Page 400 to 412
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Selection and Combining Ability Analysis of the Waxy Maize Inbred Lines with Thinner Pericarp by Phenotyping and SSR Markers

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Abstract

The thinner pericarp of the kernel are associated with the tenderness of fresh waxy corn, we evaluated 60 waxy maize inbred lines in generation S_6-S_8. To determine thickness pericarp, we used micrometer and identified 38 out of 60 lines have favorable thickness pericarp ranged from 35 to 60 µm according to fresh quality. Using 5 SSR markers *umc2189 – ZCT131; bmc1396-mmc0143; umc2118-bmc1325; umc1757-umc1550; umc2038-dupssr28* to detect QTL control thinner pericarp of waxy maize kernel. result shown that interelation between phenotype of 21 lines containing QTL. There were nine lines origined from Vietnamese traditional maize varieties (D14, 161, 21, 22, 42, 45, 60, 61, and 90), one line origined from Laos (D18) and eleven lines origined from China (D29, 30, 31, 52, 601, 70, 71, 72, 74, 82, and 85). Based on the evaluation of phenotype and molecular marker, we selected seven elite lines that suitable agronomical characters and thninner pericarp to develop waxy maize hybrid for market fresh quality those were D29, 161, 71, 601, 61, 86, and 74. These lines were crossed in diallel according to Griffing 4 to produce 21 possible F1 hybrids. Twenty-one F1 and their seven parents planted side by side in replicated trials to evaluate agronomical characteristics on phenotype and molecular markers. We selected 10 hybrids with favorable characteristics and thinner pericarp those were THL1, TH2, TH3, TH4, TH5, TH6, TH8,TH9, TH16 and TH20. They will be further evaluated to develop waxy maize hybrid have high quality in Northern of Vietnam.

Keywords: *Selection; Inbred line; Waxy corn; Thinner pericarp; Quality*

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Introduction

Waxy maize (Z*ea mays L. var. certain* Kulesh) is a special cultivated type of maize, and was first discovered in China in 1908 and then in other Asian countries (Collins GN ,1909; Fan LJ, 2009). Waxy maize with many excellent characters in terms of starch composition and economic value has grown in China for a long history and its production has increased dramatically in recent decades (Zheng H, 2013). There is a wide genetic diversity of waxy maize in agronomic traits such as plant height, maturity, resistance to insects and diseases and yield components. According to plant morphology, geographical distribution and biological characters, combined with historical data and folklore, waxy maize was considered to be derived from one single gene mutant from flint corn (Tian ML., *et al*. 2009). Fergason (2001) indicated that waxy maize varieties are now equal in yield to 'dent' field corn, and average variations in yield do not exceed 5%. But a real breakthrough in the process of selection of new varieties over the last 20 years has been observed in China (Agnieszka Klimek-Kopyra., *et al*. 2012). Introduction waxy corn as a fresh vegetable has been cultivated throughout Southeast and East Asia for more than a century for local and export markets continue to expand (Danupol Ketthaisong., *et al*. 2014).

Although normal corn is widely cultivated and used in food, forage, industrial and bioenergy, waxy corn or sweet corn, which is a special type of cultivated maize, is only used in food production. Waxy corn has become a popular and valuable crop in East Asian countries. Both demand and consumption of waxy corn have soared in recent years (Sa., *et al*. 2010). Along with increased consumption of waxy corn, consumers are also demanding more delicious new cultivars of waxy corn (Ki Jin Park., *et al*. 2013). Such as improvement of waxy corn quality is one of the most important aims in current breeding programs. To facilitate the development of new varieties with high eating quality, it is necessary to understand the genetic basis of such traits. Eating quality of waxy maize has been extensively studied consisted tenderness; flavor, taste, sweetness and kernel color depend on the market (Danupol Ketthaisong., *et al*. 2013). Kernel pericarp thickness and ear architectural traits are important selection criteria in fresh waxy corn breeding programs as they are associated with consumer sensory and visual preferences (Eunsoo Choe, 2010). Waxy maize breeding with high quality, special tenderness is priority in Vietnam therefore we conducted this study with the aim was identifying waxy maize inbred lines and crosses have high yield and quality for fresh waxy maize production in Northern of Vietnam

Materials and Methods

Plant materials

The waxy maize inbred lines consisted of 60 lines in generation S_6 to S_8 those were developed from germplasm have deference origin by self-polination, among them 30 lines from open-polination populations of Vietnam, 7 lines from Laos, and 23 lines from China with HQ6 and HN88 are check varieties as follows:

