MOLECULAR CHARACTERIZATION AND RESPONSE OF FIVE SOYBEANS GENOTYPES TO PEG TREATMENT

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Plants are challenged by different types of stresses due to their lifestyle. water-deficient is the main stress that faces plants and may be caused by drought, heat or salinity. There is a growing demond for improving drought tolerant species that can adapt with limited water sources. The limiting factor for drought tolerance is the plant ability for conserving water content, turgidity, membranes integreity, osmoregularity, pigments contents and photosynthetic activity, under water-deficient stress (Wehner *et al.*, 2016). Acquisition of drought tolerance in plants is usually associated with re-regulation of genes encoding structural and protective protiens such as Late Embryogenesis Abundant (*LEAs*), Dehydrins (*DHNs*) (Omar *et al*., 2013) antioxidant enzymes (Omar *et al*., 2012), osmolytes molecules, amino acids (proline) to protect the cells from deleterious effects resulted in tissue dehydration. Preservation of cell membrane and photosystem component against osmosis changes and oxidative damage occurred during dehydrtion consider a great componant of tolerance (Wehner *et al.*, 2016). Induction of variety of antioxidant enzymes; superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) to scave reactive oxygen species (ROS) upon stress modulate the oxidative damage during water deficit stress (Caverzan *et al*., 2016; Omar *et al*., 2012). Stressed plants encode for low molecular weight defensive proteins, heat schok proteins (HSPs) and proline for osmo-regulation and conserving membrane structure from electron leakage and keeping photosynthetic capacity (Wang *et al.*, 2011).

Drought resulted in a contest between photosynthesis down-regulation and protective proteins up- regulation. The result of this contest determines the potential drought tolerance. Wehner *et al.* (2016) reported that there is down-regulation for developmental genes of chloroplast and photosynthesis while there is up-regulation for macromolecules degradation to recycle resources for tolerance during drought. The gene family of light-harvesting complex- PSII (LHCII) encode CAB3 protein that form a complex through binding chlorophyll and carotenoids in the thylakoids to transfer the light energy to PSII (Simpson *et al.*, 1986). The CAB3 protein level is correlated to chlorophyll presence (Nikolaeva

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et al., 2010; Simpson *et al*., 1986). *DHNs* are tolerance related genes and member of late embryogenesis abundant *(LEAs)* family which has a role in preventing cell dehydration during water deficit conditions. Plants show different strategies against drought; some adaptive genotypes may conserve photosynthetic efficiency but up-regulate protective proteins such DHN (Benešová *et al*., 2012). Others manage their water resources by lowering their water consumption through lowering transpiration rate at the early stages to save water for late stages if water-deficient still persist (Gholipoor *et al*., 2013).

The study aimed to nominate drought-adaptable varieties of soybean among the widely distributed genotypes depending on some molecular tools for screening expression of related defensive components and genes-concerning drought stress such as *CAB3*, *DHN1*, *DHN2* genes.

MATERIALS AND METHODS

I. Plant materials

Five parental soybean's genotypes (*Glycine max* L.) 2n=40 named as D89-89-40, Giza 111, Giza 83, Toano and Line 30 that are widely cultivated were used in this study

II. Methods

1. Experimental and seedlings growth conditions

The seeds of these genotypes were sown in pots containing sterilized Patmos at a greenhouse at 25°C±1. Polyethylene glycol 6000 (PEG 6000) was used to induce water stress on genotypes seedlings with concentrations of; zero, 5, 10 and 15% (w/v) to represent control; -0.05, -0.15 and -0.3 MPa (water potential), respectively.

2. Seedling length and water content

The shoot height and root depth of 30 days- old for both control and treated seedlings were measured as seedling length trait expressed in cm.. Relative water content (RWC) of seedling was determined by calculating the difference between fresh and dry weight on base of fresh weight according to Elsheery and Cao (2008). The rate of length loss was also determined

3. Estimation of photosynthetic activity and pigments content

The photosynthetic activity rate was estimated in leaf discs using the ADC-LAC4 portable system (The Analytical Development Company Ltd, Hoddesdon, Herts, UK) that based on system of infrared gas analysis. Maximum quantum yield of photosystem II (PSII) was estimated according to Genty *et al.* (1989). While pigment content of chlorophyll-a, chlorophyll-b and carotenoids ($\mu g.g^{-1}FW$) was calculated according to Lichtenthaler and Wellburn (1983)**.**

4. Estimation of lipid peroxidation products and electrolyte leakages

The thiobarbituric-reactive products (TBA), equated with

malondialdehyde (MDA), was used as indicator for lipid peroxidation (Elsheery and Cao, 2008). The amount of MDA was colorimetrically in leaves using spectrophotometer (UV190IPC) at 532 nm. The TBA-reactive products (MDA) were expressed as nmol.g^{-1}DW. Electrolyte leakages (EL) of individual seedlings were estimated using a conductivity meter (Adwa-AD32). Leakage rate of electrolytes was expressed in $\mu S \cdot cm^{-1}.FW \cdot h^{-1}$ and calculated as the net conductivity of the solution with seeds immersed for 1 h divided by the total conductivity after boiling according to Omar *et al.* (2012).

Estimation of proline

Free proline content (µg. g^{-1} DW) was estimated according to Bates *et al.* (1973).

