Principal Investigators: Krishna Jagadish, Ritesh Kumar, Sunghun Park and William Schapaugh

Checkoff Organization: Kansas Soybean Commission

Project Title: "Genetically-engineered soybean – a novel way to safeguard Kansas soybeans from severe drought and heat stress"

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Department Head: Dr Rajiv Khosla

Project Summary

Glutaredoxins (GRXs) are small ubiquitous oxidoreductase stress responsive genes, involved in floral development signaling and known to enhance tolerance to abiotic stress during reproductive development, by detoxifying the reactive oxygen species (ROS). Proof of concept, ectopic expression of Arabidopsis glutaredoxin gene (AtGRXS17) in maize substantially increased kernel-set and yield under heat stress (37°C daytime) both in greenhouse and field conditions. Similarly, Dr. Park and his team have engineered the cultivar 'Thorne', [maturity group 3, developed in the 90's from Illinois], aimed at inducing tolerance to drought and heat stress at reproductive and pod-filling stages by overexpressing the same AtGRXS17 in soybean. Three AtGRXS17-overexpressing soybean lines showed significantly higher seed-set and seed-weight under heat stress in greenhouse conditions, compared to wild-type. Hence, the major objective of the proposed project is to characterize the effectiveness of AtGRXS17-expressing soybean transformants on seed numbers and weight and seed quality under drought, heat and combined heat and drought stress using field-based heat tents and controlled-environment facilities.

Project Objectives

1) Evaluate the mechanistic basis of genetically engineered soybean lines exposed to drought and heat stress during flowering and pod-filling stages, using controlled and field-based facilities

2) Ascertain the differential level of stress alleviation during the flowering and pod-filling stages in terms of yield and quality (protein, oil and oleic acid)

Project Deliverables

1. Quantifying genetically-engineered soybean response to heat stress during flowering and pod-filling stages.
2. Using the unique phenotyping facilities drought stress will be imposed during flowering and pod-filling stages to ascertain the impact of stress on yield and quality

Progress of Work

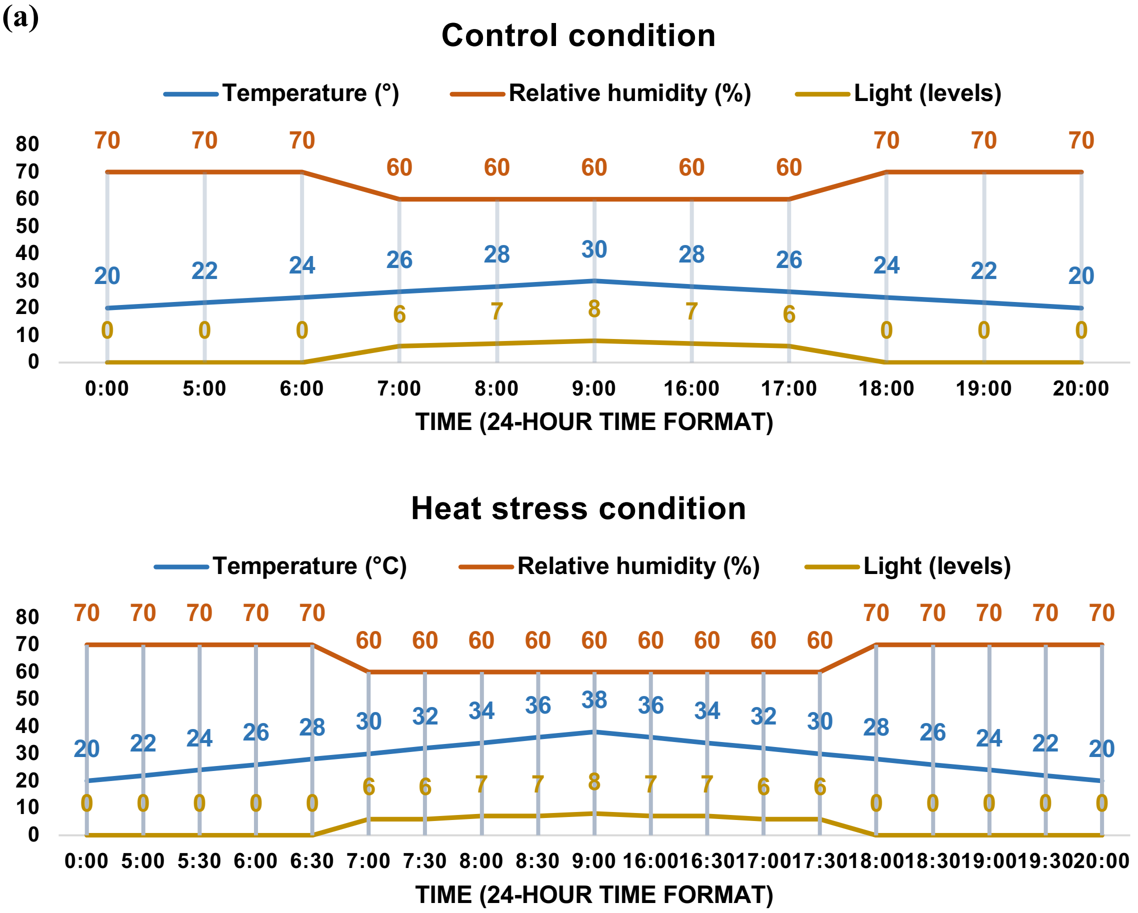
To achieve these objectives, we mainly focused on quantifying wild type (WT) and three transgenic lines overexpressing AtGRXS17 gene response to long duration heat stress during flowering and pod-filling stages. In this study, wild type and three stable soybean transformants (1035, 1039, 1041) carrying the overexpressing AtGRXS17 gene were exposed to 6 weeks of high day-time temperatures of 38°C/20°C (day/night) from 9 am to 4 pm, from start of flowering [R1] to physiological maturity [R8]) and a set of plants maintained under control temperature 30°C/20°C (day/night), using large walk-in growth chambers. Chambers conditions (air temperature, relative humidity and light levels) are shown in the Figure 1(a). To check leaves temperature of the plants during the stress thermal camera FLIR ONE Pro LT (FLIR Systems Inc., Portland, OR. USA) was used and plants images were captured from equal distance under control and heat stress condition (Figure 1(b)). The data were analysed using FLIR tools software version 2.0.2 (FLIR Systems). This allows appropriate scaling of the leaf temperature measurements for control and heat stress conditions. After giving 6 weeks of heat stress treatment yield (per plant) and yield related parameters were taken (Figures 2, 3 and 4).

