

Original Research

Physiological and Biochemical Response of Transgenic Cotton Plants to Drought Stress

Mariam Zameer¹, Shazia Shafique^{2*}, Sobiya Shafique², Usaal Tahir¹,
Nureen Zahra¹, Bushra Rashid³, Rubab Afzaal⁴

¹Institute of Molecular Biology and Biotechnology, IMBB, the University of Lahore, Pakistan

²Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan

³Centre of Excellence in Molecular Biology, University of the Punjab, Lahore 54590, Pakistan

⁴College of Earth & Environmental Sciences, University of the Punjab, Lahore, Pakistan

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Abstract

Transgenic crops are propagated to get a range of benefits like the ability to be resistant to fungi, bacteria, viruses and insects, to tolerate herbicides and to grow better under restricting environmental conditions such as drought, high salt or metal containing soils. Currently, GHSP-26 gene related to small heat shock protein HSP family (GHSP-26) was isolated from *Gossypium arboreum* and transgenic plants of *Gossypium hirsutum* were raised with inherited gene. Thus, the current study was carried out to evaluate the response of transgenic and non-transgenic plants (control) when they were subjected to 5 days stress by preventing the irrigation. Their response was characterized by assessing the morphological, physiological and biochemical alterations occurring in plants mechanism. After 5 days it was observed that the stress reduced the growth rate, relative water content, cell membrane stability, photosynthetic rate, transpiration rate, stomata conductance and chlorophyll in plants. The accumulation of proline and malondialdehyde took place in plants. The transgenic plants exhibited more tolerance as compared to non-transgenic plants towards the drought stress conditions and have better survival capacity in harsh environment due to the capability of diminishing their metabolic processes which can be retained when the conditions turn to normal. This relative study among the transgenic along with non-transgenic plants gives a better understanding of the transgene expression and its effect on different yield contributing traits, in this way, transgenic crops can be developed in future which will give better response under drought conditions.

Keywords: drought, *Gossypium hirsutum*, GHSP-26 gene, growth reduction, proline, transgenic plant

Introduction

The biotechnology application is quite helpful in developing transgenic multiple varieties of the same plant, hence, enhancing plants yields, especially for

agricultural crops [1]. Transgenic plants are genetically modified plants exhibiting more efficiency than normal plants owing to their recombinant DNA [2]. These customized plant species could withstand harsh environmental conditions and certain diseases.

Agriculture sector is one of the fundamental pillars of any country's economy owing to the production of food necessity and various raw materials for industrial

*e-mail: shazia.iags@pu.edu.pk

sector. Hence, it brings significant amount of foreign capital through the export of industrial products such as chemicals, fertilizers, textile and food [3]. In order to get optimum yield from various cultivations, one has to ensure the provision of favorable growth conditions. Plants respond negatively to any environmental stress like cold or drought conditions because of their effects on growth and other trends such as metabolism, psychological, morphological and/or biochemical mechanisms [4]. The severe drought stress results from the inhibition of water uptake from plant roots due to water shortage or increased dissolved salt contents in the soil [5]. Increased evapo-transpiration rate from plants together with decreased water availability might also lead to the stress condition for plants [6]. Sevik and Cetin [7] also reported the reduced germination rate due to the effect of water stress on the seeds of nine pieces of species for landscaping works in Kastamonu. The drought conditions are considered as key environmental stress worldwide to cotton crop also and adversely impair plant growth and development [8]. Drought stress may pose multiple side effects on plants such as altered chlorophyll content and malondialdehyde level, hence lowering down the rate of photosynthesis and subjecting leaf to turgor loss [9]. Severe shedding of leaves and decrease in the flowering in cotton plants are reported to be associated with the drought stress conditions [10]. At initial growth period, drought stress may exert serious threat to cotton plants that may become worst, if the duration of drought stress gets prolonged. Water requirement is relatively increased at this phase, whereas it is comparatively low at later, pre-flowering and post-flowering stages [11].

Furthermore, the increase in temperature during reproduction phase, poses significant adverse impact on the growth of cotton crop eventually reducing its yield [12]. Plants, depending on the ambient light and temperature conditions, and which are necessary to perform photosynthesis or respiration, directly affect the concentration of CO₂ in the local environment. The study of Sevik and Cetin [13] attempted to determine the effects of indoor plants on the concentration of CO₂ in an indoor environment under certain light conditions and found that all plants reduced the concentration of CO₂ to a certain extent during the day.

