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MOLECULAR BIOLOGY AND PHYSIOLOGY

Assessment of Cotton Leaf and Yield Responses to Water-Deficit Stress During Flowering and Boll Development

John Burke* and Mauricio Ulloa

ABSTRACT

Rainfall future events are predicted to decline to 30 to 127 mm in the majority of counties of the Texas High Plains and Rolling Plains because of climate change. Cotton (*Gossypium hirsutum* L.) is the major crop grown on the High Plains of Texas, and the lower humidity associated with the predicted reduction in rain raises the possibility of increased vegetative water-deficit stress and reproductive dehydration stress. This study assesses the vegetative and reproductive developmental processes of commercial cotton cultivar-response following water-deficit stress, specifically during flowering and boll development. Cultivars showed a significant relationship between the leaf water-deficit stress levels during boll development and final seed cotton yields. However, the cultivar Phytogen 72 (PHY72) was an exception to this observation. PHY72 exhibited excellent leaf water-deficit stress tolerance yet had reduced seed cotton yields compared with the other cultivars evaluated. Genetic analysis of the sensitivity of the PHY 72 pollen suggested a maternal deficiency in the tapetum development of the PHY 72 pollen coat resulting in increased dehydration sensitivity. Structural differences in pollen coat development in two cultivars (PHY 72 and NM67) were observed under both scanning electron and transmission electron microscopy. Predicted reduced rainfall and higher temperatures in the future, may necessitate approaches to improve not only vegetation tolerance to stress but also reproductive tolerance both of which may be important for breeding the new generation of crops.

Texas produced 49% of the United States (US) upland cotton grown in 2016 (https://www.nass.usda.gov/Statistics_by_State/Texas/Publications/Current_News_Release/2017_Rls/tx_cotton_review_2017.pdf), with most of the Texas cotton coming from the High Plains region. The High Plains is a semi-arid environment with annual rainfall of 457 mm (http://www.worldclimate.com/climate/us/texas/plains). A recent study of climate change projections for the Texas High Plains and Rolling Plains reported that both the minimum and maximum temperatures across the study region in the future showed an upward trend, with the temperatures increasing in the range of 1.9 to 2.9°C and 2.0 to 3.2°C, respectively (Modala et al., 2017). Additionally, their results predicted a decline in rainfall within a range of 30 to 127 mm in the majority of counties across the study region (Modala et al., 2017). The predicted lower humidity associated with the reduced rain potentially elevates the possibility of increased vegetative water-deficit stress and dehydration stress to cotton pollen.

A recent study, using a bioassay (Burke, 2007; Burke et al., 2010) that allows the high-throughput evaluation of leaf water-deficit stress levels, reported genetic diversity among commercial cotton varieties when water-deficit stress was applied at flowering (Burke and Ulloa, 2017). They reported that field-grown cotton was irrigated with 5 mm per day via subsurface drip until flowering. Subsequently, half of the plants were maintained on 5 mm per day irrigation, while the other half received 2.5 mm per day. The cultivars studied showed relatively low stress levels as exemplified by efficiency of quantum yield stress-test values between 1.7 and 2.3 under the 5 mm per day irrigation regime. Reducing irrigation levels from 5 mm per day to 2.5 mm per day at flowering produced a range of stress-test levels (efficiency of quantum yield referred as chlorophyll fluorescence) from 0.28 to 0.54.

Pollen has been reported to be sensitive to elevated temperatures (Abdul-Baki and Stommel, 1995; Bajaj et al., 1992, Dane et al., 1991; Halterlein et al., 1980, Herrero and Johnson, 1980, Iapichino and Loy, 1987, Kakani, et al., Kuo, et al., 1981; Ledesma and Sugiyama, 2005; Matlob and Kelly, 1973; Prasad et al., 2006; Rao et al., 1992; Sakata et al., 2000; Weaver and Timm, 1989), and is the only plant organ that does not synthesize heat shock proteins in response to heat stress (Gagliardi et al., 1995; Hopf et al., 1992; Volkov et al., 2005). Exposure

