dotakne tudi genetske podobnosti analiziranih sort hmelja.

Ključne besede: hmelj, identifikacija sort, AFLP

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1 INTRODUCTION

Hop as one of important raw material for brewing industry impart beer special bitter aroma and flavor. Different hop varieties with different chemical profile have different bitterness and aroma characters. It may influence the style of beer. It is not easy thing to determine the key compounds included in one variety which give the beer different character by the most modern chemical analytical method. Traditionally, it is the direct way to get the concept of a hop variety by pilot brewing. Thus it is important topic to identify hop varieties not only for breeders but also for brewers.

Conventional method to identify the hop variety includes morphological method and chemical method [4, 5, 6]. For fresh hop cones, it is a practical way to make use of the former method. For pellet, it is better to use the latter way. But the main analyzed objects of the two methods are easy affected by environmental conditions such as weather, disease and insect damage, fertilizer and etc. It is effective only after collecting a lot of hop information from different environment conditions.

Now the DNA fingerprinting is widely applied in a number of different fields including plant and animal breeding, variety or cultivar identification, diagnostic medicine, disease diagnosis in animals and plants, construction of genetic markers and so on. There are also some papers on application of variety identification by DNA fingerprinting technology [1, 2, 7] such as RFLPs termed as restriction fragment length polymorphism; AFLPs termed as amplified fragment length polymorphism; RAPDs termed as random amplified polymorphic DNA and SSRs termed as simple sequence repeat polymorphism or micro-satellites.

Identification of widely used hop varieties in China with AFLPs techniques are described in this paper. In a research included hop varieties were Tsingdao Flower, Kinrin Flower, Saaz, Spalt and Hallertau.

AFLPs techniques combine the techniques of RFLP and PCR. That is to amplify obtained restriction fragments with PCR method after cleaving the DNA with restriction enzymes. The fragment was modified by adding oligonucleotide linker to the ends. A relative small number of tagged restriction fragment will be amplified by selecting specific synthetic oligonucleotides. The reaction product were run on 5% denaturing polyacrylamide gels. It is possible to find the difference between hop variety by the morphological data. Hartl and Seefelder have distinguished 8 hop varieties by AFLPs techniques. However, hop cultivars ‘Saaz’, ‘Tettnanger’, ‘Spalt’ could not be discriminated [3]. Townsend and Henning have also developed an AFLPs protocol to identify 5 American hop varieties [8]. But no studies on identification of Chinese hop varieties with AFLPs were reported. The purpose of this study is to find a reliable method to identify the local and common use hop varieties in China.

2 MATERIALS AND METHODS

2.1 Plant material

The 5 hop cultivars were investigated. The Tsingdao Flower, Kirin Flower are from Xinjiang in China. The Saaz, Spalt and Hallertau are European aroma hop cultivars which are commonly used in China. Except Hallertau, all the hop samples were all dried hop cones. The Hallertau sample was in a pellet form.

2.2 DNA extraction

The dried hop cones and hop pellets were ground to powder in frozen liquid nitrogen with a mortar and a pestle. The samples were placed in a 2 mL polypropylene tube, and extracted according to the CTAB protocol by Atsushi Murakami (Technical Quarterly, 1998). The DNA concentration was estimated in comparison with a known concentration of Lambda DNA in 0.8% agarose gel.

2.3 AFLP Analysis

The restriction enzymes and reagent in this test were from Promega company. The polymerase enzyme and reagent were from Huamei company. The primers were composed by Sangon in Shanghai.

The DNA samples were digested with 5U EcoRI / MseI, ligated in the same tube with 500pmol EcoRI adapter and 50pmol MseI adapter to the restriction fragment with 2U T4-ligase for about 9 hours. After restriction enzymes were denatured at 65°C for 10 minutes. A preamplification was performed in a total volume of 20uL containing 30ng M00 and E00, 0.2mM of all four dNTP, 1×PCR buffer, 0.6U amplitaq DNA polymerase. The preamplification PCR program was 30 cycles with a following program: 94°C for 40s, 56°C for 40s, 72°C for 60s. Then the product was diluted 1:15-20 with a TE buffer.

