TEMPERATURE-SENSITIVE EXPRESSION OF LsNCED4 ENCODING AN ABA BIOSYNTHETIC ENZYME IS REQUIRED FOR THERMOINHIBITION OF LETTUCE SEEDS

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Thermoinhibition, or failure of seeds to germinate when they are imbibed at temperatures above ~30ºC, is a common phenomenon in commercial lettuce (*Lactuca sativa*) cultivars such as Salinas. In contrast, seeds of an accession of *L. serriola* (UC96US23) do not exhibit thermoinhibition. Genetic analysis of recombinant inbred lines (RIL) derived from a Salinas by UC96US23 cross revealed that a QTL associated with high temperature germination (*Htg6.1*) contained *9-cis-EPOXYCAROTENOID DIOXYGENASE-4*(*LsNCED4*), a key regulated gene in ABA biosynthesis. *LsNCED4* expression is elevated during late seed development in both Salinas and UC96US23, but increases (along with ABA content) during imbibition at high temperature only in Salinas seeds. Complementation of Arabidopsis *nced6-1nced9-1* mutants indicated that both *LsNCED4* genes from both genotypes encode functional proteins. Ectopic expression of Salinas *LsNCED4* under its native promoter resulted in thermoinhibition of UC96US23 seeds, whereas Salinas seeds in which LsNCED4 had been silenced via RNAi germinated to as high as 40ºC. Similarly, a premature stop codon mutation or missense substitution in *LsNCED4* also increased thermotolerance. Elevated *LsNCED4* expression was also induced in detached Salinas leaves by heat stress (40ºC for 1 h) but not by drought, while two other lettuce NCED genes (*LsNCED2* and *LsNCED3*) exhibited the opposite responses, indicating that NCED gene family members are expressed in response to distinct environmental signals. This work illustrates the ability to go from a QTL to a specific gene and develop native and mutant genes and markers to address a crop production limitation

A gene encoding an enzyme involved in abscisic acid biosynthesis is necessary and sufficient to enable high temperature inhibition of lettuce seed germination. Introgressed and mutant alleles of *LsNCED4* with reduced expression or enzymatic activity will allow breeding of lettuce cultivars with greater temperature tolerance during germination without compromising tolerance to water stress. The results of this study will help ecologist to understand the influence of global warming on plants.

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