JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2015. Vol. **9**(Spl. Edn. 1), p. 79-95

Genetic Variability Analysis for the Selection of Drought-Tolerant Tomato (*Lycopersicon esculentum* Mill.) Germplasm as Investigated by in Vitro Callus, Shoot, and Plantlet Cultures

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(Received: 24 February 2015; accepted: 25 April 2015)

In Arab Gulf countries, successful tomato production requires the identification of cultivars having an abiotic stress-tolerance, in particular, against drought. Under drought stress, tomato plants are exposed to numerous changes in their metabolism and gene expression, resulting in decreased yields and inferior quality of the obtained tomato fruits. Without including genetically variable stress-tolerant cultivars, breeding efforts remain ineffective to improve fruit yield and quality. Therefore, the present study aimed at the selection of drought-tolerant tomato cultivars. A total of 15 cultivars was exposed to drought treatments (0.0, 25, 50 mg L^{-1} PEG6000). For this purpose, cotyledon explants were cultured on callus induction medium, and the calli were then transferred to regeneration medium to study drought stress. The experiment was conducted following a randomized complete block design with three replications. The analysis of variance showed a highly significant two-way interaction (drought level and cultivars) ($P \leq 0.001$) for most of the parameters. Selection indices suggested five cultivarsto be tolerant to drought stress at bothstress levelsstudied, while six cultivars were found to be sensitive to drought stress, thus being only suitable for cultivation under non-drought stress. All measured traits, except for the number of leaves, negatively correlated with SSI (stress sensitivity index) and TOL (tolerance index) at both drought levels, suggesting the suitability of the used traits for drought screening. Cluster analysis based on selection indices discriminated the cultivars into three clustersaccording to their tolerance to drought. The protein profile was analyzed by SDS-PAGE, and several new protein bands at variable molecular weights (110.115, 107, 104.321, 61.900, 54.003, 46.922, 16.456, 16.130, 15.316 and 15.263 kDa) were identified in the plants grown under stress conditions. These polypeptides may be used as future markers todiscriminate stress-tolerant and -intolerant tomato plants.

Key words: Tomato, water deficit, diversity, callus, selection indices, phenotypic and genotypic variation, protein marker, cluster analysis.

Abbreviations: MS, Murashige and Skoog Medium; NAA, 1-Naphthaleneacetic Acid; 2,4-D, 2,4 Dichlorophenoxyacetic Acid; IBA, Indole-3-butyric Acid; SN, Silver Nitrate; ZEA, Zeatin; SDS-PAGE, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; SSI, Stress Sensitivity Index; STI, Stress Tolerance Index; TOL, Tolerance Index; GMP, Geometric Mean Productivity; MP, Mean Productivity; YI, Yield Index; CRG, Callus Relative Growth; CWC, Callus Water Content; CSR, Callus Survival Rate.

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Many cellular functions of plants are severely affected by water deficiency, ultimately exerting a negative impact on plant growth and reproduction (Noaman *et al.*, 2004). On average, crop yields were estimated to be reduced by 69% when plants are exposed to unfavorable stress conditions in the field (Boyer, 1982) due to a disturbed water balance of the plant body and alterations in the water uptake pattern of the plant (Waraich *et al*., 2011).Drought-tolerant plants have developed mechanisms to cope with the water deficit (Aazami *et al.*, 2010).

By harvest area and total production quantity, tomato (*Lycopersicon esculentum* L.; 2n = 12) isconsidered the second most important vegetable crop after potatoes(George *et al*., 2013).The Food and Agriculture Organization of the United Nations (FAO) estimated that worldwide tomato production reached more than 151 million metric tons per year, harvested from 441.280 ha (FAOSTAT, 2012). Sincetomato has considerable economic importance for all tropical and sub-tropical regions of the world, the main objective for plant breeders is to increase its productivity. However, in most commercially cultivated tomato cultivars resistance to abiotic stress isinsufficient (Ragab *et al*., 2007), representing a challenging hurdle to increase productivity. Thus, crossing of currently cultivated tomato cultivars with potentially drought tolerant lines might represent a promising approach to enhance productivity (Pena and Hughes, 2007).

