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IMPROVEMENT OF LARGE-SEEDED WAXY CORN (*ZEA MAYS* L. VAR. 'CERATINA KULESH') POPULATIONS FOR FOOD AND INDUSTRIAL PURPOSES

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SUMMARY

Improving large-seeded waxy corn for food and industrial purposes is one way to increase the value and utilization of new corn cultivars in Thailand. This study aimed to evaluate the agronomic traits, yield and yield components, and response to downy mildew (DM) disease caused by *Peronosclerospora sorghi* of the developed breeding lines of large-seeded waxy corn. A total of 33 waxy corn breeding lines with different seed sizes, row numbers, and kernel colors were selected from 2015 to 2018. The yield experiment was conducted at the Khon Kaen University, Khon Kaen, Thailand in the 2019/20 dry season and the 2020 rainy season. The breeding lines were also screened for DM resistance through artificial inoculation with spore suspension at farmers' fields in two locations, namely, Sok Muang and Nong Bua, in the 2020 rainy season. Genotype had a significant effect on yields, yield components, and agronomic traits. However, the interactions between genotype and season were not significant for 1000-kernel dry weight and the number of rows per ear. 'DRP-4', RLW-4', 'RLW-6', 'RLW-7', and 'RLW-10' showed the highest 1000-kernel dry weight and the optimal number of rows. Corn grown at Sok Muang Village had higher disease severity than that grown at Nong Bua Village due to the high rainfall, low temperature, and high humidity at the area. The resistant check cultivar 'Nei9008' exhibited moderate resistance to DM at both locations. Although the inbred lines were susceptible to DM disease, they appeared to be a good source of large seeds. Therefore, the backcross breeding method is still needed to obtain DM-resistant germplasm for the further development of waxy corn cultivars.

Keywords: Agronomic traits, breeding lines, large kernel, number of rows per ear, downy mildew

Key findings: Improvement for industrial utilization is one way to increase the economic value of waxy corn. Breeding lines were evaluated under field conditions, and some promising breeding lines with good yield and large kernels were identified for further use.

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INTRODUCTION

Waxy corn (*Zea mays* L. var. 'Ceratina Kulesh') is one of the popular maize cultivars cultivated in Thailand and adjacent regions. It possesses a unique sticky kernel texture because of its kernel starch properties, such as high amylopectin content (Ferguson *et al*., 2001). In Thailand, numerous waxy corn populations with different overall plant growth characteristics, ear sizes, kernel colors, textures, and eating qualities have been improved and cultivated for various purposes. For example, the waxy corn type with small seed size (SS) and good eating qualities were usually consumed in the form of steamed fresh ears, whereas the other type with large seed size (LS) and poor eating quality was used for the preparation of snacks and industrial materials. Additionally, an open-pollinated population and an F_1 hybrid with purple kernels and high anthocyanin content were used for the production of corn nuts and health food.

The seed size of waxy corn is a major factor that controls seed quantity (Kesavan *et al*., 2012). For example, the grain yield per hectare of large-seeded genotypes is 20%–25% higher than that of medium- and small-seeded genotypes. Kesavan *et al.* (2012) suggested that the improvement of seed size could indirectly contribute to grain yield (Kara, 2011). Furthermore, corn genotypes with LS could increase seed emergence percentage and seedling vigor (El-Abady, and Telekalo, 2015) or indirectly enhance maize yields due by maximizing emergence m^{-2}, plant height, and number of plants m^{-2} and reducing mortality rates (Khan *et al*., 2005). Thus, large-seeded waxy corn cultivars are used as raw materials for food and industrial purposes.

Seed size is quantitatively inherited and controlled by multiple genes. LS with seed depths of 4–7 mm is positively correlated with seed number per row and yield (Palamarchuk and Telekalo, 2018). However, in corn, the heritability of seed size is higher than that of grain yield (Austin and Lee, 1998). The divergent selection for seed size could be improve by mass selection method (Odhiambo and Compton, 1987). In addition, sources of pollen (from SS or LS) affect the seed size of the female plant (Odhiambo and Compton, 1987). The results of a previous study suggested that the controlled pollination of selected plants could increase selection efficiency (Odhiambo and Compton, 1987).

Among the selection methods for maize population improvement, ear-to-row and S1 selection methods are often used to improve yield and agricultural characteristics. Given that the S_1 selection method can be completed in one growing season, it is possibly more economic in terms of time and resources than other methods (Galarreta and Álvarez, 2007). A simple recurrent selection method has been utilized to improve the grain yield of populations. This method increased 1000-kernel dry weight to 349 g in purple waxy corn (Duangpapeng *et al.*, 2015) and to 368 g in field maize (Odhiambo and Compton, 1987). In addition, the response of the correlated trait to selection is important for the development of multiple traits. Seed size is positively correlated with ear length and grain yield (Gan *et al*., 2005; Lima *et al*., 2005; Hirsch *et al*., 2014; Sulewska *et al*., 2014). Although it merely promoted biomass and related traits in some studies, the improvement of kernel size with related traits is a challenge that must be met to fill gaps in maize breeding programs. Disease resistance is presumably included.

The downy mildew (DM) of maize caused by the oomycete *Peronosclerospora* is one of the most destructive diseases in maize production (Telle *et al*., 2011). It has spread widely over tropical regions, such as South- and Southeast Asia, with favorable conditions, e.g., at least 89% relative humidity and daily temperatures of 20 °C–24 °C (George *et al*., 2003). Most waxy corn populations have been reported as susceptible to at least six species of the genus *Peronosclerospora* (Lukman *et al*., 2013). *Peronosclerospora sorghi* has been recorded in maize fields throughout Thailand (Yao *et al*., 1991). Nowadays, several approaches have been implemented to mitigate DM. These approaches include chemical control via fungicide; metalaxyl seed treatment (Anahosur and Patil, 1980); cultural practices, such as crop rotation (Sadoma *et al*., 2011); and resistant cultivars (Ajala *et al*., 2003; Dermail *et al*., 2018). Thus, DM-resistant cultivars are required for use in areas with high incidences of disease outbreaks.

During 2015 to 2018, the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand, developed an open-pollinated population with white and large kernels by using LS corn genotypes, flour corn germplasm (Duangpapeng *et al*., 2015, 2020), a DM-resistant population from a LS waxy corn genotype, and three disease-resistant waxy corn genotypes. Therefore, this study aimed to evaluate 33 waxy corn breeding lines that were developed for large kernel size in yield trials and assess their resistance to DM via artificial inoculation under field conditions. The data obtained from this study will be useful for the further breeding of LS waxy corn for food and industrial uses.

