RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2013.39.001

ScienceAsia 39 (2013): **1–11**

Expression of drought tolerance in transgenic cotton

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Received 13 Feb 2012
Accepted 15 Nov 2012

ABSTRACT: Tolerance against drought in T1 progeny of transgenic cotton (*Gossypium hirsutum*) previously transformed with *GHSP26* (Heat Shock Protein Gene), *GUSP1* (Universal Stress Protein Gene), and *Phyto-B* (Phytochrome-B Gene) was investigated at the vegetative, squaring, and boll formation stage. Detection of transgenes into the progeny plants through PCR showed a Mendelian inheritance pattern (3:1). Real-time PCR quantified the expression of *GHSP26* as transgenic plants which tolerated drought stress for 10–12 days at an average of 18 fold more at the vegetative stage which was increased to 19 fold at the squaring stage, while control plants withstood drought period only for 6 days. The gene expression was reduced to 13-fold more (13–15 days) in transgenic lines on an average basis as compared to control plants. Expression of *GUSP1* in transgenic progeny at the vegetative and squaring stage was quantified as 22 fold more (11–13 days) and decreased to 15 fold when compared to the control plants which withstood the drought period only for 6 days. Average relative fold expression of *Phyto-B* transgene as compared to the control was 0.67 more (8 days) at the vegetative stage. Thereafter, expression was elevated to 0.85 fold under drought stress conditions at squaring stage and continued to increase to 3.5 fold higher (14–15 days) at boll formation stage when compared to that of control plants. Most notably, the number of bolls per plant, single boll weight, and seed cotton yield of our transgenic lines were greater than those of non-transgenic plants under drought stress, which is of immense worth.

KEYWORDS: *Gossypium hirsutum*, water stress, transgenic inheritance, heat shock protein, universal stress protein, *Phyto-B* gene

INTRODUCTION

Cotton is an important crop in the world due to its most valuable fibre production and oilseeds[1]. The genus *Gossypium* contains about 50 diverse species, four of which are cultivated. *G. hirsutum* L. and *G. barbadense* L. are tetraploid ($2n = 4x = 52$) and *G. arboreum* L. and *G. herbaceum* L. are diploid ($2n = 2x = 26$). There is 5% reduction in the cotton yield due to various reasons, including shortage of irrigation water[2].

The diploid cotton species is not only the reservoir of important biotic and abiotic stress resistance genes, but it also offers better opportunities to study gene structure and function through techniques of gene knockouts[3]. As compared with classical breeding, transgenic technology not only introduced valuable genes into cotton and other agriculturally important crops, but also made it possible to study function and regulation of such genes[4,5].

Abiotic stresses, such as drought, salinity, and heat, currently have a massive impact on crop productivity and agricultural supply. Global water scarcities and quality issues are reaching crisis proportions not only in developing countries but across the world[6]. It is revealed that low yield of certain crops resulting from water scarcity demands the use of modern/advanced biological techniques to resolve this problem. Recently, research into the molecular mechanisms of stress responses has started to bear fruit and, in parallel, genetic modification of stress tolerance has also shown promising results that may ultimately apply to agriculturally and ecologically important plants[7]. Under drought stress, plants generally display many physiological responses which result in accumulation of certain differentially, expressed gene products[8–10]. Drought is also the major limiting factors for fibre and lint quality after flowering. Therefore it is important to understand the genes expressed during drought stress[11] and ultimately we will require drought resistant cotton varieties.

Genes that have been successful in improving drought tolerance include those encoding heat-shock proteins (HSPs). These chaperones play a crucial role in protecting plants against stress by re-establishing normal protein conformation and thus normal cellular homeostasis[12,13]. The Universal Stress Protein (USP) super-family encompasses an ancient and conserved

group of proteins that are found in bacteria, archaea, fungi, flies, and plants[14]. These proteins GUSP1 and GUSP2 show specific expression patterns during drought stress[15,16].

Light play a fundamental role in the existence of plants as a wide array of genes is transcriptionally regulating the circadian clock in *Arabidopsis thaliana*[17]. So phytochrome controls the transition from vegetative to reproductive growth, seedling establishment, and entrainment of circadian clock[18,19]. The primary photoreceptors involved in regulating the red/far-red light-induced responses are the phytochrome pigments. Phytochrome A (PHYA) is a light-liable phytochrome that predominates in dark-grown tissues whereas PHYB is light stable and predominates in light-grown tissue. The other Phytochromes (PHYC, PHYD, and PHYE) are also light stable and have complex overlapping and differential roles relative to PHYA and PHYB.

The present study has been conducted to assess the overexpression of the transgenes *GUSP1*, *GHSP26*[20], and *Phytochrome-B*[21] which had been driven under the *CaMV 35S* promoter and previously transformed in cotton (*G. hirsutum*). Over expression of genes in the transgenic progeny plants at three different growth stages (vegetative, squaring, and boll formation) has been studied under drought stress condition.

