



Project Plan
FY2015
(Fiscal Years 2016-2017)

1. **Title:** A survey of abiotic stress tolerance variability in cotton germplasm
2. **Investigators:** Principle Investigator: Jane Dever, Texas A&M AgriLife Research
Co-Investigator: Paxton Payton, USDA-ARS-CSRL
Cooperator: Todd Campbell, USDA-ARS-CPRC
Cooperator: Reagan Anders, Texas Tech University
3. **Summary Abstract:**

Management strategies to reduce water withdrawals for irrigation and maintain agriculture industry viability and vitality of the Southern Ogallala Aquifer Region include developing crop cultivars that produce economic returns with less water. Knowledge to identify and develop profitable crop cultivars is needed now, as areas in the region transition to dryland cropping systems. We have initiated a phenotyping experiment using accessions from the National Cotton Germplasm Collection. An initial subset of 50 entries was screened for phenotypic characteristics that could be associated with differences to drought response, including taproot length, total root system dry weight, shoot dry weight, total dry weight and ratio of shoot dry weight to root dry weight, change in chlorophyll fluorescence yield. PI Dever has screened more than 400 accessions from the Texas race collection in seedling growth studies. Variability identified in various growth parameters provides information on contrasting phenotypes to be used for molecular tool and condition-specific cultivar development. Co-PI Payton and collaborator, Campbell, are currently screening multiple accessions and cultivars under field conditions. Initial trials were carried out at the Texas Tech University Quaker Research Farm with collaborator Anders to evaluate ten diverse genotypes of cotton *Gossypium hirsutum* species. These include six wild accessions and four cultivars. The accessions were characterized by phenotype using several qualitative and quantitative parameters of plant, leaf, flower, boll, and plant growth habit under irrigated and rain-fed conditions. Preliminary analysis of photosynthetic response to irrigation treatment was also performed and revealed significant differences in leaf-level gas-exchange among the selected genotypes. Based on the gas-exchange information, an acclimation effect was observed between the dry and irrigated trials in the months of June, July, August, and September. We extended evaluation of these selected genotypes, and added six more genotypes in 2014 with common seed source increased in 2013. Fifty-six crosses were made to initiate segregating populations for molecular analyses once field and greenhouse studies identify most appropriate contrasting phenotypes. The goal of this proposal is to develop the needed germplasm resources required for an integrated improvement program, and to identify a small subset of contrasting phenotypes that could practically be utilized in large-scale management studies targeted at best use of limited water resources, with cultivar response being one of the management factors.
4. **Project Narrative:**
 - a. **Objectives:** Our principal hypothesis is that identification of novel sources of germplasm and associated genes will allow for introgression of novel alleles for enhanced stress tolerance by conventional breeding or direct gene transfer. An integrated program, employing molecular, physiological, germplasm, and breeding resources affords the best chance for success in developing improved stress-tolerant cultivars. Project objectives are: 1) identify accessions with contrasting stress tolerance phenotypes by screening a wide-range of cotton germplasm for physiological, morphological, and phenological responses to water-

deficit and thermal stress and 2) create breeding populations segregating for abiotic stress tolerance, and 3) identify/develop accessions with differences in water amount/timing response for large-scale water management research. The following assumptions underlie our work: 1) multiple sources of abiotic stress tolerance exist in cotton, but tolerance is quantitative, 2) physiological and morphological screening under relevant field conditions can uncover multiple methods of adaptation that can be missed by laboratory screening alone or by yield-based selection alone, 3) efficient production in the long-term will rely upon the cotton crop's ability to yield under decreased water availability and oftentimes critical heat stress, and 4) an integrated molecular, physiological, and breeding program for tolerance will give the greatest prospects for success.

- b. Rationale/Literature Review/Conceptual Framework: Water scarcity and the escalating effects from climate change (IPCC, 2001) are the main environmental factors limiting crop production worldwide and are likely to remain as the most critical barriers to food production in the future (Chaves et al. 2003; Flexas et al. 2006). Abiotic stresses, particularly water deficit and high temperature, account for more than a 50% reduction in yields and annual economic losses in excess of \$10 billion worldwide (Kramer 1980; Boyer 1982; Sharma and Lavanya, 2002). Compounding reduced yields is growth in world population, expected to exceed 8 billion by 2030, requiring a significant increase in world food production (FAO 2006; Lobell et al., 2008). Most arable land is already under agricultural production which accounts for more than 75% of global freshwater consumption, a rate that is unsustainable with an increasing population (Shiklomanov and Rodda, 2003; Pimentel et al., 2004; Pfister et al., 2011). Achieving sustainability in global agriculture will ultimately be determined by the ability to provide adequate water resources to grow crops – whether through suitable and timely rainfall, efficient irrigation application, and/or *highly stress tolerant varieties for production under these limited water scenarios*.