No.	Symbol	Origin and population name	No.	Symbol	Origin and population name
1	D2	Khau lion lun, Thanh An, Dien Bien, Viet Nam	31	D40	Na phoc II - Laos
2	D3	Khau lion lun, Thanh An, Dien Bien, Viet Nam	32	D41	Bap Nu Xam Bum - Nhan Muc Ham Yen Tuyen Quang, Viet Nam
3	D4	Khau lion lun, Thanh An, Dien Bien, Viet Nam	33	D42	Ngo nep, Tra Linh, Cao Bang
4	D5	Khau lion lun, Thanh An, Dien Bien, Viet Nam	34	D45	Khau lion lun, Thanh An, Dien Bien, Viet Nam
5	D13	Pooc cu lau, Muong Phang, Dien Bien, Viet Nam	35	D52	Jingxi - China
6	D14	Sli Lo, Muong Phang, Dien Bien, Viet Nam	36	D60	Bap Nu , Na Kham, Nang Kha, Na Hang, Tuyen Quang
7	D15	Jingxi - China	37	D68	MX10 – Viet Nam
8	D16	Nep Nuong, Na Tau, Dien Bien , Viet Nam	38	D61	Viet Nam
9	D161	MX10 – Viet Nam	39	D601	601 - China
10	D18	Nep Lao 3 – Laos	40	D70	China
11	D19	Nep Lao 4 – Laos	41	D71	China

12	D20	Ngo nep Khanh Hoa –Phuc Loi, Luc Yen, Yen Bai	42	D72	China
13	D21	Ngo nep Khanh Hoa – Khanh Hoa, Luc Yen, Yen Bai	43	D73	China
14	D22	Ngo nep Khanh Hoa – Khanh Hoa, Luc Yen, Yen Bai	44	D74	China
15	D23	Pooc cu lau, Muong Phang, Dien Bien, Viet Nam	45	D75	Viet Nam
16	D24	Nep Trang, Lao Cai	46	D76	China
17	D25	Nep Trang, Lao Cai	47	D77	China
18	D26	Phon Kham I - Laos	48	D78	China
19	D27	Phon sa may I -, Laos	49	D79	China
20	D28	China	50	D80	China
21	D29	China	51	D81	China
22	D30	China	52	D82	China
23	D31	China	53	D83	China
24	D32	Mai plot – Tat Lia, Coc Dan, Ngan Son, Bac Kan	54	D84	China
25	D33	Bap Nu – Na Kham, Nang Kha, Na Hang, Tuyen Quang	55	D85	China
26	D34	Bap Nu Xam Bum - Nhan Muc Ham Yen Tuyen Quang, Viet Nam	56	D86	China
27	D35	Mai plot – Tat Lia, Coc Dan, Ngan Son, Bac Kan	57	D87	Phon sa may II - Laos
28	D36	MX10 - Viet Nam	58	D88	Duong ta , Laos
29	D38	Bap Nu Xam Bum - Nhan Muc Ham Yen Tuyen Quang, Viet Nam	59	D89	Ngo nep Khanh Hoa –Khanh Hoa, Luc Yen, Yen Bai
30	D39	Ngo 601 - China	60	D90	Khau lion lun, Thanh An, Dien Bien, Viet Nam

Table 1

Methods

The field experiment was implemented at Crop Resaerch and Development Institute (CRDI),Vietnam National University of Agriculture following the method of Gomez, K. A., & Gomez, A. A. (1984).

Waxy inbred lines were evaluated in spring 2014, treatments were designed in randomized complete block design(RCBD) with two replications, plot area was 10m^2, spacing 60cm x 25cm, plant density was maintained at approximately 67,000 plants per hectare. A seven inbred lines namely: D29, D61, D71, D601, D161, D86 and D74 were selected based on the performance that shown in inbred lines experiment in spring 2014, aiming to identify the ncombinations between parental lines with high and low perfomance in traits related to eating quality and thickness pericarp traits. Parental lines were crossed in Griffing 4 diallel cross to produce 21 possible F_1 hybrids introduced in trail with designed in randomized complete block design (RCBD) with three replications, plot area is 14m^2 with a row-to-row distance of 70 cm and plant-to-plant distance of 25 cm. Plant density was maintained at approximately 57,000 plants per hectare. Standard agronomic practices were applied to all trials by Vietnamese testing regulation QCVN01-56:2011/BNNPTNT.

Measuring thickness of mature corn pericarp by micrometer method Kernels were steeped in water for 3 to 4 hours at room temperature, crown cap and tip cap were cut and removed. Pericarps were peeled, excised pericarp strips were placed in a 1:3 water:glycerol solution (by volume) and evacuated. Strips were left in the evacuated solution for 24 hours at room temperature. Pericarp strips were removed from the liquid, blotted dry, and placed in an equilibration environment of 25oC at 50% RH for 24 hours. Pericarp thickness was measured with a micrometer (Model 105-01-0), plunger diameter 1.66 mm using a 1-g weight) at the levels. Five regions of pericarp thickness traits consisted are upper germinal (UG), lower germinal (LG), upper abgerminal (UA), lower abgerminal (LA), and Crown (CWN). In addition, in this study, taste, flovor, sweetness and kernel color evaluated by tasting method, this are parameters associated with quality of the fresh waxy maize.