Protein electrophoresis

Protein electrophoresis was carried out as SDS-PAGE according to Laemmli and Favre (1973). The total soluble protein was extracted according to Dure *et al.* (1981) and quantified according to Bradford (1976) to determine protein concentration in the tested samples.

Antioxidant enzyme profiling using one dimensional native PAGE

Native PAGE was carried out according to Weydert and Cullen (2010) for determining antioxidant isozymes activities of superoxide dismutase (SOD), Guaiacol peroxidase (POD) and ascorbate peroxidase (APX) according to Anjum *et al.* (2012).

Semi-quantitative reverse transcriptase-polymerase chain reaction (sqRT-PCR)

The cDNA transcript was synthesized using a 1 µg of total extracted RNA form each sample according to the protocol supported by using SensiFAST™ cDNA Synthesis Kit. Thereafter, a 1 µl of cDNA was used as a template in a 25 µl of reaction volume according to the instructions supporting with GoTaq® Green master Mix, 2x (Promega USA). The technique of sqRT-PCR was carried out using gene specific primers designed for selected gene sequences as expressed sequence tags (ESTs) on data base of National Center for Biotechnology Information (NCBI) as shown in Table (1). Expression levels of selected genes were semi-quantified against an internal constitutively expressed control gene (*actin* Ac.BW652479). PCR program was optimized for each gene to yield optimal transcripts differences. Optimized cycle number for each gene were 28 cycles for actin and *CAB3* genes as well as 29 cycles for *DHN1* and *DHN2* genes. The general program was 95°C for 5 min, followed by the cycles of 94°C for 1 min, 58-59°C for 1min, 72°C for 1 min and a final extension step at 72°C for 7 min.

RESULTS AND DISCUSSIONS

1. Seedling length and relative water content (RWC)

Seedling length (represented by shoot height and root depth) and RWC traits of all soybean genotypes were decreased along with drought stresses com-

pared with the control (Fig. 1). The studied genotypes showed different percent of the decreasing in seedling length and RWC (Fig. 2). The loss in total seedling length in response to the highest stress (15% PEG) was arranged in descending order as Giza 83 (51.8%), Giza 111 (27.7%), Toano (27.4%), D89-89-40 and Line 30 (23.4%) genotypes (Fig. 2a). Rate of reduction in RWC trait upon the highest stress (15% PEG) was arranged in descending order as Giza 83 (74.8%), Toano (59.5%), Giza 111 (21.1%), D89-89-40 (15.9%) and Line 30 (15.2%) (Fig. 2b). The decrease in growth parameters and RWC in our study pointed out that Giza 83 genotype was the most sensitive genotype, while Line 30 and D89-89-40 genotypes were the most tolerant ones

Drought-tolerant plant species keep high RWC compared with drought-sensitive species. The high RWC of tolerant genotypes revealed their ability for water absorption under water deficient conditions, and suggested RWC as a good indicator of plant performance under drought stress (Kumar and Sharma, 2010).

2. Photosynthetic pigments content and activity

Photosynthetic activity (Fv/Fm) and photosynthetic pigments (Ch-a, Ch-b and carotenoids) content were significantly decreased in all soybean genotypes by increasing drought stress (Fig. 3a-e). The highest decrease in photosynthetic pigments was occurred in genotypes Giza 111, Toano and Line 30 genotypes. The least reduction in chlorophylls and carotenoid contents was shown in D89-89-40 genotype which suggested its ability to withstand drought stress. The highest decrease in photosynthetic activity (Fv/Fm) was obtained by Giza 111 genotype (Fig. 3f), while the lowest decrease in Fv/Fm was obtained by Toano and Line 30 genotypes (Fig. 3f).

The reduction in photosynthetic pigments (chl-a, chl-b, chl-a+b and carotenoids) under drought stress was reported in soybean (De Ronde *et al.*, 2004; Zhang *et al.*, 2007) and in leaves of mango (Elsheery and Cao, 2008). The reduction could be attributed to membranous injury of chloroplast, swelling, lamellae vesiculation and distortion and lipids droplets appearance due to stomatal closure or other non-stomatal mechanisms (Elsheery and Cao, 2008). Thus the least reduction in photosynthetic activity and pigment content of D89-89-40 might be due to its ability to retain the RWC reduction, saving the chloroplast turgidity, preventing lipid peroxidation and membranous electrolyte leakage (Lawlor and Cornic, 2002). This was supported by the well-developed antioxidant system represented by SOD, POX, APX and high proline content. Proline accumulation in stressed plants was suggested to have osmoprotectory and antioxidant role that enable conserving chlorophyll content and preventing lipid peroxidation (Shevyakova *et al*., 2009).

3. Malondialdehyde (MDA) content and electrolytes leakage (EL) rate

Increasing drought stress in all soybean genotypes resulted in a gradual and significant increase in MDA content and the rate of EL as shown in Fig. (4a, b). Giza 83 genotype was severely affected by increasing PEG concentration where the highest values of both MDA content and EL rate were recorded. D89-89-40 genotype was suggested to be the most drought tolerant genotype as it had the ability to keep its plasma membrane from peroxidation under drought stress as it had low values of MDA content and EL rate.

