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FROM SEED TO PASTA IV

Carotenoid biofortification of durum wheat through inter-specific breeding

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INTRODUCTION

Esterification increases the accumulation and stability of carotenoids, and thus it is considered a target trait for grain biofortification. The gene *XAT-7Hch*, coding a xanthophyll acyl transferase in *Hordeum chilense* Roem. et Schultz, is responsible for carotenoid esterification in both *H. chilense* and tritordeum (amphiploid derived from the cross between *H. chilense* and durum wheat).
H. chilense genes have been successfully transferred to wheat using inter-specific breeding. At present, we are transferring the *XAT-7Hch* gene to durum wheat using a common wheat translocation line (T7HchS·7A/B) as donor material. For this purpose, a multidisciplinary approach involving classical breeding (inter-specific crosses), marker assisted selection and biochemical tools is being conducted to develop durum wheat lines with enhanced carotenoid esterification ability.

Inter-disciplinary approach

1. CLASSICAL BREEDING TECHNIQUES (Figure 1)

- Selection of the donor parent (common wheat translocation line T7HchS·7A/B harboring *XAT-7Hch* from *H. chilense*).
- Inter-specific crosses with durum wheat.
- Obtaining F1 hybrids.

2. MOLECULAR TECHNIQUES (Figure 2)

- DNA extraction.
- Marker assisted selection (MAS) using a tetra-primer ARMS PCR:

Tetra-primer ARMS PCR
- **XAT-7Hch_Fw_out:** 5’ GCTTACTGCTCCTGGCCATCTTCCTC 3’
- **XAT-7Hch_Rv_out:** 5’ TTGAACCCAATCTTTTCAGCTGCAACAA 3’
- **XAT-7Hch_Fw_inn_H290:** 5’ CATGTCGCCACAGGGAGGTTCTGCAGCT 3’
- **XAT-7Hch_Rv_inn_wild:** 5’ AACCTATGAAATCGATAAGCAGCTTACC 3’

3. PHYTOCHEMICAL TECHNIQUES (Figure 3)

- Extraction of carotenoids.
- Analysis by HPLC (High Performance Liquid Chromatography) for the characterization of the content and profile of carotenoids and esters.