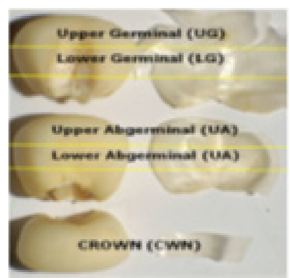

Figure 1: *Five regions of pericarp thickness traits (Eunsoo Choe, 2010).*

SSR markers were used to detect QTL for pericarp thickness traits those were five specific primers *umc2189 – ZCT131, bmc1369-mmc0143, umc2118-bmc1325, umc1757-umc1550, umc2038-dupssr28*. Eunsoo Choe, (2010) can detect thikness pericarp in 5 regions of the kernel pericarps UG, LG, UA, LA and CWN of the 60 waxy inbred lines and 21 hybrids.

DNA extraction and SSR genotyping conducted according to CIMMYT, (2005): Genomic DNA from parental and F_1 analysis to detect QTL related to thickness pericarp was isolated from young leaves. SSR amplifications were performed in a total volume of 30 µl and consisted of 20 ng genomic DNA, 1× PCR buffer, 0.3 M of forward and reverse primers, 0.2 mM deoxyribonucleotide triphosphate (dNTP) and 1 unit *Taq* Polymerase. The PCR profile consisted of a 5-minute initial denaturation period at 94°C followed by two cycles each of 1-minute denaturation at 94°C, 1-minute annealing at 65°C and 2-minute extension at 72°C. The annealing temperature was gradually decreased after the second cycle by 1°C following every second cycle until a final temperature of 55°C was reached. The last cycle was then repeated 20 times. Upon completion of the cycles, the reaction was extended for 10 minutes at 72°C. Five microliters of the final reaction product was mixed with 10 µl electrophoresis loading buffer (98% formamide, 0.02% BPH, 0.02% Xylene C and 5 mM NaOH). Remove combs only when ready to load samples. Pour enough 1X TBE buffer into the gel rig to cover the gel by at least 0.5 cm. Run samples into gel at 100 volts, constant voltage, for about 2-3 h, until the bromophenol blue dye has migrated to just above the next set of wells. Remove tray from rig and stain in 1 µg/ml ethidium bromide (100 µl of 10 mg/ml ethidium bromide in 1000 ml dH_2O) for 20 min with gentle shaking. Rinse gel in dH_2O for 20 min, slide gel onto a UV transilluminator, and photographed.

Statistical procedures and data analysis: The experimental data were analyzed using analysis of variance (ANOVA) for individual environments. Homogeneity of error variance was tested before combined analysis across environments (Gomez and Gomez, 1984). The least significant difference (LSD) analysis, analysis of the diallel for general combining ability(GCA) and specific combining ability (SCA) for yield were based on the Model 4, The statistical model for the mean value of cross (i × j) in Griffing's analysis is:

Yij= m + gi+ gj+ sij+ rij+1/b∑ eijk,

Software used for ANOVA, CV% and LSD analysis was IRRISTAT ver 5.0 and GCA, SCA used DTSL software Nguyen Dinh Hien (1995).

Results

Evaluation of the 60 waxy inbred lines in spring seasons 2014

Choe, Eunsou (2010) reported the thickness pericarp of the waxy ranged from 35 µm to 60 µm was suitable to fresh waxy maize. In this study, there were 35 inbred lines have thickness pericarps ranged from 35-60 µm and grain yield was higher 1.5 ton per hectare, among them 14 inbred lines origined from Viet Nam (D2, D14, D161, D21, D22, D33, D36, D42, D45, D60, D61, D68, D89, and D90), four inbred lines origin from Laos (D18, D26, D27, and D88), 18 inbred lines origin from China (D15, D28, D29, D30, D31, D52, D601, D70, D71, D72, D73, D74, D75,D76, D77, D78, D80, and D86. There were three inbred lines have thickness pericarp siminar check line HQ6 from Korea.

