Research article

Improving Waxy Maize, the Heritage of South East Asia

PETER STAMP*
ETH, Zurich, Switzerland
Email: peter.stamp@usys.ethz.ch

SANSERN JAMPATONG
Kasetsart University, Nakhonpathom, Thailand

HAM LE-HUY
Agricultural Genetics Institute, Hanoi, Vietnam

SEBASTIAN STREB
ETH, Zurich, Switzerland

CHOOSAK JOMPUK
Kasetsart University, Nakhonpathom, Thailand

Received 4 November 2015 Accepted 11 April 2016 (*Corresponding Author)

Abstract Amylose-free (*waxy*), i.e. amylopectin maize has been a vegetable and staple food in East and South-East Asia for centuries, resulting in hundreds of landraces (LR). Eating preferences could have resulted in the additional selection for different starch properties of *waxy* maize, of interest in the food and feed industry. But within twenty LR from Vietnam and Thailand, disappointingly no evidence was found for special starch properties. For minority ethnic groups *waxy* LR are still the main staple food, well liked for their soft grains with favorable cooking properties. But maize protein is severely lacking in quality that makes waxy maize an unsuitable staple food especially for small children. High quality protein maize (QPM) developed by the International Maize and Wheat Improvement Center (CIMMYT) has 90% of the nutritive value of milk protein. We combined the recessive *waxy* and QPM alleles into modern high yielding lines resulting in double quality grains. In a second step this double quality was introgressed into two landraces of the Hmong minority by two backcrosses. Seeds are available now of the two improved *waxy* landraces, which possess high yield potential, high protein quality and good eating quality. Consumption of double-quality *waxy* maize as staple food will improve the diets of children, a good reason to produce it.

Keywords *waxy* maize landraces, starch quality, quality protein maize, ethnic minorities

INTRODUCTION

Maize was probably introduced to Asia in its native wild-type form during the 16th century from Central America. Normal grain starch consists of two glucose polymers, amylose and amylopectin, which assemble into semi-crystalline granules. Amylopectin accounts for 60 to 90% of the granule weight. It is a highly branched polymer in which the glucose units are linked via α-1,4 and α-1,6-glucosidic bonds. In contrast, amylose consist mostly of α-1,4-linked glucose, forming long linear chains inside the starch granule.

Soon after arrival, amylose-free (*waxy*) mutants were cultivated as a common vegetable and staple food from Japan down to Myanmar. This closely follows an old consumer preference for amylose-free rice, also called glutinous or sticky rice, in Asia that dates back 2000 years (Olsen and Purugganan, 2002). The selection for the recessive *waxy* allele can be attributed to a strong preference for soft grains with more desirable cooking and flavor properties (Fergason, 2010). Sequence analysis of *waxy* landraces (LR) from southwest China, suggest independent parallel selection for the *waxy* trait in this region (Fan et al., 2008).

South East Asia was successful in reducing hunger and poverty by less rice and more meat and milk consumption (FAO, 2008). However, in hilly marginal areas the majority of people belong to diverse minority ethnic groups. Beside global poverty and low educational level, the main problems of those minority ethnic groups are the access to land, water and markets (Huynh, 2002). For them, *waxy* maize landraces still remain the staple food and meat is only rarely consumed (Swinkels and Turk, 2006). This makes an urgent task to improve their protein uptake.

Waxy maize has soft grains that are especially suitable for preparing traditional dishes such as porridge or for the consumption of immature ears as a vegetable. Sensory acceptance of *waxy* maize is mostly determined by the right degree of firmness and the absence of stickiness of cooked kernels. In turn, both firmness and stickiness are controlled by the structure and composition of starch. In *waxy* maize, starch basically consists of branched high-molecular amylopectin; the linear amylose is virtually absent. Protein of maize is lacking both in quality and quantity. For the nutrition of humans lysine is one of the most important amino acids, followed by tryptophan. But the lysine and tryptophan contents of zeins, the main protein type in maize, are quite low (FAO, 1992). Therefore it is essential to raise the protein quality in maize based diets. Breeding for maize with enhanced protein quality started in the mid of the 1960s, when mutants with an increased lysine content, like opaque2 (o2) and floury2 (fl2), were discovered; the increase in essential amino-acids results from a change of the relative amounts of different fractions constituting the maize endosperm proteins (Vasal, 2001). An agronomically superior form of o2 germplasm with hard endosperm was named high quality protein maize (QPM) by the International Maize and Wheat Improvement Center (CIMMYT, Mexico), in opposition to the soft endosperm o2, called standard o2. This novel maize type has 90% of the nutritive value of milk protein for young children (FAO, 1992).