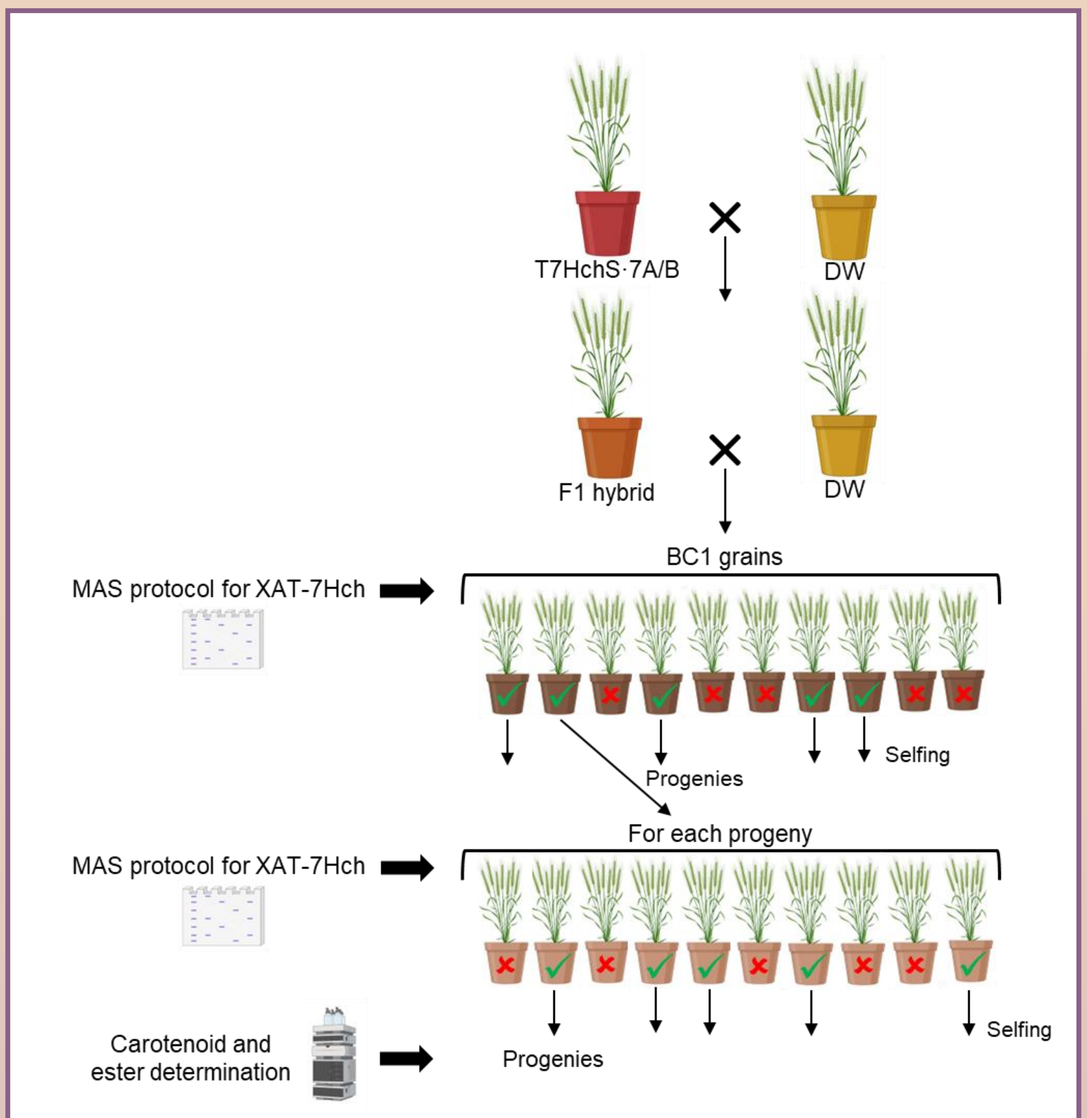

Figure 1. Scheme of crossing an selection for transferring *XAT-7Hch* to durum wheat.

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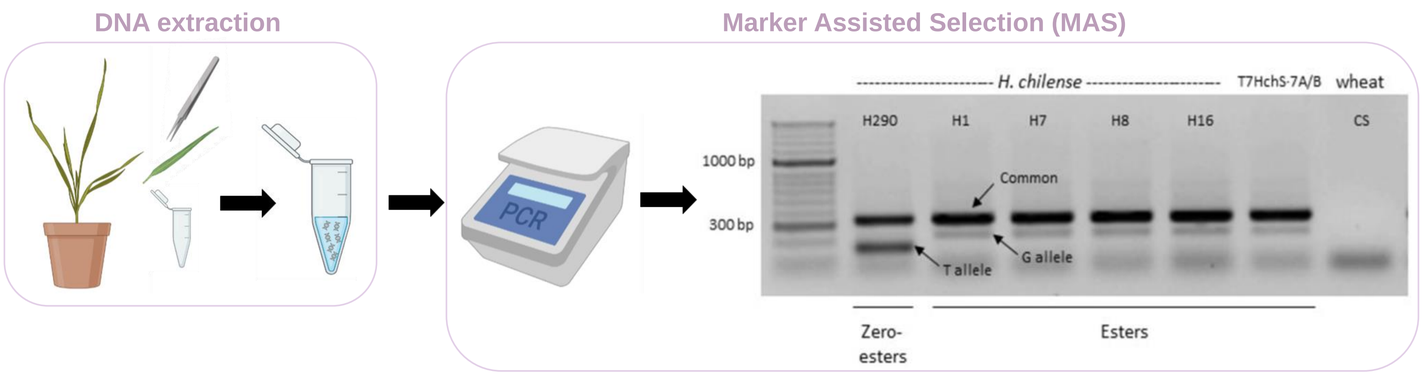

Figure 2. Molecular techniques for detection of *XAT-7Hc*h gene by tetra-primer ARMS-PCR for MAS. A common fragment of 344 bp is observed in all *H. chilense* genotypes (H290, H1, H7, H8 and H16) and in the traslocation line T7HchS·7A/B (donor parent). No amplification is observed in wheat (Chinese Spring). This marker also detects a G/T SNP by amplifyng a 163 bp fragment in the zero-esters genotype (H290) and a 237 bp fragment in the esterifying genotypes.

