Identification of Zeta-Carotene Desaturase Genes from Durum Wheat

Sherisse Reimer and Curtis Pozniak

Crop Development Centre, University of Saskatchewan, 51 Campus Drive,
Saskatoon, SK CANADA S7N 5A8

Abstract

Yellow pigment is an important quality trait in durum wheat (*Triticum turgidum* L. var *durum*). Few genes within the pathway responsible for carotenoid biosynthesis have yet to been identified in durum wheat. Zeta-carotene desaturase (ZDS) is an enzyme involved in carotenoid biosynthesis, and variation in the gene(s) coding for ZDS may partially explain the variation observed in endosperm color among durum wheat cultivars. For this study, a PCR strategy was used to clone and sequence greater than 1200 bp of the *Zds* genes from durum cultivars Kofa (high pigment) and W9262-D063 (medium pigment). Comparison of partial nucleotide sequences indicated the presence of four *Zds* genes which we temporarily designated as *tdZds1*, *tdZds2*, *tdZds3*, and *tdZds4*. Since durum wheat is a tetraploid, the presence of four genes suggests that the *Zds* gene may in fact be duplicated in the durum wheat genome, with two sets of homeologous genes. However, we have only obtained single clones of *Zds2* and *Zds4* from each of the two parents, and these results will need to be confirmed. However, we are currently in the process of mapping *Zds1* and *Zds3* to determine their role in the expression of yellow pigment concentration in durum wheat.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is predominantly grown for pasta production. Yellow pigment is an important trait in pasta production, as consumers tend to prefer a bright yellow pasta color (Hentschel et al., 2002). The desirable yellow color of durum wheat semolina and pasta products is due in part to the presence of xanthophylls, and other related carotenoid pigments in durum grain. Numerous studies have been conducted on the genetics of yellow

pigment concentration in durum wheat. In durum, this trait is largely controlled by additive gene action and is highly heritable (Elouafi et al., 2001). To date, few attempts have been made to isolate the genes associated with elevated levels of yellow pigment in durum grain. Zeta carotene desaturase (*Zds*) is an important enzyme involved in carotenoid biosynthesis (Fig. 1), and its role in the accumulation of carotenoids in maize seeds has been well characterized (Wong et al., 2004). We hypothesize that allelic variation in one or more *Zds* genes may partially explain differences in phenotypic expression of yellow pigment in durum wheat cultivars. Here we report the identification of Zds genes with the longer term objective of better understanding the quantitative variation for yellow pigment in durum wheat.

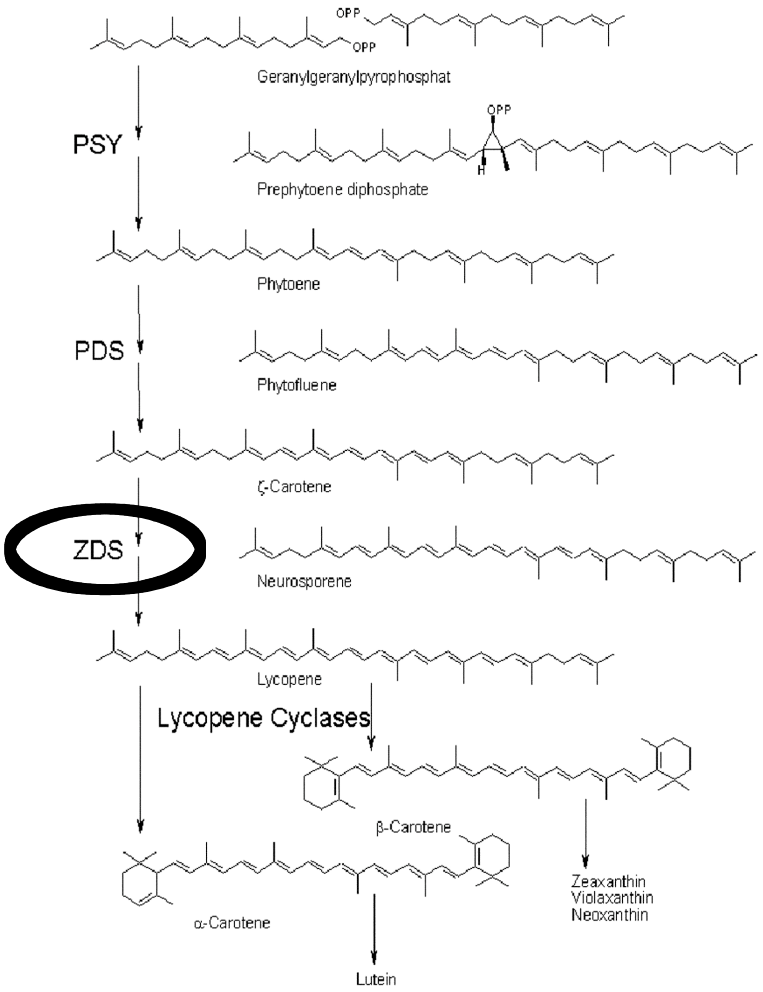

Figure 1. Biosynthesis of carotenoids in plants.

