

Quantitative Genetic Basis of Gelatinization Temperature of Rice

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ABSTRACT

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Gelatinization temperature of rice starch plays an important role in grain quality. We mapped eight loci responsible for the gelatinization parameters of rice: onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH) using a population of 242 recombinant inbred lines (RIL). Eight quantitative trait loci (QTL) were mapped onto the chromosome regions where starch-synthesis related genes are located. The *Wx* gene (granule-bound starch synthase [GBSS]), and the region of chromosome 11 played major roles in gelatinization

trait determination; and other loci were starch-branching enzyme (SBE) genes that modify the two major loci by epistasis. High-level interactions were also detected indicating a *trans* mode of action of the genes. The genetic basis for these parameters was consistent with the relationship between the thermal properties and the structure of the starch granule. This information can be helpful for the improvement of starch-producing crops, including the improvement of rice grain quality, both for food and industrial uses.

Starch gelatinization is a dynamic procedure that involves disruption of the molecular order within the starch granule on heating in excess water above a certain temperature threshold (Atwell et al 1988; Cooke and Gidley 1992; Gidley 1992). The gelatinization temperature (GT), its components, and enthalpy (ΔH) are important parameters. The peak temperature (T_p) gives a measure of crystallite quality (effectively the double helix length). Enthalpy gives an overall measure of crystallinity (quality \times quantity) and may be indicative of the loss of molecular order within the granule (Tester and Morrison 1990; Cooke and Gidley 1992; Hoover and Vasanthan 1994). Routine surveys in rice breeding usually measure GT indirectly as the alkali spreading value (ASV), assessed by extent of dispersal of whole milled rice grains in dilute alkali solution (Little et al 1958). Rice grains can be classified into four groups based on the ASV score: low (6–7), intermediate (4–5), high-intermediate (3) and high (1–2) GT (Juliano 1985). The ASV method has been successfully used in rice quality evaluation, particularly in breeding practice, because it is simple and easy. However, the ASV categories are not accurate enough to distinguish cultivars with minor thermal differences, they even have the same ASV grade by visual assessment. Also, the ASV method is inadequate to explain the multicomponent aspects of rice grain thermal quality (e.g., enthalpy) that are important factors to the food industry. Differential scanning calorimetry (DSC) has been widely used in the thermal analysis of rice starch, flour, and grain (Biliaderis et al 1980, 1986; Russell and Juliano 1983; Normand and Marshall 1989). Not only can DSC give an accurate measurement of T_p , but it can also precisely measure the enthalpy (Morrison 1995).

GT plays an important role in rice quality determination (Webb 1980; Juliano 1985; Tan and Corke *in press*). GT appears to be directly related to rice cooking times, and lower GT is desired for higher quality rice (Juliano and Perez 1983; Unnevehr et al 1992). However, few reports are available on the relationship between enthalpy and rice grain quality. A study of the gelatinization properties of the starch or flour of rice will enhance understanding of the properties of the rice grain and help in quality improvement both for the food industry and the rice breeding industry.

Genetic analysis based on dense linkage maps has become a common and powerful method to detect quantitative trait loci (QTL) (Paterson et al 1988; Lander and Botstein 1989; Tanksley 1993).

With the availability of a high-density linkage map of rice (McCouch et al 1988; Causse et al 1994; Harushima et al 1998), QTL for important agronomic traits (reviewed by Yano and Sasaki 1997), including the quality aspects of rice grain, have been mapped (Huang et al 1997; Redoña and Mackill 1998). Meanwhile, interactions among QTL were reported by several authors who showed that epistasis may play an important role in gene expression (Doebley et al 1995; Lark et al 1995; Cockerham and Zeng 1996; Li et al 1997; Yu et al 1997). Several starch-related genes have been cloned in rice (Wang et al 1990; Nakamura et al 1994; Harrington et al 1997; Fujita et al 1999) and their function in control of starch physical properties has been studied (reviewed by Martin and Smith 1995). These genes are likely to interactively affect starch properties, although few reports are available.

Our previous study showed that the ASV-based GT of rice was controlled by the *Wx* gene, or a tightly linked locus on chromosome 6 (Tan et al 1999). Because different rice cultivars from a worldwide collection (Juliano and Villareal 1993) showed different combinations of amylose content (AC), gel consistency (GC), and ASV, the results need to be confirmed. Moreover, the ASV data could not provide any information about energy during gelatinization, which is another important factor for starch properties and food industry uses. In this report, we use a genome-wide QTL analysis to map the genomic loci and to analyze the possible interactions between the loci responsible for the gelatinization parameters.

MATERIALS AND METHODS

A population of 238 F₁₀ recombinant inbred lines (RIL) derived from 240 F₂ plants by single seed descent (SSD); the two parents, Zhenshan 97 (ZB, maternal) and Minghui 63 (MH, paternal); and their F₁ hybrid were studied. All the materials were planted in the summer rice growing season of 1997 at Huazhong Agricultural University, Wuhan, China. Fields were managed according to normal agronomic procedures until natural ripening of the grain (Xing 1999). Rice grain was harvested and dried to $\approx 14\%$ moisture and stored at room temperature for at least three months. The paddy grain was husked and milled, and ground into flour as described previously (Tan et al 1999).

Differential scanning calorimetry (DSC) was used to characterize the thermal properties of the milled rice flour. A DSC20 instrument (Mettler, Naenikon-Uster, Switzerland) connected with a Mettler TC11 data analysis station was used. Flour (2.0 ± 0.1 mg) was weighed directly into a 40- μ L pan and 5.0 mg of deionized water was added to a final weight of 7.0 ± 0.1 mg of flour-water suspension. The sample pans were sealed and allowed to stand overnight at room temperature, then heated at $10^\circ\text{C}/\text{min}$ from 35 to 110°C . An empty pan was used as reference. The onset temperature (T_o), peak temperature (T_p), completion temperature (T_c), and the enthalpy of gelatinization (ΔH , J/g) were recorded.

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Basic descriptive analysis followed SAS procedures (1988 version, SAS Institute, Cary, NC). The interaction analysis among loci was done with Statistica for Windows (StatSoft, Tulsa, OK).

Linkage map construction consisted of 162 RFLP (restriction fragment length polymorphism) and 19 SSR (simple sequence repeat) markers covering 12 chromosomes (Xing 1999; Tan et al 2000).

