**Final Research Progress Report**

**MSMC Project Number 14-359-18**

1. **Title of Grant**

Improving Heat Tolerance: Development of mapping populations and identification of heat tolerance mechanisms

1. **Period Covered (date of last report to current time; for a multi-year project, identify year of work.)**

April 1, 2016 to June 30, 2018

1. **Project Leader and Co-Primary Investigator**

Felix B. Fritschi, Jason Gillman, Arun Prabhu Dhanapal

1. **Layman’s Summary (limit to one page)**

Soybean yield is strongly influenced by the weather conditions prevailing during key parts of the growing season. Among the weather factors, temperature plays a major role in the success or failure of reproductive structures, and therefore yields. Temperature conditions in Missouri can vary considerably and rapidly. In essence, there are no management practices that growers can use to mitigate soybean exposure to high temperature stress, therefore, varieties need to be developed that can yield better when temperatures are high. Developing varieties with favorable genes for heat tolerance will maintain or increase yields in the face of heat stress. This is particularly important for Missouri farmers because, even though they are located in the southern portion of the Midwestern soybean belt, little attention has been given to the targeted development of varieties tolerant to high temperatures.

We have pursued a two-pronged approach based on the identification of lines with robust heat tolerance that are used i) to make crosses and develop mapping populations, and ii) to improve our understanding of heat tolerance (and sensitivity) mechanisms. This project has built on our results from MSMC Project 14-359 and advanced an unprecedented set of recombinant inbred line populations (RIL) for targeted and efficient improvement of soybean tolerance to high temperatures. Further this project has provided us 1) germplasm with increased tolerance (and susceptibility) to heat stress, 2) progeny of crosses between heat tolerant and heat susceptible lines as part of RIL population development, 3) preliminary understanding of physiological mechanisms associated with heat tolerance and heat susceptibility. Additional efforts are necessary to advance the unique RIL populations under development to the point where they can be used for genetic mapping which is expected to lead to the identification of novel molecular markers and possibly genes and biochemical pathways that can be targeted to improve soybean heat tolerance.

1. **State your objectives in question form and discuss how your results answer these objectives.**

**Project Objectives**

1. Continue development of populations for genetic analyses (in part initiated during 2014 and 2015).
2. Identify physiological fingerprints involved in protecting soybean yield from losses during episodes of high temperature stress.

**Summary**

* More than 300 MG III soybean lines were screened for heat tolerance in high temperature greenhouse conditions during 2014 and 2015.
* Based on initial characterization of the 300 genotypes and follow-up experiments with subsets of genotypes, putative heat tolerant and heat sensitive lines were selected to make crosses to develop mapping populations. Selections were made based on overall visual rating (heat only) seed number, pod number (heat only), seed per pod (heat only), 100-seed weight, germination, and wrinkling. Initial crosses and development of F2 populations were successful, however, due to bad germination and heavy rain after planting in 2017, the number of recombinant inbred lines (RIL) was reduced to the extent that further advancement of this population is not warranted.
* Characterization of seeds harvested from heat stress and ambient experiments for a subset of 60 genotypes has been completed for seed wrinkling score, germination, 100 seed weight as well as seed dimensions.
	+ Genotypic differences for heat tolerance with regard to all seed characteristics were identified.
	+ Some genotypes previously identified as heat tolerant and heat sensitive based on reproductive success traits also appear to be more tolerant to heat stress in terms of the seed characteristics, which could represent sources of valuable alleles for future breeding efforts.
* In total, six parental lines (4 putative heat tolerant and 2 putative heat sensitive) were chosen from the 60 genotypes subset for crossing in the 2016 season at the BRC. Of the different cross combinations planted at South Farm Research Center in 2017 for advancement to F2:3, five populations of 200+ RILs have been harvested and planted for population advancement in 2018 for progression from F3 to F4. After further advancement, these populations will represent unique mapping populations which are specific for tolerance/sensitivity to elevated temperatures.
* Seed from SoyNAM parental lines that were grown in a high temperature environment were analyzed for several seed characteristics, including seed composition and seed germination. Significant genotypic variation was identified among the SoyNAM parental lines and four populations were planted at a heat nursery location. The selected NAM populations included NAM12 (Cross: IA3023 x LD02-4485), NAM23 (Cross: IA3023 x U03-100612), NAM29 (Cross: IA3023 x LG05-4464), and NAM28 (Cross: IA 3023 x LG05-4317). Unfortunately, the heat stress field experiments with these four NAM populations were compromised by iron-deficiency symptoms. As such, they will not provide reliable data on heat stress tolerance or sensitivity. Thus, these experiments would have to be planted again as part of a future project.

