Comparison of Glutathione Reductase Gene Expression in Drought-sensitive and Tolerant Soybean Lines using Real-time PCR

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Abstract

Background: Global climate change and associated adverse abiotic stress conditions, such as drought, salinity, heavy metals, waterlogging, extreme temperatures, oxygen deprivation, etc., greatly influence plant growth and development, ultimately affecting crop yield and quality, as well as agricultural sustainability in general. This study provides new insights into the analysis of the function of soybean genes in abiotic stress. Drought is one of the significant constraints that limit agricultural productivity. Some factors, including climate changes and acreage expansion, indicate the need for developing drought-tolerant Genotypes.

Materials and methods: The study of the expression Glutathione Reductase (GR) gene in soybean drought-tolerant and sensitive cultivars using real-time PCR. Seeds from (drought-sensitive) and (drought-tolerant) lines were planted under specific temperature conditions drought stress treatment, in the research greenhouse of Islamic Azad University of Arak, Iran. Changes in gene expression compared to reference genes were recorded using the formula $2^{-\Delta\Delta CT}$. Three technical replications were given for each cDNA sample related to each sampling and used to analyze test data from MINITAB16 software.

Results: The results showed that the threshold expression of gene expression (Glutathione) in the Pyramid line had the highest expression of drought resistance and the lowest expression of the Glutathione Reductase gene belonging to the Will line. The theory is also true that chaperone proteins produced during the plant growth cycle are not destroyed to express the Glutathione Reductase gene. The expression cycle of the Glutathione Reductase gene shows that the proteins produced by this gene have a high rate of expression and increase in cell drought stress. This gene expression continues until the pressure ends. The results showed that lines and cultivars with a weak expression against drought stress could have a high expression at the beginning of drought stress but a decrease in gene expression rate during stress. Drought stress-sensitive lines have a decreasing expression in the middle and end of stress during the stress period.

Introduction

Global climate change and associated adverse abiotic stress conditions, such as drought, salinity, heavy metals, waterlogging, extreme temperatures, oxygen deprivation, etc., greatly influence plant growth and development, ultimately affecting crop yield and quality, as well as agricultural sustainability in general. Plant cells produce oxygen radicals and their derivatives, so-called reactive oxygen species (ROS), during various processes associated with abiotic stress. Moreover, the generation of ROS is a fundamental process in higher plants and employs to transmit cellular signaling information in response to the changing environmental conditions. One of the most important consequences of abiotic stress is the disturbance of the equilibrium between the generation of ROS and antioxidant defense systems triggering the excessive accumulation of ROS and inducing oxidative stress in plants. Notably, the balance between the detoxification and generation of ROS is maintained by both enzymatic and nonenzymatic antioxidant defense systems under extreme environmental stresses. Although this field of research has attracted tremendous interest, it largely remains unexplored, and our understanding of ROS signaling remains poorly understood [1]. during stress conditions, overgeneration of ROS demolishes the equilibrium and causes

cellular damage, leading to programmed cell death (PCD) and decreasing plant productivity[2]. Drought is an adverse environmental factor affecting crops growth, development, and yield. Promising genotypes and genes with improved tolerance to drought are probably effective ways to alleviate the situation [3]. Drought is considered a significant threat to soybean production worldwide[4]. Drought is a severe but infrequent stressor affecting soybean production[5]. Abiotic stresses severely affect the growth, development, and ultimately yield of the plant, which results in heavy economic losses and food crises. Oxidative stress, associated with almost all the abiotic stresses, is due to the overproduction of toxic reactive oxygen species (ROS) ,including superoxide ions, hydrogen peroxide, and hydroxyl radicals. Plants combat oxidative stress via enzymatic and nonenzymatic machinery[6]. Drought stress reduced the chlorophyll content and relative water content in the soybean leaves and increased the osmolyte contents, antioxidant potential, and peroxidation of the membrane lipids [7]. GR is an increase in the activity of plant stress markers in the plant[8] By measuring glutathione Reductase, the degree of stress sensitivity in the plant can be measured. glutathione, Reductase is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays a key role in the cellular control of reactive oxygen species. Reactive oxygen species act as intracellular and extracellular signaling molecules and complex cross-talk between levels of reactive oxygen species, levels of oxidized and reduced glutathione and other thiols, and antioxidant enzymes such as glutathione Reductase determine the most suitable conditions for redox control within a cell; In general, insects and kinetoplastids (a group of protozoa, including Plasmodia and Trypanosoma) do not express glutathione Reductase or Glutathione biosynthetic enzymes [9]. The evidence indicates an an essential role of Glutathione, Reductase, and Glutathione Reductase as components of a peroxide-scavenging mechanism in the soybean cell body[9].