MATERIAL AND METHODS

Seed source, sterilization, and germination

Seeds of T1 transgenic progeny of *G. hirsutum* previously transformed with *GUSP1*, *GHSP26*, and *Phyto-B* genes were obtained from seed bank of Centre of Excellence in Molecular Biology, Lahore, Pakistan (*GUSP1*, *GHSP26*[20], and *Phytochrome-B*[21]). The plasmid construct used in this study was made as pCambia 1301 T-DNA region contained a GUS gene along with hygromycin plant selection gene driven by the *CaMV 35S* promoter. Lint was removed from the seeds with concentrated H_2SO_4 and washed with tap water. The seeds which floated at the surface of water were discarded. Seeds sterilization was done by using 0.1% $HgCl_2$ for 5–10 min followed by 5 washings with autoclaved distilled water and kept for germination in dark at 30 °C on moist filter paper for 72 h. After germination seedlings were grown in composite soil (soil, sand, peat moss 1:1:1) for 40 days in green house at 30 ± 2 °C and relative humidity near 50%[22]. Metal halide illumination lamps (400 W) were used to supplement natural radiation. Light radiation reached a maximum of 1500 µmol m^2 s^{-1} at the top of canopy at midday. Complete randomized design was used for this study.

Amplification of foreign genes through PCR in progeny of transgenic cotton

Transgenes (*GUSP1*, *GHSP26*, and *Phyto-B*) were amplified through polymerase chain reaction (PCR) in the T1 progeny of transgenic cotton plants. This was done only for the initial screening for amplification of the foreign genes into the transgenic plants. For this purpose genomic DNA was isolated as described earlier[23] with some modifications. The sequence of *GUSP1* primer was F 5′-CTTCGACTGTATCTTG CTCATTTTC-3′ and R 5′-CCAAAGCTGGATTC CATATTAGAAG-3′, that of *GHSP26* was F 5′-GG CTGAGCATCTGGTAGCTT-3′ and R 5′-AATCC AAACCGTGGACAATG-3′, and that of *Phyto-B*, F 5′-GGATCATGGTTTCCGGAGTCGG-3′ and R 5′-GGATCTAATATGGCATCATCAGCA-3′. The PCR was carried out with the above primers to amplify the 710 bp, 510 bp and 646 bp fragments of *GHSP26*, *GUSP1 and Phyto-B* genes, respectively. PCR reactions were performed in volume of 25 µl with 2.5 U Taq DNA Polymerase, 0.2 mM dNTP's, 10 pM of each primer, and 50 ng of DNA template. The PCR temperature to amplify *GHSP26* and *GUSP1* genes was kept at 94 °C for 4 min, 94 °C for 30 s 60 °C for 30 s, and 72 °C for 1 min followed by 40 times. PCR temperature to amplify *Phyto-B* gene was kept at 95 °C for 4 min, 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min followed by 40 times. PCR products were resolved on 1% agarose gel and observed under UV light.

Drought stress treatment to transgenic plants and inheritance studies

To evaluate the temporal gene expression of candidate genes for drought tolerance, transgenic and non transgenic plants were kept without watering for maximum 15 days for drought stress treatments. First drought stress was applied to the plants at vegetative growth stage (after 40–45 days of germination), second drought stress was applied at square formation stage (55–60 days old plants) and third drought stress was applied at boll formation stage (120–130 days old plants). Chi square (χ^2) test was also applied for inheritance of the candidate genes in T1 progeny of the transgenic plants (Table 1).

Total RNA extraction and cDNA synthesis

Total RNA was extracted as the method described earlier with some modifications[24]. Fresh young leaf samples were taken from the main terminal branch

Table 1 Inheritance studies of foreign genes in T1 transgenic cotton progeny after drought stress treatment.

Gene	N	O	E	χ^2
GHSP26	21	14	15.75	0.78
GUSP1	32	23	24.00	0.17
Phyto-B	14	10	10.50	0.10

N: total number of plants. O: drought tolerant plants. E: expected drought tolerant plants.
$\chi^2 = \sum(O-E)^2/E$. The calculated value is in all the cases less than the tabulated value for $\mathrm{dof} = 1$ at 5% level of probability (3.84), showing the inheritance of these genes in accordance with Mendelian ratio for single gene inheritance (3:1).

parts and used for RNA extraction. RNA was quantified with the help of Nanodrop ND-1000 spectrophotometer by measuring absorbance at 260 nm and 280 nm wavelength. RNA quality was checked by resolving the sample on 1% agarose gel. cDNA was synthesized by using Fermentas cDNA synthesis kit (Cat # 1632).