The researchers to cope this issue such as employing osmoprotectants and soil amendments including organic and inorganic mineral nutrients applications have adapted different methodologies [14]. However, in recent years, the development of transgenic drought resistant plant varieties to enhance crop yield have been evolved as an optimal alternative strategy [15]. For this purpose, an ideal gene that possesses required stress tolerance characteristics must be determined and incorporated into plants. As heat shock proteins are effective to combat drought stress in cotton plants, the genes of these proteins are inserted in cotton plants making them heat resistant [16]. The present study investigated the possibility of employing genetic

engineering as a solution towards drought stress in cotton crop. Small heat shock protein sHSP gene (GHSP-26) was isolated from *Gossypium arboreum* and inoculated into *Gossypium hirsutum* to develop transgenic plant. Thus, the main aim of this study was to investigate and understand the morphological, physiological and biochemical responses of drought stress on transgenic and wild plants. The study will help to identify stress related genes, enhancing the quality and quantity of crop to provide benefits to the farmer, consumer and industry, ultimately assisting the global cotton cultivation in water stressed conditions.

Experimental Procedures

Seeds Collection and Processing

Pre-transformed seeds of *Gossypium hirsutum* were collected from the seed bank of Centre of Excellence in Molecular Biology (CEMB), university of the Punjab, Lahore which contained the drought tolerant gene GHSP-26 isolated from *Gossypium arboreum* [17].

Concentrated H₂SO₄ was employed to delint the seeds with 100 mL kg⁻¹ volume to weight ratio, along with stirring for 10-15 minutes until the shiny surface appeared. The treated seeds were then surface sterilized with 1% HgCl₂ for 15 minutes and were subjected to washing thrice, with sterilized distilled water.

Confirmation of Transgenic Plants

The seeds were sown in plastic pots, perforated at bottom, containing soil mixture comprising of clay, sand and peat moss (1:1:1) moistened well with distilled water [18]. Plants were kept in greenhouse at 30±2°C temperature and 250-300 µmol m⁻²s⁻¹ light intensity. After two weeks, the seedlings were transferred to new pots, containing one seedling per pot.

PCR Analysis

For confirmation of inoculation of gene of interest, fresh leaves of both transgenic and non-transgenic selected plants were taken and their DNA was extracted separately by adding 750 µL of lysis buffer into 0.1 g of dried sample. The sample was vortexed and incubated for 1 hour at 65°C and regular vortexing after 15 minutes was carried out. Afterwards, chloroform:alcohol (24:1) was added. Samples were vortexed and centrifuged at 14 rpm for 20 minutes at 4°C continuously. Supernatant obtained from this process was collected in other tubes. Afterwards, isopropanol (0.6 µL) was added and placed over night at 4°C and then samples were centrifuged at same temperature for 15 minutes. DNA pellets were formed and separated by discarding supernatant. Pellets were washed with 500 µL of 70% ethanol and centrifuged again. Then pellets were removed and ethanol was discarded [19].

DNA purity and concentrations were also measured using Nano drop spectrophotometer method. Polymerase chain reaction (PCR) was employed to amplify transgenes (GHSP-26) in T3 progeny of transgenic cotton plants. This was done for initial screening of transgenic plants with desired genes using HSP-F (5'-CTTCGACTGTATCTTGCTCATTTC-3') and HSP-R (5'-CCAAAGCTGGATTCCATATTAGAAG-3') primers. The first step of PCR was the denaturation of DNA at 94°C for 3 minutes that convert the double stranded DNA into linear strands. The process was followed by 35 cycles of denaturation at 94°C for 30 seconds. The next step was the annealing at 57°C for 30 seconds. The third step was the amplification of the strands. The extension occurred at 72°C for 30 seconds and the final extension was given for 10 minutes at 72°C [20].

Application of Drought Stress

Transgenic and non-transgenic plants at control conditions were watered well regularly up to 60 days and sampled for the analysis of all the parameters to determine their response before application of stress. Transgenic and non-transgenic plants were subjected to 5 days stress level by stopping the irrigation for 5 days and these plants were sampled for the analysis of morphological characters, physiological changes and biochemical responses of plants toward the drought stress. These responses help to understand the survival mechanism of cotton plant under drought stress.

Morphological Parameters

The morphological analysis was carried out to determine the total leaf area, number of leaves, shoot length, plant color.

Physiological Parameter

Physiological analysis was conducted by calculating leaf relative water content, cell membrane stability, photosynthesis and transpiration rate.