MATERIALS AND METHODS

Plant material

Four groups of maize populations were used in this study (Table 1). The first group consisted of three lines. They originated from the cross between a purple waxy corn cultivar ('KND') and 'KND' and 'H53'/'Ki56'/'NS3'/'NSX' with resistance to three major diseases of corn, *viz*., DM, northern corn leaf blight (NCLB), and rust (R). These three populations had different kernel colors, such as white (DNRW) and yellow (DNRY). The second group consisted of 18 lines. They were derived from the cross between 'KND' and 'H53'/'Ki56'/'NS3'/'NSX' with resistance to DM and R. This group differed in terms of kernel colors, *viz*., white (DRW), yellow (DRY), and purple (DRP). The third group consisted of 11 lines. They came from the cross between DRP/BBQ and DRW/DRY/BBQ, with BBQ being an open-pollinated and large-kernel flour corn cultivar. These populations had disease resistance, large kernels, and different kernel colors, *viz*., white (RLW), yellow (RLY), and purple (RLP). The last group consisted of only one line from the cross 'KND'/'BBQ'/'Cuzco' (F_2) and purple sweet waxy corn ('KGW'; F_1). 'Cuzco' is a large-kernel maize cultivar, and 'KGW' is a purple sweet-waxy corn cultivar, namely 'Kam Whan.' These lines have large purple kernels (LP).

Table 1. Four corn cultivars used in this study.

Cultivar groups	Pedigree	No of lines	Kernel color	1000-kernel dry weight (g)
DNR	H53/Ki56/NS3/NSX × KND	3	W/Y	200– 240 (M)
DR	KNDxH53/Ki56/NS3/NSX	18	W/P/Y	195–280 (M)
RL	DRP/DRW/DRY × BBQ	11	W/P/Y	200–350 (L)
L	KND/BBQ/Cuzco × KGW	1	P	200–300 (L)

DNR = Resistance to downy mildew, northern corn leaf blight, and rust; DR = Resistance to Downy mildew and rust; RL = Disease-resistance and large kernel; L = Large kernel; W = White kernel; Y = Yellow kernel; P = Purple kernel; M = Medium kernel.

Generation of base populations and line development

The base populations that were developed in several projects, including large kernel waxy corn that is suitable for food and industrial purposes (Duangpapeng *et al*., 2015), disease-resistant LS waxy corn (Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand), and disease-resistant waxy corn genotypes (Chalorcharoenying *et al*., 2016), were used in this study.

Four seasons of selection were carried out during the 2017/2018 dry season to 2019 rainy season at the Khon Kaen University, Khon Kaen, Thailand (Figure 1). At the initial step, base populations (F_2) were planted. Then, the plants in each population were selfed. After that the plants in each population were randomly mated in the second season without selection. In the next two seasons, the seeds were planted, and selected plants were self-pollinated. The major selection criteria were based on kernel size; row number; ear size; and agronomic traits, such as plant height, ear height, and disease-free appearance. Potassium iodide staining was also carried out to select for waxy phenotypes.

In the final step, S_3 breeding lines were generated. During the dry season of 2019/2020, S_3 seeds were duplicated for evaluation in two experiments. The first experiment involved evaluation of maize populations for yield, yield components, and agronomic traits at the Khon Kaen University, Khon Kaen, Thailand. The second experiment involved the assessment of DM resistance under field conditions at local farmers' fields. The detailed schematic for population generation and line development are presented in Figure 1.

Field experiment for yield trials and crop management

Experimental design and crop management

The 33 S_3 lines derived from the four cultivar groups of waxy corn populations; one F_1 hybrid

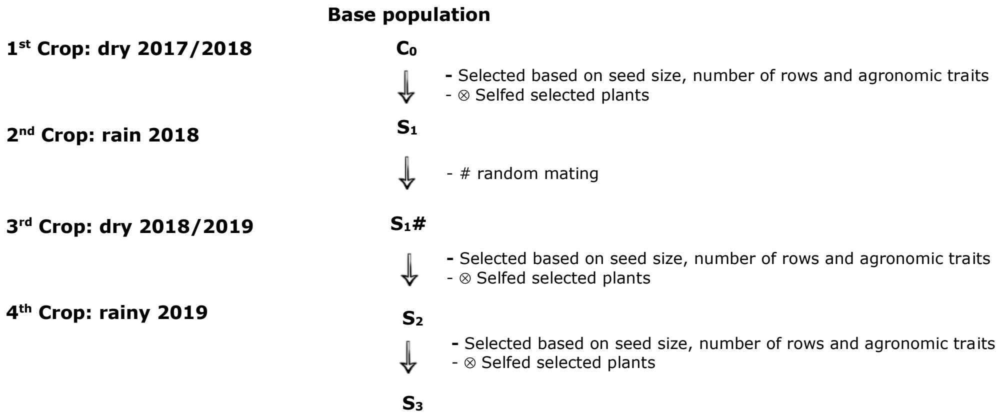


Figure 1. Schematic of the improvement of waxy corn populations in terms of seed size, row number, and agronomic traits from the 2017/2018 dry season to the 2019 rainy season.

