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Effect of chromosome 7H^{ch} from *Hordeum chilense* Roem. et Schultz. on carotenoid content and lutein esterification in common wheat

Mattera MG[1,2], Hornero-Méndez D[3], Ávila CM[4], Atienza SG[1], Cabrera A[2]

[1]Dpto. Genética, Universidad de Córdoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, 14071 Córdoba, Spain
[2]Instituto de Agricultura Sostenible (IAS-CSIC), Apdo. 4084, 14080 Córdoba, Spain
[3]Instituto de la Grasa (IG-CSIC), Av. Padre García Tejero 4, 41012 Seville, Spain
[4]Área de Mejora y Biotecnología, IFAPA-Centro Alameda del Obispo, Apdo. 3092, 14080 Córdoba, Spain
Corresponding author: sgatienza@ias.csic.es

Introduction

Yellow colour due to carotenoids has become an important selection criterion in durum wheat (*Triticum turgidum* spp. *durum*, 2n=4x=28, AABB) due to its importance for the production in pasta. On the contrary, white flour is traditionally preferred by consumers for the bread-making using common wheat (*T. aestivum* L. 2n=6x=42, AABBDD). However, lutein also contributes in part to the yellow colour of yellow alkaline noodles which is promoting the development of high lutein materials (Ahmad et al 2013).

The wild barley *H. chilense* has potential for the improvement of the seed carotenoid content in wheat. Indeed hexaploid tritordeum (the amphiploid derived from the cross *H. chilense* × durum wheat) has up to 8-fold carotenoid content compared to wheat (Atienza et al. 2007) due to the specific action of carotenogenic genes from *H. chilense* (Rodríguez-Suárez et al. 2014). The aims of this work were to develop new introgressions of the chromosome 7H^{ch} of *H. chilense* into common wheat and to study the effect of chromosome 7H^{ch} in the seed carotenoid content.

Material and methods

Plant material

A total of fifteen genotypes were used in this work (Table 1). Four common wheat (Chinese Spring, CS)-*H. chilense* introgression lines for 7H^{ch} developed at the University of Córdoba were used. These lines were obtained by pollinating tritordeum line HT31 (amphiploid between *H. chilense* and durum wheat) with a disomic addition line for chromosome 2C from *Ae. cylindrica* Host. F_1 plants were backcrossed to CS followed by five generations of selfing. In addition, selected CS / *H. chilense* substitutions developed at the John Innes Centre (JIC) (https://www.jic.ac.uk/germplasm/Wheat-Precise-Genetic_stocks-Aliens.pdf) were included. Finally, durum wheat 'Kofa', tritordeum 'HT621' and CS completed the set.

The four wheat-*H. chilense* lines were characterized at cytological (chromosome counting and FISH) and molecular levels (EST and COS markers). All the lines were genotyped for the presence of *Phytoene synthase 1* from *H. chilense*.

Table 1. List of plant materials used in this work

Source	Genotype	AB	Hch	D	2n
	CS	28	-	14	42
	HT621	28	14	-	42
	Kofa	28	-	-	28
JIC	CS+7Hch	28	2	14	42 + 2
JIC	CS (5A)5Hch	26	2	14	42
JIC	CS (5B)5Hch	26	2	14	42
JIC	CS (5D)5Hch	28	2	12	42
JIC	CS (7A)7Hch	26	2	14	42
JIC	CS (7B) 7Hch	26	2	14	42
JIC	CS (7D) 7Hch	28	2	12	42
This work	CS (7D) 7Hch	28	2	12	42
This work	CS Dt7HchL	28	2 telos	14	42 + 2t
This work	T7HchS·7AL	26 + 2T	2T	14	42
This work	T7HchS·7DL	28	2T	10+2T+2 telos	40 + 2t
JIC	CS (7D) 7HchS	28	2 telos	12	40 + 2t

JIC = John Innes Centre

Carotenoid extraction

Grains were milled with a spice hand mill, and 1 g of the resulting flour was used for carotenoid extraction by using the protocol proposed by Atienza et al. (2007) with minor modifications. Canthaxanthin was used as internal standard for all samples. Following the extraction, the solvent was evaporated under a nitrogen stream and the pigments were dissolved in 0.5 mL of acetone. All operations were carried out under dimmed light to prevent isomerisation and photo-degradation of carotenoids. The analysis were carried out in quadruplicate.

