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Research Application Summary

Applications and benefits of marker-assisted introgression of the opaque-2 gene in quality protein maize breeding

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Abstract

Maize (*Zea mays*) is a crucial crop in Africa serving as one of the chief carbohydrate sources used as food and feed. However, maize is deficient in essential amino acids, lysine and tryptophan. Protein malnutrition is a major challenge as a result, especially in developing countries where maize is a staple crop. The discovery of the *opaque-2* maize mutant, high in lysine and tryptophan, has offered an avenue for maize protein quality improvement. Quality protein maize (QPM) is a product of the extensive development of the *opaque-2* mutant. It stands as an affordable and viable option to overcome the scourge of protein malnutrition in humans and monogastric livestock. The conventional QPM breeding methods are based on phenotypic selection to identify materials carrying two copies of the recessive *opaque-2* allele. However, phenotypic selection in QPM breeding programs is influenced by the environment and has huge drain on resources such as time, money and labour with low genetic gains. Marker assisted introgression of the *opaque-2* gene with simple sequence repeat markers for foreground selection is a molecular technology based methodology of accelerating the Quality protein maize breeding process. Colorimetric tryptophan analysis stands as a crucial step in the QPM breeding process as it validates the elevated levels of tryptophan and the action amino acid modifier loci. Knowledge of the combining ability of the genetic materials used in the crosses made is pivotal in any QPM breeding programme.

Key words: Combining ability, foreground selection, simple sequence repeat markers

Résumé

Le maïs (*Zea mays*) est une culture cruciale en Afrique considéré comme la source première des glucides utilisés comme aliments destinés aux humains et animaux. Cependant, le maïs est déficient en acides aminés essentiels, lysine te tryptophane. La malnutrition en protéine est, par conséquent, un défi majeur plus particulièrement dans les pays en développement où le maïs est une culture de de base. La découverte du maïs mutant opaque-2, élevé en lysine et tryptophane, a offert la possibilité pour l'amélioration de la qualité de protéine du maïs. Quality protein maize (QPM) est un

produit du développement extensif du mutant opaque-2. C'est une option moins-chère et viable pour surmonter l'énorme carence en protéine chez les humains et les animaux monogastriques. Les méthodes de sélection conventionnelle pour le QPM se basent sur la sélection phénotypique consistant à identifier les matériels possédant deux copies de l'allèle récessive opaque-2. Néanmoins, la sélection phénotypique du QPM est influencée par l'environnement et épuise beaucoup de ressources comme le temps, ressources financières et de labeur avec de faibles gains génétiques. L'introgression du gène opaque-2 assistée de marqueurs à séquence répétée pour une sélection avant-plan est une méthodologie basée sur la technologie moléculaire pour l'accélérer le processus d'amélioration génétique du QPM. L'analyse colorimétrique de tryptophane est une étape cruciale du processus d'amélioration génétique du QPM comme cela valide les niveaux élevés de tryptophane et l'action des loci modificateur d'acides aminés. La connaissance de l'aptitude à combiner des matériels génétiques utilisés lors des croisements effectués est primordiale pour le programme d'amélioration génétique du QPM.

Mots-clés : Aptitude à combiner, sélection avant-plan, marqueurs à simples séquences répétées

Background

In many African countries, including Zimbabwe, maize is a crucial crop which serves as the chief source of carbohydrate used as food and feed (Scott *et al.,* 2004). Despite the high carbohydrate content, maize has low quality protein since it lacks essential amino acids, lysine and tryptophan (Lauderdale, 2000; Babu *et al.,* 2005). The low protein quality undermine nutrition of humans and livestock that feed on maize (Vasal, 2000). Most people who depend on maize are prone to protein deficiency related disorders particularly if they do not have access to other cheaper sources of dietary protein. Hence, over-dependence on maize based diets in the absence of other complementary protein sources leads to protein deficiency related diseases, such as kwashiorkor in young children (Babu *et al.,* 2005).

The breakthrough in maize protein quality improvement

The discovery of a natural maize mutant, later named the *opaque-2* mutant was a breakthrough in maize improvement with respect to protein quality (Vivek *et al.,* 2008). The mutant has elevated levels of the essential amino acids, tryptophan and lysine due to the expression of the *opaque-2* gene in its homozygous recessive state. This mutant underwent intensive development to eliminate some of the deleterious effects associated with the *opaque-2* gene, such as increased disease susceptibility as well as low yield and was later termed quality protein maize (QPM) by the International Maize and Wheat Improvement Centre (CIMMYT) (Lauderdale, 2000). QPM is an economically feasible and viable option to alleviate protein malnutrition and reduce animal feed costs, given that its grain protein contains more than double the lysine and tryptophan levels of normal maize (Zaidi *et al.,* 2008). It has been widely used to convert

non-QPM maize germplasm to QPM using the conventional direct backcross method (Vivek *et al.,* 2008).