The whole genome was scanned for quantitative trait loci (QTL) for the traits using MAPMAKER/QTL 1.0 (Paterson et al 1988; Lincoln et al 1992) with a LOD threshold of 2.4 (Lander and Botstein 1989; van Ooijen 1999). If two or more QTL were detected from the scanning, the QTL with the largest effect was fixed to rescan the whole genome. Additionally, QTL Cartographer v. 1.13 was used for composite interval mapping (Zeng 1993, 1994; Basten et al 1999). Only QTL detected by both methods were retained, and all QTL for a specific trait were combined for the calculation of the total likelihood and variance contribution.

RESULTS

Typical gelatinization profiles of the sample flour (Fig. 1) show that MH had a narrow peak, whereas ZB and the F₁ had wider peak curves, although F₁ was more open than ZB. In a comparison of the parameters (Table I), MH expressed significantly higher values than ZB and the F₁ for all four parameters, except T_o, where MH and ZB were similar. ZB and F₁ had similar values for T_p, T_c, and ΔH, indicating similar properties between the maternal parent and the hybrid. This also implied a dominance-maternal effect for the gelatinization trait, consistent with our former report based on ASV (Tan et al 1999).

To study the genetic basis of the parameters during gelatinization, we first surveyed their distributions. Three parameters showed bimodal distributions with a biased tail that consisted of eight lines of the population. The distribution of ΔH closely followed a normal distribution, but there was still a gap at ≈7.5 J/g (Fig. 2). The bimodal distribution of the parameters implied segregation for a single gene, confirmed by the χ² test (Table II). The biased tails of the distributions suggested possible modification by minor genes.

To detect possible linkage between the markers and parameters, one-way analysis of variance (ANOVA) of the traits was made using the marker genotypes as groups. Results indicated that several chromosome segments may be involved (Table III). The predominant region is around the Wx gene located in the short arm of chromosome 6. The second region for significant loci is near the end of chromosome 11 (Causse et al 1994). The other groups of loci are distributed on chromosome 2, the long arm of chromosome 6 and chromosome 8, respectively (Table III and Fig. 3).

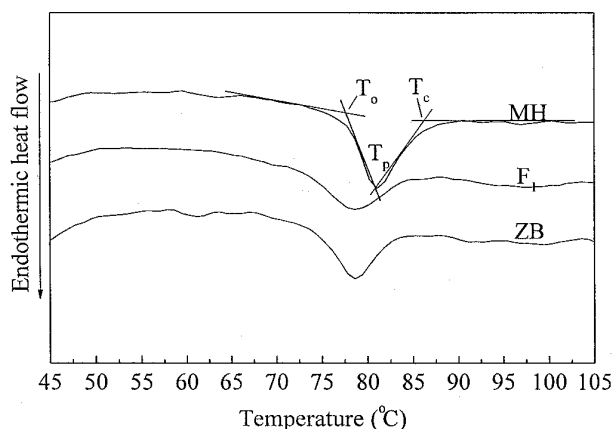


Fig. 1. Typical gelatinization endotherms of parents Minghui 63 (MH) and Zhenshan 97 (ZB) and their hybrid (F₁). Onset (T_o), peak (T_p), and conclusion temperatures (T_c) and gelatinization enthalpy (ΔH).

QTL Mapping

The results from one-way ANOVA imply that more than one gene may be involved, which seems to contradict the bimodal distributions and the χ² test (Fig. 2 and Table II). A whole genome scan was made using the MAPMAKER/QTL1.0 software package to calculate the contribution from all the loci involved (Paterson et al 1988; Lincoln et al 1992). In addition, composite interval mapping (Zeng 1993, 1994; Basten et al 1999) was applied to scan the chromosome regions to confirm the reality of QTL resolved in the chromosome regions. Three main regions were detected on chromosomes 2, 6, and 11, respectively (Table IV and Fig. 3).

It is obvious that the Wx region plays the major role in flour gelatinization behavior with the explained variances ranging from 37.4% (for ΔH) to 53.9% (for T_p). Alleles from MH at the Wx gene all increased the value of T_o, T_p, T_c, and ΔH. The region marked by RZ324 has a small effect on T_o and T_c, with explained variances of 5.6 and 7.2%, respectively. Though LOD for T_o is only 2.2, which is slightly lower than the threshold setting 2.4, we still deduce that this locus had an effect on T_o, which can be confirmed by LOD = 2.9 for T_c from the same region. It is interesting that the allele from MH at this locus decreased values of the parameters, that is, acted in the opposite direction to the Wx gene. The region on chromosome 11 affects T_p and T_c with 5.3 and 5.0% of explained variance, respectively. Alleles from MH at this locus all increase the parameter values in the same direction as the Wx gene. However, two marker regions that were detected as having significant effects on the parameters by one-way ANOVA were not confirmed by interval mapping or composite interval mapping. One is in the region of the long arm of chromosome 6 (marked by RG653), and the other is on chromosome 8 (marked by G1149) (Table IV).

Combining the results of ANOVA, interval mapping, and composite interval mapping, we observed that the detected regions were all in the vicinity of known starch-synthesis related genes. They are the granule-bound starch synthase or Wx gene on the short arm of chromosome 6 (GBSS, EC 2.4.1.11) (Wang et al 1990), Q-enzyme I (QE1) on the long arm of chromosome 6 (marked by RG653/G342) (Nakamura et al 1994), starch-branching enzyme III (SBEIII) on chromosome 2 (marked by RZ324) (Harrington et al 1997), and the isoamylase gene on chromosome 8 (marked by G1149) (Fujita et al 1999) (Table III).

QTL Interaction

Because of the transgressive segregations in the RIL population (Figs. 2 and 3), and previous results based on ASV value (Tan et al 1999), we further analyzed higher level interaction among the markers covering the whole genome. Special attention was focused

TABLE I
Statistical Analysis of Flour Thermal Characteristics of Rice^a

| Source ^b | T _o (°C) | T _p (°C) | T _c (°C) | ΔH (J/g) |
|---------------------|---------------------|---------------------|---------------------|------------|
| MH | 73.7 ± 1.70 | 81.0 ± 0.18 | 88.3 ± 1.30 | 9.6 ± 0.73 |
| ZB | 72.4 ± 0.83 | 78.4 ± 0.12 | 85.1 ± 1.45 | 7.1 ± 0.30 |
| F ₁ | 69.9 ± 1.67 | 78.4 ± 0.14 | 86.2 ± 1.94 | 7.6 ± 0.62 |

^a Onset (T_o), peak (T_p), and conclusion temperature (T_c) and enthalpy (ΔH).

