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Quality protein introduced into waxy maize landraces of ethnic minorities

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Abstract

Ethnic minorities in South East Asia use waxy maize as a staple food, lacking in essential amino acids. Recently, we combined the recessive *waxy* and *opaque2* alleles to double quality grains (w/o, pure amylopectin, high quality protein), which still must be introgressed into germplasm acceptable to ethnic minorities. Two w/o lines of Chinese and Thai background, respectively, were crossed once with two Vietnamese waxy landraces of good taste, WVN 3 and WVN 10. At the preferred harvest time for eating, dough stage, homozygous w/o F_2 offspring with WVN 3 were equal in dehusked ear yield with commercial waxy hybrids and 40% superior in yield compared with WVN 10 F_2 offsprings. In WVN 3 F_2 crosses and F_2 backcrosses with WVN 3, all w/o dehusked ears were equal in eating quality, grain protein content and a good leaf health; but the yield of dehusked ears and the grain tryptophan content was highest in the topcross. High quality germplasm is available now as a source of high quality protein for ethnic minorities. The two original w/o lines led to equal results in crosses with landraces, but their test hybrid was extremely high-yielding, indicating a good potential to breed for commercial high protein quality snacks in South East Asia.

Keywords: *Zea mays* L, ethnic minorities, South East Asia, protein quality

Introduction

In general, South East Asia has been quite successful in reducing hunger and poverty for their populations, due to the reduction of the rice fraction and the increase in meat and milk consumption (FAO, 2008). However, in hilly marginal areas many people belong to diverse minority ethnic groups. 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 and other monogastrics 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*. It could be proven that *o2* maize has 90% of the nutritive value of milk protein for young children (FAO, 1992).

In recent years we demonstrated that it is possible to combine the 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

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were chosen as targets that excelled by good eating taste. The objectives were to boost protein quality of waxy maize landraces by introgressing QPM genes, generating w/o germplasm that can be implemented into the food chain of malnourished people in South East Asia.

Materials and Methods

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 two parental w/o lines were crossed with each other as well to form a test hybrid. In Experiment 1 the four w/o crosses plus the w/o test hybrids were cultivated together with WVN3 and WVN 10, and the Thai waxy hybrid Max 1 in 2011. As the w/o lines had no impact on agronomic performance and as WVN 10 was much inferior in yield (see Table 1), the WVN 3 topcrosses were intermated and then backcrossed once to WVN 3, selected thereafter again for w/o homozygosity. In Experiment 2 the WVN w/o cross and backcross were tested in 2013.

The experiments were conducted at Suwan Farm, Thailand (14.5°N, 101°E, 360 m above sea level; lowland climate; Rhodic Kandiustox Oxisol (USDA taxonomy)). The experiments were arranged in four blocks. Each plot consisted of four rows, 5 m long and 0.75 m apart, with 21 plants per row. The distance between adjacent plants in a row was 0.25 m, resulting in a population density of 3.9 m^{-2}. Two seeds were sown manually in each mound, and redundant plants were removed at the 3-leaf stage. Prior to sowing, 25 kg N ha^{-1} and 30 kg P ha^{-1} were applied, with an additional 115 kg N ha^{-1} one month after sowing. Herbicides and insecticides were applied as required according to local practices. The experiments were sprinkler-irrigated four times during the first three weeks after sowing. Thereafter, the experiments were furrow-irrigated once a week (~30 mm). The grains were harvested at dough stage for fresh eating maturity and at full grain maturity for protein related traits.

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). Young and healthy leaves of individual four-week-old plants were sampled for DNA extraction. The *opaque-2* allele the waxy alleles were identified by the SSR markers phi057 and phi022, respectively. An initial polymorphism analysis of both parental lines had been performed for *opaque-2*. Primer sequences and their repeat motifs are available in the Maize Genomic Database (http://www.maizegdb.org/).

Twenty-five seeds per plant were soaked in distilled water for 25 min before removing pericarps and embryos. The endosperms were air-dried overnight, ground in a cyclone mill and wrapped in a commercial filter-paper envelope to remove the fat with 100% hexane in a Soxhlet-type continuous extractor. The ground samples were then analyzed for tryptophan content using a spectrophotometer as described by Nurit et al (2009); the protein content was measured according to the micro-Kjeldahl method.

Data of the quality and agronomic traits were analyzed according to a randomized complete block design (RCBD). The significance of individual lines was calculated with the F-test.

Results and Discussion

In a first step, 48 waxy maize landraces, 12 from Vietnam and 36 from Thailand, were tested at the National Corn and Sorghum Research Center for general agronomic performance (data not shown). From these, superior Vietnamese landraces of good taste two were crossed with two waxy x QPM (w/o) inbred lines, one with a southern Chinese and one with a Thai background. From the best performing topcrosses four w/o crosses were generated; these were tested together with a commercial Thai waxy hybrid, the two original Vietnamese landraces and the test hybrid between the parental w/o lines in Experiment 1 (Table 1). The days from planting to silking varied from 48 to 55. Plant heights were moderate on average, quite low in the original landraces and highest in

Table 1 - Agronomic field data of homozygous waxy x quality protein maize in 2011.

