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Starch swelling power and amylose content of triticale and *Triticum timopheevii* germplasm

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ABSTRACT

Triticale (× *Triticosecale* Whittmack) and *Triticum timopheevii* have undergone little selection relative to other grains for quality characters, including starch amylose content. Using starch swelling power (SSP) in water and spectrophotometric analysis of the iodine binding ratio, 247 lines of triticale and 20 lines of *T. timopheevii* were screened for amylose content. Following this, the expression of the starch-forming protein granule-bound starch synthase (GBSS) in triticale was investigated by SDS-PAGE in the eight highest and eight lowest SSP lines. A strong correlation ($R^2 = 0.8174$) was found between iodine binding and SSP. The SSP of *T. timopheevii* lines ranged from 13.7 to 16.7, indicating an approximate range of amylose content from 28.1 to 33.8%: a small range within typical results from commercial wheat cultivars. The SSP of triticale ranged from 12.5 to 23.6 suggesting amylose content ranged from 12.8 to 35.1%: a much wider range reflecting the contribution of both the wheat and rye genomes. It appeared that expression of GBSS-4A was down-regulated in low amylose lines. Therefore there is significant potential to select for amylose content in triticale to increase quality in both the animal and human feed markets.

1. Introduction

The starch composition of cereal grains plays a major part in the digestibility and bread-making quality of flour. In general, lower starch amylose content corresponds to higher peak viscosity of paste, lower peak viscosity temperature and greater resistance to retrogradation (as reviewed in Pham Van et al., 2006). This has a variety of implications for product quality, including better Japanese udon noodle texture (Oda et al., 1980; Toyokawa et al., 1989), greater bread loaf volume, more porous crumb structure (Lee et al., 2001) and reduced bread staling (Lee et al., 2001; Morita et al., 2002). Furthermore, high starch amylose content is preferred for human and ruminant consumption owing to slower digestion and absorption (Mikulikova and Kraic, 2006).

Triticale (× *Triticosecale* Whittmack, AABBRR genome) has relatively poor bread and noodle-making qualities, owing partly to a short history of selection for quality. Similarly, *Triticum timopheevii* (AAGG genome), a wild-type wheat, has also had little selection for grain quality. Relatively little is published regarding the starch content of either species, although extensive studies have been conducted in bread wheat (Lehmann and Robin, 2007; Pham Van et al., 2006; Svihus et al., 2005).

The amylose content of triticale appears to be similar to that of wheat. Sharma et al. (2002) observed a range of approximately 20–30% in triticales grown in Australia when comparing partially waxy to normal types. The amylose content in Australian hexaploid wheat cultivars was found to range from 23.5 to 38.9%, with a similar range of 17.6–34.0% for exotic cultivars (Regina, 2000).

Most of the genes involved in starch synthesis have been identified in *Zea maize*, *Hordeum vulgare*, *Triticum durum*, *Triticum aestivum* and *Oryza sativa* (Hayden, 1999; He et al., 2006; Paschall and Whistler, 1965; Regina, 2000). These enzymes fall into four main groups: ADP-Glc pyrophosphorylase (EC 2.7.7.27), starch synthase (EC 2.4.1.21), branching enzyme (EC 2.4.1.18), and debranching enzyme (EC 3.2.1.41 and 3.2.1.68). The starch synthase enzymes can be separated into two distinct groups: granule-bound starch synthases (GBSS, waxy protein, EC 2.4.1.242) and soluble starch synthases (SS). GBSS is involved in the production of linear amylose molecules of starch and is located exclusively within the starch granule (SG) (Echt and Schwartz, 1981; Tsai, 1974). Soluble SS enzymes are involved in the synthesis of branched amylopectin molecules and vary in distribution between the soluble and SG

Abbreviations: GBSS, granule-bound starch synthase; HMW, high molecular weight; LMW, low molecular weight; SD, standard deviation; SDS, sodium dodecyl-sulfate; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; SG, starch granules; SSP, starch swelling power.

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protein fractions (Ball and Morell, 2003). Starch branching enzymes (SBE, BE) also play a crucial role in amylopectin synthesis as they catalyse a transferase reaction that cleaves a donor linear 1,4-glucan chain (either amylose or a linear region of amylopectin) and attaches it to a recipient chain via an α-1,6 linkage, producing a branch in a growing amylopectin molecule.

The presence or absence of expression of the starch synthesis enzymes such as GBSS can have a marked effect on starch quality traits such as SSP. In hexaploid wheat, three waxy loci have been identified on the short arms of chromosomes 7A (*Wx-A1*) and 7D (*Wx-D1*) and the long arm of chromosome 4A (*Wx-B1*) (Chao et al., 1989; Nakamura et al., 1993; Yamamori et al., 1994; Zhao and Sharp, 1996). The three isoforms of GBSS encoded by these genes (GBSS-7A, GBSS-7D and GBSS-4A respectively) vary in their effects on the amylose content of hexaploid wheat. In general, the absence of expression of GBSS-4A has been found to cause a larger reduction in amylose content than the absence of either GBSS-7A or 7D (Miura et al., 1994; Yamamori et al., 1994). The results, however, are often confounded by differences in the genetic background of lines being compared. One study using nullisomic–tetrasomic lines of the experimental wheat 'Chinese Spring' found that the absence of GBSS-4A expression caused a reduction of amylose content from 25.5 to 22.5% (Miura and Sugawara, 1996). Lines with an absence of GBSS-7A or 7D also had a reduction in amylose content that was less dramatic than that observed in the 4A null lines. Sharma et al. (2002) documented a difference in the average amylose contents of triticale with normal genotypes (26%) and lines with null alleles at GBSS-4A (21.5%). Several studies have produced mutant wheat breeding lines with null alleles for all three waxy genes (Kiribuchi-Otobe et al., 1997; Nakamura et al., 1995; Yasui et al., 1997). The resulting lines all have a waxy phenotype, with virtually no amylose present.

