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Carotenoid accumulation and agronomic performance of maize hybrids involving parental combinations from different marker-based groups

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ABSTRACT

The present study examined the effect of crossing parental lines from two AFLP-based groups on carotenoid accumulation and agronomic performance in hybrids, which were tested in four environments in Nigeria. Environments, hybrids and hybrid × environment interactions had significant effects on carotenoid content. Hybrids had consistent carotenoid levels across test environments. The correlations between carotenoids produced in a specific branch of the biosynthetic pathway were significant and positive. Environments, hybrids and hybrid × environment interactions had significant effects on grain yield and other traits in this study. Several hybrids with high provitamin A content that were competitive to a commercial hybrid in grain yield and other traits were identified in this study. Selection of parental lines with high provitamin A content and desirable agronomic traits from different molecular-based groups may serve as the basis for developing hybrids with greater expression of heterosis in productivity and concentrations of provitamin A carotenoids.

1. Introduction

Millions of people in Africa subsist on cereal-based diets with low levels of vitamin A and thus run a risk of vitamin A deficiency (Ruel, 2003). Vitamin A deficiency affects more than 43 million children younger than 5 years of age in sub-Saharan Africa (Aguayo & Baker, 2005), which retards physical growth, depresses immune function, increases susceptibility to infectious diseases, diminishes possibility of survival from serious illness, and night or complete blindness (Sommer, 2008). Maize (*Zea mays* L.) is one of the major staple food crops in sub-Saharan Africa, which is used in a variety of traditional foods, as green maize, and as a major component in local weaning foods. Maize kernels naturally accumulate carotenoids with provitamin A activity and xanthophylls that also are beneficial to human health, making the crop a suitable dietary source of these nutrients (Harjes et al., 2008). As the commonly cultivated maize cultivars contain low levels of provitamin A in their kernels (Harjes et al., 2008), considerable efforts have been made to increase accumulation of this nutrient in maize through conventional breeding to contribute to alleviation of vitamin A deficiency in areas with limited access to animal products, fruits and vegetables (Pfeiffer & McClafferty, 2007).

Pfeiffer and McClafferty (2007) proposed exploitation of heterosis as a viable option to enhance carotenoid accumulation in maize kernels. The critical step in a hybrid development program is the selection of adapted orange and yellow endosperm maize inbred lines with diverse genetic backgrounds and carotenoid concentrations to help maximise exploitation of heterosis for enhanced provitamin A content and superior agronomic performance. A total of 421 adapted orange and yellow endosperm maize inbred lines derived from crosses, backcrosses, and broad-based populations grown in four independent trials were thus assayed for carotenoid composition and content (Menkir, Liu, White, Maziya-Dixon, & Rocheford, 2008). Close to 10% of these inbred lines, had provitamin A content exceeding the respective trial average by 50–171%. The parental lines used to create source populations of these inbred lines were selected primarily based on desirable agronomic and adaptive traits without due consideration of their heterotic patterns. Such source populations may give rise to several related or unrelated lines as well as some lines with mixed origin.

Lack of knowledge about the heterotic patterns of these adapted yellow and orange endosperm maize inbred lines may limit their use in hybrid development. When clearly defined heterotic groups are absent, Melchinger and Gumber (1998) recommend use of markers as potential tools to characterise and organise maize germplasm for research and product development purposes. Several studies found amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers effective for assessing

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the extent of genetic diversity amongst maize inbred lines and for defining their heterotic groups (Barbosa, Geraldi, Benchimol, Garcia, & Souza, 2003; Warburton et al., 2002). Thirty-eight inbred lines with varying levels of carotenoids were selected from those lines surveyed by Menkir et al. (2008) and genotyped with AFLP and SSR markers, which detected considerable genetic diversity and separated them into two major groups consistent with their genetic backgrounds (Adeyemo, Menkir, Gedil, & Omidiji, 2011). As the 38 maize inbred lines possess genes for adaptation and defensive traits, they represent potential parents to develop the first generation of hybrids that combine intermediate provitamin A levels with desirable agronomic traits.

Adapted maize inbred lines belonging to different groups may carry complementary alleles for carotenoid composition and content as well as desirable agronomic traits. Barbosa et al. (2003) found that AFLP markers were more efficient in assigning lines to heterotic groups and for predicting single cross performance than SSR markers. Understanding the effect of determining parental combinations based on AFLP-based groups on carotenoid concentrations and agronomic performance may then facilitate the development of productive and provitamin A enriched maize hybrids. Crosses between parents of diverse origin produce higher grain yields than crosses amongst lines with the same genetic background (Melchinger & Gumber, 1998). Maize hybrids combining high provitamin A levels with high yield potential and other desirable agronomic and adaptive traits will be attractive to farmers and may likely be widely adopted in areas affected by vitamin A deficiency. The present study was, therefore, conducted to assess the potential usefulness of selecting parental lines from different AFLP-based groups on carotenoid concentrations and agronomic performance in hybrids.