**Research Progress**

***Objective I:******Continue development of populations for genetic analyses (in part initiated during 2014 and 2015)***

*Initial selection of genotypes for population development:*

Initial selections from 2014 data were made using seed set, pod set, and seed per pod data from heat stressed plants relative to an ambient control. For each trait, values were sorted and the bottom and top 10 lines were selected. From these lists, 3 lines were chosen with preference given to lines that appeared in multiple top or bottom 10 lists for each trait. Yield data from USDA-GRIN were taken into consideration by eliminating lines that had values <1500 kg/ha. The lists of the top and bottom 3 lines for each trait were consolidated, leaving 6 putative heat tolerant lines and 4 putative heat sensitive lines. These genotypes were planted in five replications in individual pots and were grown under ambient conditions at the BRC in 2015. Putative heat sensitivity or tolerance was confirmed when seed production under high temperatures was compared to that under ambient conditions, where putative heat sensitive genotypes generally had lower relative seed number than the putative heat tolerant genotypes (Table 1 and Figure 1). These 10 genotypes were considered to be the most promising initial candidates to make crosses for genetic analyses and development of more heat tolerant germplasm, and ultimately cultivars.

Table 1. List of heat tolerant and heat sensitive lines with traits based on which they were classified

|  |  |
| --- | --- |
| **Heat Tolerant Lines** | **Trait for Classification** |
| PI518757 | Seed Set |
| PI507491 | Seed Set/Pod Set |
| PI360841 | Seed Set/Pod Set |
| PI398694 | Pod Set |
| PI548313 | Seed per Pod |
| PI548632 | Seed per Pod |
|  |  |
| **Heat Sensitive Lines**  | **Trait for Classification** |
| PI438335 | Seed Set/Seed per Pod |
| PI438357B | Seed Set/Seed per Pod |
| PI438427 | Pod Set/ Rel Seed per Pod |
| PI561292A | Pod Set |

**Figure 1.** Relative seed number of individual plants grown under high temperature conditions compared to those grown under ambient/field conditions. Total seed was counted for individual plants grown in the greenhouse, while seed collected from the field was estimated using 100-seed weight and divided by the total number of plants in each plot. Each genotype in the greenhouse had five replicate pots and each pot had 1-2 individual plants. Numbers represent the mean of total seed number of each plant for all five replicate pots divided by the mean total seed number for three replicates from the ambient/field plots. Tolerant (orange) or sensitive (blue) genotypes were selected based on data collected in 2014.

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*Background on selection of genotypes for crossing:*

Three new and one old (PI360841) putative heat tolerant genotypes and two putative heat sensitive genotypes were selected based on additional characterization and confirmations of diverse genotypes with the goal of creating 8 crosses from promising contrasting genotypes (genotypes selected from the 60 genotypes subset described in Objective II) (Table 2). Although field conditions were not ideal in the spring, attempts were made on 7 of the 8 planned crosses. From 30 single pods that were collected from these attempts, 18 of them comprising 6 different crosses were kept. Seed from all the six crosses were grown in a greenhouse over the winter to maximize single seed descent for large future mapping population sizes to be advanced in the upcoming seasons (Table 3). Further, two additional crosses were made with the heat tolerant PI 518757 as male and PI548523/Pella and or Williams 82 as female (Table 3).

Table 2. List of heat tolerant and heat sensitive genotypes with traits based on which they were classified. This list resulted from characterization of a subset of promising lines identified in the original screening.

|  |  |
| --- | --- |
| **Heat Tolerant Lines** | **Trait Selected** |
| PI360841 | High rel. seed, rel. germination, and visual rating |
| PI370055 | High seed/pod, rel. seed weight, and visual rating |
| PI437317 | High rel. seed, pods, and visual rating |
| PI567510A | High rel. seed, seed/pod, rel. seed weight |
|  |  |
| **Heat Sensitive Lines**  | **Trait Selected** |
| PI561202A | Low pods, seed/pod, rel. germination, rel. seed weight; high visual rating |
| PI437367 | Low rel. seed, seed/pod, rel. seed weight, visual rating |

Table 3. List of crosses between genotypes contrasting in heat tolerance as well as between a heat tolerant genotype and advanced lines.

