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Research Application Summary

Exploiting genome synteny in breeding for Protein Quality and Waxiness in maize and sorghum

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Abstract

Maize and sorghum are important staple cereals in sub-Saharan Africa. Nonetheless, their utility and remunerability is compromised by a low available lysine content and digestiblitiy of both protein and starch in the endosperm. Over the decades, progress has been made in enhancing the lysine content of maize and sorghum by introgression of the *opaque2* gene into elite materials. Also, feeding trials have indicated more gain from utilising materials introgressed with the waxy gene. Pleiotropic effects are common place in breeding for protein quality and waxiness and molecular markers including SSRs have been used in marker assisted breeding for both traits complimented by biochemical analyses and visual assessment. Recent genetic studies have implicated the waxy locus to be involved in modification in quality protein maize (QPM). In this study, we intend to develop functional genetic markers based on expressed genes involved in the starch biosynthetic pathway. The main objective will be to identify single nucleotide polymorphisms in the expressed regions of interest and explore their potential for use as markers and determine their transferability between the two cereal species.

Key words: Genetic markers, nucleotide polymorphism, *opaque2* gene, quality protein maize

Résumé

Le maïs et le sorgho sont les céréales importantes constituant des aliments de base en Afrique Sub-saharienne. Néanmoins, leur utilité et leur rémunérabilité sont compromises par un faible contenu disponible de lysine et une digestibilité de la protéine et de l'amidon dans l'endosperme. Pendant des décennies, le progrès a été réalisé en augmentant la teneur en lysine du maïs et du sorgho par l'incorporation du gène *opaque2* dans des matériaux d'élite. En outre, les essais d'alimentation ont indiqué de meilleur rendement en utilisant des matériaux incorporés

avec le gène cireux. Les effets pléiotropiques sont courants dans l'élevage pour la qualité de protéine et la teneur en cire et les marqueurs moléculaires comprenant SSRs ont été employés dans l'élevage assisté par marqueur pour les deux traits complimentés par les analyses biochimiques et l'évaluation visuelle. Les études génétiques récentes ont impliqué le locus cireux à être impliqué dans la modification en maïs de protéine de qualité (QPM). Dans cette étude, nous avons l'intention de développer les marqueurs génétiques fonctionnels basés sur les gènes déclarés impliqués dans la voie biosynthétique d'amidon. L'objectif principal sera d'identifier des polymorphismes simples de nucléotide dans les régions déclarées d'intérêt et d'explorer leur potentiel pour l'usage comme marqueurs et de déterminer leur transférabilité entre les deux espèces de céréale.

Mots clés: Marqueurs génétiques, polymorphisme de nucléotide, gène *opaque2*, maïs de protéine de qualité

Background

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolour* (L) Moench) are important staple cereals in most of sub-Saharan Africa accounting for approximately 15 to 56% of the daily caloric intake (Prasanna *et al.*, 2001). Nonetheless, both crops are low in their nutritional and processing qualities. With more than a third of Africa's population (over 250 million people) dependent on both cereals, the risk of nutrient deficiency-based diseases such as various forms of anaemia, rubella and kwashiorkor are eminent (AHBFI, 2007). Since the discovery of mutations *opaque2 (o2)* and *waxy (wx)* in maize and sorghum, progress has been made to improve the nutritional and grain quality traits in both crops.*Opaque2* (*o2*) enhances the lysine content in maize and sorghum and its interaction with modifier loci results in genotypes with modified vitreous kernels while maintaining an enhanced protein profile. The waxy mutation (*wx*) in cereals alters starch content, mostly the accumulation of amylopectin up to 100% and subsequently results in better digestibility and processing qualities of the grain. Due to the pleiotropic effects associated with the two mutations, genetic studies have attributed the modified kernel phenotype to direct or indirect starch modification in the grain (Gibbon *et al*., 2003). In particular, activities of granule bound starch synthase I (GBSSI), starch branching enzymes (SBEs) and starch debranching enzymes (SDBEs).

Molecular markers including simple sequence repeats (SSR) based on polymorphism in DNA sequences flanking regions of interest are widely used to tag both traits in maize. Vast

information on expressed sequence tag (EST) data for maize and sorghum is available in public databases and has been exploited to develop functional markers for other traits based on the large syntenic segements in maize and sorghum. This study aims to develop single nucleotide polymorphism (SNP) markers in maize and sorghum with respect to the *o2* and *wx* loci. Potential for transferability of the developed markers in the two grass species will be explored.