Quantitative real-time PCR

The primers used for real-time PCR for *GUSP1* were F 5′-TCGGAGTTCAGAGAGAAGGAAG-3′ and R 5′-CTGGCATCACCCCAGTAAAT-3′, for *GHSP26*, F 5′-CCTAAACGGTTGGCTATGGA-3′ and R 5′-TGTCATTGCGTCCTCGAATA-3′, and for *Phyto-B*, F 5′-CTCCTGGCTGAGTTTCTGC T-3′ and R 5′-GCTTGTCCACCTGCTGCTAT-3′. Real-time PCR reactions were carried out with iQ5 cycler (BIO-RAD) in 96-well plate using the IQTM SYBR Green Super Mix. Different concentrations of the plasmid containing P2T1-4 were used as standard to validate the iQ5 Cycler reaction and to determine the range of quantification (standard curve). cDNA (50 ng) from the transgenic cotton plants transformed with *GUSP1*, *GHSP26* and *Phyto-B* genes was used at vegetative, squaring and boll formation stages of plant growth. The reaction conditions for genes *GHSP1* and *GUSP26* were as follows: denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 40 s, and final elongation step at 72 °C for 10 min. For *Phyto-B*: denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, and final elongation step at 72 °C for 10 min. Melting curve was analysed by continuous monitoring of fluorescence between 60 °C and 95 °C with 0.5 °C increments after every 30 s. Cotton glyceraldehyde-3-phosphate dehydrogenase (GAPDH) house-keeping gene was used as a control. Gene specific primers were designed using BIOEDIT software.

Effect of drought stress on yield

Productivity of transgenic cotton progeny was estimated as number of bolls per plant was counted, average single boll weight was measured and seed cotton yield per plant was hand collected. Seed cotton was collected, dry petals and trash was removed and the yield per plant was measured by weighing balance.

RESULTS AND DISCUSSION

Prevalence of drought stress is inconsistent under field conditions and plants may perhaps be exposed to this abiotic stress at any time throughout their life. Cotton is comparatively drought-tolerant, but severe water losses can slowdown plant maturity, affecting bolls and ultimately reduce yield. This study demonstrates that cotton plants of transgenic lines of progeny transformed with *GUSP1*, *GHSP26*, and *Phyto-B* genes, showed improved drought tolerance at three plant growth stages (vegetative, squaring, and boll formation) that are key water stress periods during plant growth. All the transgenic lines showed germination 60–100%.

Amplification of foreign genes in the T1 progeny of transgenic cotton plants

PCR showed the amplification of GHSP26 gene in T1 transgenic progeny of transgenic cotton (Fig. 1a). Out of 21 plants, 14 showed amplification of 710 bp fragment of this gene. GUSP1 gene was also amplified in the progeny plants in the form of 510 bp with full length primers (Fig. 1b). Out of 32 plants, 23 were confirmed for the amplification of GUSP1 gene. Similarly PCR confirmed the amplification of Phyto-B gene in T1 progeny (Fig. 1c) and out of 7 plants, 6 amplified the 646 bp fragment of Phyto-B gene. Plasmid construct used in this study was made as pCambia 1301 T-DNA region contained a GUS gene along with hygromycin plant selection gene driven by the *CaMV 35S* promoter.

Drought stress treatment to transgenic plants and inheritance studies

Although this study is based on the analysis of T1 generation which is expected not to be homozygous, but it was done to investigate the initial studies related to inheritance and integration of the transgenes into the progeny plants. According to Mendelian inheritance pattern for gene integration, the ratio of

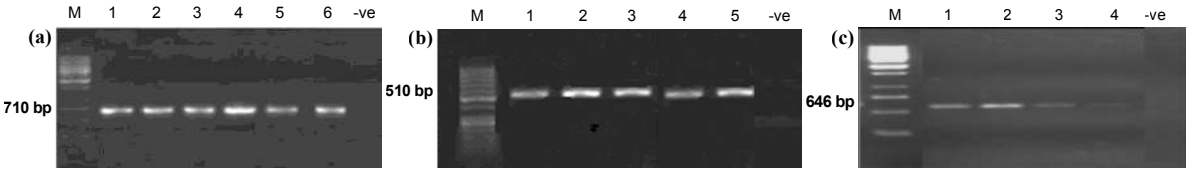

Fig. 1 PCR amplification of different drought tolerant genes in T1 transgenic cotton lines. (a) PCR product of *GHSP26* from transgenic plants. Lane M is a 1KB DNA ladder, lanes 1–6 transgenic plants, -ve negative control. (b) PCR product of *GUSP1* from transgenic plants. Lane M is a 50 bp DNA ladder, lanes 1–5 transgenic plants, -ve negative control. (c) PCR product of *Phyto-B* from transgenic plants. Lane M is a 1KB DNA ladder, lanes 1–4 transgenic plants, -ve negative control.

transgene containing plants to control or non transgenic ones should fit the expected 3:1 in T1 generation, which was also consistent with our statistical results. It is probably due to random chance or for some other factors influencing the experimental results (Table 1). There are many reports in context of inheritance and expression stability of foreign incorporated genes in transgenic crops. The previous studies revealed consistent Mendelian ratios for single gene inheritance in certain crops like, alfalfa[25], rice[26], maize[27], cotton[28–31], and in cow pea[32]. However, in many cases, instead of Mendelian segregation, the complicated segregation profiles for the genes have also been reported[33–38]. Nevertheless, in the present study, Mendelian ratio of single gene inheritance was followed.