|  |  |  |
| --- | --- | --- |
| **Cross** | **Cross** | **Cross Advancement**  |
| FF5 | PI360841 (104) x PI561292A (87) | Abandoned due small population size |
| FF6 | PI360841 (104) x PI437367 (275) | Advanced  |
| FF8 | PI370055 (243) x PI437367 (275) | Abandoned due small population size |
| FF9 | PI437317 (271) x PI561292A (87) | Advanced |
| FF10 | PI437317 (271) x PI437367 (275) | Advanced |
| FF11 | PI567510A (359) x PI561292A (87) | Abandoned due small population size |
| JGx16-9 | PI518757 x PI548523/Pella | Advanced |
| JGx16-10 | PI518757 x Williams 82 | Advanced |

*Heat tolerance/sensitivity population development:*

Unfortunately, unfavorable conditions at germination and heavy rain after planting in 2017 reduced the number of RILs originating from the first crosses that were made as part of this project (derived from crosses with PI507491 and PI360841). Since the number of remaining RILs was less than 100, the power for robust mapping of QTL was lost, therefore, we had to abandoned advancement of these populations.

However, we made additional crosses for population development using heat tolerant (HT) and heat sensitive (HS) lines as follows: FF6 - PI360841 (HT, male) x PI437367 (HS, female), FF9 PI437317 (HT, male) x PI561292A (HS, female), and FF10 - PI437317 (HT, male) x PI437367 (HS, female). Further, two additional crosses were made with the heat tolerant PI518757, JGx16-9 – PI518757 x PI548523/Pella and JGx16-10 – PI518756 x Williams 82. Seed from these crosses were planted in the greenhouse in January 2017. Each plant was sampled at V2 and tested for genetic markers to confirm the cross. Of the 33 total seeds planted, 27 were confirmed crosses and F2 seeds were harvested from 24 F1 greenhouse plants from five distinct (tolerant x sensitive or tolerant x advanced) crosses; one genetic cross produced F1 with extremely few seed and was dropped. F1:2 seed were planted in 2017 and F3 seeds were harvested from individual F2 plants. These populations under development [FF6 - PI360841 (HT, male) x PI437367 (HS, female), FF9 - PI437317 (HT, male) x PI561292A (HS, female), and FF10 - PI437317 (HT, male) x PI437367 (HS, female); JGx16-9 - PI518757 (HT, male) x PI548523/Pella (advanced); JGx16-10 - PI518757 (HT, male) x Williams 82 were planted in 2018 for advancement from F3 to F4. We will continue the advancement of these RILs to generate F5- or F6-derived F8 mapping populations. Ultimately, these five populations will be used in genetic mapping studies to understand heat tolerance mechanisms in the future, and would not have been possible without this project.

Table 4. Mapping populations under development and status in summer 2018.

|  |  |  |  |
| --- | --- | --- | --- |
| ***Populations*** | ***Cross*** | ***Population status (2018)*** | ***# RIL from multiple F1 plts* †** |
| FF6 | PI360841 x PI437367 | F3:4 | 146 |
| FF9 | PI437317 x PI561292A | F3:4 | 215 |
| FF10 | PI437317 x PI437367  | F3:4 | 220 |
| JGx16-9 | PI518757 x PI548523/Pella | F3:4 | 446 |
| JGx16-10 | PI518757 x Williams 82 | F3:4 | 512 |

**†** *To achieve a sufficiently large number of RILs we are combining seed from multiple F1 plants.*

***Objective II: Identify physiological fingerprints involved in protecting soybean yield from losses during episodes of high temperature stress***

*Background on selection of genotypes for studies in 2016:*

Field plots were planted in 2015 at the BRC in three replications for 384 of the 400 genotypes originally selected for this project. These plots were used to assess genotype characteristics under ambient conditions and to provide seed for follow-up studies. Total seed number per plot was determined based on 100-seed weight, and germination tests were completed for each plot. In addition, seed size and seed wrinkling were evaluated from pictures of the seed taken before germination. These results were compared to seed size, seed wrinkling, and seed germination of samples obtained from the same genotypes but grown in the greenhouse under high temperature conditions (described below).