Electrolyte leakage and MDA are related to osmosis disturbance and oxidation of the cell by trigged ROS upon stress. The ROS that produced upon stress may act as a signal in tolerant species or cause membranes peroxidation in sensitive species, depending on the antioxidant system efficiency in ROS scavenging (Caverzan *et al*., 2016). Accordingly, low MDA level was correlated to the highest expression level of total antioxidant activity observed at D89-89-40 genotype (11 isozymes) and line 30 (10 isozymes) which suggested them as tolerant one among the studied genotypes. D89-89-40 was suggested to be the most drought tolerant genotype as it had the ability to keep its plasma membrane from peroxidation under drought stress as it had low values of MDA and EL. Low concentration of MDA has been associated with drought tolerance in chickpea (Moussa and Abdel-Aziz, 2008). In contrast, Giza83 had high oxidation level that was correlated to its low antioxidant activity (9 isozymes) and drought sensitivity.

4. Proline content

Proline content was significantly varied among the genotypes (Fig. 4c). Proline content increased by drought stress and reached its highest level with 5% PEG in Giza 111 genotype, with 10% PEG in Line 30 genotype and with 15% PEG in D89-89-40.genotype. Giza 83 genotype had constructive elevated levels of proline content in response to the increasing of water stress. On the other hand proline content decreased gradually in Toano genotype by increasing drought stress.

Increasing in proline content in D89-89-40 genotype (with 15% PEG) followed by Line 30 genotype (with 10%) and Giza 111 genotype (5%) while decreased in Toano suggested that D89-89-40 and Line 30 genotypes (with superiority to D89-89-40) are drought tolerant, while Giza 83 and Toano genotypes are sensitive ones based on antioxidant activity and proline content. This suggestion endorsed by Wang *et al.* (2011), who correlated stress tolerance/ sensitivity to the genotype antioxidant activity and osmosis regulation by proline. The increase of proline content is endorsed by Kim *et al.* (2004) who noticed an increase in the levels of free amino acids normally present in protein such as glutamine, histidine, isoleucine, leucine, phenylalanine, proline and valine upon water stress

due to the hydrolysis of protein in stressed conditions. Proline has a role in stress tolerance as it acts as osmotic-stabilizing macromolecules for membranes, besides detoxifying tissues from excess nitrogenous compounds such ammonia (Rabe, 1999). Drought stress forces plants to do hydrolysis for their proteins to amino acids; mostly proline leading to soluble proteins reduction (Kim *et al.,* 2004). The decrease in proline content in Toano with all stress treatments might be due to formation of new proteins during adaptation process of the plant to stress**.** However, Toano is suggested to be a drought-sensitive genotype due to the reduction in its proline content under stress. This suggestion is confirmed by Wang *et al.* (2011) who reported proline as osmoregulatory solutes that enable *J. curcas* for adaptation to drought and poor conditions.

5. Total protein fractionation

SDS-Protein fractions of the treated and non-treated (c) soybean seedlings can categorize the genotypes into three groups according to their response to drought treatment (Fig. 5). Group "I" was represented by Giza 111 and Giza 83 genotypes which revealed a great loss for a number of bands almost locates at range of 21~25 KDa, 37~48 KDa and 90~137 KDa, especially with 5% PEG treatment. In regard to protein biosynthesis, Giza 83 showed more depressive response to drought treatment than Giza 111 genotype which showed an induced band at 19 KDa with 15% PEG treatment. Moreover, the quantitative level of protein biosynthesis in both Giza 111 and Giza 83 genotypes showed a reduction as revealed by the band intensities, especially with 5% and 15% PEG treatment. Group "II" was represented by Toano and Line 30 genotype which characterized by elevated level of protein biosynthesis as bands intensities increased. Besides induction of new three protein bands which ranged from 19 KDa to 37 KDa, there was no loss for any band and the response was mainly obvious with 15% PEG treatment. Group "III" was represented by D89-89-40 genotype which its response was stable to some extent as it neither showed loss or induction of bands but only increased concentrations of protein intensities. This result suggested that D89-89-40 genotype might do the osmoregulation of its cellular content with a different mechanism that did not affect its protein content.

Under stress, proteins may be hydrolyzed as a result to osmotic adjustment changes in the cell (Sankar *et al*., 2007). Also, the decrease of a certain protein accumulation or loss of certain protein could occur (Mohammadkhani and Heidari, 2008). Newly synthesized proteins in the profiling pattern of the studied soybean genotypes may associate in the acquisition of drought tolerance. Abdelgawad *et al.* (2015) reported that newly appeared protein bands upon drought stress occurred in different drought tolerance barley genotypes. Also the protein content may be increased at the expense of amino acids under stress that affects these compounds inter conversion.

6. Antioxidant enzymes activities

Differential expression pattern of the studied antioxidant enzyme activities showed different responses in all tested genotypes as shown in Fig. (6). The banding pattern for SOD (Fig. 6a) showed that there was no induction for new isoforms for both Mn-SOD and Fe-SOD, while the enzyme activity regard to expression mostly increased in different genotypes as their band intensities became intensive. D89-89-40 and Line 30 genotypes showed the highest Mn-SOD activity with all PEG treatments. D89-89-40 genotype was distinctive in its naturally content of Fe-SOD where it was expressed six isomers (Fe-SOD_{1-6}) than other genotypes that expressed only four isomers (Fe-$SOD_{1\sim4}$). D89-89-40 and Line 30 genotypes revealed a general Fe-SOD induction attitude with regard to Fe-SOD expression with all PEG concentrations. The other genotypes could be arranged according to Fe-SOD expression as Giza 111, Toano and Giza 83 where the Fe-SOD induction was repressed with some PEG concentrations.

