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Evaluation of five peanut (*Arachis hypogaea*) genotypes to identify drought responsive mechanisms utilising candidate-gene approach

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Abstract. Drought can significantly limit yield and quality in peanut (*Arachis hypogaea* L.), depending on its timing, duration and severity. The objective of this study was to identify potential molecular mechanism(s) utilising a candidate-gene approach in five peanut genotypes with contrasting drought responses. An early season drought stress treatment was applied under environmentally controlled rain-out shelters. When water was completely withheld for 3 weeks, no physical differences were observed for treated plants compared with their fully irrigated counterparts as indicated by relative water content; however, yield, grades (total sound mature kernel, TSMK), specific leaf area, and leaf dry matter content showed significant differences. Comparing expression levels of candidate genes, 'C76–16' exhibited significantly higher levels for CuZnSOD, NsLTP and drought protein 1 week earlier compared to the other genotypes, followed by significantly lower levels for the same genes. This suggested an early recognition of drought in C76–16 followed by an acclimation response. Cultivar 'Georgia Green' showed different patterns of gene-expression than C76–16. AP-3, a susceptible genotype, showed generally lower levels of gene-expression than C76–16 and Georgia Green. *Myo*-inositol phosphate synthase gene-expression showed high levels in irrigated treatment, ranging from 4-fold for 08T-12 to 12-fold for Georgia Green, but were significantly inhibited in drought treatment after 2 weeks of drought and after recovery.

Additional keywords: abiotic stress, breeding, gene-expression.

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Introduction

Drought is a major abiotic stress that reduces yield and can lead to higher levels of aflatoxin infection in peanut (*Arachis hypogaea* L.). However, strong environmental responses make plant selection with consistent drought tolerance traits very difficult. Recent studies indicate that many major and minor quantitative trait loci (QTL) control drought tolerance in peanut (Ravi *et al*. 2011; Gautami *et al*. 2012); thus, it would take breeders many years to stack multiple drought tolerant mechanisms into a single plant. Different criteria have been utilised for selection of drought tolerant peanuts, including larger root system (Jongrungklang *et al*. 2011, 2012), physiological traits such as specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR) and harvest index (HI) (Girdthai *et al*. 2012), or nitrogen fixation (Pimratch *et al*. 2009). Because peanuts are grown in semiarid environments and usually under rain-fed conditions, multiple mechanisms of drought response may exist within the same plant.

Early-season or pre-flowering stage drought does not significantly reduce yield and quality, but rather, can lead to increased yields (Nageswara Rao *et al*. 1985; Puangbut *et al*. 2010; Jongrungklang *et al*. 2011). It is thought that the recognition of drought in the early stages of plant development can facilitate plant adaptation by the development of a larger and more extensive root system (Songsri *et al*. 2008) or induction of gene expression such as transcription factors (Dang *et al*. 2012) to activate a cascade of molecular responses. Because of the variations in levels and severity of drought, plants have evolved complex molecular signalling mechanisms. The precise timing and level of gene expression for specific genes orchestrate plant drought tolerance or adaptive strategies.

Several genes that are involved in different aspects of plant drought response have been identified. Early light-induced proteins (ELIPs) transcripts were shown to accumulate directly proportional to increasing light intensity and high temperature to the degree of photoinactivation (Hutin *et al*.

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2003; Pinto *et al*. 2011). Thioredoxin, a class of small proteins, acts as disulfide reductases to modulate redox status of many proteins in wide range of organisms (Meyer *et al*. 2009). Target proteins by thioredoxin have been implicated in different aspects of plant growth, development, and adaptation to environmental stresses (Meyer *et al*. 2012). The 70 kilodalton heat shock protein (HSP70) is a ubiquitous protein that is present in virtually all organisms and plays an important role to protect protein folding, especially from environmental stresses. Recently, HSP70 has been shown to be elevated to protect *Aloe barbadensis* (L.) Burm.f. from high temperatures (Huerta *et al*. 2013). Rubisco activase is a nuclear-encoded chloroplast enzyme that maintains Rubisco activity by active removal of sugar phosphates from Rubisco catalytic sites (Parry *et al*. 2008). Reduction in photosynthesis has been correlated with Rubisco inactivation due to inhibition of Rubisco activase under moderate heat stress **(**Kurek *et al*. 2007**).** Overexpression in transgenic rice showed that Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature (Yamori *et al*. 2012). Under environmental stresses, such as high temperature and drought, plants produce reactive oxygen species (ROS) that cause oxidative damage to cellular and genetic components. Superoxide dismutase (SOD), a Cu and Zn isoform, is an important antioxidant that scavenges (ROS) in plant cells and ectopic expression of CuZnSOD, along with ascorbate peroxidase, has been shown to protect potato from oxidative stress and high temperature (Kim *et al*. 2010). Plant non-specific lipid-transfer proteins (LTPs) are small, basic proteins that are able to transfer different lipids to other molecules and can bind to fatty acids. Several LTPs have recently been shown to confer resistance to low temperature and drought stress (Guo *et al*. 2013). Late embryogenic abundant (LEA) proteins have been identified in many plants and were associated with protection of cellular proteins under various stresses, including heat and drought stress. A LEA protein from pea seed mitochondria was shown to protect enzymes in drying (Grelet *et al*. 2005). Tonoplast intrinsic proteins (TIP) is a type of aquaporin that facilitates the transport of water and small molecules across biological membranes. A review by Zhao *et al*. (2008) talks about structure and function of aquaporins and how they are related to water stress. Drought protein was identified by a peanut transcriptome sequencing (Guo *et al*. 2009) that has homology to RD22 drought related gene from *Arabidopsis*. Metallothionein is a family of cysteine-rich and low molecular weight proteins with metal binding ability, providing protection against heavy metal toxicity and oxidative stress. Overexpression of *OsMT1e-P*, a metallothionein from rice, conferred multiple abiotic stresses through ROS scavenging (Kumar *et al*. 2012). *Myo*-inositol is an important signal molecule for many eukaryotic biochemical pathways, and *myo*-inositol phosphate synthase is a key enzyme in the *myo*-inositol biosynthetic pathway. Overexpression of *CaMIP2* in *Arabidopsis* enhanced tolerance to salt and drought stress (Kaur *et al*. 2013).