A total of 240 genotypes were planted in three replications in individual pots in 2015. Pots remained at the BRC until R1 or shortly before, at which time they were moved to two greenhouse bays set for ~43/27 ˚C (~110/80 ˚F) daily maximum/minimum temperature. The plants remained under high temperatures until maturity. Pods were collected at the time of harvest, and the total number was recorded. Total seed number was counted after seed cleaning. Germination was determined as mentioned above with pictures taken of each sample for seed size and seed wrinkling assessment.

For our purposes, we consider pod number, seed number, and seed per pod the main yield characteristics upon which to focus for selection of differential genotypes. Other characteristics assessed and taken into consideration for selections were seed weight, seed size, seed wrinkling, germination, and visual rating of the plant.

*Selection and assessment of genotypes in 2016:*

A subset of 60 genotypes was selected based on pod number (under high temperatures only), seed number (heat vs. ambient), seed per pod (under high temperatures only), 100-seed weight (heat vs. ambient), germination (heat vs. ambient), wrinkling (heat vs. ambient), and overall visual score (under high temperatures only) as either being heat tolerant or heat sensitive (~30 each) from the 2015 data collection described above. For each trait, values were sorted and the top and bottom 25 genotypes were selected. This resulted in top and bottom 25 lists for each of the seven traits listed above. These lists were compared to determine which genotypes appeared on multiple lists. The genotypes selected where either tolerant or sensitive for three of the seven traits, but not all of the genotypes were tolerant or sensitive for the same traits.

Six replicates of these genotypes were planted in pots in the open field at the BRC in 2016. The pots remained at the BRC until shortly before R1 (June 28th, 2016) when they were moved to a greenhouse set for ~43/27 ˚C (~110/80 ˚F) daily maximum/minimum temperature. Field plots were planted at the BRC in 2016 in three replications for 380 genotypes. These plots were used to assess genotypes under ambient conditions with a focus on the 60 selected genotypes and to provide seed for follow-up studies. When plants were at R7/8, two plants per plot were harvested in all three replications of all 60 genotypes that were grown in the greenhouse experiment described above. Pod number and total seed for each of the two harvested plants was counted.

Pod number, seed number and seed per pod data between plants grown in ambient and high temperature conditions. Selections for pod number and seed per pod were based on extreme genotypes (lowest and highest 25 values for each trait) under high temperatures only. Selections for seed number were mostly based on relative numbers (heat:ambient), where tolerant genotypes had relative values from ~0.8 – 1.5, and sensitive genotypes had values ≤0.5. Relative pod number data collected in 2016 showed that sensitive and tolerant genotypes shared the entire range (Figure 2). However, certain genotypes were consistently tolerant (*e.g.*, orange bars ≥1.0) or sensitive, (*e.g.*, blue bars ≤0.5).

**Figure 2.** Relative pod number of individual plants grown under high temperature conditions in the greenhouse compared to those grown under ambient/field conditions in 2016. Pods were counted for individual plants grown in the greenhouse or determined from two plants per plot under ambient conditions. Numbers represent the mean of total pod number of each plant in the greenhouse (n = 6) divided by the mean total pod number of plants from field plots (n = 3). Tolerant (orange) or Sensitive (blue) genotype classification is based on 2015 data.



**Figure 3.** Relative seed number of individual plants grown under high temperature conditions in the greenhouse compared to those grown under ambient/field conditions in 2016. Total seed number was counted for individual plants grown in the greenhouse or determined from two plants per plot under ambient conditions. Numbers represent the mean of total seed number of each plant in the greenhouse (n = 6) divided by the mean total seed number of plants from field plots (n = 3). Tolerant (orange) or Sensitive (blue) genotype classification is based on 2015 data.



Relative seed number followed a similar trend as relative pod number (Figure 3). In 2015, seed number from plants grown under ambient conditions was estimated from the 100-seed weight for each plot. For sensitive genotypes this revealed a striking difference between the number of seed produced under ambient conditions compared to high temperatures, where there was significantly more seed produced under ambient conditions for sensitive genotypes. Several genotypes produced more seed under ambient than high temperature conditions (<1.0); however, many of the genotypes showed similar numbers under both conditions (Figure 3). Although these differences were not as pronounced, there were genotypes that consistently were sensitivity (*e.g.*, PI417309A orPI548624) and genotypes that consistently were tolerant (*e.g.*, PI399077, PI518757, PI567510A) in 2015 and 2016. For the number of seeds per pod, the majority of genotypes showed similar or higher numbers under high temperatures than under ambient conditions (Figure 4).

