

EVALUATION OF PURPLE WAXY CORN LINES FOR HYBRID VARIETY DEVELOPMENT

Pham Quang Tuan¹, Nguyen The Hung², Nguyen Viet Long²,
Nguyen Thi Nguyet Anh¹, Vu Van Liet^{2*}

¹*Crop research and Development Institute, Vietnam National University of Agriculture*

²*Faculty of Agronomy, Vietnam National University of Agriculture*

Email*: vvliet@vnua.edu.vn

Received date: 01.12.2015

Accepted date: 20.03.2016

ABSTRACT

This study was carried out to evaluate and select superior purple waxy corn lines derived from self-pollination (S_3 to S_6 generation) with high grain yield, marketable fresh cob yield, anthocyanin content, good eating quality and desirable agronomical characteristics. These lines were extracted from exotic and domestic germplasm. Phenotypic data collected included growth and developmental characteristic, yield and yield components, marketable fresh cob yield, total anthocyanin content, eating quality, pericarp thickness, sugar content, tenderness and taste. Eighteen purple waxy corn lines out of 45 lines were selected based on selection index computed from ideotype plant analysis with 12 traits. These lines had high anthocyanin content (22.4 to 260.10 $\mu\text{g/L}$), acceptable grain yield (2.0 to 3.5 t/ha) and marketable fresh cob yield (3.8 to 6.4 t/ha), good eating quality and suitable agronomical characteristics. These lines were recommended for further purple waxy corn inbred line and hybrid development.

Keywords: Anthocyanin, purple waxy corn.

Đánh giá các dòng ngô nếp tím phục vụ chọn tạo giống ngô nếp lai

TÓM TẮT

Nghiên cứu đánh giá và chọn lọc các dòng ngô nếp tím tự phối đời S_3 đến S_6 tốt nhất có năng suất hạt, năng suất bắp tươi thương phẩm, hàm lượng anthocyanin cao, chất lượng ăn uống tốt và đặc điểm nông sinh học phù hợp. Những dòng nghiên cứu phát triển từ nguồn gen trong nước và nhập nội. Số liệu kiểu hình thu thập trong thí nghiệm đồng ruộng gồm các đặc điểm sinh trưởng phát triển, năng suất, yếu tố cấu thành năng suất, năng suất bắp tươi thương phẩm. Phân tích hàm lượng anthocyanin bằng phương pháp pH vi sai, độ dày vỏ hạt đo bằng vi trắc kế, hàm lượng đường bằng máy đo độ brix, đánh giá chất lượng ăn uống độ mềm, độ đậm bằng thử nếm. Chọn lọc dòng ưu tú dựa trên chỉ số chọn lọc mô hình cây lý tưởng với 12 tính trạng. Kết quả đã chọn được 18 dòng ưu tú nhất cho nghiên cứu tiếp theo. Các dòng này có hàm lượng anthocyanin cao từ 22,4 đến 260,10 $\mu\text{g/L}$, năng suất hạt từ 2,0 đến 3,5 t/ha và năng suất bắp tươi thương phẩm từ 3,8 đến 6,4 t/ha, chất lượng ăn uống tốt và đặc điểm nông sinh học phù hợp để tiếp tục tự phối phát triển dòng thuần cho tạo giống ngô nếp tím ưu thế lai. Nghiên cứu cũng cung cấp thông tin đầu tiên về hàm lượng anthocyanin trong nguồn gen ngô nếp tím ở Việt Nam.

Từ khóa: Chọn lọc, hàm lượng anthocyanin, ngô nếp tím.

1. INTRODUCTION

Purple waxy corn (*Zea mays* L. *ceritina* Kulesh.) is edible with high anthocyanin content (Harakotr *et al.*, 2014). Pigments may possess valuable potentials in health product industries as they also contain the bioactive

compounds (Limsitthichaikoon *et al.*, 2014). There are number of special cultivars that contain colored pigments and give rise to numerous varieties of black and purple corn. The dark purple color of corn is caused by high content of anthocyanins located in the pericarp layers and cob. Anthocyanin pigment was found

in all parts of purple corn, but it was found at particularly high concentration in the husk and cob (Li *et al.*, 2008). Kernel pigments like anthocyanins and carotenoids have numerous nutritional functions in animals and human beings. Increasing the levels of these compositional traits and pigments in kernels should increase the nutritional quality of maize (Si Hwan Ryu, 2010). Purple waxy corn cob is one of the interesting agricultural wastes for health products because of aqueous extracts from those were shown to have potential valuable contents for health use (Limsitthichaikoon *et al.*, 2014).

High yield is still a primary goal of most plant breeding programs (Fehr, 1987). Pest resistance, stalk strength, uniformity, kernel quality and early maturity are also important in corn and waxy corn breeding programs. Purple waxy corn is the main source of low priced anthocyanins compared with other phytochemical plants (Abdel-Aal and Hucl, 1999). Therefore, the development of purple waxy corn products for lifestyle of the modern consumer for health benefit was expected to rapidly increase in the market share (Amnueysit *et al.*, 2010). Almost purple waxy corn varieties in Asian countries and Viet Nam are open-pollinated varieties (OPVs) and imported from other countries. Generally, grain yield of OPVs purple waxy corn is slightly lower than ordinary corn varieties because of small grains and ears. Therefore, improvement of existing open pollinated varieties (OPVs) and selection of domestic collected genetic resources and exotic germplasm are important step for purple waxy hybrid development in order to achieve higher yield and economic values. The objectives of the present study were evaluation and selection of superior purple waxy corn lines of potential for hybrid development.

