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Molecular breeding approach for crop improvement of quality traits

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ABSTRACT

Plant breeding and molecular maker assisted selection (MAS) approaches have been used to produce plants carrying the desired traits. Molecular marker based breeding strategies have been used to accelerate the process of moving trait genes into high-yielding germplasm for commercialization. These products are being tested for applications in food, feed and industrial markets. Clearly, DNA based marker techniques are not a replacement for classical breeding and selection techniques. Rather they have become a powerful accelerating tool to improve the rate of selection for quality traits. The specificity of molecular monitoring provides the confidence in trait selection and overall cost-effectiveness for improvement for these complex quality traits.

Key Words: *Gene discovery, MAS, Quality traits, QTL*

INTRODUCTION

There are many aspects of crop quality, which can be defined differently according to, for example, crop species, geographical region and intended use of crop or crop product. Some attributes that define crop quality include: nutritional value (amino acid composition, protein content, micronutrients, vitamins, secondary metabolites, nutraceuticals, etc.), consumer preference (flavour, texture, colour, grain size/shape), pre- and post-harvest and industrial/technological characteristics (fibre traits, sucrose content, storage quality, sprouting, oil content, starches, processing, bread-making). The market value of most crops is determined by various factors. Quality is a subjective, complex trait, with many different components determined by the growing environment and the target market. Although HYV varieties in many crops have contributed greatly to world agriculture in the past decades, its potential to improve grain quality further is being questioned.

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However, to meet the challenges posed by severe crop damage by pests and diseases, the extensive use of pesticides and chemical fertilizers, and a shortage of water and energy, more elite cultivars are needed in different crops. In recent years, world has seen continued improvements in crop genetics, powered by functional genomics as a way forward to safeguard its food production. Biotic and abiotic stresses have a negative impact on product quality. Indeed, in developing countries with low input agricultural systems or reduced access to plant protection compounds, such stresses can have devastating effects on crop quality. Resistances to such quality-affecting stresses should be essential components of breeding programmes aimed at crop quality improvement. Therefore, quality traits are usually complex, controlled by the action of several genes, and are also subject to environmental influences. The complex genetics of quality traits has led to their being difficult to improve through conventional plant breeding. Furthermore, the complexity in assessing some quality characters aggravates the difficulties in improving crop quality. Breeding activities that rely on use of genotypic rather than phenotypic selection have the

potential to overcome these limitations. For example, traits controlled by recessive alleles, environmentally-sensitive characters, traits that are expensive or difficult to score, or expressed late in development will benefit greatly from application of genotypic selection. The advent of molecular marker techniques now makes it possible to tag alleles conferring desirable quality traits. For many crop plants large numbers of molecular markers of different types (RFLP, SSR, AFLP etc) are now available. These vary widely in cost of development and deployment and the choice of markers to be used will depend largely on the breeder's objectives and the available facilities. Development of markers tightly linked to quality traits or resistance to quality-affecting stresses should enable breeders to select on a genotypic basis. Marker assisted selection (MAS) offers great promise for both the selection of individual quality traits and the pyramiding of a number of important quality characters simultaneously into the same improved genotype. Breeding programmes could benefit from the implementation of MAS for gene pyramiding in terms of time saving and reduction of cost as compared to conventional breeding. MAS should be considered as complementary to conventional breeding, in that it can be used to replace certain phenotypic assessments, rather than as an alternative to selection based on phenotype. Despite their great potential, DNA-based markers have not yet been widely implemented in breeding for quality, particularly in developing countries, due to economic constrain and cost effectiveness.

Improvement of quality traits depends on the availability of sufficient variability for the targeted traits. In the past the level of variability in some quality traits has been increased using mutagenesis. However, the full potential of induced mutations has not been realized in plant breeding for quality traits because of the difficulty in screening large mutagenised populations for infrequent mutations generating desirable crop quality alleles. The exploitation of mutated genes has been restricted to those that have easily identifiable phenotypes (eg. waxy mutation in rice, barley and other crops). Recent development of screening procedures for quality traits in mutagenised populations will allow the more efficient identification of novel and useful variants. Such screening procedures and the development of markers linked to the induced mutant alleles will facilitate their incorporation into breeding programmes (Shu 2004). Researches at different labs now use biotechnology to introduce various desired quality traits in different crops and assure that new varieties have those components at the DNA level. So far the technology has found the DNA markers for starch quality and for resistance to blast, a fungal plant disease that takes its toll on rice yields throughout the growing regions of the world. It's faster, cheaper and better to use this technology in breeding new varieties. And it is being put to use in the field more quickly than research findings often are with the DNA sequences of every gene known, researchers now are trying to delve further into the code to "mark" what each gene expresses in the plant (Phillips 2001).

Marker-assisted (or molecular-assisted) breeding provides a dramatic improvement in the efficiency with which breeders can select plants with desirable combinations of genes. A marker is a "genetic tags" that identifies a particular location within a plant's DNA sequences. Markers can be used in transferring a single gene into a new cultivar or in testing plants for the inheritance of many genes at once (Suslow *et al* 2001). Advanced breeding technologies that meet these goals have resulted from recent research in plant biotechnology. The techniques have been utilized for some time using a bar-code-like fingerprint of differentiating enzymes and proteins (the products of the genes) for many important quality traits to monitor or reveal inheritance in the same way (Suslow and Bradford 1999). By providing quick and efficient tests for many different genes, DNA markers have become valuable new tools for breeding crop varieties having optimal combinations of desirable genes.

DNA markers have been used for transferring quality genes to cultivated varieties, assisting selection of complex multi-gene traits (such as flavor), aiding evaluation of regionally and seasonally optimized varieties (Suslow *et al* 2001).

Rice is the most important food crop and the challenge to produce enough rice for the future, however, remains daunting, as the current rate of population growth outpaces that of increases in rice production. Examining molecular, genetic and cellular techniques, it considers recent advances in four research approaches for increasing yields and improving the nutritional quality of rice. Plant genomics: knowing the identity and location of each gene in the rice genome is of immense value in all aspects of rice science and cultivar improvement. Molecular biological approaches to increase yield: to produce more biomass by increasing photosynthetic rate and duration and by improving grain filling.