GHSP26 gene expression

For temporal gene expression pattern studies, total RNA was extracted from leaves under drought stress condition at different growth stages, Vegetative stage (after 40–45 days germination), Square formation (after 55–60 days germination) and Boll formation (after 120–130 days germination). The products were visualized as a smear along with two distinct ribosomal RNA bands, 28S and 18S. Real-time PCR detected the expression level of foreign genes in the transgenic progeny. Different lines exhibited different gene expression in response to drought stress at vegetative stage. An optimum cotton crop at 40 days after planting is thought to be a picture of health. In addition to being drought stress-free, the crop would exhibit healthy leaves, with roots extending into the row middles, and plants growing rapidly and uniformly. Among 6 lines of the GHSP26 transformed lines, line 5 on average basis showed maximum tolerance (12 days) to the drought stress showing maximum expression (30 fold expression) of GHSP26 followed by line 3 and 6 which contended drought for 10 days each, while control (non-transgenic) withstood drought period only for 6 days (Fig. 2a, b). Following the formation of first reproductive branch, new branches will develop every 3 days after approximately. The first square is formed on the lowest reproductive branch of the plant. Drought tolerance was continued to increase at squaring stage, line 5 on average basis showed maximum tolerance (12 days) and exhibited maximum expression (34 fold expression) of HSP26 followed by line 3 and 1 which endured for 10 days each as compared to 6 days of control (non-transgenic under the aforesaid conditions) (Fig. 2c, d).

After fertilization, seeds are developed in a cell like structure, called boll. First bolls generally begin to open 125–130 days after sowing and irrigation during this period is thought to be critical. Transformed lines were once again subjected to drought stress and expression of gene at boll formation revealed that transgenic line 5 on average basis showed maximum drought tolerance (15 days) showing maximum expression (22 fold expression) of HSP26 followed by line 1 and 3 which endured for 14 and 13 days, respectively, (Fig. 2e, f). Control (non-transgenic) tolerated drought stress for 7 days. Expression of GHSP26 has been found reduced with the plant growth and found at peak at earlier stage[39–41], which is in contrast to previous reports[7,42] as the elevated expression of Taldo1 gene at lateral stage of plant growth was observed as compared to juvenile stage where the expression level was very less but this change in gene expression could be genotype dependent.

GUSP1 gene expression

Universal stress protein play role in survival of cells affected by different abiotic stresses[43]. Progeny of transformed lines consists of GUSP1 subjected to drought stress at vegetative stage and among 10 lines of the GUSP1 transformed lines, line 1 on average basis showed maximum tolerance (13 days) to the drought showing maximum expression (70 fold expression) of GUSP1 (Fig. 3a, b) followed by line 6 and 8 which contended for 11 and 10 days, respectively,