**Figure 4**. Relative seed per pod of individual plants grown under high temperature conditions in the greenhouse compared to those grown under ambient/field conditions in 2016. Total seed per pod was counted for individual plants grown in the greenhouse or determined from two plants per plot under ambient conditions by dividing the total number of seeds by the total number of pods. Numbers represent the mean of seeds per pod of each plant in the greenhouse (n = 6) divided by the mean of seeds per pod of plants from field plots (n = 3). Tolerant (orange) or Sensitive (blue) genotype classification is based on 2015 data.

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*Preliminary pollen heat tolerance/sensitivity studies*

Six genotypes were planted in replicated pots (x4) at the BRC. Two of four replicates were moved into a greenhouse set at ~43/27 ˚C (~110/80 ˚F) daily maximum/minimum temperature at R1. Two replicates remained at the BRC to serve as ambient controls. These six genotypes are a few of the same tolerant/sensitive lines used to make the crosses in a winter nursery in Costa Rica or those used for crossing this past season (2016). Flower collection and pollen germination data were determined using the protocol described in Djanaguiraman et al. 2013. Pollen germination is highly variable, however at least one putative heat sensitive line had preliminarily lower pollen germination than most of the putative heat tolerant lines (Figure 5). Further assessing pollen germination as a characteristic of heat tolerance or sensitivity will require a higher replication and standardization of flower collection regarding its position on the plant and time of flowering. This could explain the very low pollen germination seen in PI437317 (Figure 5). These results are not discouraging, as higher pollen germination may not in every case be the mechanism for heat tolerance for a given heat tolerant genotype.

**Figure 5**. Percent pollen germination for putative heat tolerant and heat sensitive soybean lines. Flowers were collected on two separate occasions from plants grown under high temperature conditions. Each time pollen from 5 flowers per genotype were dusted onto slides containing germination medium. 100-200 pollen grains were counted per flower collected. Numbers represent the mean percent pollen germination for each genotype (n=10). Sensitive and Tolerant classifications based on 2015 data.



*Assessment of selected diverse genotypes in 2016/2017:*

 Genotypes were classified as sensitive or tolerant using data collected in the initial characterization experiments (as described above). Tolerant or sensitive genotypes were classified as such if they showed tolerance or sensitivity in at least three of the seven traits assessed. This means that not all genotypes were tolerant or sensitive in terms of pod number or seed number, and therefore, genotypes can show varying tolerance or sensitivity for a trait that they weren’t selected for. Also, inconsistent results are not uncommon due to varying field conditions from year-to-year.

Pod number, seed number, seed per pod, and visual ratings were the traits primarily used for selection of genotypes for development of mapping populations. Additionally, data were collected for 100 seed weight (Figure 6), seed germination (Figure 7) and seed characteristics assessment (Figure 8) for the selected 60 genotypes grown in field and high-temperature greenhouse conditions. Similar to pod number, seed number, seed per pod, and visual ratings, significant genotypic variation was also found for all examined seed traits presented below. Not surprisingly, these results also confirm that heat tolerance with respect to one trait does not necessarily mean that a particular genotype is also heat tolerant with respect to a different seed characteristic. Nonetheless, some genotypes appear to be superior to others on the basis of several traits (Figs. 6, 7, and 8).

**Figure 6.** (A) 100 Seed Weight of individual plants grown under high temperature (heat stress) conditions and field (ambient) conditions. (B) 100 Seed Weight of selected plants grown under high temperature conditions and field conditions. Genotypes were categorized into tolerant and sensitive groups based on previous data and not based on 100 seed weight. Total seed weight was counted for individual plants grown in the greenhouse, while seed collected from the field was estimated using 100-seed weight obtained from two plants per plot. Each genotype in the greenhouse had six replicate pots and each pot had 1-2 individual plants. Numbers represent the mean of 100 seed weight of each plant in the greenhouse (n = 6) and the mean of 100 seed weight of two plants in the field (n = 3).

**Genotypes**

**100 Seed Weight**

**A**

**B**

**Genotypes**

**Sensitive**

**Tolerant**

**100 Seed Weight**

**Figure 7.** (A) Germination percentage of 50 seeds from individual plants grown under high temperature (heat stress) conditions and field (ambient) conditions. (B) Germination percentage of 50 seeds from selected plants grown under high temperature conditions and field conditions. Genotypes were categorized into tolerant and sensitive groups based on previous data and not based on % germination. Germination percentage of 50 seeds were counted for individual plants grown in the greenhouse, while seed collected from the field was estimated using germination percentage obtained from two plants per plot. Each genotype in the greenhouse had six replicate pots and each pot had 1-2 individual plants. Numbers represent the mean of germination percentage of each plant in the greenhouse (n = 6) and the mean germination percentage of two plants from the field (n = 3).