Enhancing tolerance to biotic and abiotic stresses: with new DNA array technologies, it is now possible to assess global genomic response to stresses. Understanding the relationships among stress pathways may create new opportunities for gene manipulation to enhance tolerance to multiple biotic and abiotic stresses. Improving nutritional quality in the grain: knowledge of the biosynthesis of micronutrients in plants permits genetic engineering of metabolic pathways to enhance the availability of micronutrients. Recent advances in rice genomics research and completion of the rice genome sequence have made it possible to identify and map precisely a number of genes through linkage to DNA markers. Noteworthy examples of some of the genes tightly linked to markers are resistance to or tolerance of blast, bacterial blight, improved agronomic and grain quality traits etc. MAS can be used for monitoring the presence or absence of these genes in breeding populations and can be combined with conventional breeding approaches. The use of cost-effective DNA markers derived from the fine mapped position of the genes for important agronomic traits and MAS strategies will provide opportunities for breeders to develop high-yielding, stress-resistant, and better-quality rice cultivars (Jena and Mackill 2008). Phillips (2001) found the first marker (after rice genome sequencing) which regulates amylose, a component of starch. In rice, high amylose means that the grains are firm and separate and low starch means we can eat it with chopsticks because it sticks together. A problem in breeding new varieties is that the air temperature while rice is growing influences the amount of amylose that the plant produces. By diagnosing rice in breeding programs with DNA markers, however, scientists can accurately decide whether to keep working with progeny from a cross, or to cease selection. Breeders don't have to worry about unusual weather giving false reads on a potentially good variety.

Long-te-fu (LTF) and Zhan-shan 97 (ZS) are two key female parents for the generation of indica hybrid rice, which have greatly contributed to the achievement of rice production in China. However, the high amylose content (AC) in the endosperm, controlled by the Waxy (Wx) gene encoded granule-bound starch synthase I, of both lines results in poor cooking and eating quality of the milled rice. Previous studies have shown that AC was correlated with the ability to excise intron 1 from the leader sequence of the Wx transcript, and which is responsible by a single nucleotide polymorphism (G or T) located at the first nucleotide of the splice donor site of Wx intron 1. Thus, a CAPS marker was subsequently developed, and with this MAS, Qiao-Quan *et al* (2006) successfully introgressed the Wx-TT locus of rice cultivars with good quality intermediate AC into LTF-B and ZS-B. These were subsequently introduced into their relevant male-sterile lines (LTF-A and ZS-A) to generate improved indica hybrids. In the selected lines LTF (tt)-B and ZS (tt)-B, the AC was reduced to a relatively low level (15%). Consequently, the hybrids crossed from the

selected lines had dramatically reduced amylose levels. The ability to transfer cloned genes allows plant breeders to use genes from essentially any source as tools for crop improvement. For example, to enable rice grains to accumulate beta-carotene (which is converted into vitamin A when consumed by animals) and create the so-called "Golden rice," scientists used genes from daffodil, pea, a bacterium and a virus. Transgenic plant methods enable these four well characterized genes to be inserted into a transgenic plant, producing a highly specific change in only the trait of interest. Woody tree and vine crops are exceedingly difficult to improve by traditional breeding technology because it takes a number of years for a seedling to begin flowering, and the unique traits of specific varieties are hard to regain after sexual crosses (Suslow *et al* 2001). Golden rice was created by Ingo Potrykus of the Institute of Plant Sciences at the Swiss Federal Institute of Technology, working with Peter Beyer of the University of Freiburg. The project started in 1992 and at the time of publication in 2000, golden rice was considered a significant breakthrough in biotechnology as the researchers had engineered an entire biosynthetic pathway.

Golden rice was created by transforming rice with two beta-carotene biosynthesis genes:

1. Psy (Phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)
2. Crt1 from the soil bacterium *Erwinia uredovora*

(The insertion of a lyc (lycopene cyclase) gene was thought to be needed but further research showed that it is already being produced in wild-type rice endosperm).

The psy and crt1 genes were transformed into the rice nuclear genome and placed under the control of an endosperm specific promoter, so that they are only expressed in the endosperm. The exogenous lyc gene has a transit peptide sequence attached so that it is targeted to the plastid, where geranylgeranyl diphosphate formation occurs. The bacterial crt1 gene was an important inclusion to complete the pathway, since it can catalyze multiple steps in the synthesis of carotenoids, while these steps require more than one enzyme in plants (Hirschberg 2001). The end product of the engineered pathway is lycopene, but if the plant accumulated lycopene the rice would be red. Recent analysis has shown that the plant's endogenous enzymes process the lycopene to beta-carotene in the endosperm, giving the rice the distinctive yellow colour for which it is named (Schaub *et al* 2005). The original Golden rice was called SGR1, and under greenhouse conditions it produced 1.6 µg/g of carotenoids.

Golden rice was developed as a fortified food to be used in areas where there is a shortage of dietary vitamin A. In 2005 a new variety called Golden Rice 2 was announced which produces up to 23 times more beta-carotene than the original variety of golden rice (Paine *et al* 2005). Neither variety is currently available for human consumption.

Molecular marker technology is playing an increasingly important role in the selection of wheat lines with improved quality attributes. This is due to the identification of molecular markers tightly linked to chromosome regions involved in the control of important quality characteristics such as dough properties, grain hardness, semolina and flour colour, grain protein content and starch composition, which strongly influence wheat end use, and its nutritional and market value. Moreover, the implementation of MAS allows the selection of individuals carrying the favourable alleles at the target loci, and also the pyramiding of favourable quantitative trait loci (QTL) alleles from different sources and for different traits. This not withstanding, the progress obtained until now in applying MAS to quality characteristics has been slow compared to other traits (Lafiandra *et al* 2007).