Line	Mean of the TP	Yield	Taste	Tenderness	Flovor	Line	Mean of the TP	Yield	Taste	Tenderness	Flovor
	(µm)	(t/ha)	(scale)	(scale)	(scale)		(µm)	(t/ha)	(scale)	(scale)	(scale)
D2	55.6	1.56	2.7	1.7	1.6	D40	103.9	1.32	2.3	2.7	2.9
D3	76.1	0.93	2.5	2.8	4.1	D41	71.3	1.55	2.2	3.3	2.9
D4	77.1	1.12	1.7	2.2	2.6	D42	59.6	1.04	2.3	3.0	2.3
D5	64.3	1.65	2.7	2.8	3.1	D45	59.8	2.92	1.3	2.7	2.6
D13	63.4	1.01	1.3	4.3	2.8	D52	59.8	1.82	2.8	2.7	2.6
D14	63.4	1.15	2.5	2.8	2.2	D60	58.9	2.70	1.8	1.8	2.0
D15	57.5	2.04	1.2	2.7	2.9	D601	54.1	2.18	2.2	4.3	2.8
D16	57.3	2.52	2.7	2.3	1.6	D61	59.1	2.45	1.9	1.6	1.9
D161	62.5	1.24	1.7	2.8	2.1	D68	53.6	2.33	1.3	4.3	2.8
D18	54.3	2.78	1.2	4.8	3.1	D70	59.1	2.01	2.2	2.7	2.6
D19	59.9	1.71	2.2	1.2	0.4	D71	59.6	2.09	2.0	2.0	2.8
D20	74.1	1.91	2.5	1.2	1.4	D72	52.7	2.06	2.1	2.2	1.9
D21	75.9	1.18	2.5	2.0	2.8	D73	58.2	2.92	2.1	2.3	2.3
D22	59.6	2.52	1.8	1.5	2.4	D74	54.5	2.24	2.0	2.1	2.0
D23	59.5	1.66	2.0	3.8	2.4	D75	54.7	2.50	2.2	2.3	2.0
D24	85.8	1.25	1.2	1.2	1.6	D76	56.9	2.56	2.3	3.7	2.9
D25	64.0	2.14	1.2	2.3	2.1	D77	58.9	1.91	2.2	2.2	1.4
D26	54.4	1.45	2.0	1.7	1.8	D78	56.8	2.93	2.2	1.2	1.6
D27	54.3	2.83	1.2	2.5	2.1	D79	58.7	2.33	1.2	3.0	2.6
D28	58.7	1.56	2.1	2.3	2.3	D80	57.0	1.45	1.8	2.7	2.9
D29	47.2	1.92	1.7	1.7	2.1	D81	59.3	2.37	2.5	2.7	3.6
D30	45.7	1.92	2.3	2.8	2.4	D82	60.0	1.18	2.3	3.2	3.1
D31	54.6	1.70	1.7	1.2	1.6	D83	59.1	1.91	1.2	3.0	2.3
D32	50.9	1.83	1.3	1.2	1.4	D84	74.5	2.89	2.3	1.5	2.4
D33	55.7	1.06	1.3	2.3	1.6	D85	67.2	2.38	2.3	1.7	1.6
D34	60.0	1.69	2.2	2.5	2.3	D86	57.8	1.56	2.5	2.3	2.3
D35	74.3	1.33	2.0	2.2	2.9	D87	57.1	2.82	2.0	2.5	2.6

D36	59.0	10.8	2.7	2.2	2.4	D88	93.4	1.93	2.0	1.7	1.8
D38	59.3	1.55	1.3	3.2	3.4	D89	54.3	2.83	2.0	2.2	2.5
D39	69.2	1.88	1.2	3.8	1.4	D90	56.9	2.56	2.7	2.2	2.4
HQ6	59.1	1.83	2.1	1.9	2.6	HQ6	59.1	1.83	2.1	1.9	2.6
CV%	2.7	7.3				CV%	2.7	7.3			
$LSD_{0.05}$	3.9	0.25				$LSD_{0.05}$	3.9	0.25			

Table 2: *Fresh yield, eating quality and thickness pericarp of the 60 waxy inbred lines in spring season 2014 at CRDI, Gia Lam, Ha Noi.*

Indentification of the waxy inbred lines that contained QTLs control for pericarp thickness traits referance from Choe Eunsoo., (2010) with 5 primers flanked QTLs, which located at the chromosome 1, 2, 3 and 4. Detection of the QTLs in a set of waxy maize inbred lines include 60 inbred lines and check inbred line HQ6. Result was showed in table 3

Marker	Region	Allele No.	Lines lacked Allele
umc 2189	Deteced the thickness on the 3 regions are UA, LA and CWN	51	D2,D23,D26,D27,D35,D38,D39,D75, D77
ztc131		59	D87
umc 2118	Deteced the thickness on the 5 regions are UG, LG, UA, LA and CWN	59	D76
bmc 1325		58	D33, D34
bmc 1369		60	0
mmc 0143		47	D3,D4,D15,D16,D19,D20, D27, D28, D73, D75, D81, D83, D86
dupsr 28	Deteced the thickness on the UG	59	D88
umc 2038		60	0
umc 1550	Deteced the thickness on the 2 regions are UG and LG	50	D2, D25, D26, D27, D28, D32, D36, D78, D79, D84
umc 1757		51	D34, D40, D41, D68, D73, D80, D84, D87, D89