The method to determine water content was described by Turner [21]. Leaf samples (0.1 g) from both control and drought stressed plants (transgenic) were taken. Firstly, leaves were cut into pieces and then their fresh weight (FW) was determined. These leaves were immersed in deionized water for 24 hours and again their weight as determined that was the full turgid weight (TW). These samples were taken to oven for drying at 70°C overnight. Weight calculated after drying process was the dried weight (DW). Then leaf relative water content was calculated by the formula:

$$\text{Leaf relative water content} = \frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}} \times 100$$

The major environmental stress impact was analyzed in cell membrane and its stability was

calculated [22]. For the determination of cell membrane stability samples were blotted on filter paper and soaked in 25 mL of deionized water for 24 hours. Electrical conductivity of sample was measured (EC1). Then electrical conductivity (EC2) was measured after autoclaving of leaf samples as autoclaving kills tissues. Cell membrane stability (%) was determined by the formula [23].

$$\begin{aligned} & \text{Cell membrane stability (\%)} \\ & = \frac{\text{EC1} - \text{EC0}}{\text{EC2} - \text{EC0}} \times 100 \end{aligned}$$

Where

EC0 = Electrical conductivity of only deionized water

Photosynthesis and transpiration rates were calculated in the leaf with a Hand-held Refractometer RGA and gas exchange system (CI-340 Handheld Photosynthesis System; CID Bio-Science, Inc.). Net photosynthesis rate was measured by leaf area unit (Pn; in $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, while transpiration rate (E) in $\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ and stomatal conductance to water vapor (c) in $\mu\text{mol m}^{-2}\text{s}^{-1}$ were determined [23].

Biochemical Parameter

Estimation of proline, malondialdehyde and chlorophyll were carried out to determine the biochemical response of plants under condition of droughts.

Leaf tissues were collected for the estimation of proline content. The leaves of equal size and weight were taken. Approximately 1 g of leaf sample was used to find the proline concentration. Leaves were homogenized in 10 mL of 3% sulphosalicylic acid. This homogenized mixture was filtered through Whatman No. 1 filter paper. Then 2 mL of filtrate was taken in new tube and 2 mL of acid ninhydrin was added in it. The solution was then incubated at 100°C for 1 hour. Solution had developed color. The reaction was terminated in ice bath. The solution was then treated with 4 mL toluene. Separate layers were appeared. The intensity of red color was measured at 520 nm wavelength using spectrophotometer. The concentration of proline was obtained by comparing the values to the standard curve which was obtained by dissolving the known concentration of L-proline in sulphosalicylic acid [24].

The level of Malondialdehyde (lipid peroxidation) was measured in transgenic and non-transgenic plants (control). Cotton leaves under drought stress and control conditions were ground with 5 mL of 10% trichloroacetic acid. The leaves samples were centrifuged at 12000 rpm for 10 minutes. The supernatant was collected and 4 mL of 0.6% thiobarbituric acid was added. The reaction mixture was placed in water bath for incubation at high temperature for 15 minutes. The reaction was ended

in room temperature. It was centrifuged at 12000 rpm for 10 minutes. The supernatant was collected and the absorbance was measured at 450, 532 and 600 nm with spectrophotometer [25]. The concentration was calculated by given formula:

$$C (\mu\text{mol L}^{-1}) = 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56\text{OD}_{450}$$

For measuring the chlorophyll content, photosynthetic rate can be estimated. Pre weighed leaves samples were homogenized in 10 mL of 80% acetone to obtain the concentration of photosynthetic pigment (chlorophyll a and b) according to Arnon & Whately [26]. These homogenized samples were left overnight at room temperature. The two layers appeared. The upper layer was collected for the chlorophyll estimation. The absorbance of the extract was observed at 663 nm and 645nm. The concentration of chlorophyll a, b and total chlorophyll (mg gm⁻¹ fresh weight) was measured using Arnon's equations.

- Chlorophyll a = 12.7 (OD663)-2.6 (OD645)
- Chlorophyll b = 22.9 (OD645) - 4.7(OD663)
- Total Chlorophyll = Chlorophyll a + Chlorophyll b.

Results and Discussion

Confirmation of Gene (GHSP-26) in T3 Transgenic Cotton Progeny

Transgenic cotton plants being the first commercially genetically modified of their types are employed to enhance crop yield owing to their better immunity against drought stress. The history of development of such plants dates back to 1983 whereby first tobacco plant was genetically modified later on seeing the production of modified crop plant as well in 1987 at united states [27] Since then many plant species such as maize, soybean, cotton, and potato have undergone modification through incorporation of stress tolerant genes such as heat shock proteins HSPs, ultimately

increasing their yield [5]. These chaperones play a vital role in protecting plants against stress by rebuilding the normal protein and normal cellular homeostasis [28]. Currently the DNA of transgenic and non-transgenic plant (control) was isolated. The quantification of DNA was done by nanodrop spectrophotometer in 1 μL of sample. Then PCR product was analyzed on 1% agarose gel electrophoresis. DNA ladder (1000bp) was loaded in wells along the samples. A DNA band of 260bp was visualized on the gel in transgenic plants representing (GHSP-26) gene that is documented in Fig. 1. The samples which exhibited positive results were selected for further analysis.

