Gene Introgression by Plant Breeding and Effect of LsNCED4 Gene on Lettuce Seed Germination

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Introduction

Cultivated lettuce seed germination is inhibited at high temperatures (thermoinhibition) well below the biological upper limit of seedling growth. Thermoinhibition of lettuce seeds can lead to poor stand establishment and uneven crop growth which could lead to economic losses. The hormones associated with lettuce seed germination are abscisic acid (ABA), gibberellic acid (GA), and ethylene. ABA inhibits germination and GA and ethylene promote germination (Nascimento, et al., 2000; Gonai, et al., 2004; Toyomasu, et al., 1994). Genetic analysis of thermoinhibition on a recombinant inbred line (RIL) population from the cross of thermosensitive *L. sativa* cv. Salinas and thermotolerant *L. serriola* UC96US23 revealed a quantitative trait loci (QTL) *HTG 6.1* originating from *L. serriola* UC96US23 which was responsible for 25% of the variation (Argyris, et al., 2005). The LsNCED4 gene, a gene involved in the ABA biosynthetic pathway, was collocated with *HTG 6.1*. Studies have demonstrated that thermoinhibited seeds also have increased expression of the LsNCED4 gene and higher ABA levels while seeds with the *L. serriola* UC96US23 allele do not express up-regulation of LsNCED4 and have lower ABA levels leading to seed thermotolerance (Argyris, et al., 2008a; Argyris, et al., 2008b). The results from the research above have lead to conclude that the LsNCED4 gene regulates thermoinhibition in lettuce seed.

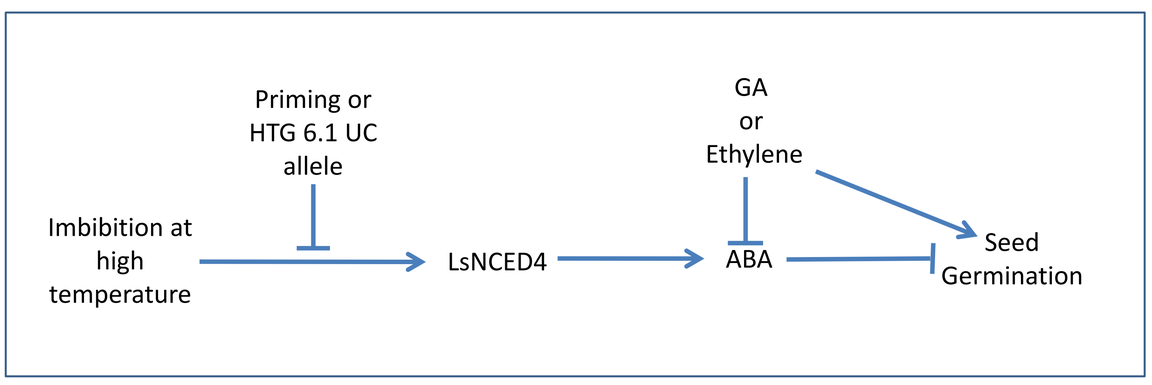

Figure 1. Proposed thermoinhibition scheme.

Thermosensitive lettuce cultivars Margarita and Twostar were crossed with 9 near isogenic lines (NIL) with the introgressed UC96US23 LsNCED4 allele. The purpose of this project is to assay if the introgressed LsNCED4 has any affect in seed germination at thermoinhibiting temperatures.

Materials and Methods

F2 seeds from introgressed lines, cultivar, and NIL parents were germinated at 20°C red light and 31°C red light. Seeds were placed in numbered petridishes with 30 seeds in each petridish and 3 replications from each introgressed lines. The seeds were imbibed with 4.5 mL of deionized water and counted at time intervals of 14, 24, 38, 48, 62, and 72 hours after inbibition. While counting the seeds they were exposed to green light; room temperature was raised to at least 30C and petridishes were taken out 6 at a time from the incubator to minimize time exposure of the seeds to temperature change. From the introgressed lines 5 germinated seeds were selected at random and 5 non-germinated seeds were selected at random. After germinating the non-geminated seed DNA was extracted from all seedlings using Quickextract™ protocol and real-time qPCR was done on the DNA samples using Taqman probes for allele discrimination. The alleles are single nucleotide polymorphism (SNP).