The mechanisms underlying some quality traits in wheat are now understood. Examples include the

role of high and low molecular weight glutenins in contributing to strength and extensibility of wheat doughs, puroindolines that affect grain texture, and variation in granule-bound starch synthase that produces starches with altered amylose content and physical properties. This knowledge, coupled with the availability of the DNA sequences of various alleles of the genes encoding these proteins and the wide application of the polymerase chain reaction, has enabled the design of diagnostic DNA markers for these quality traits. Such markers are now being used by wheat breeders to select lines with the required quality attributes, without the need for the direct measurement of those traits in early generation screening. DNA markers may be implemented on leaf tissue from individual plants, for a number of independent traits, with results that are independent of environmental variation. The use of a common platform for all marker assays and the potential for multiplexing or parallel analysis of many different markers will further increase the efficiency and speed of the development of improved cultivars in the future (Gale 2005).

Wheat quality is a subjective, complex trait, with many different components determined by the growing environment and the target market. Using Australian germplasm Schmidt *et al* (2004) have identified molecular markers for milling yield, water absorption, flour colour and specialised dough development properties required for expansion into markets where sponge and dough style baking dominates. Novel marker-trait associations were identified for milling yield, dough strength and dough development time. A novel sponge and dough QTL has been located on chromosome 4B and further characterization of this locus is still underway. Previous studies have resulted in identification of molecular markers for many general interest traits such as milling yield (Parker *et al* 1999), flour colour (Parker *et al* 1998, Mares and Campbell 2001), protein content and dough strength (Metakovsky *et al* 1997), and implementation of these markers in Australian breeding programmes will provide many benefits for growers.

Wheat (*Triticum aestivum*) gluten contains both high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits. The high molecular weight glutenin subunits (HMW-GS) are key factors in bread making quality since they are major contributors of glutenin elasticity and polymer formation of wheat dough. The effects of (HMW-GS) on dough properties (strength and elasticity) may be additive or synergistic with significant interactions with (LMW-GS) subunits (Beasley *et al* 2002). The HMW-GS are encoded by polymorphic genes at the Glu 1 loci that are present on the long arm of group 1 chromosome (Payne and Lawrence 1983). At each locus (Glu-A1, GluB1, GluD1) there are two tightly linked HMW-GS genes, one of them is x-type with higher molecular weight and the other is y-type. For instance, at the Glu-1 there are subunits 1, 2 and null (there is no y allele), at the Glu-B1 locus there are Bx17+By18, Bx7+By8, Bx7+By9, Bx6+By8 subunits, and at the Glu-D1 locus, there are Dx5+Dy10, Dx2+Dy12, Dx3+Dy12, Dx4+Dy12 subunits.

The presence of different allelic composition of the HMW-GS in one specific wheat variety is one of the most important genetic factors in determining the bread making quality (Payne *et al* 1987). For example, wheat varieties containing allelic compositions of (Dx5 paired with Dy10) at the Glu-D1 locus will form stronger dough than those containing (Dx2 paired with Dy12). Due to the large contribution of allelic interaction in bread making quality, Békés *et al* (2006) suggested targeting different allelic combinations rather than individual glutenin allele in developing new lines with certain quality attributes. Baking quality is a major target in wheat breeding programs. However, due to small population size of wheat that can be obtained in early generations of breeding programs, full-scale mill and bakery testing is not routinely

feasible. Alternatively, DNA marker screening of these quality traits may be performed on leaf materials at early stage and before grain setting (De Bustos *et al* 2000).

DNA markers for HMW-GS allelic composition have proven to be superior to electrophoresis of SDS-PAGE in some instances (D'Ovidio and Anderson 1994). Moreover, this assay can be used to select individual plants within populations and to overcome the environmental variation originating from both field and laboratory. Data presented by Uthayakumaran *et al* (2006) showed that the MAS for the HMW-GS are especially valuable tool for breeding programs since the information about the bread making quality can be obtained at an earliest stages of breeding program, thus poor-quality lines are not propagated. Moreover, MAS for the HMW and LMW glutenin alleles is widely performed by breeding programs throughout the world to select for improved dough characters. Blechl *et al* (2007) suggested that contribution of allele–allele interactions, and different allelic combinations should be targeted rather than the individual glutenin alleles in breeding program to develop new lines with certain quality attributes, especially to improve extensibility. The main reason given was the significant differences found among the values, which described the contribution of HMW-GS×LMW-GS interactions on extensibility.

The association between molecular markers and bread-making quality (BMQ) was investigated by Manifesto *et al* (1998) in a cross between two wheat cultivars with the same high M_r-glutenin subunits but significantly different BMQ. A segregant F_2 population was generated after crossing Klein 32 and Chinese Spring, and the BMQ of each F_2-derived F_3 family was estimated using sodium dodecyl sulfate (SDS) sedimentation and mixograms. The same families were characterized for 11 polymorphic loci using restriction fragment length polymorphisms (RFLP) and simple sequence repeats (SSR). These loci were specifically selected for their complete or close linkage to storage protein gene families. No significant differences in BMQ were detected at XGlu-B1 and XGlu-A1 loci using RFLP markers. Highly significant ($P<0.01$) differences in all BMQ parameters were detected for XGli-B1 and XGlu-B3 loci on chromosome arm 1 BS. The increase in the number of Klein 32 alleles at these loci determined a linear increase in sedimentation and mixogram values. It was not possible to differentiate the effect of XGli1 from that of XGlu3 because of the close linkage between these two loci. These two loci, considered together, explained from 11 to 15% of the variation in BMQ observed in this cross.

Similarly Gale (2005) reported that some diagnostic markers had been used by wheat breeders in identifying the BMQ of varieties without the need for the direct measurement of those traits in early generation screening. DNA markers applied on leaf tissue from individual plants will further increase the efficiency and speed of the development of improved cultivars in the future. MAS could increase the genetic gain and economic efficiency of a specific breeding strategy.

