การประเมินความบริสุทธิ์และความแปรปรวนทางพันธุกรรมของข้าวโพดข้าวเหนียวด้วยเครื่องหมายเอสเอสอาร์

Assessment of Genetic Purity and Variation of Waxy Corn (*Zea mays* L. *ceratina*) Using SSR Markers

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บทคัดย่อ

การศึกษาการประเมินความบริสุทธิ์และความแปรปรวนทางพันธุกรรมของข้าวโพดข้าวเหนียวด้วยเครื่องหมายเอสเอสอาร์ ปลูกข้าวโพดข้าวเหนียว 6 พันธุ์ ได้แก่ แม็กซ์วัน บิ๊กไวท์-852 ไข่มุก-49 ทับทิมสยาม รัชตะ และเหนียวสวรรค์ วางแผนการทดลองแบบ Randomized Complete Block Design (RCBD) จำนวน 4 ซ้ำ บันทึกลักษณะทางการเกษตร ผลผลิตและองค์ประกอบผลผลิตของข้าวโพดข้าวเหนียว นำต้นกล้าจำนวน 100 ต้น ของแต่ละพันธุ์มาสกัดดีเอ็นเอด้วยวิธี CTAB จากนั้นประเมินความบริสุทธิ์และความแปรปรวนทางพันธุกรรมของข้าวโพดข้าวเหนียว 6 พันธุ์ ด้วยเครื่องหมายเอสเอสอาร์ โดยใช้ 6 ไพรเมอร์ ได้แก่ bnlg 1012, bnlg 1017, bnlg 1055, bnlg 1118, bnlg 1188 และ UMC 1086 พบว่า ทั้ง 6 ไพรเมอร์แสดงรูปแบบแถบที่คล้ายคลึงกันของข้าวโพดข้าวเหนียวแต่ละสายพันธุ์ จากนั้นวิเคราะห์ข้อมูลเพื่อหาความสัมพันธ์ทางพันธุกรรมของพันธุ์ข้าวโพดข้าวเหนียวที่ศึกษาด้วยการคำนวณค่า Dice's similarity coefficient และจัดกลุ่มสายพันธุ์ข้าวโพดข้าวเหนียวด้วยวิธี Unweighted Pair Group Method with Arithmetic mean (UPGMA) พบว่า ข้าวโพดข้าวเหนียวกลุ่มที่ 1 ได้แก่ พันธุ์เหนียวสวรรค์ และไข่มุก-49 กลุ่มที่ 2 ได้แก่ พันธุ์รัชตะ ทับทิมสยาม และแม็กซ์วัน และกลุ่มที่ 3 ได้แก่ พันธุ์บิ๊กไวท์-852 ซึ่งพันธุ์บิ๊กไวท์-852 มีวันออกดอกตัวผู้ 50 เปอร์เซ็นต์ วันออกไหม 50 เปอร์เซ็นต์ ช้ากว่าพันธุ์อื่นๆ จากการศึกษานี้แสดงให้เห็นว่าเครื่องหมายเอสเอสอาร์มีประสิทธิภาพและสามารถทดสอบความบริสุทธิ์ทางพันธุกรรมของข้าวโพดข้าวเหนียวได้

ABSTRACT

Simple sequence repeat (SSRs) analysis was done to assess genetic purity and variation of waxy corn. Six waxy corn varieties; Maxone, Bigwhite-852, Kaimook-49, Tabtim Siam, Ratchata and Neawsawan were grown under randomized complete block design with 4 replications. Agronomic characteristics, yield and yield component were recorded. Total genomic DNA of 100 seedlings of each

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variety was extracted using the modified CTAB method. Simple sequence repeat (SSR) marker was used to evaluate genetic purity and variation of six waxy corn varieties using 6 SSR primers (bnlg 1012, bnlg 1017, bnlg 1055, bnlg 1118, bnlg 1188 and umc 1086). All of 6 primers exhibited similar banding pattern of each waxy corn variety. The pair wise comparisons of the varieties based on the proportion of unique and shared amplification products were used to measure the genetic similarity by Dice's Similarity Coefficient and were grouped by Unweighted Pair Group Method with Arithmetic mean (UPGMA). It was found that waxy corn varieties as Neawsawan and Kaimook-49 were clustered together in group 1. Relationships among seed samples clustered into group 2 were Ratchata, Tabtim Siam and Maxone. Bigwhite-852 could not be grouped to any group because it was delayed in days to 50% tasselling and 50% silking than other cultivars. The study showed that SSR markers are efficient and reproducible for genetics purity testing of waxy corn.