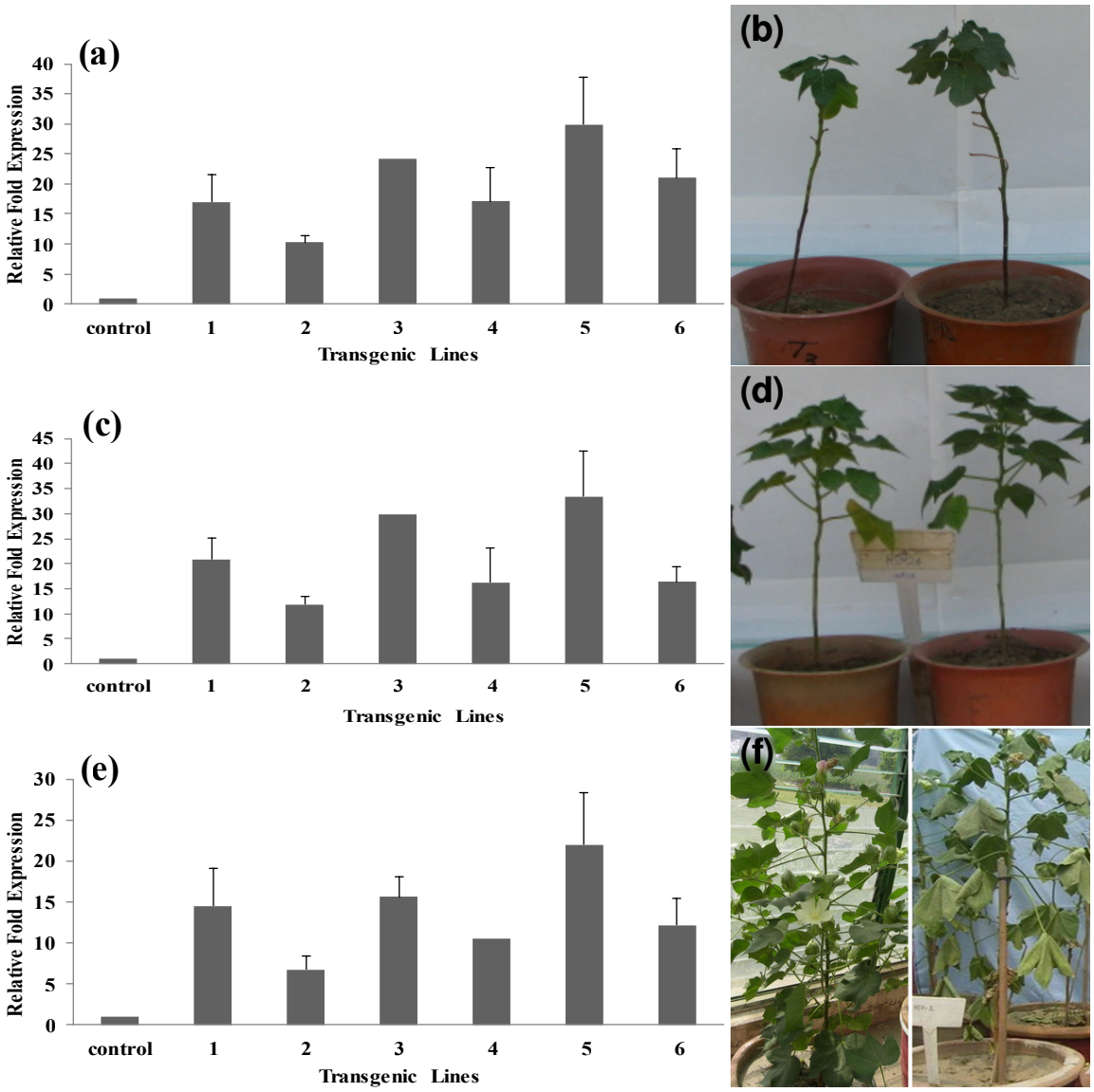

Fig. 2 Relative fold expression of GHSP26 in T1 progeny and transgenic cotton plants at different growth stages. Expression of GHSP26 in leaves at (a) vegetative stage, (c) squaring stage, and (e) boll formation, and phenotype of transgenic (right) and control (left) plants at (b) vegetative stage, (d) squaring stage, and (f) boll formation stage. Plants were kept under water withheld condition for 15 days at each developmental stage. The data was normalized with reference to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as internal control. The solid bars are the standard deviation calculated from three repeats.

while control (non-transgenic) withstood drought period only for 6 days. After the vegetative stage, a temporal expression study of the drought tolerant GUSP1 gene was studied at the square formation stage. Different lines showed different level of gene expression in response to drought stress at this stage as well but among 10 lines of the GUSP1 transformed lines, line 1 on average basis showed maximum tolerance (12 days) to the drought and exhibited maximum expression (82-fold expression) of GUSP1 followed by line 6 and 8 which endured for and 11 days each as compared to 6 days of control (non-transgenic) under the aforesaid conditions (Fig. 3c, d). Transgenic lines exhibited different level of drought tolerance in response to drought stress at boll formation stage. Among 10 lines of the GUSP1 transformed lines, maximum drought tolerance (16 days) on average basis was observed in line 1 with maximum gene