Primers	Forward	Reverse
LsNCED4	AGC CAA TTG GCG AAC TGC	CCG GCT ACA CCT CTT GCA TAA
Taqman Probes	**Salinas**	**UC96US23**
LsNCED4	/56-FAM/CTT GGT TT**A** GCT CGG CT/3IABkFQ/	/5MAXN/TGG TTT **G**GC TCG GCT /3IABkFQ/

Table 1. Primer and. Taqman probe sequences.

Cultivars	NILs	Introgressed Lines (F2)
Margarita	38_128	Margarita x 38_128
Twostar	39_132	Margarita x 39_132
	40_134	Margarita x 40_134
	41_143	Margarita x 41_143
	42_118	Margarita x 42_118
	43_120	Margarita x 43_120
	44_123	Margarita x 44_123
	45_126	Margarita x 45_126
	46_144	Margarita x 46_144
		Twostar x 43_120
		Twostar x 44_123

Table 2. List of genotypes used in experiment.

Results

Inbibition at 20°C red light had resulted in 100% germination for all seeds indicating that the seeds were viable and not dormant. Inbibition at 31°C red light resulted in thermoinhibition of cultivars Margarita and Diplomat; the NIL parents germinated at least 89% or higher while the introgressed lines showed germination between 35.6% and 70% with a mean germination of 47.7%. The sample of germinated seeds consisted of 58.2% UC96US23 homozygotes , 32.7% of heterozygotes (UC96US23/ Salinas), and 9.1% Salinas homozygotes. The sample of non- germinated seeds consisted of 3.7% UC96US23 homozygotes , 66.7% heterozygotes (UC96US23/ Salinas), and 29.6% Salinas homozygotes.

