# A NEW *VRS1* ALLELE IDENTIFIED IN 2-ROW SPANISH LANDRACES

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## INTRODUCTION

*Vrs1*, the gene determining the type of spike in barley, has been extensively studied. The wild dominant allele encodes a homeodomain-leucine zipper transcription factor whose activity results in a two-rowed spike, whereas the recessive allele produces a six-row phenotype (Komatsuda et al. 2007). At least three alleles in two-rowed types and four different alleles in six-rowed barleys have been described.

Previous results using MWG699, a marker closely linked to *Vrs1*, suggested different geographic origins for six-row alleles. Among them, the *vrs1.a2* allele originated in the Western Mediterranean. A large proportion of Spanish and Moroccan six-row landraces, some two-row landraces as well as three wild barleys, all shared the same haplotype in MWG699. We analyze *Vrs1* sequence variation in those materials.

## METHODOLOGY

**PLANT MATERIAL.** This study involved 215 accessions: 177 Spanish landraces (51 two-rowed and 126 six-rowed); 7 Moroccan landraces (3 two-rowed, 4 six-rowed), 3 wild, *Hordeum spontaneum* from Morocco and 28 barley cultivars (15 two-rowed and 13 six-rowed).

**GENOTYPING.** The haplotype for MWG699/TaqI (A, D or K) was analyzed in all the samples as reported (Tanno et al. 2002; Casas et al. 2005). Also, the accessions were genotyped with a panel of 322 polymorphic SNP markers, including two within the *Vrs1* sequence. To confirm the obtained results, *Vrs1* was sequenced in selected genotypes.

**SEQUENCE COMPARISONS.** Multiple alignment of protein sequences was performed with Clustal Omega. The haplotype network was computed with TCS.

**POPULATION STRUCTURE.** The software STRUCTURE was used to infer the population structure under an admixture model. Principal Coordinate Analysis was conducted, on the same dataset, using the software DARwin, and the Simple Matching coefficient.

## RESULTS

Nine polymorphisms identified within the *Vrs1* locus allow differentiating seven alleles (4 two-rowed and 3 six-rowed), including novel allele *Vrs1.b5*.


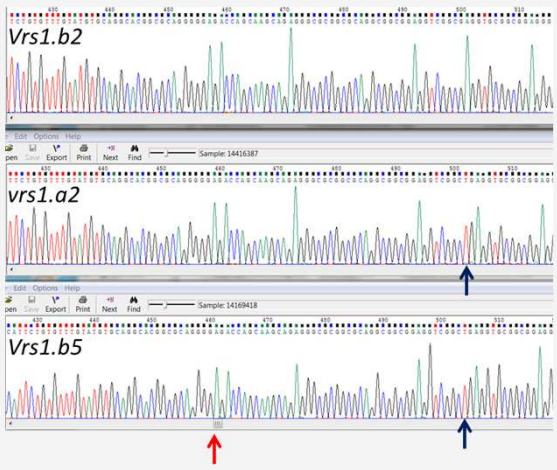


**Figure 1.** Partial nucleotide sequences showing polymorphisms scored in three alleles of *Vrs1*. Arrows point to indels affecting the open reading frame.

Most Spanish landraces are six-rowed and carry the *vrs1.a2* allele (ex. SBCC037), characterized by insertion of a 'T' in exon 2, causing a frame shift (Figure 1, 2nd arrow). These lines have the D haplotype in MWG699. This same haplotype was shared by wild barleys and two-rowed landraces from Morocco, all *Vrs1.b2*. Seven two-rowed Spanish landraces (ex. SBCC153), all with the D haplotype, had a new *Vrs1.b5* allele. Interestingly, this sequence contains the same 'T' found in the six-rowed *vrs1.a2*, but has an additional upstream deletion (Figure 1, 1st arrow) that results in the change of 15 amino acids and a potentially functional protein (Figure 2).

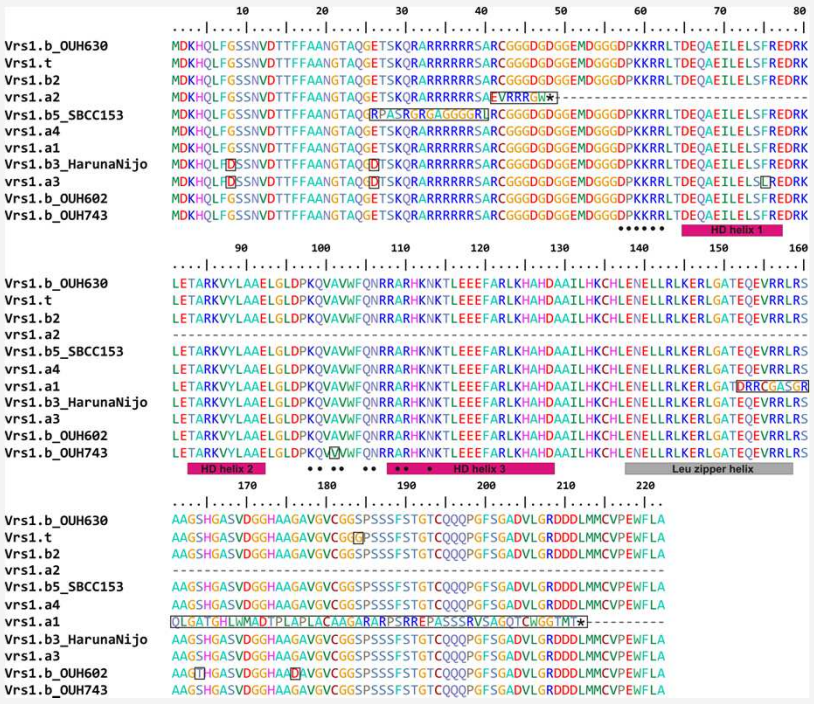


**Figure 2.** Multiple alignment of protein sequences of different *Vrs1* alleles. Boxed sequences highlight differences among alleles and asterisks show premature stop codons. Solid circles are protein-DNA interface residues.

