Germplasm Enhancement and Breeding Strategies for Crop Quality in Japan

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Abstract: Rice is the staple food for most Asians. Breeding efforts at the national and international levels have resulted in high-yielding varieties with resistance/tolerance to biotic and abiotic constraints. Consequently Asia has enjoyed rice self-sufficiency in recent years. Now in some countries over-production of rice has occurred, partly because of reduced rice consumption. For instance, in 1962 Japan had a per capita rice consumption of 118.3 kg and then this rapidly declined to about 60 kg in 2003. Imbalances between production and consumption in rice and other crops have promoted a paradigm shift of breeding objectives oriented from producers to consumers. Germplasm enhancement (pre-breeding) and breeding strategies now focus on a broad range of crop and food qualities, which are closely associated with industrial and processing properties and human health and nutrition. In particular, physiological functions of chemical compounds involved in crop products are being studied as a part of breeding programs. Diverse plant genetic resources and advances in plant genome research have contributed to successful breeding strategies to improve and manage crop and food quality. Recent progress in germplasm enhancement and breeding strategies for quality improvement of rice, wheat, soybean and sweet potato in Japan is discussed.

Key words: Genetic improvement, Rice, Soybean, Sweet potato, Wheat.

This paper summarizes recent progress in breeding strategies for improving processing quality and nutritional values of rice, wheat, soybean and sweet potato in Japan. The physiological function of foods is also a recent highlight in breeding science and technology. Diverse plant genetic resources and advances in breeding tools have been integrated to accelerate breeding efficiency and effectiveness.

Rice

Since the late 1980’s as a result of over production of rice in Japan, rice breeding has focused on new grain types to diversify rice processing and use. Amylose content in starch deposited in rice endosperm is closely related to cooking and eating quality of rice and varies depending on genotypes and environmental factors. Lower amylose content is related to the stickiness of cooked rice and East Asian people prefer stickier rice than people from South Asia. A gene, du, lowering amylose content was screened from induced mutant stocks (Okuno et al., 1983) and used as a gene source in rice breeding programs throughout Japan. Two different genes, du and Wx alleles, have been used to broaden the genetic variation of amylose content. The function of the wx locus is structural for starch granule-bound glucosyltransferase that is responsible for amylose synthesis; whereas du genes regulate gene expression of the non-waxy allele at the wx locus. Since the first release of a low amylose
variety in 1991, 17 new low amylose varieties have been developed. Amylose content is dramatically affected by temperature during the early grain filling period, especially 20 days after flowering, suggesting that the expression of du and Wx alleles are sensitive to temperatures during the grain filling period from anthesis to 20 days after flowering (Asaoka et al., 1989, Okuno et al., 1993). Since the response of du and Wx alleles are sensitive to temperature during the grain-filling period, growing location is a key factor in selecting the most suitable genes to lower amylose content. For uniform rice quality it is necessary to grow rice adapted to different temperature conditions. Five du loci have been identified (Yano et al., 1988) and choosing allelic combinations at the wx locus and du loci can provide varieties that produce a range of variation in amylose content in rice endosperms.

Storage proteins in rice endosperms are accumulated in two different protein bodies, PBI and PBII. The digestibility and protein polypeptides accumulated in PBI and PBII are quite different. Glutelin is a primary protein polypeptide and accounts for 80% of the total storage protein. Glutelin is easily digested and is accumulated in PBI. Prolamine is a major protein polypeptide and accounts for 20% of total rice storage protein. Prolamine is difficult to digest and is accumulated in PBI. In Japan the number of patients who suffer from kidney disease and require dialysis is estimated to be about 650,000. As kidney disease patients requiring dialysis are limited in their daily protein intake, either reduced content of total protein or easily digested glutelin polypeptide is required in their diets. Low glutelin rice varieties have been developed (Kumamaru et al., 1988, Iida et al., 1993) and tested for their effect on patients suffering from kidney disease. Varieties with a lower content of both glutelin and amylose were also bred to improve eating quality. Food allergies cause human health problems. Allergy against rice protein is caused by 16kDa globulin, a major allergen in rice grains. A mutant with lower 16kDa globulin content has been produced (Iida and Nishio 1993), however, its effect on allergy suppression is uncertain.