POX banding pattern (Fig. 6b) showed that D89-89-40 genotype had only three isomers against Line 30 genotype which had four isomers, while Giza 111, Giza 83 and Toano genotypes had four isomers. This pattern changed upon all PEG treatments which induced a new isomer in both D89-89-40 and Giza 111 genotypes. Additionally, these two genotypes had an induced peroxidase expression level upon all PEG treatments but reached the maximum level with 10% PEG for Giza 111 genotype and with 15% for D89-89-40 genotype. Thus, D89-89-40 and Giza 111 genotypes patterns were distinctive with expression regard to POX expression. Induction of new POX isoforms occurred in Toano genotype only by 15% PEG treatment, while 10% PEG resulted in a turn off expression of POX_2. Giza 83 genotype showed an aggressive reduction in expression level of all POX isoforms when raising PEG treatments as measured by band intensity which didn't show any induction/suppression for appearance of new isomers. Toano and Line 30 genotype showed a gradual decrease in expression level along with raising PEG concentration.

Banding pattern of APX activity in different soybeans genotypes occurred as a single isoform showed changes in intensity according to PEG treatment (Fig. 6c). A gradual decrease in APX expression level was obtained by increasing the concentration of PEG treatments, the decrease incidence was moderate in Giza 111 genotype, but was severe and reached to the basal level of expression less than control in Giza 83 and Toano genotypes with 15% PEG treatment. Line 30 and D89-89-40 genotypes exhibited an increase in APX expression level, depending to the PEG concentration. In Line 30 genotype, the enzyme activity increased gradually with 5% and 10% PEG treatment but decreased with 15% PEG treatment. In D89-89-40 genotype, the enzyme

level increased with 5% PEG (maximum increase) and 15% PEG treatments but decreased with 10% PEG treatment.

The highest number of total antioxidants isozyme was detected in D89-89-40 genotype (12 isoforms with control and all PEG concentrations), followed by Line 30 genotype (10 isoforms with control and 15% PEG), Giza 111 genotype (10 isoforms with all PEG concentrations), Toano genotype (10 isoforms with only 15% PEG), Giza 83 genotype (9 isoforms with control and all PEG concentrations).

D89-89-40 and Line 30 are more adaptive genotypes for drought since their successes in maintaining their elevated antioxidant system response to high PEG concentrations. The importance of the antioxidant system in keeping cell vitality and protecting the cell from ROS produced in response to stress (Omar *et al*., 2012). The efficiency of antioxidant system in scavenging ROS is a limiting factor in stress-tolerance because it determines the action of ROS either in oxidative damage for the cells or in trigging the defense system by signaling (Caverzan *et al.*, 2016). Increased levels of SOD, POX and APX in water-stressed plants were reported by Weydert and Cullen (2010). The efficient antioxidant system that D89-89-40 genotype possess might have endorsed its tolerance through preventing the oxidative damage of the cell which was osmo-regulated by high level of proline even under high drought stress (Caverzan *et al.*, 2016; Wang *et al*., 2011).

sqRT-PCR of CAB3 and DHNs

Photosynthetic-related gene (*CAB3*) and drought-related genes (*DHN1* and *DHN2*) showed a wide range of expression patterns among the studied soybean genotypes under 15% PEG treatment, compared to the control and referenced by actin expression (Fig. 7). Based on the band intensities of the stressed plants, the genotypes were classified into two groups. Group "I" included Giza 111, Giza 83, and Toano genotypes; while group "II" included Line 30 and D89-89-40 genotypes.

The incidence of water-stressed conditions by 15% PEG treatment resulted in partial suppression of *CAB3* and *DHN1* expression in both groups "I" and II but group "II" was less affected. With regard to *DHN2* expression, it decreased in group "I" while increased in D89-89-40 genotype but didn't change at line 30 genotype. This suggested that both D89-89-40 and Line 30 genotypes were relatively drought-tolerant with superiority to D89-89-40 genotype which showed more of *DHN2* induction under stress conditions with slightly suppression to *DHN1* transcript and a moderate reduction to *CAB3* transcript.

The CAB3 protein binds and assembles chlorophyll into the light-harvesting complex (Simpson *et al.*, 1986). All genotypes got a reduction in both photosynthetic activity (maximum in

