

CRA-MAC
Unità di Ricerca per la Maiscoltura
Via Stezzano 24, 24126 Bergamo (Italy)

Screening of favourable alleles for β -carotene content in maize inbred lines

Alfieri, M, Berardo, N, Redaelli, R

e.mail: michela.alfieri@entecra.it

Carotenoids are natural compounds that play an important role for human nutrition and health; among them β -carotene is quite relevant, being a precursor of vitamin A. Deficiency of this compound is a global health problem that involves many people, especially children, in the South of the world. The biosynthetic pathway of carotenoids has been extensively studied and described by many authors (DellaPenna and Pogson, *Annu Rev Plant Biol* 57:711-738, 2006). The precursor of both monooxygenated carotenoids, i.e. carotenes, and their oxygenated derivatives (xanthophylls), is lycopene. From it two biosynthetic branches derive, distinguished by a different cyclic-end group. Two beta rings lead to the β, β branch (β -carotene and its derivatives: β -cryptoxanthin, zeaxanthin) whereas one beta and one epsilon ring define the β, ϵ branch (α -carotene and its derivatives: zeinoxanthin and lutein). The gene *hydroxylase3* (*HDY3*) controls the synthesis of one of the hydroxylases involved in the conversion of provitamin A carotenes to non-provitamin A xanthophylls.

Recently, a simple and fast PCR assay was developed, that allows to identify the alleles of *HDY3* potentially associated with an enhanced or reduced provitamin A content (Vallabhaneni *et al.*, *Plant Physiol* 151: 1635-1645, 2009). Allele A was found to be correlated with a low-medium content of provitamin A, whereas alleles B and C appeared to improve provitamin A content, resulting therefore more interesting from the nutritional point of view. This PCR assay was used to screen a group of genotypes (100 Italian inbred lines and nine public lines) to identify the lines carrying the optimal alleles. Among the Italian germplasm, 91 lines presented allele A, three lines showed allele B, one line had alleles AC, and five lines carried alleles AB. Among the public lines, five presented allele B, the others had allele A. To verify the introgression of optimal alleles in hybrid seeds, 20 crosses among the lines were carried out in 2011. As expected, hybrid grains contained the alleles of both parents.

Some of the lines tested with molecular assay had been previously selected for a breeding program focused on maize nutritional quality; they were therefore used for the extraction and quantification of total carotenoids. The extraction procedure was based on the protocol described by Schaub *et al.*

(http://www.cropsci.uiuc.edu/faculty/rocheford/quick_carotenoid_analysis_protocol.pdf, 2004); the content of total carotenoids was quantified by spectrophotometer using the Lambert-Beer equation and expressed on a dry matter basis. The quantification of total carotenoids in the 13 inbred lines showed a range of variation from 23.16 (Lo1189) to 50.10 (Lo59) $\mu\text{g/g}$ d.m. with a mean value of 33.21 ± 8.9 $\mu\text{g/g}$ d.m. Both the mean value and the range of variation resulted to be quite high as compared to the data reported for inbred lines in recent works (Chander *et al.*, J Agric Food Chem 56: 6506-6511, 2008; Ibrahim and Juvik, J Agric Food Chem 57: 4636-4644, 2009; Vallabhaneni *et al.*, Plant Physiol 151: 1635-1645, 2009; Kuhn *et al.*, J Sci Food Agric, 91: 1548-1553, 2011). The results confirmed those obtained in previous papers about the richness in total carotenoids of traditional Italian maize germplasm (Berardo *et al.*, Innov Food Sci Emerg Technol, 5: 393-394, 2004; Berardo *et al.*, J Agric Food Chem, 57: 2378-2384, 2009). On the other hand, the five public lines analyzed showed a narrower range of variation, from 14.67 (F2) to 36.66 (Oh43) $\mu\text{g/g}$ d.m. and a lower mean value (23.93 ± 8.7 $\mu\text{g/g}$ d.m.). The carotenoids content was quantified also in hybrid grains; the results were quite different, depending on the genetic composition of the hybrid, and the amount of carotenoids ranged from 31.88 to 64.97 $\mu\text{g/g}$ d.m. Finally, quantification of the carotenoid components by HPLC will help to verify if the amount of β -carotene in these genotypes is related to the content predicted on the basis of *HYD3* alleles.