A molecular phylogeny positioned the new *Vrs1.b5* allele as sister to the six-rowed *vrs1.a2* allele (Fig. 3A), with both sharing a common ancestor with *Vrs1.b2*. The haplotype network on Fig. 3B places *Vrs1.b5* and *vrs1.a2* one step apart (indel in Figure 1).

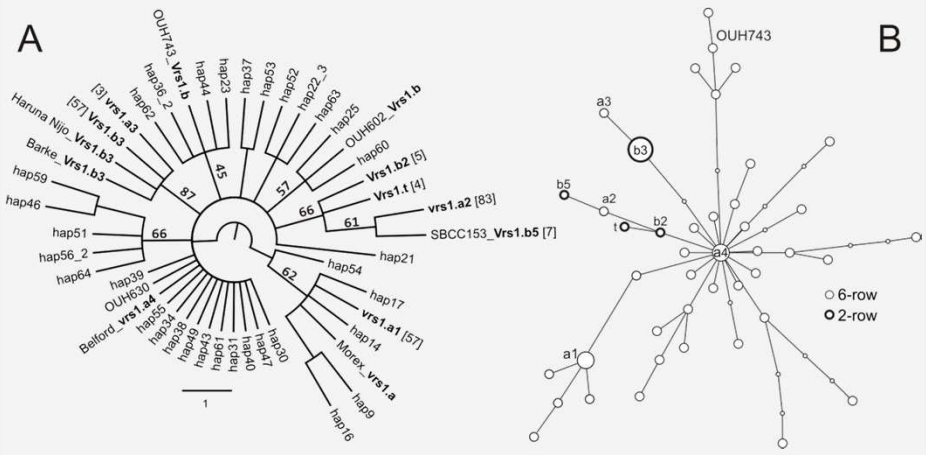


**Figure 3.** A) Parsimony tree with bootstrap values. B) Haplotype network. Two-row barley accessions are displayed as thick circles.

PCoA separates two-rowed (left) from six-rowed (right) accessions. Further subdivision within those groups is apparent (Figure 4). A distinct group of two-row Spanish landraces stands out.

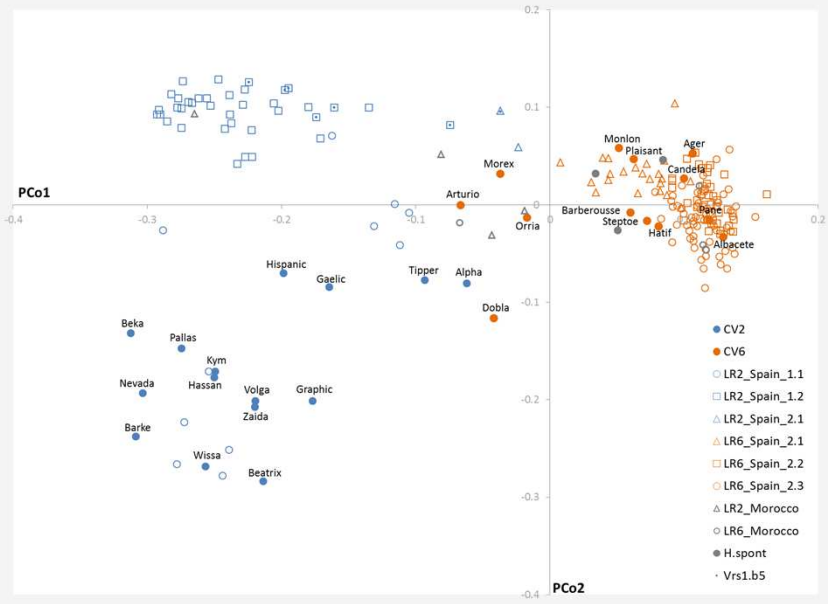


**Figure 4.** First two principal coordinates from PCoA dividing two-row lines on the left and six-row lines on the right.

## CONCLUSIONS

- A new *Vrs1.b5* allele was identified in two-row Spanish landraces.
- Phylogenies suggest that loss-of-function allele *vrs1.a2* is derived from *Vrs1.b2*. Some time later, a deletion in *vrs1.a2* gave rise to *Vrs1.b5*, which restored the ORF and reverted the two-rowed phenotype.
- PCoA separates the landraces in subpopulations also differing in the *Vrs1* allele (*.b3*/*.b3+.b5*/*.b2+.a1+.a2*/*.a1*/*.a2*). Similar results were obtained with microsatellites (Yahiaoui et al. 2008).

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