Estimation of Drought Stress to Cotton Plants T3 Progeny

Plants become resistant by accumulating specific stress-associated proteins in order to survive in adverse environmental conditions [29]. Currently, after 60 days of planting, the transgenic and non-transgenic plants were employed 5 days drought stress for the evaluation of morphological parameters, physiological changes and biochemical responses of plants toward the drought stress. The visible effect of drought observed in transgenic and non-transgenic plants was depicted in Fig. 2.

Morphological Parameters

Present research was carried out to investigate the comparative performances of both transgenic and non-transgenic plants under drought stress conditions of 0DS and 5 DS, respectively. These conditions could primarily be associated to the impaired germination. The variation in responses was evident in all the selected morphological parameters. The visual analysis revealed that transgenic plants were more vigorous and lush green in colour as compared to non-transgenic plants.

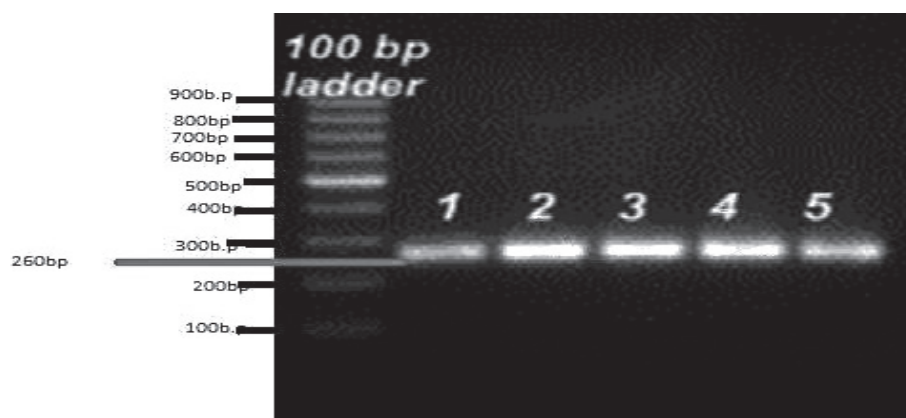


Fig. 1. PCR analysis of transgenic plants. Lane 1-5 amplified on 260 bp representing GHSP-26 gene in transgenic plants.

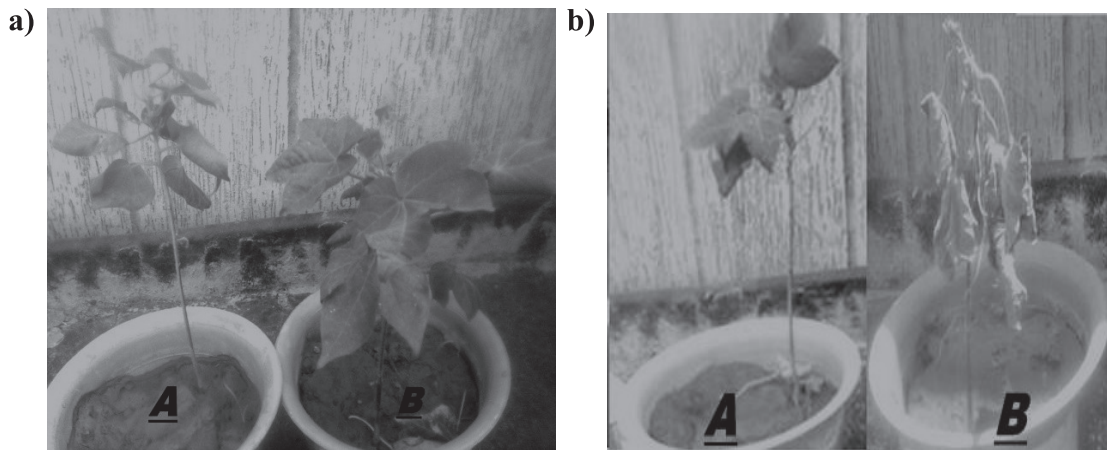


Fig. 2. Effect of drought stress on *Gossypium hirsutum*. In a) The transgenic (A) and non-transgenic (B) plants before the drought stress and in b) The transgenic (A) and non-transgenic plant (B) after the drought stress.

Number of Leaves

In the study of morphological parameters, it was observed that at both stress levels i.e., 0DS and 5DS transgenic plants exhibited better growth in terms of number of leaves as they were found to be healthier. It was observed that transgenic plants produced a greater number of leaves as compared to non-transgenic plants as the average number of leaves was found to be 13 and 14 in transgenic plants and 11 and 8 in non-transgenic plants, at 0DS and 5DS plants, respectively (Fig. 3a).