J. Burke* and M. Ulloa, USDA Cropping Systems Research Laboratory, 3810 4th Street, Lubbock, Texas 79415.
*Corresponding author: john.burke@ars.usda.gov

to elevated temperatures during flower development also inhibits pollen formation and induces flower sterility (Fisher, 1975). Because of this, reproductive stress responses reported in the literature focus primarily on flower abortion and flower sterility (Percy et al., 2006). In an attempt to address the heat sensitivity of pollen, Burke and Chen (2015) investigated the enhancement of tobacco and cotton pollen heat tolerance through expression of an *Arabidopsis thaliana* heat shock protein 101 (AtHSP101) that is not normally expressed in pollen but is reported to play a crucial role in thermotolerance of vegetative tissues. Pollen from AtHSP101 transgenic tobacco and cotton lines exhibited significantly higher germination rates and much greater pollen tube elongation under elevated temperatures or after a heat exposure than non-transgenic plants. In addition, significant increases in boll set and seed numbers were also observed in transgenic cotton lines exposed to elevated day and night temperatures in both greenhouse and field studies (Burke and Chen, 2015). These findings suggested that heat stress injury to pollen could be alleviated, in part, by expression of HSP101 and/or possible additional gene expression interactions.

Exposure to low humidity poses an equally serious threat to cotton pollen viability. It was originally reported that most plant species cope with arid environments by releasing pollen from the anther in a partially dehydrated state (Heslop-Harrison, 1979). Later, Nepi et al. (2001) reported that several species, including the Malvaceae Family, had partially hydrated pollen. Burke (2011) investigated the hydration status of cotton (G*ossypium hirsutum* L.), another member of the Malvaceae Family, and reported that it too had partially hydrated pollen grains. Burke concluded that these findings have implications for gene flow from plant to plant that required further investigation. In a recent study, Burke (2016) utilized "red" and "green" pigmented cotton, in addition to gossypol glanded and glandless cotton cultivars, to study pollen movement in the field. Genetic diversity in outcrossing was observed among the six cultivars tested. Differences in outcrossing of 5 to 15% were observed under both irrigated and dryland production systems. Cotton cultivars with mature pollen sensitive to low humidity exhibited reduced outcrossing compared to cultivars that were less sensitive to low humidity.

The present study investigated the vegetative and reproductive water-deficit stress tolerance of commercial cultivars. New insights into the disconnect between stress tolerances of leaves and reproductive stress tolerances will be provided.

METHODS AND MATERIALS

Crop Management: 2013. The soil type was an Amarillo fine sandy loam, and the fields were located in Lubbock, TX. Four 15 m rows of Nitro44, Phytogen 375, STV 4946GLB2, All-Tex_Epic RF, FM 8270GLB2, All-Tex_Edge, NexGen 1511B2RF, DP 1212, Phytogen 367, Phytogen 499WRF, FM 2011GT, Phytogen 72 and DP 1219B2RF cotton were planted in a North-South orientation per replication of a randomized complete block design on Day of Year 169 (18 June 2013) using a John Deere 7300 MaxEmerge 2 VacuMeter Planter. The field was part of an annual sorghum-cotton rotation. The plots were pre-plant irrigated by furrow irrigation and the plots subsequently irrigated with sub-surface drip. All plants received 5 mm/day from sub-surface drip in the furrows on 80-inch centers until initiation of flowering. Irrigation was then reduced to 2.5 mm/day on half of the plots (four replicates), while maintaining the 5 mm/day irrigation on the remaining plots (four replicates).

Stress Test Bioassay. A 1-cm^2 leaf punch was harvested from a source leaf (in cotton this is the fifth main stem leaf from the top) using a leaf punch. This was repeated on five separate plants per plot. The punches were transferred to a well in a Costar® 3524 24-well cell culture cluster (Corning Inc., Corning, NY) that had been half filled with water. The lid was returned to the cell culture plate immediately following addition of the leaf punches. This process was repeated until samples from all treatments had been harvested.