Development of high-yielding wheat varieties with good end-use quality has always been a major concern for wheat breeders. To genetically dissect QTLs for quality traits such as grain and flour protein content, gluten strength as evaluated by mixograph and SDS sedimentation volume, an F_1-derived doubled haploid (DH) population of 185 individuals was developed from a cross between a Canadian wheat variety "AC Karma" and a breeding line 87E03-S2B1. A genetic map was constructed based on 167 marker loci, consisting of 160 microsatellite loci, three HMW glutenin subunit loci: Glu-Al, Glu-B1 and Glu-D1, and four STS-PCR markers. QTL analyses were performed using composite interval mapping. A total of 26 QTLs for quality-related traits were identified. The largest QTL clusters, consisting of up to nine

QTLs, were found on chromosomes 1D and 4D. HMW glutenin subunits at Glu-1 loci had the largest effect on BMQ. However, other genomic regions also contributed genetically to bread making quality (Huang *et al* 2006).

Breseghello *et al* (2005) conducted a study to identify genomic regions related to differences in milling and baking quality between a soft and a hard cultivar of hexaploid wheat. A population of 101 double-haploid lines was generated from a cross between Grandin, a hard spring wheat variety, and AC Reed, a soft spring wheat variety. The genetic map included 320 markers in 43 linkage groups and spanned 3555 cM. The effect of qualitative variation for kernel texture, caused by the segregation of the Hardness gene, was controlled by regression on texture class. The residual variance was used for composite interval mapping, and QTLs on 1A, 1B, 1A/D, 2A, 2B, 2D, 3A/B, 4B, 5B and 6B were detected. The effect of some QTLs was opposite to the direction expected on the basis of parental phenotypes. The hard wheat parent contributed alleles favorable for soft wheat varieties at QTLs on 1AS,L, 1BL-2, and 6B, whereas the soft parent contributed alleles for higher protein content at QTLs on 2BL-1, 4B-1, and 6B and higher flour yield on 2BL-2 and 4B-2. Their results indicated that hard x soft wheat crosses have considerable potential for improving milling and baking quality of either class.

A set of 187 doubled haploid lines derived from the cross between cvs. Courtot and Chinese Spring was explored for QTLs for three bread-making quality tests: hardness, protein content and strength of the dough (W of alveograph) by Perretant *et al* (2000). About 350 molecular and biochemical markers were used to establish the genetic map, and technological criteria were evaluated in 1 to 3 years. QTL detection was performed by the "marker regression" method. For hardness, they confirmed a previously tagged major QTL on chromosome 5DS, and two additional minor QTLs were found on chromosome 1A and 6D, respectively. For protein content two main QTLs were identified on chromosomes 1B and 6A, respectively. For W, three consistent QTLs were detected: two at the same location as those for hardness, on chromosomes 1A and 5D; the third one on chromosome 3B.

Gene discovery to improve quality related traits Maize is clearly a diverse crop with many specialty uses and types. These types have evolved from a rich past of selection based on recognition of unique properties associated with various genetic variants. The continued analysis of genetic variation has provided additional resources for the refinement and development of specialty corn. The ability of the geneticists to discover new genes and to manipulate genetic variation at the level of specific genes offers the potential to tailor genetic variation for the production of precisely designed specialty maize in the future.

By utilizing genetic variation, the composition of the kernel can be altered for both the quantity and quality (structure and chemical diversity) of starch, protein and oil throughout kernel development. The ability of future generations of plant breeders/plant scientists to use existing genetic variation and to identify and manipulate commercially important genes will open new avenues for designing novel variation in grain composition. This will provide the basis for the development of the next generation of specialty maize and of new products to meet future needs. Developing plants with improved grain quality traits involves overcoming a variety of technical challenges inherent in metabolic engineering programs. Advances in plant genetics and in technologies for genome-wide studies and for large-scale gene expression analysis are contributing to the acceleration of gene discovery for product development (Motto *et al* 2003).

Developing plants with improved grain quality traits involves overcoming a variety of technical

challenges inherent in metabolic engineering programs. Advances in plant genetics and genomic technologies are contributing to the acceleration of gene discovery for product development. The complexity of the maize genome, particularly the abundance of repetitive sequences, makes direct genome sequencing for gene discovery difficult; the segmental allotetraploid origin of maize suggests that this species may contain more genes than a true diploid. Estimates of gene number for maize range from 50,000 to 80,000 distributed in a 2.3 x 109 base pairs present in ten chromosomes (Gai *et al* 2000).

In the past few years there has been much progress in the development of strategies to discover new plant genes. In large part, these developments derive from four experimental approaches:

1. Genetic and physical mapping in plants and the associated ability to use map-based gene isolation strategies (Coe *et al* 2002),

2. Transposon tagging which allows the direct isolation of a gene via forward and reserve genetic strategies (Walbot 2000),

3. Protein-protein interaction cloning that permits the isolation of multiple genes contributing to a single pathway or metabolic process (Pelletier and Sidhu 2001) and

4. Through bioinformatics/genomics, the development and use of large expressed sequence tags (ESTs) databases, which are easier to generate than long tracts of genomic sequences and provide large-scale information on the gene complement of maize (Fernandes *et al* 2002).

While extensive collections of maize ESTs have been assembled by the commercial sector, approximately 154,500 ESTs derived from 20 cDNA libraries have been deposited in the ZMDB database http://www.zmdb.iastate.edu/zmdb/EST/) of the NSF Maize Gene Discovery Project. DNA microarray technology (Brown and Botstein 1999) represents a collection of promising tools for the discovery of mRNA level controls of complex pathways and may shed light on pathway interactions, the understanding of which is essential for successful metabolic engineering of crop plants.

DNA microarray analysis has been used to study gene expression in a wide range of organisms including yeast (Spellman *et al* 1998), humans (Schena *et al* 1996) and Arabidopsis seed (Girke *et al* 2000). Progress in these four areas will permit the isolation of many new genes and regulatory control points that will have a major impact on the improvement of the maize grain cell factory. Hunter *et al* (2002) assayed the pattern of gene expression in normal and opaque endosperm patterns by profiling endosperm mRNA transcripts with an Affinetrix GeneChip containing approximately 1,400 selected maize gene sequences. The results revealed distinct, as well as shared, gene expression pattern in these mutants, and they provide a framework for investigating a common mechanism that underlines the opaque kernel phenotype.