Total Leaf Area

Data analysis of total leaf area revealed that transgenic plants were less affected by drought stress as compared to non-transgenic plants. In general, transgenic plants were more resistant to drought stress as compared to non-transgenic (control) which indicated variable response of both genotypes and suggested more stress resistant behavior of transgenic plants under drought stress. Total leaf area was found to be reduced from 88cm to 85cm and 90cm to 83cm in transgenic and non-transgenic plants at 0DS and 5DS, respectively (Fig. 3b).

Shoot Length

In comparative analysis of initial shoot length, the transgenic plants demonstrated more increase in shoot length as compared to non-transgenic plants. Data presented in Fig. 3c) depicted about 12% increase in shoot length at 0DS in transgenic plants. However, drought didn't induce any stress in transgenic as well as non-transgenic plants for instance similar trend in increase in shoot length was noticed at 5DS. Drought stress leads to reduce in number of leaves per plant and leaf size and height, length of leaf; shoot length and total leaf area as the soil moisture declines. Encouraging results were marked in the case of transgenic plants

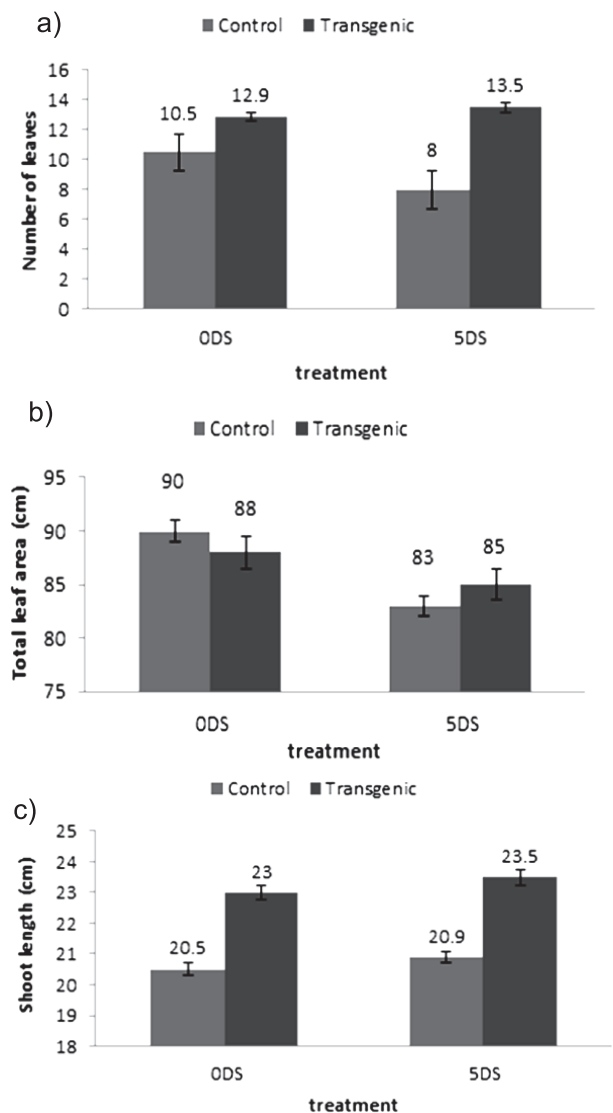


Fig. 3. Effect of drought stress on different morphological parameters in T3 progeny of cotton plants. a) Number of leaves, b) Total leaf area and c) Shoot length.

showing better adaptation and survival capabilities under stress conditions as evident from less variation in the morphological parameters during the study period i.e. wilting, shredding of leaves and decrease in growth rate [28]. The percentage increase in number of leaves, total leaf area and shoot length were well controlled in transgenic plants that helps them to survive in stressed environment. Comparatively less reduction in RWC in the transgenic plants could be attributed to the good osmotic adjustment.

Physiological Parameter

Drought stressed related effects are complex and interrelated on psychological processes of plants. Stomatal aperture is controlled by relative water content, and affects CO₂ diffusion and photosynthesis is directly affected by stomatal conductance.

Relative Water Content

The results of physiological analysis revealed that transgenic plants exhibited more water retention ability than non-transgenic ones as transgenic plants depicted approximately 14% more relative water content in contrast to non-transgenic plants. It was observed by the results demonstrated in Fig. 4a) that after the application of the stress of 5 DS, the loss of relative water content (RCW) in transgenic plants was less i.e., 6% however, non-transgenic plants were found to be severely affected by showing a significant reduction of about 26% in relative water content. Previously, in drought conditions maize (*Zea mays* L.) genotypes sensitivity to dehydration was studied and a decrease in stomatal conductance was recorded [30]. In the present study, Cotton plants have developed many adaptive traits for their survival during water scarcity mainly reduced tissue and cell dehydration. Stomatal opening and closing were more significantly affected under water deficit conditions with some affects being observed in case of cell membrane functions such as penetrability and decreased stability. Formerly water and drought stress were examined in Cotton genotype RAHS 187 [31].