In recent years we were successful in combining two recessive traits, *waxy* and QPM (w/o), into one new kernel type with normal aspects, i.e. suitable for staple food (Dang et al., 2011; Sinkangam et al., 2012). Those findings were recently confirmed (Zhang et al., 2013). This provides now a firm basis to tackle the problem of protein malnutrition in a double approach, improving the staple *waxy* maize agronomically as well as nutritionally: as our new w/o lines are derived from modern agronomic stock, they should be suitable to upgrade by introgression the protein quality of traditional landraces and to improve the yield by residual effects. *Waxy* maize landraces from the Hmong minority in Vietnam were chosen as targets that excelled by good eating taste.

OBJECTIVE

The very low indigenous protein quality of *waxy* maize landraces must be improved by incorporating quality protein (QPM) genes. This requires that two grain mutants are expressed together without impairing the vitality of the grain. This double quality breeding material can then be implemented into the food chain of malnourished people in South East Asia. In a second approach, the starch quality of a representative set of Thai and Vietnamese *waxy* landraces was going to be analyzed for the potential of new types that could be of value for the food and feed industry.

METHODOLOGY

Starch Quality

Waxy maize landraces (LR) were chosen (Fig. 1) that are or had been used by ethnic majorities and minorities in Thailand (12 LR; Anonymous, 1983) and in Vietnam (8 LR; Maize germplasm section - National Maize Research Institute, 1997) in representative regions of cultivation. A commercial *waxy* feed grain hybrid (PR38A22, DuPont/Pioneer) was used as a control.

For amylose quantification, differential scanning calorimetry (DSC) and starch granule size distribution was utilized, extracted starch was analyzed for amylose content according to Hostettler et al. (2011). Starch granule size was quantified by digital image analysis coupled to light microscopy (Wilson, Bechtel, et al., 2006). Gelatinization temperature and enthalpy of starch suspensions were determined using a differential scanning calorimeter (DSC 2010) from TA instruments (Jacquier, Kar, et al., 2006).

Pearson product-moment correlation coefficient (r) for the relationships between starch properties and the level of significance (p) for two-tailed test were calculated in Excel.

Quality Protein Maize (QPM)

Two *waxy* Hmong landraces of good taste (White *waxy* Cao Bang 3-Highland, North Vietnam; WVN 3 and White *waxy* S2- Highland, North Vietnam; WVN 10), were crossed with two *waxy* x QPM (w/o) lines of modern agronomic background, ETH w/o (southern Chinese origin; Dang et al., 2011) and Agron w/o (Thai origin; Sinkangam et al., 2012), w/o types were selected from sib-mated plants. The resulting top crosses were then backcrossed once again to the parental *waxy* LR. Field experiments were conducted at Suwan Farm, Thailand (14.5°N, 101°E, 360 m above sea level; lowland climate) in 2011, 2013 and 2014. For further technical information see Sinkangam et al. 2012. Molecular analyses were needed to assay for the recessive opaque-2 and the *waxy* alleles and carried out according to methods described in detail by Sinkangam et al. (2012). Data of the quality and agronomic traits were analyzed according to a randomized complete block design (RCBD).

Table 1 Ear yield, eating and protein quality of improved landrace (LR) S2 after crossing with ETH W/O and AGRON W/O and back-crossings to LR S2

Pedigree	QPM = Yes QPM = No	Ear fresh weight	Bite test	Tryptophan in protein
		t/ha	(1-9)	%
Ratchata 1 (Hybrid)	No	7.81a	6.7a	0.48b
S2 (LR Vietnam)	No	6.41b	5.0b	0.41b
S2 *waxy* x QPM	Yes	7.73a	6.3a	0.82a

Rating score of bite test (1-9) = 1 is poor and 9 is good.