The project "A Functional Blueprint of the *Zea mays* Endosperm Cell Factory" funded by the EU examines in considerable detail the transcriptome and proteome of the developing maize endosperm. This information will be used to target distinctive, previously uncharacterized endosperm specific genes which will be knocked out via Mutator transposon tagging. Characterization of the normal and modified endosperm will provide further research material for the academic laboratories involved, including this laboratory, as well as material for the plant breeders and food processors to include in their respective research or product development pipelines.

Breeding for improved protein quality in maize began in the mid-1960s with the discovery of mutants, such as opaque-2, that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm proteins. However,

adverse pleiotropic effects imposed severe constraints on successful exploitation of these mutants. Interdisciplinary and concerted research efforts led to amelioration of the negative features of the opaque phenotype, and the rebirth of 'Quality Protein Maize' (QPM). QPM holds superior nutritional and biological value and is essentially interchangeable with normal maize in cultivation and kernel phenotype (Prasanna *et al* 2001).

A set of 23 Quality Protein Maize (QPM) lines, including 13 lines developed in India and 10 lines at CIMMYT (International Maize and Wheat Improvement Center), Mexico, was analyzed by Kassahun and Prassana (2003) using microsatellite or simple sequence repeat (SSR) markers. Polymorphic profiles for 36 SSR loci have aided in differentiating the QPM inbred lines. The study resulted in identification of SSR markers, such as bnlg439, phi037, bnlg125, dupssr34 and bnlg105, with high polymorphism information content in the selected QPM genotypes. Detection of 30 unique/rare SSR alleles could contribute to effective differentiation of 14 of the 23 QPM inbreds. An opaque-2 specific microsatellite marker, phi057, also facilitated differentiation of opaque-2 carrying QPM inbreds from the non-opaque genotypes. Analysis using SSR markers indicated high levels of heterozygosity in majority of the Indian QPM lines and in one CIMMYT QPM inbred, CML188. Cluster analysis using SSR data, followed by canonical discriminant analysis, clearly distinguished the Indian QPM inbreds from those developed at CIMMYT. The cluster patterns were largely in congruence with the available pedigree information of the QPM inbreds studied. The study demonstrates the effectiveness of SSR markers in QPM genotype discrimination and analysis of genetic relationships.

Brewers are reluctant to change malting barley (*Hordeum vulgare* L.) cultivars due to concerns of altered flavor and brewing procedures. Selection for malting quality in breeding programs by micro-malting and micro-mashing is time consuming, and resource-intensive and is a major breeding objective for any breeding programs. Characters that affect malting quality (i.e. malt extract content, α- and β-amylase activity, diastatic power, malt β-glucan content, malt β-glucanase activity, grain protein content, kernel plumpness, and dormancy) are quantitatively inherited and variously influenced by the environment (E) (Zale *et al* 2000). With the advent of molecular markers, it is possible to map and tag the loci affecting malting quality.

Considerable QTL analyses have been performed in recent years on a number of crosses. A minimum of 168 malting quality QTLs representing 19 malting quality traits have been mapped in nine mapping populations. QTL regions are spread across each of the seven barley chromosomes with concentrations especially within chromosomes 1, 2, 4, 5 and 7. Whereas, there is remarkable QTL conservation in some chromosome regions among crosses, some regions hold unique QTLs as well. It is also noteworthy that there are many overlapping QTLs, especially but not surprisingly, of related traits. Malt extract QTLs are almost always coincident with component traits such as carbohydrate hydrolytic enzyme activities. Diastatic power QTLs are often associated with α- and/or β-amylase activity QTLs. In some cases widely conserved QTL chromosome regions may be targets for selection to maintain malting quality, but selection for unique regions may lead to new improvements (Zale *et al* 2000). Two major QTL regions in six-row barley for malt extract percentage, α-amylase activity, diastatic power, and malt β-glucan content on chromosomes 1 (QTL1) and 4 (QTL2) have been previously identified. The flanking markers, Brz and Amy2, and WG622 and BCD402B, for these two major QTL regions were used in MAS (Han *et al* 1997). Schmierer *et al* (2005) wanted to develop high yielding near isogenic lines that maintain traditional malting quality characteristics by transferring QTL

associated with yield, via molecular marker-assisted backcrossing, from the high yielding cv. Baronesse to the North American two-row malting barley industry standard cv. Harrington. For transfer, they targeted Baronesse chromosome 2HL and 3HL fragments presumed to contain QTL that affect yield. Analysis of genotype and yield data suggests that QTL reside at two regions, one on 2HL (ABG461C-MWG699) and one on 3HL (MWG571A-MWG961). Based on yield trials conducted over 22 environments and malting analyses from 6 environments, they selected one isogenic line (00-170) that has consistently produced yields equal to Baronesse while maintaining a Harrington-like malting quality profile.

QTLs associated with malting quality traits were mapped in 2 populations derived from parents with elite malting quality by Panozzo *et al* (2007). Progenies were tested for grain protein percentage, α-amylase activity, diastatic power, hot water extract, wort viscosity, wort β-glucan, β-glucanase, and free α-amino acids. QTLs for malting quality traits were detected on all chromosomes and for both populations. There were many coincident QTLs for traits that are expected to be related such as diastatic power and α-amylase activity, wort β-glucan and wort viscosity and for some traits that are not expected to be related such as hot water extract and malt viscosity (Panozzo *et al* 2007).

Germplasm improvement in cotton has been practiced since ancient spinners selected plants that produced fiber with improved properties for cloth production. More recently, breeding approaches for traditional pedigree breeding and biotechnology have been employed in cotton to address the quality of output fiber quality. Traditional approaches include germplasm access with a focus on fiber quality, intense selection pressure throughout the breeding process, modifications to pedigree breeding methodology and the development of a fiber selection index that includes more measurable fiber properties. Future approaches to fiber quality improvement will also include plant biotechnology beyond recombinant DNA techniques, molecular breeding and plant-based biotechnology solutions beyond input traits.