In the laboratory, the punches were placed on moistened Model 583 Gel Dryer Filter Paper (Bio-Rad Laboratories, Hercules, CA) in a Pyrex baking dish. The leaf punches and filter paper were covered with Glad® ClingWrap [CO_2 permeable] (The Glad Products Company, Oakland, CA) and pressed flat with a speedball roller for Microseal film (MJ Research, Inc., Waltham, MA) to remove air bubbles and ensure good contact between the tissue and filter paper. Initial chlorophyll fluorescence yield of quantum efficiency (Fv/Fm') levels of Photosystem II were determined using an Opti-Science OS1-FL Modulated Fluorometer and then samples were placed in the dark in a VWR Model 2005 incubator (Sheldon Manufacturing, Inc., Cornelius, OR) set to 39°C. The samples were heat treated for 30 min at 39°C, and then removed from the incubator and placed on the bench top at 25°C for 30-min. The

decline in fluorescence yield (Fv/Fm') was used as a relative measure of the stress level of the plant (a slight decline occurring in tissue from very stressed plants, and a greater decline occurring in tissue from less stressed plants).

Reproductive Structure – Pollen. Phytogen 72 (PHY72) and New Mexico 67 (NM 67), F1 PHY 72 x NM 67, NM 67 x PHY 72 developed populations were used to investigate the reproductive structure of pollen under electron and transmission microscopy. Additionally, the time course of pollen swelling and rupturing (popping pollen assay) in 0.8 M sucrose was determined. Traditional breeding crossing nomenclature was used to identify maternal (first) x paternal (second) derived progeny or populations. The popping pollen assay protocol is as follows: three to four drops of 0.8 M sucrose were placed onto a glass microscope slide. Cotton flowers were harvested following pollen dehiscence, the petals bent down toward the stem, and the anthers dipped into the sucrose drops. A timer was started, and a coverslip was placed over the sucrose drops. The slide was placed on a Leica MDG28 binocular microscope equipped with a DFC420 camera. The pollen grains were monitored at a magnification allowing 50 to 100 pollen grains to be in the field of view. The time for the first pollen grain to rupture and release the cytoplasm into the sucrose solution was determined.

Cotton Harvesting. When the cotton cultivars averaged >60% open bolls the plots were sprayed with Ginstar (Bayer CropScience, RTP, NC) and Prep (Bayer CropScience, RTP, NC) according to manufacturers' instructions on Day of Year 280, and the plots were harvested on Day of Year 305. Plots were harvested with a John Deere 484 two-row plot stripper and evaluated for yield. Seed cotton weights per row of plot were measured for eight replicates per line per treatment.

Experimental Design and Statistical Analysis. The field experiment used a randomized complete block design with four replications. Statistical significance between genotypes and treatments were analyzed with studentized t-test through the statistical applications of Social Science Statistics (http://www.socscistatistics.com/tests/Default.aspx). Graphs were created using KaleidaGraph Version 4.1.3 (Synergy Software, Reading, PA).

Meteorological Measurements. The USDA - PSWC Meteorological Tower is located immediately adjacent to the experimental plots. Five-minute measurements of temperature (C) were collected and hourly averages calculated.

RESULTS

Table 1 provides the efficiency of quantum yield (Fv/Fm') values for thirteen commercial cotton varieties grown under differential irrigation from initiation of flowering throughout boll development. The water-deficit stress treatment was a 50% reduction in the amount of water applied through the subsurface drip system. The varieties grown under irrigation applications of 5 mm/day had low efficiency of quantum yield values associated with well-watered cotton ranging from 0.167 to 0.226. NexGen 1511B2RF had the lowest efficiency of quantum yield of 0.167 and AllTex Edge exhibited the highest efficiency of quantum yield value of 0.226. When irrigation was reduced to 2.5 mm/day a subsequent increase in efficiency of quantum yield values was observed for all varieties tested (Table 1). The varieties grown under irrigation applications of 2.5 mm/day had higher efficiency of quantum yield values ranging from 0.299 to 0.531 typical of water-deficit stressed cotton. DeltaPine 1219B2RF had the lowest efficiency of quantum yield of 0.299 and Phytogen 367 exhibited the highest efficiency of quantum yield of 0.531.