The goal of this research was to identify potential molecular mechanism(s) utilising peanut genotypes with contrasting drought responses and a candidate-gene approach. The identification of drought regulated genes, determination of their specific gene-expression patterns, and the association with the levels of drought tolerance will provide a basis for drought tolerant plant selection in peanut breeding programs.

Materials and methods

Plant selection and growth conditions

Two peanut cultivars and three advanced breeding lines were utilised for the study. Cultivar 'Georgia Green' (GG) (Branch 1996) had been the accepted agronomic standard and was planted on a majority of acres in the South-eastern United States (Georgia, Alabama, Florida) because of its moderate tolerance to drought in late 1990s and early 2000s. The more recently released cultivar 'AP-3′ (Gorbet 2007) showed a weak drought tolerance in the previous study (Dang *et al*. 2012). 'C76–16' and 'A-104' are advanced breeding lines that were identified as drought tolerant (Holbrook *et al*. 2009). '08T-12' was identified as drought tolerant from Auburn-USDA National Peanut Research Laboratory (NPRL) peanut breeding program under non-irrigated condition for drought tolerance in Brownfield, TX. Peanuts were planted in environmental controlled rain-out shelters (5.5 × 12.2 m) (Blankenship *et al*. 1989) in the middle of May in 2010 at NPRL, Dawson, GA, USA. Each genotype was planted in a single row plot of 5.5 m long and 0.76 m wide with three replications using seed rate of 20 seeds m^{-1}. The plots were irrigated before planting to provide uniform germination. At 10–14 days after planting (DAP), germinated seeds were counted for all plots to ensure consistent germination efficiency. Each genotype was arranged in split plot design, with irrigation treatment (main split: full irrigation, 30 DAP stress, and non-irrigation (rain-fed)) by genotype with three replications in 2010. All tested entries were considered to have similar maturity requirement, thus digging date was determined by the hull scrape method (Williams and Drexler 1981) of GG in each respective treatment block. Irrigation and agronomic management inputs were applied according to University of Georgia best management practices for peanut. Irrigation was based on evapotranspiration (ET) replacement method described by Stansell *et al*. (1976). Watermark moisture sensors (Irrometer, Riverside, CA, USA) were placed at 10 and 20 cm depths and read every 4th day. Plots were irrigated at –60 kPa, based on an average moisture reading of both 10 and 20 cm depths. For drought treated plots, no irrigation was applied for 3 weeks and then re-irrigated. Irrigated plots were fully-watered throughout the growing season.

Sample collection and physiological measurements

Samples were collected once a week for the entire period of the experiment: stage 1, 43 DAP; stage 2, 50 DAP; stage 3, 58 DAP; stage 4, 64 DAP; and stage 5, 71 DAP. Stage 1 was the start of the experiment with no drought treatment; stages 2–4 represent 3 weeks of drought treatment and followed by a recovery stage 5 after 1 week of re-irrigation. Fully expanded leaves were collected from the main stems of two randomly selected plants per 5.5 m row, put into individual plastic zip lock bags and placed on ice. For RNA analysis, samples were immediately frozen and stored at −80°C until processed. For physiological measurements, fresh leaves were weighed then were fully submerged in deionised water and placed under white light lamp for 2 h to ensure tissues become completely turgid.

Leaves were blotted dry and weighed. Leaf area was measured using LI-3100 area meter (Li-Cor Biosciences, Lincoln, NE, USA). Relative water content (RWC) was determined based on a formula (Barrs and Weatherley 1962): RWC (%) = ((FW – DW)/(TW – DW)) × 100, where TW is turgid weight. SLA is the ratio of leaf area to leaf dry mass and leaf dry matter content (LDMC) is the ratio of leaf dry mass to saturated fresh mass.

RNA extraction

Mini-prep RNA isolation was performed utilising Omni Bead Ruptor 24 (Kennesaw, GA, USA) in a 2 mL tube preloaded with 0.28 mm ceramic beads for cell disruption and extraction method described by Chomczynski and Sacchi (1987) with the addition of 1% polyvinyl polypyrrolidone (PVPP) in homogenisation solution. RNA was quantified using Nanodrop 2000c spectrophotomer (Thermo Scientific, Wilmington, DE, USA) and quality was evaluated through RNA gel electrophoresis (Sambrook *et al*. 1989). RNA was DNase-treated with Turbo DNA-free (Ambion, Austin, TX, USA) before reverse-transcription for cDNA synthesis.