HPLC analysis of carotenoids

Carotenoids were analyzed by HPLC according to the method of Mínguez-Mosquera and Hornero Méndez (1993) with some modification of the elution gradient (Atienza et al. 2007). The HPLC system consisted of a Waters 2690 Alliance fitted with a Waters 2990 photodiode array detector, and controlled with Millennium32 software. A C18 reverse phase column (Mediterranea SEA18, 3µm, 20x0.46 cm; Teknokroma, Barcelona, Spain) was used. An injection volume of 10 µL and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and online spectra were acquired in the 350-600 nm wavelength range. Quantification was carried out using a calibration curve obtained with lutein standard. This calibration curve was used to quantify both free lutein and esterified lutein. According to our previous works (Atienza et al. 2007; Mellado Ortega & Hornero-Méndez 2012) these chromatographic conditions allowed distinguishing three different lutein fractions regarding to the degree of esterification, that is free, monoesterified and diesterified lutein.

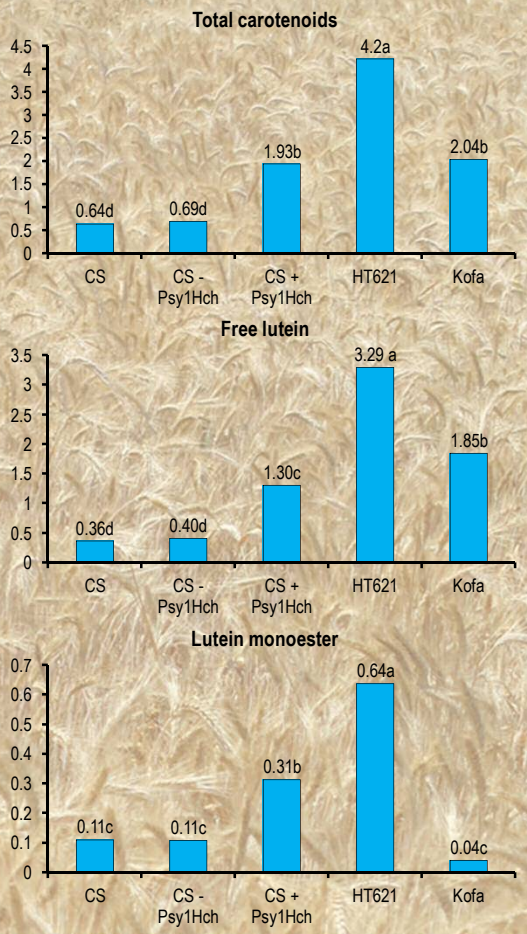
Results & discussion

The genetic composition of the four genotypes developed in this work was elucidated after cytological and molecular analyses. One line was a substitution, two were translocations and the last one consisted in a ditelosomic addition (Table 1). All of them carry the *Psy1* gene from *H. chilense* with the exception of the ditelosomic line. The JIC lines carrying chromosome 7H^{ch} or 7H^{ch}S also carry *Psy1_Hch*. Lines with 5H^{ch} have both *Psy2* and *Psy3*.

The ANOVA revealed that the lines with *Psy1* had a higher seed carotenoid content, free lutein and lutein monoester than CS (Figure 1). On the contrary, the lines without *Psy1* did not differed from the control for any of these traits. No significant differences were found between these groups for diesterified lutein. The *Psy1+* group did not reach the carotenoid content of tritordeum which indicates the important role of other genes as shown by Rodríguez-Suárez et al. (2014).

These results show the potential of 7H^{ch} to increase the seed carotenoid content in wheat. Similar results have been achieved with other alien resources (Ahmad et al. 2013; Zhang et al. 2005). The durum wheat 'Cincinnato' is an example of the potential of alien introgresions to improve YPC (Ceoloni et al. 2014).

Figure 1. Effect of Phytoene synthase 1 on the seed carotenoid content (µg / g) in wheat background



Conclusions

The addition of *Psy1* increases total carotenoid content, total free lutein and total monoester. Disaggregate analyses are still required to study in detail the differences among the genotypes used in this work.

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