The selective amplification reaction was carried out in a total volume of 20uL comprising 40ng selective EcorRI and MseI primer with 0.23mM of all dNTP and 0.6U amplitaq DNA polymerase. The selective amplification program of the first 12 PCR cycle was used with a step-down of the annealing temperature with each cycle that is 94°C for 35s; 65°C for 35s; 72°C for 60s. The annealing temperature was reduced by 0.7°C within each cycle. The final 30 cycle were carried out at a constant annealing temperature, that is 95°C for 40s; 56°C for 40s and 72°C for 60s.

The detailed primer and adapter sequence are listed in Table 1.

Table 1: List of primers and adapters

Electorphoresis and gel analysis

Primers/adapters	Sequences（5-3）
EcoRI adapter	CTCGTAGACTGCGTACC TACTCAGGACTCAT
E00	GACTGCGTACCAATTC
E15	E00+CA
E32	E00+AAC
E33	E00+AAG
E36	E00+ACC
E38	E00+ACT
MseI adapter	CTCGTAGACTGCGTACC CTGACGCATGGTTAA
M00	GATGAGTCCTGAGTAA
M31	M00+AAA
M37	M00+ACG
M47	M00+CAA
M49	M00+CAG
M58	M00+CGT
M59	M00+CTA
M61	M00+CTG
M62	M00+CTT

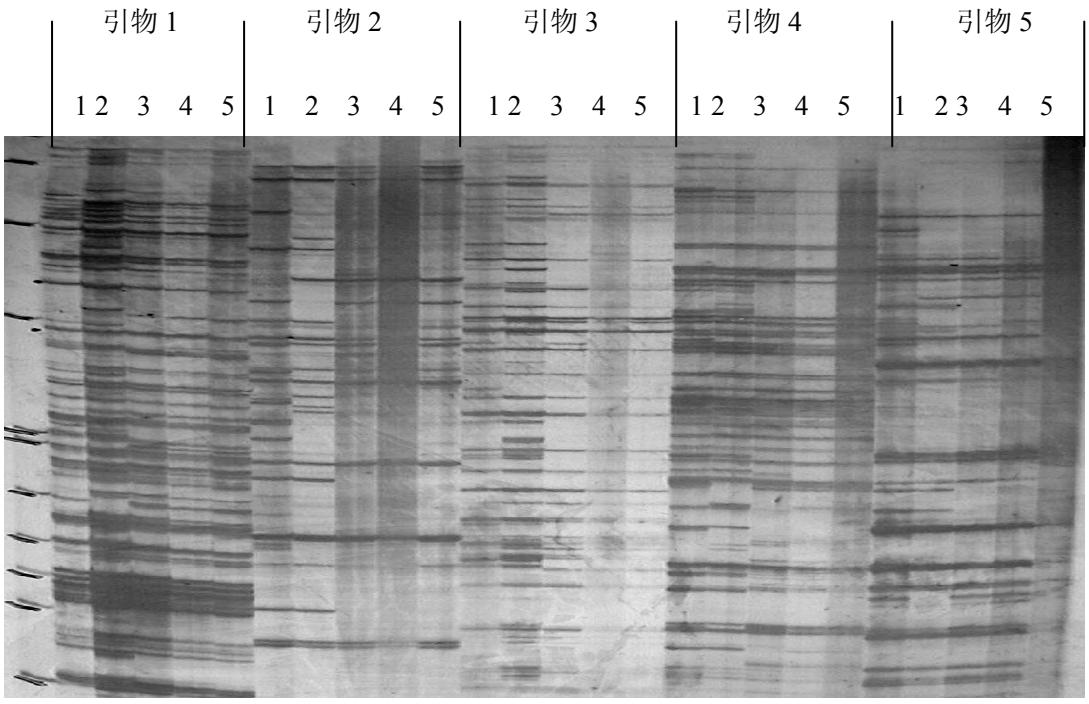

Table 2: Genetic similarity for five hop varieties based on the formula of Dice

	Qingdao flower	Kirin flower	Saaz	Spalt	Hallertau
Qingdao flower	1.000				
Kirin flower	0.835	1.000			
Saaz	0.764	0.808	1.000		
Spalt	0.741	0.777	0.937	1.000	
Hallertau	0.797	0.813	0.973	0.986	1.000