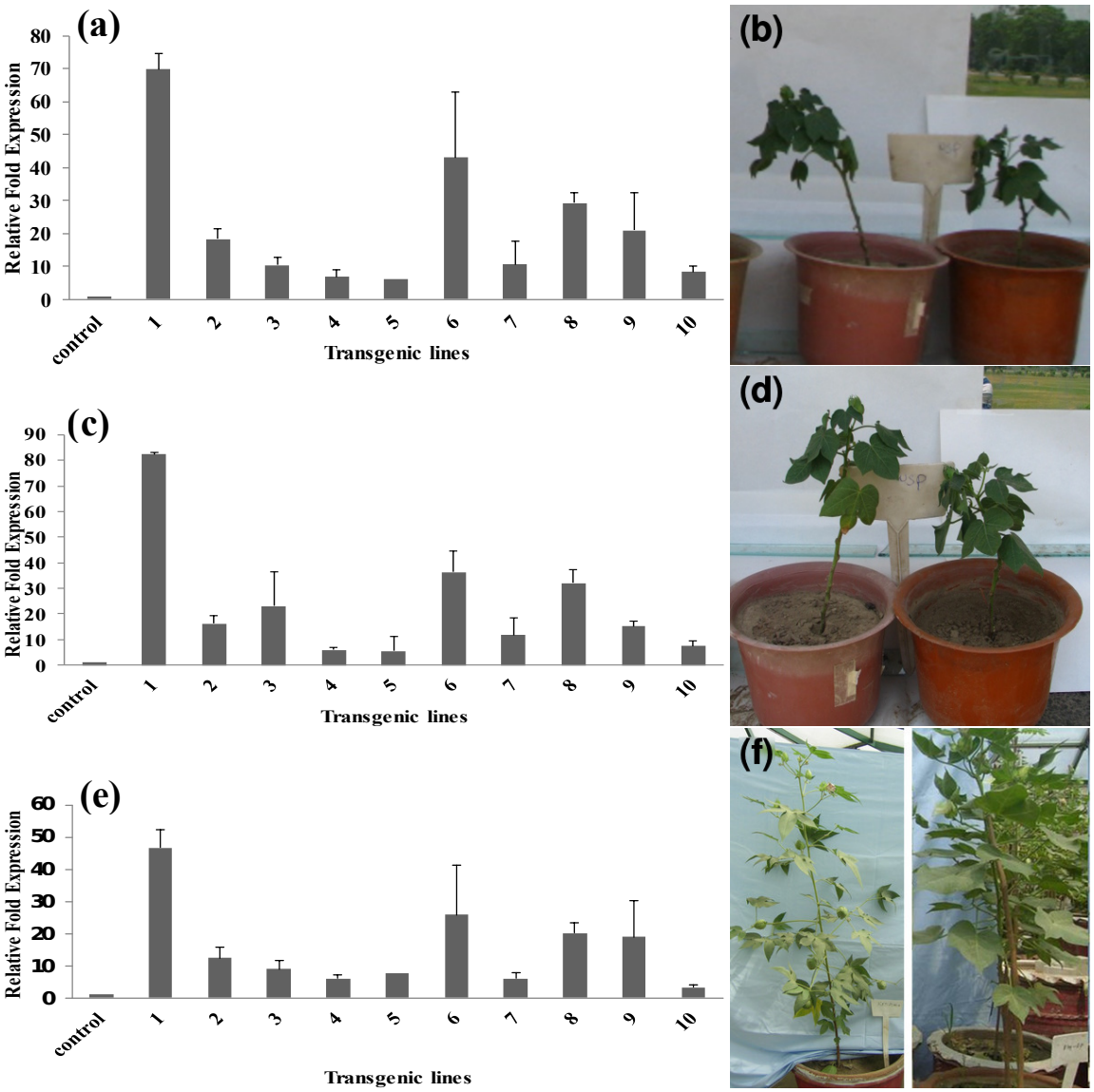

Fig. 3 Relative fold expression of GUSP1 in T1 progeny and transgenic cotton plants at different growth stages. Expression of GUSP1 in leaves at (a) vegetative stage, (c) squaring stage, and (e) boll formation, and phenotype of transgenic (right) and control (left) plants at (b) vegetative stage, (d) squaring stage, and (f) boll formation stage. Plants were kept under water withheld condition for 15 days at each developmental stage. The data was normalized with reference to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as internal control. The solid bars are the standard deviation calculated from three repeats.

expression (47 fold expression) of GUSP1 followed by line 6 and 8 which endured for 14 days each (Fig. 3e, f). Control plants tolerated drought stress only for 7 days. As the expression of GUSP1 has found to be decrease with the advancement in plant growth but even then it has expressed and helped the plant to tolerate the drought which indicate that this gene might function as a switch in adaptation of drought stress. Possible explanation for elevated expression of *GUSP1* gene at earlier stages may be the meristematic activity of the plant[44]. Another reason may be the high copy number of transgene in transgenic plants[45].

Phyto-B gene expression

Transgenic lines of Phyto-B were studied for gene expression in response to drought stress at vegetative stage. Among 2 lines of the Phyto-B transformed