Table 2 Ear fresh yield, eating and protein quality of two test hybrids and a commercial hybrid

Pedigree	QPM = Yes QPM = No	Ear fresh weight	Bite test	Tryptophan In protein
		t/ha	(1-9)	%
Big White 852 (commercial hybrid)	No	7.39b	7.0a	1.19b
SW14D-B7-1055-11 x 34	Yes	12.35a	6,3ab	1,75a
SW14D-B7-1055-14 x 34	Yes	12.05a	5,7b	2,04a

Rating score of bite test (1-9) = 1 is poor and 9 is good.

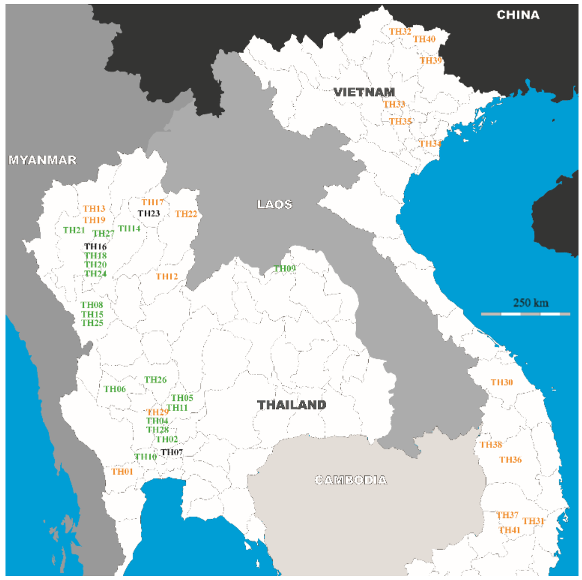

Fig. 1 Origin and purity of Thai and Vietnamese landraces used in the study
Amylose free landraces (waxy/waxy) are shown in yellow, landraces with mixed background (WAXY/waxy) in green and wild-type plants (WAXY/WAXY) in black.

RESULTS AND DISCUSSION

Starch

Genetic resources, like the hundreds or more, probably thousands, of *waxy* LR in South-East and East Asia, cannot be easily accessed for research due to their well protected status. On the other hand, they never made it to the international genebank of CIMMYT; their conservation unluckily depends on activities of individual nations. We had access to collections in Thailand and Vietnam but no information existed for tests on *waxy* purity, and for the maintenance history. The more recent Vietnamese collection was pure *waxy*. Within the Thai collection, three LR were wrongly classified as *waxy* and some other were mixed or heterogeneous (Fig. 1). A practical approach was successfully taken to regenerate pure *waxy* genotypes within the LR of mixed status. This was done by iodine staining for lack of amylose. This experience with national or local conservation of germplasm highlights the challenge to maintain essential but recessive traits.

For the now pure *waxy* LR, starch quality tests were done in comparison with a commercial *waxy* fodder maize from France for gelatinization fine structure of amylopectin and starch granule size. No significant differences existed for these traits within the Asian LR and between them and the commercial hybrid (data not shown).

The Thai and Vietnamese collections of *waxy* maize landraces (LR) have probably not been complete, considering the varied traditions in the mountainous areas of Southeast Asia. Zheng et al. (2013) analyzed the genetic diversity of Chinese *waxy* landraces, and modern *waxy* inbred lines proving a wide genetic diversity.

We identified four different *waxy* mutations in our LRs. Two of these are known from South Chinese genetic resources (Fan, Quan, et al., 2008), the other two have been first found here in South-East Asian LR. This suggests a strong and parallel selection for amylose free varieties by local farmers throughout the last centuries, as nowhere else in the world these rare mutations have been targeted for specialty food before varieties from Asia had been described (Collins, 1909; Weatherwax, 1922), and later on used for the production of quality feed. We investigated if the remaining amylopectin was also targeted by selection rendering its structure to meet the eating preferences. However, we could not identify alteration in amylopectin in the LRs. Therefore, our hypothesis of concomitant changes in the branching pattern of amylopectin in *waxy* LR, leading to altered starch traits of economic interest had to be rejected.