Rice mutants with giant embryos have been induced (Satoh and Omura 1981) and are being used to develop rice with new physiological functions. Gamma-aminobutyric acid (GABA) which is a free amino acid and functions as an inhibitory neuro-transmitter, accumulates mainly in rice embryos. There is more GABA in pre-germinated brown rice than dried brown rice (Saikusa et al., 1994a). The content of GABA rapidly increases when water is imbibed by rice embryos (Saikusa et al., 1994b). The weight of embryos in giant embryo varieties is 7-8% of whole grain, which is about three times larger than that of normal rice embryos. The amount of GABA in giant embryos is three times as much as that in normal embryos. GABA is effective in normalizing blood pressure and aids recovery from disorders of the autonomic nervous system. Germinating brown rice, containing higher content of GABA, has been increasingly commercialized as physiologically functional food material. Commercial varieties with giant embryos adapted to a wide range of local environments in Japan have been produced. Since a total of 33 millions people in Japan are estimated to suffer from high blood pressure, new rice varieties with giant embryos are expected to assist normalize blood pressure as part of the diet of these people.

Colored and aromatic rice varieties have also been developed using exotic germplasm. Purple-black and red colored rices contain anthocyanin and tannin in the pericarp of their rice grains. These rice varieties are rich in vitamins, iron and other minerals and may contribute to diversified uses of rice.

Recent rice breeding programs in Japan have also focused on the development of new varieties for whole crop silage (WCS). Cultivation of WCS rice is a key technology for using paddy fields for new rice products. In Japan WCS rice in 2003 covered 4900ha of paddy fields compared with 500ha in 2000. Rice varieties for WCS are characterized by high yield of total rice plants, resistance to diseases and insects, high adaptability for heavy manuring culture and high quality as a cattle feed. A total of four WCS varieties for early, medium, medium-late and late maturing cultures have been released to farmers and can be cultivated in northern and southern parts of Japan. Total plant yield in WCS varieties shows an increase of 12-40% compared with grain varieties. Total digestible nutrient (TDN) content of WCS rice varieties is similar to that of ordinary varieties. However, TDN yield in WCS varieties was about 10t/ha, an increase of 20-30% compared with 8.4t/ha in grain varieties. Feed efficiency for milk production of cows was compared between WCS rice and the forage grass, timothy (Phleum pratense). Using WCS rice as forage, cows produced 26.5kg/day of milk, which was comparable to milk production (28.5kg/day) when timothy was used for feeding cows as forage. The quality of milk produced using WCS rice to feed cows was better than that produced using timothy. It seems that cultivation of WCS rice varieties in paddy fields is a promising practices for future rice cropping in Japan to efficiently use paddy fields and resolve the problem of over production of rice and under production of forage crops.

Wheat

Low amylose and glutinous (waxy) wheat varieties are expected to meet new demands from consumers and the flour industry. The major portion of wheat flour is storage starch consisting of 75% amylopectin and 25% amylose. Amylose content affects the
glutinosity of Japanese 'udon' noodles. Glutinosity is a key factor in the palatability or textural quality of Japanese noodles in which some degree of stickiness is desirable. The level of Wx protein bound to starch granules is correlated to the amylose content. A modified SDS-PAGE can separate the wheat Wx proteins into two bands, a high molecular weight band consisting of the Wx-A1 protein and a low molecular weight band comprising two proteins, Wx-B1 and Wx-D1 (Nakamura et al., 1992). The geographical distribution of nulls for Wx-A1, B1 and D1 proteins was determined in 2,000 wheat accessions (Yamamori et al., 1994). Gel electrophoretic analysis revealed that the null allele for the Wx-A1 protein occurred frequently in Korean, Japanese and Turkish varieties. About 48% of varieties from Australia and India were deficient for the Wx-B1 protein. Only one Chinese variety lacked the Wx-D1 protein. While 9 Japanese varieties were deficient in both the Wx-A1 and Wx-B1 proteins, no varieties lacked both the Wx-A1 and Wx-D1 proteins, both the Wx-B1 and Wx-D1 proteins or all the three Wx proteins. On the basis of the presence or absence of three Wx proteins, wheat varieties can be classified into 8 types with different amylose content (Table 1) (Nakamura et al., 2002). The relation between amylose content and starch pasting properties was analyzed using varieties classified into the 8 wheat types (Yamamori and Quynh 2000). Densitometric analysis indicated that the amylose content was related to the amount of Wx protein in the 8 types. Parameters in the Rapid Visco Analyzer test (RVA) and swelling power (SP) were correlated to amylose content. Consequently, amylose content and pasting properties of endosperm starch were influenced most by the lack of the Wx-B1 protein, followed by lack of Wx-D1 and Wx-A1 proteins, suggesting the differential effects of the three null alleles for the Wx proteins. Amylose content and parameters based on RVA and SP are related to eating quality such as softness and elasticity of Japanese noodles. The results suggest that lower amylose flour or starch with higher peak viscosity in RVA and higher SP gained higher eating quality scores in noodle sensory tests. Mechanical properties of noodles were also analyzed using near-isogenic lines with different Wx protein deficiency (Ishida et al., 2003). The results indicated that mechanical properties of noodles are primarily determined by the amylose content of flour and properties of starch gel. Sensory evaluation of noodles showed that the noodles made using single-null types lacking either Wx-B1 or Wx-D1 proteins and double-null type lacking both Wx-A1 and Wx-D1 proteins had desirable textures for noodle making. Future research will focus on detecting combinations of the three null alleles for Wx proteins that influence improved noodle-making quality through differential control of amylose content in wheat starch or flour.