2. MATERIALS AND METHODS

2.1. Materials

The materials consisted of 45 purple waxy corn lines (S3-S6) and one white waxy corn inbred line (S6) used as check because of purity

and low anthocyanin content. These purple waxy corn lines were developed at CRDI (Crop Research and Development Institute) of VNUA (Vietnam National University of Agriculture) by self-pollination to S₃ to S₆ three to six generation from OPV and hybrids imported from China, Korea and Thailand (Table 1).

2.2. Field experiment

The experiment was conducted in 2015 spring season at the CRDI, VNUA. Forty and six purple waxy corns lines (S₃ to S₆) were evaluated in a randomized complete block design with three replications. The plot size was a four-row plot with five meters in length and plant spacing of 0.70 × 0.25 m. Conventional tillage was practiced for soil preparation and total dose of fertilizers consisted of 120 kg ha⁻¹ nitrogen, 70 kg ha⁻¹ phosphorus and 91 kg ha⁻¹ potassium. Irrigation was supplied regularly to avoid drought stress, and insect pests, diseases and weed were appropriately managed to obtain optimum crop growth and yield.

2.3. Data collection

Data were recorded for whole ear yield, marketable-husked yield, ear diameter, ear length, plant height, ear height, days to tasseling and days to silking. Days to 50% tasseling and silking were recorded from total number of plants in each plot. After pollination, plant height and ear height were recorded from 10 continuously chosen plants in each plot without borders. Harvest time was determined at 20 days after pollination (R₄ growth stage) to determine eating quality and fresh yield. All ears from the two center rows or 40 plants were harvested and weighed. Ear diameter, ear length and marketable-husked yield were recorded based on National technical regulation on testing for Value of Cultivation and Use of Maize varieties (VCU QCVN01-56-2011/BNNPTNT).

2.4. Anthocyanin determination

Total anthocyanin content was measured by the pH method according to Wrolstad *et al.*

Table 1. Purple waxy corn inbred lines and their respective original parents

No.	Code	Pedigree	Inbreeding Generation	Country of origin	No.	Code	Pedigree	Inbreeding Generation	Country of origin
1	NT1	NNT.1	S6	Dienbien, VN	24	NT24	NT11.2.6	S4	Thailand
2	NT2	NNT.2	S3	Korea	25	NT25	NT11.4	S3	Thailand
3	NT3	NNT.2.1	S4	Korea	26	NT26	NT12.2.1	S4	Thailand
4	NT4	NNT.2.6	S4	Korea	27	NT27	NT12.2.2	S4	Thailand
5	NT5	NNT.3	S3	Korea	28	NT28	NT12.2.3	S4	Thailand
6	NT6	NNT.5.1	S4	China	29	NT29	NT12.2.5	S4	Thailand
7	NT7	NNT.5.2	S4	China	30	NT30	NNT13.1	S3	Korea
8	NT8	NNT.5.3	S4	China	31	NT31	NNT13.2	S3	Korea
9	NT9	NNT10.1	S4	Thailand	32	NT32	NNT13.3	S3	Korea
10	NT10	NNT10.2	S4	Thailand	33	NT33	NNT13.7	S3	Korea
11	NT11	NNT10.3	S4	Thailand	34	NT34	NNT4.1	S3	Korea
12	NT12	NNT10.4	S4	Thailand	35	NT35	NNT4.2	S3	Korea
13	NT13	NNT10.5	S4	Thailand	36	NT36	NNT4.3	S3	Korea
14	NT14	NNT10.6	S4	Thailand	37	NT37	NNT4.5	S3	Korea
15	NT15	NNT10.7	S4	Thailand	38	NT38	NNT6.1	S3	China
16	NT16	NNT10.8	S4	Thailand	39	NT39	NNT6.2	S3	China
17	NT17	NNT10.9	S4	Thailand	40	NT40	NNT6.2.4	S4	China
18	NT18	NNT11.1	S4	Thailand	41	NT41	NNT6.3	S3	China
19	NT19	NNT11.2	S4	Thailand	42	NT42	NNT6.6	S3	China
20	NT20	NNT11.2.1	S4	Thailand	43	NT43	NNT6.8	S3	China
21	NT21	NNT11.2.2	S4	Thailand	44	NT44	NNT7.5	S3	China
22	NT22	NNT11.2.4	S4	Thailand	45	NT45	NNT9.1	S3	China
23	NT23	NNT11.2.5	S4	Thailand	46	Check	F46	S6	White waxy corn