Identification of candidate genes and primer design

Peanut transcriptome sequencing project identified candidate genes that may have a role in biotic stresses (Guo *et al*. 2009). DNA sequences analysis was performed by Sequencher ver. 5.0 (Gene Codes, Ann Arbor, MI, USA), and sequences were searched against NCBI databases (BLASTn and BLASTx) to identify potential gene function (Table 1). Primer Express ver. 3 (Applied Biosystems, Foster City, CA, USA) was utilised to design real-time PCR primers. A regression analysis was performed on Ct values generated from real-time PCR plotted against log-transformed dilution (1 : 2) series to determine primer efficiency.

Reverse transcriptase and real-time PCR

A total RNA samples of 1 µg was utilised as a template for cDNA synthesis utilising SuperScript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) in the presence of 50 µM oligo dT according to manufacturer's instructions. The reaction mix was incubated at 42°C for 1 h, heated to 85°C for 5 min to terminate the reaction, diluted 1 : 100 with 10 mM Tris pH 7.5, and stored at 4°C until use. Real-time PCR data was generated on an ABI 7500 real-time PCR utilising RT^2 SYBR Green qPCR Mastermix with ROX (Qiagen, Valencia, CA, USA). A 4 µL diluted cDNA was added to a 21 µL reaction mixture consisting of 0.4 µM of each primer and 12.5 µL of qPCR mastermix. PCR cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, and a dissociation curve analysis which consisted of 15 s at 95°C, 20 s at 58°C and 15 s at 95°C. For each sample, three independent reactions were performed.

Yield and grade

Peanut yield and grade characteristics were determined according to the USDA (1998) standard method and yield was reported after moisture was adjusted to 7% moisture. Percentage of total sound mature kernel (TSMK), as one of the most valuable grade characteristics, is also reported in this study.

Statistical analysis

Raw Ct data from the real-time PCR experiments were evaluated based on a method described by Livak and Schmittgen (2001). All samples were first normalised to *Actin* gene as an internal control then transformed data were normalised to stage 1 of GG and plotted to determine relative fold changes in gene-expression. Analysis of variance was conducted in SAS (SAS Institute 2009) with PROC GLM under the general linear model. Treatment effect *F*-tests were conducted against their specific error source. Means were separated using Fisher's protected l.s.d. or Student's paired *t*-test method at $P < 0.05$.

Results

Physiological measurements

Measurements of RWC, SLA and LDMC of plants challenged with drought compared with irrigated controls can indicate relative level of stress (Girdthai *et al*. 2010). In this experiment, no signs of physical stress were observed in drought treated compared with irrigate peanuts throughout the test period. Similar patterns of RWC were observed in the range of 0.95 and 1.0 for plants in irrigated (Fig. 1*a*) and drought treatment (Fig. 1*b*). No differences were observed at stages 3 and 4 comparing drought to irrigated treatment when soil water content was significantly reduced from –70 to –150 kPa at 10 cm and –59 to –79 kPa at 20 cm depth. 08T-12 was significantly higher for SLA than the other genotypes at stage 3 with irrigated treatment (Fig. 1*c*) and C76–16 was significantly lower than the other genotypes at stage 4. At stage 3 for SLA with drought treatment (Fig. 1*d*), C76–16 and A-104 were significantly lower than the other three genotypes. At stage 4, A-104 was significantly lower than GG, 08T-12, and AP-3. 08T-12 was significantly lower than the other genotypes at stage 3 for LDMC under irrigated treatment (Fig. 1*e*) and 08T-12, GG, and A-104 were significantly lower than the other 2 genotypes at stage 4. At stage 3 for LDMC under drought treatment (Fig. 1*f*), C76–16 and A-104 were significantly higher than the other genotypes. C76–16, A-104, and 08T-12 were significantly higher than GG and AP-3 at stage 4.

Quantitative PCR results

The relative fold changes ranged from 0 to 4.5 for genes analysed, except for *myo*-inositol phosphate synthase (MIPS), which ranged from 0 to 16. Full irrigation treatment was compared with drought treatment for 21 days drought (stages 1 to 4) and recovery (stage 5). For ELIP with full irrigation treatment (Fig. 2*a*), fluctuation of gene-expression showed a negative trend starting with an average from 1.5-fold to the lowest expression level at stage 3 (0.74) for four genotypes and followed by an increasing trend (1.5). C76–16 showed a negative trend from 1.9 at stage 1 to 0.64 at stage 4 and an increasing trend to 1.2 at stage 5. For drought treatment (Fig. 2*b*), similar trends were observed for all five genotypes compared with irrigated, but only a slight upwards trend was observed from stages 3 to 5. Similar trends were observed with thioredoxin for irrigated (Fig. 2*c*) and drought treatment (Fig. 2*d*) for stage 1 (1.1 and 1.0) to stage 3 (0.63 and 0.63), but no upward trend was observed for drought treatment at stage 4 (0.53). For HSP70 with full irrigation treatment, C76–17 (2.0) was significantly

Table 1. Primers and PCR characteristics for 11 candidate-genes and one internal control actin gene