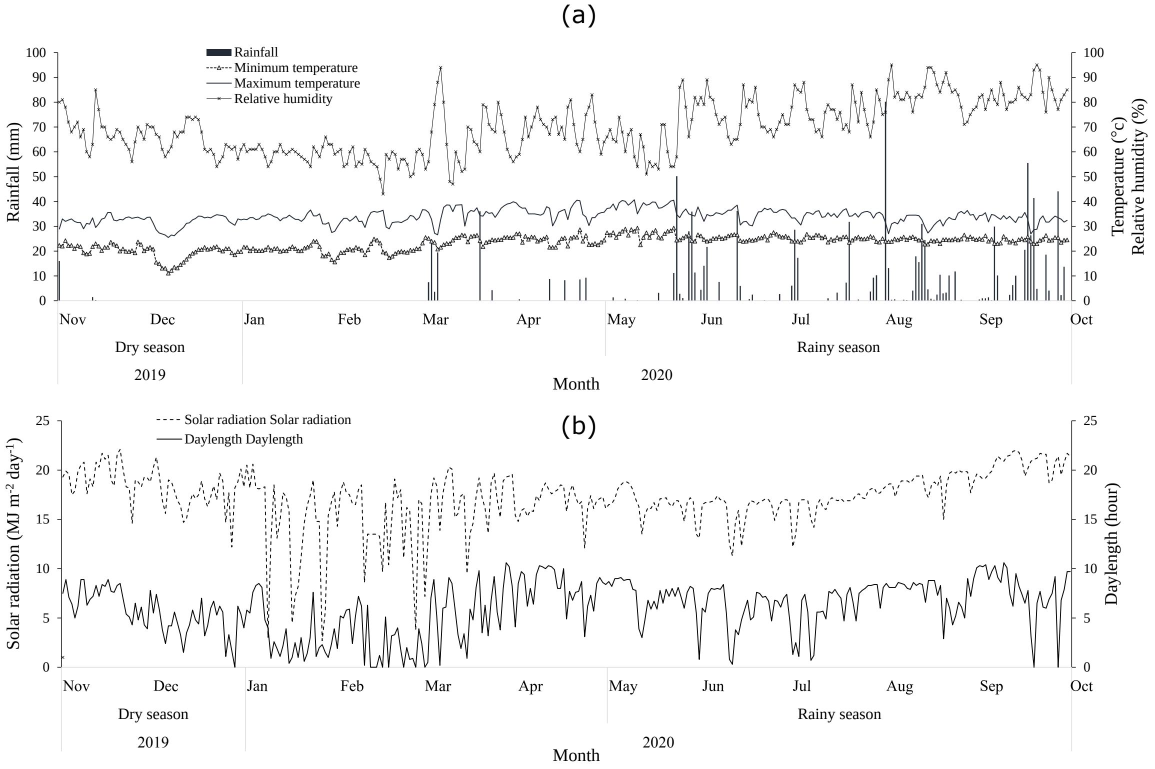


Figure 2. Daily rainfall, relative humidity, maximum and minimum temperatures (a), solar radiation, and day length (b) during yield evaluation and disease assessment at Khon Kaen Province, Thailand, 2019–2020.

of field corn (‘Takfa1’); one F_1 hybrid of purple sweet waxy corn (‘KGW’), namely ‘Kam Whan’; and one inbred line of field corn (‘Nei9008’) were evaluated in a randomized complete block design (RCBD) with two replications. The field experiment was conducted in two seasons, i.e., the 2019/2020 dry season and the 2020 rainy season at the Khon Kaen University, Khon Kaen, Thailand (16°28'27.7" N, 102°48'36.5" E; 190 m above sea level). Each plot consisted of two rows 5 m long with a spacing of 0.8 m between rows and 0.25 m between plants within a row.

The soil had a sandy texture; pH of 6.53; low organic matter content (<1%); and relatively low available soil macronutrients, *viz*., total nitrogen (0.03%), available phosphorus (48.7 ppm), and exchangeable potassium (42.3 ppm). The weather information during evaluation and corn growing during the 2019/2020 dry season and 2020 rainy season are shown in Figure 2. The accumulated rainfall, average minimum air temperature, average maximum air temperature, and average relative humidity during the dry season were lower than those during the rainy season.

The soil was amended with chicken manure applied three times at the rate of 3.2 tons ha^{-1} before tillage. Two seeds were sown in each hill. Then, thinning was performed 2 weeks after sowing to obtain one plant per hill. NPK fertilizer (15–15–15) was incorporated into the soil at the rate of 125 kg ha^{-1} during planting. Nitrogen fertilizer in the form of 46% urea was applied at the rate of 320 kg ha^{-1} at 14 and 30 days after planting (DAP). In addition, NPK (13–13–21) chemical fertilizer was applied at the rate of 160 kg ha^{-1} at 50 DAP. The crop was managed in accordance with the recommendations for the commercial production of corn in Thailand.

Data collection

Data were recorded for days to anthesis, plant height (cm), ear height (cm), husk yield, cob yield, and grain yield. Days to anthesis were observed as the number of days between planting and 50% of pollen shed. Plant height was measured from the ground level to the base of the tassel, and ear height was recorded from the ground level to the ear-bearing node of the uppermost ear. The plant and ear heights of 10 plants in each plot were randomly collected after the reproductive stage. Yield components were derived from dry husk mass and cob mass per plot and converted into kg per hectare. The crop was harvested at the dry stage, unhusked, and hot-air dried until the seed moisture content reached 14%. Then, the ears were sampled for the determination of kernel number and 1000-kernel weight.

Pathogen inoculation, field experiment, and disease assessment

Field experimental design and disease inoculation

The 33 breeding lines and three check cultivars were evaluated for DM resistance by using the spreader row technique at two locations, including Nong Bua Village (16°27'45.2"N 102°37'10.7"E) and Sok Muang Village (16°28'14.5"N 102°34'28.5"E) in Ban Phang District, Khon Kaen Province, Thailand during the 2020 rainy season.

The susceptible genotypes, which included ‘Tuxpeno’ (Phumichai *et al*., 2008) and ‘Niew Muang Tam’ were planted as spreader rows to increase disease inocula (George *et al*., 2003). Two rows were planted at the spacing of 70 cm × 25 cm and at the seeding rate of two seeds per hill. The rows surrounded the experimental sites and were inserted between every 10 rows of the tested genotypes. The spreader rows were artificially inoculated 7 days after germination. The spore suspension of DM was sprayed by hand on the spreader rows eight times at 3-day intervals at Nong Bua Village because of low DM disease severity (DS). The natural inoculation of spreader rows at Sok Muang Village was carried out starting at seed germination due to high DM DS. The tested genotypes were planted after DM symptoms were observed in the spreader rows.