Table 1. Efficiency of quantum yield values and standard errors of leaf tissues following a 30 min 39 °C treatment and a 25 °C recovery period for commercial cotton receiving either 2.5 mm/DAY or 5 mm/DAY subsurface drip irrigation during flowering and boll set. Values provided are average Fv/Fm' readings with standard errors of 4 replications

VARIETY	2.5 mm/DAY Fv/Fm	5 mm/DAY Fv/Fm
Phytogen_367	0.531 ± 0.032	0.197 ± 0.017
All_Tex_Edge	0.464 ± 0.053	0.226 ± 0.011
All_Tex_EpicRF	0.452 ± 0.031	0.214 ± 0.020
DP_1212	0.440 ± 0.020	0.211 ± 0.010
Phy_499WRF	0.426 ± 0.020	0.217 ± 0.011
Nitro44	0.400 ± 0.035	0.179 ± 0.008
ST4946GLB2	0.387 ± 0.029	0.210 ± 0.011
FM_8270GLB2	0.375 ± 0.021	0.182 ± 0.010
Phytogen_375	0.364 ± 0.022	0.188 ± 0.018
NG_1511B2RF	0.335 ± 0.021	0.167 ± 0.008
FM_2011GT	0.312 ± 0.014	0.203 ± 0.110
Phytogen_72	0.301 ± 0.021	0.202 ± 0.014
DP_1219B2RF	0.299 ± 0.031	0.196 ± 0.100

The relationship of the efficiency of quantum yield values provided in the leaf stress bioassay and seed cotton yields is shown in Figure 1. Twelve of the commercial lines exhibited a significant relationship (R^2=0.74) between the vegetative water-deficit stress bioassay value during boll filling and the final yields harvested. The Phytogen 72 variety had excellent vegetative stress tolerance as exhibited by the value of 0.301 in the 2.5 mm/day treatment (Table 1), but, had low seed cotton yields (Figure 1) compared with the vegetative stress tolerant varieties DeltaPine 1219B2RF and FM2011GT.

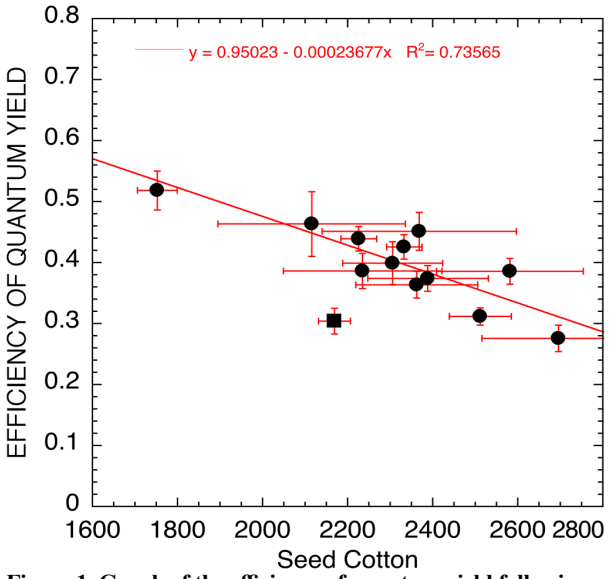

Figure 1. Graph of the efficiency of quantum yield following a 30 min 39 °C treatment followed by 30 min 25 °C incubation compared to seed cotton yields. Cultivars evaluated included ALL TEX EDGE, ALL TEX EPIC RF, DP 1212, FM _2011GT, FM 8270, NEX GEN 1511, NITRO 44, Phy 367, PHY 375, PHY 499, STV 4946, NEX GEN 4111 (closed circles) and PHY 72 (closed square). Irrigation treatment was 2.5 mm/ Day. Error bars represent standard errors of 4 replications.