^b Two parents, Zhenshan 97 (ZB, maternal) and Minghui 63 (MH, paternal); and their F₁ hybrid.

TABLE II
Distributions and χ² Tests^a for Thermal Characteristics in Recombinant Inbred Lines (RIL) Showing a Single Locus Segregation Mode

| Trait ^b | Number of Lines | χ ² | P | |
|--------------------|-----------------|-----------------|-------|-------|
| T _o | 113 (≤ 73.8 °C) | 125 (> 73.8 °C) | 0.508 | 0.476 |
| T _p | 108 (≤ 80.5 °C) | 130 (> 80.5 °C) | 1.853 | 0.173 |
| T _c | 113 (≤ 85.4 °C) | 125 (> 85.4 °C) | 0.508 | 0.476 |
| ΔH | 127 (≤ 7.5 J/g) | 111 (> 7.5 J/g) | 0.945 | 0.331 |

^a Chi-square distribution (χ²) and probability distribution (P).

^b Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH). Expected ratio for all characteristics is 1:1.

on the chromosome regions where the starch-synthesis related genes are located.

As amylose content (AC) is also determined by the *Wx* gene (Tan et al 1999), we first surveyed the relationship between AC and T_p . The scatter distribution of AC vs. T_p (Fig. 4) showed two main groups, one composed of lines with high GT ($\approx 82^\circ\text{C}$) but low AC ($\approx 12\%$). The paternal (MH) type and the other group composed of the maternal (ZB) type had lower GT ($\approx 78^\circ\text{C}$) but higher AC ($\approx 30\%$). This bimodal segregation is not surprising because the material is a recombinant inbred line population derived by single seed descent (SSD). However, a third group composed of eight lines was also observed with AC $\approx 26\text{--}34\%$ and $T_p \approx 68\text{--}70^\circ\text{C}$. The T_p value is much lower than that of either of the two parents (Table I). Similar situations were observed between AC and the other parameters (data not shown). As environmental factors can have some effect on GT of starch (Asaoka et al 1985; Tester et al 1999), we first carefully checked the heading date of the eight lines. No significant difference was observed for heading date or days-to-ripening (data not shown), indicating the effect of temperature during the milky stage of grain development could be excluded. We postulated that the phenomenon could be due to the difference of the fine structure of the amylopectin leading to a difference in granule crystallinity. Longer chains of amylopectin branches may act like amylose to bind iodine during potentiometric titration. A large proportion of short "long" chains gives a higher reading of amylose content and leads to the defective crystalline structure contributing to a lower GT (Asaoka et al 1985; Jane et al 1999; Tester et al 1999; Waigh et al 2000b). Another possibility is epistasis of different loci as the synthesis of amylopectin involves many genes besides the *Wx* gene, although we could not exclude a possible mutation during the 10 generations of selfing for this transgression.

We subsequently analyzed interaction between the *Wx* gene and other marker loci spanning the whole genome. No significant interaction was detected between *Wx* and other markers (data not shown) except those linked to other starch-synthesis related genes. Of the significant interactions among the related four marker loci, none was significant by one-way ANOVA or interval mapping (Table V). It is clear that three out of the four interactions are related to the *Wx* gene, and no interaction is observed between two or more starch-synthesis related genes. This indicates that interaction only occurred between genes with relatively large effects and genes with minor effects.

The phenotypes for each of the 16 combinations at the four loci (Table VI) show that the *Wx* gene plays the major role in determining all the parameters. Combinations with AA allele at the *Wx* locus all had significantly higher values than those with BB allele at the *Wx* gene, and no significant difference was detected for each of the parameters among the AA allele groups at the *Wx* locus.

Among the genotypes containing the BB allele of the *Wx* gene, only the BB-AA-BB-BB combination at the four loci (*Wx*, G342, RZ324, and Y6855RA) has significantly lower values than the other seven combinations, except for ΔH . The eight lines with extreme values in the tail of the distributions are included in this group (Fig. 2). Further analysis showed that the lower values could be attributed to these eight extreme lines (data not shown).

The first significant interaction was detected between the *Wx* gene and RZ324 for T_c . At the genotype BB (allele from ZB) of the *Wx* gene, the T_c difference can be 1.1°C between the two alleles of RZ324. At this locus, genotype BB showed higher T_c than genotype AA (allele from MH). The two-locus genotype combination AA-BB (MH allele at *Wx* gene and ZB allele at RZ324) had the highest T_c (Fig. 5A).