Giza 111) and pigments content under stress but the pigments reduction was non-significant for D89-89-40 and highly significant for Giza 83. This was correlated to the *CAB3* gene expression that reduced in all genotypes with less reduction in D89-89-40 and Line 30 compared to Giza 111, Giza 83 and Toano. Decreasing photosynthetic pigments and activity might be attributed to reduced synthesis of the main chlorophyll pigment complexes that bind by PSII CAB protein encoded by the *CAB* gene family (Nikolaeva *et al.*, 2010). Consequently, CAB protein amount is correlated to chlorophyll amount in plant leaves, as CAB protein decreases in case of chlorophyll depletion in stressed plants through transcriptional level regulation for its mRNA (Simpson *et al*., 1986). Reduction in *CAB3* under drought stress is usually correlated to the elevated levels of abscise acid that synthesized under water-deficient conditions (Li *et al*., 2017). All genotypes showed regression in *DHN1* expression upon PEG treatment with least decrease in D89-89-40 and Line 30. It is likely to believe that D89-89-40 followed by Line 30 are more drought tolerant than other genotypes. This suggestion is endorsed by the protective function of DHNs where it stabilizes macromolecules and structural proteins such in membranes (Savitri *et al*., 2013). The plants respond to stress by re-regulation of genes that encode for *LEAs* transcripts, *DHNs*, osmoprotectants, ROS and apoptosis signals that lead to differential expression challenge between two groups of genes which either accelerate or delay leaf senescence that determine drought tolerance potentiality (Wehner *et al.*, 2016). This endorsed the differential expression of *DHN1, DHN2,* ROS and proline as oxidative- and osmo- protectants in studied soybean genotypes. DHNs may act as antioxidative defensive proteins or scavenger for ROS, or indirectly through detoxifying metals by binding to prevent ROS production (Hara *et al*., 2005). Drought-tolerance is limited by accumulated DHN quantities (Lopez *et al.*, 2002). DHNs proteins are responsible for recovering cell from dehydration (Fuganti *et al*., 2017). Thus, drought-sensitive genotypes may insufficiently accumulate DHNs (Savage, 2003). For example, *DHN1* and *DHN2* expression was significantly increased in maize under stress condition (Klimešová *et al.*, 2017).

In the present study, the tolerance potentiality varied among the soybean genotypes and PEG concentrations. Increasing water stress cause lipid peroxidation changes in terms of MDA consequently increasing rate of membrane EL, so PEG treatments were impressive to all soybean genotypes. D89-89-40 might be the most adaptive genotype among species followed by Line 30 genotype, while Giza 83 followed by Toano genotypes are the most sensitive one but Giza 111 was moderate under high PEG concentrations. The D89-89-40 genotype is likely suggested to follow a strategy of conserving its membrane stability and photosynthetic efficiency by decreasing the severe effects of ROS produced under drought stress, developing scavenging system like anti-

oxidant enzymes and increasing the transcription rate of protective proteins as *DHN2* and *CAB3*.

SUMMARY

Improving of drought tolerant genotypes becomes a principle demand, especially for economical crops like soybean. Five soybean genotypes were treated with different polyethylene glycol 6000 concentrations (5%, 10% and 15%) to determine the potential tolerance of the genotypes under maximum water-deficient conditions. Among the studied genotypes, D89-89-40 and Line 30 were found to be more adaptive to 15% and 10% PEG treatments, respectively. They showed the highest expression for proline content as well as superoxide dismutase, peroxidase and ascorbate peroxidase under water stress. This enabled that D89-89-40 and Line 30 genotypes kept the decrease in lipid peroxidation and electrolyte leakage, consequently the decrease in relative water content to a basal level in response to drought treatments was observed. Semi quantitative analysis of *CAB3*, *DHN1* and *DHN2* genes showed increase in the expression level of *DHN2* gene in D89-89-40 genotype but kept steady in Line 30 genotype. The slightest reduction in *DHN1* and *CAB3* expressions among genotypes was recorded in D89-89-40 and Line 30 genotypes accompanied with the slightest decrease in photosynthetic activity and pigment content. The study suggested that D89-89-40 and Line 30 genotypes as drought-tolerant genotypes and nominated them for wide spreading cultivation. The results also assumed that the expression level of *DHN2* gene could be used as a selection marker for drought tolerance screening in soybean genotypes.

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Table (1): The three used genes and actin as housekeeping gene: names, accession no (NCBI) and primers sequences.

Genes	Accession no.	Primer sequence 5'----------3'
DHN1 Dehydrin-COR47-like	NM_001253177	F- 5'-TCATCGCCACCGAGTTTCAA-3' R- 5'-CTGTCTTCTTGTGCCCTGGT- 3'
DHN2 *LEA-D11*	AM421515	F- 5'-TCGCAAGGTCGACGAGTATG-3', R-5'- TTCTTCTCGTGCTGACCACC- 3'
CAB3 Chlorophyll a/b-binding protein	NM_001248354	F-5'-GGTCCCAGATCTTCAGCGAG-3' R 5'-TAGGCCCAGGCATTGTTGTT-3'
Actin	BW652479	F-5'-ATCTTGACTGAGCGTGGTTATTCC-3' R-5'GCTGGTCCTGGCTGTCTCC-3'