(2005). Anthocyanins reversibly change color with pH, which limits their effective use as food colorants for many applications, but also provides an easy and convenient method for measuring total pigment concentration (Giusti & Wrolstad, 2001). Ten gram grain samples of purple waxy corn after dried and kept 1 day in freeze Dryer was grinded by Multi-Beads Shoker machine. 0.5 ± 0.0001g powder are diluted with aqueous solutions of pH 1.0 and 4.5 buffer and absorbance measurements were taken at the wavelength of maximum absorbance of the pH 1.0 solution. The difference in absorbance between the two buffer solutions is due to the monomeric anthocyanin pigments. Calculation for determining total monomeric anthocyanin was as follows:

$$\frac{\text{Total anthocyanin (mg/g)}}{\epsilon \times l} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

$$A = (A_{\max} - A_{700\text{nm}})pH_{1.0} - (A_{\max} - A_{700\text{nm}})pH_{4.5}$$

MW = Molecular Weight

DF = Dilution Factor

ε = molar extinction coefficient, L x mol⁻¹ x cm⁻¹

l = pathlength (1 cm)

Pericarp thickness was measured by micrometer, sugar content by Brix meter, and tenderness and taste were evaluated by eating test expressed in score rankings from 1 (poor quality) to 9 (better quality). Selection of the elite lines was based on the distance from ideotype (de Carvalho *et al.*, 2002) calculated by the following formula:

$$IDI = \sqrt{\frac{1}{n} \sum_{j=1}^n (y_{ij} - V_{o_j})^2}$$

Where: IDI is the index based on the distance from ideotype, n is the number of traits included in the index, and j is the relative importance for the j_{th} trait. The ideotype was defined as the accession, not necessarily evaluated, presenting a mean phenotypic value for each trait equal to the respective V_{o_j} -the optimum phenotypic value standardized and weighted according to the square root of the relative importance of the j_{th} trait.; Y_{ij} is the ij_{th} transformed mean phenotypic value standardized and weighted according to the square root of the relative importance of the j_{th} trait.

2.5. Data analysis

Analysis of variance was performed for each character (Gomez and Gomez, 1984). Significant differences compared to the check line were assessed by least significant difference (LSD) at 0.05 probability level, and all analyses were carried out using IRRISTAT software ver. 5.0. Selection index was calculated using DTSL software (Nguyen Dinh Hien, 1995).

3. RESULTS AND DISCUSSIONS

The growth and development characteristics among 45 purple waxy lines (Table 2) revealed that date from sowing to harvest ranged from 95 to 104 days. Two lines having significant shorter growth duration than F46 (check), days to tasseling of the purple waxy lines ranged from 66 to 72 days and day to silking ranged from 67 to 72 days equivalent to check varieties and belong to early and medium maturity group. There was no significant difference found among genotypes domestic and exotic germplasm for these characteristics. Such growth duration suitable for fresh waxy production in Northern of Viet Nam, especially in winter season of the Red River Delta with the cropping pattern: rice-rice-winter crop. The purple waxy corn lines had shorter Anthesis-silking-interval (ASI) trait and therefore better, because ASI involving adaptation to abiotic

stress condition and yield lines. Most lines have ASI ranged from 0 to 3 days. T17, T39 and T42 are three lines have 4 days larger ASI than other lines (Table 2). The lines have ASI shorter could be effectively utilized for developing maize hybrid suitable for drought/rainfed conditions (Shadakshari and Shanthakumar, 2015) and also applying for purple waxy corn breeding.

The leaf number per plant ranged 16 to 17 leaves. There were 16 lines with number of leaves significantly higher than check (table 3). Plant and ear height is one of the most important selection criteria in most maize breeding program. Especially, ear and plant height is of importance in relation to logging; high ear position is likely to be more susceptible to logging (Ji *et al.*, 2006). Plant height of the studied lines ranged from 85.3 cm (NT16) to 160 cm (NT43). Among 45 lines studied 29 lines were shorter than the check at 5% significant level, and most lines ranged 110 to 130 cm. The ear height to plant height ratio was 50% and lower (appropriate for inbred line according to Ji *et al.*, 2010). There were 8 lines: NT4, NT19, NT30, NT31, NT32, NT35, NT40 and NT44 having ratio of plant height to ear height above 50% and higher than check F46 and they are unsuitable maize breeding for logging resistance. There were 7 lines, NT4, NT19, NT35, NT37, NT40 and NT44 having ear height higher than the check.

Analysis of variance indicated significant differences for number ear per plant (EP), number of kernel row per ear (RE), 1000-kernel weight (KW), grain yield (GY) and marketable husked yield (MHY) among the inbred lines studied. Results showed considerable diversity among the set of inbred lines in this study for studied traits (Table 4). Number of ear per plant ranged from 0.9 (NT27, NT33 and NT43 line) to 1.3 (NT2, NT23 and NT37 line). Ear length ranged from 10.7cm (NT9) to 16.3 cm (NT13). Two lines (NT12 and NT13) had EL longer than the check ($p \leq 5\%$). The ear diameter of 45 lines was of the medium size (3.53 cm to 4.82 cm). There were 4 lines (NT19, NT21,