**% Germination**

**Genotypes**

**B**

**Sensitive**

**Tolerant**

**Genotypes**

**% Germination**

**Figure 8.** (A) Circularity of 25 seeds from individual plants grown under high temperature (heat stress) conditions and field (ambient) conditions. (B) Circularity of 25 seeds from of selected plants grown under high temperature conditions and field conditions. Genotypes were categorized into tolerant and sensitive groups based on previous data and not based on circularity. Circularity of 25 seeds were counted for individual plants grown in the greenhouse, while seed collected from the field was estimated using germination percentage obtained from two plants per plot. Each genotype in the greenhouse had six replicate pots and each pot had 1-2 individual plants. Numbers represent the mean of circularity of 25 seeds of each plant in the greenhouse (n = 6) and mean circularity of 25 seeds from two plants from the field (n = 3). For the purposes of this study, the difference circularity between ambient and heat stress treatments is taken as a surrogate measure of heat induced changes in seed shape (e.g. such as caused by seed wrinkling).

**A**

**Genotypes**

**Circularity**

**B**

**Sensitive**

**Tolerant**

**Circularity**

**Genotypes**

*Assessment of SoyNAM parental genotypes*

The SoyNAM populations represent an incredible genetic resource created through previous projects funded by the United Soybean Board, where 40 diverse lines were all crossed to a single hub parental line, and populations and genotypic information were made available to the general soybean research community. As such, any phenotypic information is readily correlated with previously produced genotypic data. We included all NAM parental lines in our screening studies (visualized in different colors in Fig. 9), and identified significant differences for traits important for seed yield formation and seed quality. We have completed the analysis of NAM line seed production in Columbia (control) and at a heat nursery location. Important genotypic differences were observed, including contrasting susceptible/ resistant genotypes for reduction in seed protein/oil (Fig. 9A, 9B) and seed weight (Fig. 9B) in response to elevated temperature.

**Figure 9:** A) Response of seed composition (protein + oil) among NAM genotypes in a control (blue line) and heat stress nursery location (orange line). Putative tolerant and sensitive NAM lines were identified. B) General response of seed composition and 100-seed weight to control and heat stress among three planting dates (PD1-3).



We identified both putatively tolerant and sensitive genotypes, with the hub parent (IA 3023) being almost perfect in the middle of the response range. Germination analyses from different planting dates (PD1, PD2, and PD3) at the heat nursery also reveal significant genotypic differences in germinability of seeds that developed under high temperatures (Figure 10). As such, we are poised to perform a full genetic mapping study using the NAM lines in the immediate future to identify genomic regions and/or genes responsible for tolerance to elevated temperatures. Because the NAM parents contain elite high yielding public cultivars, it is likely that these results will be directly applicable to breeding efforts already underway in the public sector, and potentially also in the private sector.

**Figure 10.** Response of seed germination % on the analysis of NAM lines at a heat nursery location. Important genotypic differences were observed, including contrasting susceptible/ resistant genotypes for reduction in seed germination in PD-1(Fig. 10A), PD-2 (Fig. 10B) and PD-3 (Fig. 10C) and Across Planting dates (Fig. 10D) in response to elevated temperature. Genotypes were categorized into tolerant and sensitive groups based on % germination.

**NAM lines**

**% Germination**

On the basis of the significant genotypic variation found among SoyNAM parental lines, four NAM populations were selected and planted at a heat nursery location in 2018: NAM12 (Cross: IA3023 x LD02-4485), NAM23 (Cross: IA3023 x U03-100612), NAM29 (Cross: IA3023 x LG05-4464), and NAM28 (Cross: IA 3023 x LG05-4317). Unfortunately, the heat stress field experiment with these four NAM populations were compromised by iron-deficiency symptoms. As such, this 2018 field experiment will not provide reliable data on heat stress tolerance or sensitivity. We hope to garner funding to continue this work in the future.