Cell Membrane Stability

The transgenic plants demonstrated more cell membrane stability as demonstrated by the less affected metabolism than non-transgenic plants at 0DS. An inverse relation in cell membrane stability and drought stress was noticed i.e., with an increase in stress duration the constancy of cell membrane was found to be impeded. However; the response of transgenic plants was again significantly better as their cell membranes were found to be 14% more stable as compared to non-transgenic plants (Fig. 4b). One of the first indicators of water stress was reduced turgor pressure which is eventually expressed as wilting [32].

Photosynthesis

In physiological analysis, the process of photosynthesis exhibited similar trend to the previous parameters studied. The drought stress exhibited significant reduction in photosynthetic rate in both transgenic and non-transgenic plants (Fig. 4c). The photosynthesis rate lowered down from 12.4 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to 10.8 $\mu\text{mol m}^{-2}\text{s}^{-1}$ showing 13% arrest in photosynthesis in case of transgenic plants and from 10.2 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to 8 $\mu\text{mol m}^{-2}\text{s}^{-1}$ depicting approximately 26% reduction in case of non-transgenic plants when stress mounted from 0 DS to 5DS. The reason behind the reduction of the photosynthesis rate could be attributed to the variation in the factors such as stomatal conductance, transpiration rate which are interlinked and affect each other. Two near-isogenic species of rice (*Oryza sativa* L.), w-14 and w-20 were used to analyze the photosynthetic rate under drought stress on external application of silicon [33]. Under mild to moderate drought Stomata closure is responsible for decreased photosynthesis. Plants perform stomatal closure action to prevent further water loss during stress through transpiration. So, CO₂ diffusion is reduced [34]. Transgenic plants have shown less deviation in photosynthetic rate, transpiration and stomatal conductance as compared to non-transgenic ones.

Stomatal Conductance

Data analysis of stomatal conductance revealed an inverse pattern in comparison to other parameters. It was visualized in Fig. 4d) that the stomatal conductance at 0 DS and 5 DS was 3.1 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 3.2 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in case of transgenic plant at, and in the case of non-transgenic plants it was 3.7 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 3.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 0DS and 5DS, respectively. Transgenic plants showed negligible effect on stomatal conductance in stressed conditions.

Transpiration

Physiological analysis revealed that the rate of transpiration was decreased in both sets of plants after drought stress as water uptake was affected. Transpiration rate was 6.1 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 5.8 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in case of transgenic plants at 0 DS and 5 DS, respectively and in case of non-transgenic plants, it was 5.9 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 4.2 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 0DS and 5 DS, respectively. However, it was evident from the results that the transgenic plants portrayed less reduction of about 4% in transpiration rate as compared to the non-transgenic plants which exhibited approximately 28% impede in transpiration rate (Fig. 4e). It was due to ability of transgenic plants having small heat shock protein gene that was playing its protective role in the sustainability by enhancing the tolerance level of *G. hirsutum* making it more resistant to wilting [17].