Materials and Methods

A PCR based approach was utilized to clone *Zds* genes from durum wheat cultivar Kofa (high pigment) and a breeding line designated as W9262-260D3 (medium pigment). Forward primer ZDSF1 (5'-GGCTGCATGTCTTCTTTGGT-3') and reverse primer ZDSR1 (5'-ATCAGGTGAGCCCTTTAGCA-3') were previously reported to amplify durum wheat BAC clones containing putative Zds genes (Cenci et al., 2004) and were used in a PCR containing 300 ng of Kofa and W9262-260D3 genomic DNA. Following electrophoresis, fragments approximately 1250 bp in size were gel purified, cloned into the Topo Zero-Blunt vector (Invitrogen, Canada) and sequenced. A minimum of 25 clones were sequenced from each parent. Consensus sequences were generated using multiple-sequence comparisons to verify single-nucleotide differences and were used to construct a sequence similarity dendogram using the neighbor joining method (Saitou and Nei, 1987). To differentiate homeologous sequences, PCR of plasmid DNA was performed using primers ZDS1F/ZDS1R, and the resulting amplicons digested simultaneous with *AleI* and *EcoRI* and separated on a 1% (w/v) agarose gel.

Results and Discussion

Given the importance of *Zds* in carotenoid biosynthesis, we set out to determine if allelic differences in the *Zds* gene(s) would explain, at least in part, the phenotypic variation observed in yellow pigment concentration among durum wheat cultivars. In other species studied, only single copies of *Zds* have been reported, but since durum wheat is an allotetraploid, two genes, one from the A genome and one from the B genome, would be expected. However, sequencing of the amplicons derived from PCR primers ZDSF1 and ZDSR1 resulted in four unique sequences from both Kofa and W9262-260D3 (Fig. 2) which displayed sequence homology to the rice and maize *Zds* genes. Based on sequence homology, clones from Kofa and W9262-260D3 were assigned into 1 of 4 sequences groups designated as *Zds1, Zds2, Zds3* and *Zds4*.

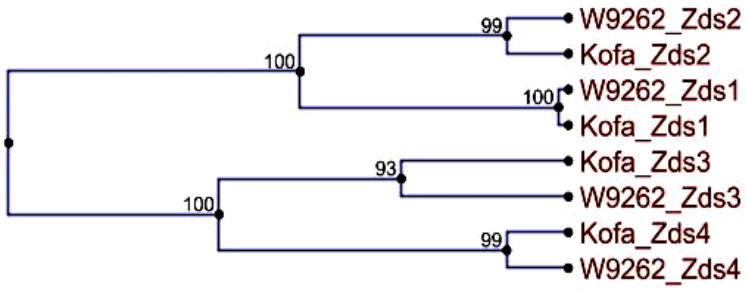

Figure 2. Similarity dendogram of *Zds* sequences from Kofa and W9262-260D3. Bootstrap values are shown at as percentages of 1,000 replicates. Clade strength was tested using 1000 replications.

Restriction analysis of clones derived from Kofa and W9262-260D3 with *EcoRI* and *AleI* resulted in four unique restriction patterns (Fig. 3), confirming the presence of four unique *Zds* clones from each parental genotype. To date, we have only identified single clones of *Zds2* and *Zds4* from each of the two parents, and further experiments will need to be conducted to confirm these sequences. However, a minimum of 10 clones each of *Zds1* and *Zds3* were sequenced from both Kofa and W9262-260D3. We have mapped a QTL for yellow pigment to chromosome 2A in a doubled haploid population derived from the cross W9262-260D3/Kofa (Fig. 4).

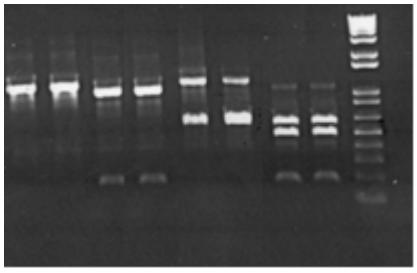

Fig. 3. *EcoRI / AleI* double digestion of PCR fragments derived from clones derived from Kofa and W9262-260D3. Four clone types were identified from each parent and were temporarily designated as *Zds1* (lanes 1 and 2), *Zds2* (Lanes 3 and 4), *Zds3* (Lanes 5 and 6), and *Zds4* (Lanes 7 and 8). Lane 9 represents the DNA size standard. For each grouping, amplicon digests are from clones derived from Kofa (K) and W9262-260D3 (W), respectively

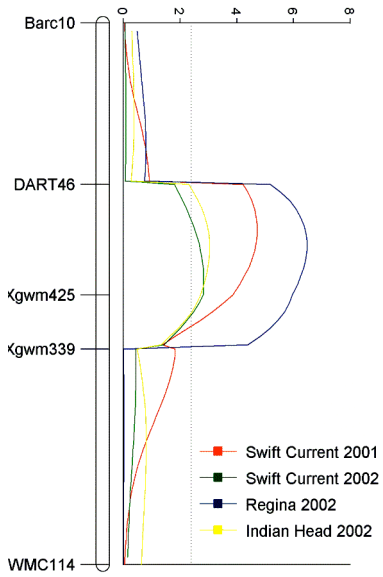

Fig. 4. Partial genetic map of chromosome 2A and location of a QTL for yellow pigment. The QTL spans 12 cM.

Cenci et al. (2004) mapped BAC clones containing putative *Zds* genes to the group 2 chromosomes and thus the *Zds* genes identified in this study represent good candidate gene(s) for the 2A yellow pigment QTL. Since the greatest nucleotide variation between Kofa and W9262-260D3 was observed at the *Zds3* locus (Fig. 2), we hypothesize that *Zds3* maybe associated with this QTL. Based on allelic variation between Kofa and W9262-260D3, we are currently in the process of mapping *Zds*3 to confirm this hypothesis.

Acknowledgements

This research was funded in part from research grants provided by the Western Grains Research Foundation (WGRF) Endowment Fund, the National Science and Engineering Research Council of Canada (NSERC) and a scholarship to S. Reimer from the Canadian Wheat Board

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