QPM

This work started with collections of *waxy* maize landraces (LR) from Thailand and Vietnam (Fig. 1). From 48 initial LR, two vigorous Vietnamese of good taste were selected according to field vigor and excellent taste. They were crossed with two *waxy* x QPM lines that had been developed by us (Dang et al., 2011; Sinkangam et al., 2012), one with a southern Chinese and one with a Thai background. Their offspring was further selected in several generations at Kasetsart University, Thailand. Today, seeds are available from open pollinated varieties that are close to the original parental LR, but with the improved protein of QPM and quite some agronomic advantages.

As *waxy* maize is usually eaten as a vegetable, the right harvest time is at dough stage. In a tropical climate this was reached three weeks after flowering. On average almost 80% of the maximum grain yield was attained at this time with grain moisture content at about 45%. An example for the yield and quality potential of the new material is presented in Table 1. The protein content was 11% without differences between the genotypes. The commercial *waxy* hybrid had the highest yield, but the protein-improved LR S2 achieved similar values. Despite the good ratings for eating quality in Vietnam, the original LR was inferior in the test for grain softness by the biting test to the hybrid and the improved LR. Tryptophan is one of the essential amino acids lacking in cereal protein, it is well correlated with the quality of the whole protein fraction. Its content in protein was doubled in the grains in the improved LR S2. This is a great success indeed as it was not known before if two mutations, *waxy* and QPM could be combined within one grain without damaging the grain development.

As *waxy* maize is still an important vegetable or snack all over South East Asia to South Korea, we started a second approach on protein improved (QPM) hybrids that combine agronomic vigor with excellent agronomic performance. For this we used the *waxy* x QPM lines that had been originally developed by us (Dang et al., 2011; Sinkangam et al., 2012), one with a southern Chinese and one with a Thailandese background. The lines have been further adapted for a good seed production and a good combining ability in the last years, providing a promising platform to utilize general breeding progress in Thailand and southern China. Up to date 44 test hybrids have been analyzed for their field performance. Data are presented for two advanced test hybrids (Table 2). Both protein improved test hybrids are very high yielding in comparison to a commercial *waxy* hybrid, though a little bit inferior to eating quality as indicated by the biting test. However, the goal of fundamentally upgrading the protein quality was reached, as indicated by very high tryptophan contents in protein.

As both of our two *waxy* lines for QPM had a modern agronomic background, a positive residual effect on agronomic performance was expected and indeed achieved; which is a plus for convincing the ethnic minorities to accept new varieties bio-fortified for high protein quality (Dang et al., 2011; Sinkangam et al., 2012). The "Bite test" is a quality test using human preference. A "9" indicates a thin pericarp, a good tenderness and good aroma. It has to be kept in mind that this test has been developed for vegetable waxy maize, cooked at the doughy stage. Ethnic minorities, however, use a large proportion of their harvest as a staple, therefore the quality requirements for preparing food may vary. The protein content of mature grains had reached good levels above 11%, without significant differences between entries. Lysine and tryptophan contents are closely correlated in maize grains (Sinkangam et al., 2012). The high values in crosses corresponded well with findings by Vasal (2001) and Prasanna et al. (2001) who indicated that QPM genotypes had almost double the amount of tryptophan compared to normal maize, but were similar in overall protein content.

Based on this new w/o germplasm, the next step must be an introduction to carefully selected regions of ethnic minorities in Vietnam. The Hmong people, as a large minority, are the main target, often eating three times per day *waxy* maize as a staple. This germplasm has the genetic potential to be further adapted as it is still genetically broad, due to its Chinese and Thailandese QPM background. A successful integration of *waxy* x QPM could provide an extra advantage for local communities with new market chances selling healthy snacks.