Waxy (amylose-free) wheat variety was developed

| Types | Wx-A1 | Wx-B1 | Wx-D1 | AM(%) |
|-------|-------|-------|-------|-------|
| Type 1 | +     | +     | +     | 28.7  |
| Type 2 | -     | +     | +     | 28.5  |
| Type 3 | +     | -     | +     | 27.1  |
| Type 4 | +     | -     | -     | 28.0  |
| Type 5 | +     | -     | -     | 19.8  |
| Type 6 | +     | -     | -     | 25.8  |
| Type 7 | +     | -     | -     | 22.9  |
| Type 8 | -     | -     | -     | 0.9   |

* Percentage based on 100 mg flour.
from the cross between the low amylose Japanese variety, Kanto 107, lacking Wx-A1 and Wx-B1 proteins and the Chinese variety, Bai Huo, missing Wx-D1 protein (Hoshino et al., 1996, Nakamura et al., 1995). Waxy starch of wheat flour is expected to contribute to improving glutinosity, resistance to gel formation and retrogradation. Waxy wheat will be used as materials for enhancing qualities of noodle and bread, for new food diversification and non-food use. The viscoelastic properties and molecular structure of the starch isolated from waxy (amylose-free) hexaploid wheat have been investigated (Hayakawa et al., 1997). Waxy starch generally showed lower gelatinization onset temperature, peak viscosity, and setback than starch isolated from normal hexaploid wheat. Differential scanning calorimetry (DSC) showed that waxy wheat starch had higher transition temperatures and enthalpy than normal wheat starch. However, enthalpy of waxy wheat starch based on amylpectin content was almost the same as that of its parental varieties. Typical A-type X-ray diffraction pattern, higher crystallinity and greater retrogradation resistance also characterized waxy wheat starch. There was little difference in the structure of amylpectin between waxy line and its parental varieties, Kanto 107 and Bai Huo. Further studies are needed to exploit the physicochemical properties specified for waxy starch of wheat compared to waxy starch of maize and rice.

**Soybean**

Since 1995 soybean-growing areas have rapidly increased in Japan due to rice production controls. Most of soybean crops are cultivated in fields converted from paddy fields. A total of one million tons of soybeans are consumed for food use and 4 million tons of soybeans are for non-food uses. Japanese soybean production accounts for one-fourth of soybeans consumed for food use (240,000 tons). The production of soybean has increased 3-4 times over the past decade. Domestic soybeans are mainly used as raw material for traditional processed food such as tofu, miso and natto. The production of soybean in Japan has the following constraints; yield instability, high production costs and food processing quality. Since the lectin deficient variation was discovered, germplasm screening has searched for null mutations for trypsin inhibitors, lypoxygenase isoforms, globulin subunits and acetyl saponins.

Soybean seeds contain three isozymes of lipoxygenase (LOX, L-1, L-2, L-3) that catalyzes peroxidation of lipids and is related to the formation of volatile compounds causing unpleasant soybean flavor. Varieties lacking each of these three lipoxygenase isozymes have been detected. There is a clear difference in the formation of volatile flavor compounds between tofu produced using normal and LOX-deficient soybeans. The deficiency for each LOX isozyme is independently controlled by a single gene (Hildebrand and Hymowitz 1982, Kitamura et al., 1983, 1985). Since 1996, several LOX deficient varieties have been released from soybean breeding programs in Japan (Hajika et al., 1991). These varieties showed no pleiotropic effects on main agronomic traits. LOX-free soybeans are useful raw material for the production of soymilk, tofu and other soybean fresh products. One of the problems in post-harvest preparation of LOX deficient varieties is the contamination between LOX deficient and normal soybean seeds. 7S and 11S globulin protein accounts for 70% of total storage proteins in soybean seeds. 7S globulin (beta-conglycinin) consists of alpha, alpha’ and beta subunits, while 11S globulin forms disulfide bond comprising group I and II subunits. 11S globulin contains more sulphur-containing amino acids compared with 7S globulin. There is also a difference in the content of sulphur-containing amino acids between the 2 groups of 11S globulin. Soybean breeding has focused on decreasing 7S globulin or increasing 11S globulin to improve amino acids constitution in soybean seeds. Mutations lacking alpha and alpha’ subunits of 7S globulin were induced by gamma-ray treatment (Takahashi et al., 1994) and were released as a higher content 11S globulin variety, Yumeminori, in 2001. This variety is characterized by 30-50% higher content of the sulfur-containing amino acids, methionine and cystine compared to standard soybean varieties.