Results

Using thermal imaging we found that the leaf temperature ranges from 28-29°C for control and 35-36°C for heat stressed plants. After observing plant morphology it was found that heat stress plants have less number of pods at the top of the main stems (late flowers) as shown in the figure 2. Further we found presence of wrinkled-shape seeds due to heat stress in the WT and the transgenic lines (figure 3). Seed weight per plant is also found to be decreased due to heat stress in all plants WT and transgenics.

Figures

(a)



(b)

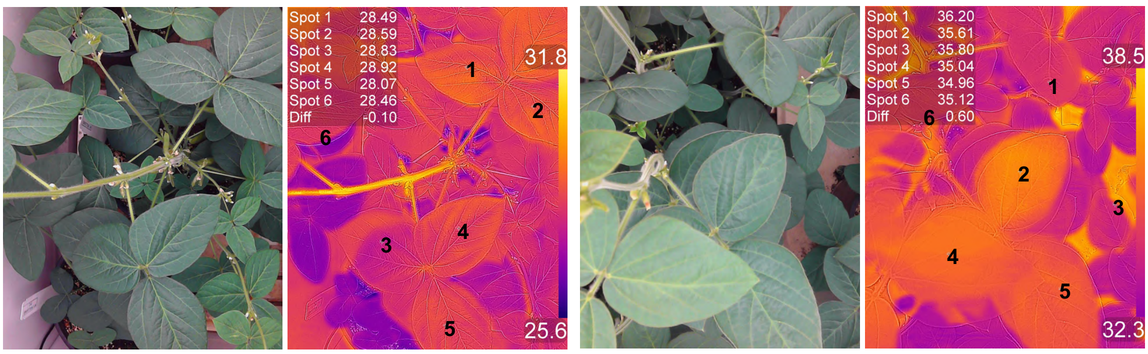


Figure 1: (a) Figure showing temperature (°C), relative humidity (%) and light (light levels) used for control and heat stress condition in temperature controlled chambers. (b) Figure showing thermal images of soybean plants under control and heat stress during the day (picture taken after 18 days of heat stress). Temperature at six different spots on the soybean leaves are shown in the figure.