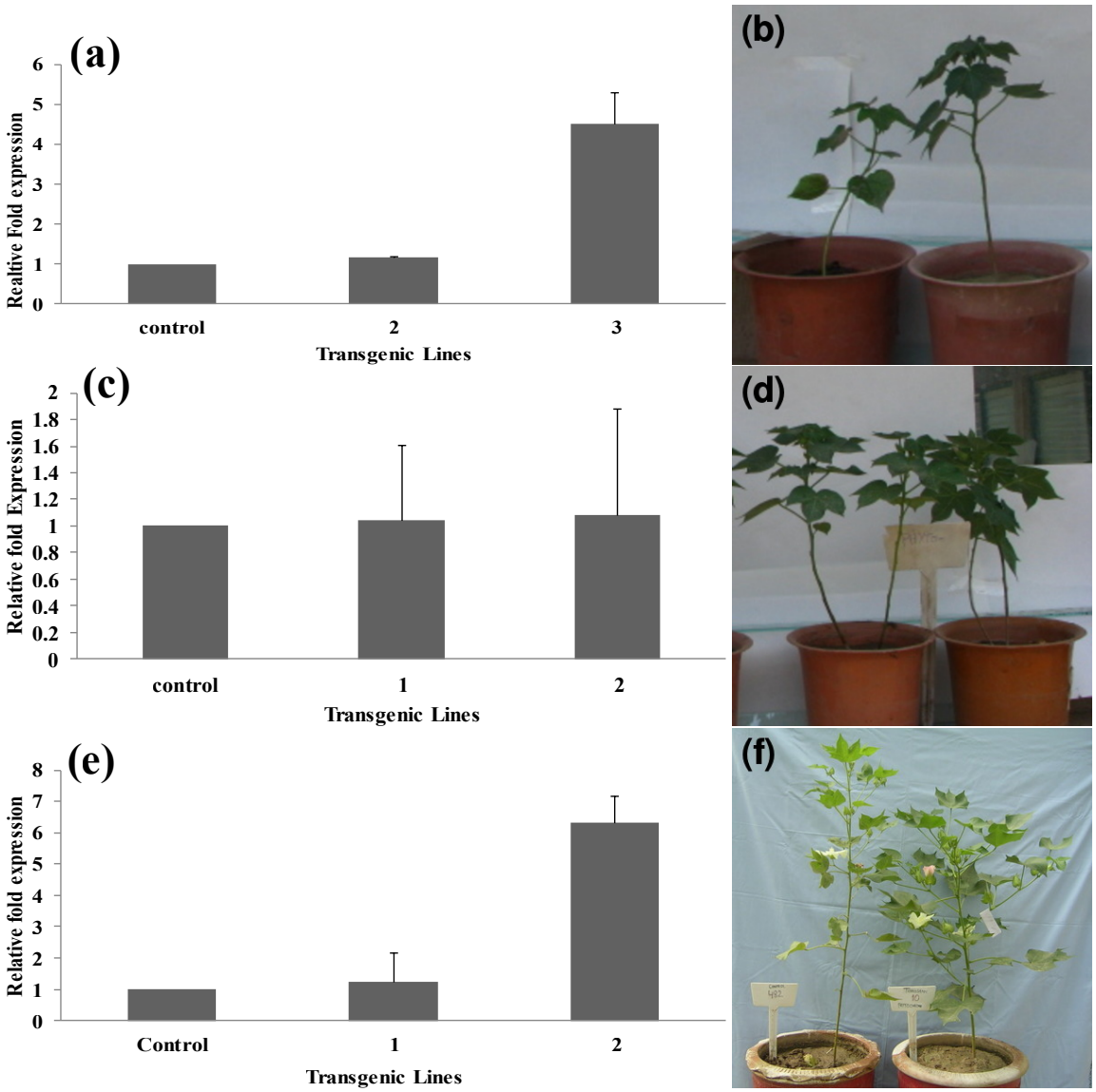

Fig. 4 Relative fold expression of PHYTO-B in T1 progeny and transgenic cotton plants at different growth stages. Expression of GUSP1 in leaves at (a) vegetative stage, (c) squaring stage, and (e) boll formation, and phenotype of transgenic (right) and control (left) plants at (b) vegetative stage, (d) squaring stage, and (f) boll formation stage. Plant kept under water withheld condition for 15 days at each developmental stage. The data was normalized with reference to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as internal control. The solid bars are the standard deviation calculated from three repeats.

lines, line 2 showed maximum tolerance (8 days) to the drought stress showing maximum expression (1.2 fold expression) of Phyto-B (Fig. 4a, b) as compared to control which withstood drought tolerance only for 6 days.

Transgenic lines were subjected to drought stress at square formation stage and temporal analysis of the gene showed a little bit elevated expression pattern but almost equal expression as that of control under drought stress condition (Fig. 4c, d). This elevated expression was continued to increase in transgenic lines in response to drought stress for 14–15 days at boll formation stage. Line 2 on average basis showed 6.3 fold higher expressions as compared to that of control (non-transgene) (Fig. 4e, f).

Phytochrome may manipulate plant response to drought suggesting particular role in ABA regulation and stomatal conductance. Phyto-B gene restricts

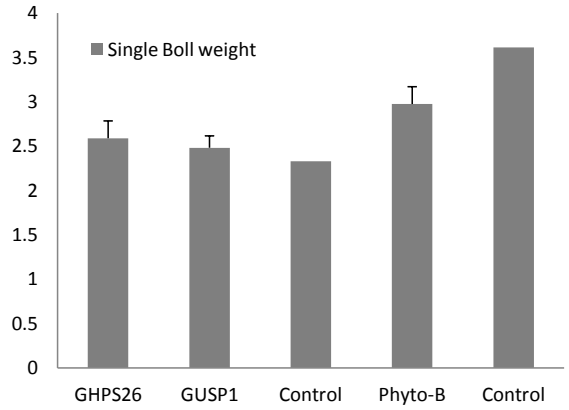

Fig. 5 Single boll weight of T1 cotton progeny transformed with GHSP26, GUSP1 and Phyto-B genes. The solid bars are the standard deviation calculated from three repeats.

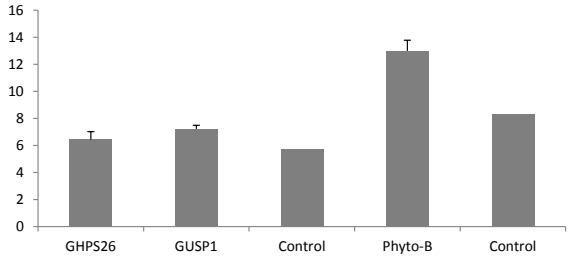

Fig. 6 Number of bolls of T1 cotton progeny transformed with GHSP26, GUSP1 and Phyto-B genes. The solid bars are the standard deviation calculated from three repeats.