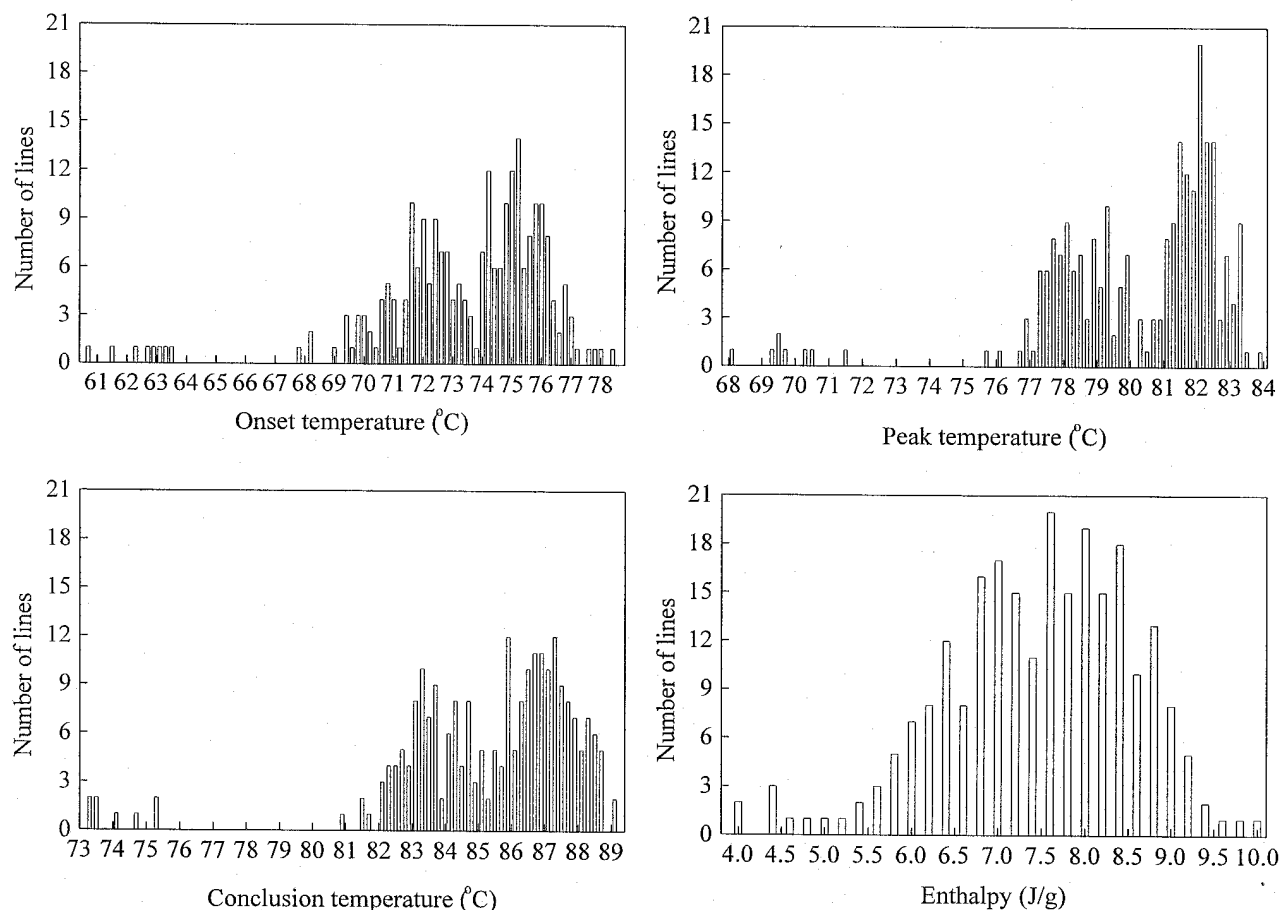


Fig. 2. Distributions of onset (T_o), peak (T_p), and conclusion temperatures (T_c) and gelatinization enthalpy (ΔH) in recombinant inbred lines (RIL).

The second significant interaction was detected between RZ324 and Y6855RA for T_o (Fig. 5B) and T_c (Fig. 5C). For this two-locus combination the genotypes AA at RZ324 and BB at Y6855RA had the lowest T_o and T_c , with differences of 1.8°C and 1.7°C respectively, whereas genotypes BB at RZ324 and AA at Y6855RA showed the highest T_o and T_c , respectively.

Two significant three-way interactions were observed (Fig. 6). The first significant interaction is among the Wx gene, G342, and Y6855RA for T_o . The difference between the two genotypes at each locus of these three markers is significant by one-way analysis (Table III). Though no two-loci interactions among the three markers are significant, the genotype combination BB-BB-BB (alleles at the three loci are all from ZB) has the lowest T_o when compared with any other combinations. When the allele of the Wx gene comes from MH, the lines would show high T_o (AA allele from the Wx gene is sufficient to display high T_o) (Fig. 6). The other three-way interaction is among the Wx gene, RZ324, and Y6855RA for T_o and T_p (Fig. 6B). Similarly, within the BB groups at the Wx locus, the genotype combination BB-AA-BB for Wx , RZ324, and Y6855RA displayed significantly lower T_o (70.5°C) and T_p (76.9°C) than other combinations. No significant difference was observed among the eight groups with allele AA at the Wx gene locus. The results imply that alleles at the Wx gene locus are necessarily critical for the low or high values of the parameters. Alleles from other loci may play some roles in modifying the expression of the Wx gene.

ΔH exhibits slightly different behavior when compared with other parameters. This might be due to the physical differences between the enthalpy and gelatinization temperatures. Morrison (1995) points out that GT are qualitative indices of the crystalline structure, but the enthalpy reflects the molecular order of the double helix (Cooke and Gidley 1992). This difference can also be attributed to the significant difference in the amylopectin (and amylose) content between the two parents leading to the differences in the crystalline structure

and molecular order. Other components such as protein and lipids, etc., may also have some effects. The amylose-lipid complex can act as water inhibitor during swelling and gelatinization (Tester and Morrison 1990a). Removal of lipid and protein can facilitate water entering the interior of the rice kernel, leading to significant changes of starch gelatinization endotherm and the related parameters (Champagne et al 1990; Marshall et al 1990).

DISCUSSION

Starch is composed of amorphous and crystalline regions within the granule, forming the lamellae that can be observed microscopically. A structural model postulated for the starch granule suggests

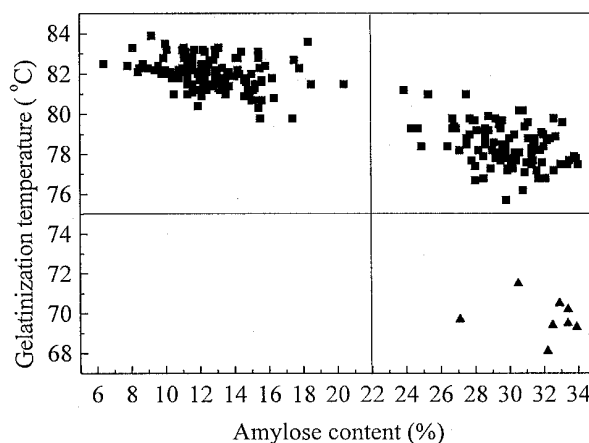


Fig. 4. Scatter distribution of amylose content vs. peak temperature (T_p); ▲ = 8 extreme lines with GT significantly lower than the parents.

