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. 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.

Media summary

Recent progress in Japan in germplasm enhancement and breeding for improving quality in rice, wheat, soybean and sweet potato is discussed.

Key words

rice, wheat, soybean, sweet potato, genetic improvement

Introduction

Asia covers geographically broad crop-growing areas from the tropics to temperate regions. Crops that originated in Asia are widely cultivated in diverse environmental conditions. Sustainable farming practices over millennia have enabled high human populations with different lifestyles to be supported.

Rice is the staple food for most Asians. Since the Green Revolution in the 1960's, breeding efforts in Asia have provided high-yielding varieties with resistance/tolerance to biotic and abiotic constraints. As a result many countries that were previously importing rice have become self-sufficient for rice, or rice exporting. In Japan, economic development has resulted in a major change in rice consumption habits. In 1962, per capita consumption of rice was 118.3 kg and then it rapidly declined to about 60 kg in 2003. Future crop improvement in Asia will follow two trends. Firstly, increasing crop production to satisfy future demand for food security where this has not been achieved. Secondly, improving crop quality to satisfy changes in lifestyles to provide high-quality food. Recent research has been directed to the physiological function of crop and food constituents. Research has also focused on how crop improvement can address some major public health problems. In Japan there has been a paradigm shift in thinking concerning future food demand that will have a great impact on germplasm enhancement (pre-breeding) and breeding strategies for strengthening or improving food quality of crops.

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 endosperms 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, PB1 and PB2. The digestibility of protein polypeptides accumulated in PB1 and PB2 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 PB2. Prolamine is a major protein polypeptide and accounts for 20% of total rice storage protein. Prolamine is difficult to digest and is accumulated in PB1. 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 16 kDa globulin, a major allergen in rice grains. A mutant with lower 16 kDa 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), a free amino acid that 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 represent 7-8% of whole grain, which is about three times larger than 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 a 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 million people in Japan are estimated to suffer from high blood pressure, new rice varieties with giant embryos are expected to assist normalizing 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 4900 ha of paddy fields compared with 500 ha 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 rice varieties shows an increase of 12-40% compared with rice 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 1 t/ha, an increase of 20-30% compared with 0.84 t/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 26.5 kg/day of milk and this was comparable to milk production when timothy was used for feeding cows as forage, 28.5 kg/day. 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 practice 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 nine 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 eight types with different amylose content (Table 1) (Nakamura *et al.* 2002). Compared with 28.7% in Type 1 with three Wx proteins, the amylose content in Type 3 (deficient in Wx-B1), 5 (deficient in Wx-B1 and Wx-D1), 6 (deficient in Wx-A1 and Wx-D1) and 7 (deficient in Wx-A1 and Wx-B1) significantly decreased to 19.8-27.1%. Type 8 (waxy or glutinous wheat), lacking all the three Wx proteins, was not detected through evaluation of wheat germplasm collections. A Chinese variety, Bai Huo, was found to lack the Wx-D1 protein, making it possible to breed a waxy or glutinous phenotype in cross combination with type 7 varieties which lack Wx-A1 and Wx-B1 proteins. Wx-B1 protein has a greater affect on decreasing amylose content in wheat endosperm starch than Wx-A1 and Wx-D1 proteins.

Table 1. Classification of partial waxy wheats according to the presence (+) or absence (-) of each waxy protein, and amylose contents (AM) of each class.