Key Words: genetic purity, genetic variation, SSR markers, waxy corn

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INTRODUCTION

Waxy corn (*Zea mays* L. *ceratina*) has been commercially grown due to its tough, low sugar conten and tender texture (Neuff *et.al*., 1968). Local waxy corn variety is no consistency in yield and quality. Therefore, commercial waxy corn hybrids have been developed. Higher genetic purity is essential prerequisite for the commercialization of hybrid seeds. The use of seeds with low genetic purity results in segregation of the traits, lower yields and genetic deterioration of varieties. Therefore, it is critical for seed suppliers to control genetic purity before marketing. The grow-out test is traditionally method to determine the genetic purity test based on morphological traits that are time consuming and environmental effects. The biochemical markers such as isozyme and protein profile limited polymorphism and did not discriminate between related inbred lines (Lucchese, *et al.,* 1999)

It is essential to develop a more rapid, accurate and cost-effective method for assessment genetic purity of hybrids. DNA marker provide an opportunity for assessing genetic purity by simple sequence repeats (SSRs) that are the great importance for parental line seed purity (Yashitola *et al*., 2002). The use of SSR markers for assessing seed purity has been reported in rice (Nandakumar *et al*., 2004; Sundaram *et al*., 2008), maize (Mingsheng *et al*., 2006), sunflower (Antonova *et al*., 2006) and horticultural crops such as tomato (Smith and Register, 1998), cabbage (Liu *et al*., 2007) and melon (Jianli *et al*., 2006). However, the utility of SSR marker in the genetic purity testing of waxy corn has not been reported. Therefore, the objective of the present study was to assess genetic purity and variation of waxy corn using SSR markers.

MATERIALS AND METHODS

Six commercial waxy corn varieties consisted of Maxone, Bigwhite-852, Kaimook-49, Tabtim Siam, Ratchata and Neawsawan were grown in the field of Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University based on random complete block design (RCBD) with four replications. For each variety, 100 seedlings were planted in the spacing of 25 x 75 cm^2. Plant height, days to 50 % tassel, days to 50 % silking, ear height, ear length, ear width, number of seed row, ear weight, seed weight per ear and shelling percentage were recorded.

Total genomic DNA was using the modified method by Agrawal *et al*. (1992). About 2 g of young leaf tissue from each sample was homogenized in liquid nitrogen and incubated at 60 °C for 60 min with 700 µl of CTAB buffer [2% hexadecyltrimethyl ammoniumbromide, 2.8 M NaCl, 40 mM EDTA (pH 8.0), 200 mM Tris-HCl (pH 8.0)]. Then 700 µl of chloroform:isoamyl alcohol mixture (24:1) was added and blend thoroughly. After centrifuge (15 min, 12,000 rpm), supernatant layer was put into a new microtube and approximately equal volume of chilled ethanol was added. After storage at -20°C for 30 min, precipitated DNA was centrifuged, vacuum dried and finally stored in TE buffer.

Six SSR primer pairs were selected for genetic purity analysis. PCR reactions were performed in a total volume of 20 µl containing 50 ng of genomic DNA, 10×*Taq* buffer, 25 mM $MgCl_2$, 2 mMdNTP, H_2O, 0.2 µM of each forward and reverse primersand 1U of *Taq* DNA polymerase in a Thermal Cyclear. The thermocycling program was 94°C for initial denaturation, 72°C for final extension and 38-40 cycles of 94 °C for denaturation, 55-60°C for annealing and 72°C extension. PCR products (2µl) were separated on a 2% agarose gel and stained with ethidium bromide and photographed using Gel documentation unit under UV light.

