SCSB Quarterly Report

General Information

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Proposal Information

Title: Development of Molecular Markers to Facilitate Breeding for Heat-Tolerance in Soybean

Progress Assessment and Next Steps

Soybean seeds comprise up to 40% protein and 20% oil (lipids) (Liu et al. 2019). Lipids are also the major constituents of biological membranes, which act as the interface between the cell and the environment. The structure and function of cells are dependent on the fluidity and stability of membranes, which are determined by lipid composition and unsaturation levels. *Cis* double bonds commonly present in most plant cell membrane fatty acyl chains introduce bends in the chains and reduce compact packing of adjacent lipid molecules. The compactness of packing is also reduced upon exposure to heat stress. Therefore, decreasing the number of double bonds at high temperatures can be an adaptive mechanism in plants to maintain the optimal lipid packing, fluidity, and integrity of membranes, which is critical for maintaining the normal functioning of the photosynthetic-machinery under stress (Narayanan et al. 2020).

Fatty acid desaturases (FADs) introduce *cis* double bonds into the hydrocarbon chain of fatty acids to produce unsaturated fatty acids and hence serve critical roles in plant development and environmental adaptation. The two major classes of FADs include oleate desaturase (ω-6 fatty acid desaturase) that produce moderately-unsaturated fatty acids from mono-unsaturated fatty acids by introducing a second double bond in the fatty acid acyl chain and linoleate desaturase (ω-3 fatty acid desaturase) that installs the third double bond in the fatty acid chains to give rise to poly-unsaturated fatty acids (Fig. 1).

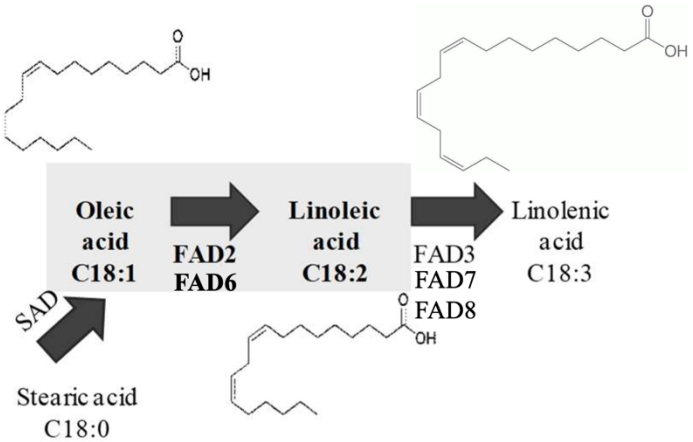
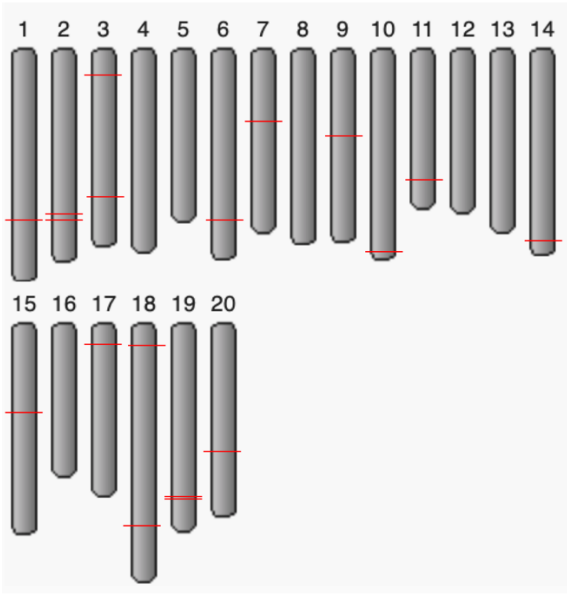


Fig. 1. diagrammatic illustration of the fatty acid desaturation process, which involves oleate and linoleate desaturases.