CONCLUSION

The understanding of the uses and requirements of *waxy* maize LR may open new possibilities for its cultivation of this traditional maize type. Major changes had been expected in the structure of amylopectin as different mutations for the *waxy* mutation had occurred in Asia in the past; this would be of high interest for industry or specialty production. According to our investigations, however, there are no indications that *waxy* maize with additional new specific starch properties exist for economic purposes.

On the contrary, we are excited that we have developed two types of QPM x *waxy* materials, directly to be used to alleviate the protein deficiency for ethnic minorities depending on a maize diet, and secondly as high-yielding hybrids that can be used as a protein improved vegetable or healthy snacks, especially for school kids.

REFERENCES

Anonymous, 1983. Exploration and collection of indigenous strains of glutinous corn, popcorn and Job's tears in Thailand. Mission Team Field Research Institute, Department of Agriculture, Bangkok, Thailand, 1982 /1983.

Collins, G.N. 1909. A new type of Indian corn from China. U.S. Department of Agriculture, Bureau of Agriculture-BULIEflNNO.16L.

Dang, N.C., Munsch, M., Aulinger, I., Renlai, W., Le-Huy, H., Jampatong, S. and Stamp, P. 2011. Composition of starch and protein in the endosperm of newly generated double recessive waxy and opaque 2 maize (*Zea mays* L.) Genotypes. J Agric Sci Technol B 1, 631-637.

Fan, L.J., Quan, L.Y., Leng, X.D., Guo, X.Y., Hu, W.M., Ruan, S.L., Ma, H.S. and Zeng, M.Q. 2008. Molecular evidence for post-domestication selection in the waxy gene of Chinese waxy maize. Molecular Breeding, 22, 329-338. doi:10.1007/s11032-008-9178-2.

FAO. 1992. Maize in human nutrition. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO. 2008. The state of food and agriculture in Asia and the Pacific region. FAO/Regional Office for Asia and the Pacific, Bangkok, Thailand.

Fergason, V. 2001. High amylose and waxy corns. In: Hallauer AR, (Ed). Specialty corns. Boca Raton, CRC Press, 63-84.

Huynh, T.B., Duong, B.H., Bui, T.C., and Plant, R. 2002. Indigenous peoples/Ethnic minorities and povery reduction, Vietnam. Asian Development Bank.

Jacquier, J.C., Kar, A., Lyng, J.G., Morgan, D.J. and McKenna, B.M. 2006. Influence of granule size on the flow behaviour of heated rice starch dispersions in excess water. Carbohydr Polym,66, 425-434. doi:10.1016 /j.carbpol.2006.03.029.

Olsen, K.M. and Purugganan, M.D. 2002. Molecular evidence on the origin and evolution of glutinous rice. Genetics, 162, 941-950.

Prasana, B.M., Vasal, S.K., Kassahun, B. and Singh, N.N. 2001. Quality protein maize. Current Science, 8, 1308-1319.

Sinkangam, B., Stamp, P., Srinives, P., Jompuk, P., Mongkol, W., Porniyom, A., Dang, N.C. and Jompuk, C. 2012. Integration of quality protein in waxy maize (*Zea mays* L.) by means of SSR markers. Crop Sci, 51, 2499-2504.

Swinkels, R., and Turk, C. 2006. Explaining ethnic minority poverty in Vietnam: A summary of recent trends and current challenges Hanoi – Vietnam. Worldbank.

Vasal, S.K. 2001. High quality protein corn. Specialty corns. Hallauer, A. R., CRC Press LLC.

Weatherwax, F. 1922. A rare carbohydrate in waxy maize. Genetics, 7, 568-572.
Wilson, J.D., Bechtel, D.B., Todd, T.C. and Seib, P.A. 2006. Measurement of wheat starch granule size distribution using image analysis and laser diffraction technology. Cereal Chem, 83, 259-268. doi: 10.1094/Cc-83-0259.
Zhang, W., Yang, W., Wang, M., Wang, W., Zeng, G., Chen, Z. and Cai, Y. 2013. Increasing lysine content of waxy maize through introgression of opaque-2 and opaque-16 genes using molecular assisted and biochemical development. PLoS ONE 8 (2), e56227. doi:10.1371/journal.pone.0056227.