Table 3: *Details of polymorphisms and genetic analysis of five microsatellite markers across the 60 waxy maize inbred lines.*

Screening methods by marker SSR for identifying QTL control thickness pericarp traits, the marker *umc*2189 detected QTL relate to thickness pericarp traits in three regions are UA, LA and CWN was identified 51 bands with size ranged 100 to 200bp, such as 51 lines among the 60 lines contained QTL for thickness pericarp trait in three regions of the kernel pericarp. Marker ztc131 detected 59 bands with size from 100 to 200bp, correspond to 59 lines have contained QTL control thickness pericarp traits in three regions are UA, LA and CWN among 60 waxy inbred lines. Marker *umc*2118 identifed 59 bands with size ranged from 100 to 200bp. There were 59 inbred lines contained QTL control thickness pericarp traits of the five regions of the kernel pericarp (UG, LG, UA, LA and CWN). Marker bmc1325 identified 58 bands with size ranged 100 to 200bp, this marker identified 58 lines have contained QTLs control thickness pericarp in five regions of kernel pericarp. Another markers *bmc*1369 identified 59 lines contained QTL in fieve regions of kernel pericarp. Marker *mmc* 0143 identified 41 lines, Marker *dupsr*28 indentifed 59 lines (figure 2).

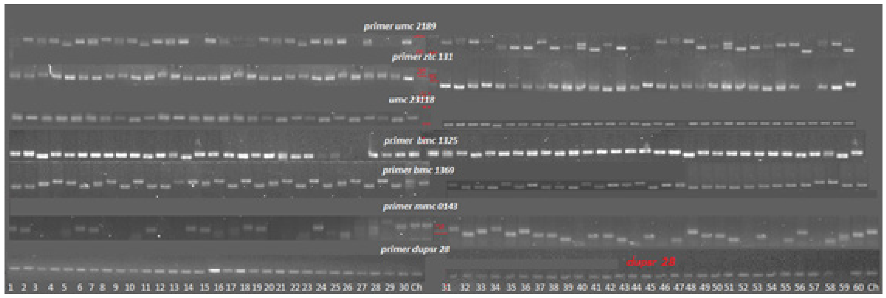

Figure 2: *PCR products of the markers are umc 2189, ztc131, umc2118, bmc1325, bmc1369, mmc 0143 and dupsr28 primer detected for thickness pericarp-related traits in 60 waxy inbred lines.*

Marker umc2038 identified 55 bands in 55 lines those contained QTL control thickness pericarp trait in two regions (UA, LA). Among 60 waxy inbred lines, marker *bmc* 1550 identifed 50 bands such as correspondence to 55 lines have contained QTL control thickness pericarp trait in region (LA). Among 60 waxy inbred lines, marker *umc*1757 identifed 51 lines have contained QTL control thichkness pericarp in region LA of kernel (Figure 3).

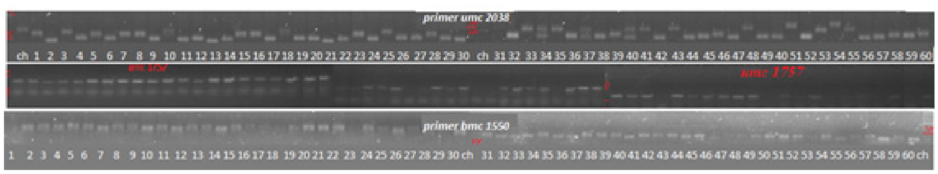

Figure 3: *PCR products of the markers are umc2038, bmc 1550, umc1757 and umc1757 primer detected for thickness pericarp-related trait in 60 waxy inbred lines.*

Correlation between phenotypic and molecular markers was ditermined in 24 out of 60 lines those have thinner pericarp trait. Combination of the thinner pericarp trait and yield was selected 21 lines within 24 lines those were considered promising lines, 9 lines origined from Viet Nam (D14, D161,D 21, D22, D42, D45, D60, D61 and D90), one line from Laos (D18) and 11 lines from China (D29, D30, D31, D52, D601, D70, D71, D72, D74, D82 and D85).

In addition, selected based on the other quality traits as taste, flovor, tenderness were picked out 7 lines put into combining ability evaluation were D29 (1,71); D61 (3,74); D71 (4,27); D601 (4,28); D161 (4,62); D86 (4,46); D74 (4,48). These lines have pericarp thickness trait values and selected best lines based on pericarp thickness phenotype and the selected best line based on pericarp thickness favorable QTL alleles on each line measured in spring season 2014.