The aim of this study was to determine the range of amylose content in triticale and identify the potential of lines with particularly high or low amylose content for future breeding programs. A further aim is to observe the range of swelling powers of a random selection of *T. timopheevii* lines and deduce the potential of *T. timopheevii* as a source of high or low amylose germplasm. This study also evaluates how the wheat and rye genome may interact to genetically control starch content in triticale.

2. Experimental

2.1. Materials

A selection of 247 triticale lines from CIMMYT (33rd ITSN and 33rd ITYN) were grown in the field in 2004 at the Plant Breeding Institute, Cobbitty along with 20 *T. timopheevii* lines sourced from the Australian Winter Cereals Collection (Tamworth, NSW). Waxy wheat was provided by Dr Xiaochun Zhao, Plant Breeding Institute, Cobbitty. Maize standards of 0% and 27% amylose were obtained from Sigma–Aldrich (Castle Hill, Australia) and 50% amylose maize starch was a gift from Penford Australia Ltd. (Lane Cove, Australia).

2.2. Starch extraction

Purification of starch was based on Stoddard (1999). Between 15 and 20 seeds were manually cracked and soaked overnight at 4 °C in approximately 2.5 mL 0.5 M NaCl, then manually mashed. The supernatant was centrifuged at 4700 rpm for 1 min, then decanted. This was followed by addition of 500 μL of 100% w/v CsCl to the pellet, vortexed at 1400 rpm for 1 min, centrifuging at 4700 rpm for 1 min then decanting. This process was repeated with 500 μL of 100% w/v CsCl, followed by 1 mL distilled water (twice), 1 mL 2% w/v sodium dodecyl sulfate (SDS), 1 mL distilled water (twice) then 1 mL 95% v/v ethanol. Subsequently, 1 mL of 85% v/v methanol was added and samples were mixed in a vortex mixer for 1 min at 1400 rpm before being placed in a water-bath at 65 °C for 20 min. Samples were again centrifuged for 1 min at 4700 rpm, then placed in desiccators after the supernatant was discarded.

2.3. Starch swelling power assay

Initial assay for amylose content was by swelling power (Konik-Rose et al., 2001). Subsamples of 25 mg of starch were weighed to the nearest 0.3 mg then mixed with 600 μL of 0.1% w/v $AgNO_3$ in a vortex mixer at 1300 rpm for 1 min to inhibit alpha-amylase activity (Blazek and Copeland, 2008). They were then heated in a water-bath for 30 min at 92.5 °C with manual shaking at 1 min intervals for the first 5 min, in 2.5 min intervals for the next 5 min and then every 5 min for the remaining time. Following this, they were placed in cold water for 5 min and then centrifuged for 5 min at 7400 rpm. The supernatant was carefully removed using a pipette, then the tubes weighed. Swelling power was calculated by the following formula:

$$\text{Swelling power} = \frac{\text{Mass of swollen sample}}{\text{Initial mass (0.025 g)}}$$

Maize standards were included in every assay as controls. However, they can only be used to estimate the amylose content of triticale due to differences in the swelling power/amylose relationship between triticale and maize.

To confirm the results of the 12 highest and 26 lowest triticale lines, a confirmation swelling was performed (and repeated for some lines) using the same method apart from increasing the volume of 0.1% w/v $AgNO_3$ to 1 mL. All replicates from the same line were averaged for analysis.

2.4. Iodine staining and spectrophotometric analysis

The lines with the highest and lowest swelling powers were stained to determine if any lines were truly waxy (contained no amylose). One to two drops of iodine solution (0.2 g iodine, 2 g potassium iodide and 250 mL distilled water) were added to a freshly cut surface of grain endosperm. Blue colour indicated normal amylose content whilst a poorly defined reddish colour indicated no amylose.

To determine the amount of amylose in the lines with the 10 highest and 10 lowest swelling powers their iodine binding ratio was evaluated (Zhu et al., 2008). Two replicates of 5 mg starch extract were each mixed with 75 μL of 95% v/v ethanol and 450 μL of 1 M NaOH in a vortex mixer, then heated to 100 °C in a water-bath for 30 min. After cooling, 50 μL were removed from each replicate and 500< μL of 0.1 M citric acid was added, then replicates were stained with 200 μL of iodine solution made as above. Samples were diluted with 2.25 mL of distilled water then refrigerated for 40 min. The absorption of the iodine–amylose complex was measured in two 200 μL replicates at 620 and 535 nm. The ratio of the A620/A535 absorbance was calculated to determine the amount of amylose based on a standard curve created using 0–50% maize standards. These two wavelengths allow both the highest and lowest points on the absorbance spectra to be taken into account and thus increase the precision of the linear regression equation (Zhu et al., 2008). Data analysis used GenStat version 7.0.

2.5. SDS-PAGE analysis of triticale protein extracts

Eight high and eight low SPP triticale lines were selected for protein analysis by SDS-PAGE (Table 1). Starch was prepared from