Disease scoring and data collection

DM was evaluated at 14, 21, 28, 35, and 42 days after inoculation by using five levels of visual rating scores: 0 = no symptoms, 2 = 20% of leaves showing mosaic symptoms and just starting of mosaic symptoms, 4 = 50% of leaves on the plant showing mosaic symptoms, 6 = 60% of leaves showing mosaic symptoms and sporulation just starting, 8 = 80% of leaves showing mosaic symptoms, and 10 = 100% of all leaves showing spore and mosaic symptoms on the plant. DS was calculated from disease rating scores in reference to Raupach *et al*. (1996):

$$\text{DS}\ (\%) = \Sigma\left[\frac{\text{rating score} \times \text{number of plants in rating}}{\text{total number of sampled plants} \times \text{highest rating}}\right] \times 100$$

The reactions of the tested genotypes to DM were classified into five levels, including 0% infection (no symptoms) = highly resistant, 1%–10% infection = resistant, 11%–25% infection = moderately resistant, 26%–50% infection = moderately susceptible, 51%–75% infection = susceptible, and 76%–100% infection = highly susceptible (Craig, 1982; Nagabhushan *et al*., 2014). Disease incidence (DI) was calculated as described by Anfoka (2000):

$$\text{DI}\ (\%) = \left(\frac{\text{number of infected plant}}{\text{total number of plants}}\right) \times 100$$

The area under the disease progress curve (AUDPC) was calculated for DI (AUDPC-DI) and DS (AUDPC-DS) by using the following equation (Ojiambo *et al.*, 1998):

$$\text{AUDPC} = \sum_{i-1}^{n-1} \left[\frac{y_i + y_{i+1}}{2}\right] \times (t_{i+1} - t_i)$$

where Y_i = the DM intensity of the *i*th note, Y_{i+1}= DM intensity of the *i+1*th note, $(t_{i+1}-t_i)$ = the number of days between the *i*th note and the *i+1*th note.

Statistical analysis

Data for individual seasons were statistically analyzed via analysis of variance (ANOVA) in an RCBD, and error variances were tested for homogeneity (Gomez and Gomez 1984). Error variances were homogeneous for all of the characters under study. Therefore, the data from the two seasons were combined in the combined ANOVA. The least significant difference (LSD) was used to compare mean differences at the 0.05 probability level. All calculations were done by using the computer software STATISTIX10 software program (version 10.0, Analytical Software, Tallahassee, FL, USA).

RESULTS

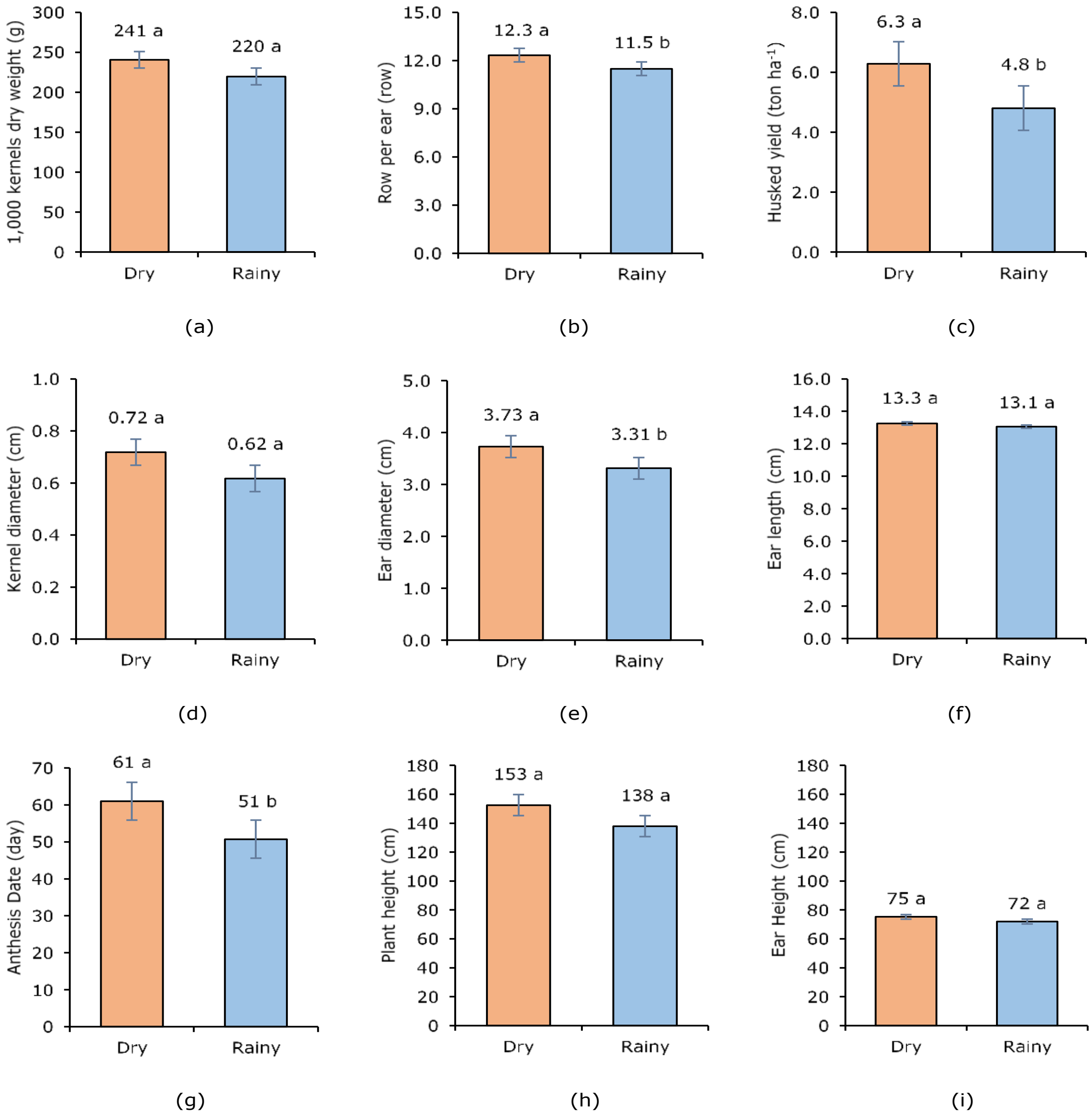
Yield and agronomic traits

The combined ANOVA for yields, yield components, and agronomic traits of 36 waxy corn cultivars in the 2019/20 dry season and the 2020 rainy season are shown in Table 2. The effects of season were not significant ($P \leq 0.05$ and 0.01) on most traits except for husked yield, ear diameter, rows per ear, and anthesis date. The effects of genotypes were significant ($P \leq 0.05$ and 0.01) on all traits, indicating that genetic variation existed among these breeding lines. The interactions between genotypes and season were significant ($P \leq 0.05$ and 0.01) for most traits, except for 1000-kernel dry weight, kernel diameter, number of rows per ear, and ear height. Notably, genotype × season interactions were not significant for the two major traits 1000-kernel dry weight and number of rows per ear investigated in this study, suggesting that selection for these two traits may be conducted only in selected representative seasons or locations.

Table 2. Mean squares for 1000-kernel dry weight, husked yield, ear diameter, ear length, kernel diameter, days to anthesis, plant height, and ear height of 36 waxy corn cultivars in the 2019/20 dry season and the 2020 rainy season.