More than 90% of cotton's economic value resides in the fiber. Drought reduces yield and fiber quality, costing producers millions of dollars in revenues each year. Water availability has a direct effect on plant growth and development and is the primary limiting factor to productivity in the majority of U.S. cotton growing regions. Vegetative biomass (leaf area index) decreases as water availability becomes limited. Ultimately, this leads to a reduction in photosynthetic capacity (Jackson and Gerik, 1990; Morrow and Krieg, 1990). Though plants with fewer bolls can compensate to some degree producing larger bolls, the number of bolls per unit area is the most significant yield component (Wells and Meredith, 1984). Arid conditions during boll and fiber development also affect fiber quality characteristics such as fiber maturity, length, strength, and micronaire (Guinn and Mauney, 1984a, b; Antony and Kutty, 1975). Erosion of genetic diversity in breeding germplasm (Meredith 2000), further exacerbated by intensive selection pressures under irrigated conditions, has inadvertently decreased genetic variability for drought resistance (Rosenow *et al.* 1983). Additionally, Helms (2000) reports increased yield variations over recent decades and attributes this to the use of few genetic backgrounds for breeding. Despite this trend, considerable variation persists for resistance to drought (Yakir *et al.* 1990; Saranga *et al.* 1998) and genetic mapping identified 33 drought-related QTL that influence plant productivity only in water-limited treatments (Saranga *et al.* 2001, 2004). Although genetic diversity within cultivated species of cotton is narrow, there exists considerable diversity in wild accessions; and in the case of *G. hirsutum* and *G. barbadense* across species that may be exploited for improvement of yield and stress tolerance (Rosenow et al., 1983; Brubaker and Wendel, 1994; Small 1999). These results hold promise for breeding efforts via introgression.

In semi-arid cotton production regions of the Southwestern U.S., insufficient groundwater replenishment, increasing salinity of irrigation water, and energy costs for pumping imperil long-term viability of production. In years of adequate rainfall, rain-fed cotton lint yields on

the Texas High Plains often exceed 780 kg ha⁻¹, while in years of inadequate rainfall yields drop below 475 kg ha⁻¹, a 40% reduction (NASS, 2010). While efficient irrigation technologies help to reduce the gap between potential and actual yield, there are clear limits to stimulating agricultural productivity using existing water resources. The majority of growers in the southern Ogallala currently have 200-400 gpm well capacities, allowing for maximum daily applications of 0.6-1.3 inches and irrigation intervals between 9 and 14 days. Therefore, the sustainability of irrigation as a yield-increasing practice is questionable at best. This is a paradigm change that shifts the research focus on improving crop productivity under water-limited conditions toward improvements in germplasm and the development of alternative crops. Furthermore, while significant gains can be made through improved crop management strategies, water delivery, crop models, and tillage practices, ***the critical need is for significant improvement in germplasm to fit new paradigms of low-input production.***

Development of drought-tolerant cotton is handicapped at several levels: 1) there is little understanding of the genetic mechanism for drought tolerance; 2) selection for fiber yield has unintentionally narrowed genetic variability for drought tolerance and water-use-efficiency; and 3) information and resources related to drought tolerance are derived mainly from model systems that are first and foremost, not necessarily drought-tolerant species, and secondly, studies are conducted under artificial conditions that do not easily translate to crop plants under field conditions.

- c. Proposed Activities: Sixteen genotypes, with common seed source (all increased during the same experiment in the greenhouse during 2013) were extensively phenotyped during 2014 (and 2015) over time, with the same 10 plants/plot measured weekly from squaring to boll development cessation. Four-row plots were arranged in a randomized complete block with 4 replications and two water treatments; non-destructive phenotyping was conducted in the center two rows, and the outer rows were reserved for destructive phenotyping. Yield and quality were measured from the center two rows. Following 2015 field trials, data will be analyzed, and a subset of genotypes selected that exhibit differences in response to water over time. Seed will be increased of these genotypes so additional water parameters (amount and timing) can be included in the experimental design. Fifty-six cross combinations among phenotyped accessions have been completed. Field data will be used to select parents representing contrasting water response. F₁ seed of crosses from those parents will be increased to initiate breeding tool and cultivar development trials.