NT38 and NT39) with ear diameter significantly larger than check F46 (4.2cm). There was large variation for number of kernel rows per ear, ranging from 10.0 to 19.2; similar result was found for the number of kernels per row (14.4 to 28.0 kernels/row) (Table 4). Most of the studied lines had 1000-grain weight lower than the check line F46 except for NT12 (317g) and NT31 (288g). The variance of ear traits was similarly reported before in the Arido-American maize accessions in Ohio University (Si Hwan Ryu, 2010). In general, grain yield of the 45 lines was low and ranged from 1.5 t/ha (NT14) to 3.7 t/ha (NT12). There were 11 lines: NT3,

NT4, NT12, NT17, NT19, NT31, NT38, NT39, NT40, NT42 and NT43 having significant grain yield higher than check F46 line. These lines are valuable resources for hybrid waxy corn breeding. Other lines had grain yield comparable to or lower than the check line (Table 4). Marketable husked yield (MHY) is an important target in fresh waxy corn breeding. This study identified 10 lines: NT3, NT4, NT12, NT17, NT19, NT31, NT38, NT40, NT42 and NT43, with fresh ear yield higher than F46 check line. NT3 had the highest marketable husked yield (6.4 tons/ha) followed by NT31 and NT42 (6.1 tons/ha).

Table 2. Growth duration and days to tasseling and silking of purple waxy corn lines in 2015 spring season at Gia Lam, Ha Noi

Line	Growth duration (day)	Days to tasseling	Days to silking	ASI ¹ (day)	Line	Growth duration (day)	Days to tasseling	Days to silking	ASI ¹ (day)
NT1	98	68	70	2	NT24	100	69	70	1
NT2	100	69	70	1	NT25	101*	70	70	0
NT3	95*	66	68	2	NT26	100	69	70	1
NT4	96	67	68	1	NT27	98	68	69	1
NT5	98	67	70	3	NT28	96	67	68	1
NT6	98	68	69	1	NT29	98	68	69	1
NT7	102*	70	71	1	NT30	98	67	70	3
NT8	100	69	70	1	NT31	97	67	69	2
NT9	99	68	70	2	NT32	97	67	69	2
NT10	98	67	70	3	NT33	98	67	70	3
NT11	100	70	69	-1	NT34	104*	72	71	-1
NT12	96	67	68	1	NT35	102*	70	71	1
NT13	100	68	71	3	NT36	103*	70	72	2
NT14	100	69	70	1	NT37	104*	72	71	-1
NT15	104*	72	71	-1	NT38	103*	71	71	0
NT16	103*	71	71	0	NT39	99	67	71	4
NT17	99	67	71	4	NT40	95*	67	67	0
NT18	100	70	69	-1	NT41	101*	70	70	0
NT19	103*	71	71	0	NT42	99	67	71	4
NT20	101*	70	70	0	NT43	102*	70	71	1
NT21	102*	70	71	1	NT44	98	68	69	1
NT22	102*	70	71	1	NT45	97	67	69	2
NT23	103*	70	72	2	F46	98	66	68	2
CV%	5.4	-	-	-		5.4	-	-	-
LSD _{0.05}	2.6	-	-	-		2.6	-	-	-

Note: 1: ASI = anthesis-silking interval; *Significant at the 0.05 probability level.

Quality traits and total anthocyanin content of studied lines are presented in the Table 5. Anthocyanin content measured by the pH method showed large variation, ranging from 1.3 mg/L (NT4) to 490.2 mg/L (NT25). Si Hwan Ryu (2010) also found large variation of total anthocyanin content between purple corn germplasm accessions between from 0.8 - 111.7 mg/100 g. There were 14 lines having anthocyanin content

higher than 100 mg/L: NT6 (260.1), NT8 (162.1), NT9 (103.4), NT16 (144.6), NT19 (119.4), NT20 (103.6), NT21 (211.1), NT25 (490.2), NT32 (167.9), NT34 (287.7), NT35 (118.3), NT36 (129.0), NT38 (110.6) and NT41 (205.6).

Other components related to fresh waxy corn quality are tenderness, pericarp thickness, taste and sugar content. Average pericarp thickness measured by Micrometer with 10

Table 3. Main agronomic characteristics of purple waxy corn lines in Spring season 2015 at Gia Lam, Ha Noi