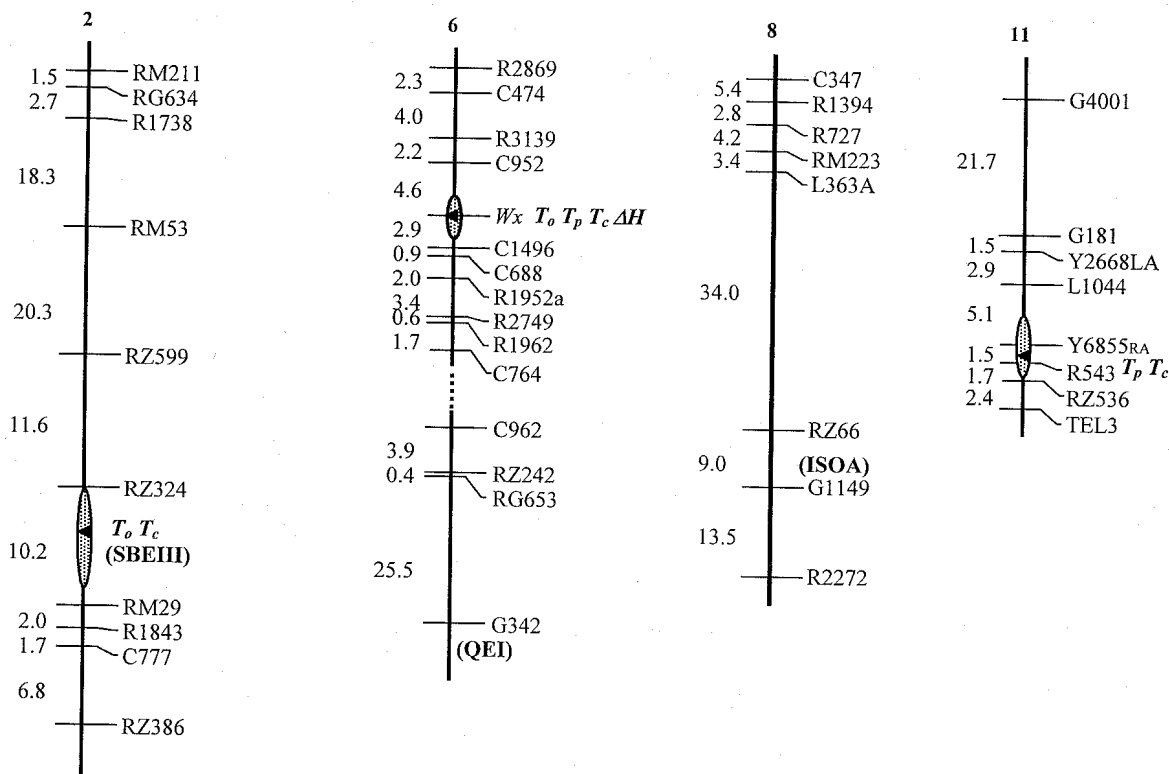


Fig. 3. Quantitative trait loci (QTL) for gelatinization parameters of rice flour (onset [T_o], peak [T_p], and conclusion temperatures [T_c] and gelatinization enthalpy [ΔH]). Only the portions of the linkage maps where QTL were detected are shown. Map distance to left of chromosomes by Kosambi function (Kosambi 1944). Top number indicates chromosome. Ovals indicate 1-LOD support intervals of QTL. Small triangles indicate peaks of LOD contours. Two starch branching enzymes (SBE III and SBE I) and isoamylase gene (ISAO) are indicated in brackets in their approximate positions based on Harrington et al (1997) Nakamura et al (1994), and Fujita et al (1999), respectively.

that the linear chains formed by α -1,4 linkage of the amylopectin are responsible for the crystalline lamella of the granular growth rings, and most of the α -1,6 branches form the amorphous portions (French 1984; Ball et al 1996; Wang et al 1998). Thus, branching leads to the amorphous regions, and the unbranched segments of the comparatively short free ends of A- and B-chains form the crystalline clusters. The disordering of the clusters is the principal process during gelatinization. It has been postulated that high-GT starches have longer chains and, hence, more crystallites than low-GT starches. Low-GT starches have more amorphous and less crystalline material than the high-GT starches. Crystallite perfection may be the principal mechanism controlling GT in the starch (Hizukuri 1985; Tester and Morrison 1990). Biochemically, the structures of the two lamellae are discontinuously synthesized by the joint activities of the starch synthases and the branching enzymes during the development of the starch granules (Manners 1989; Smith and Martin 1995; Ball et al 1996; Wang et al 1998). Our results provide direct genetic evidence supporting this model both for the starch granule structure and the process of gelatinization.

The *Wx* gene coding the granule-bound starch synthase (GBSS, EC 2.4.1.21) is responsible for the synthesis of amylose and amylopectin. The amylose content of rice is controlled by the *Wx* gene, and no evidence is found that other starch-synthesis related genes influence AC (Tan et al 1999). Many reports are available on the individual genes responsible for the specific mechanisms of starch synthesis (reviewed by Smith and Martin 1995). The com-

plex role of GBSS and SBE in amylose and amylopectin synthesis is not fully understood. However, the regulation in *cis* mode within the *Wx* gene of rice has been reported (Wang et al 1995; Bligh et al 1998; Cai et al 1998; Hirano et al 1998; Isshiki et al 1999). In this report, genes with minor effect seem to regulate the expression of the major gene (*Wx* locus in *trans* mode) because the chromosomal locations are different. This is consistent with the result of Kawasaki et al (1996) that the *flo-2* locus *trans*-regulates the starch branching enzyme (SBE3) gene of chromosome 6. However, we did not detect any effect or interaction of the SBE3 with other loci. We note that our results are different from those of Lanceras et al (2000). The loci on chromosome 2 are obviously not allelic because the two QTL intervals are at least 50 cM apart (Causse et al 1994). Although the QTL they detected as having the largest effect was also mapped into chromosome 6, it was located in the vicinity of the *Wx* gene (Table II and Fig. 4 of Lanceras et al 2000). Those differences may be due to the different materials they used (a cross between *indica* and *japonica*, two major subspecies of rice), whereas we used a cross between two *indica* varieties. Another reason might be due to the differences of testing methods between ASV and DSC because ASV is based on categorical visual assessment. Another interesting point is that the segment of chromosome 11 that we detected as playing an important role in the determination of GT was not reported previously. A cluster of resistance genes are located within this region (Causse et al 1994). The nature of the genetic role of this region needs further investi-

