

## RESUMEN

El trigo candeal (*Triticum turgidum* spp. *durum*) es un cereal importante en la alimentación humana y constituye la materia prima para la elaboración de pastas y otras comidas tradicionales. En Argentina el cultivo ocupa el sur de la provincia de Buenos Aires de clima sub-húmedo/semiárido, inviernos marcados y temperaturas mínimas variables en fecha e intensidad. Las heladas de julio a noviembre afectan el cultivo en estadio vegetativo y reproductivo.

El germoplasma cultivado de candeal en nuestro país está reducido a unas pocas variedades de hábito primaveral, con escasa descripción de los determinantes de la fecha de floración y del comportamiento frente a bajas temperaturas. La complejidad del carácter ha limitado la efectividad del mejoramiento tradicional. El análisis de secuencias de ADN y de expresión génica abre nuevas perspectivas. Se identificaron genes en trigo pan que intervienen en la determinación de la longitud del ciclo y en los procesos de aclimatación y adquisición de tolerancia a heladas.

El objetivo de este estudio fue determinar la variabilidad en la tolerancia a bajas temperaturas en una colección de trigo candeal en etapa vegetativa y reproductiva y caracterizar genes candidatos. Se evaluaron 72 accesiones a campo en tres ambientes (Cabildo 2008, Cabildo 2009, Barrow 2009) donde se registró días a espigazón y se cuantificó el porcentaje de daño en hoja y en espigas (blanca, deformada y afectada por zonas). Se observó correlación entre ambientes para la variable días a espigazón. El daño en espiga varió entre máximos de 45% en Barrow y 12% en Cabildo hasta un mínimo de <1% en ambos, siendo la espiga afectada por zonas la forma de daño más frecuente.

Un set de 32 genotipos se seleccionó para evaluar floración, tolerancia a helada en condiciones controladas y para análisis molecular. Se observaron diferencias en la respuesta a vernalización y fotoperíodo y variación en los genes involucrados. De los 32 genotipos, 28 mostraron el alelo primaveral *VRN-A1* y los restantes el alelo invernal *vrn-A1*. Sólo una accesión presentó requerimiento estricto de vernalización. Se reconocieron hábitos de crecimiento primaveral, invernal y facultativo. El estudio de expresión de *VRN-A1* mostró inducción por vernalización en todos los materiales y estadios. En condiciones no vernalizantes, los materiales facultativos mostraron una

expresión más tardía de *VRN-A1*, mientras que en el genotipo invernal no se detectó expresión. En cuanto al fotoperíodo, ocho genotipos presentaron el alelo de insensibilidad en el locus *Ppd-A1* y 24 el alelo de sensibilidad. En todos los genotipos excepto en dos, la evaluación fenotípica coincidió con la caracterización molecular del promotor de *Ppd-A1*.

Las evaluaciones de tolerancia a heladas en cámara basadas en el porcentaje de daño en hoja, discriminaron genotipos tolerantes y susceptibles destacándose dos líneas que corresponden al un material invernal y a uno primaveral. En cuanto a los genes de respuesta a frío, se obtuvieron secuencias de *TdDREB-A1* y *TdCBF14*. En el primer caso se identificaron polimorfismos cuya combinación originó seis haplotipos, uno de los cuales resultó específico del material invernal tolerante. En *TdCBF14* se encontró un polimorfismo asociado a tolerancia a frío en cebada. El análisis de expresión de genes *COR* en ocho genotipos mostró diferencias entre materiales y tratamientos, aunque la relación entre los polimorfismos moleculares y la tolerancia requieren de experimentos adicionales.

La integración de la información obtenida representa un aporte a la caracterización de los recursos genéticos de trigo candeal, de utilidad al momento de elegir los materiales con ciclos más apropiados para cada sitio de cultivo.

## ABSTRACT

Durum wheat (*Triticum turgidum* spp. *durum*) is an important cereal for human consumption and constitutes the raw material for pasta manufacturing and others traditional foods. Cultivation in Argentina comprises the South of Buenos Aires province where the climate is sub-humid/semi-arid, hard winters and variable minimum temperatures in date and intensity. Frost days from July to December can affect the crop both during vegetative and reproductive stage.

Cultivated germplasm in our country is reduced to a few varieties with spring habit, with scarce description about determinants of flowering date and performance under low temperatures. The complex inheritance of this trait has limited the effectiveness of traditional breeding programs. The analysis of DNA sequences and gene expression opens new perspectives. Genes involved in cycle length, cold acclimation and the acquisition of frost tolerance has been identified in bread wheat.

The objective of this study was to determine the variability in the tolerance to low temperatures in a durum wheat collection at vegetative and reproductive stage and to characterize candidate genes.

Seventy-two accessions were field evaluated in three environments (Cabildo2008, Cabildo2009, Barrow2009) where ear emergence date was registered and percentage of damage in leaves and spikes were quantified (white spikes, deformed spikes and affected by zones). Days to ear emergence showed to be correlated across environments. Ear damage varied between maximum values of 45% in Barrow and 12% in Cabildo up to a minimum of <1% in both environments, being the spike affected by zones the most frequent class of damage.

A set of 32 genotypes was selected to evaluate flowering date and frost tolerance under controlled conditions and for molecular analysis. Differences at vernalization and photoperiod response together with allelic variability at the involved genes were observed. Within the 32 accessions, 28 showed the *VRN-A1* spring allele and the others the winter *vrn-A1* allele. Only one accession presented a strict vernalization requirement. Spring, winter and facultative growth habits were observed.

The study of *VRN-A1* gene expression showed vernalization induction in all the materials and stages. Under not-vernalized conditions, the facultative materials showed delayed expression of *VRN-A1* whereas in the winter genotype expression was not detected at all.

Regarding photoperiod response, eight genotypes presented the allele of insensitivity at the locus *Ppd-A1* and 24 genotypes possessed the allele of sensitivity. In all the genotypes except in two, the phenotypic evaluation coincided with molecular characterization of the *Ppd-A1* promoter.

The evaluation of frost tolerance at growth chamber based on the percentage of leaf damage, discriminated between tolerant and susceptible genotypes where two lines, one winter and one spring were prominent.

Concerning the genes involved in cold response, sequences of *TdDREB-A1* and *TdCBF14* were obtained. In the first case, polymorphisms were identified and their combination generated six haplotypes, one of them showed to be unique for winter tolerant material. A polymorphism detected in *TdCBF14* has been previously associated with cold tolerance in barley. The analysis of expression of gene *COR* in eight

genotypes showed differences across genotypes and treatments, although the relation between molecular polymorphisms and cold tolerance requires additional experiments.

The integration of the obtained information represents a contribution to the durum wheat genetic resource characterization that hopefully facilitates the choosing of the best performance genotypes at each site.

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