Materials And Methods

Drought-resistant and drought-sensitive seeds were obtained from Karaj Seed Breeding Research Institute, which are imported cultivars (Table 1). Seeds were sown in drought stress treatment at temperature ($30 \pm 2^{\circ}$ °C) and 16 hours of light ($20 \pm 2^{\circ}$ °C) and 8 hours of darkness, in the research greenhouse of Islamic Azad University, Arak city, Markazi province. Leaf was up to 7 days (prolonged stress). To examine the expression of drought stress genes on day 5, leaf sampling was performed by observing the tubularity of the leaves (simple leaves are open enough). After sampling, the samples were placed in liquid nitrogen and stored at -80° °C to extract total RNA. Total RNA was extracted using a Sina clone gene RNA extraction kit. Sinapure-RNA (cell culture) PR891620-EX6031. Using Nanodrop to determine the concentration (NanoDrop 1000 spectrometer), the absorbance of each sample was recorded at 230, 260, and 280 nm. CDNA (Sinnacolon First Strand DNA Synthesis Kit-50T) Sina Clone was used to remove genomic DNA and synthesize the first strand of CDNA. 2 µl of extraction buffer was poured into each sample in a 0.2 µl microtube. For 16 samples the material was mixed and, an additional unit (test unit) was added to the extraction solution (34 µl in total). 0.5 µl RT (RevertAid MMuLVReverse Transcriptase) per sample was added and, a complete additional unit of 8.5 µL was slowly inverted. 0.5 µl was added to each sample and, one RNase inhibitor unit was added to the microtube (total 8.5 µL) and inverted slowly for 30 seconds. 2 µl per sample and one unit more than the total dNTP mixture was added to the 34 µL tube and slowly inverted. Finally, 85 µl of DEPC water was added to

the tube for 16 samples, and another unit. The tube containing the CDNA solution was placed in Banmarry for 60 minutes at + 42°C and stopped the reaction at 85°C for 5 minutes. Samples were stored at -20°C. To design the primer the gene sequences of Glutathione Reductase were downloaded from the NCBI Gene Bank (National Center for Biotechnology Information, 2001). Dedicated primers were designed using Oligo7 software and the Primer 3 Plus site (Table 2). The primers were synthesized by SinaClone Company. PCR and electrophoresis were performed to ensure the specific performance of the primers. PCR products were observed on 1% agarose gel to provide specific amplification of genes less than 200 bp (Figure 1). Genes have introns. CDNA product size difference confirmed. CDNA was without contamination with genomic DNA. Real-time PCR used the specific primers in Table 1 to amplify the Target Gene and House Kipping Gene. The reaction components were placed in the form of Cyber green Master Mix (12.5 µl), one µl each, one µl of CDNA sample, 8.5 µl of Deps Water, and a total volume of 25 µl in glass lid tubes. The real-time reaction was performed in 40 cycles on the model device (China Bioer) FQD 48A. Changes in gene expression compared to reference genes were recorded using the formula $2^{-\Delta\Delta CT}$. Three technical replications were given for each cDNA sample related to each sampling and used to analyze test data from MINITAB16, Excel software.