Field Trials: Four contrasting accessions, representing water response differences expected among commercial cultivars, and four water treatments with different amounts/timing of application, will be arranged in a factorial design with four replications. Data collected over two years will include weekly assessments of plant height, square, flower, and boll initiation and retention, node development; and monthly chlorophyll fluorescence ratio readings and comprehensive plant mapping. Detailed temperature, rainfall, and soil moisture data will be kept at each location, and chlorophyll fluorescence ratio readings and plant mapping will be measured at 4-week-intervals during the growing season. Yield, agronomic properties, and fiber quality will be determined using established standard, small-plot harvesting and sampling methods.

Breeding Population and Tool Development: Crosses from selected parents will be increased in the greenhouse to provide enough F₂ seed to characterize ~500 individuals. Depending on space limitations and number of crosses selected, individual plants will be characterized in greenhouse tube experiments, or grown in the field under supplemental irrigation. Leaf samples will be collected for DNA extraction, and seed will be harvested from each plant. Seed of each plant will be divided and progeny rows planted under two different water regimes, to validate differences in water response for productivity and fiber development. Data from F₂ plants and F₃ progeny rows will be available to use for tool development. Selections will be made in the progeny rows showing most desirable water

response to advance development of distinct, uniform, and stable breeding lines to be used in breeding cultivars improved for limited irrigation and dryland farming systems.

Timelines:

Fall 2015	Increase accession seed (greenhouse)	Select parents for segregating populations	Plant F ₁ seed of selected populations (greenhouse)	Harvest and process 2015 field trial
Spring 2016	Analyze 2014 and 2015 field and greenhouse data	Select subset of accessions for field trials	Harvest seed increase of selected accessions; plant field trials	Harvest F ₂ seed and plant segregating populations
Fall 2016	Extract DNA from F ₂ plants	Complete phenotyping and harvest field trials	Analyze preliminary data	Present preliminary results (poster)
Spring 2017	Analyze greenhouse and field trial data	Repeat seed increase	Repeat field trial	Plant F ₃ progeny rows
Fall 2017	Harvest large-scale field trials	Harvest and sample F ₃ progeny rows	Analyze phenotype data	Present results (full paper)
Spring 2018	Complete F ₃ validation trials	Plant F ₄ progeny rows	Prepare manuscripts	Submit final report to OAP

- d. Expected Outcomes: We will provide comprehensive data concerning the stress-tolerance phenotype of public cotton germplasm to a newly constructed stress response database. Selected materials will be identified for trait introgression through conventional and marker-assisted breeding to improve cotton tolerance to water deficit and thermal stress. We will conduct grower-scale commercial test plots at the CSRL, Texas Tech Quaker Avenue Research Farm and Texas A&M AgriLife at Lubbock and host field days to educate growers and inform industrial partners of our latest research findings. All screening data will be deposited in the Cotton Heat and Drought Stress Database currently being developed by Locy, Campbell, and Payton. Water availability is the most limiting factor in agricultural and cotton production, and this drives the desire to commit grower resources to irrigation as a means of increasing production. Improvements in abiotic stress tolerance characteristics of this important economic crop will have lasting impacts on the U.S. economy. Data differentials between water treatments will be a valuable tool to assess economic impacts.

5. Relevant Publications:

Hinze, L. L., **J. K. Dever**, and R. G. Percy. 2012. Molecular variation among and within improved cultivars in the U.S. Cotton Germplasm Collection. *Crop Science* 52(1): 222- 230.

Eng, E. H., K. Jernigan, W. Smith, E. Hequet, **J. K. Dever**, S. Hague, and A. Ibrahim. 2013. Stability analysis of upland cotton in Texas. *Crop Science* 53(4): 1347-1355.

Niu, G., D. Rodriguez, **J. K. Dever**, and J. Zhang. 2013. Responses of five cotton genotypes to sodium chloride and sodium sulfate saline water irrigation. *Journal of Cotton Science* 17(3): 233-244.

Zeng, L., W. R. Meredith, B. T. Campbell, **J. K. Dever**, J. Zhang, K. Glass, A. Jones, G. Myers, and F. Bourland. 2014. Genotype X environment interaction effects on lint yield of cotton cultivars across major regions of the U. S. cotton belt. *Journal of Cotton Science*

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Kothari, N., **J. K. Dever**, S. Hague and E. F. Hequet. 2014. Evaluating intra plant cotton fiber variability. *Crop Science* 55(2):564-570.

Hinze, L., P. Horn, N. Kothari, J. Frelichowski, **J. K. Dever**, K. Chapman, and R. Percy. 2014. Non-destructive measurements of cottonseed nutritional trait diversity in the US National Cotton Germplasm Collection. *Crop Science* 55:770-782.

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Guin, G. and Mauney, J. 1984a. Fruiting of cotton. I. Effects of plant moisture stress on flowering. *Agron. J.*, 76:90-94.

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