TABLE III
One-Way Analysis of Variance of Gelatinization Temperature Parameters of Recombinant Inbred Lines (RIL)
Using Randomly Selected Marker Genotypes as Groups

| Parameter ^a | Locus | Chromosome | MS Effect ^b | MS Error | df Error | F | P | |
|------------------------|--------------------|------------|------------------------|----------|----------|-------|-------|-------|
| T_o | RG173 | 1 | 24.4 | 8.8 | 235 | 2.8 | 0.097 | |
| | RZ599 ^c | 2 | 40.2 | 8.0 | 233 | 5.0 | 0.026 | |
| | RG393 | 3 | 4.7 | 8.8 | 236 | 0.5 | 0.468 | |
| | <i>Wx</i> | 6 | 931.8 | 4.9 | 236 | 190.2 | 0.000 | |
| | RZ667 | 6 | 49.3 | 8.6 | 236 | 5.7 | 0.018 | |
| | RG653 ^d | 6 | 32.6 | 8.7 | 235 | 3.7 | 0.054 | |
| | G1149 ^e | 8 | 43.3 | 8.7 | 236 | 5.0 | 0.026 | |
| | RZ536 | 11 | 67.1 | 8.6 | 236 | 7.8 | 0.006 | |
| | G4001 | 11 | 7.6 | 8.8 | 236 | 0.9 | 0.353 | |
| | T_p | RG173 | 1 | 18.7 | 7.5 | 235 | 2.5 | 0.115 |
| | | RZ599 | 2 | 34.3 | 7.0 | 233 | 4.9 | 0.027 |
| RG393 | | 3 | 8.4 | 7.5 | 236 | 1.1 | 0.291 | |
| <i>Wx</i> | | 6 | 919.1 | 3.6 | 236 | 253.7 | 0.000 | |
| RZ667 | | 6 | 45.8 | 7.3 | 236 | 6.3 | 0.013 | |
| RG653 | | 6 | 32.0 | 7.4 | 235 | 4.3 | 0.039 | |
| G1149 | | 8 | 34.7 | 7.4 | 236 | 4.7 | 0.031 | |
| RZ536 | | 11 | 84.8 | 7.2 | 236 | 11.9 | 0.001 | |
| G4001 | | 11 | 6.1 | 7.5 | 236 | 0.8 | 0.368 | |
| T_c | | RG173 | 1 | 21.1 | 7.2 | 235 | 2.9 | 0.089 |
| | | RZ599 | 2 | 52.3 | 6.5 | 233 | 8.0 | 0.000 |
| | RG393 | 3 | 5.8 | 7.3 | 236 | 0.8 | 0.372 | |
| | <i>Wx</i> | 6 | 835.2 | 3.7 | 236 | 223.1 | 0.000 | |
| | RZ667 | 6 | 43.4 | 7.1 | 236 | 6.1 | 0.014 | |
| | RG653 | 6 | 26.5 | 7.2 | 235 | 3.7 | 0.056 | |
| | G1149 | 8 | 27.1 | 7.2 | 236 | 3.8 | 0.053 | |
| | RZ536 | 11 | 75.0 | 7.0 | 236 | 10.8 | 0.001 | |
| | G4001 | 11 | 5.8 | 7.3 | 236 | 0.8 | 0.373 | |
| | ΔH | RG173 | 1 | 4.7 | 1.3 | 235 | 3.7 | 0.056 |
| | | RZ599 | 2 | 2.6 | 1.3 | 233 | 2.1 | 0.150 |
| RG393 | | 3 | 0.1 | 0.8 | 236 | 0.1 | 0.763 | |
| <i>Wx</i> | | 6 | 109.1 | 1.2 | 236 | 131.1 | 0.000 | |
| RZ667 | | 6 | 10.8 | 1.3 | 236 | 8.6 | 0.004 | |
| RG653 | | 6 | 8.4 | 1.3 | 235 | 6.7 | 0.010 | |
| G1149 | | 8 | 0.0 | 1.3 | 236 | 0.0 | 0.967 | |
| RZ536 | | 11 | 8.0 | 1.3 | 236 | 6.4 | 0.012 | |
| G4001 | | 11 | 0.3 | 1.3 | 236 | 0.3 | 0.606 | |

^a Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH).

^b Mean square effect. One degree of freedom associated with the test for each effect.

^c Marker from the short arm of chromosome 2 where the SBE III is located (Harrington et al 1997).

^d Marker from the distal end of chromosome 6 where the gene for SBE I is located (Nakamura et al 1994).

^e Marker from the short arm of chromosome 8 where the gene for Isoamylase is located (Fujita et al 1999).

gation. Understanding the genetic mechanism of the starch synthesis as well as the starch granule structure could be accelerated after the whole rice genome sequence is available.

The *Wx* gene plays a major role in determining gelatinization parameters, with modification by other genes. This strongly implies the existence of genic interactions (epistasis), which has also been detected by Lanceras et al (2000) and for several other traits in rice including grain yield and its components (Li et al 1997; Yu et

al 1997) and fertility (Wang et al 1998). The magnitude of the gene interaction is not strong. The variance explained by the loci other than the *Wx* locus is relatively small, and the probability of false positives for interactions between or among the loci are relatively high. This may be due to both the experiment design and the method. Although a population size of 242 RIL lines was used, a skewed distribution in the population was detected (Yu et al 1997; Xing 1999). The use of flour rather than purified starch could introduce

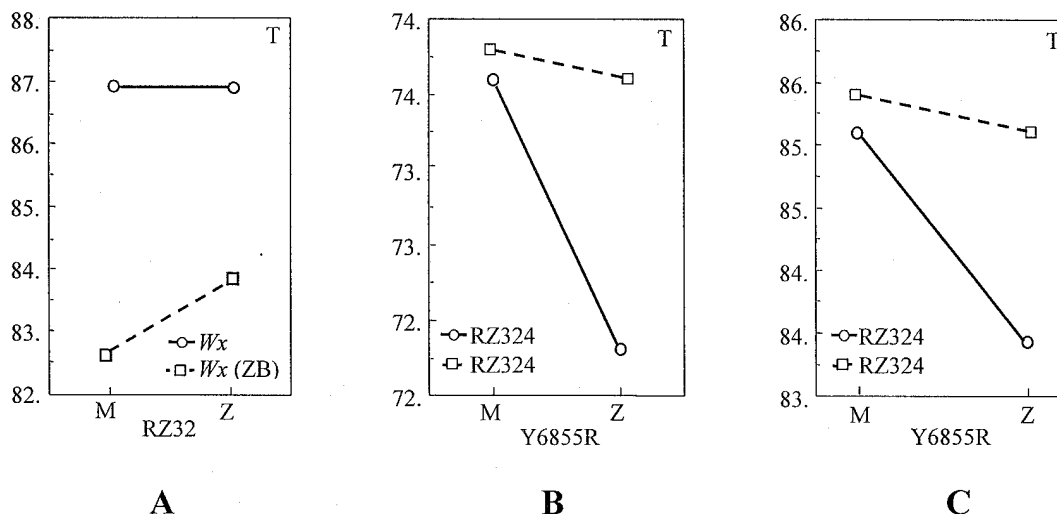


Fig. 5. Interaction between two loci of parents Minghui 63 (MH) and Zhenshan 97 (ZB) conditioning onset temperature (T_0) by *Wx* and RZ322 (A), and T_0 by Y6855RA and RZ322 (B), and conclusion temperature (T_c) by Y6855RA and RZ322 (C), respectively.