SOV	Season (S)	Genotype (G)	G × S	Pooled error	CV (%)
Df	1	35	35	70	
1000-kernel dry weight	14625^{ns}	6014**	1682^{ns}	1711	18.0
Husked yield	78*	9**	6**	2	25.5
Ear diameter	6.25*	0.37**	0.25*	0.14	10.7
Ear length	1.5^{ns}	11.5**	7.1*	3.9	15.0
Kernel diameter	0.32^{ns}	0.03^{*}	0.02^{ns}	0.02	21.7
Row per ear	25.6*	6.0**	1.3^{ns}	1.3	9.5
Kernel per ear	411^{ns}	102**	61*	32	25.3
Anthesis date	3749**	44**	23**	7	4.6
Plant height	7582^{ns}	725*	679*	409	13.9
Ear height	377^{ns}	256*	142^{ns}	141	16.2

ns, * and ** = not significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 3. Seasonal effect on the grand means of 33 breeding lines and three check cultivars for (a) 1000-kernel dry weight, (b) husked yield, (c) row per ear, (d) kernel diameter, (e) ear diameter, (f) ear length, (g) anthesis date, (h) plant height, and (i) ear height evaluated in the 2019/2020 dry season and 2020 rainy season at the Khon Kaen University, Khon Kaen, Thailnad. Means followed by different letters across bars are significantly different based on LSD at *P ≤ 0.05*.

Overall, no significant difference in the 1000-kernel dry weight, kernel diameter, ear length, plant height, and ear height of the tested breeding lines between the dry and the rainy seasons were observed (Figure 3). However, the row per ear, husked yield, and ear diameter of the breeding lines grown in the dry season were significantly higher than those of the breeding lines grown during the rainy season (12.3 vs. 11.5, 6.3 ton ha^{-1} vs. 4.8 ton ha^{-1}, and 3.73 cm vs. 3.31 cm, respectively) (Figure 3b,c,e). Moreover, the anthesis periods of the majority of the breeding lines planted during the dry season were longer than those of the breeding lines planted during the rainy season (61 days vs. 51 days)(Figure 3g). The average temperature during the growing season in the dry season was lower than in the rainy season (data not shown).

Table 3. Means for the 1000-kernel dry weight (g), row number per ear, husked yield (ton ha^{-1}), kernel diameter (cm), ear diameter (cm), ear length (cm), days to anthesis (days), plant height (cm), and ear height (cm) of 33 S_3 breeding lines of waxy corn and three check cultivars in the dry season 2019/20 and the rainy season 2020.

Genotypes	1,000 weights	Row per ear	Husked yield	Kernel diameter	Ear diameter	Ear length	Anthesis date	Plant height	Ear height
Medium kernel									
DNRW-1	200 f–h	13.1 a–e	3.5 jk	0.47 g	2.93 k	11.5 g–l	59 bc	157 a–g	80 a–e
DNRY-1	230 d–f	12.9 a–f	3.8 i–k	0.57 d–g	3.03 j–k	13.8 b–h	63 a	123 hi	56 h
DNRY-2	215 d–g	11.4 f–l	3.8 i–k	0.68 b–f	3.15 i–k	12.6 d–k	57 b–f	120 i	59 gh
DRW-11	205 e–h	11.8 e–i	5.6 c–i	0.66 b–g	3.53 b–j	13.6 b–i	53 h–j	156 a–g	79 a–e
DRW-12	200 f–h	12.1 d–h	4.6 g–k	0.59 d–g	3.38 d–k	12.3 e–k	56 b–h	158 a–f	81 a–d
DRW-15	210 e–h	13.7 a–c	5.4 c–j	0.66 b–g	3.69 b–h	14.2 a–g	58 b–d	143 b–i	77 a–f
DRW-17	220 d–f	11.8 e–j	4.2 h–k	0.59 d–g	3.43 c–k	12.1 e–k	59 bc	137 e–i	65 d–h
DRW-19	230 d–f	13.0 a–f	5.4 c–j	0.67 b–g	3.78 b–e	11.4 h–l	59 b–d	132 f–i	67 c–h
DRW-20	215 d–g	13.8 ab	7.3 bc	0.66 b–g	3.89 a–d	14.5 a–f	56 b–i	150 a–h	72 b–h
DRW-22	195 f–h	12.4 b–g	3.8 i–k	0.56 e–g	3.15 i–k	11.4 h–l	58 b–f	124 hi	64 e–h
DRW-24	200 f–h	12.6 a–g	5.1 d–k	0.67 b–g	3.40 c–k	13.8 b–h	59 bc	127 hi	60 gh
DRW-25	230 d–f	11.5 e–k	5.0 d–k	0.75 a–e	3.45 c–k	13.7 b–i	59 b	146 b–i	80 a–e
DRW-27	225 d–f	11.5 e–j	5.7 c–i	0.65 b–g	3.68 b–i	13.1 c–k	54 e–j	146 b–i	73 a–g
DRW-28	260 b–e	13.4 a–d	5.9 b–h	0.65 b–g	3.74 b–f	12.6 d–k	56 b–h	140 b–i	76 a–g
DRP-3	260 b–e	12.6 a–g	5.9 b–h	0.57 d–g	3.55 b–j	14.0 a–h	53 h–j	146 b–i	77 a–f
DRP-4	270 b–d	12.1 d–h	7.3 bc	0.74 a–e	4.03 ab	14.3 a–f	56 b–i	169 a–b	83 a–c
DRP-5	210 e–h	9.9 l	3.8 i–k	0.85 ab	3.28 e–k	10.8 j–l	57 b–g	139 c–i	79 a–e
DRP-7	250 b–f	11.9 d–h	4.7 f–k	0.62 c–g	3.28 e–k	12.2 e–k	59 b	148 a–i	78 a–f
DRP-12	230 d–f	12.5 b–g	3.1 k	0.52 fg	3.18 h–k	9.1 l	59 bc	140 c–i	70 c–h
DRY-1	270 b–d	10.2 i–l	3.8 i–k	0.60 d–g	3.15 i–k	10.6 kl	56 c–i	132 f–i	73 a–g
DRY-3	230 d–f	13.9 ab	4.2 h–k	0.67 b–f	3.55 b–j	10.5 kl	58 b–d	128 g–i	65 d–h
Large kernel									
RLW-3	215 d–g	9.9 kl	6.3 b–g	0.94 a	3.69 b–h	13.3 b–j	53 g–j	176 a	87 ab
RLW-4	300 ab	10.2 j–l	5.9 b–h	0.81 a–c	3.63 b–i	12.8 c–k	53 h–j	149 a–i	71 b–h
RLW-6	270 b–d	10.7 h–l	6.6 b–f	0.75 a–e	3.73 b–g	14.0 a–h	56 b–i	165a–e	89 a
RLW-7	295 a–c	11.5 e–j	7.3 bc	0.77 a–e	3.93 a–c	15.3 a–d	54 f–j	150 a–h	75 a–g
RLW-8	270 b–d	10.7 h–l	6.5 b–g	0.58 d–g	3.53 b–j	15.9 ab	58 bc	150 a–h	79 a–e
RLW-10	335 a	10.8 h–l	6.9 b–e	0.77 a–e	3.80 b–e	11.8 f–l	57 b–g	157 a–f	78 a–e
RLP-2	240 c–f	12.1 c–h	7.8 b	0.73 a–e	3.55 b–j	14.3 a–g	58 bc	137 e–i	72 b–h
RLP-3	245 b–f	11.5 e–j	6.5 b–g	0.64 c–g	3.50 b–j	14.6 a–e	58 b–e	138 d–i	70 b–h
RLP-4	250 b–f	12.7 a–g	7.0 b–d	0.71 b–f	3.88 a–d	13.6 b–i	54 e–j	166 a–d	78 a–e
RLY-1	215 d–g	11.5 e–j	7.3 bc	0.73 a–e	3.75 b–f	15.1 a–d	55 d–i	167 a–c	87 ab
RLY-3	215 d–g	9.9 l	4.3 h–k	0.66 b–g	3.40 c–k	10.9 i–l	53 ij	135 e–i	61 f–h
LP-2	210 e–h	10.6 h–l	5.0 e–k	0.60 d–g	3.43 c–k	13.6 b–i	56 c–i	146 b–i	78 a–f
KGW	160 g–h	13.7 ab	10.5 a	0.67 b–g	4.35 a	16.7 a	44 k	143 b–i	69 c–h
Takfa1	155 h	11.3 g–l	5.2 d–j	0.65 b–g	3.20 g–k	15.4 a–c	51 j	142 b–i	71 b–h
Nei9008	155 h	14.1 a	5.1 d–j	0.70 b–f	3.23 f–k	14.3 a–f	51 j	148 a–i	72 a–h
F-value	**	**	**	*	**	**	**	*	ns
C.V. (%)	20.1	7.8	28.1	30.0	11.7	10.3	10.6	23.0	20.5