Some potential causes for the observed yield reduction in Phytogen 72 include sensitivity of pollen development, issues associated with mature pollen, or shedding of bolls in response to the imposed water-deficit stress. Pollen development and pollen viability are sensitive to elevated temperatures that can result in flower sterility. Average daily air temperatures in Lubbock, Texas in 2013 (Figure 2) reached 30 °C in July; however, the temperatures in August and September only averaged 25 °C suggesting a favorable thermal environment for pollen development. Observation of hundreds of Phytogen 72 flowers revealed normal pollen dehiscence (Figure 3) under the reduced irrigation. We next evaluated mature pollen structure under scanning electron and transmission microscopy. Figure 4 contains scanning electron micrographs from New Mexico 67 – NM67 (A, B) and Phytogen 72 (C, D) pollen. Clear differences in the pollen coat are apparent. The NM 67 pollen had a relatively smooth pollen coat, while the Phytogen 72 pollen had a rough surface with numerous breaks in the outer coating. This may explain, in part, why Phytogen 72 pollen swells more rapidly in 0.8 M sucrose than NM 67 pollen. Figure 5A and 5B are photomicrographs of Phytogen 72 pollen placed in 0.8 M sucrose. Figure 5A shows the pollen immediately after addition to the sucrose solution. Figure 5B shows two pollen grains rupturing in the upper left-hand corner of the photograph 1-min after exposure to the sucrose solution. Figure 5C shows NM 67 pollen 2-min after exposure to the sucrose solution, and Figure 5D shows NM 67 pollen 3-min after exposure to the sucrose solution. A pollen grain rupture is apparent in the upper center of Figure 5D following the 3-min exposure. It is interesting to note that particles of the pollen coat are being expelled from the NM 67 surface as the pollen grain swells (arrows Figure 5C). The phenomenon was not observed in the Phytogen 72 pollen treatment (Figure 5 A, B) even with prolonged sucrose exposure. Further analyses with transmission electron microscopy revealed significant differences in the pollen coats of NM 67 (Figure 5E) and Phytogen 72 (Figure 5F). The NM 67 extracellular lipidic matrix is seen pulling away from the exine surface and little material remains in the interstices of the exine (Figure 5E). Phytogen 72 pollen, on the other hand, has a tightly bound extracellular matrix on the surface of the exine and within the interstices of the exine (Figure 5F).

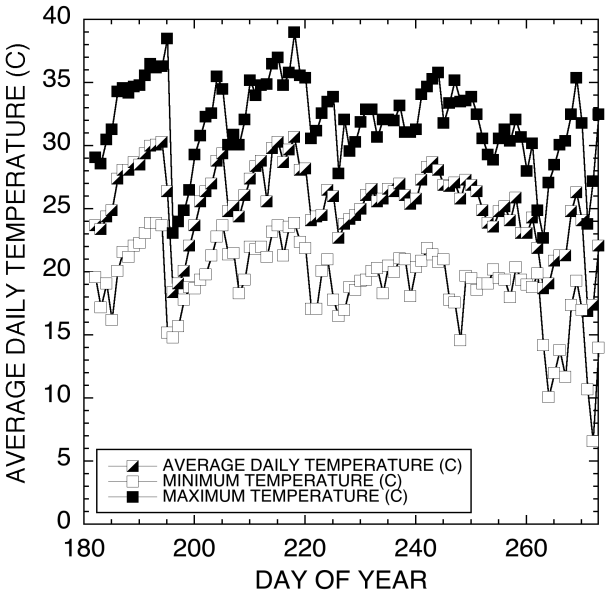

Figure 2. Graph of air temperatures in Lubbock, Texas during the 2013 growing season. Maximum air temperature = closed square, minimum air temperature = open square, and average daily temperature = open/closed square.

Figure 3. Photograph of a Phytogen 72 flower showing dehisced anthers with numerous pollen grains.

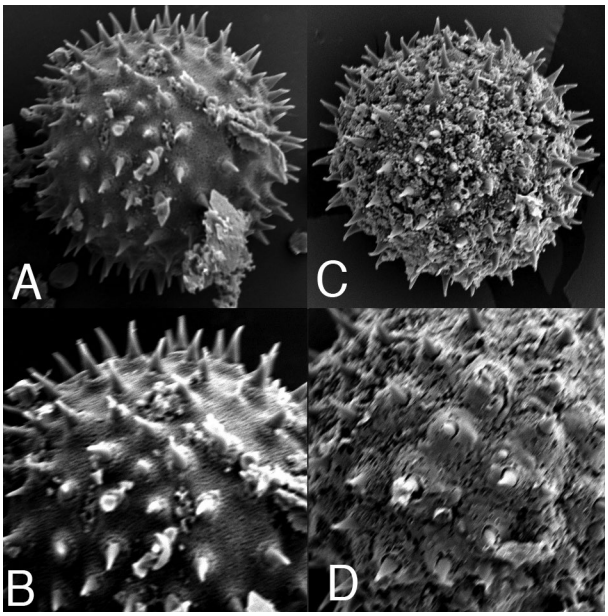

Figure 4. Scanning electron micrographs of NM 67 pollen (A, B) and PHY 72 pollen (C, D).