Currently, there is a disparity compared to some other agronomic crops in terms of availability of effective molecular tools for use in a targeted molecular breeding approach. Cotton is behind in molecular breeding approaches because of its complex and large genome, its low genetic variability and the complexity of priority traits such as fiber properties. It is expected that cotton breeding will evolve along the same lines as breeding has evolved in other crops over the past ten years. Looking further into the future, the ultimate breeding approach is design based. In this view, accumulated genomic information will allow assembly of an ideal genotype from available allelic variants for all known genes. However, as a guiding principle for the development and implementation of a molecular breeding technology platform, it is a very valuable concept.

Until very recently, little was known about the molecular aspects leading to specific cotton fiber properties and few research tools were available to probe cotton fiber quality. The initial focus is on improving fiber characteristics such as fiber length, uniformity and strength that are important in spinning. Approaches are also being explored to improve properties of cotton fiber that would add value to the overall fiber processing industry and be of real benefit to consumers (Dever and Hamill 2005). Although potential genetic diversity exists in Gossypium genus, it is largely ‘underutilized’ due to photoperiodism and the lack of innovative tools to overcome such challenges. The application of linkage disequilibrium (LD) based association mapping is an alternative powerful molecular tool to dissect and exploit the natural genetic diversity conserved within cotton germplasm collections,

greatly accelerating still 'lagging' cotton MAS programs.

Abdurakhmonov *et al* (2008) reported the extent of genome-wide LD and association mapping of fiber quality traits by using a 95 core set of microsatellite markers in a total of 285 exotic Gossypium hirsutum accessions, comprising of 208 landrace stocks and 77 photoperiodic variety accessions. They demonstrated the existence of useful genetic diversity within exotic cotton germplasm. In their germplasm set, 11–12% of SSR loci pairs revealed a significant LD. At the significance threshold ($r^2 \geq 0.1$), a genome-wide average of LD declines within the genetic distance at < 10 cM in the landrace stocks germplasm and > 30 cM in variety germplasm. Genome wide LD at $r^2 \geq 0.2$ was reduced on average to ~ 1–2 cM in the landrace stock germplasm and 6–8 cM in variety germplasm, providing evidence of the potential for association mapping of agronomically important traits in cotton. They observed significant population structure and relatedness in assayed germplasm. Consequently, the application of the mixed liner model (MLM), considering both kinship (K) and population structure (Q) detected between 6% and 13% of SSR markers associated with the main fiber quality traits in cotton. Their results highlight for the first time the feasibility and potential of association mapping, with consideration of the population structure and stratification existing in cotton germplasm resources. The number of SSR markers associated with fiber quality traits in diverse cotton germplasm, which broadly covered many historical meiotic events, should be useful to effectively exploit potentially new genetic variation by using MAS programs.

The evaluation of organoleptic quality of tomato fruit requires physical, chemical and sensory analyses, which are expensive and difficult to assess. Therefore, their practical use in phenotypic selection is difficult. Five chromosome regions strongly involved in organoleptic quality attributes were then chosen to be introgressed into three different recipient lines through MAS. A marker-assisted backcross (MABC) strategy was performed by Lecomte *et al* (2004), as all the favorable alleles for quality traits were provided by the same parental tomato line, whose fruit weight (FW) and firmness were much lower than those of the lines commonly used to develop fresh market varieties. Three improved lines were obtained after three backcrossing and two selfing generations. Breeding efficiency strongly varied according to the recipient parent, and significant interactions between QTLs and genetic backgrounds were shown for all of the traits studied.

Improving organoleptic quality of fresh market tomato fruit has become an important objective for tomato breeders. Several QTLs controlling the variation of tomato quality traits have been detected using a recombinant inbred line population derived from a cross between a cherry tomato chosen for its good flavor and a line with bigger fruits but poor taste. A MAS scheme was then set in order to transfer the five most important QTLs involved in fruit quality into three recurrent lines. The backcross optimisation (population size, number and position of markers) is used, taking into account both theoretical and practical aspects (Causse *et al* 2004).

Color is among the most important attributes of tomato for processing into whole and diced products. Both color and color uniformity are greatly affected by Yellow Shoulder Disorder (YSD), a ripening disorder that results in discoloration of the proximal end tissues of the fruit. Darrigues *et al* (2008) show that lycopene and beta-carotene concentrations are reduced by 18% and 22%, respectively, in fruits affected by YSD. Variance partitioning suggests that YSD incidence and severity is affected by both genetics and environment. In order to elucidate the genetic basis of YSD, they developed single nucleotide polymorphisms (SNPs) as molecular markers for

application in three inbred backcross populations derived from either *Solanum lycopersicum* × *S. lycopersicum* or *S. lycopersicum* × *S. pimpinellifolium* crosses. SNP discovery for application in these populations is based on both analyses of large public EST databases and on hybridization to a custom oligonucleotide array. The array was hybridized with target cDNA from *S. lycopersicum* (Ohio 7814) and *S. pimpinellifolium* (LA1589). They developed algorithms to detect outliers and identified 1,296 potential SNPs. These putative SNPs are being verified by sequencing, screened for utility as markers on a collection of 99 S. lycopersicum lines and wild relatives and applied to the genetic dissection of YSD. Implementing SNP-based marker technology has the potential to dramatically alter our approach to genetic characterization. This study will facilitate the use of population structures that favor simultaneous genetic analysis and crop improvement.

As one of the most versatile food crops, the potato (*Solanum tuberosum*) is used worldwide for human and animal consumption, and as raw material for starch and alcohol production. Nowadays, one of the most important aspects of potato production is tuber quality, that includes biological traits (e.g. proteins, carbohydrates, and minerals), sensorial traits (e.g. flavour, texture); and industrial traits (e.g. tuber shape, cold sweetening, starch quality). Since most quality traits are genetically controlled, breeding work can successfully meet the needs of a changing and demanding world. Exploitation of tuber bearing Solanum species as source of valuable quality traits/allelic diversity is favored by the possibility to manipulate whole chromosome sets through sexual hybridization. Genetic engineering is an additional tool to produce new genetic variability and to study important metabolic pathways. Examples are there on the use of this strategy to produce starches with modified amylose to amylopectin ratio, and potatoes with a higher nutritional value (Carputo *et al* 2005).