Conventional Quality Protein Maize breeding

In Quality Protein Maize breeding three genetic systems are involved (Vasal, 2000). These are the *opaque-2* gene which is the central component, the endosperm modification and the action of amino acid modifiers which are key in tryptophan and lysine level elevation (Babu *et al.,* 2005). The *opaque-2* gene is responsible for supressing zein synthesis while simultaneously elevating the synthesis of other non-zein proteins that are richer sources of tryptophan and lysine (Nuss *et al.,* 2011). Endosperm modification is a quantitatively inherited trait and since it has complex genetic control and lacks reliable molecular markers linked to the endosperm modifier loci, a light table is presently used to physically select for endosperm hardness (Hossain *et al.,* 2008). Colorimetric tryptophan analysis is used to determine and monitor tryptophan levels in QPM breeding programmes by the use of biochemical methods to quantify tryptophan levels Vivek *et al.* (2008). Since the *opaque-2* gene is the central component in QPM breeding, selection of individuals carrying two copies of *opaque-2* allele in the homozygous recessive state is based on phenotypic selection on a light table. Breeding for a recessive gene such as the *opaque-2* and reliance on the phenotype for selection is greatly influenced by the environment and takes time to produce the desired product thus reducing the genetic gains. This has a huge drain on resources such as time, money and labour. This means that the plant breeder would deal with a large plant population just to select a few individuals to proceed, more people to do the pollinations and field related operations with a huge waste of time and other resources thereby lengthening the QPM development process. A more efficient yet economically feasible technology need to be employed to accelerate foreground selection in the breeding process to quickly deliver QPM for release as varieties. Since QPM is not yet fully established in Zimbabwe, the use of molecular markers can accelerate the breeding of QPM for use and as much strengthening its utilisation.

Marker assisted Quality Protein Maize breeding

Marker assisted introgression of the *opaque-2* gene with simple sequence repeat (SSR) markers for foreground selection (Maphosa, 2008) can be employed to complement conventional methods thus quickening the selection process in QPM breeding. The use of these molecular markers compliment conventional breeding methods by improving the accuracy of crosses as well as the quality of the genetic materials gained (Stubber *et al.,* 1999). Due to their simplicity and low cost of use simple sequence repeat markers are widely applied in maize breeding (Kostadinovic *et al.,* 2014). These are short nucleotide sequences usually from two to six bases in their length that are arranged in repetitive tandem arrays. Polymorphisms that can be amplified are unveiled due to the differences in the number of tandem repeats which lie between sequences that are conserved for each locus. These polymorphisms occur naturally in sequences of DNA such as deletions, additions and substitutions (Gupta *et al.,* 1999; Kostadinovic *et al.,* 2014). In QPM breeding three simple sequence repeat markers (phi057, phi112 and umc 1066)

situated as internal repetitive sequences within the *opaque-2* gene are being used as foreground selection markers for the *opaque-2* gene (Kostadinovic *et al.,* 2014). Marker assisted selection has the capacity to increase reliability and efficiency as well as reducing time and costs to attain QPM (Babu *et al.,* 2004). In QPM breeding programs, the effects of general combining ability (GCA) and specific combining ability (SCA) are crucial indicators of potential value for inbred lines and their derived hybrid combinations.

Conclusion

Marker assisted introgression of the *opaque-2* gene with the use of simple sequence repeat makers makes foreground selection efficient. Merits such as reduced population size per breeding generation, selection at any developmental stage and reduced time to completion accrue to the breeder. A more efficient selection methodology results in more efficient breeding programmes, leading to more reliable and guaranteed results. This expedites the breeding process making QPM available for release as varieties to farmers. When these varieties finally reach the farmers, a good, sound and cheap biofortified protein source is established thus providing a better diet for both people and monogastric livestock. By so doing, QPM utilisation will be strengthened in Zimbabwe. Colorimetric analysis of tryptophan during breeding monitors and validates the elevation of the tryptophan and lysine (since these two amino acids are correlated) in the genotypes with the *opaque-2* gene. After carrying out the combining ability analysis, inbred lines found with favourable general combining ability effects and hybrids with favourable specific combining ability effects are selected for use in other breeding programmes as testers and to be released as hybrid varieties respectively. These inbred lines can be used as testers in QPM breeding and the hybrids could proceed for further evaluation, multiplication and may end up as varieties on the market. Yield drag analysis will provide information on the extent of yield loss after having introduced the *opaque-2* gene into maize genotypes. This will present the economic feasibility of the utilisation of the QPM genotypes to farmers and having these genotypes replace some of the non-QPM maize genotypes grown by farmers in Zimbabwe.

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