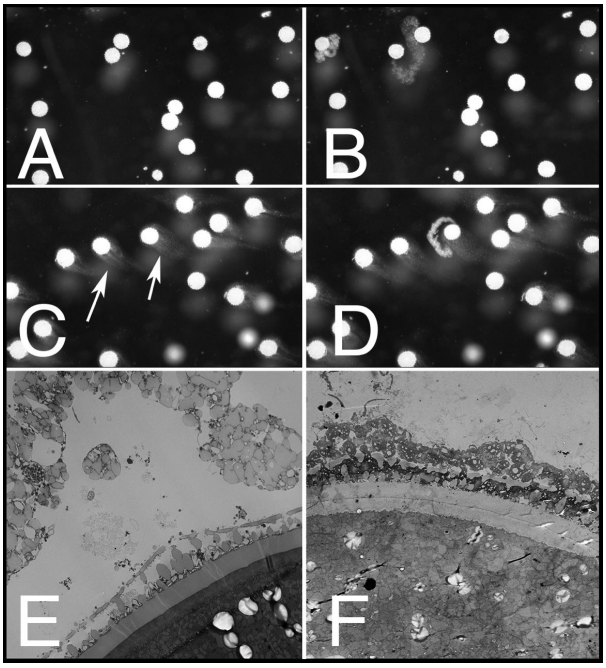

Figure 5. Photographs of PHY 72 pollen grains in 0.8 M sucrose (A=0 min, B=1 min) and photographs of NM 67 pollen grains in 0.8 M sucrose (C=2 min, D=3 min). Transmission electron micrographs of NM 67 (E) and PHY 72 (F) pollen grain cell walls and pollen coats.

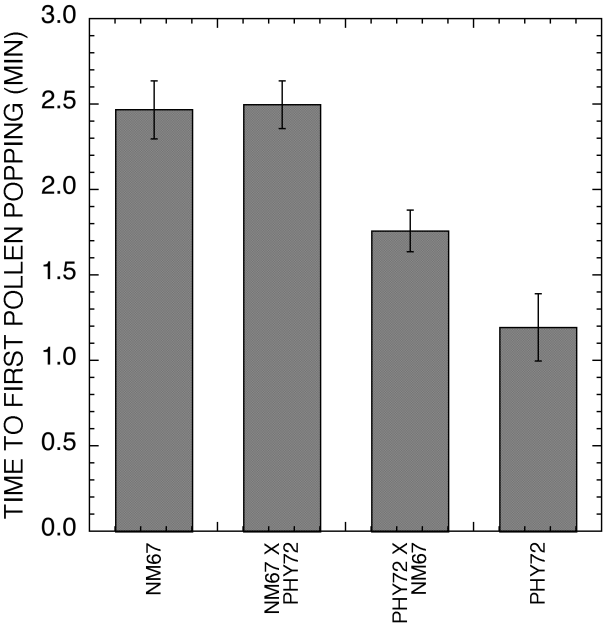

Figure 6. Graph of the time (min) to first pollen popping for NM 67, NM 67 X PHY 72, PHY72 X NM67, and PHY 72. Error bars represent standard errors.

The structural data (Figures 4 and 5) suggests a possible defect in the laying down of the pollen coat in the Phytogen 72 pollen. Because the pollen coat is derived from the tapetum (Piffanelli and Murphy, 1998) the defect in pollen coat development would be maternally inherited. To test the hypothesis that the Phytogen 72 has a defect in the tapetum transfer of pollen coat material we performed reciprocal crosses between NM 67 and Phytogen 72. F1 seeds were harvested and plants grown for pollen evaluation. We used the timing of the pollen rupturing in 0.8 M sucrose as an easy measure of pollen coat makeup. Figure 5 is a graph of the time to the first pollen grain popping for NM 67, NM 67 x PHY 72, PHY 72 x NM 67, and PHY 72. When NM 67 is the female parent, the offspring have average pollen popping times similar to the NM 67 parent. When the PHY 72 was used as the female parent, the offspring had average pollen popping times intermediate between the two parents. The data supports the hypothesis that there exists a defect in the way that the Phytogen 72 tapetum lays down the pollen coat.