Figure 2: Morphology of three AtGRXS17-transformed lines (1035, 1039 and 1041) and wild type plants post-heat stress treatment. Labels used for wild type control condition (WT_C) AtGRXS17-transformed lines control condition 1035_C, 1039_C and 1041_C and heat stress wild type plants WT_HS and AtGRXS17-transformed lines 1035_HS, 1039_HS and 1041_HS. Rep 1 is presented here with all the other replicates being highly uniform.

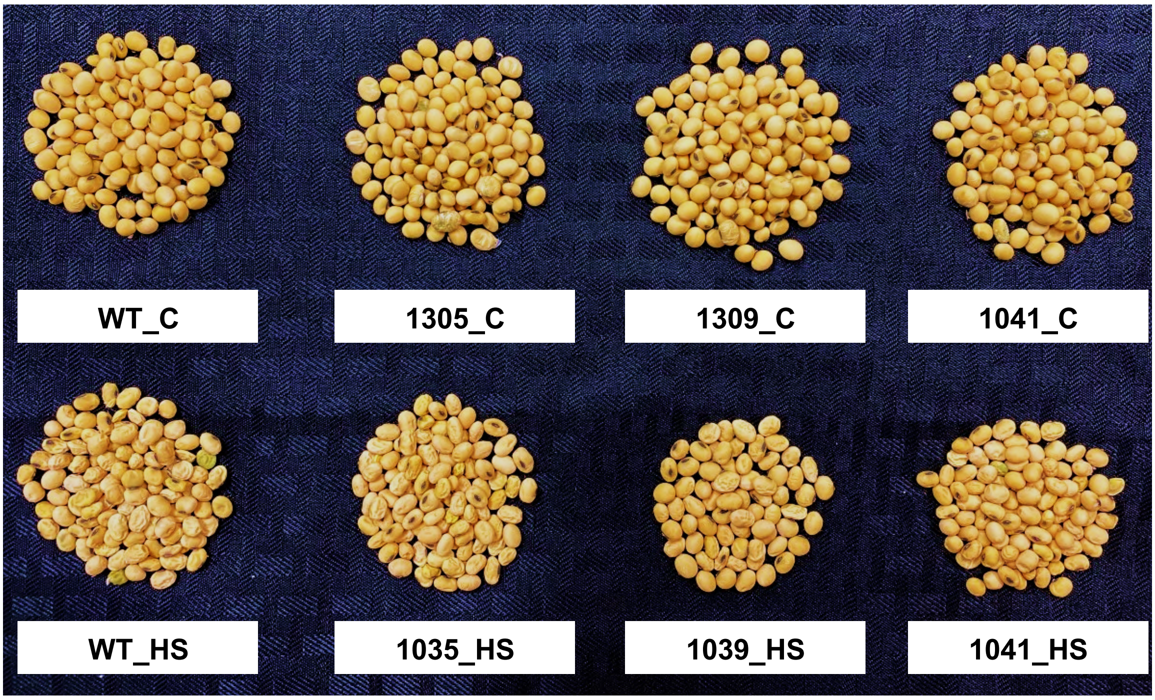


Figure 3: Seed morphology of wild type (WT) and ATGRXS17 transgenic lines (1305, 1309 and 1041) under control and heat stress.