Line	No. leaves	Plant height (cm)	Ear height (cm)	Line	No. leaves	Plant height (cm)	Ear height (cm)
NT1	16.6	123.5 [*]	57.6	NT24	16.7	109.4 [*]	46.3 [*]
NT2	17.2 [*]	144.0	68.0	NT25	16.7	131.3	58.7 [*]
NT3	16.9 [*]	141.9	62.2	NT26	16.6	112.2 [*]	51.8 [*]
NT4	16.9 [*]	137.2	77.2 [*]	NT27	15.6	105.5 [*]	42.6 [*]
NT5	16.7	117.6 [*]	49.0	NT28	16.7	150.9 [*]	66.0
NT6	16.6	136.5	60.9	NT29	16.2	113.6 [*]	48.6 [*]
NT7	16.6	118.3 [*]	47.8	NT30	16.9 [*]	108.9 [*]	59.7 [*]
NT8	16.7	118.8 [*]	42.6	NT31	17.0 [*]	108.0 [*]	61.2 [*]
NT9	16.4	91.6 [*]	37.0	NT32	16.6	98.8 [*]	52.2 [*]
NT10	16.6	104.5 [*]	41.2	NT33	16.8 [*]	104.7 [*]	43.6 [*]
NT11	16.7	91.0 [*]	41.2	NT34	16.9 [*]	134.0	59.4 [*]
NT12	16.6	140.2	65.6	NT35	16.9 [*]	143.4	74.5 [*]
NT13	16.5	127.8 [*]	53.0	NT36	17.0 [*]	143.3	54.4 [*]
NT14	16.6	104.7 [*]	43.4	NT37	17.0 [*]	148.4 [*]	71.2 [*]
NT15	16.8 [*]	111.7 [*]	55.0	NT38	16.5	126.0 [*]	59.8 [*]
NT16	16.8 [*]	85.3 [*]	40.1	NT39	16.8 [*]	126.3 [*]	63.2
NT17	16.6	87.3 [*]	40.4	NT40	17.0 [*]	151.8 [*]	77.8 [*]
NT18	16.6	113.9 [*]	56.5	NT41	16.5	132.7	52.1 [*]
NT19	16.4	142.3	73.4 [*]	NT42	17.1 [*]	139.4	67.4
NT20	16.7	115.9 [*]	50.6	NT43	16.7	160.0 [*]	58.4 [*]
NT21	16.6	101.4 [*]	49.3	NT44	16.8	129.6 [*]	70.7 [*]
NT22	16.6	112.5 [*]	49.6	NT45	16.8 [*]	108.1 [*]	42.2 [*]
NT23	16.3	104.3 [*]	44.5	F46	16.2	138.5	66.4
CV%	0.80	7.54	4.00	cv%	0.80	7.54	4.12
LSD _{0.05}	0.59	7.24	3.40	LSD _{0.05}	0.59	7.24	3.40

Note: *Significant at the 0.05 probability level.

Table 4. Yield and yield components of the purple waxy corn lines in Spring season 2015 at Gia Lam, Ha Noi

Line	EP	EL (cm)	ED (cm)	RE	KE	KW (g)	Grain Yield (t/ha)	MHY (t/ha)
NT1	1.0	12.4 [*]	4.09	14.8	18.0 [*]	159.0 [*]	1.8 [*]	2.9 [*]
NT2	1.3	12.2 [*]	3.58 [*]	10.6 [*]	22.2	213.0 [*]	2.1 [*]	4.1 [*]
NT3	1.2	13.7	4.46	15.5	25.5	209.0 [*]	3.5 [*]	6.4 [*]
NT4	1.0	13.8	4.56	14.3	21.5	247.0	3.3 [*]	5.8 [*]
NT5	1.2	12.3 [*]	4.17	12.8	19.8 [*]	219.0 [*]	2.4	4.3 [*]
NT6	1.0	11.9 [*]	3.70 [*]	11.2 [*]	18.8 [*]	226.0 [*]	2.0 [*]	3.8
NT7	1.0	14.1	4.00	12.7 [*]	24.0	195.0 [*]	2.5	4.8
NT8	1.0	12.0 [*]	4.06	14.8	23.6	171.0 [*]	2.6	4.6
NT9	1.0	10.7 [*]	4.22	16.0	23.0	164.0 [*]	2.6	4.6
NT10	1.0	12.0 [*]	4.09	13.0	24.0	205.0 [*]	2.7	5.2
NT11	1.0	11.8 [*]	4.20	14.7	21.3	197.0 [*]	2.6	4.8
NT12	1.0	15.6 [*]	4.27	13.3	27.0 [*]	317.0 [*]	3.7 [*]	5.9
NT13	1.0	16.3 [*]	4.50	14.8	25.6	174.0 [*]	2.8	5.4
NT14	1.0	12.3 [*]	3.53 [*]	12.0 [*]	19.6 [*]	149.0 [*]	1.5 [*]	3.2 [*]
NT15	1.0	11.2 [*]	4.06	12.7 [*]	20.2	187.0 [*]	2.0 [*]	3.9 [*]
NT16	1.1	11.6 [*]	4.21	14.0	22.7	177.0 [*]	2.4	4.3 [*]
NT17	1.1	14.0	4.26	13.2	25.4	217.0 [*]	3.1 [*]	5.5 [*]
NT18	1.0	10.0 [*]	3.93	13.6	17.0 [*]	168.0 [*]	1.7 [*]	3.4 [*]
NT19	1.0	14.8	4.62 [*]	14.7	27.5 [*]	194.0 [*]	3.1 [*]	5.6 [*]
NT20	1.1	11.8 [*]	4.53	14.7	18.5 [*]	199.0 [*]	2.3	4.1 [*]
NT21	1.0	12.1 [*]	4.91 [*]	16.0	21.0	171.0 [*]	2.5	4.6
NT22	1.0	11.9 [*]	4.52	16.7 [*]	16.0 [*]	206.0 [*]	2.3	4.3 [*]
NT23	1.3	13.2	3.88	10.0 [*]	28.0 [*]	188.0 [*]	2.3	4.0 [*]
NT24	1.0	13.5	4.11	12.0 [*]	19.6 [*]	232.0 [*]	2.3	4.2 [*]
NT25	1.0	11.2 [*]	4.05	15.3	16.0 [*]	176.0 [*]	1.8 [*]	3.5 [*]
NT26	1.0	12.5 [*]	4.39	12.8	20.4	243.0 [*]	2.7	4.8
NT27	0.9	12.3 [*]	4.13	13.5	21.9	176.0 [*]	2.2 [*]	4.3 [*]
NT28	1.1	13.7	4.34	13.6	20.4	216.0 [*]	2.6	4.7
NT29	1.2	11.9 [*]	4.36	15.0	19.3 [*]	219.0 [*]	2.7	4.8
NT30	1.3	12.1 [*]	4.31	15.6	20.8	142.0 [*]	2.0 [*]	3.8 [*]
NT31	1.0	10.9 [*]	4.48	16.7 [*]	18.7 [*]	288.0 [*]	3.2 [*]	6.1 [*]
NT32	1.0	11.2 [*]	4.44	19.2 [*]	23.4	179.0 [*]	2.7	5.2
NT33	0.9	11.0 [*]	4.39	16.8 [*]	14.4 [*]	187.0 [*]	1.9 [*]	3.7 [*]
NT34	1.1	11.6 [*]	4.08	14.4	20.0 [*]	133.0 [*]	1.6 [*]	3.5 [*]
NT35	1.0	11.8 [*]	4.12	13.2	24.2	193.0 [*]	2.6	4.8
NT36	1.0	13.3	3.98	12.4 [*]	22.4	161.0 [*]	1.9 [*]	3.6 [*]
NT37	1.3	13.6	4.58	16.0	25.4	152.0 [*]	2.6	4.8
NT38	1.1	13.4	4.82 [*]	15.6	24.0	194.0 [*]	3.1 [*]	5.5 [*]
NT39	1.0	11.0 [*]	4.62 [*]	17.6 [*]	20.4	191.0 [*]	2.9	5.3
NT40	1.0	13.2	4.45	15.3	21.0	227.0 [*]	3.1 [*]	5.6 [*]
NT41	1.1	11.5 [*]	4.02	13.6	18.2 [*]	152.0 [*]	1.6 [*]	3.6 [*]
NT42	1.0	11.4 [*]	4.19	14.8	26.0	195.0 [*]	3.2 [*]	6.1 [*]
NT43	0.9	14.0	4.54	14.0	23.8	251.0	3.0 [*]	5.5 [*]
NT44	1.0	11.5 [*]	4.09	14.2	23.2	171.0 [*]	2.4	4.3 [*]
NT45	1.0	12.7 [*]	4.05	13.2	19.4 [*]	208.0 [*]	2.3	4.1 [*]
F46	1.0	14.3	4.20	14.5	23.2	250.0	2.6	4.9
CV%	-	10.82	8.50	11.70	14.40	8.63	7.04	6.45
LSD _{0.05}	-	1.32	0.38	1.67	3.14	3.64	0.29	0.58