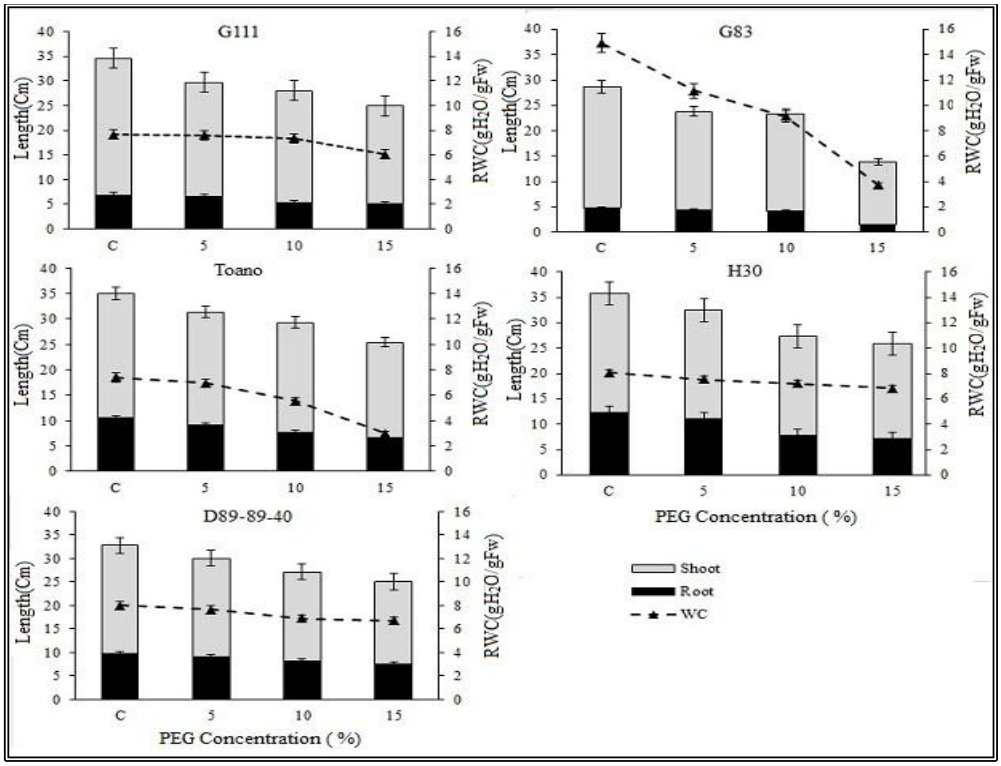

Fig. (1): Changes in shoot/root lengths and water content of the screened soybean genotypes under control (C) and different PEG concentrations (5%, 10%, and 15%).

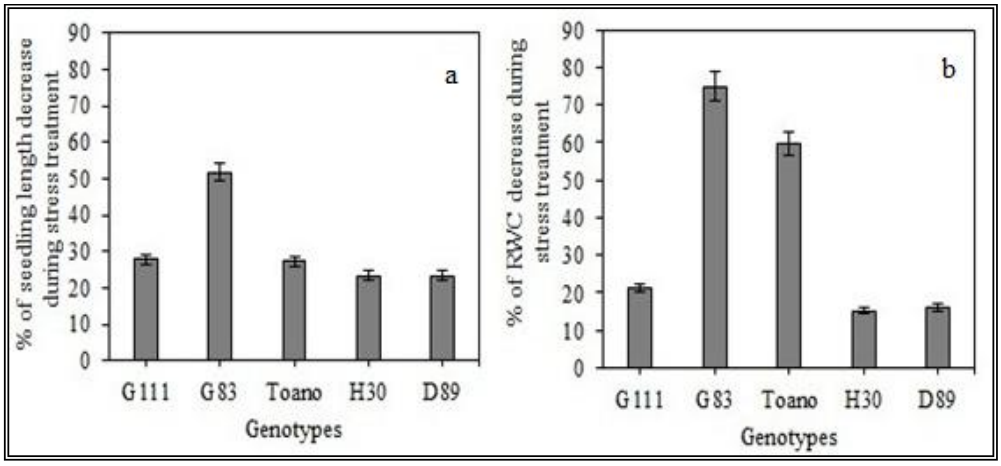

Fig. (2): Percentages of the decrease in seedling length (a), and water content (b) in all studied genotypes. The percentage was calculated as the difference between the mean of values at control conditions and at 15% PEG on the base of value at the control condition.