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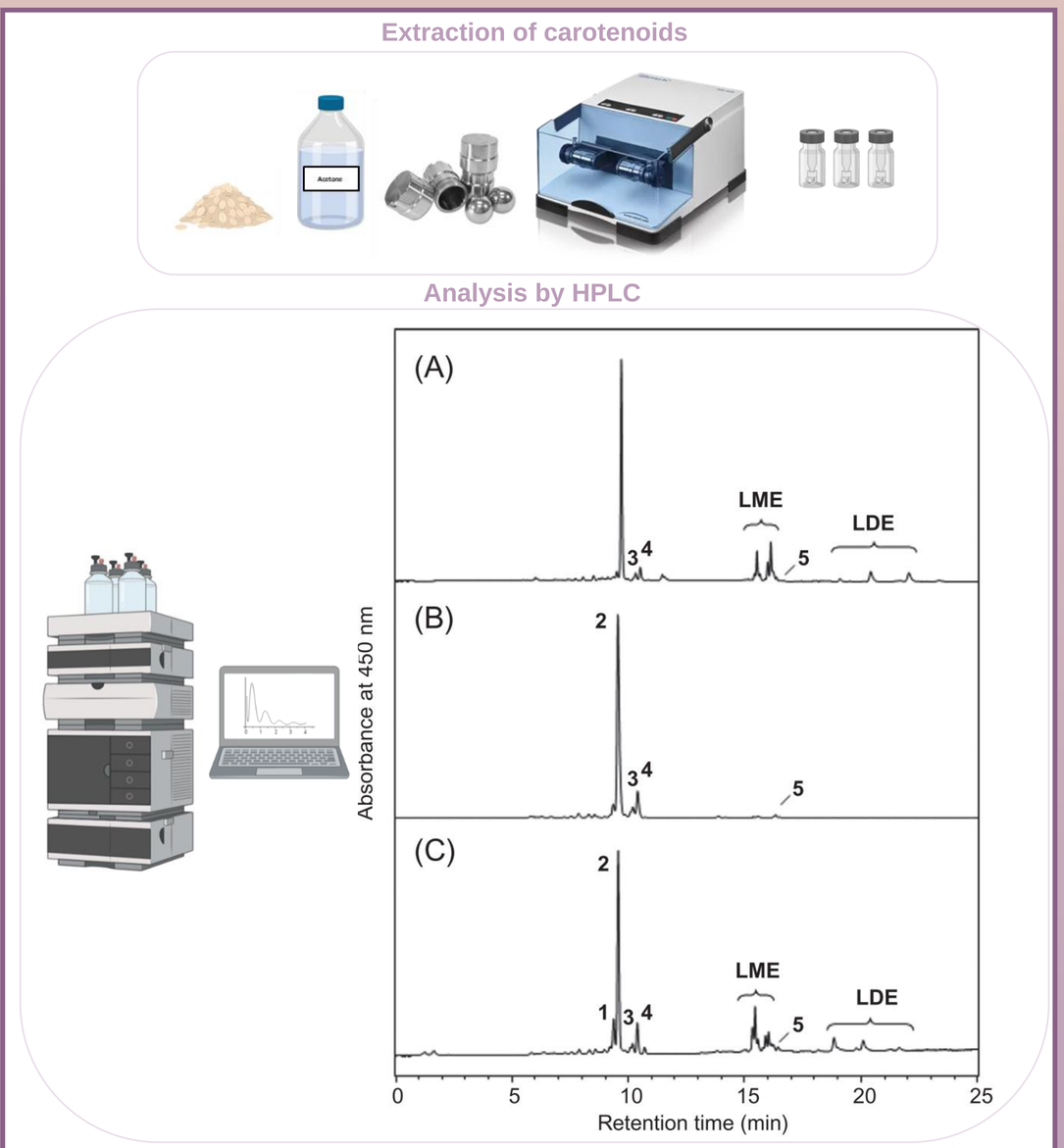

Figure 3. Phytochemicals techniques. HPLC chromatograms corresponding to the carotenoid extract obtained from grains of tritordeum (HT621(A), durum wheat (’Kofa’) (B) and durum wheat landrace producing lutein esters (C) (accession BGE047536 from the Spanish Inventory of Plant Genetic Resources). Peaks: 1. (all-E)-zeaxanthin; 2. (all-E)-lutein; 3. (9Z)-lutein; 4. (13Z)-lutein; LME (lutein monoesters); 5. (all-E)-β-carotene; LDE (lutein diesters). Detection wavelength was set at 450nm.

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CONCLUSION

Carotenoid esterification is a promising target for the enhancement of carotenoid levels in cereals. The utilization of this approach requires the application of classical breeding, molecular genetics and biochemistry tools and thus multidisciplinary teams should be involved to get satisfactory results.

Rodríguez-Suárez C, Requena-Ramírez MD, Hornero-Méndez D, Atienza SG. Chapter 4: The breeder’s tool-box for enhancing the content of esterified carotenoids in wheat: From extraction and profiling of carotenoids to marker-assisted selection of candidate genes. En: Carotenoids: Carotenoid and apocarotenoid biosynthesis, metabolic engineering and synthetic biology. pp. 99-125. Editor. Eleanore T. Wurtzel. Book series: Methods in Enzymology. Editorial: Academic Press. Elsevier.

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