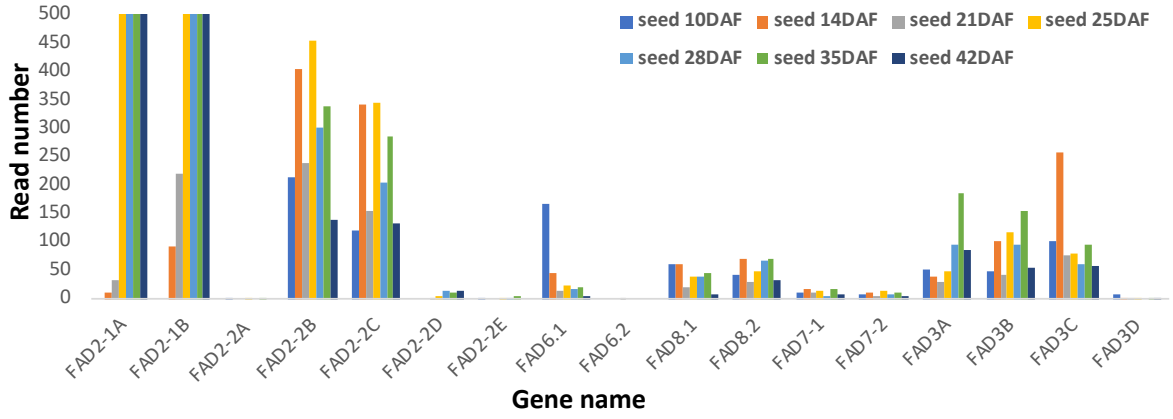
Genetic mapping of the soybean *FAD* genes. Soybean possesses at least 18 *FAD* genes, including ten oleate desaturases and eight linoleate desaturases. This study assigned these genes to 14 soybean chromosomes via database searchers (Fig. 2). Products of twelve *FAD* genes, namely *FAD2-1A, FAD2-1B, FAD2-2A, FAD2-2B, FAD2-2C, FAD2-2D, FAD2-2E, FAD2-3, FAD3A, FAD3B, FAD3C*, and *FAD3D*, localize to the endoplasmic reticulum (ER) and six *FAD* genes to plastids (*FAD6.1, FAD6.2, FAD8.1, FAD8.2, FAD7-1*, and *FAD7-2*), which suggest their site of action within the plant cell.



Chromosome number	Physical length (Mb)	Gene (chromosome location)
1	55.9	*FAD8.1* (41.33 Mb)
2	51.7	*FAD6.1* (38.87 Mb); *FAD3B* (41.41 Mb)
3	47.8	*FAD2-2C* (36.01 Mb); *FAD8.2* (7.80 Mb)
4	49.2	
5	41.9	
6	50.7	*FAD2-3* (42.04 Mb)
7	44.6	*FAD7-2* (18.35 Mb)
8	47.0	
9	46.8	*FAD2-2D* (22.17 Mb)
10	51.0	*FAD2-1A* (50.01 Mb)
11	39.2	*FAD3D* (32.20 Mb)
12	40.1	
13	44.4	
14	49.7	*FAD3A* (45.93 Mb)
15	50.9	*FAD2-2E* (22.30 Mb)
16	37.4	
17	41.9	*FAD6.2* (5.82 Mb)
18	62.3	*FAD7-1* (48.36 Mb); *FAD3C* (5.64 Mb)
19	50.6	*FAD2-2A* (40.81 Mb); *FAD2-2B* (40.81 Mb)
20	46.8	*FAD2-1B* (35.31 Mb)
Total/average	950.0	

Fig. 2. Genomic distribution of the ten oleate desaturases and eight linoleate desaturase genes in the soybean genome.

Expression profiling of the soybean *FAD* genes. Additionally, we studied the expression patterns of the *FAD* genes (except *FAD2-3*) throughout soybean development. The expression profiling results are based on exploring the RNA-Seq Atlas of *Glycine max* genotype A81-356022 available at http://www.soybase.org/soyseq. This genotype is not tested for heat-tolerant; therefore, we treated it as heat-susceptible in this report. *FAD2-2B* and *FAD2-2C* exhibited constitutive expression throughout soybean development (Fig. 3). In an earlier study, the transcript abundance of *FAD2-2C* was reported to increase in pods grown in cool conditions relative to those grown in warmer conditions. *FAD2-3* was also shown to express in both vegetative and developing seed tissues constitutively but with no significant change in transcript abundance in cold-stressed leaves (Li et al. 2007). The linoleate desaturases, i.e., *FAD3A*, *FAD3B*, *FAD3C*, *FAD3D*, *FAD7-1*, *FAD7-2*, *FAD8-1*, and *FAD8-2*, also exhibited constitutive but reduced expression in comparison to the *FAD2* genes (Fig. 3).