*Significant at the 0.05 probability level.

EP: number of ear per plant; EL: ear length; ED: ear diameter; RE: number of row per ear; KE: number of kernel per row; KW: weight of 1000 seeds, MHY: marketable husk yield

Table 5. Quality of the purple waxy lines in Spring season 2015 at Gia Lam, Ha Noi

Line	Anthocyanin content (mg/L)	Pericarp thickness (μm)	Tenderness (1- 9)	Sugar content (%Bx)	Taste (1- 9)
NT1	35.5 [*]	64.6	2	12.7	2
NT2	31.2 [*]	70.7 [*]	3	12.6	3
NT3	56.4 [*]	75.7 [*]	3	12.6	3
NT4	1.3 ^{ns}	77.3 [*]	2	12.2	3
NT5	64.5 [*]	97.0 [*]	2	10.7	2
NT6	260.1 [*]	67.5 [*]	3	14.3	3
NT7	23.1 [*]	69.4 [*]	2	14.7	2
NT8	162.1 [*]	78.6 [*]	3	12.7	3
NT9	103.4 [*]	76.8 [*]	4	12.7	4
NT10	15.5 [*]	68.8 [*]	3	12.5	3
NT11	57.7 [*]	69.9 [*]	3	11.2	3
NT12	43.2 [*]	72.0 [*]	3	10.8	3
NT13	52.1 [*]	56.3 [*]	3	13.3	3
NT14	26.6 [*]	78.0 [*]	3	11.2	3
NT15	59.2 [*]	70.8 [*]	3	11.8	3
NT16	144.6 [*]	122.4 [*]	3	13.0	3
NT17	24.2 [*]	96.4 [*]	2	11.9	2
NT18	27.9 [*]	85.0 [*]	3	14.0	3
NT19	119.4 [*]	101.9 [*]	2	10.9	3
NT20	103.6 [*]	68.7 [*]	3	11.2	3
NT21	211.1 [*]	79.6 [*]	3	13.7	3
NT22	57.8 [*]	59.2 [*]	3	13.2	3
NT23	66.8 [*]	58.6 [*]	3	12.5	4
NT24	29.2 [*]	70.8 [*]	4	12.8	3
NT25	490.2 [*]	68.6 [*]	3	12.9	2
NT26	72.0 [*]	70.8 [*]	3	13.1	2
NT27	34.0 [*]	74.2 [*]	3	12.6	3
NT28	49.9 [*]	77.3 [*]	3	13.2	3
NT29	75.5 [*]	78.1 [*]	3	13.4	3
NT30	10.4 [*]	77.6 [*]	2	12.8	3
NT31	48.4 [*]	66.5	3	13.0	3
NT32	167.9 [*]	60.7 [*]	2	12.8	3
NT33	20.1 [*]	88.1 [*]	3	13.1	3
NT34	287.7 [*]	86.8 [*]	3	12.3	3
NT35	118.3 [*]	78.2 [*]	2	12.0	3
NT36	129.0 [*]	60.2 [*]	2	13.0	3
NT37	73.3 [*]	66.2	3	13.2	3
NT38	110.6 [*]	74.7 [*]	2	12.2	2
NT39	33.3 [*]	61.5 [*]	2	8.8	3
NT40	33.4 [*]	66.1	3	11.7	3
NT41	205.6 [*]	76.1 [*]	3	11.7	3
NT42	22.4 [*]	55.2 [*]	4	13.6	3
NT43	75.0 [*]	63.7	2	12.5	2
NT44	39.1 [*]	99.2 [*]	3	13.8	3
NT45	39.6 [*]	60.9 [*]	2	13.3	3
F46	0.7	64.4	3	14.8	3
cv%	16.3	5.4	-	-	-
LSD _{0.05}	9.0	2.4	-	-	-