Combining ability analysis of the waxy maize inbred lines and detected QTL related thickness pericarp in F1 progenies in autunm-winter season 2014

The seven waxy inbred lines were crossed in diallels designe by Griffing 4 method to produced 21 hybrids. The experiments were evaluated 21 hybrids in a complete randomized block design with three replications, where each plot consisted of two 10-m long rows. Rows were spaced 0.7 m and plants 0.25 apart, with a total area of $10m^2$. Recorded data on the fresh yield, average thickness pericarp and some quality charecteristics showed that the fresh yield of the hybrid ranged from 8.16 t/ha (THL4) to 11.58t/ha (THL8), there were 16 THLs have fresh yield equal check variety HN88. The thickness pericarp was thinly ranged from 45.1 µm (THL15) to 60.0 µm, equal check variety HN88 and all hybrids belong to thickness pericarp appropriated to fresh waxy maize market (Eunsoo Choe, 2010) and accepted consumers in Viet Nam. Another quality traits flavor, tenderness, taste in similar to check variety

Hybrid	Crosses	Average thickness pericarp (μm)	Fresh yield (t/ha)	Tenderness (scale)	Flovor (scale)	Taste (scale)
THL1	D29/D161	51.9	10.91	2.3	2.2	2.5
THL2	D29/D71	55.7	11.42	2.0	2.6	2.1
THL3	D29/D601	58.2	10.89	3.0	2.0	2.0
THL4	D29/D61	56.9	8.16	1.0	2.0	1.0
THL5	D29/D86	59.1	11.33	2.0	2.2	1.8
THL6	D29/D74	54.6	10.63	1.3	2.0	2.0
THL7	D161/D71	57.9	9.64	3.0	3.0	2.3
THL8	D161/D601	58.6	11.58	3.0	3.2	2.0
THL9	D161/D61	51.3	8.16	2.1	3.2	2.1
THL10	D161/D86	55.1	10.91	2.0	2.5	2.0
THL11	D161/D74	57.1	9.89	3.0	3.2	2.1
THL12	D71/D601	59.9	10.91	2.0	3.2	2.2
THL13	D71/D61	54.1	11.15	1.0	2.2	1.3
THL14	D71/D86	51.7	10.48	2.0	2.5	2.0
THL15	D71/D74	45.1	11.65	2.0	3.0	2.1
THL16	D601/D61	59.5	11.18	2.0	2.2	2.0
THL17	D601/D86	56.6	11.01	2.5	2.4	2.3
THL18	D601/D74	58.5	11.25	1.7	2.1	1.8
THL19	D61/D86	55.3	11.38	2.3	2.3	2.1
THL20	D61/D74	60.0	11.01	2.4	2.0	2.4
THL21	D86/D74	59.3	9.51	1.2	1.9	2.2
HN88 (đ/c)		56.3	10.82	1.5	1.9	2.1
CV%			5.4			
$LSD_{0.05}$			0.70			

Table 4: *Yield and eating qulity of the 21 hybrids in autunm-winter season 2014 at Gia Lam, Ha Noi.*

Diallel analysis of combining ability was carried out using Method 4 of Griffing (1956). Test of significant difference from zero for GCA and SCA effects was performed using t-test. Combining ability analysis of the purple maize waxy inbred lines is important in hybrid waxy maize (Shieh., *et al*. 2004). Estimation of combining ability to identify candidates for promising hybrid combinations to develop new hybrid variety (R.N. Mahto and D.K.Ganguli, 2003). Combining ability analysis of seven waxy maize inbred lines of variance and means for grain yield with three replications. Significant differences ($P < 0.05$) between genotypes were detected in the combined analysis with mean squares 207.3 and Ft is 656.75 as table 5a.

Source of variation	SS	df	MS	Ft
Whole	4146.94	41	101.14	
Genotype	4140.64	20	207.03	656.75
Rep.	1.49	1	0.75	0.25
Error	6.36	20	0.32	

Table 5a: *Combined analysis of variance and means for grain yield across replications of 21 hybrid waxy corn genotypes evaluated for autumn-winter seasons in 2014.*

Mean squares from GCA analysis between genotypes (hybrids) for fresh yield is 94.363 and Ft was 598.676 , and SCA was 107.439 and Ft was 681.637 such significant differences ($P < 0.05$) between genotypes were detected in the combined analysis as table 5b.

Source of variation	SS	df	MS	Ft
Whole	2073,47	41	50,572	
Hybrid	2070,32	20	103,576	328,374
GCA	566,18	6	94,363	598,676
SCA	1504,14	14	107,439	681,637
Error	3,152	20	0,158	

Table 5b: *Combined analysis of variance and means for grain yield across replications of 21 hybrid waxy corn genotypes evaluated for autumn-winter seasons in 2014.*

For maize yield, we found that GCA was relative more important than SCA for unselected inbred lines, whereas SCA was more important than GCA for previously selected lines (Luciano Lourenço Nass et al., 2000). This study was identified 7 inbred lines consisted of 6 lines have GCA value positive (D29, D161, D61, D86, D601 and D74), but only two lines (D601 and D74) have GCA value was not higher LSD.01 level and there were four lines have GCA value over LSD0.5 at significant level (D29, D161, D61 and D86). Among them D61 have GCA value highest was (+5.444) and one line have GCA at negative value (-3,156) not further utilization for hybrid breeding.