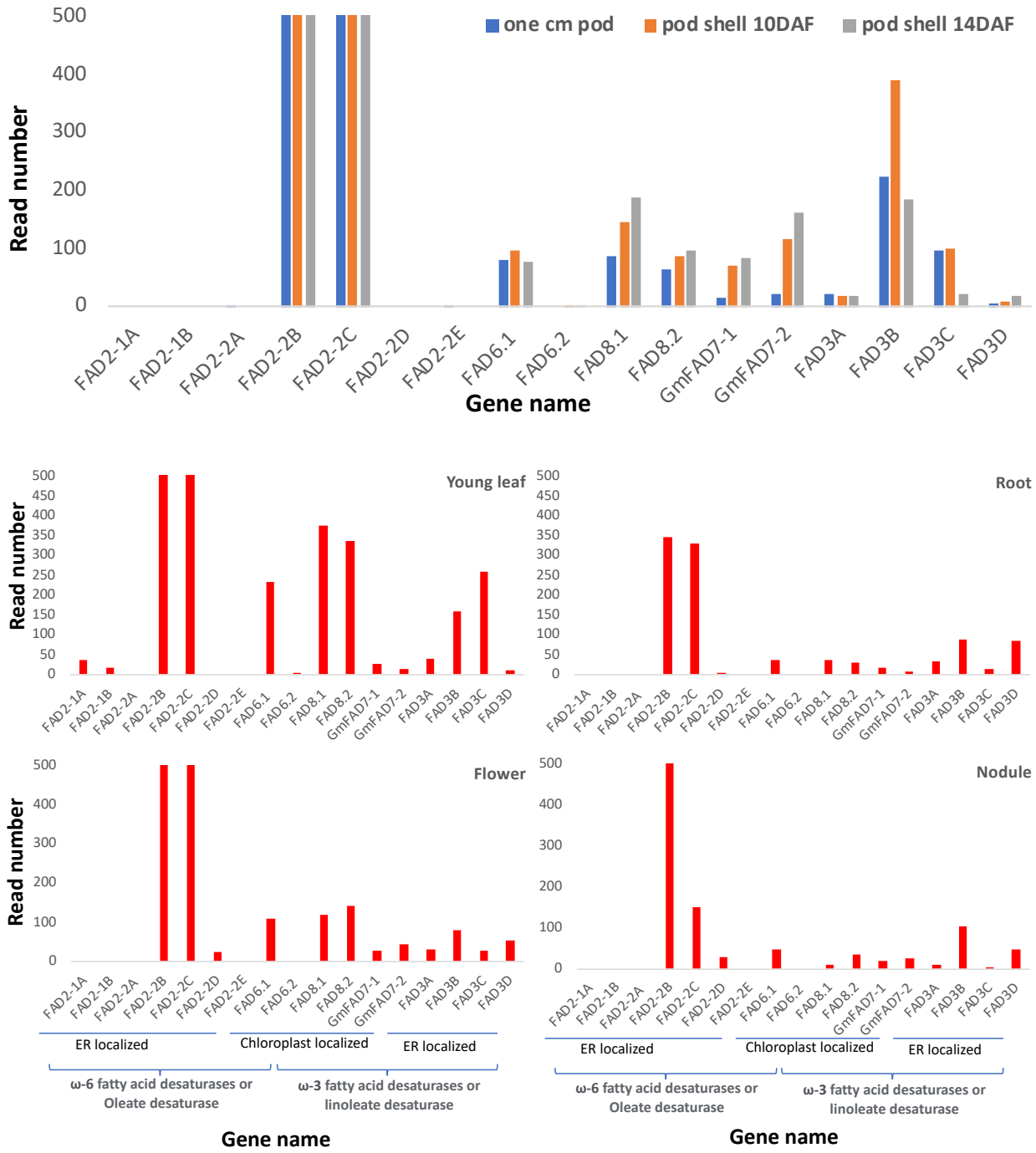
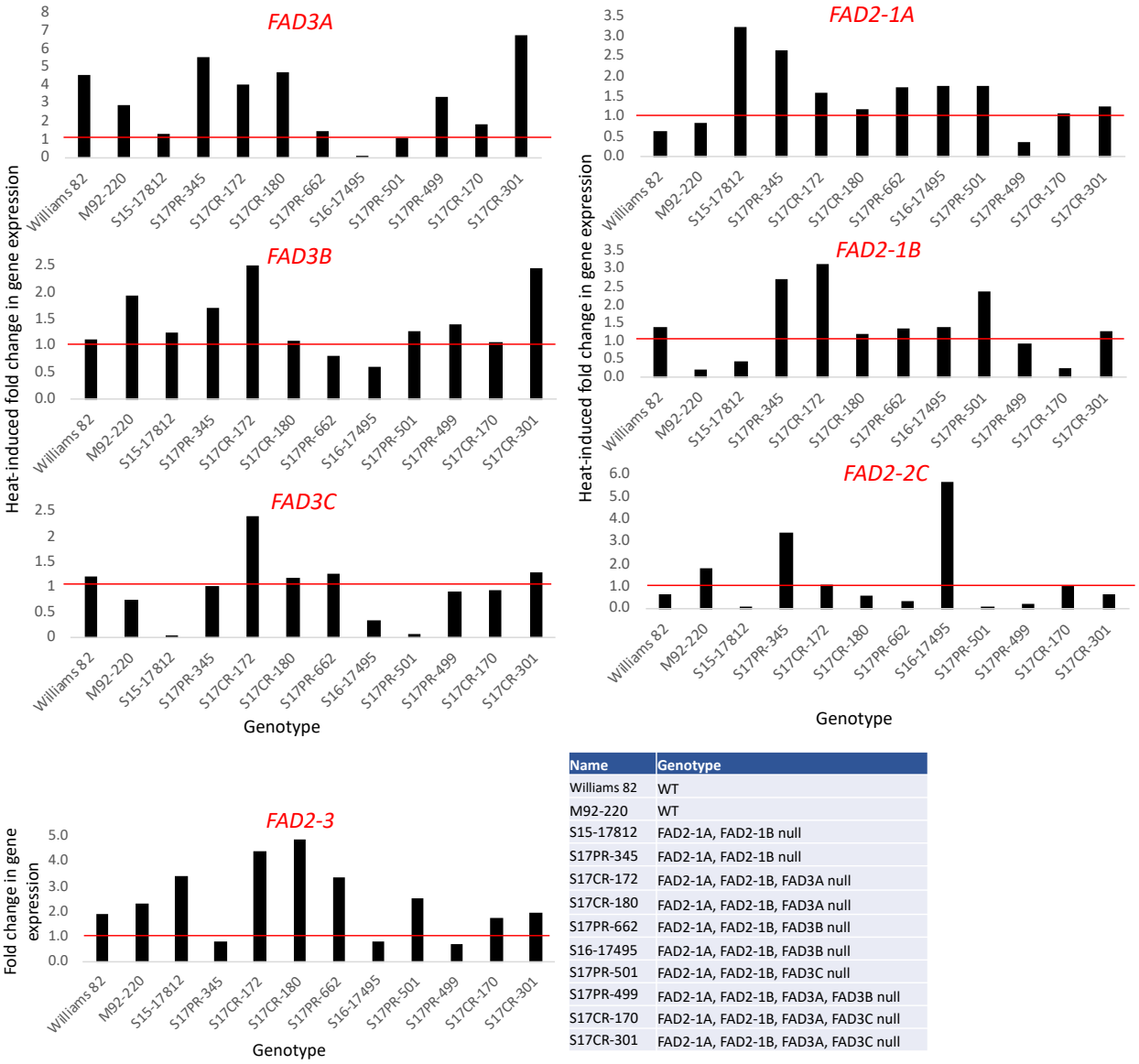