Entries	Silking time (days)	Plant height (cm)	Ear fresh weight (t ha^{-1})
ETH x Agron (w/o*)	54	185	9.19
WVN 3 x ETH (w/o)	49	168	8.72
WVN 3 x Agron (w/o)	48	179	8.76
WVN 10 x ETH (w/o)	55	167	5.60
WVN 10 x Agron (w/o)	52	168	6.49
WVN 3	50	154	4.62
WVN 10	55	155	2.78
Max 1	52	170	7.14
Big White 852	48	136	6.41
LSD (0.05)	2	24.9	1.76

* w/o = Homozygous waxy x QPM

the w/o hybrid. As waxy maize is usually eaten as a vegetable, the right harvest time is at dough stage. In a tropical climate this was reached at 21 days after silking for each genotype tested although for individual ones the optimum quality date varied plus/minus 1.5 days. On average almost 80% of the maximum grain yield was attained at this time with grain moisture content at about 45%. On the basis of dehusked ears the yields of w/o crosses with WVN 3 were more than 2 t ha^{-1} higher than those with WVN 10, reflecting well the differences in yield of the respective landraces themselves. With this level WVN 3 topcrosses out-yielded the commercial hybrid as well, exhibiting a probably strong heterosis, which has not been further analyzed. Such a good performance of the w/o crosses had not been expected as the introduction of a double recessive grain quality change was never attempted at a landrace level before. An excellent combining ability was proven for the QPM-delivering parental w/o lines, too, their experimental hybrid achieved a yield quite superior to commercial waxy hybrids; a potential suitability as a marketable high protein quality snack was acknowledged but not further explored as the seed production on the two parental lines was low. In all w/o crosses mature kernels were fully filled and smooth but had no vitreous aspect, indicating that an additional modifier gene present in QPM was not expressed in association with the *waxy* gene; according to Dang et al (2011), this is anyhow desirable for acceptance as waxy maize is preferred due to its soft grains. Zhang et al (2013) demonstrated that plump and smooth grains as found here cannot be taken as granted, depressed and wrinkled grains occurred in their studies in specific w/o genotype combinations.

In Experiment 2, 2013, the WVN 3 w/o cross outperformed the WVN 3 w/o backcross in yield and plant height; but significant differences existed just between values of the topcross and the parental landrace (Table 2). As both w/o lines for QPM had a modern agronomic background, a positive residual effect was expected (Dang et al, 2011; Sinkangam et al, 2012). But the yield penalty by backcrossing was relatively small despite the rather low yield level in landraces. The resistance level against foliar diseases, prevalent was southern rust disease, was acceptable in both cross and backcross but WVN 3 had a significantly lower disease resistance than its top- and backcrosses. A clear impact of modern breeding in general vigor compared to landraces has been proven for other maize germplasm as well (Schneider et al, 2011). 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, with less importance of sugar contents. According to Table 2, the Bite test values were all acceptable with 6.3 to 6.7. The protein content of mature grains had reached good levels above 11%, without significant differences between entries, too. Lysine and tryptophan contents are closely correlated in maize grains (Sinkangam et al, 2012). Here the tryptophan content is presented that was at the same very high level in the topcross, similar to the Thai w/o line; but in the backcross the tryptophan content was disappointingly low, not much higher than in the original landrace. The tryptophan analyses were therefore performed on mature grains of the corresponding cross and backcross with the second landrace, WVN 10; the values were in the same range as with WVN 3, with 0.82, 0.61, and 0.41 for topcross, backcross and WVN 10, respectively. 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 amounts 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 Thai QPM background. A successful integration of waxy x QPM could provide an extra advantage for local communities with new market chances selling healthy snacks.

Table 2 - Agronomic and grain quality field data of homozygous waxy x quality protein maize in 2013.

Pedigree	Ear fresh weight kg ha^{-1}	Plant height cm	Foliar. dis (1-5)	Bite test (1-9)	Protein %	Tryptophan/ protein %
WVN 3 (w/o*) Topcross**	7.39	178	2.5	6.7	11.69	0.81
WVN 3 (w/o) Backcross***	6.06	166	2.7	6.7	11.32	0.63
WVN 3 (w)	5.36	148	3.3	6.3	11.32	0.54
LSD (0.05)	1.28	19	0.4	0.9	ns	0.2

Rating score foliar disease (1-5) = 1 is good and 5 is poor. Rating score bite test (1-9) = 1 is poor and 9 is good.* w/o = Homozygous waxy x QPM; ** = Topcross with ETH x Agron; *** = Topcross backcrossed to WV.

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