D29	D161	D71	D601	D61	D86	D74
4.644	5.444	-3.156	0.216	5.564	1.856	0.426

Table 6a: *General combining ability value for fresh yield.*

D29	D161	D71	D601	D61	D86	D74
19.388	23.613	9.932	7.608	30.934	3.417	0.154

Table 6b: *General combining ability variance.*

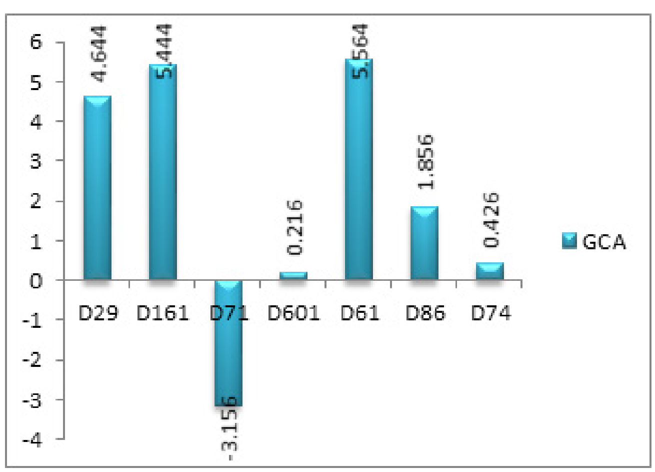

Figure 4: *General combining ability value of the seven waxy maize inbred lines in autumn-winter 2014 at Gia Lam, Ha Noi.*

The combining ability analysis of diallel data across replications showed highly significant effects (P < 0.01). Specific combining ability analysis showed that the largest positive and negative SCA effects were observed with line D29 x line D161 (9.47), next was D161 x D61 (8.70), D161 x D601 (8.21) and D61 x D86 (5.94). Crosses between two inbred lines from difference origin showed SCA value higher as lLine D29 have origin from China, D161 from Viet Nam, line 601 have origin China. Han., *et al*. (1991), Vasal., *et al*. (1992), and Gama., *et al*. (1995) reported that, on average, crosses produced by crossing interpopulation lines have more positive SCA effects than those produced by crossing intrapopulation lines which tend to have more negative SCA effects.

	D29	D161	D71	D601	D61	D86	D74
D29	-	9.47	5.17	-2,58	7.12	0.92	0.75
D161		-	-7.82	8.21	8.70	-7.82	-2.99
D71			-	-5.93	2.39	-5.92	0.11
D601				-	5.08	-4.33	-0.45
D61					-	5.94	3.23
D86						-	-13.64
D74							-

Table 7: *Spesific combining ability value of the seven waxy maize inbred lines in autumn-winter 2014 at Gia Lam, Ha Noi.*

The waxy maize inbred lines while GCA and having SCA are D29, D161 and D61, among them lines conformable female are D29 and D161, line D61 is conformable to male line in order to develop new waxy maize hybrid. Three elite lines were selected from this study can used further ion waxy maize breeding programme.

The utilization of molecular markers for identifying QTLs related to thinner pericarp

QTL for thinner pericarp traits of the 21 waxy maize hybrids detected by 4 specific primers according to Eunsoo Choe, (2010) and have high effect detected QTL control thinner pericarp in experiment evaluated 60 waxy maize inbred lines previous mention, marker pair umc2189-ZCT131 used for detection of QTL control thickness pericarp in UA (Upper Abgerminal) and LA (Lower Abgerminal) and CWN (Crown). Marker pair umc2118-bmc1325 used to detecte QTL control thickness pericarp in CWN, UA, LA, LG (Lower Germinal) and UG (Upper Germinal). Marker pair bmc1396-mmc0143 used to detecte QTL control thickness pericarp in UG, LG, UA, LA and CWN. Marker pair umc1550-phi096 used to detecte QTL control for LG.