Fig. 3. Expression profile of the soybean *FAD* genes in vegetative and reproductive tissues and across different developmental stages. Notice the constitutive expression of the *FAD2-2B* and *FAD2-2C* genes and also the *FAD3A-D* genes; however, they respectively exhibited different expression levels, high and low. In contrast, the *FAD2-1A* and *FAD2-1B* genes exhibited tissue-specific expression patterns, and the *FAD2-2A* gene exhibited no expression. To compare the expression pattern of different genes, the scale on the y-axis was adjusted by truncating at 500 normalized reads number. DAF = days after flowering.

Expression profiling of the soybean *FAD* gene under heat stress. In this experiment, we studied the effect of heat stress on the expression pattern of the *FAD* genes in the soybean double, triple, and quadruple mutants (carrying mutations in two, three, and four *FAD* genes) and two wild type lines, 'William 82' and M92-220. The heat stress was imposed on the 15-day old plants for 15 days, and leaf samples were collected seven days after the start of heat treatment for RNA extraction, cDNA synthesis, and quantitative RT-PCR analysis. Based on our previous results, we anticipated that the heat-induced reduction in the expression of the *FAD3A* and *FAD3B* genes is associated with heat-stress tolerance, as the heat stress-tolerant soybean genotype DS25-1 showed reduced accumulation of *FAD3A* and *FAD3B* genes and concomitant reduction in the level of the poly-unsaturated lipids.