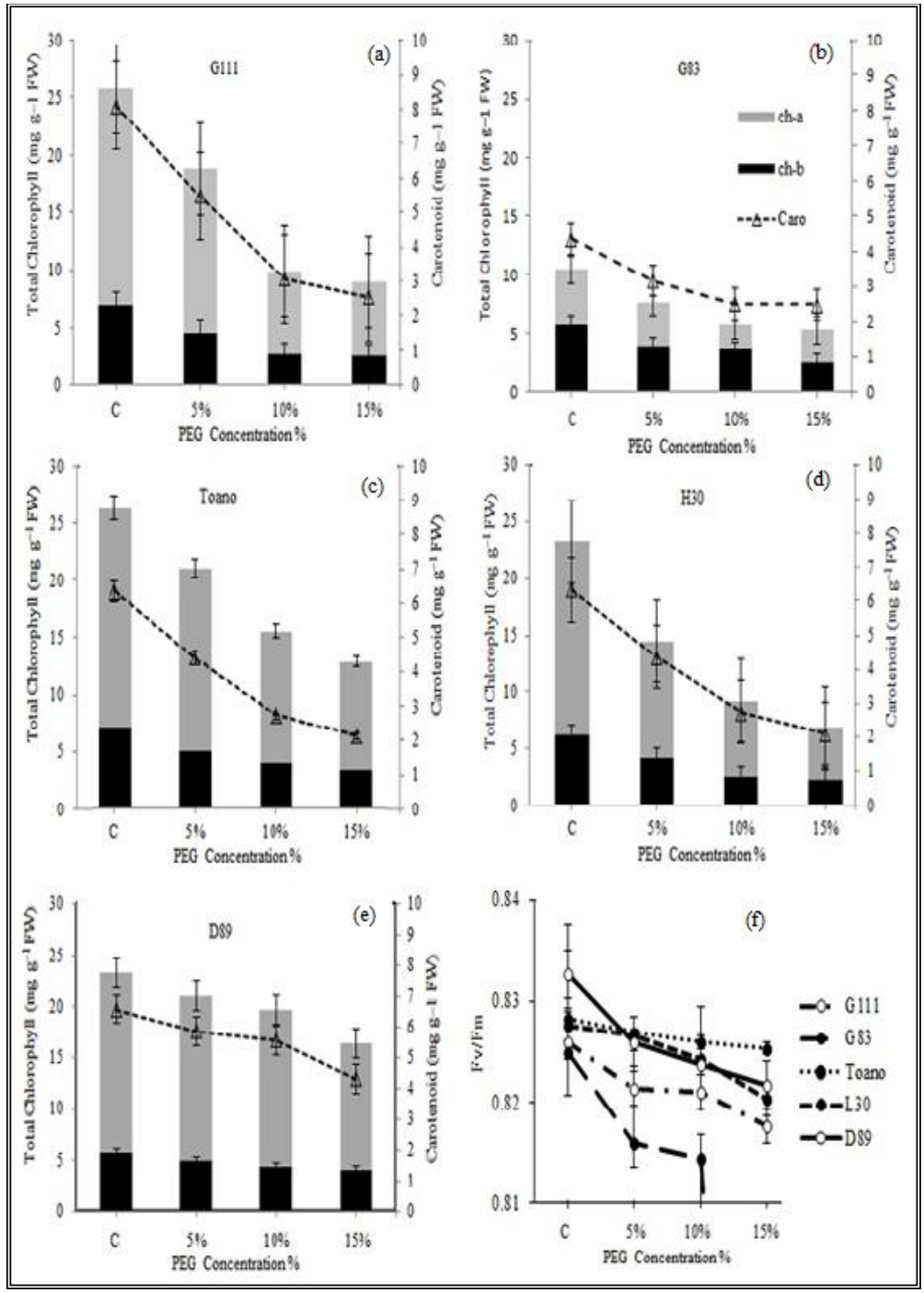

Fig. (3): Changes in photosynthetic pigments Ch a, Ch b, carotenoid content (a-e) and photosynthetic activity (f) of studied genotypes under control (C) and different PEG concentrations (5%, 10% and 15%).

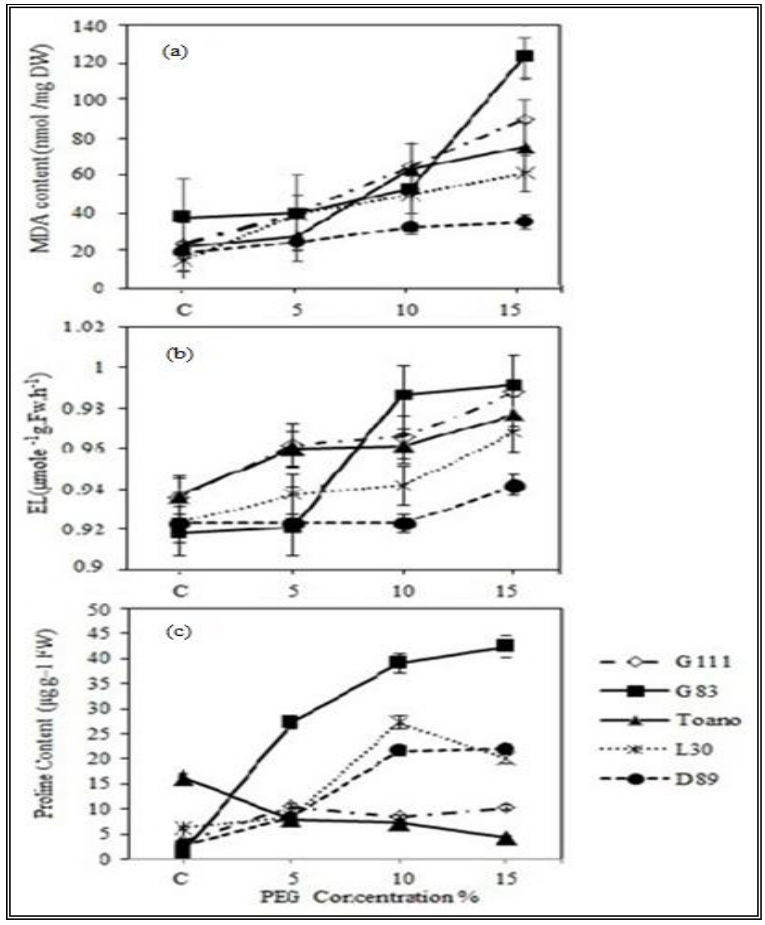

Fig. (4): Changes in MDA content (a), rate of electrolyte leakage (b) and proline content of studied genotypes under control (C) and different PEG concentrations (5%, 10% and 15%).