* Genotypes were grouped on the basis of seed size.
Means followed by different letters within the same column are significantly different based on LSD at $P \leq 0.05$.
P represents purple waxy corn, W represents white waxy corn, Y represents yellow waxy corn, and L represents large kernel.

The yields and agronomic traits of the 33 breeding lines and three check cultivars in this study are presented in Table 3. The 1000-kernel dry weights ranged between 195 and 335 g. Among the tested genotypes, five lines, namely, 'DRP-4', 'RLW-4', 'RLW-6', 'RLW-7', and 'RLW-10' had the highest 1000-kernel dry weights of 270, 300, 270, 295 and 335 g, respectively. The number of rows per ear ranged between 9.9 and 14.1. The row numbers of most tested genotypes were similar to those of the check cultivars and ranged from 11.3 to 14.1. However, some genotypes had lower row numbers than others.

Husked yields ranged between 2.7 and 10.5 tons ha^{-1} (Table 3). Among the tested lines, 'RLW-7', 'RLW-10', and 'RLW-4' had the highest husked yields of 7.3, 7.3, and 6.9 tons ha^{-1}, respectively. However, the yields of these lines were lower than those of 'KGW' (10.5 tons ha^{-}) but higher than those of 'Takfa1' (5.2 tons ha^{-1}) and 'Nei9008' (5.1 tons ha^{-1}). The kernel diameters of the tested lines and checks were between 0.45 and 1.00 cm. The widest kernel diameters of the tested lines were shown by 'RLW-10' (0.78 cm), 'RLW-4' (0.75 cm), and 'RLW-7' (0.75 cm) and were larger than the kernel diameters of 'KGW' (0.65 cm), 'Takfa1' (0.65 cm), and 'Nei9008' (0.70 cm). Therefore, 'RLW-4', 'RLW-7' and 'RLW-10' were selected as promising waxy corn lines with large kernels. These breeding lines can be further used for hybrid development.

The ear diameters of the tested lines and checks ranged between 2.93 and 4.35 cm (Table 3). 'KGW' had the largest ear diameter of 4.35 cm. 'DRP-4' and 'DRW-20' had the highest ear diameters of 4.03 and 3.89 cm, respectively. Although these breeding lines had higher ear diameters than 'Takfa1' (3.20 cm) and 'Nei9008' (3.23 cm), they had lower ear diameters than 'KGW' (4.35 cm). The ear lengths of the tested lines and checks ranged between 9.1 and 16.7 cm. The highest ear length of 16.7 cm was shown by 'KGW', and the highest ear lengths were shown by 'RLW-8' (15.9 cm) and 'RLW-7' (15.3 cm) and were similar to the ear length of 'Takfa1' (15.4 cm).

Days to anthesis ranged from 44 days ('KGW') to 63 days ('DNRY-1'). The days to anthesis of the other two checks ('Takfa1' and 'Nei9008') were 51 days (Table 3). All tested lines had higher days to anthesis, which ranged from 53 days to 63 days, than the checks. The plant heights of the tested lines ranged between 120 and 176 cm, whereas the plant heights of the checks were between 142 and 148 cm. Most tested lines were taller than the checks. The ear heights of the tested lines were between 56 and 95 cm, whereas the plant heights of the checks were between 69 and 72 cm. Most tested lines had higher ear placement positions than the checks.

Among all cultivars, five namely, 'DRP-4', 'RLW-4', 'RLW-6', 'RLW-7', and 'RLW-10' showed the highest overall yields, yield component, and agronomic traits (Figure 4). The 'DRP-4', 'RLW-4', 'RLW-6', 'RLW-7' and 'RLW-10' had the highest 1000-kernel weights (270, 300, 270, 295, and 335 g, respectively), anthesis dates (56, 53, 56, 54, and 57 days, respectively), row number per ear (12.1, 10.2, 10.7, 11.5, and 10.8 rows, respectively), husked yield (7.3, 5.9, 6.6, 7.3, and 6.9 ton ha^{-1}, respectively) and kernel diameter (0.74, 0.81, 0.75, 0.77, and 0.77 cm, respectively).