2. Materials and methods

2.1. Genetic materials and field trial

Genetic diversity assessment of 38 orange and yellow endosperm maize inbred lines using AFLP markers separated the lines into two groups (Adeyemo et al., 2011). These AFLP-based groups were used as the basis to identify unrelated parental lines with varying levels of provitamin A (Table 1) to develop hybrids that were included in the present study. Eight inbred lines selected from each AFLP-based group were divided into two sets each of four inbred lines (Table 1). The four inbred lines in each set selected from the first AFLP-based group were used as females and crossed with the four inbred lines in another set selected from the second AFLP-based group as males using a factorial mating scheme (Hallauer & Miranda, 1988). Each inbred line was used as a female parent in one set of crosses and as a male parent in the second set of crosses (Set 1 × Set 2, Set 2 × Set 4, Set 3 × Set 1, and Set 4 × Set 3) to form hybrids. Two hybrids were not formed due to lack of synchrony between pollen shed and silking of the parental lines. The resulting 62 hybrids along with duplicate entries of an orange endosperm commercial hybrid (Oba Super II) in Nigeria used as a check were arranged in an 8 × 8 simple lattice design and were grown at Ikenne (3°42′E, 6°54′N, altitude 30 m), Saminaka (8°39′E, 10°34′N, altitude 760 m), and Zaria (7°45′E, 11°8′N, altitude 622 m) in Nigeria during the 2009 and 2010 cropping seasons. Each hybrid included in this study was planted in a single 5 m long row with 0.75 m spacing between rows and 0.5 m spacing between plants within a row. Fertilizer and field management practices recommended for optimum maize production were used for all the trials grown at these locations. A minimum of five representative plants were self pollinated in each row at Saminaka and Zaria to avoid contamination with pollen from other maize genotypes and the harvested ears were threshed to make a composite sample for carotenoid analysis.

2.2. Analysis of carotenoids

A 10-g sample drawn from each hybrid was sent to the University of Wisconsin-Madison for carotenoid analysis using HPLC. Each 10-g seed sample was ground to a fine powder. A 0.5-g sample was transferred to a 50-ml glass centrifuge tube. Then 6 ml ethanol containing 0.01% butylated hydroxyl toluene was added to the glass tube, which was capped and mixed with a vortex for 15 s. The glass tube was then placed in 85 °C water bath for 5 min. The glass tubes were removed from the water bath and 500 μl of 80% potassium hydroxide was added to the tubes, which were then placed in the water bath for 10 min, after which they were removed and mixed with a vortex for about 15 s. The samples were then moved immediately into ice and 3 ml cold deionized water was added and the tube was mixed with a vortex for 15 s. To each sample, 200 μl internal standard b-Apo 8′-carotenal (Sigma Aldrich, Saint Louis, Missouri, USA) and 4 ml hexane were added, vortexed and then centrifuged. The hexane fraction was extracted and transferred into a new glass tube. Extraction of the hexane fraction was repeated twice by adding 3 ml hexane each time. The hexane extract was then dried down under nitrogen using a concentrator (Organomation Associates, Inc., Berlin, Massachuset, USA). The samples were reconstituted in 500 μl of 50:50 methanol/dichloromethane and the tube was capped immediately. The extracts were vortexed, rotated and centrifuged. The extracts were placed in HPLC vials and 50 μl aliquots were injected into an HPLC (Water Corporation, Milford, Massachuset, USA) for analyses of α-carotene, β-carotene (*cis* and *trans* isomers), β-cryptoxanthin, lutein, and zeaxanthin. The Water's HPLC components, operated with Empower 1 Software, included a 717 Plus autosampler with temperature control set at 5 °C, Waters 1525 binary HPLC pump, and a 2996 photodiode array detector for carotenoid quantification. Carotenoids were separated on a 5-μm C30 Carotenoid Column (4.6 × 250 mm; 3 μl) eluted by a mobile phase 30 min gradient from 70% methanol/water (92:8 v/v) with 10 mM ammonium acetate and 30% methyl tertiary butyl ether (100%) to 40% methanol/water and 60% methyl tertiary butyl ether. The flow rate was 1.0 ml/min and the solvents were HPLC grade. To maximise detection of carotenoids, absorbance was measured at 450 nm. Provitamin A was defined as the sum of β-carotene, β-cryptoxanthin and α-carotene, with α-carotene and β-cryptoxanthin contributing 50% of the value of β-carotene (U. S. Institute of Medicine, 2001).

2.3. Agronomic data recording

Days from planting to anthesis and silking were recorded in each plot as the number of days from planting to when 50% of the plants were shedding pollen and displaying visible silks, respectively. Plant and ear heights were measured in cm as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant aspect was rated on a scale of 1–5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Ear aspect was scored on a 1–5 scale, where 1 = clean, uniform and large ears and 5 = rotten, variable and small ears. Southern corn rust, southern corn leaf blight, and *Curvularia* leaf spot were scored at Ikenne for two seasons on a scale of 1–5, where 1 = slight leaf infection and 5 = severe leaf infection. All ears harvested from each plot were weighed and representative samples of ears were shelled to determine percent moisture. Grain yield adjusted to 15% moisture was computed from ear weight assuming a shelling percentage of 80%.