Note: *Significant at the 0.05 probability level, ns:non-significant; 1: bad (low), 9: good (high).

Table 6. Superior lines selected based on IDI (index based on the distance from ideotype) values on the 12 of phenotypic traits and 40% selection pressure

Line	IDI	An (mg/L)	PER (μm)	Ten (1-9)	Su (%Bx)	Tas (1-9)	EL (cm)	ED (cm)	RE	KR	KW (g)	GY (t/ha)	MHY (t/ha)
NT21	7.16	211.10	79.6	3	13.70	3	12.1	4.91	16.0	21.0	171	2.5	4.6
NT6	7.58	260.10	67.5	3	14.30	3	11.9	3.70	11.2	18.8	226	2.0	3.8
NT9	7.66	103.40	76.8	4	12.70	4	10.7	4.22	16.0	23.0	164	2.6	4.6
NT8	7.67	162.10	78.6	3	12.70	3	12.0	4.06	14.8	23.6	171	2.6	4.6
NT3	7.81	56.40	75.7	3	12.60	3	13.7	4.46	15.5	25.5	209	3.5	6.4
NT37	7.84	73.30	66.2	3	13.20	3	13.6	4.58	16.0	25.4	152	2.6	4.8
NT29	7.98	75.50	78.1	3	13.40	3	11.9	4.36	15.0	19.3	219	2.7	4.8
NT19	8.2	119.40	101.9	2	10.90	3	14.8	4.62	14.7	27.5	194	3.1	5.6
NT13	8.21	52.10	56.3	3	13.30	3	16.3	4.50	14.8	25.6	174	2.8	5.4
NT28	8.35	49.90	77.3	3	13.20	3	13.7	4.34	13.6	20.4	216	2.6	4.7
NT32	8.71	167.90	60.7	2	12.80	3	11.2	4.44	19.2	23.4	179	2.7	5.2
NT44	8.84	39.10	99.2	3	13.80	3	11.5	4.09	14.2	23.2	171	2.4	4.3
NT31	8.86	48.40	66.5	3	13.00	3	10.9	4.48	16.7	18.7	288	3.2	6.1
NT42	9.01	22.40	55.2	4	13.60	3	11.4	4.19	14.8	26.0	195	3.2	6.1
NT40	9.06	33.40	66.1	3	11.70	3	13.2	4.45	15.3	21.0	227	3.1	5.6
NT35	9.18	118.30	78.2	2	12.00	3	11.8	4.12	13.2	24.2	193	2.6	4.8
NT12	9.36	43.20	72.0	3	10.80	3	15.6	4.27	13.3	27.0	317	3.7	5.9
NT38	9.54	110.60	74.7	2	12.20	2	13.4	4.82	15.6	24.0	194	3.1	5.5

Note: IDI: index based on the distance from ideotype

grains of each line ranged from 55.2 to 122.4 μm . This study identified 8 lines having PER \leq 60 μm (NT13, NT22, NT23, NT32, NT36, NT39, NT42 and NT45) comparable to the check line (64.4 μm) and these lines are useful in waxy corn breeding. Sweetness measured by Brix meter ranged from 8.8 (NT39) to 14.7 (NT7) and all purple waxy corn lines had sugar content lower than check line (F46). Tenderness and taste measurement evaluated by eating test were rated with score range 2-4 comparable to the check line (Table 5).

