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Review

Diagnostic DNA markers for quality traits in wheat

K.R. Gale*

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

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Abstract

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. This review provides an overview of diagnostic DNA markers that are currently available for the selection of quality traits in wheat.

Keywords: DNA markers; Wheat quality; Glutenins; Granule-bound starch synthase 1; Puroindolines; Marker assisted selection; Review

1. Introduction

Wheat is one of the major food crops utilised worldwide. Modern cultivars of bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* var. durum) are the result of extensive selection by breeders to meet the agronomic and quality requirements of the diverse environments under which they are cultivated and the wide range of products for which they are utilised. The current challenges for wheat breeding programs around the world are to maintain or improve agronomic performance and to improve wheat quality, thus maintaining competitiveness in the increasingly discriminating international marketplace. Wheat cultivars may be either targeted or blended for specific end-uses such as traditional pan, sponge and dough pan, flat or steamed breads, frozen doughs, yellow alkaline or Udon style noodles, confectionary, biscuits, cakes and high-quality pastas.

In the past, selection of breeding lines with enhanced performance has relied on the direct measurement of the traits of interest. Clearly, for simple morphological traits such as plant height, it is easy to make selections from large numbers of progeny. The scoring of most traits, however, requires laboratory testing or bioassay. Many traits are laborious to measure (grain dormancy and late maturity amylase, for example, Mares and Mrva, 2001; Mrva and Mares, 2001) and thus impose a major constraint on the speed and scale of selections that are feasible with the resources available to breeders. In such cases, the availability of markers, that represent an indirect indicator of the trait of interest and that can be scored with relative ease, are of significant value to wheat breeders. Markers may be linked (i.e. have a probability of being co-inherited with the trait of interest that is dependent on the genetic proximity of the marker and the gene influencing the trait) or diagnostic (also

Abbreviations: A-PAGE, acidic-polyacrylamide gel electrophoresis; ELISA, enzyme linked immunosorbent assay; GBSS1, granule bound starch synthase1; GSP, grain softness protein; HMV-GS, high molecular weight-glutenin subunits; HPLC, high pressure liquid chromatography; LMW-GS, low molecular weight-glutenin subunits; PCR, polymerisation chain reaction; RT-, real time; RP-, reversed phase; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SKCS, Single Kernel Characterisation System; SNP, single nucleotide polymorphism.

* Tel.: +61 2 6246 5317; fax: +61 2 6246 5345.
E-mail address: kevin.gale@csiro.au.

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known as perfect) if the marker is directly associated with the gene that influences the trait. Diagnostic markers have the significant advantages that they do not require independent validation for each parental line used in a breeding program and have absolute linkage with the trait being selected.

There are several types of markers used by plant breeders. A small number of morphological characteristics are linked to specific traits, for example, psuedo black chaff linked to *Sr2* stem rust resistance (Brown, 1993) and red glume colour linked to *Yr10* stripe rust resistance (Metzger and Silbaugh, 1970). Biochemical markers have found wide application in wheat breeding with the earlier observations that wheat endosperm storage proteins (prolamins), that may be prepared using a simple alcohol extraction, are extremely polymorphic between wheat lines (Gupta and Shepherd, 1990; Lawrence and Shepherd, 1980; Payne et al., 1981; Payne and Lawrence, 1983). These polymorphisms may be scored by acidic polyacrylamide gel electrophoresis (A-PAGE) in the case of the monomeric gliadin fraction (Bushuk and Zillman, 1978), or SDS-PAGE in the case of the polymeric glutenin fraction (Gupta and Shepherd, 1990; Gupta and MacRitchie, 1991; Bean and Lookhart, 2000). Reversed phase (RP-) high performance liquid chromatography (HPLC) has been used to score glutenin content (Huebner and Bietz, 1985), with the advantage that HPLC may also be used to quantify the expression levels of specific proteins and this information may be informative for some quality traits, such as dough strength (Marchylo et al., 1989a). Isozyme variation has been reported for many wheat enzymes (http://wheat.pw.usda.gov/ggpages/wgc/98/Contents.htm) and whilst these have been mapped to specific loci, their implementation as markers in breeding programs has not been reported. The biochemical quantification of pigments such as xanthophylls and flavonoids has been directly linked to colour traits in durum and bread wheat (Mares and Campbell, 2001; Parker et al., 1998; Troccoli et al., 2000). In some studies, colour stability has been linked directly with polyphenol oxidase (PPO) activity in noodles (Mares and Campbell, 2001; Mares and Panozzo, 1999).