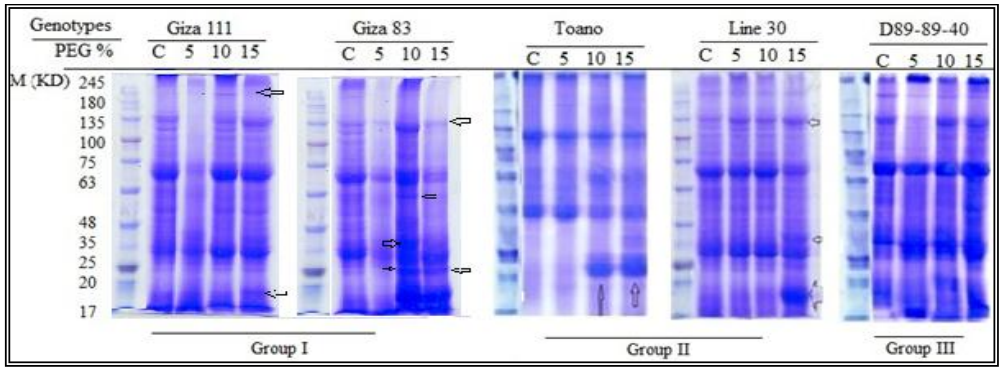

Fig. (5): SDS-protein profiles of the studied soybean genotypes under control (C) and different PEG concentrations (5%, 10%, and 15%)

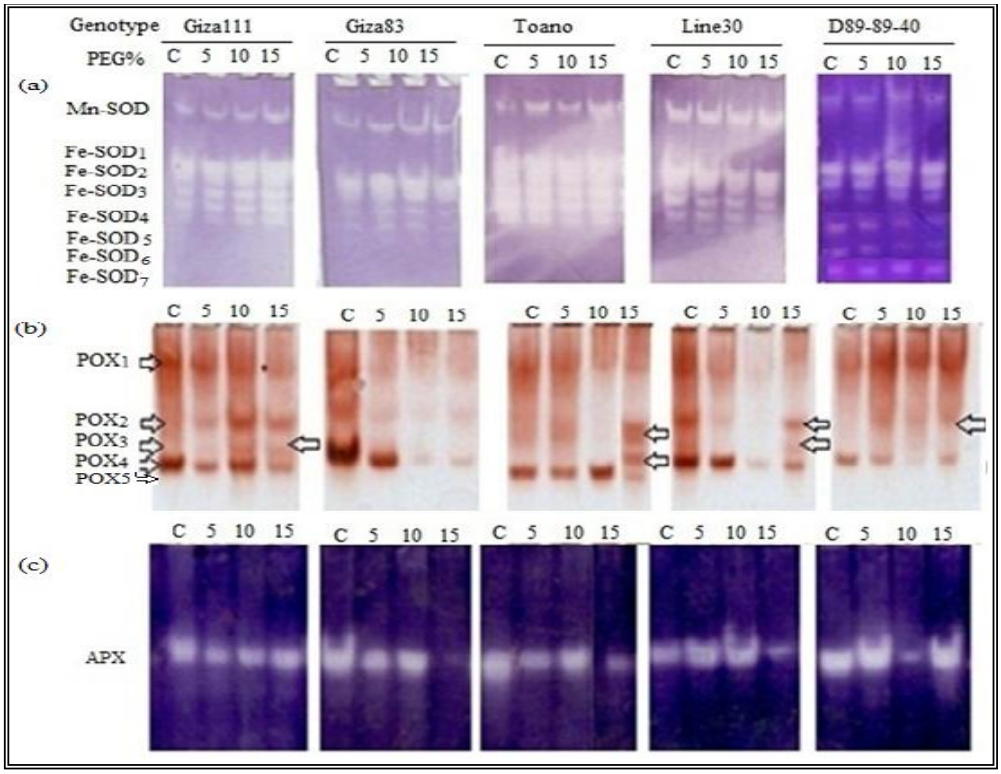

Fig. (6): Changes in the activity of antioxidant enzymes: (a) superoxide dismutase (SOD), (b) peroxidase (POX) and (c) ascorbate peroxidase (APX).of the studied soybean genotypes under control (c) and different PEG concentrations (5%, 10%, and 15%).

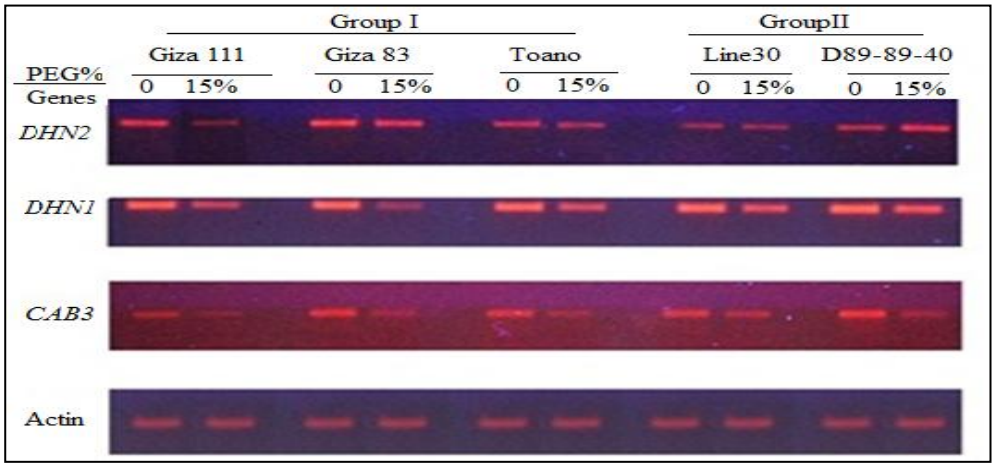

Fig. (7): Cchanges in levels of expression of CAB3, DNH1 and DNH2 of studied soybean genotypes under control (C) and different PEG concentrations (5%, 10% and 15%). Transcriptional level measured as the amplified cDNA against standardized actin transcript.