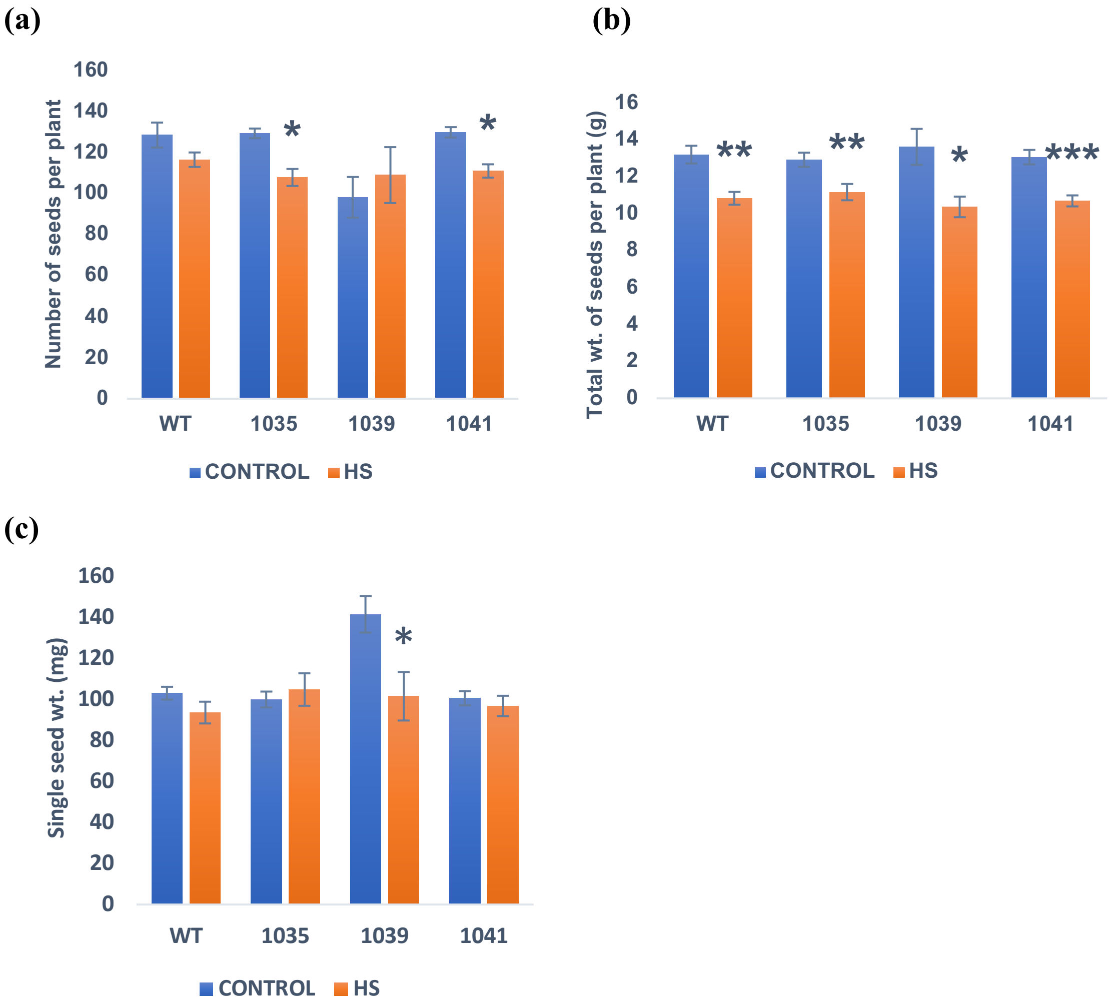


Figure 4: Graphs showing (a) number of seeds per plant, (b) total weight of seed per plant (in grams) and (c) single seed weight (in milligrams) in Control (C) and heat stressed (HS) of wild type (WT) and ATGRXS17 transgenic lines (1035, 1039 and 1041). Data are mean ± SE (n = 6 for each group). Statistical significance is calculated by applying unpaired t.test where *, **, *** represents p-value less than 0.05, 0.01 and 0.001 respectively.

Summary of results

- Heat stress imposed was captured through thermal imaging which indicated that the tissue temperature was close to the target stress temperature, consistently at different time points during the 6 weeks of stress exposure.
- The objective to determine the level of resilience to long duration heat stress encompassing both the flowering and the entire grain-filling duration was severe as both the wild type and the transgenics recorded similar levels of loss in seed numbers, morphology and yield per plant.

- However, among the transgenics 1039 had a numerical increase in number of seeds (11.03% over control; although non-significant), but the other two transgenics 1035, 1041 had a significantly reduction (16.62% and 14.50%, respectively) in number of seeds per plant.
- Although all the entries including the transgenics and the wild type had similar levels of decrease in seed weight per plant, 1035 (13.54%) and 1041 (18.13%) had no significantly reduction on single seed grain weight, while wild type had 9.16% and a highly significant reduction of 28.22% was observed in the transgenic 1039.
- The morphology of the formed grains was highly shrivelled equally across the wild type and the transgenic lines (Fig. 3) and so no clear distinction could be derived.

Current planned experiment

- To ensure a more comparative and differentiable response, the same set of lines will be exposed to the same stress conditions but for different durations i.e. for 2 weeks from the start of flowering and 2 weeks after flowering till physiological maturity. The aim would be to delineate the responses for heat stress in transgenics in their ability to retain seed number (for example in 1039) and seed weight (1035).
- Simultaneously, a lysimeter-based experiment will be initiated to determine the impact of drought stress on the ability of the transgenics to retain both grain numbers and grain weight from stress coinciding with flowering and grain filling, respectively
- Seeds from the presented experiment and the future heat and drought stress experiments, will be used to quantify protein and oil concentration using the NIR curves developed from a previous project supported by the Kansas Soybean Commission.