		Wx proteins		
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

About 40% of the Australian wheats carried the null allele *Wx-B1b*. The Australian Standard White (ASW), which is a wheat brand composed of several Australian varieties, showed a lower amylose content of flour and better glutinosity for noodles than standard Japanese wheat varieties. Since the null allele *Wx-B1b* seems to lower amylose content, it could be also responsible for the low amylose of ASW. Two wheat varieties suitable for Japanese noodles, Kanto 79 (22.3% of amylose content) and Kanto 107 (21.6%), have been released and lack Wx-A1 (*Wx-A1b*) and Wx-B1 (*Wx-B1b*) proteins that correspond to type 7. The relation between amylose content and starch pasting properties was analyzed using varieties classified into the eight wheat types (Yamamori and Quynh 2000). Densitometric analysis indicated that the amylose content was related to the amount of Wx protein in the eight 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 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 material 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 amylopectin 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 amylopectin between waxy line and its parental varieties, Kanto 107 and Bai Huo. Further studies are needed to exploit the physiochemical 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 the 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 catalyze the 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 (β-conglysinin) consists of α, α' and βsubunits, 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 the amino acid constitution in soybean seeds. Mutations lacking α and α' subunits of 7S globulin induced by gamma-ray treatment (Takahashi *et al.* 1994) were released as a higher content 11S globulin variety, Yumeminori, in 2001. This variety is characterized by 30-50% higher content of the sulphur-containing amino acids, methionine and cystine compared to standard soybean varieties.

The main allergenic proteins in soybean are Gly m Bd30K, Gly m Bd28K and 7S globulin α subunits. Yumeminori has a lower content of allergenic proteins, in particular it lacks one of three main allergenic proteins, Gly m Bd28K. As disulfide bonds formed between Gly m Bd30K and 7S globulin α/α' 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 α/α' subunits, treatment with reducing reagents is unnecessary to remove allergenic Gly m Bd30K protein. Low allergen soybean can be used as diet for soybean-allergenic 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 α, β, γ and δ tocopherols with different vitamin E activity. Soybean oil is high in total content of tocopherols but its major component is γ-Toc and only 5% in α-Toc which has the highest vitamin E activity. Germplasm screening has succeeded in discovering germplasm with increased content of α-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 5^{th} in sweet potato production. The area of sweet potato cultivation has been decreasing and by year 2000 only 50,000 ha were planted. In contrast, the yield of sweet potato has rapidly increased to 25 t/ha. Since World War 2, 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, β-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 the sweet potato is mainly derived from maltose that is formed from starch by β-amylase. Normal sweet potato starch is gelatinized above 70 ^{o}C. β-amylase acts on gelatinized starch but it lacks this activity at around 70 ^{o}C. Starch with lower gelatinization temperature or heat tolerant β-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”.

References

Asaoka M, Okuno K, Hara K, Oba M and Fuwa H (1989). Effects of environmental temperature at the early developmental stage of seeds on the characteristics of endosperm starches of rice (*Oryza sativa* L.). Denpun Kagaku (Starch Science) 36, 1-8.

Hajika M, Igita K and Kitamura K (1991). A line lacking all the seed lipoxygenase isozymes in soybean (*Glycine max* (L.) Merrill) induced by gamma-ray irradiation. Japanese Journal of Breeding 41, 507-509.

Hajika M, Takahashi M, Sakai S and Igita K (1996). A new genotype of 7S globulin (β-conglycinin) detected in wild soybean (*Glycine soja* Sieb. et Zucc.). Japanese Journal of Breeding 46, 385-386.

Hayakawa K, Tanaka K, Nakamura T, Endo S and Hoshino T (1997). Quality characteristics of waxy hexaploid wheat (*Triticum aestivum* L.): Properties of starch gelatinization and retrogradation. Cereal Chemistry 74, 576-580.

Hildebrand DF and Hymowitz T (1982). Inheritance of lipoxygenase-1 activity in soybean seeds. Crop Science 22, 851-853.

Hoshino T, Ito S, Hatta K, Nakamura T and Yamamori M (1996). Development of waxy common wheat by haploid breeding. Breeding Science 46, 185-188.

Iida S, Amano E and Nishio T (1993). A rice (*Oryza sativa* L.) mutant having a low content of glutelin and a high content of prolamine. Theoretical and Applied Genetics 87, 374-378.

Iida S and Nishio T (1993). Mutants having a low content of 16-kDa allergenic protein in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 86, 317-321.