The phenotyping data of 45 purple waxy corn lines were used to compute selection index and to identify the superior lines. Analysis was performed by considering 12 purple waxy corn traits and selection was done according to de Carvalho *et al.* (2002) and Luz *et al.* (2014). Our study used 40% selection pressure on the components of yield and yield and several quality traits as tenderness, taste, sugar, and

anthocyanin content. The IDI values from 7.16 to 9.54 were used to select 18 lines from total 45 of purple waxy corn lines in this study (Table 6). The selections showed high the anthocyanin content: 22.40 to 260.10 $\mu\text{g/L}$ with grain yield from 2.0 - 3.5 t/ha and marketable husk yield from 3.8 to 6.4 t/ha.

The 18 lines selected showed several traits closely to ideotype plant model. In addition, the selected lines had high anthocyanin content, thinner pericarp, more tenderness, and high grain yield and fresh ear yield. These lines are recommended for further self-pollination to develop inbred lines and hybrid purple waxy corn breeding program.

4. CONCLUSION

In conclusion, this study showed the evaluation and selection of purple waxy corn lines in generation from S_3 to S_6 on the

agronomical traits such as growth duration, ASI, plant height, ear height, yield and yield components to identify lines suitable for inbred line development is possible in Viet Nam. This study also provided information on the eating quality traits as pericarp thickness, tenderness, sugar content, taste and anthocyanin content to select elite line for development hybrid variety.

Among 45 purple waxy corn lines evaluated 18 lines were selected. The selections have high yield marketable husk yield and anthocyanin content and good eating quality in terms of thinner pericarp, more tenderness. These lines can be used for development of inbred lines through selfing and hybrid development.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Vietnam National University of Agriculture - Belgium Institutional University cooperation for the funding support of this study. We would like to sincerely thank Mr. Nguyen Quoc Trung, Faculty of Biotechnology and Ms. Nguyen Thi Huyen Chang for their help in plant phenotyping in this study.

REFERENCES

- Abdel-Aal E-SM, Hucl P. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry*, 76: 350-354.
- Amnueysit, P.; Tatakul, T.; Chalermnan, N.; Amnueysit, K. (2010). Effects of purple field corn anthocyanins on broiler heart weight. *Asian Journal of Food and Agro-Industry*, 3: 319-327
- Claudio Guilherme Portela de Carvalho, Cosme Damião Cruz; José Marcelo Soriano Viana and Derly José Henriques da Silva (2002). Selection based on distances from ideotype. *Crop Breeding and Applied Biotechnology*, 2: 171-178.
- Choe, E., and Rocheford, T. (2012). Genetic and QTL analysis of pericarp thickness and ear architecture traits of Korean waxy corn germplasm. *Euphytica*, 183: 243-260.
- Fehr, W.R. (1987). *Principle of Cultivars Development*, Volume 1. MacMillan, New York.
- Gomez Kwanchai A. and Gomez Arturo A. (1984). *Statistical Procedures for Agricultural Research*, Willy & Sons. Inc.
- Giusti, M. M., and Wrolstad, R. E. (2001). Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In "Current Protocols in Food Analytical Chemistry". John Wiley & Sons, Inc.
- Harakotr, B., Suriarn, B., Tangwongchai, R., Scott, M. P., and Lertrat, K. (2014). Anthocyanin, phenolics and antioxidant activity changes in purple waxy corn as affected by traditional cooking. *Food Chemistry*, 164: 510-517.
- Luz LN, Santos RC, Melo Filho PA, Gonçalves LSA (2014). Combined selection and multivariate analysis in early generations of intraspecific progenies of peanuts. *Chilean Journal of Agricultural Research*, 74: 16-22.
- Li, C.-Y., Kim, H.-W., Won, S. R., Min, H.-K., Park, K.-J., Park, J.-Y., Ahn, M.-S., and Rhee, H.-I. (2008). Corn Husk as a Potential Source of Anthocyanins. *Journal of Agricultural and Food Chemistry*, 56: 11413-11416.
- Ji HeeChung; Cho JinWoong; Yamakawa, T. (2006). Diallel analysis of plant and ear heights in tropical maize (*Zea mays* L.). *Journal of the Faculty of Agriculture, Kyushu University*, 51(2): 233-238.
- Ji HeeChung, Lee HeeBong and Takeo Yamakawa (2010). Major Agricultural Characteristics and Antioxidants Analysis of the New Developed Colored Waxy Corn Hybrids. *Journal of the Faculty of Agriculture, Kyushu University*, 55(1): 55-59.
- Sucharat Limsitthichaikoon, Bhattanitch, Khampaenjiraroach, Kedsarin Saodaeng, Thithima Rimdusit and Suthasinee Thapphasaraphong (2014). Quality evaluation of purple waxy corn cobs for health use, *The Official Journal of Asian Association of Schools of Pharmacy*, 3: 326-332.
- Shadakshari and G.Shanthakumar (2015). Evaluation of maize inbred lines for drought tolerance under contrasting soil moisture regimes. *Karnataka Journal of Agricultural Sciences*, 28(2): 142-146.
- Si Hwan Ryu, M.S. (2010). Genetic Study of Compositional and Physical Kernel Quality Traits in Diverse Maize (*Zea mays* L.) Germplasm Thesis for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University.
- VCU QCVN01-56-2011/BNNPTNT. National technical regulation on testing for Value of Cultivation and Use of Maize varieties.
- Wrolstad, R. E., Durst, R. W., and Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science & Technology*, 16: 423-428.