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Zeaxanthin biofortification of sweet-corn and factors affecting zeaxanthin accumulation and colour change

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ABSTRACT

Zeaxanthin, along with its isomer lutein, are the major carotenoids contributing to the characteristic colour of yellow sweet-corn. From a human health perspective, these two carotenoids are also specifically accumulated in the human macula, and are thought to protect the photoreceptor cells of the eye from blue light oxidative damage and to improve visual acuity. As humans cannot synthesise these compounds, they must be accumulated from dietary components containing zeaxanthin and lutein. In comparison to most dietary sources, yellow sweet-corn (*Zea mays* var. *rugosa*) is a particularly good source of zeaxanthin, although the concentration of zeaxanthin is still fairly low in comparison to what is considered a supplementary dose to improve macular pigment concentration (2 mg/person/day). In our present project, we have increased zeaxanthin concentration in sweet-corn kernels from 0.2 to 0.3 mg/100 g FW to greater than 2.0 mg/100 g FW at sweet-corn eating-stage, substantially reducing the amount of corn required to provide the same dosage of zeaxanthin. This was achieved by altering the carotenoid synthesis pathway to more than double total carotenoid synthesis and to redirect carotenoid synthesis towards the β-arm of the pathway where zeaxanthin is synthesised. This resulted in a proportional increase of zeaxanthin from 22% to 70% of the total carotenoid present. As kernels increase in physiological maturity, carotenoid concentration also significantly increases, mainly due to increased synthesis but also due to a decline in moisture content of the kernels. When fully mature, dried kernels can reach zeaxanthin and β-carotene concentrations of 8.7 mg/100 g and 2.6 mg/100 g, respectively. Although kernels continue to increase in zeaxanthin when harvested past their normal harvest maturity stage, the texture of these 'over-mature' kernels is tough, making them less appealing for fresh consumption. Increase in zeaxanthin concentration and other orange carotenoids such as β-carotene also results in a decline in kernel hue angle of fresh sweet-corn from approximately 90° (yellow) to as low as 75° (orange-yellow). This enables high-zeaxanthin sweet-corn to be visually-distinguishable from standard yellow sweet-corn, which is predominantly pigmented by lutein.

Introduction

Amongst all carotenoids, zeaxanthin and its isomer lutein are the sole dietary carotenoids that are actively absorbed and accumulated in the human macula [6]. There is increasing evidence that the yellow and orange carotenoids, lutein, zeaxanthin, and meso-zeaxanthin are concentrated in the macula to protect against blue-light oxidation and subsequent cellular damage [3,12,21,24], as well as increasing visual acuity [16,19,22]. Increased carotenoid consumption has been directly linked to an increase in macular pigment concentrations [4]. Zeaxanthin is much rarer in the human diet relative to lutein, but is accumulated to a much higher degree than lutein in the central macula [2], perhaps indicating greater relative importance for photo-protection. Photoreceptor damage in the form of macular degeneration is the leading cause of blindness in Australia and other developed countries [1,14]. Although sweet-corn (*Zea mays* var. *rugosa*) is a good source of zeaxanthin relative to other foods [8,20], the concentration of zeaxanthin used in supplemental doses is substantially higher (2 mg/person/day) [5] and would require a daily consumption of 4–11 cobs of corn, 1.2 kg of kale, or 1–9 kg of eggs to achieve an equivalent concentration.

The purpose of our project was therefore to increase zeaxanthin levels in sweet-corn through conventional breeding within a tropical sweet-corn breeding population, such that consumption of a

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single cob or 100 g kernels will deliver approximately 2 mg zeaxanthin, ideally minimising the need for artificial supplements.

Materials and methods

Breeding and selection for high zeaxanthin sweet-corn

Approximately 200 breeding lines of sweet-corn were assessed for their carotenoid profile by HPLC using the methodology described in Fanning et al. [7]. Plants were grown at Kairi Research Station (Queensland, Australia) and cobs harvested at sweet-corn eating stage (21 days after pollination), frozen at −20 °C and transported to the laboratory for analysis. Lines with highest zeaxanthin concentration were identified and their carotenoid profiles compared with regular sweet-corn lines.

The lines exhibiting highest zeaxanthin concentrations (HZ)[1] were subsequently self-pollinated over several generations with continuous selection for lines with highest zeaxanthin concentration and total carotenoid synthesis. During the selection process, it was established that zeaxanthin concentration was correlated with kernel colour [7], such that kernels with a hue angle greater than 85° were unlikely to have zeaxanthin concentrations greater than 4 µg/g FW, and could therefore be removed prior to HPLC analysis.

Lines with high total carotenoid synthesis were subsequently combined with lines favoring β-arm carotenoid synthesis (the arm of the carotenoid synthesis pathway in which zeaxanthin is produced) to produce lines with a combination of both characteristics to maximise zeaxanthin concentration. From the initial cross which produced cobs containing kernels of varying colour, kernels with the deepest orange colour were selected. Subsampling of these deep-orange coloured kernels for HPLC analysis established that they possessed both high total carotenoid synthesis and increased β-arm carotenoid synthesis. The deep-orange coloured kernels were subsequently inbred to establish uniformly deep-orange coloured cobs.