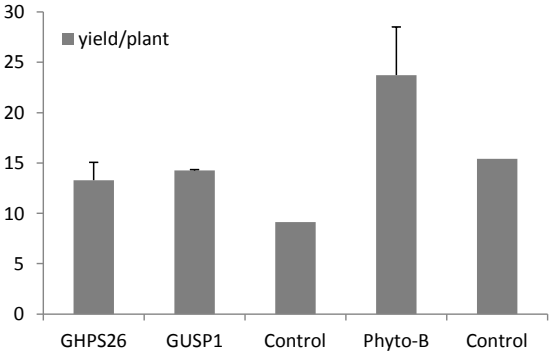

Fig. 7 Yield/plant of T1 cotton progeny transformed with GHSP26, GUSP1, and Phyto-B genes. The solid bars are the standard deviation calculated from three repeats.

the fruit shedding in cotton crop and fruit shedding is directly related to the production levels of ABA, therefore it is assumed that certain phytochrome genes may suppress particular drought response under specific environment[21,46]. Gene expression of Phyto-B was higher at the latter plant growth stage and lower at the earlier stage similar as reported earlier[12,42]. Such difference in gene expression at different plant developmental stages may be due to strong influence of transcriptional activation of transgene, its site of integration and genotype dependence.

Effect of drought stress on yield

Application of plant biotechnology is one of the strategies for crop improvement and food production. The ultimate objective of this study is to get good yield even after the reduced irrigation and we observed that the number of bolls in transgenic plants have been increased as compared to non transgenic or control plants. The boll weight has also been increased on the transgenic lines as compared to the non-transgenic. Single boll weight of the transgenic progeny of plants transformed with GHSP26 and GUSP1 was increased by 23% and 36%, respectively (Fig. 5). Interestingly the single boll weight of the transgenic progeny of Phyto-B was found a little bit reduced on transgenic progeny as compared to the control plants (Fig. 5). However, this reduction in boll weight is only 5%. Phyto-B gene is reported to increased photosynthetic activity and enhancement of other physiological traits in plants, therefore it is assumed that the increased vegetative growth may have suppressed the boll formation process and ultimately the fold expression for drought tolerance at vegetative and boll formation, respectively, is less (1.2 and 6.3) as compared to the GHSP26 and GUSP1. Number of bolls per plant of the transgenic progeny of GHSP26, GUSP1, and Phyto-B was found to be increased by 25%, 36%, and 71%, respectively, as compared to non-transgenic plants (Fig. 6). The data collected for the seed cotton yield per plant of the transgenic plants of GHSP26 of the same progeny was also found increased by 54% while compared with non-transgenic plants. In the same way seed cotton yield per plant for transgenic progeny of GUSP1 was increased by 63%. Similarly the yield data for transgenic progeny of PHYTO-B showed an increase of 62% seedcotton yield per plant (Fig. 7). It has been observed that as the fold expression for drought tolerance is increased, the yield is also found to be increased which shows that the transgene is expressing at its maximum.

Seasonal variations affect the transgene expression in different cotton lines. This variation for the transgene efficacy is correlated with the promoter activity[47,48]. The other factors reported for variation

in the level of gene expression, might be alteration in nucleotide sequence, gene integration point, copy number, cellular changes, and environmental factors[21,49]. Transgenic plants expressing the drought tolerant genes are morphologically better and growing larger than the control plants under water withheld condition. Moreover, roots of GHSP26, GUSP1, and Phyto-B expressing plants expected to be larger than non transgenic plants. Therefore, fresh shoot and root biomass of transgenic plants may be higher than control plants. Three types of transgenic plants, when compared for the drought tolerance and the yield (in terms of single boll weight, number of bolls and seed cotton yield), it is observed that the GUSP1 is the best as showing 70–80 fold expression of drought tolerance for 13–12 days at vegetative and squaring stage, respectively, and 47 fold expression of drought tolerance for 16 days and yield was 63% in terms of seed cotton. Yield of other transgene expressing plants is also significantly higher than that of control plants after drought treatment. These results are consistent with the other reports as more bolls and fibre formation in the transgenic plants expressing AVP1 gene[50]. Transcription factors also play an important role in abiotic stress tolerance mechanisms and so far a number of transcription factors have been found to be involved in abiotic stress tolerance pathways. Genes encoding transcription factors may considerably increase the drought tolerance by regulating (over expression or suppressing) the function of some genes.

Irrigation is very important after first blooming and by regulating the irrigation period after blooming yield could be increased[51]. Severe drought conditions affect the cotton plant's development and may cause small bolls and squares to shed. Improved maintenance of stomatal conductance under water stress ensures the higher photosynthesis, better growth and yield[52]. Exogenous application of Glycinebetaine improves the growth and production of cotton plants under drought stress[53].

In conclusion, most notably, the seed cotton yield of our transgenic lines was greater than that of non-transgenic plants under drought stress, which is of great worth. Therefore, it is expected that our results may promote strategies to improve crop yields in arid and semiarid areas.

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