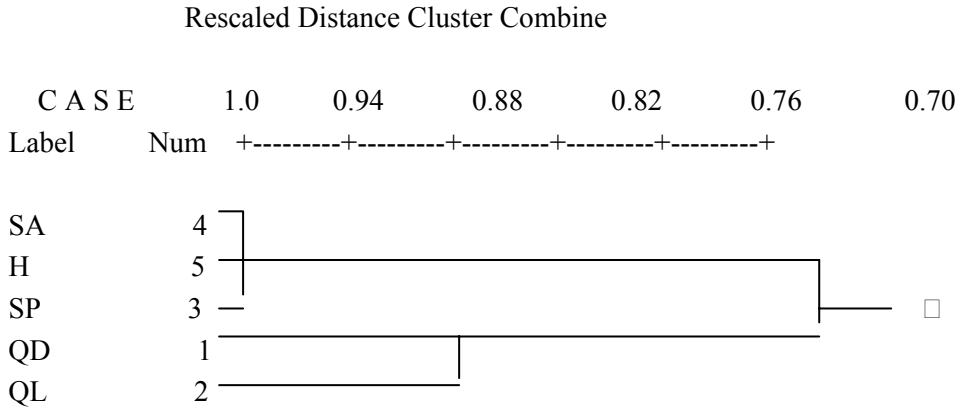

Fig. 1: Dendrogram of the five hop varieties derived from the genetic similarity matrix

2.4 Electorphoresis and gel analysis

The reaction product was mixed with 20uL loading dye and denatured at 95°C for 5 min, then chilled on ice. The sample was immediately transferred to 5% denaturing polyacrylamide gel. The gel was prerun at a constant 70 w for 30 min, then the sample was loaded into well. The gel was run at a constant 70 w for about 1.5 hour. At last stain the gel was with silver nitrate and photographed with a Kodak DC290.

The clearly visible markers were scored in a binary data matrix. The genetic similarities between each two varieties were estimated. A dendrogram was generated using Rescaled Distance cluster combine by a spass program.

3 RESULTS AND DISCUSSION

In this study, five Ecor I primers and eight Mse I primers, each with three selective bases, were used to generate AFLP within five hop varieties. Out of all primer combinations, five primer combinations were selected for their reliable and clear banding pattern (figure 1). The five primer combinations are E33M61, E38M58, E38M59, E32M49 and E36M49. The total number of amplified bands by each of the 5 AFLP primer combinations is between 28-47 bands. The five primer combinations amplified 165 DNA fragments where 74 of them were polymorphic. 14.8 polymorphic fragments per primer combination were found. More bands were conducted by E33M612, but the most polymorphic bands were generated by E33M58.

It's possible to differentiate the five hop varieties with the five primer pair combinations. There is big difference between Qingdao Flower and Kirin Flower with other varieties. In other words, one can easily find the difference between Qingdao Flower and Kirin Flower with other varieties by each of the primer combination. The difference between Saaz and Spalt is very little. That was also mentioned by Hartland and Seefelder (1998). But one can still find one different band between them using one of the five primer combinations.

The Qingdao Flower and Kinrin Flower had the lowest genetic similarity 0.835, while the Saaz and Spalt had higher genetic similarity 0.937. The genetic similarity between Saaz and Hallertau; Spalt and Hallertau were the highest.

It was concluded that the DNA fingerprint technique is a useful method for identification of hop varieties. In this research, five primer combinations were selected to identify the common used hop varieties in China. But it is much more complicated for breweries to apply such a technique. More simple and easier methods ought to be developed in the future.

4 LITERATURE

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