Genes	Accession number	Putative function	Reference	Direction	Primers (5′–3′)	Size (bp)	T_m (°C)	Efficiency	Regression coefficient R2
ELIP	AF479309	Photoprotection	Pinto *et al.* (2011)	F	ATGGAAGGCTTGCAATGATTG	120	59	101.4	0.996
				R	CCACACTAGTCCCCAAGAACCA				
Thioredoxin	JR555818	Redox and light regulation	Meyer *et al.* (2012)	F	CACCCAATGGTGTGGTCCTT	120	59	93.6	0.990
				R	TTTGCCAATGGCTTGTTCTCT				
Actin	EZ723877	Internal Control	Dang *et al.* (2012)	F	CACATGCCATCCTTCGATTG	150	59	98.5	0.988
				R	CCAAGGCAACATATGCAAGCT				
HSP70	EZ733089	Heat and water stress protection	Huerta *et al.* (2013)	F	AAGGACATAAGTGGCAACCCTAGA	120	58	94.6	0.997
				R	TCCCTCATACAGCGAATCAATTT				
Rubisco activase	EZ721324	Photosynthesis	Kurek *et al.* (2007)	F	TAGACGCAGGAGCAGGTCGTA	120	60	88.0	0.998
				R	ACATCCCTGGGAGCTGAACA				
CuZn SOD	EZ722218	Reactive oxygen scavenger	Kim *et al.* (2010)	F	GAAATGGTCCAACCACTGTGACT	120	59	83.5	0.995
				R	TGAAATGCGGTCCAGTTGAC				
nsLTP	FS980595	Adaptation to stress	Guo *et al.* (2013)	F	GATGAAGAAGGTGTGTGCAGTGTT	120	60	89.0	0.903
				R	GTGATGGCCCCAAGACATG				
LEA	HM543589	Tolerance to water stress	Grelet *et al.* (2005)	F	ATCACTTCAAACAAAACGAGCTTTAA	125	60	86.7	0.950
				R	TTGTGCTGTTGTCGCATATCC				
TIP	EZ751999	Osmotic stress adjustment	Zhao *et al.* (2008)	F	TAGCTGCTGGCCCATTCAGT	125	62	90.9	0.998
				R	CAAGCCCCCACCAATCAAC				
Drought protein	ES753436	Unknown	Peanut ESTs	F	TCACGAAATCCATGGAACCA	125	59	84.7	0.997
				R	TTTCATAAAGAACCTCCGCATTC				
Metallothionein	EZ735993	Reactive oxygen scavenger	Kumar *et al.* (2012)	F	ACTGCGACTGCGCTGACA	125	62	80.6	0.997
				R	GCACTTGCATCCGTCGTTCT				
MIPS	ES752304	Polar auxin transport	Kaur *et al.* (2013)	F	CGCTCCAAGGAAATCTCCAA	125	60	86.5	0.987
				R	TCCCCCACATATGGCACATA				

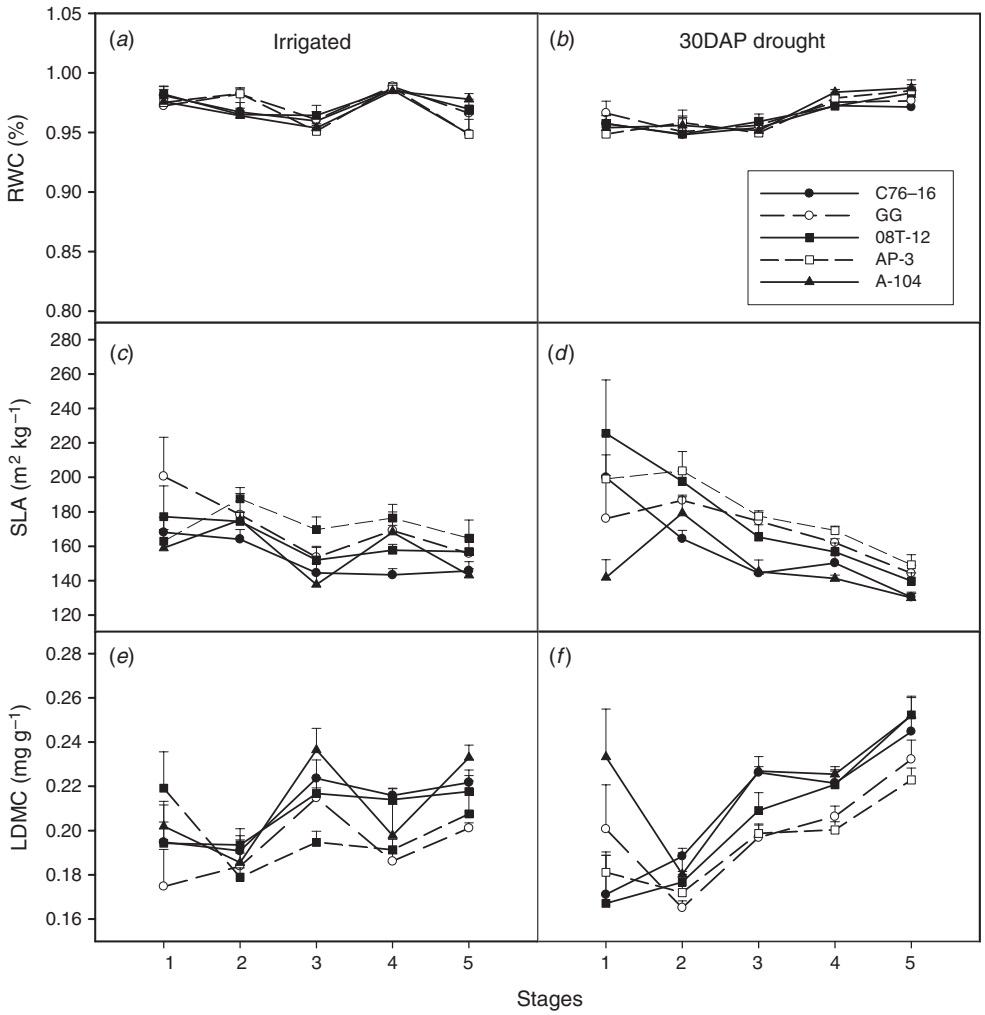

Fig. 1. Relative water content (RWC), specific leaf area (SLA) and leaf dry matter content (LDMC) measurements of irrigated (*a*, *c*, *e*) and drought treatment (*b*, *d*, *f*) of five peanut genotypes at drought stages (1–4) and recovery (5).