Results And Discussion

Real-time PCR can quantify cDNA by amplifying energy using a threshold cycle that continues to resist. Real-time PCR programs to detect different types of stresses in resistance-specific resistance-specific sensitivity and specificity programs. In addition, real-time PCR performance requires test protocol performance by measuring sensitivity, specificity, accuracy, and reproducibility. Approved real-time PCR is an easy, fast, and accurate way to monitor the results of diagnosis and treatment in a stressful environment[10]. Real-time PCR can analyze hundreds of samples in a day[11]. The results of analysis of variance tables of CT[1]s obtained from the analysis of real-time PCR data showed a significant difference at the level of 1% between the studied lines (Table 3). The results of comparing the threshold cycles of drought-sensitive and tolerant lines in soybean showed that the pyramid line had the highest gene expression and showed a significant difference at the level of 1% with other lines. This reported that the pyramid line is also resistant to living stresses[12]. Williams, Columbus, and Douglas lines did not differ significantly in the expression of drought resistance gene, these lines had the highest expression of GR gene to drought stress after pyramid line, called semi-drought resistance lines. The lowest expression of the GR gene and the most sensitive to drought stress will line. Although tiffin, Xiaowuyie, and Amcor89 lines did not differ significantly in GR gene expression, these lines showed high sensitivity to drought stress (Figure 2). In the study of drought stress resistance and sensitivity using cluster analysis and using Euclidean distances, it can be concluded that Pace and will lines were placed in a separate cluster. The pyramid line, which had the highest expression of GR gene in drought resistance expression, was placed in a cluster with s39-99, Columbus, and PI475822a lines. Lines in a cluster with the pyramid line have the genetic potential to increase drought stress resistance. The Amcor89 and xiaowuyie lines were in a separate cluster, and the will, Douglas, and Crowford lines were in a separate cluster. The Euclidean distance diagram (Figure 3) for future crossings in improving and increasing the expression of the GR gene is forward-looking, showing the best location for subsequent crossings by the dashed line. The results of the plot matrix for comparing the first and second threshold cycles (CT1 and CT2) according to the formula $2^{-\Delta\Delta CT}$ showed that the most significant difference in the

first and second threshold cycles is related to the Dare line. On the other hand, the high expression results of the Pyramid line gene start in the threshold 22 cycles, while in the threshold cycle, the Dare line starts in the 30th cycle. Comparison of GR gene expression in comparing the first and second threshold cycles (CT1 and CT2) showed that GR gene expression was strongly reduced in Crawford and Will lines. In comparison, the expression of the GR gene in the CT1 and CT3 threshold cycles of the Douglas line was decreasing, but in the CT3 threshold cycle, this gene expression was increasing. The results of comparing the GR gene expression threshold cycle in the second threshold cycle showed that they would line acted out of expression, meaning that due to the lack of GR gene expression in the will line, the real-time PCR device could not calculate this amount of gene expression (Figure 4). Comparing the second and third threshold cycles in GR gene expression in the 22-line will cycle again showed the lowest gene expression. The lines that are expressed earlier are indeed the lines that feel the drought stress sooner and have to release their genes and enzymes sooner, but this gene expression and stress identification must continue while lines like tiffin, Amcore and, Xiaowuyie had earlier gene expression, but this gene expression is not continuous, so it is observed in stress-sensitive cells. Clearance of free radicals in cells by resistance genes Clearing them is acceptable when gene expression continues until stress is relieved. Lines that express their stress resistance genes earlier are not the reason for their stable resistance, but a line should have both fast and high gene expression and continuous expression of that gene. Resistance in lines is acceptable when the expression of amino acids and resistance-building proteins is done to the end of stress relief. Accelerated gene expression is not a sufficient reason for resistance to drought stress. Sometimes some lines have a high rate of gene expression, but it is observed that the cell does not show sufficient gene expression, and the line will not be sensitive to stress, or the cell will lose its vital activity due to stress and will disappear. Compared to the first and third threshold cycles (Figure 5), the odel line had the fastest expression in cycle 20, but the result of continued expression of the GR gene was again decreasing. As mentioned, a line can be called resistant that continues to express its stability against drought stress. The lines that have their threshold changes compared in Figure 6 and are outside the standard line of incremental gene expression are the lines that have decreased threshold changes. The will line had the lowest expression with a decreasing trend compared to the second and third thresholds. In environments where water is limited, genetic improvement of a crop for drought resistance is an economically attractive option [13]. However, despite the extensive resources committed to soybean breeding, progress in improving drought resistance has been slow for several reasons. Identifying lines with the highest yield potential under optimum moisture conditions is an important selection criterion in soybean. Conversely, evaluating lines from low-yielding environments under drought conditions is often not considered, because small yield differences among lines do not separate high yielding genotypes from low yielding genotypes [14]. Historically, the emphasis in soybean breeding was upon resistance to biotic stress rather than abiotic stress such as drought, due to the complexity of trait evaluation. This unfortunately, resulted in a narrow genetic base for initiating drought resistance breeding programs [15]