DISCUSSION

The impetus for this study was an apparent disconnect between the water-deficit stress tolerance of cultivar Phytogen 72 measured via the vegetative bioassay described by Burke (2007, 2010) and the measured seed cotton yields obtained following a water-deficit stress during 2013 field study at the United States Department of Agriculture-Agricultural Research Services (USDA-ARS) Plant Stress and Water Conservation Laboratory, Lubbock, Texas. Other cultivars evaluated in this study exhibited a significant relationship (R^2=0.74) between the efficiency of quantum yield values from the stress-test bioassay and the final cotton yield (Figure 1). Phytogen 72 exhibited excellent leaf water-deficit stress tolerance (Table 1). In this bioassay, because of the prior 30 min heat treatment, the lower the efficiency of quantum yield (Fv/Fm), the lower the original stress level of the tissue.

'Phytogen 72' (PHY 72; PVP 200100115) is often included in germplasm trials as a high fiber quality check (Percy et al., 2006). PHY 72 derives from the cross of the cultivar 'Acala Prema' (PVP8800171) and Acala 1517D (National Plant Germplasm System, 2009). Percy et al. (2010) compared lint yields of cotton cultivar checks PHY 72, SG 747, FM 958 and FP 393 with that of CRB 252 in 13 tests conducted at Florence, SC; Blackville, SC; Tifton, GA; Plains, GA; Alexandria, LA; Maricopa, AZ; and Shafter, CA in 2007 and 2008. The PHY 72 had the lowest yields across these environments with an average lint yield of 1336 kg ha^{-1} compared with the 1632 kg ha^{-1} for DP 393. In the present study, the reduced cotton yields despite the low vegetative water-deficit stress level of 0.301 identified by the efficiency of quantum yield bioassay was unexpected. The results of this study also showed reduced cotton yields compared with other commercial cultivars (Figure 1).

Potential reasons for the reduced PHY 72 yields included stress-related issues associated with pollen development, mature pollen viability, or stress-related boll shedding. Percy et al. (2006) evaluated pollen sterility due to heat stress at the Maricopa Arizona location in 2004. They used a rating scale of 1 = 100% fertility to 5 = total sterility. Averaged across three dates, lines AGC85, AGC208, and AGC375 exhibited pollen sterility rates (1.8, 2.0, and 1.8, respectively) higher than that of SG747 (1.3) but significantly lower than those of the high fiber quality cultivars Fibermax 958 (2.8) and Phytogen 72 (2.7). High temperatures can affect different stages of the reproductive process, such as pollen formation, pollen germination, and subsequent fertilization, as well as ovule longevity, resulting in boll shedding and yield reduction. Reddy et al. (1991) reported that most squares and flowers in cotton plants were aborted when day/night temperatures were above 30°C/20°C for 13 h. Clearly PHY 72 exhibited high temperature-induced sterility in the Arizona study as evidenced by the pollen sac nondehiscence (Percy et al., 2006). Average air temperatures in Lubbock, Texas during the 2013-growing season (Figure 2) seldom reached 30°C and the plants did not experience temperatures at which pollen development would normally be impaired. PHY 72 pollen dehiscence was observed in the 2013 study (Figure 3) therefore we decided to analyze the mature pollen released by the plant.

The tapetum is a single layer of secretory cells that encloses the anther locule and sustains pollen development and maturation (Rejón et al., 2016). Upon apoptosis, the remnants of the tapetal cells fill the pits of the sculpted exine to form the bulk of the pollen coat. This extracellular matrix forms an impermeable barrier that protects the male gametophyte from water loss and UV light (Rejón et al., 2016). The pollen coat both prevents further water

loss from dehydrated pollen grains during their brief autonomous existence and facilitates the uptake of water once the pollen grain lands on a receptive stigmatic surface (Piffanelli et al., 1998). Wu et al. (1997) described a novel class of organelles, which they term tapetosomes. Murphy and Ross (1998) characterized the biosynthesis, targeting and processing of some of the major protein components of the pollen coat, or tryphine, of *Brassica napus*. They described a targeting mechanism for the delivery of tapetosomes from the tapetum to the pollen wall and their subsequent 'dismantling' to form the pollen coat (Murphy and Ross, 1998).