1. Please answer the following.
2. How do your results benefit Missouri soybean growers?

The germplasm identified and populations we develop can be used by breeders for incorporation into improved varieties. In the long term, Missouri soybean farmers will benefit from the availability of heat tolerant germplasm, advanced breeding lines, and stress tolerant cultivars. Developing varieties with favorable genes for heat tolerance will maintain or increase yields in the face of heat stress

1. Estimate financial return for the average Missouri soybean producer.

Currently, no reliable data are available to accurately estimate the yield reductions associated with high temperature stress in Missouri. Therefore, we are not in a position to provide an appropriate estimate with regard to the financial return for the average Missouri soybean producer. However, periods of high temperatures occur every year in Missouri and can result in significant yield losses, which are often overlooked or ignored. Due to the frequency of heat waves and extended periods of above-optimal temperatures, the availability of more heat-tolerant soybean varieties could result in tremendous economic benefits for soybean growers for decades to come.

1. Do your results benefit the environment?

No direct benefits to the environment are expected.

1. What products or processes can be commercialized from this research?
2. List disclosure(s) of inventions or plant varieties submitted to the MU Tech Transfer Office.

None to report at this time

1. Identify potential disclosure(s) of inventions or plant varieties. *Please note that credit must be given to MSMC for any inventions or discoveries resulting from this research*.

None to report at this time

1. How would you commercialize these products or processes?

Our work is screening public germplasm and the identification of methods to select for heat tolerant accessions to inform a breeding operation. The eventual results may result in patentable and/or commercial cultivars with stress tolerance (or potentially, the genes responsible). However, physiological understanding and population development are in the early stages and commercialization and/or patenting will likely not be imminent.

1. If no specific products or processes were produced, how do you plan to make your results available to producers or industry?

Our results will be made available through both presentations, eventual publications (several of which are currently being drafted) and through sharing of genetic mapping results/populations in the future.

1. Is additional time or research required before your results can be used by producers and industry?

Yes, we will need additional time as our germplasm characterization and population development are in the early stages.

1. List publications by type (popular press, thesis, journals, other) written or planned.
* A poster entitled “Evaluation of diverse soybean genotypes for reproductive success under high temperature conditions” with A.P. Dhanapal, J.J. Biever, J. Gillman, and F.B. Fritschi as authors, was presented at the ASA, CSSA, and SSSA International Annual Meetings Oct. 22-25, 2017, Tampa, FL.
* A study initiated with USDA-ARS funds, aimed at gaining insight into the molecular mechanisms associated with heat tolerance has been completed. Towards this end, gene expression has been interrogated in mature seed of a heat tolerant and a heat sensitive genotype (with respect to seed germinability) from plants grown in contrasting stress and unstressed environments. Data analysis has been completed and a manuscript for publication in a peer-reviewed journal is currently being drafted for submission in the near future.
* A publication describing the results from the examination and characterization of diverse soybean genotypes to high temperature relative to control temperature conditions is planned. Data analysis is ongoing.
1. List cost of original project and actual expenditures. *The U.S. Department of Agriculture requires that we ask for budget information, including the number of hours spent on the project, the number of dollars remaining on account, as well as a breakdown of expenses. You are required to provide this information in your report.* Please also include names and titles/positions of those whose time has been charged to this project.

Dr. Arun Dhanapal joined the project as research scientist on June 2017 and has been working full time on this project since his arrival. Prior to Dr. Dhanapal joining the project, Dr. Jessica Biever was the full-time postdoctoral researcher on this project. Dr. Hua Bai, and graduate students Charles Krueger and Matt Herritt helped bridge the gap between the departure of Dr. Jessica Biever and the transition of Dr. Dhanapal to this project. In addition, the following undergraduate students / hourly works have assisted with various aspects of this project: Chris Hanrahan, Sarah Dixon, Andrew Loehnig, and Nicholas Rector. Drs. Fritschi and Gillman spent significant time on this project, but neither were paid from this project.

**Budget: April 1, 2016 to June 30, 2018:**

|  |  |  |
| --- | --- | --- |
| **Budget Item** | **Budgeted** | **Spent** |
| Salary | $53,876 | $60,093.13 |
| Benefits | $16,792 | $17,434.93 |
| Operating | $32,000 | $25,138.99 |
| **Total** | **$102,668** | **$102,667.05** |

1. List equipment purchased with MSMC funds, identifying inventory and serial number. (It is not considered equipment unless it costs $500 or more and has a life expectancy of at least 2 years.) Indicate current and future use of this equipment in support of soybean research.

 No equipment was purchased with funding from this project.