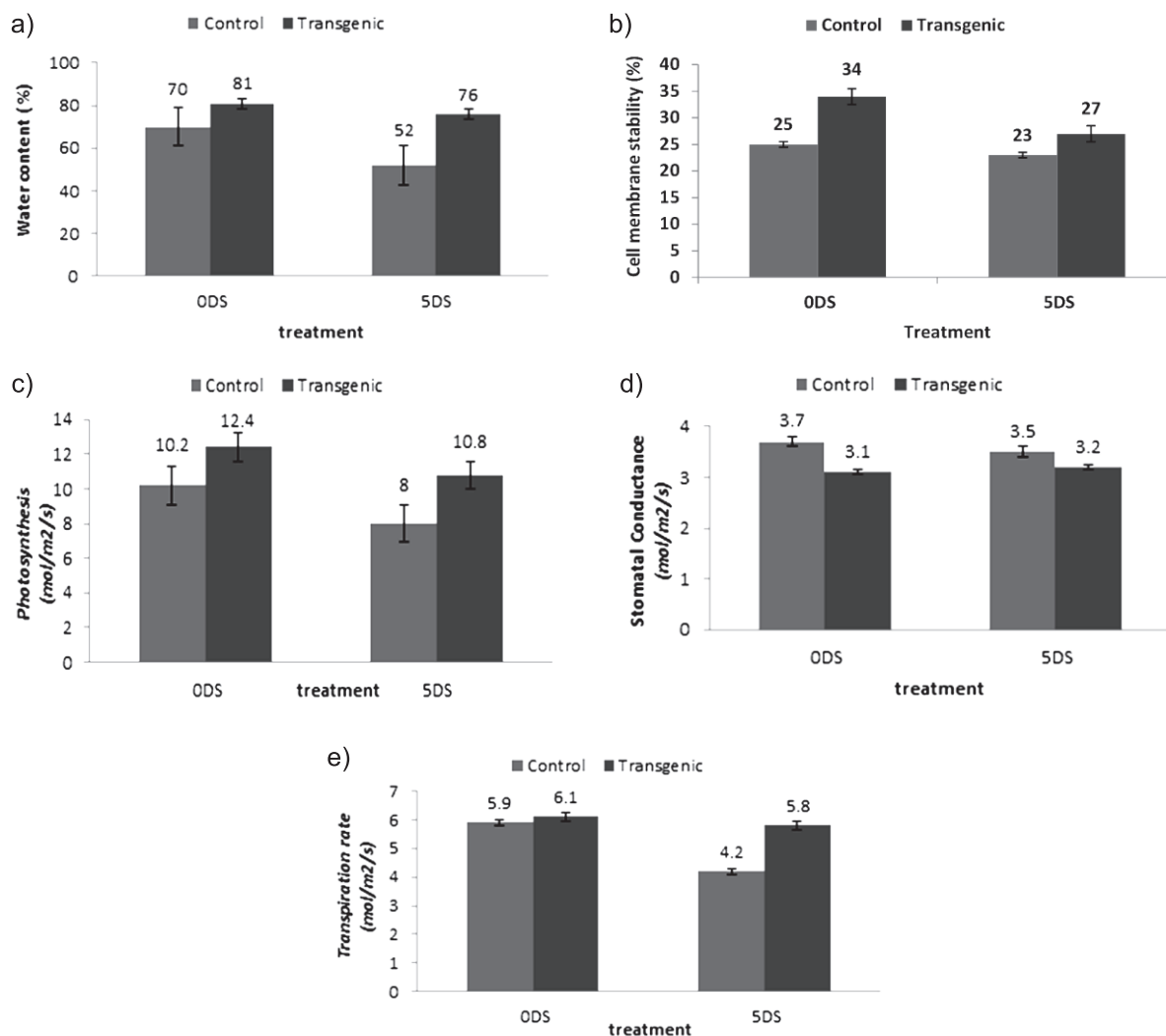


Fig. 4. Effect of drought stress on different physiological parameters in T3 progeny of cotton plants. a) Relative water content, b) Cell membrane stability, c) Photosynthesis, d) Stomatal conductance and e) Transpiration.

Biochemical Parameters

Proline Contents

In general, the proline content was low before application of stress in both genotypes and after the application of stress it was found to be increased. Non-transgenic plants exhibited more proline content at both ODS and 5DS than the transgenic plants. It was observed that high proline content is related to less membrane stability during stress conditions. In the study of biochemical parameters 0.35 mg/g proline content was calculated in non-transgenic plants at ODS that was significantly increased (approximately 67%) up to 0.58 mg/g by employing the drought stress. Conversely, in case of transgenic plants the proline content was 0.26 mg/g and 0.34 mg/g at ODS and 5DS, respectively, by exhibiting about 27% increase in proline content (Fig. 5a). Proline an osmolyte and organic in nature is present in the cells and tend not be harmful for plant cells even at high concentration.

Its synthesis and accumulation vary from plant to plant and generally a progressive increase in drought stress triggered the accumulation of proline in water stressed cotton plants. Zandalinas and co-workers analyzed chickpea and studied proline content and some other response towards drought stress [28]. In present study, the proline accumulation in transgenic plant was low as compared to the non-transgenic plants at 5 DS indicating their high tolerance and survival ability under stress condition and stability in harsh climates. However, the current findings are in contrast to those of [35], where transgenic Indica rice plants enhance its proline content in response to drought stress.

Malondialdehyde

Biochemical analysis revealed that the transgenic plants have shown low level of Malondialdehyde (MDA) as compared to non-transgenic plants because of many reactive oxygen species were produced that

damages cellular components such as lipid during stressed conditions i.e., lipid peroxidation. MDA was recorded 0.26 nmol/g and 0.8 nmole/g in transgenic plants at 0DS and 5DS, respectively, while in non-transgenic plants high level of MDA was recorded i.e., 0.48nmol/g and 1.22 nmol/g at 0DS and 5DS treatment, respectively (Fig. 5b). Thus, the transgenic plants were proved to be comparatively resistant to damage of lipids and triggered low lipid peroxidation by producing low quantity of Malondialdehyde (MDA) at 0DS as well as 5DS in relation to non-transgenic plants. In response to any environmental stress such as drought, the reactive oxygen species (ROS) is progressive which plays a destructive role by damaging the lipids, nucleic acid and proteins ultimately affecting the plant integrity [36]. This state is contributed to increased lipid peroxidation, protein degradation and DNA fragmentation leading to cells death. Along with overproduction of ROS during water stress conditions is also attributed to increased MDA content as MDA acts as an indicator of oxidative damage exclusively for lipid peroxidation [37].