higher than the other four genotypes (1.2) at stage 3 and followed a similar trend at stages 4 and 5. For drought treatment (Fig. 2*f*), increasing trends were observed from stage 1 (1.1) to the highest point at stage 4 (1.6) followed by a negative trend at stage 5 (1.1). In contrast with the full irrigation treatment, C76–16 (0.49) was significantly lower than the other genotypes (1.4) at stage 3. Similar trends were observed for Rubisco activase (Fig. 2*g*) compared with thioredoxin gene-expression patterns at full irrigation treatment. Continuous negative trend from stage 1 (1.1) to stage 5 (0.82) was observed for Rubisco activase with drought treatment (Fig. 2*h*) with no increasing trend. For CuZnSOD with irrigated treatment (Fig. 2*i*), an increasing trend was observed with C76–16 (3.5) significantly higher than the other genotypes (1.5) at stage 3, followed by GG (2.0) significantly higher than the other three genotypes. A negative trend was observed for CuZnSOD under drought treatment (Fig. 2*j*) from 1.1 at stage 1, with the lowest point at stage 4 (0.58) followed by a slight increasing trend after irrigation (0.76). Among the five genotypes, the most significant change from irrigation treatment to drought stress was observed in C76–16 at stage 3 (3.5 to 0.5) with CuZnSOD gene-expression, in contrast with AP-3, which had the least change (1.3 to 0.8), indicating that C76–16 was strongly responsive to drought. For NsLTP with full irrigation treatment (Fig. 2*k*), similar trends were observed between genotypes. For NsLTP gene-expression with drought treatment, C76–16 (2.2) and GG (2.0) were significantly higher than the other three genotypes (1.1) at stage 2, followed by a decreasing trend (0.50) at stage 4 and an increasing trend (1.1) after irrigation at stage 5. For LEA with irrigated treatment (Fig. 2*m*), C76–16 (3.4) was significantly higher than the other genotypes (1.0) at stage 1, followed by decreasing trend (0.70) to stage 3 and increasing trend at stages 4 (0.85) and 5 (1.8). LEA gene-expression under drought treatment (Fig. 2*n*) showed a general negative trend from stage 1 (1.5) to stage 4 (0.34), followed increased gene-expression level at recovery stage 5 (1.2). GG (1.4) was significantly higher than the other genotypes (0.29) at stage 3. For TIP under irrigated treatment (Fig. 2*o*), similar gene-expression patterns were observed compared with thioredoxin and Rubisco activase gene-expression patterns. With drought treatment (Fig. 2*p*),