Ishida N, Miura H, Noda T and Yamauchi H (2003). Mechanical properties of white salted noodles from near-isogenic wheat lines with different Wx protein-deficiency. Starch 55, 390-396.

Islam MS, Yoshimoto M, Yahara S, Okuno S, Ishiguro K and Yamakawa O (2002). Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) genotypes. Journal of Agricultural and Food Chemistry 50, 3718-3722.

Kikuchi A, Tsukamoto C, Tabuchi K, Adachi T and Okubo K (1999). Inheritance and characterization of a null allele for group A acetyl saponins found in a mutant soybean (*Glycine max* (L.) Merrill). Breeding Science 49, 167-171.

Kitamura K, Davies CS, Kaizuma N and Nielsen NC (1983). Genetic analysis of a null-allele for lipoxygenase-3 in soybean seeds. Crop Science 23, 924-927.

Kitamura K, Kumagai T and Kikuchi A (1985) Inheritance of lipoxygenase-2 and genetic relations among genes for lipoxygenase-1, 2 and -3 isozymes in soybean seeds. Japanese Journal of Breeding 35, 413-420.

Kumamaru T, Satoh H, Iwata N, Omura T, Ogawa M and Tanaka K (1988). Mutants for rice storage proteins. Theoretical and Applied Genetics 76, 11-16.

Nakamura T, Yamamori M, Hidaka S and Hoshino T (1992). Expression of HMW Wx protein in Japanese common wheat (*Triticum aestivum* L.) cultivars. Japanese Journal of Breeding 42, 681-685.

Nakamura T, Yamamori M, Hirano H and Hidaka S (1993). Decrease of waxy (Wx) protain in two common wheat cultivars with low amylose content. Plant Breeding 111, 99-105.

Nakamura T, Yamamori M, Hirano H and Hidaka S, Nagamine T (1995). Production of waxy (amylose–free) wheats. Molecular and General Genetics 248, 253-259.

Nakamura T, Yamamori M, Hirano H and Hidaka S (1993). Decrease of waxy (Wx) protain in two common wheat cultivars with low amylose content. Plant Breeding 111, 99-105.

Okuno K, Fuwa H and Yano M (1983). A new mutant gene lowering amylase content in endosperm starch of rice, *Oryza sativa* L. Japanese Journal of Breeding 33, 387-394.

Okuno K, Nagamine T, Oka M, Kawase M, Katsuta M, Egawa Y and Nakagahra M (1993). New lines harboring *du* genes for low amylase content in endosperm starch of rice. Japan Agricultural Research Quarterly 27, 102-105.

Satoh H and Omura T (1981) New endosperm mutations induced by chemical mutagens in rice, *Oryza sativa* L. Japanese Journal of Breeding 31, 316-326.

Saikusa T, Horino T and Mori Y (1994a). Distribution of free amino acids in the rice kernel and kernel fractions and effect of water soaking on the distribution. Journal of Agriculture and Food Chemistry 42, 1122-1125.

Saikusa T, Horino T and Mori Y (1994b). Accumulation of γ-aminobutyric acid (Gaba) in the rice germ during water soaking. Bioscience, Biotechnology and Biochemistry 58, 2291-2292.

Takahashi K, Banba H, Kikuchi A, Ito M and Nakamura S (1994). An induced mutant line lacking the α-subunit of β-conglycinin in soybean (*Glycine max* (L.) Merrill). Breeding Science 44, 65-66.

Yamamori M, Nakamura T, Endo TR and Nagamine T (1994). Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theoretical and Applied Genetics 89, 179-184.

Yamamori M and Quynh NT (2000). Differential effects of Wx-A1, -B1 and –D1 protein deficiencies on apparent amylase content and starch pasting properties in common wheat. Theoretical and Applied Genetics 100, 32-38.

Yano M, Okuno K, Satoh H and Omura T (1988). Chromosomal location of genes conditioning low amylase content of endosperm starches in rice , *Oryza sativa,* L. Theoretical and Applied Genetics 76, 183-189.