Marker	No. band	No. polymorphirm band	Hybrid without band
*umc*2189	17	0	THL11,THL 13, THL18, THL19
*ztc*131	21	0	0
*umc*2118	20	0	THL 21
*bmc*1325	19	0	THL14, THL15
*bmc*1369	17	2	THL 7,THL11, THL13, THL17
*mmc*1043	20	8	THL 10
*mmc*1550	18	0	THL11,THL12, THL17
*phi*096	17	0	THL 10, THL11, THL12,THL14

Table 8: *Result PCR analysis with markers detected QTLs control thickness pericarp of the 21 hybrids.*

Marker pair *umc 2189-ztc131* used to detecte 17 per 21 hybrids contained QTL control thickness pericarp with size allele ranged from 100 to 200bp. Marker pair *umc 2118- bmc 1325* used to detecte 20 hybrids that contained QTL. Marker pair bmc 1369 - mmc 0143 used to detecte 17 hybrids contanined QTL and marker pair *mmc 1550 - dupssr28* used to detecte 18 hybrids contained QTL control the thickness pericarp. Basically, there were 10 hybrids among 21 hybrids contained QTLs control thinner pericarp in five regions of kernel pericarp were THL1 (D29/D161), THL2 (D29/D71), THL3 (D29/D601), THL4 (D29/D61), THL5 (D29/D86), THL6 (D29/D74), THL8 (D161/D601), THL9 (D161/D61), THL16 (D601/D61), THL20 (D61/D74). Another hybrids contained QTL control thinner pericarp only in two to three regions of kernel pericarps.

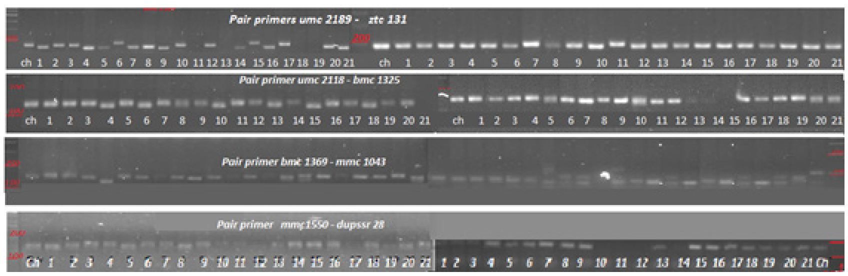

Figure 5: *PCR products of the markers detected for thickness pericarp-related traits in 21 waxy maize hybrids on the agarose gel-based SSR marker.*

In this study, the additive effect of QTL was negative for thickness pericarp therefore markers were choice to detect QTL control thickness pericarp were suitable and can utilized for MAS in hybrid waxy maize breeding with thinner pericarp trait. Marker assisted selection (MAS) may be useful for validating QTL effects and pyramiding favorable alleles in a fresh waxy corn breeding program. All five pericarp thickness traits were highly positively correlated, ranging from 0.78 to 0.93. Hybrids were varies significantly for different regions within a single kernel, and differs some among positions within a single ear. This indicated that some aspects of phenotypic breeding for pericarp thickness have difficulties due to experimental measuring errors. Therefore, early generation selection of QTL with additive effects for pericarp thickness traits may be promising for simultaneous indirect improvement of TP.

Conclusions

Evaluation of the growth, development, agronomical characteristics and fresh yield of 60 waxy maize inbred lines, we identified 35 inbred lines have thickness pericarps ranged from 35-60 μm and grain yield attained was higher 1.5 ton per hectare. Among them, 14 inbred lines origined from Viet Nam (D2, D14, D161, D21, D22, D33, D36, D42, D45, D60, D61, D68, D89, and D90), four inbred lines origined from Laos (D18, D26, D27, and D88), and 18 inbred lines origined from China (D15, D28, D29, D30, D31, D52, D601, D70, D71, D72, D73, D74, D75,D76, D77, D78, D80, and D86).

Using SSR markers showed that the correlation between phenotypic screening and molecular markers was ditermined in 24 out of 60 lines that have thinner pericarp traits. Combination of the thinner pericarp traits and yield was selected 21 lines within 24 lines considered promising lines, in there 9 lines origined from Viet Nam were D14, D161,D 21, D22, D42, D45, D60, D61 and D90, one line from Laos was D18 and 11 lines from China were D29, D30, D31, D52, D601, D70, D71, D72, D74, D82 and D85.

The waxy maize inbred lines have GCA and SCA are D29, D161 and D61, among them lines conformable female were D29 and D161, line D61 was conformable to male line in order to develop new waxy maize hybrid. Three elite lines were selected from this study can be used further ion waxy maize breeding programme. There were 10 hybrids among to 21 hybrids contained QTLs control thinner pericarp in five regions of kernel pericarp were THL1 (D29/D161), THL2 (D29/D71), THL3 (D29/D601), THL4 (D29/D61), THL5 (D29/D86), THL6 (D29/D74), THL8 (D161/D601), THL9 (D161/D61), THL16 (D601/D61), THL20 (D61/D74). Through evaluating lines and hybrids base on phenotype and SSR markers was evidence that the QTL information could be utilized through MAS to reduce pericarp thickness while maintaining favorable ear traits important to fresh waxy corn hybrid breeding.

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