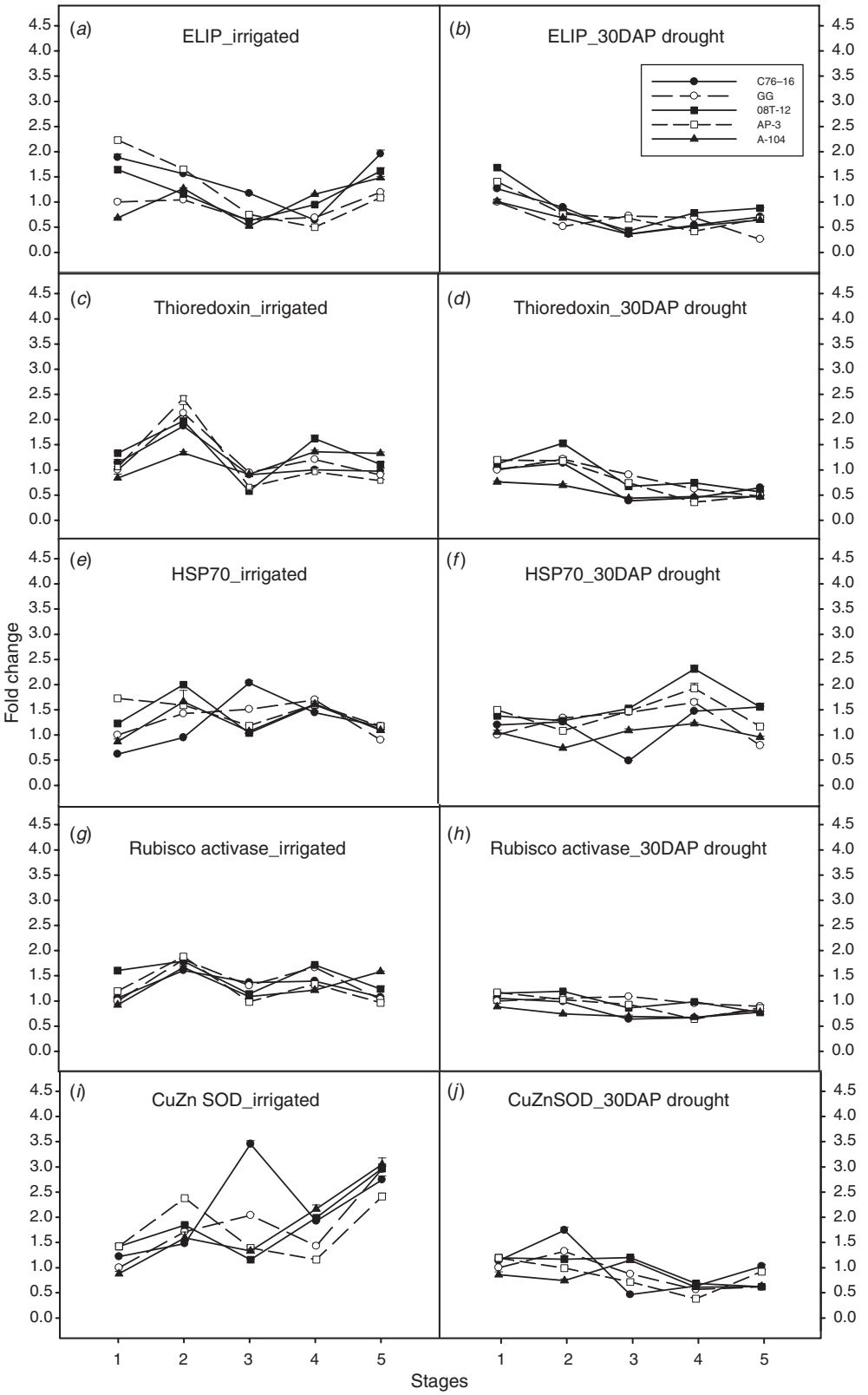

Fig. 2. Quantitative PCR results of eleven candidate-genes compared with an actin reference gene. Relative fold changes in gene-expression in irrigated (*a*, *c*, *e*, *g*, *i*, *k*, *m*, *o*, *q*, *s*, *u*) and drought treatments (*b*, *d*, *f*, *h*, *j*, *l*, *n*, *p*, *r*, *t*, *v*) of five peanut genotypes at drought stages (1–4) and recovery (5).

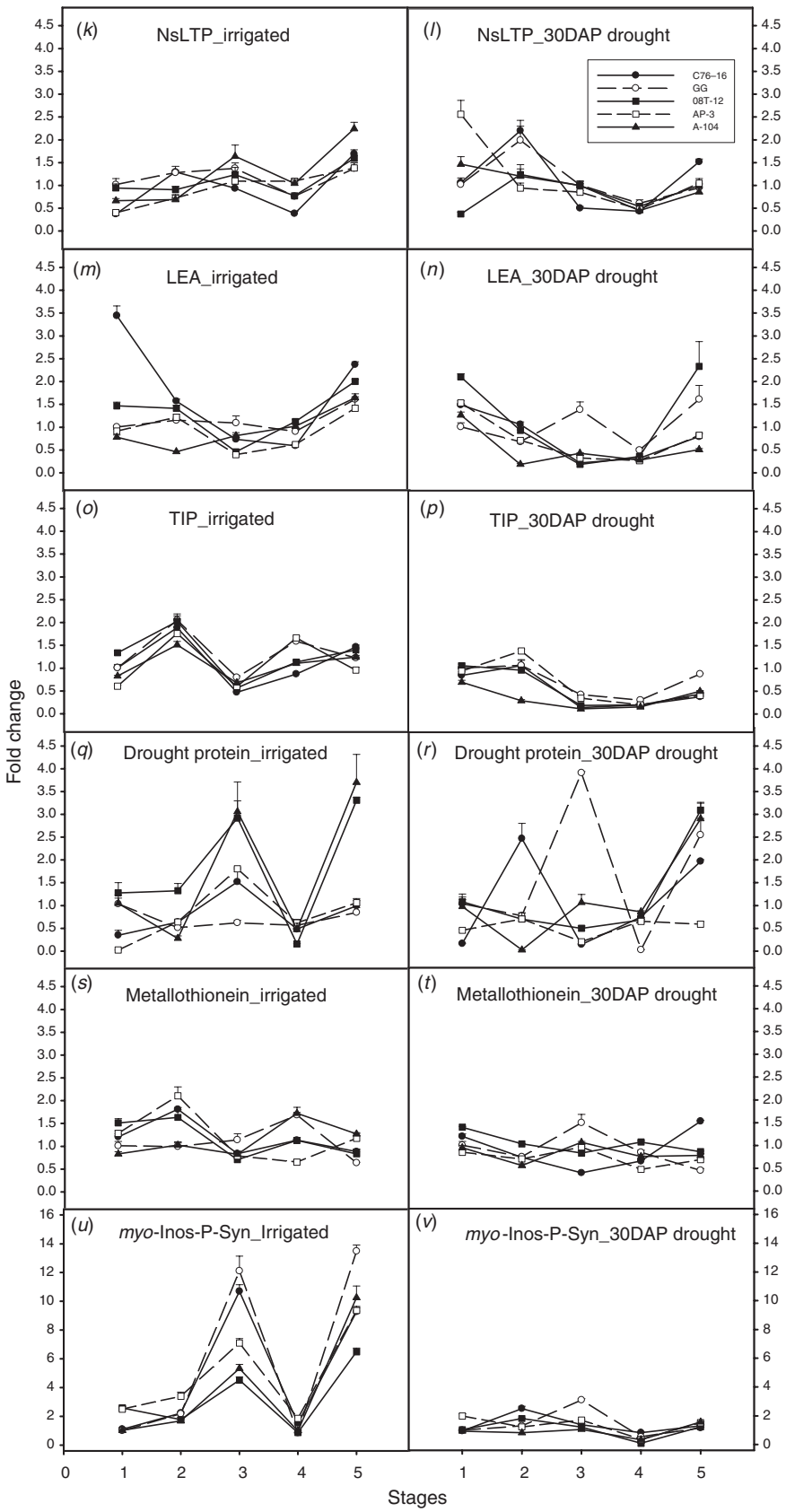

Fig. 2. (*continued*)