Analysis of DNA banding pattern was carried out on Statistica Package version 2.14. The pair-wise comparisons of the varieties based on the proportion of unique and shared amplification products were used to measure the genetic similarity by Dice's Similarity Coefficient (1945) and were grouped by Unweighted Pair Group Method with Arithmetic mean (UPGMA).

RESULTS

Agronomic characteristics

Six waxy corn varieties were different in agronomic characteristics excepting for ear height. Maxone and Bigwhite-852 were not different in days to 50% tassel (50.83 and 49.68 days, respectively) whereas Tabtim siam showed the earliest in days to 50% tassel (44.37 days). Ratchata, Neawsawan and Kaimook-49 had 46.50, 46.12 and 45.96 days to 50% tassel, respectively. Maxone and Bigwhite-852 had 52.40 and 51.67 days to 50% silking, respectively. Ratchata, Kaimook-49 and Neawsawan had 49.60, 48.89 and 47.98 days to 50% silking, respectively. Whereas, Tabtim siam had the earliest in days to 50% silking (44.37 days).

Neawsawan, Kaimook-49 and Tabtim siam were not significantly different in plant height of 182.66, 182.58 and 180.48 cm, respectively. Maxone showed the lowest in plant height (176.75 cm) and were not significantly different to Ratchata and Bigwhite-852. However, all varieties were not significantly different in ear height (Table 1).

Table 1 Agronomic characteristics of 6 waxy corn varieties

Cultivars	Agronomic characteristics			
	Days to 50% tassel	Days to 50% silking	Plant height (cm)	Ear height (cm)
Tabtim siam	44.37 c	46.59 d	180.48 ab	94.65
Ratchata	46.50 b	49.60 b	179.21 bc	94.14
Maxone	50.83 a	52.40 a	176.75 c	92.64
Neawsawan	46.12 b	47.98 cd	182.66 a	94.49
Kaimook-49	45.96 b	48.89 bc	182.58 a	93.26
Bigwhite-852	49.68 a	51.67 a	179.13 bc	95.65
Mean	47.24	49.52	180.14	94.14
C.V. (%)	1.72	2.03	0.91	2.23
F-test	**	**	**	ns

ns = Non-significant ** = Significant at $p < 0.01$

Yield and yield components

Six waxy corn varieties were not significantly different in ear length and shelling percentage (Table 2). Maxone showed the highest in ear width (4.92 cm) and number of seed row (16.70). Ratchata and Neawsawan were not significantly different in ear width (4.65 and 4.63 cm, respectively). Whereas, Bigwhite-852 had the lowest in ear width (4.27 cm) and number of seed row (12.51). Neawsawan, Tabtim siam, Ratchata and Kaimook-49 had not significantly different in number of seed row (14.42, 13.83, 13.37 and 13.32, respectively). Maxone had the highest in ear weight of 128.87 g and seed weight per ear (115.07 g). However, the ear weight and seed weight per ear of Maxone were not significantly different from Ratchata (125.46 and 112.97g, respectively). Ear weight of Neawsawan, Tabtim siam, Bigwhite-852 and Kaimook-49 were 120.33, 115.37, 111.96 and 106.21 g, respectively. Neawsawan, Tabtim siam, Bigwhite-852 and Kaimook-49 had 108.77, 100.97, 99.93 and 94.89 g of seed weight per ear, respectively (Table 2).