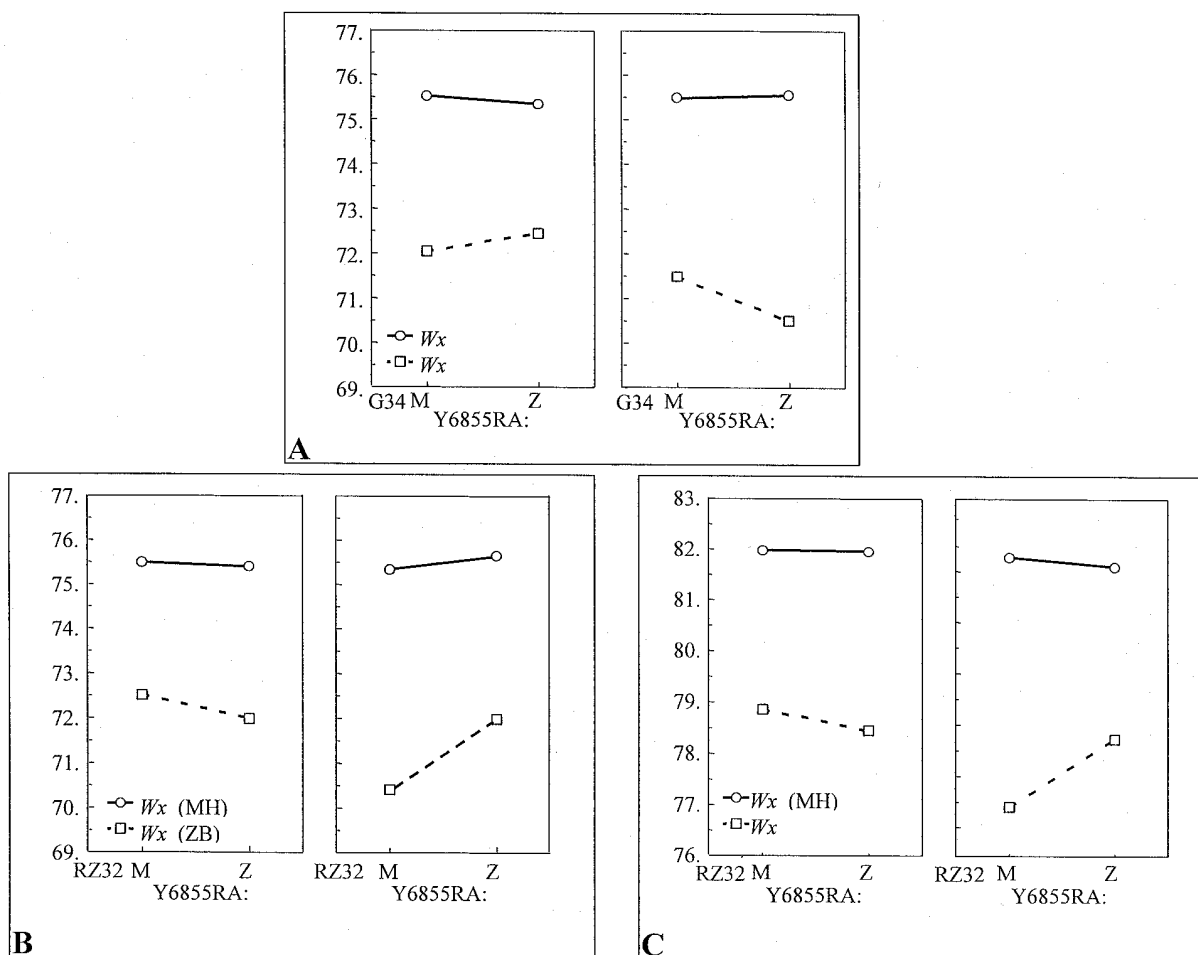


Fig. 6. Interaction among three loci of parents Minghui 63 (MH) and Zhenshan 97 (ZB) conditioning the onset temperature (T_0) by *Wx*, Y6855RA and G342 (A), and among three loci conditioning T_0 (B) and peak temperature (T_p) (C) by *Wx*, Y6855RA, and RZ322.

bias because gelatinization of starch is affected by factors such as lipid and protein (French 1984; Tester and Morrison 1990a; Gidley 1992; Morrison 1995). The *Wx* gene is also responsible for the protein content of the rice flour (Tan et al 2000). The disposition of lipid and protein in the granule are not well known but could partially account for the transgressive distributions of the parameters. Removal of lipid and protein can lower the parameters (Champagne et al

1990; Marshall et al 1990). The roles of lipid and protein should be further investigated.

Gelatinization temperature of rice grain plays an important role in rice grain quality (Juliano 1985). The genetic basis can be resolved into the starch-synthesis related genes, providing the potential for quality improvement by biotechnology. This could include breeding with marker-assisted selection focused on the genes concerned.

TABLE IV

Quantitative Trait Loci (QTL) Information for Thermal Characteristics^a of Rice Flour by Interval Mapping with Recombinant Inbred Lines (RIL)^b

| Trait/Chromosome | Interval | A ^c | LOD | VE (%) ^d | Positive Allele | |
|------------------|----------|----------------|-------|---------------------|-----------------|----|
| T_o | 2 | RZ324 – RM29 | 1.40 | 2.2 | 5.6 | ZB |
| | 6 | C952 – Wx | -4.10 | 31.3 | 47.3 | MH |
| | Total | | | 31.7 | 48.0 | |
| T_p | 6 | C952 – Wx | -4.03 | 38.2 | 53.9 | MH |
| | 11 | Y6855RA– R543 | -1.26 | 2.8 | 5.3 | MH |
| | Total | | | 40.0 | 55.5 | |
| T_c | 2 | RZ324 – RM29 | 1.44 | 2.9 | 7.2 | ZB |
| | 6 | C952 – Wx | -3.89 | 35.4 | 51.7 | MH |
| | 11 | Y6855RA – R543 | -1.20 | 2.6 | 5.0 | MH |
| | Total | | | 37.7 | 54.3 | |
| ΔH | 6 | C952 – Wx | -1.40 | 23.12 | 37.4 | MH |
| | Total | | | 23.12 | 37.4 | |

^a Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy (ΔH).