Effect of harvest maturity on zeaxanthin

Early in the breeding program, the effect of harvest maturity on carotenoid development was investigated. Cobs harvested at early maturity (16 days after pollination, DAP), normal maturity (20 DAP), and late maturity (24 DAP) of a line with higher concentrations of zeaxanthin (HZ) and a commercial yellow sweet-corn (Hybrix5) line were compared for carotenoid accumulation. Five replicates were taken at each harvest-date. A 10 g sample of kernels was taken from each cob and analysed for carotenoids according to the method of Fanning et al. [7]. Carotenoids were calculated on a fresh-weight and dry-weight basis. Moisture content was determined by blending 5 g kernels and measuring weight before and after evaporating moisture for 24 h in a vacuum oven at 70 °C.

Effect of zeaxanthin concentration on kernel colour

Sweet-corn kernels from 150 cobs over a range of zeaxanthin concentrations (2–25 µg/g FW) were assessed for colour (hue angle) at eating-stage maturity (20 DAP) using a Minolta Chromameter (CR-400). Hue angle was compared with individual carotenoid profiles and regression analysis conducted using Genstat 8.0.

Results

Initial screening of 200 existing tropical breeding lines resulted in zeaxanthin concentrations ranging from 0.1 to 4.8 µg/g FW. Comparison of the carotenoid profiles of the highest zeaxanthin accessions (>4.5 µg/g FW) with the standard commercial yellow cultivar, 'Hybrix5' (2–3 µg/g FW) identified that higher zeaxanthin concentration was due to a combination of higher overall carotenoid synthesis and a higher proportion of zeaxanthin relative to other carotenoids present (Fig. 1). Zeaxanthin constituted approximately 43% of carotenoids present in the highest zeaxanthin accession, compared to 21% in Hybrix5.

Subsequent increases in zeaxanthin through inbreeding ('selfing') were associated with further increases in total carotenoid production, rather than an increase in the proportion of zeaxanthin. Through this series of inbreeding steps, zeaxanthin concentration was increased to approximately 11 µg/g FW. Recombining this line with a line possessing a higher proportion of zeaxanthin (approximately 75% of carotenoids present) resulted in a further significant increase in zeaxanthin after further selection of segregants (Fig. 1). Zeaxanthin concentration was increased up to 25 µg/g FW in kernels at sweet-corn eating-stage, constituting approximately 70% of the total carotenoids present (Fig. 2).

Kernel colour was also observed to change with increasing zeaxanthin concentration (Fig. 2). As an objective measurement of colour, hue angle was measured to decline with increasing concentration of zeaxanthin, such that kernel colour changed from yellow (hue ∼90°) to a more orange-yellow colour (hue ∼75°) (Fig. 3). Change in hue angle was found to be most closely correlated ($r^2 = 0.84$) with the total sum of orange β-carotenoids (i.e. zeaxanthin, β-carotene, β-cryptoxanthin), rather than zeaxanthin alone.

Generally, all kernel carotenoids, including zeaxanthin and β-carotene (Table 1), were observed to increase with later harvest date. Although kernels slightly declined in moisture content with increasing physiological maturity (16 DAP: 77.7%; 20 DAP: 76.6%; 24 DAP: 74.7%), carotenoids still continued to increase in concentration when measured on either a fresh-weight or dry-weight basis (Table 1). Leaving cobs to fully mature on the plant (60 DAP) allowed zeaxanthin and β-carotene concentration of HZ kernels to further increase, reaching up to 87.1 µg/g FW and 25.8 µg/g FW, respectively.

Discussion

The zeaxanthin concentration achieved in the present breeding program (up to 25 µg/g FW) appears to be significantly greater than that reported for sweet-corn in the scientific literature. Zeaxanthin concentration in sweet-corn has been previously reported as ranging from 0.02 to 6.83 µg/kg FW [13,18,23]. This

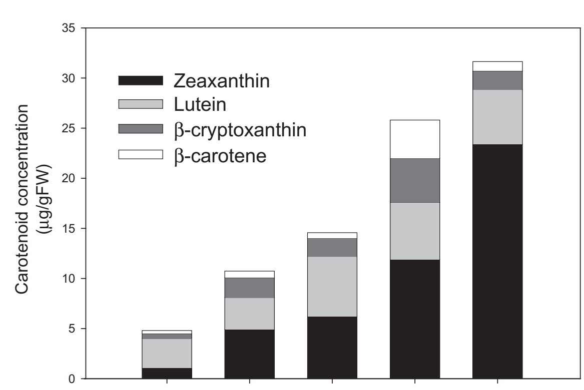

Fig. 1. Incremental increase in zeaxanthin concentration through increase in total carotenoid synthesis and increase in the proportion of zeaxanthin relative to other carotenoids.

[1] *Abbreviations used:* HZ, high zeaxanthin selection; DAP, days after pollination.