Literature Summary

Quality Protein Maize (QPM) contains nearly twice as much usable than normal (Konstantinov and Mladenovic, 2007). Pertinent to the *o2* trait is a set of modifier genes (*mo2*) that interact with the *o2* locus resulting in kernels with a vitreous phenotype. However, not much is understood yet of the mode of action or identity of these modifier genes thereby retarding the breeding process (Gibbon and Larkins, 2005). The waxy trait in cereals is attributed to a defect in metabolism precluding the synthesis of amylose in the endosperm and is observed to exhibit similar pleiotropic properties as *o2* (Perdersen *et al*., 2005). Expression of the waxy trait is in the endosperm and selection of waxy lines has often been done by visual assessment of the endosperm fracture patterns and more recently by potassium iodide staining. Central to both *o2* and *wx* mutations is the modification of starch in the endosperm which is alluded to increased amylopectin branching. Recent reports on modifier loci have implicated the involvement of the waxy locus in modification of QPM (Holding *et al.*, 2008). The waxy locus has been cloned in maize, rice and other cereals including sorghum (Shure *et al*., 1983; Okagaki and Wessler, 1988; Wang *et al.*, 1990; Selahattin, 2004). In rice, up to 8 starch synthase and 2 GBSS gene isoforms are involved in amylopectin and amylose synthesis; that is SSI, SSIIa, SSIIb, SSIIc, SSIIIa, SSIIIb,SSIVa and SSIVb; GBSSI and GBSSII. In comparision with maize, phylogenetic studies among starch synthesis genes did not find a homologue for one of the synthases, SSIIc. Starch synthase isoforms may be tissue specific or ubiquitously expressed in the plant. For purposes of functional marker development, genes that are expressed ubiquitously offer the greatest benefit. Currently, SSI, SSIVa, SSIVb, BEI, BEIIa and GBSS could be the likely targets (Ohdan *et al.*, 2005). Mining for single nucleotide polymorphisms (SNP) using expressed sequence tag (EST) data has been carried out in maize and other cereals (Kantety *et al.*, 2002). Thus, given the large syntenic segments of conserved gene order among the grass family, exploration of already available ESTs could provide an avenue for the development of functional genetic markers to

be integrated in breeding for protein quality and waxiness in maize and sorghum.

Study Description

A set of three near isogenic maize lines will be used in this study and differ only in the *o2* and *wx* genes. The sorghum inbred lines include BTx630, BTxARG1 and RTx29071 were chosen for their differential expression of the waxy trait.

EST sequence data for maize and sorghum will be accessed from dbEST/GenBank (http://ncbi.nlm.nih/entrez). Additional maize EST sequences will also be accessed from ZmDB (http://www.zmdb.iastate.edu) and will be merged into one FASTA format file. Targeted genes will include starch branching enzymes (SBE), debranching enzymes (DBE) and granule bound starch synthase I (GBSS). Maize genotype B73 will be used as the EST source from the database. Also, sequences from rice will be downloaded and aligned for comparison. The merged FASTA format file will be assembled into groups of similar, overlapping sequences called contigs using CAP3 (Huang and Madan, 1999) at a stringency level of 95% homology. The output file from CAP3 will be processed through a BioPerl module (A. Walsh; http://www.agr.gc.ca/science/winnipeg/mg_biomf_e.htm) that converts the "*.ace" file into a padded FASTA file that is readable by JalView (M. Clamp; http://www.ebi.ac.uk/~michele/jalview). JalView is a JavaScript applet that displays the CAP3-based alignments and allows editing of the alignment, including changing the sequence order, deleting sequences, and colouring the individual nucleotides. Only contigs containing 11–60 EST members will be considered for further analysis. This is because contigs with 10 or fewer ESTs are not informative enough and it becomes difficult to view and edit contigs with >60 ESTs using JalView (Somers *et al.*, 2003). The final sequence alignments will be visually inspected to identify homoeologue sequence variants (HSVs) that defined homoeologous EST clusters and SNPs within the homoeologue EST cluster. Primers for allele-specific amplification will be designed from a random selection of identified contigs with 11–60 EST members in each. A homoeologue EST cluster will be visually scanned to identify SNPs between the different maize and sorghum genotypes. Two or three primers in each of the forward and reverse directions will be designed to test multiple primer combinations for allele-specific amplification. All primers will be designed to at least 20 bp lengths. All PCRs reactions will be run in 25 ìL reactions and amplicons separated in 1.5% w/v Tris–acetate–EDTA (TAE) agarose gels then visualized

by ethidium bromide staining. Allele-specific primer pairs from selected contigs will be tested with the *wx* and *o2* genotypes to identify robust PCR primer pairs. To further validate identified SNPs, primers will be tested on segregating population of a cross between *waxy* and *opaque2*.

Research Application

Current approaches to marker assisted breeding with respect to both *opaque2* and *waxy* is based on polymorphism of DNA sequences flanking the region of interest; the most widely used markers being simple sequence repeats (SSRs). These however need to be complimented by biochemical analysis and visual assessment of the kernel and the iodine test in the case of waxy genotypes. Given the complexity in breeding for both recessive traits in association with modifier genes, developing functional markers based on EST data provides a user-friendly and cost efficient marker assisted breeding approach. We also envisage the identification of an SNP that is transferable between maize and sorghum in breeding for both protein quality and waxiness.

Recommendation

With the advances in molecular biology little is yet known of the identity and number of modifier loci that interact with the *opaque2* gene to produce a modified kernel phenotype in QPM. Similarly, not much has been done on the interaction of the waxy locus and possible modifier loci that lead to a modified kernel phenotype in waxy genotypes of maize and sorghum. Several studies suggest a possible interaction of the altered starch structure with proteins in the kernel and more recently, suggestions on the possible involvement of the waxy locus provides insight into the modification complex observed in QPM. The identification of SNPs in expressed genes and their integration in a marker assisted breeding programme will not only expedite the breeding process and precision of gene introgression but also provide a simpler and cost-efficient means for selection in breeding for protein quality and waxiness in maize and sorghum.

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