

**Effect of High Temperature Shock During Grain
Maturation On Dormancy Of Wheat (*Triticum
aestivum* L.) and Analysis of *TaDOG1***

Submitted by,

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Statement of authorship

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Abstract

Pre-harvest sprouting (PHS) is the germination of seed under wet environmental conditions whilst still on the mother plant prior to harvest. In wheat, PHS causes farmers substantial economic losses due to quality downgrading. A high level of dormancy is regarded as an important mechanism of resistance to PHS in cereal species, such as wheat (*Triticum aestivum* L.). Many dormancy quantitative trait loci (QTL) have been identified and the corresponding genes that impart tolerance to PHS are actively being sought. Besides genetic factors, environmental conditions during grain maturation have been shown to have profound effects on dormancy. These environmental factors include temperature, light, drought and nutrients.

This project aimed to determine the role of high temperature shock during wheat seed maturation on its dormancy at harvest-ripeness. The results of these experiments showed that seed of dormant or intermediate dormant wheat genotypes may have lower dormancy levels after experiencing a high temperature shock (5 days of 40°C). The highest sensitivity towards high temperature shock is limited to a short “window” of approximately five days starting around 25 dpa. The sensitivity increases and reaches a peak at 25 to 30 days post anthesis (dpa), causing an effective and quicker release of dormancy. The sensitivity later decreases and high temperature becomes less influential on dormancy. For the dormant genotype SUN325B, release from dormancy occurred 35 days earlier if treated with temperature shock. There was no significant correlation between the timing of the peak of sensitivity and factors such as daily temperatures before temperature shock, humidity or subsequent grain moisture losses. However, the stronger the intensity (40°C *versus* 35°C) and longer duration (5 days *versus* <5 days) of temperature shock seem to influence dormancy more significantly. Changes in embryonic and endospermic abscisic acid (ABA) concentration following temperature shock could not explain the change in dormancy levels in genotype SUN325B. Instead, the change in

dormancy levels following the temperature shock could potentially be ascribed to the loss of ABA sensitivity.

A comparison of the dormancy response to temperature shock of 23 genotypes that harbour different combinations of known dormancy QTL was performed. Strongly dormant genotypes, P07.683, 50213/Cunn798 and DM1073 #31, did not show an increased germination index at 65 dpa. Other genotypes, ranging from dormant to intermediate dormant phenotype under control conditions, i.e. displaying germination index less than 0.6 at 65 dpa, responded strongly to temperature shock. Dormancy levels significantly decreased, reflected by increases of germination index ranging from 0.4 to 0.9. It is highly likely that a temperature-shock-induced decrease in dormancy is a common response of these genotypes, and a higher number of combinations of QTL are required to resist dormancy changes.

The identification of *Delay of Germination 1 (DOG1)* as the gene underlying a major dormancy QTL in *Arabidopsis thaliana* has led to stronger understanding of the mechanism of dormancy. Based on results of studies of the *AtDOG1* and *TaDOG1-like* genes in *Arabidopsis* and cereal species respectively, and their functional conservation and responsiveness towards temperature changes during seed development, it seems likely that the *TaDOG1-like* genes in wheat might play a role in temperature shock-regulated dormancy levels.

A search of publicly available bioinformatics databases revealed three expressed sequence tags (ESTs) with accession numbers *AK336217*, *X56782* and *D12921* that could be *TaDOG1-like* genes in wheat additional to the five previously reported by Ashikawa *et al.* (2010, 2014). Protein motif analysis showed that the orthologs of *DOG1* possess combinations of protein motifs specific to each clade. Promoter analysis revealed RY repeats (CATGCAT) and (ACGTG)-core-containing ABA-responsive elements-like sequences in the promoters of these genes. Comparative mapping analysis also showed that one *TaDOG1-like* gene (accession:

X56782, could reside in dormancy QTL regions in wheat and similarly, two rice putative orthologs (*Os01g0159000* and *Os05g0560200*) reside in dormancy QTL regions in rice.

In a reverse transcription quantitative PCR (RT-qPCR) analysis, no significant expression of two *TaDOG1-like* genes (accession: *AB555729* and *AK332921* or *TaDOG1-L1* and *TaDOG1-L2* respectively) were detected in either embryos or de-embryonated wheat grains of SUN325B at 45 and 60 dpa. However, both genes were detected to be expressed at 25 dpa and 30 dpa in the excised embryo tissues, signifying the mRNA to be short-lived. *TaDOG1-L1* showed a non-significant increase following temperature shock, hence failed to explain the increase in germination index. *TaDOG1-L2* was significantly down-regulated at 30 dpa following temperature shock, which could cause a lower protein accumulation towards 65 dpa. Further investigation could focus on the role of *TaDOG1-L2* in dormancy in white wheat genotypes to better explain the effect of temperature shock during the sensitive “window” on dormancy in general.