Table 1

Drought stress lines for determination of drought stress resistance using Glutathione Reductase gene expression

LINE NAME	Pace	Dare	odell	Prohio
LINE NAME	S39-99	crowford	Douglas	tiffin
LINE NAME	pella	PI 475822 A	Columbus	will
LINE NAME	Williams	Amcor 89	Xiao Wuyie	pyramid

Table 2

Primer characteristics used and reference gene

Primer name	Primer sequence		N.N	Primer size	T.m	%GC	M.W g/mol
Glutathione Reductase	F	GCGCCCGAGTCACTCATCA	19	216	62.36	63.16	5733.8
	R	GCGACCCAACCAAATCACAGTCAA	24		64.06	50	7268.8
FBOX	F	CTAATGGCAATTGCAGCTCTC	21	93	53	47.6	6381.2
Glyma12g05510	R	AGATAGGGAAATGGTGCAGGT	21		56	47.6	6599.3
Forward: F							
Rivers: R							
Molecular Weight:M.W							
Number of Nucleotides:N.N							

Table 3

Analysis of variance for CTs obtained from PCR real-time analysis

S.O.V	DF	SS	MS
Line	15	461.122	30.741**
Error	32	58.627	
Total	47		
** significant 1%			

Declarations

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Conflict of interest

The authors declare that they do not have any conflict of interest.

Consent to Participate (Ethics)

I undertake to submit a standard and standard article to that journal and, if necessary, provide raw data for this research. This article is taken from my student doctoral dissertation and if I have used the research of others, their names are mentioned in the article. I know that it is against the law to submit this article to several journals or articles published or accepted in other journals at the same time. I know references, including all books, magazines, and websites, and other personal research is carefully conducted. I know the person in charge and his colleagues in the article and I take care if there are no unrelated names. The person in charge of the article has played a key role in the preparation, preparation, design, and execution of the article, and other authors have also been mentioned as collaborators in the article. I am sure that there is no other name than the authors of the article. I also know that all the authors of the article have read it and agreed to submit it. In the article, I have explicitly mentioned the dangers that humans or other creatures face due to the research of any author. I undertake to notify the journal whenever I find an error or inaccuracy in my article, correct it or return the article. This article has not been published in any other source. Also, all authors are informed and satisfied with their publication and position in the article. Submitting this article also means that the research institute or sponsor has given its consent to publish the research to the responsible author. I also undertake not to submit this article or article with similar results in Persian or English or any other language to any other publishing authority during the review or acceptance process. Otherwise, the publisher reserves the right to complain. I undertake not to enter any source or content or data in the article without providing and mentioning the source, and legal permission for the mentioned sources has been obtained from the original source.

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Author contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mohammad Hassanvand. The first draft of the manuscript was written by Mohammad Hassanvand and I commented on previous versions of the manuscript. I read and approved the final manuscript.

Taxonomy:

• Conceptualization: Mohammad Hassanvand; Methodology: Shahab Khaghani; Formal analysis and investigation Mohammad Hassanvand and ezatollah sedaghatfar; Writing - original draft preparation: Mohammad Hassanvand; Writing - review and editing: Mahdi Changizi and Masoud Gomarian; Resources: Mohammad Hassanvand; Supervision: Shahab Khaghani.

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Figures

Figure 1

Confirmation of CDNA specific amplification for Glutathione Reductase (GR) and R.G. reference genes: FBOX Glyma12g05510

Figure 2

Comparison Fold Chang of lines in Glutathione Reductase gene expression

Figure 3

Dendrogram for hybridization that is associated with increased Glutathione Reductase levels

Figure 4

Comparison of the threshold cycle of the first cycle data with the second cycle

Figure 5

Comparison of the threshold cycle of the first cycle data with the third cycle

Figure 6

Comparison of the threshold cycle of the second cycle data with the third cycle

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