Table 2 Yield and yield components of 6 waxy corn varieties

Cultivars	Yield and yield components					
	Ear length (cm)	Ear width (cm)	No. row of seed	Ear weight (g)	Seed weight/ear (g)	Shelling (%)
Tabtim siam	12.73	4.43 de	13.83 b	115.37 c	100.97 c	90.41
Ratchata	14.85	4.65 b	13.37 bc	125.46 a	112.97 ab	90.04
Maxone	16.34	4.92 a	16.70 a	128.87 a	115.07 a	89.34
Neawsawan	13.19	4.63 bc	14.42 b	120.33 b	108.77 b	89.30
Kaimook-49	14.16	4.47 cd	13.32 bc	106.21 d	94.89 d	89.29
Bigwhite-852	14.12	4.27 e	12.51 c	111.96 c	99.93 c	87.46
Mean	14.23	4.56	14.23	118.03	105.43	89.31
C.V. (%)	16.4	2.31	5.58	2.25	2.92	1.79
F-test	ns	**	**	**	**	ns

ns = Non-significant ** = Significant at $p < 0.01$

Genetic purity testing using SSR markers

In this study all varieties were genotyped with six SSR primer pairs (bnlg 1012, bnlg 1017, bnlg 1055, bnlg 1118, bnlg 1188 and umc 1086). A total of 22 clear and reproducible bands were generated. Only the primers which amplified bands specific of each variety were considered for assessing the purity of these waxy corns. The SSR markers such as bnlg 1188 and umc 1086 showed polymorphism by producing the specific alleles (Figure 1, Figure 2). The details on number of off type and genetic purity tested by different SSR markers along with morphological traits results are given in Table 1 and 2.

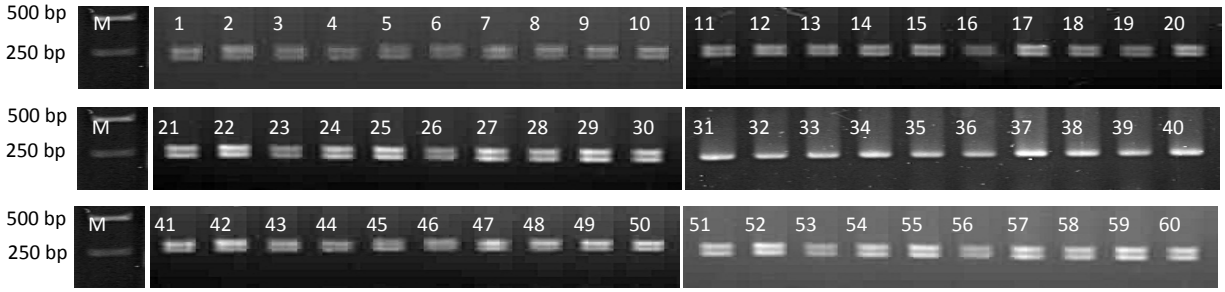

Figure 1 Genetic purity analysis of Tabtim siam (1-10), Neawsawan (11-20), Ratchata(21-30), Maxone (31-40), Bigwhite-852 (41-50) and Kaimook-49 (51-60) using SSR primer bnlg 1188. M-DNA ladder

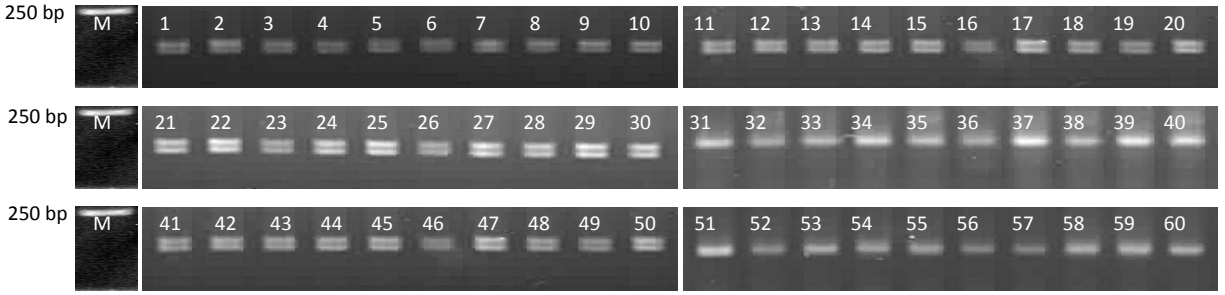

Figure 2 Genetic purity analysis of Tabtim siam (1-10), Neawsawan (11-20), Ratchata(21-30), Maxone (31-40),