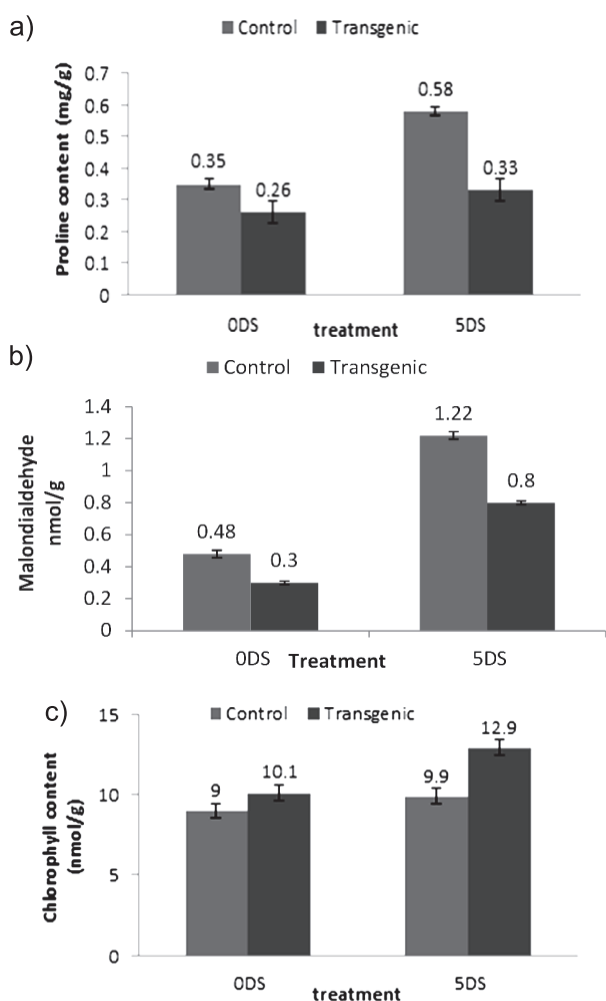


Fig. 5. Effect of drought stress on different biochemical parameters in T3 progeny of cotton plants. a) Proline content, b) Malondialdehyde content, and c) Chlorophyll content.

Chlorophyll Content

Data analysis regarding chlorophyll content documented that during drought stressed conditions chlorophyll content was increased both in transgenic and non-transgenic plants but transgenic plants showed better performance by exhibiting approximately 28% increment in chlorophyll content than non-transgenic plants which depicted only about 10% augmentation in chlorophyll content. Chlorophyll content at 0DS and 5DS was calculated as 9mg/g and 9.9 mg/g in non-transgenic plants while 10.1mg/g and 12.9 mg/g in transgenic plants at 0DS and 5DS, respectively (Fig. 5c). ROS were responsible for the reduction of chlorophyll content which were produced during drought stressed conditions. They have a direct relation with photosynthetic rate. This could also be linked to the plant's response towards drought stress whereby the relative water content in plant decreases hence affecting the plant turgor that ultimately causes the stomatal closure and decreasing the transpiration rate ultimately inhibiting the process of photosynthesis. A study was conducted by Ni et al. [37] on a gene GmNFYA3 in soybeans (*Glycine max* L.) focusing the chlorophyll content response to stress. It was concluded after the biochemical analysis that rate of decrease of chlorophyll content, in transgenic plant was comparatively less than non-transgenic plants indicating high stress tolerance level in transgenic plant. Survival and productivity of cotton plants depends on their ability to develop adaptive mechanisms to tolerate stress during stressed conditions.

Conclusion

It can be concluded that transgenic plants that contain GHSP 26 gene has more tolerance as compared to non-transgenic plants towards the drought stress conditions and have better survival capacity in harsh environment due to the capability of diminishing their metabolic processes which can be retained when the conditions turn to normal. Depending upon the degree of stress resistant genotypes generally survive and recover soon than susceptible ones due to rehydration. Non-transgenic plants show limited development that maintain their biological functions. Overall, one might presume on the basis of encouraging results of the experimental that transgenic plants are important alternative solution to the problems of the drought stress to be expected in water stress and water scarce regions of the world, that would be further triggered in the near future by climate change impacts.

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Conflict of Interests

The authors have no conflict of interests to declare.

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