As PCR techniques have developed over the last 15 years, a wealth of new DNA marker technologies have arisen which have enabled the generation of high-density molecular maps for all the major Brassica crop species. For numerous qualitative traits, traditional mapping approaches have led to the development of MAS strategies in oilseed Brassica breeding, and in some cases to map-based cloning of the responsible genes. Because Brassica species represent the closest crop plant relatives to the model plant *Arabidopsis thaliana*, significant progress will be achieved in the coming years through integration of candidate gene approaches in crop brassicas, using the detailed information now available for the Arabidopsis genome. Integration of information from the model plant with the increasing supply of data from physical mapping and sequencing of the diploid Brassica genomes will undoubtedly give great insight into the genetics underlying both simple and complex traits in oilseed rape (Snowdon and Friedt 2004).

Cheung *et al* (1998) used a genetic map of *B. juncea* to localize genes and QTLs for a number of seed quality traits in this species. The map was constructed using a segregating population of 119 F_1 microspore derived doubled haploid (DH) lines from a cross between a high oil B. juncea breeding line of AAFC Saskatoon (derived from a high oil Russian line) and an adapted canola quality B. juncea line. The map consists of 343 RFLP markers distributed in 18 linkage groups and five short unassigned segments covering a distance of 2073 cM. The seed quality traits concerning contents of oil, erucic acid, linolenic acid, total alkenyl glucosinolates and individual classes of alkenyl glucosinolates of the mapping population were evaluated by field trials. The data were analyzed using a QTL-interval mapping approach.

Rines *et al* (2006) reported the development of PCR-based Sequence Characterized Amplified Region (SCAR) and Cleaved Amplified

Polymorphic Sequence (CAPS) molecular markers in oat for application in genetic studies and marker assisted breeding. There are 8 marker sets known for oil content (Orr and Molnar 2007) and 15 sets of markers for beta-glucan and protein content (Orr and Molnar 2008). The more robust markers were developed from Random Amplified Polymorphic DNA (RAPD) markers mapped in the Kanota x Ogle (Wight *et al* 2003) or the Terra x Marion (De Koeyer *et al* 2004) recombinant inbred populations and associated with QTLs for the traits of interest. While many of the new markers map to the same loci as the original RAPD markers, others map to homologous or homoeologous genomic regions and still others to regions not known to be orthologous to the original RAPD regions. These markers have potential to define homologous and homoeologous relationships in oat, to investigate the complex genetics of these grain quality traits, and for marker assisted oat breeding.

Soybean is undoubtedly the most important legume crop in the world. In the past decade, plant genome analyses using model organisms have provided a large amount of genomic information and various categories of knowledge for gene functions: genome sequence, collection of expressed sequence tags (ESTs) and DNA markers are widely exploited for the discovery of genes. Soybean has a large genome of 1,115 Mbp with 2n = 40 that contains complex regional duplications. Several types of genome projects including linkage map production have been initiated to uncover its complex genomic structure and to help gene discovery. As for the EST collection, soybean has the sixth largest collection of more than 390,000 sequences (http://www.ncbi.nlm.gov/dbEST). Transcriptional maps with single nucleotide polymorphism (SNP) markers have been published using EST information. Recently, whole genome sequencing of soybean has started in the USA, using a shotgun sequencing strategy (http://soybeangenome.siu.edu, http://www.agbionetwork.orgsoybeangenome/GSA.php). For the genetic and genomic analyses of the soybean genome, precise genetic and physical maps are important. Indeed, various types of physical maps have so far been reported using RFLP, RAPD, SSR and AFLP markers. Sequencing of the soybean genome and the model legume species will provide us with a large amount of information for useful genes. Recently, a soybean genome sequence project was also started in Japan using a Japanese cultivar 'Enrei'. More than 10,000 independent full-length cDNAs have been collected from various organs and stress-treated soybean. The reports in this issue will be useful for future functional analysis of genes involved in soybean productivity.

Seed calcium content is an important quality attribute of specialty soybean [Glycine max (L.) Merr] for soyfoods. However, analyzing seed for calcium content is time consuming and labor intensive. Knowing QTL for seed calcium will facilitate the development of elite cultivars with proper calcium content through MAS. Calcium content was tested in 178 F2:3 and 157 F2:4 lines derived from the cross of SS-516 (low calcium) x Camp (high calcium) by Zhang et al (2008). Four QTL designated as Ca1, Ca2, Ca3, and Ca4 on linkage groups (LGs) A2, I and M were identified by both single-marker analysis and composite-interval mapping, and the QTL accounted for 10.7%, 16.3%, 14.9%, and 9.7% of calcium content variation, respectively. In addition, multiple-interval mapping analysis revealed a significant dominant-by-dominant interaction effect between Ca1 and Ca3, which accounted for 4.3% calcium content variation. These QTL will facilitate the implementation of MAS for calcium content in soybean breeding programs.

Forage quality depends on the digestibility of fodder, and can be directly measured by the intake and metabolic conversion in animal trials. It is not possible to study thousands of plant genotypes, as required in breeding programs. Therefore, several indirect methods including near-infrared reflectance

spectroscopy (NIRS) have been established to overcome this limitation. However, the ideal indirect system for the prediction of forage performance would be based on gene-derived "functional" DNA markers, allowing early selection ultimately without need of field trials, and being environment independent. In addition, once identified relevant genes controlling forage quality are targets for transgenic approaches. Substantial progress has recently been achieved in the development and application of genomic tools both in model species and major forage crops such as ryegrass and alfalfa. Key genes involved in developmental and biochemical pathways affecting forage quality such as cell-wall, lignin, fructan, and tannin biosynthesis have been isolated and characterized. For some of these genes, allelic variation has been studied in detail and sequence motifs with likely effect on forage quality have been identified by association studies. Moreover, transgenic approaches substantiated the effect of several of these genes on forage quality. Perspectives and limitations of findings for forage crop breeding needs to be discussed given expected further progress in forage crop genomics, but also the complexity of the trait complex forage quality, since typically species mixtures of heterogeneous and heterozygous genotypes are grown in the field (Lübberstedt 2007).

Perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*L. multiflorum*) are regarded as ideal grass species for use as animal forage in temperate grassland agriculture. However, their use is restricted as they lack persistency, especially in marginal areas and locations that are subject to summer and winter stresses and drought stress. Close relative species from within genus Festuca are much better adapted to such abiotic stresses but, by contrast, do not compare well in animal forage provision to Lolium species as they show poor establishment and comparatively lower quality characteristics. Lolium and Festuca species hybridize naturally and exhibit high frequencies of gene exchange in the hybrid condition. Intergeneric hybrids (Festulolium) between Lolium and Festuca species are being used to broaden the gene pool and to provide the plant breeder with options to combine high quality traits with broad adaptations to a range of environmental constraints. Festulolium varieties have promise as novel grasses with high forage quality and resistance to environmental stress and can thereby improve grassland productivity, persistency and benefit incomes.

Conventional forage grass breeding programs rely on basis observable phenotypes using the natural genetic variation found between and within varieties or ecotypes. Genetic improvement of forage grasses by conventional breeding programs is very slow due to the obligate outbreeding and perennial nature of grasses. Advances in genomics and gene manipulation can complement and enhance conventional plant breeding programs. Many studies concerning the implementation of DNA markers, high-throughout gene discovery, genome-wide gene expression analysis and gene manipulation are currently being conducted for forage grasses (Yamada *et al* 2005).

Molecular and biochemical studies were undertaken to elucidate gene/product relationships which influence key fruit quality traits in cultivated strawberry (Fragaria × ananassa Duch.). Comparative transcription profiling experiments in selected genotypes pointed out a number of differentially-expressed genes, possibly related to important fruit quality traits as aroma and fruit firmness. Some of the altered cDNAs encode putative cinnamyl alcohol dehydrogenase, cinnamoyl CoA reductase, cellulase and expansin genes, involved in the early steps of lignin biosynthesis and in cell wall structure, respectively. Parallel biochemical analyses studied the spectra of volatile compounds by Proton Transfer Reaction-Mass Spectrometry (PTR-MS) and alcohol acyl transferase (AAT) specific activity in red fruits. A

correlation between the expression of an aat gene, total AAT activity and the presence of related esters in fruit headspace was found by Fabrizio *et al* (2006).

The seed industry is beginning to use DNA based markers to develop "fingerprint" patterns for grouping or clustering complex beneficial traits. This "cluster analysis" will allow breeders to predict regional performance or environmental adaptation based on shared marker patterns. For example, processing tomatoes adapted for California conditions cluster separately from varieties that perform best in Ohio. The efficiency and outcome of selection strategies can be greatly enhanced by optimizing breeding crosses to a targeted DNA marker cluster. Another practical application is to identify seed lot purity in asparagus or in any seed lot. Molecular markers were used to show that some seed lots used for crown production contained over 70% poor yielding types and types with low postharvest quality. This can be critical information for a perennial crop that is expected to be productive over a 10-12 year span (Suslow and Bradford 1999).

4-Ketocarotenoids in Flower Petals (Patent # WO03080849).

The formation of a carotenoid compound containing a 4-keto-beta-ionene ring such as astaxanthin or canthaxanthin in flowers, and particularly in the corolla and reproductive parts of a flower of a higher plant whose flowers produce a carotenoid compound containing a beta-ionene ring such as beta-carotene or zeaxanthin, but otherwise do not produce astaxanthin or canthaxanthin is disclosed. One or more genes controlled by a promoter are inserted (transformed) into a higher plant. The inserted gene encodes a chimeric enzyme including (a) a carotenoid-forming enzyme that is at least a ketolase. That gene is operatively linked to (b) a plastid-directed transit peptide. Some higher plants to be transformed produce at least zeaxanthin or beta-carotene in their flowers prior to transformation, whereas other plants produce little if any coloured carotenoid pigments prior to transformation and are transformed with a cassette of carotenoids-forming genes (Hauptmann *et al* 2003).

The present invention provides a new method for promoting the synthesis of fatty acid in a plant. The amount of protein of carboxyl transferase and beta subunit encoded by accD gene is increased by introducing a promoter sequence of a gene highly expressed in chloroplast at the upstream of an E.coli type acetyl CoA carboxylase accD gene by chloroplast transformation technique. The amount of protein of other subunit constituting acetyl CoA carboxylase is also increased by this process. Since acetyl CoA carboxylase is the key enzyme of the first stage of fatty acid synthesis, the synthesis of fatty acid can be promoted by the method of the present invention. The transformed vegetable produced by the method exhibits remarkable promotion of fatty acid synthesis, prolonged life of the leaf, increased yield of seeds and improved productivity of the plant body (Sasaki *et al* 2002). Preparing Transgenic Leguminous Plants with Increased Protein Content (Patent # WO0175128). The invention relates to a method for the production of leguminous plants with increased protein content in the seeds and longer seed filling duration, by means of introduction of recombinant DNA molecules. Said recombinant DNA molecules are introduced into the plant, by means of a transformation system and comprise a DNA sequence from the plant, expressed in plants, the genetic product of which inhibits a protein in the seed with the enzymatic activity of an ADP glucose pyrophosphorylase (AGP) and/or a plastid phosphoglucomutase (pPGM) and, optionally, the regulatory sequence of a seed-specific promoter in leguminous plants. Furthermore, at least one selection marker gene is separately transferred, which is subsequently removed again. The plants

which display increased protein content and a lengthier seed-filling duration are chosen (Weber *et al* 2001).

Crop quality improvement is gaining unprecedented importance in both developed and developing countries. Products with improved quality give the farmer added value and a competitive market advantage, which in turn will result in improved human welfare and increased farm income. Thus, the improvement of quality characters in crop plants has great potential to alleviate problems caused by poverty and malnutrition through both direct (food quality and quantity) and indirect effects (income stability, etc) that affect farmer's social and economic status.

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