TIP showed a general decline from stage 1 (0.91) to stage 4 (0.21) and a slight increase of gene-expression at stage 5 (0.52), with GG (0.88) significantly higher than the other genotypes (0.43) at stage 5. For drought protein gene-expression with irrigated treatment (Fig. 2*q*), an increasing trend was observed from stage 1 (0.74) to stage 3 (2.0), with A104 (3.1) and 08T-12 (2.9) significantly higher than the other three genotypes (1.31), followed by AP-3 (1.8) and C76–16 (1.5) significantly higher than GG (0.62). At stage 5, A-104 (3.7) and 08T-12 (3.3) were significantly higher than the other three genotypes (0.97). For drought protein under drought treatment (Fig. 2*r*), C76–16 (2.5) was significantly higher than the other genotypes (0.55) at stage 2. GG (3.9) was significantly higher at stage 3 compared with the other genotypes (0.48), significantly lower (0.03) at stage 4 compared with the other genotypes (0.73), and similar to the other genotypes at stage 5 (2.1). When comparing full irrigation and drought stress among five genotypes for drought protein gene-expression, GG had a positive increase from irrigation to drought stress at stage 3 (0.5–4.0) whereas the other four genotypes had negative increases with varied degrees. This demonstrated that the drought protein gene expression in GG is unique. For metallothionein, similar trends were observed in irrigated treatment (Fig. 2*s*) compared with Rubisco activase gene-expression patterns (Fig. 2*g*). For metallothionein gene-expression under drought treatment (Fig. 2*t*), GG (1.5) was significantly higher than the other genotypes (0.81) at stage 3 whereas C76–16 (0.41) was significantly lower than the other genotypes. For *myo*-inositol phosphate synthase gene-expression in irrigated treatment (Fig. 2*u*), C76–16 (10.7) and GG (12.1) were significant higher at stage 3 compared with the other three genotypes (5.6); whereas with drought treatment (Fig. 2*v*), *myo*-inositol phosphate synthase gene expression was significantly inhibited at stages 3 and 5 for all genotypes. Fluctuation of high gene expression was observed at stage 3 (7.9), low at stage 4 (1.2), and high at stage 5 (9.7).

Yield and grade components

Analysis (*t*-tests) indicated that there were no differences for yield and grade between full irrigation and 30 DAP drought stress, but significant differences were observed for yield and grade between 30 DAP drought stress and non-irrigation; and between full-irrigation and non-irrigation (Table 2). There were significant differences of yield for genotypes under three treatments (ranging from 4240 to 5206 kg ha^{-1}, 2561 to 4549 kg ha^{-1}, and 4283 to 5467 kg ha^{-1} under 30 DAP, non-irrigation and full-irrigation conditions respectively) (Table 2), indicating that the five tested genotypes were genetically different in responses to environment. Among the five genotypes, AP-3 had the most yield deduction (–1774 kg ha^{-1}) from full irrigation to non-irrigation indicating AP-3 is a susceptible variety. C76–16 had the least yield deduction (–918 kg ha^{-1}) and the best yield performances in all tested conditions, indicating C76–16 is a drought tolerant genotype (Table 2).

Discussion

Growing in their native environment, plants are constantly exposed to abiotic stresses such as drought and high temperature that can limit productivity. As a result, plants have evolved different mechanisms such as escape, avoidance, or tolerance to minimise exposure to stress and re-establish optimal growth and productivity. Plants exhibiting escape mechanism will have early flower and maturing characteristics; avoidance mechanism includes changes such as thicker waxes or numerous hair, larger or deeper root system, efficient water use (WUE), high harvest index (HI), high relative water content (RWC), low specific leaf area (SLA), high osmotic potential; and tolerance mechanism involves gene induction of plant protection systems for survival, which may limit productivity (Bueckert and Clarke 2013).

Cultivated peanut appears to exhibit avoidance mechanism and recent drought research has concentrated on different physical and physiological characteristics (Songsri *et al*. 2008; Girdthai *et al*. 2012; Hamidou *et al*. 2013). It was observed that some genotypes can withstand early season drought to produce yields similar to or even higher levels compared with full irrigation treatment (Nageswara Rao *et al*. 1985; Nautiyal *et al*. 1999). It is proposed that plants respond to early season drought stress by modifying root distribution to obtain deeper water sources (Songsri *et al*. 2008) or induce specific gene-expression (Dang *et al*. 2012) to maintain optimal growth and development. In this experiment, an early season drought was applied to five peanut genotypes. When water was completely withheld for 3 weeks, no physical differences were observed for treated plants compared with their fully irrigated counterparts. RWC showed no significant differences between irrigated and drought treatment and among genotypes (Fig. 1*a*, *b*), suggesting that only a mild drought stress was applied. Our results showed no significant yield differences between drought and irrigated

Table 2. Yield (kg ha^{-1}) performance and total sound mature kernel (TSMK) of five genotypes at three treatments in 2010

Means within different treatments followed by the same letter are not significantly different by *t*-test at $P < 0.05$. DAP, days after planting