Figure 4 shows scanning electron micrographs of NM 67 (Figure 4A, 4B) and PHY 72 (Figure 4C, 4D) pollen surfaces covered by the pollen coat. Structural differences between NM 67 and PHY 72 pollen surfaces are apparent from both the scanning electron micrographs (Figure 4) and the transmission electron micrographs (Figure 5). The PHY 72 pollen surface is rough, with numerous cracks in the pollen coat. Additionally, the pollen coat that is present remains tightly associated with the exine (Figure 5F) compared with the pollen coat of the NM 67 (Figure 5E). These structural differences may help to explain the differential rate of water uptake from a 0.8 M sucrose solution between NM67 and PHY 72 (Figure 5 A-D, Figure 6). The genetic analyses of the rapid pollen popping trait observed in PHY 72 showed pollen popping times of F1 plants where PHY 72 was the male parent were identical to the popping times for the NM 67 parent similar to Mendelian dominance gene action. Additionally, when the PHY 72 was the female parent, the F1 offspring exhibited more rapid pollen popping times similar to those of PHY 72 (Figure 6) also known as similar to Mendelian intermedium to recessive gene action. These findings support the hypothesis that PHY 72 has a defect in the development of the pollen coat that may be associated with the functioning of the tapetum during pollen development.

The increased sensitivity of the PHY 72 pollen to dehydration was first reported by Burke (2007) where *in situ* pollen dehydration of cotton pollen from NM67 and Phy72 cotton was evaluated following removal of the flower petals and a 6.5 h exposure of the dehisced anthers and pollen to a 25% relative humidity. In vitro pollen germination was then evaluated at 28C and 80% relative humidity according to the procedure described by Burke et al. (2004). The percent reduction in pollen germination of the low humidity treated pollen was compared with the germination of freshly dehisced pollen (control), and the Phy72 pollen showed a 46% reduction in pollen germination, while the NM67 pollen showed a 30% increase in pollen germination following the drying experiment. Burke (2016) investigated the impact of pollen sensitivity to low humidity on the amount of outcrossing to neighboring plants. NM67 had the greatest level of outcrossing with 15% in the dryland plot and 13% in the irrigated plots. PHY72 showed the lowest level of outcrossing with the dryland and irrigated plots showing 8 to 9% outcrossing. Burke (2016) concluded that cotton cultivars with mature pollen sensitive to low humidity exhibited reduced outcrossing compared to cultivars that were less sensitive to low humidity.

Developing cultivars with tolerance to the impact of water deficits or drought tolerance may be more difficult than originally thought because researchers and plant breeders must explore innovative ways to identify new traits and mine new genetic diversity that can alleviate lint-yield losses (Ulloa et al. 2007; Ulloa, 2014; Hinze et al. 2016). Like most crops, in cotton the focus is on selecting the highest yielding cultivar following with the best possible fiber quality. Physiological traits usually involve many genes, which are part of multiple pathways, making it difficult to directly relate these traits to yield. Due to these factors, identifying a small number of traits to target in a breeding program may be difficult. Currently there is a lack of information on a selection method or traits to further improve the drought tolerance of cotton.

CONCLUSION

This study evaluated commercial cotton cultivars responses to water-deficit stress during flowering and showed a significant relationship between the vegetative water-deficit stress levels and final seed cotton yields for all cultivars, except PHY72. Phytogen 72 was an exception as it exhibited excellent low vegetative water-deficit stress level of 0.301 identified by the efficiency of quantum yield bioassay, yet the variety exhibited reduced seed cotton yields compared with the other cultivars evaluated. Structural differences in pollen coat development in PHY 72 were observed under both scanning electron and transmission electron microscopy. Genetic analysis of the sensitivity of the PHY 72 pollen suggested a maternal deficiency in the tapetum development of

the PHY 72 pollen coat resulting in increased dehydration sensitivity. With the predictions of climate change, less rainfall and higher temperatures in the future, approaches to improve not only vegetative structures but also reproductive structures will be important for breeding the new generation of crops.

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DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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