Main allergenic proteins in soybean are Gly m Bd30K, Gly m Bd28K and 7S globulin alpha subunits. Yumeminori has a lower content of allergenic proteins, and particularly it lacks one of three main allergenic proteins, Gly m Bd28K. As disulfide bonds formed between Gly m Bd30K and 7S globulin alpha/alpha’ subunits in normal soybeans, treatment by reducing agents is needed to remove the allergen from proteins in soybean processing. However, in Yumeminori which is deficient in both Gly m Bd28K protein and 7S globulin alpha/alpha’ subunits, treatment with reducing reagents is unnecessary to remove allergenic Gly m Bd30K protein. Low allergen soybean can be used as diet for soybean-allergic patients and also as raw materials to produce allergen free soybean food products.

Research on soybean quality has recently focused on genetic variability of tocopherol composition. Tocopherols are fat-soluble vitamin E and protect food from oxidation. Tocopherols consist of alpha, beta, gamma and delta tocopherols with different vitamin E activity. Soybean oil is high in total content of tocopherols but its major component is gamma-Toc with only 5% in alpha-Toc which has highest vitamin E activity. Germplasm screening has succeeded in discovering germplasm that has increased content of alpha-Toc (Kitamura et al., personal communication).
Sweet potato

Sweet potato is a major root crop in Asia. Japan produces about 1,300,000 ton of sweet potato annually and has a world ranking of 5th in sweet potato production. The area of sweet potato cultivation has been decreasing and by year 2000 only 50,000ha were planted. In contrast, the yield of sweet potato has rapidly increased to 25 t/ha. Since World War II sweet potato production in Japan has changed emphasis from home consumption to industrial or pre-processing use. A recent trend in consumption of sweet potato is its use as a vegetable and for processing. Breeding strategies for sweet potato have shifted from emphasizing the crop grower to the consumer. The key words in sweet potato breeding are quality improvement for human health and convenience. Regarding human health breeding efforts have been made to improve the content of functional components such as anthocyanins, beta-carotene, polyphenols, dietary fibers, vitamins and minerals. New varieties with higher anthocyanin content have been released. These varieties showed physiological functions associated with anti-oxidation. Purple sweet potato varieties have contributed to creating new processed foods. As leaves and stems of sweet potato are rich in vitamins, minerals and polyphenols, varieties suitable for eating fresh as a green vegetable and food processing have been also developed. Leaf extracts from one variety, Suioh, showed strong anti-mutagenic activity. Leaf extracts from sweet potato were compared with those from spinach, lettuce and cabbage regarding inhibitory effect of their extracts on HIV virus proliferation. The result indicated sweet potato extract most effectively prevented HIV virus from proliferation (Islam et al., 2002).

Convenience in terms of sweet potato breeding refers to speed and sweetness in cooking. Sweetness of sweet potato is mainly derived from maltose that is formed from starch by beta-amylase. Normal sweet potato starch is gelatinized above 70°C. Beta-amylase acts on gelatinized starch but it lacks this activity at around 70°C. Starch with lower gelatinization temperature or heat tolerant beta-amylase is required for quick and sweet cooking property. A newly released variety, QuickSweet, has much lower pasting temperature and unique DSC gelatinization properties compared with standard varieties. This variety can be cooked in a shorter steaming time, probably due to the lower pasting temperature of its starch caused by the change in the structure of amylopectin. Sweet potato starch with lower gelatinization temperature may be useful material for the starch industry because of reduced energy input when starch is converted into alcohol or biodegradable plastic.

Conclusions

Crop breeding in Japan has used a number of approaches, including germplasm screening and mutagenesis to find new and useful properties in major crops. Successful incorporation of these useful traits using traditional breeding methods assisted by new biotechnologies is enabling Japanese agricultural scientists to rapidly address changing demands from industry and consumers in a “convenience-store age”.

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