A small number of diagnostic antibody markers linked to wheat quality are available and these offer the advantage of being applicable in a simple, high throughput format such as the enzyme-linked immunosorbent assay (ELISA). Antibody-based marker assays are currently available for the detection of granule-bound synthase 1 (GBSS1) null lines with increased flour swelling volume, that are preferable for Udon noodle wheats (Gale et al., 2001; Graybosch et al., 1998a), the prediction of grain hardness (Partridge et al., 2002) and the presence of wheat/rye chromosome translocations previously used to introduce disease resistance alleles into wheat (Andrews et al., 1996; Skerritt et al., 1996). A post-harvest antibody-based test for the prediction of starch quality by measurement of amylase levels expressed in wheat grain has been developed (Skerritt and Heywood, 2000; Verity et al., 1999). Antibody-based tests have also been developed for the detection of bread wheat adulteration of durum pasta based on the detection of friabilin (Durotest, Rhone Poulenc Diagnostics Ltd, European patent, EP540432-A1) and the testing of gluten-free foods to ensure their safety for coeliac sufferers (reviewed by Denery-Papini et al., 1999).

A DNA marker may be defined as an assay for the detection of polymorphism in DNA sequence between samples. A number of different DNA marker systems have been described (Gupta et al., 1999) most of which now rely on the sensitivity and specificity of the polymerase chain reaction (PCR, Saiki et al., 1985). The first demonstration of the application of PCR-based DNA markers to the analysis of polymorphism in wheat was made by D'Ovidio et al. (1990) using PCR amplification of γ-gliadin sequences from durum wheat. DNA markers have now become the markers of choice for most marker-assisted breeding applications (Eagles et al., 2001; Gupta et al., 1999). DNA markers offer the advantages of being applicable to any developmental stage and any tissue of the plant, and of producing results that are independent of environmental conditions. This review focuses on diagnostic DNA markers for wheat quality traits.

2. High molecular weight glutenin subunits and the glutenin polymer

High molecular weight glutenin subunits (HMW-GS) are components of the glutenin polymer and therefore play a major role in the determination of the unique visco-elastic properties of wheat doughs (Halford et al., 1992; Nieto-Taladriz et al., 1994; Payne et al., 1987). The effects of the HMW-GS on dough properties, such as dough strength and elasticity, may be additive or synergistic, with significant interactions with the low molecular weight glutenin subunits (LMW-GS, Beasley et al., 2002). The HMW-GS are encoded by polymorphic genes at the *Glu-1* loci present on the long arms of the group 1 chromosomes (Payne and Lawrence, 1983). At each locus (*Glu-A1*, *Glu-B1* and *Glu-D1*), there are two tightly linked HMW-GS genes, one x-type and one y-type, with the y-type encoding HMW-GS of lower molecular weight than the x-type. The y-type genes of the A genome are not usually expressed in hexaploid wheat (Payne et al., 1981; Payne and Lawrence, 1983). The HMW-GS proteins have mobilities by SDS-PAGE in the range 80–130 kDa, although their true molecular weights deduced from amino acid sequences are in the range 60–90 kDa (Bunce et al., 1985). HMW-GS contains unique N- and C-terminal domains and a large central repetitive domain rich in glycine, proline and glutamine residues. Intermolecular disulphide bonding between the sulfhydryl groups of cysteine residues contained within the N- and C-terminal domains plays a major role in the formation of the glutenin macro-polymer (reviewed by Gras et al., 2001). The sequences of many HMW-GS genes and their allelic