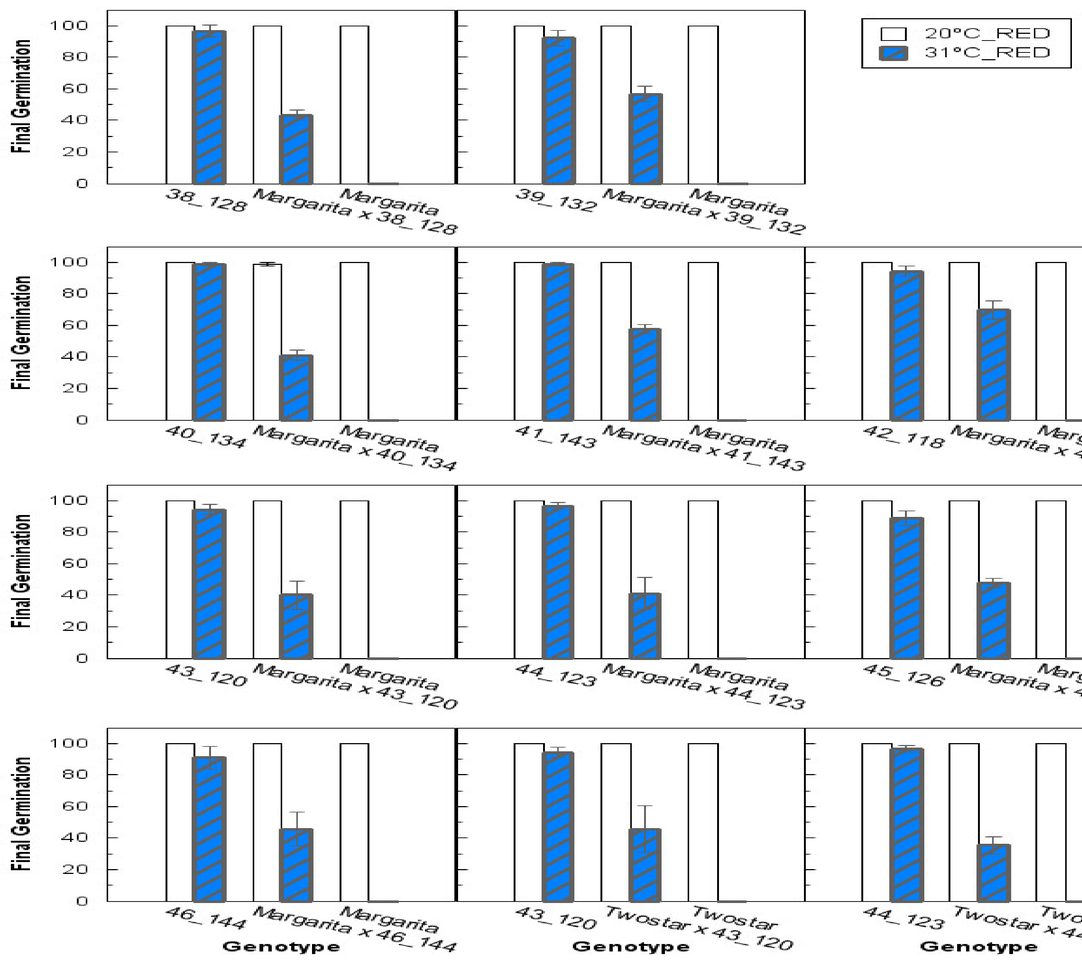

Figure 2. Final germination of introgressed lines compared with their respective parental lines.

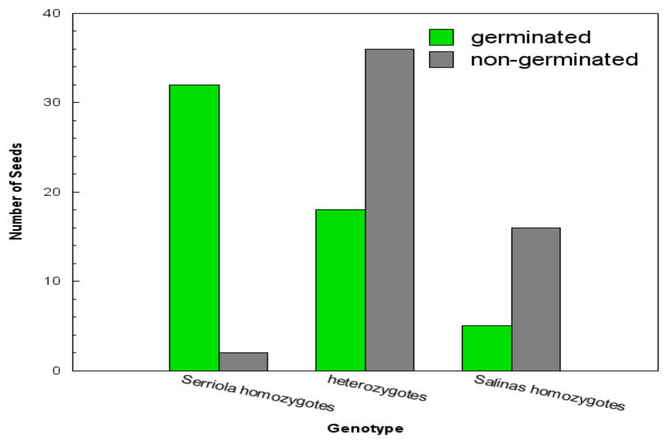

Figure 3. Distribution of genotyopes in sample population of germinated seeds and non-germinated seeds.

Discussion and Conclusion

According to the data, introgressed lines germinated more than the cultivated varieties. The introgression of UC96US23 LsNCED4 allele had a significant influence on germination based the genotyping data using two-factor Chi square analysis ($P < 0.001$). Homozyous UC96US23 seeds were significantly more abundant in the germinated seed sample population than in the sample population of non-germinated seeds. Homozygous UC96US23 seeds germinated 84.4% more than homozygous Salinas seeds at thermoinhibiting temperatures (31°C). This indicates that the introgression of the UC96US23 alleles improved germination in temperatures that caused thermoinhibition . The gene did not behaved as a dominant/ recessive gene, because heterozygotes germinated at thermoinhibiting temperatures; this was not consistent with data by Argyris (2008a). Further experimentation should be done with seed populations that have been backcrossed and have a homozygous UC96US23 allele to assay if the gene can cause thermotolerance comparable to the NILs. This experiment should also be reproduced in cultivated varieties that are less sensitive to high temperature without the UC96US23 allele to see if the introgression of the gene would further improve germination at higher temperatures.

References

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