Genotype	Yield			TSMK		
	30 DAP	Non-irrigated	Irrigated	30 DAP	Non-irrigated	Irrigated
08T-12	4746	3236	4283	0.71	0.72	0.74
A-104	4540	3275	4380	0.75	0.71	0.75
AP-3	4408	2561	4335	0.7	0.67	0.7
C76–16	5206	4549	5467	0.71	0.7	0.73
GG	4240	3841	4988	0.76	0.75	0.78
Mean	4628a	3492b	4691a	0.73a	0.71b	0.74a

treatment among genotypes (Table 2), except for GG. Grades (TSMK) were also not significantly different among genotypes, except for A-104. Because yield is a complex trait with many factors interacting specifically to different stages of plant development, a non-irrigated (rain-fed) treatment was included to observe yield potential for multiple and fluctuating drought stress responses. The experiment was conducted using environmental control plots and standard agronomic practices similarly for all plots with precise irrigation controls to minimise experimental errors. A moderate and short-term drought treatment was applied to identify gene-expression differences among peanut genotypes, focusing on plant responses that maintain high productivity rather than a severe and long-term drought which can illicit survival mechanism. Cultivar C76–16 was identified as drought tolerant genotype with highest yields in all three treatments; drought, irrigated, and non-irrigated. GG was identified as medium drought tolerant genotype with the second highest yield in non-irrigated treatment, followed by 08T-12 and A-104, and AP-3 as drought-susceptible genotype with the lowest yield. AP-3 was also shown to have the lowest yield in another study, contributing to the inability of this genotype to convert prolific number of flowers at reproduction stage to pods at maturity stage (Rowland *et al*. 2010).

In regards to the utilisation of physiological measurements as plant selection for drought tolerance, identification of plants with low SLA has been applied in breeding program (Girdthai *et al*. 2012). Reduction in SLA, a measurement of leaf thickness, is assumed to enhance WUE because thicker leaves usually have higher chlorophyll contents and greater photosynthetic capacity than thinner leaves and high LDMC, a function of SLA that predicts leaf nitrogen content and soil fertility. Fundamentally, SLA and LDMC reflects two contrasting plant function between a rapid production of biomass (high SLA, low LDMC) and increased drought tolerance (low SLA, high LDMC) (Monty *et al*. 2013). C76–16 and A-104 had the lowest SLA and highest LDMC after 2 weeks of drought compared with the other three genotypes, with C76–16 positively correlated with yield. Significant year affect and genotype × environment interactions were observed for drought tolerance traits in peanut (Hamidou *et al*. 2013), making plant selection difficult.

Gene-expression studies, utilising drought tolerant versus susceptible genotypes under environmental controlled shelters, should facilitate a more precise plant selection for drought tolerance. Eleven candidate genes were selected to evaluate five genotypes with different responses to drought. Originally, C76–16 was identified as a resistant genotype through application of late season drought stress utilising a screening method developed by Holbrook *et al*. (2009). For the irrigated treatment, C76–16 showed significantly higher levels of gene-expression observed at stage 3 for HSP70, CuZnSOD, drought protein, and *my*-inositol phosphate synthase. For C76–16 drought treatment, significant higher levels of gene-expression were observed at stage 2 for CuZnSOD, NsLTP and drought protein compared with the other genotypes, followed by significantly lowest level at stage 3 for the same genes. HSP70 gene-expression level in C76–16 was significantly lower at stage 3 than the other genotypes. This could suggest an early recognition of drought, after 1 week of treatment, followed by a plant response that alleviated the mild stress. Dang *et al*. (2012) showed that specific transcription factors were highly induced under drought in peanuts and C76–16 can recognise drought stress 1 week earlier and has a stronger recovery response than AP-3. GG showed different patterns of gene-expression than C76–16. Gene-expression for drought protein was significantly lower than the other genotypes in irrigated treatment but was significant higher than the other genotypes under drought treatment (Fig. 2*r*). This gene was identified through BLASTx matching a RD22 drought induced gene in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki 1993) that contained a BURB domain with unknown function. High level of gene-expression in GG after 2 weeks of drought suggests that drought protein is important for plant protection. Similar to C76–16, GG showed a significantly higher level of *myo*-inositol phosphate synthase gene-expression compared with the other three genotypes. Recently, overexpression of *MfMIPS*1, a *myo*-inositol phosphate synthase cloned from *Medicago falcate* and transformed into tobacco, conferred plant tolerance to cold, drought, and salt stress (Tan *et al*. 2013). We noted that there were significant differences of *myo*-inositol phosphate synthase gene-expression among the five genotypes in irrigated treatment, ranging from 4-fold for 08T-12 and 12-fold for GG, but significantly reduced in drought treatment at stage 3 and after recovery. If this gene expression was confirmed to be associated with drought tolerance, this result can be applied to marker assisted selection in peanut breeding program for drought tolerance under a normal field condition other than controlled plot. In drought treatment, GG showed significantly higher levels of gene-expression for LEA, metallothionein, and *myo*-inositol phosphate synthase at stage 3 than the other genotypes, indicating a better drought response than 08T-12, A-104, and AP-3, but not C76–16.

Results from this experiment re-emphasised that early-season drought stress does not reduce peanut yield and quality. Fluctuations in the level of specific gene-expression are observed during normal development for the candidate genes, which can indicate a trend for other genes as well. Comparison of irrigated and drought treatment patterns of gene expression identified two genes that have the potential to be utilised in breeding programs. In general, a single or multiple drought adaptive responses can occur in peanut. The application of candidate gene approach for drought tolerant plant selection will facilitate the development of peanut varieties with a combination of drought tolerant mechanisms.

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