Bigwhite-852 (41-50) and Kaimook-49 (51-60) using SSR primer umc 1086. M-DNA ladder

Dendrogram was generated by cluster analysis based on genetic variation and UPGMA method. Three groups are evident in the dendrogram (Fig. 3). Neawsawan and Kaimook-49 were clustered together in group 1 using the similarity coefficient of 0.78 as a standard. Also, the varieties in group 1 tended to have the rarest state for at least one of the characteristics (days to 50 % tassel, ear length, no. row of seed, seed weight/ear). Relationships among seed samples clustered into group 2 were Maxone, Tabtim Siam and Ratchata. All the tested samples indicating closed relationship and had high purity level. The last group, Bigwhite-852 was clearly different from the other groups. For phenotypic difference, Bigwhite-852 had days to 50 % tassel and silking later than other varieties.

This result revealed a clear demarcation between most of the genotype indicating differentiation of the varieties into groups due to genetic variation and their source. The broad genetic variation detected within and among the seed samples demonstrates the genetic purity that exists among the tested samples as well as the potentials of using SSR markers for genetic purity analysis in waxy corn.

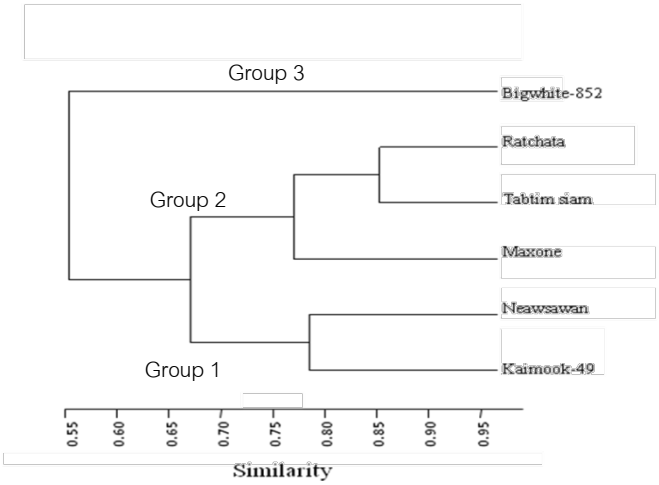

Figure 3 Dendrogram generated by SSR data of six waxy corn cultivars using PAST software

DISCUSSIONS

Assessment of genetic purity is one of the most important quality control components in hybrid seed production. Traditionally, the purity has been employed to assess of hybrid seed using morphological traits. The traits such as days to 50% tessel, days to 50% silk, plant height, ear height, ear length, ear width, number row of seed, ear weight, seed weight per ear and shelling were studied to distinguish the interspecific hybrid from off type. Though morphological traits is used to determine genetic purity of waxy corn that is tedious space demanding and time consuming and often does not allow the unequivocal identification of genotypes.

Hence, a recent development in DNA markers has been suggested for genetic purity testing since they are used to assess precisely the genotype but not for the phenotype (Sundaram *et al.*, 2008). Among different types of DNA markers, SSR marker found to be very useful for testing genetic purity. In

this study, morphological traits of six waxy corn varieties supported the genetic purity. Moreover, it was also supported by the results of SSR marker.

CONCLUSIONS

Waxy corn hybrids and open pollinated variety could be distinguishable clearly using SSR markers. SSR primer bnlg 1012, bnlg 1017, bnlg 1055, bnlg 1118, bnlg 1188 and umc 1086 can be used to identify the genetic purity of waxy corn varieties. The analysis of Dice similarity coefficient can be classified waxy corn cultivars into 3 groups.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support from Corn Excellent Center, Kasetsart University. Authors are also very grateful to Crop Improvement and Biotechnology Laboratory, Department of Horticulture, Kasetsart University for providing facilitation for this work.

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