Name	Genotype
Williams 82	WT
M92-220	WT
S15-17812	FAD2-1A, FAD2-1B null
S17PR-345	FAD2-1A, FAD2-1B null
S17CR-172	FAD2-1A, FAD2-1B, FAD3A null
S17CR-180	FAD2-1A, FAD2-1B, FAD3A null
S17PR-662	FAD2-1A, FAD2-1B, FAD3B null
S16-17495	FAD2-1A, FAD2-1B, FAD3B null
S17PR-501	FAD2-1A, FAD2-1B, FAD3C null
S17PR-499	FAD2-1A, FAD2-1B, FAD3A, FAD3B null
S17CR-170	FAD2-1A, FAD2-1B, FAD3A, FAD3C null
S17CR-301	FAD2-1A, FAD2-1B, FAD3A, FAD3C null

Fig. 4. Expression profiling of the soybean double, triple, quadruple *FAD* mutants under heat stress. The bar diagrams exhibited a heat-induced fold difference in the expression pattern of the FAD genes. The red line depicts the expression pattern of the gene under optimal growth conditions. Bars taller than the threshold line show inducted expression, whereas the bars shorter

than the threshold line exhibit suppressed expression of a particular FAD gene. The genotype of each mutant line is shown in a table at the bottom right.

Here we report the expression pattern of seven *FAD* genes, namely *FAD2-1A*, *FAD2-1B*, *FAD2-2C*, *FAD2-3*, *FAD3A*, *FAD3B, and FAD3C,* in the heat-stress treated plants. The data on the above-ground plant biomass, seed characteristics (seed number, seed weight, seed coat wrinkling), and seed germinability are currently being recorded on these plants and reported in the future. The expectation is that the lines carrying mutations in the *FAD* genes, specifically *FAD3A*, *FAD3B*, and/or *FAD3C*, will exhibit reduced accumulation of these genes and exhibit heat-tolerance. The expression analysis, completed recently, exhibited the following results:

1. Not all *FAD* mutants showed reduced *FAD* gene expression. Indeed, some mutant lines showed heat-induced overaccumulation of *FAD* gene transcripts (Fig. 4). Interestingly, S16-17495, a line carrying mutations in the soybean *FAD2-1A*, *FAD2-1B*, and *FAD3B* genes, showed reduced expression of all studied *FAD3* genes, whereas it exhibited induced expression of *the FAD2-2C* gene. In contrast, the mutant lines carrying mutations in more than one *FAD3* gene generally exhibited enhanced *FAD* gene expression under heat stress. These observations can be explained in light of previous observations, such as plants try to compensate for the loss of function by overexpressing the gene in mutant lines. Also, the sequence variations (substitutions and insertion/deletions) in the genes may not necessarily reduce their transcription, which could be the case for the *FAD* genes. Indeed, in earlier studies, a higher transcription of the *FAD* mutant allele was observed under specific circumstances (Ohlrogge et al. 2015). In the present study, several-fold higher expression of the selected FAD genes under heat-stress was observed in double, triple, and quadruple *FAD* mutants, which stack mutations in *FAD2-1A, FAD2-1B, FAD3A, FAD3B*, and/or *FAD3C* genes. Mutations in these genes determine the accumulation of lipids with monounsaturated/moderately unsaturated fatty acids over poly-unsaturated fatty acids. However, we do not yet know if the *FAD* mutants show heat-tolerance relative to the wild type line, which will become apparent as we finish recording the phenotypic data in February 2021 and analyze it.
2. The *FAD* mutations were induced and stacked in the background of a heat-susceptible genotype. According to our previous research, the heat-susceptible genotypes exhibit enhanced expression of the *FAD* genes, specifically *FAD2-1B* and *FAD3B* (Narayanan et al. 2020), probably due to the presence of the elements, such as transcription factors and/or micro RNAs, that *trans* regulate the expression of *FAD* genes and upregulate their expression.
3. Another plausible explanation could be, the tolerant (DS25-1) and susceptible (DT97-4290) soybean genotypes possess sequence variations in the *FAD* genes different from that found in the *FAD* mutants, which lead to reduced transcription. It suggests the importance of studying the *FAD* genes for variations in their sequences in the heat-tolerant and heat-susceptible genotypes and also use a genetic population derived from them to study the association between the heat-tolerance, *FAD* gene expression levels, and the sequence variations in the *FAD* genes. These are the project proposal's objectives submitted to the SC Soybean Board for consideration under the 2021 call for proposals. The genetic population, characterized under the proposed project, might also allow studying the *trans* regulation of the *FAD* genes if involved in the heat-induced expression of these genes.

References

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