Figure 4. Ear samples of the top five breeding lines ('RLW-6', 'RLW-7', 'RLW-4', 'RLW-10', and 'DRP-4') developed through S_3 progeny selection for seed size, row number, and agronomic traits for two consecutive seasons and evaluated for two seasons for yield and yield components.

Table 4. Means for AUDPC-DS, AUDPC-DI, DS index, and resistance level of DM of 33 S_3 waxy corn genotypes and three check cultivars during the rainy season 2020 at two locations (Nong Bua Village and Sok Muang Village) in Ban Phang District, Khon Kaen Province, Thailand.

Genotypes	Nong Bua village				Sok Muang village			
	AUDPC DS	AUDPC DI	DS	Resistance Level	AUDPC DS	AUDPC DI	DS	Resistance Level
Medium seed								
DNRW-1	814 f–i	2590 a–e	50.7 d–h	MS	2048 d–i	2800 a	85.9 d	HS
DNRY-1	720 h–i	2459 c–e	46.9 h	MS	1625 jk	2800 a	73.5 e	S
DNRY-2	967 e–g	2683 a–c	56.0 c–h	S	2083 d–i	2800 a	90.3 a–d	HS
DRW-11	992 d–g	2619 a–e	59.3 cd	S	2493 a	2800 a	97.0 a–d	HS
DRW-12	929 e–h	2647 a–d	52.7 d–h	S	2441 ab	2800 a	97.5 a–c	HS
DRW-15	828 f–i	2655 a–d	49.2 e–h	MS	2013 f–i	2800 a	85.9 d	HS
DRW-17	802 f–i	2528 a–e	48.3 f–h	MS	2237 a–h	2800 a	98.1 ab	HS
DRW-19	943 e–h	2653 a–d	51.4 d–h	S	2321 a–e	2800 a	96.2 a–d	HS
DRW-20	861 f–i	2631 a–e	50.2 d–h	MS	2271 a–g	2800 a	95.5 a–d	HS
DRW-22	1016 c–g	2658 a–d	62.9 bc	S	2063 d–i	2800 a	91.2 a–d	HS
DRW-24	897 e–i	2629 a–e	54.1 c–h	S	2019 e–i	2800 a	90.7 a–d	HS
DRW-25	1231 bc	2663 a–c	63.0 bc	S	2198 a–h	2800 a	97.1 a–d	HS
DRW-27	791 g–i	2411 e	53.9 c–h	S	1934 hi	2800 a	87.3 b–d	HS
DRW-28	955 e–h	2676 a–c	51.9 d–h	S	2178 b–h	2800 a	91.1 a–d	HS
DRP-3	794 g–i	2476 c–e	47.5 gh	MS	1866 ij	2787 ab	86.2 cd	HS
DRP-4	1342 ab	2734 a	74.8 a	S	2335 a–d	2800 a	100.0 a	HS
DRP-5	1220 b–d	2710 ab	63.2 bc	S	2310 a–f	2800 a	96.4 a–d	HS
DRP-7	984 e–g	2672 a–c	57.8 c–f	S	2270 a–g	2800 a	99.2 a	HS
DRP-12	936 e–h	2679 a–c	56.1 c–h	S	2351 a–d	2800 a	97.8 ab	HS
DRY-1	866 f–i	2680 a–c	52.0 d–h	S	2101 c–i	2800 a	94.4 a–d	HS
DRY-3	815 f–i	2665 a–c	48.6 f–h	MS	2414 ab	2800 a	98.1 ab	HS
Large seed								
RLW-3	1109 c–e	2637 a–e	58.8 c–e	S	2333 a–d	2800 a	97.8 ab	HS
RLW-4	903 e–i	2554 a–e	54.3 c–h	S	1987 g–i	2782 b	90.4 a–d	HS
RLW-6	854 f–i	2489 b–e	52.4 d–h	S	1945 hi	2800 a	93.8 a–d	HS
RLW-7	902 e–i	2462 c–e	53.9 c–h	S	2406 a–c	2800 a	98.8 a	HS
RLW-8	987 e–g	2589 a–e	59.1 c–e	S	2430 ab	2800 a	100.0 a	HS
RLW-10	987 e–g	2619 a–e	57.6 c–f	S	2207 a–h	2800 a	98.9 a	HS
RLP-2	1125 b–e	2623 a–e	60.2 b–d	S	2324 a–e	2782 b	94.0 a–d	HS
RLP-3	990 d–g	2643 a–d	57.0 c–g	S	2293 a–g	2800 a	100.0 a	HS
RLP-4	1007 c–g	2585 a–e	55.7 c–h	S	2286 a–g	2800 a	97.6 ab	HS
RLY-1	834 f–i	2619 a–e	51.9 d–h	S	2284 a–g	2800 a	96.2 a–d	HS
RLY-3	958 e–h	2569 a–e	58.3 c–f	S	2171 b–i	2800 a	92.3 a–d	HS
LP-2	1032 c–f	2682 a–c	57.4 c–g	S	2342 a–d	2800 a	96.6 a–d	HS
KGW	1477 a	2674 a–c	69.9 ab	S	2471 ab	2800 a	100.0 a	HS
Takfa1	727 h–i	2161 f	50.5 d–h	MS	1544 k	2749 c	53.2 f	S
Nei9008	672 i	2432 de	23.2 i	MR	960 l	2644 d	16.0 g	MR
F-value	**	*	**		**	**	**	
CV (%)	12.0	4.4	9.1		7.0	0.3	6.2	

* Genotypes were grouped based on seed size.

Means followed by different letters within the same column are significantly different based on LSD at $P \leq 0.05$.

P represents purple waxy corn, W represents white waxy corn, Y represents yellow waxy corn, L represents large kernel.

These five genotypes revealed significantly different traits (*P ≤ 0.05*) from the three check cultivars ('KGW', 'Takfa1', and 'Nei9008'). The other traits of these five cultivars, including ear diameter, ear length, and plant height, were not significantly different from those of the check cultivars (Table 3).