^b All intervals with QTL peak LOD > 2.0. Positive additive value indicated contribution from allele of Zhenshan 97 (ZB) whereas negative from allele of Minghui 63 (MH).

^c Additive effect.

^d Phenotypic variance explained by QTL.

TABLE V

Results from Analysis of Variance Using Marker Genotypes as Groups at Four Marker Loci

| Effect | T_o | | | T_p | | | T_c | | | ΔH | | |
|-------------|------------------------|-------|-------|-----------|-------|-------|-----------|-------|-------|------------|-------|-------|
| | MS Effect ^a | F | P | MS Effect | F | P | MS Effect | F | P | MS Effect | F | P |
| 1 (Wx) | 752.9 | 186.0 | 0.000 | 744.7 | 259.9 | 0.000 | 654.2 | 209.0 | 0.000 | 89.1 | 115.4 | 0.000 |
| 2 (G342) | 1.0 | 0.3 | 0.613 | 5.9 | 2.0 | 0.154 | 4.3 | 1.4 | 0.244 | 3.1 | 4.0 | 0.046 |
| 3 (RZ324) | 10.6 | 2.6 | 0.106 | 7.0 | 2.4 | 0.119 | 19.4 | 6.2 | 0.013 | 0.3 | 0.3 | 0.555 |
| 4 (Y6855RA) | 8.5 | 2.1 | 0.148 | 14.7 | 5.1 | 0.024 | 10.1 | 3.2 | 0.074 | 0.4 | 0.5 | 0.484 |
| 1×3 | 6.6 | 1.6 | 0.201 | 10.6 | 3.7 | 0.055 | 16.7 | 5.3 | 0.022 | 1.5 | 2.0 | 0.158 |
| 3×4 | 21.8 | 5.4 | 0.021 | 8.4 | 2.9 | 0.089 | 13.3 | 4.3 | 0.040 | 1.2 | 1.6 | 0.206 |
| 1×2×4 | 16.6 | 4.1 | 0.044 | 7.4 | 2.6 | 0.111 | 2.8 | 0.9 | 0.342 | 0.0 | 0.1 | 0.821 |
| 1×3×4 | 16.8 | 4.2 | 0.043 | 14.4 | 5.0 | 0.026 | 9.4 | 3.0 | 0.085 | 1.2 | 1.6 | 0.206 |
| Error | 4.0 | | | 2.9 | | | 3.1 | | | 0.8 | | |

^a There is one degree of freedom for each group as there are only two genotypes at each marker loci, and 222 degrees of freedom for error for all the parameters. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy (ΔH).

TABLE VI

Parameters of Gelatinization Temperature^a Using Four Loci Combinations

| Waxy | G342 | RZ324 | Y6855RA | N ^b | T_p | T_o | T_c | ΔH |
|----------------|------|-------|---------|----------------|--------------------|-------|-------|------------|
| A ^c | A | A | A | 17 | 82.2a ^d | 75.9a | 87.3a | 8.3a |
| A | A | A | B | 18 | 81.7a | 75.1a | 86.6a | 8.0a |
| A | A | B | A | 23 | 81.8a | 75.4a | 86.8a | 7.8ab |
| A | A | B | B | 17 | 81.8a | 75.8a | 87.1a | 8.1a |
| A | B | A | A | 12 | 81.8a | 75.0a | 86.9a | 7.9a |
| A | B | A | B | 9 | 82.1a | 75.8a | 86.9a | 7.9a |
| A | B | B | A | 23 | 82.2a | 75.5a | 87.3a | 8.2a |
| A | B | B | B | 13 | 81.6a | 75.4a | 86.7a | 7.7ab |
| B | A | A | A | 12 | 78.5b | 72.0b | 83.6b | 6.9c |
| B | A | A | B | 19 | 77.8b | 71.2b | 82.9b | 6.7cd |
| B | A | B | A | 10 | 78.7b | 71.6b | 83.9b | 6.7cd |
| B | A | B | B | 7 | 79.0b | 72.3b | 84.4b | 7.2bc |
| B | B | A | A | 9 | 78.7b | 72.7b | 83.6b | 6.6cd |
| B | B | A | B | 24 | 75.8c | 69.2c | 81.2c | 6.0d |
| B | B | B | A | 14 | 78.4b | 72.3b | 83.8b | 6.7cd |
| B | B | B | B | 11 | 78.1b | 72.2b | 83.9b | 6.7cd |

^a Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy (ΔH).

^b Number of lines in the population.

^c Genotype “A” at each locus indicated an allele from Minghui 63 and “B” indicated an allele from Zhenshan 97, respectively.

^d Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Though amylose content plays a major role in rice grain quality, the other loci should not be neglected. Using closely linked DNA markers, target chromosome segments can be homologously and simultaneously replaced after a short period of selection (Tanksley et al 1989). A second method could be genetic engineering by modifying the gene structure or its regulation. Visser et al (1991, 1993) reported that the amylose content of potato can be reduced through antisense RNA. Edwards et al (1999) have generated a chimeric antisense construct to reduce the starch synthase activity in amylopectin synthesis. These examples illustrate the potential for starch modification through genetic engineering, although other factors could not be neglected, such as the structure of the starch granule, the effects of physical principles of self-organization of the higher order structure of starch granules, and the process of gelatinization (Waigh et al 2000a,b).

Rice is a model organism in molecular biology. With the available sequence of the *Wx* gene (Wang et al 1990), and now the whole genome, the genetic basis of amylose content and GT, as well as the roles of the other genes involved, should be easier to understand. In turn, this can help us to understand the structure and properties of the rice starch with implications for understanding starch synthesis in other *Graminae*, and in other starch-producing crops such as potato, cassava, and peas (Martin and Smith 1995). This information can be helpful for the improvement of starch-producing crops including the improvement of rice grain quality, both for food and industrial uses.

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