DM disease assessment

The DM disease assessment of the tested corn cultivars at two field conditions was summarized into the AUDPC on the basis of DS and DI data (Table 4). Generally, all inoculated corn cultivars grown at Nong Bua Village and Sok Muang showed DM symptoms. However, DS at Sok Muang was higher than that at Nong Bua Village. The DS of the tested breeding lines at Nong Bua Village ranged from 46.9% to 74.8% and from 73.5% to 100.0% at Sok Muang. AUDPC-DS at Nong Bua Village ranged from 720 to 1342 and from 1625 to 2493 with an average of 274 at Sok Muang. AUDPC-DI ranged from 2411 to 2734 at Nong Bua Village and from 2782 to 2800 at Sok Muang.

The susceptible check ('KGW') remained susceptible to DM with the DS of 69.9% and 100.0% at Nong Bua Village and Sok Muang, respectively (Table 4). Only one resistant check cultivar ('Nei9008') consistently expressed moderate resistance to DM at both local fields with the DS of 23.0% and 26.2%. A resistant check cultivar ('Takfa1') expressed moderate susceptibility (DS = 50.5%) and susceptibility (DS = 53.2%) at Nong Bua Village and Sok Muang, respectively.

The breeding lines of waxy corn at Nong Bua Village were classified into two groups *viz*., moderately susceptible and susceptible. However, the breeding lines grown at Sok Muang exhibited high susceptibility to DM. In accordance with the AUDPC-DI, AUDPC-DS, and DS, five selected waxy corn cultivars with the highest overall yields, yield component, and agronomic traits showed susceptibility to DM at Nong Bua Village: 'RLW-4' (903, 2554, and 54.3%), 'RLW-6' (854, 2489, and 52.4%), 'RLW-7' (902, 2462, and 53.9%), 'RLW-10' (987, 2619, and 57.6%) and 'DRP-4' (1342, 2734, and 74.8%) (Table 4). These five cultivars exhibited high susceptibility when tested at Sok Muang. These results showed that no breeding lines that were screened in both locations had DM resistance.

DISCUSSION

In this study, the inbreeding progeny selection method (S_3) for seed size improvement in waxy corn was successful, resulting in 33 progenies for yield trial evaluation and DM assessment. After the plants with desirable traits were selected, they were self-pollinated and further grown for ear and waxy phenotypes. Selection for the waxy phenotype was completed in the first season because the recessive waxy allele is fixed (Hunt *et al*., 2010). The selected ears were planted during the second season, and selection was repeated without the progeny test. The selection cycle was completed in two seasons.

Selection for seed size is essential for the yield improvement of cereal crops (Kesavan *et al*., 2012). LS genotypes have several advantages over SS genotypes; these advantages include good germination, rapid early growth, and good yield (Ambika *et al*., 2014). Moreover, large kernel size is linked to kernel number per row and yield (Harakotr *et al*., 2016; Palamarchuk *et al*., 2018).

The empirical evidence was observed from the purple field corn population (Khamphasan *et al*., 2020) and yellow small-ear waxy corn germplasm (Sukto *et al*., 2020). In this study, husked yield, ear diameter, anthesis date, and row per ear significantly differed between seasons. The tested genotypes grown in the dry season showed higher values of large kernel size and husked yield than those grown during the rainy season. Extremely high rainfall during the rainy season (Figure 2) after sowing may be a constraint of seed germination and maize production (Li *et al*., 2019). These results verified similar findings on maize caused by waterlogging and flooding (Xiong *et al*., 2020). In addition, genetics in terms of plant genotype or cultivars principally controls specific corn traits, such as large kernel size (Palamarchuk *et al*., 2018). Moreover, in this study, 1000-kernel weight, husked yield, and some other traits varied among the investigated waxy corn cultivars. Thus, genotype contributed to the largest proportion of phenotypic variation for large kernel size and most traits, suggesting that these traits are selectable in waxy corn.

The interaction between season and genotype (G × S) also affected some traits of maize, such as kernel diameter and row per ear, as found in inbred maize lines (Senete *et* *al*., 2011). In this study, G × S had an effect on husked yield and ear diameter. However, the effect of S was reduced when the variance of 1000-kernel dry weight described by G × S was lower than all other terms in this model because the contribution of the genotype to the total variance of 1000-kernel dry weight or large kernel size was increased. Although kernel size is quantitatively inherited, controlled by multiple genes, and susceptible to environmental effect (Austin and Lee, 1998), this trait can be improved through recurrent selection. The top five genotypes (Figure 4) in terms of yield, yield components, and agronomic traits were observed in this study. They had large kernels with 1000-kernel dry weights of approximately 270–335 g, which corresponded to the previously reported value of 302.7–330.0 g (Duangpapeng *et al*., 2015). These results confirmed the findings of a similar report on other traits, such as the grain yield of maize (Kara, 2011). Another report showed that field corn derived from various cultivars had very large kernel sizes (368.9 g) (Odhiambo and Compton, 1987; Kalapakdee *et al*., 2020).

The DM disease severity in the 33 tested waxy corn genotypes differed between the two locations and was reliant on the disease triangle (Islam *et al*., 2017), which comprises three factors: the virulent pathogen, the susceptible host, and a favorable environment (Agrios, 2005). Although the same set of corn genotypes was used for the two local fields, the weather during the experiment at the Sok Muang location was more optimal for DM disease development than at Nong Bua Village. DM symptoms occurred at the end of the vegetative stage at Nong Bua but appeared after germination at Sok Muang. All genotypes in this study, except for ‘Nei9008’, were susceptible to DM likely due to the loss of DM resistance genes during the development of the 33 breeding lines. Essentially, most corn cultivars were not evaluated for DM resistance during line development and in this study because susceptible lines usually die at an early stage (Ajala *et al*., 2003). Thus, disease assessment was conducted after desirable yield and agronomic traits were obtained.

CONCLUSIONS

The improvement of large kernel size in waxy corn is the major objective of this research. Five genotypes, namely, ‘DRP-4’, ‘RLW-4’, ‘RLW-6’, ‘RLW-7’, and ‘RLW-10’ were chosen on the basis of kernel size, yield, and agronomic characteristics. These genotypes possessed high 1000-kernel dry weight and thus exhibited high productivity. However, they remained inferior in some characters. For example, they had low yield per plant, late maturity, tall plant height, and high ear placement. These characters should be improved in the next breeding cycles. These breeding lines will be self-pollinated for generation advance and used as parents in all combinations of crosses to generate experimental hybrids for hybrid breeding. These lines were also screened for DM resistance in local fields under artificial inoculation. Given that the disease screening experiment was not able to identify resistant lines, another breeding program, such as one involving backcross breeding, is still required. All of the five selected lines will be useful for developing large-seeded waxy corn for food and industrial uses.

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