To date, the selection process based on conventional plant breeding methods is highly time- and resource-consuming. Consequently, regarding environmental stress toleranceof crops, only little improvement has been achieved so far.(Rai *et al.*, 2011). During past years,*in vitro*cultures were shown to be instrumental in enhancing the tolerance to water or drought stress by selection of plant tolerance to drought stress (Ania 2003; Manaj *et al.*, 2011; Bradar-Jakanovic *et al.*, 2014; Soliman and Mohamed, 2013; Metwali *et al.*, 2014). Forthe selection of drought-tolerant genotypes, polyethylene glycol (PEG) as an osmoticum is added to the cell culture media to induce osmotic stress by reducing the availability of free water to the cells, leading to a loss of cell turgor and cell hydration,which ultimately results in reduced growth without direct physiological damage (El-Houssine and Mohammed 2012).In addition, PEG isa non-penetrating inert and non-phytotoxic osmoticum (Hassan *et al.*, 2004; Adachi *et al.*, 2014).

While several physiological growth parameters of the resulting callus, shoots, or plants may be directly used to evaluate the stress-tolerance of the studied cultivar, several workers have proposed to study the proteome of the plant cells. By these means, a number of proteins have been shown to be induced by abiotic stress such as drought, reflecting the complexity of biochemical and physiological responses (Chen *et al.*, 1992; Arefian *et al.*, 2014). These changes in protein expression are directly associated with the biological changes, being responsible for the increased performance of stress-tolerant cultivars. In fact, several previous studies reported that the electrophoretic protein profiles could be used as genotype markers in tomato due to their high stability and independence of the ecological conditions (Azeez and Morakingo, 2004; Elizabeta *et al.*, 2008; Furdi 2012; Hameed *et al.*, 2014). However, unambiguous identification of potential marker proteins indicating the drought-tolerance of a cultivar is still pending.

Thus, the main goal of this workwas to investigate the drought-induced behavior of 15 different tomato cultivars, using *in vitro* callus, shoot, and plantlet cultures as well as SDS-PAGE for protein characterization. We aim at improving tomato fruit production by a cultivar screening using biotechnological approaches.

MATERIALS AND METHODS

Plant material

Seeds of 15 tomato (*Lycopersiconesculentum* Mill.) cultivars used in this study were provided from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.Table 1 their commercial names, botanical classification , and country of origin are listed. This study was conducted during the period from February 2014 to January 2015 at Tissue culture and Genomic Lab, Biological Science Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA incooperation with Institute of Food Science and

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Callus Induction

Seeds were three times washed with tap water and surface sterilized by soaking in 70% (v/v) aqueous ethanol for one minute followed by a thorough rinsing with sterile distilled water. Afterdippinginto a commercial bleaching solution (2.5% w/v aqueous NaOCl) enriched with 5 drops of Tween-20 as a wetting agent, the seeds were immediately rinsed three times (each for one minute)with sterile distilled waterfor the complete removal of sodium hypochlorite. Completing the sterilization procedures, the sterilized seeds were cultured *in vitro* under aseptic conditions in a laminar airflow hood, using a hormone free germination medium (M1) composed of 4.4 g L^{-1} MS salts(Murashige and Skoog, 1962), 3% sucrose, 100 mg L^{-1}*myo*-inositol, 1mg L^{-1}thiamin-HCl) and solidified by 0.25% (w/v) Phytagel. The pH was adjusted to 5.7 by either 1 M NaOH or HCl, prior to autoclaving at 121 °C for 20 min. Cultured seeds were kept in an incubator at 25±1 °C in the dark for one week to induce seed germination. Subsequently, they weremaintained under a daily 16 h photoperiod under 60 µmol $m^{-2}s^{-1}$ illumination supplied by cool, white fluorescent light for three weeks. The cotyledon explants excised from 27 days-old growing seedlings, were cut into halves with a scalpel blade and placed abaxial side down facing the callus induction medium according to Metwali *et al.* (2015). The composition of the callus induction medium M2 was as follows: 4.4 g L^{-1} MS salts, 3 % sucrose, 0.25 % Phytagel, 100 mg L^{-1} *myo*-inositol, 1 mg L^{-1} thiamin-HCl, 0.4 mg L^{-1} 1-naphthaleneacetic acid (NAA), 2 mg L^{-1} kinetin, and 1 mg L^{-1}2,4 dichlorophenoxyacetic acid (2.4-D) according to El-Sayed *et al.,* (2004). Different concentrations (0.0, 25.0 and 50.0 g L^{-1}) of polyethylene glycol (PEG6000) were added to the callus induction medium to establish an osmotic gradient. A total of 5 explants per jar and ten jars per treatment was used following a completely randomized blocked design (CRD), which was repeated three times with different arrangement. The callus cultures were incubated in total darkness at 25±1 °C for 4-5 weeks for recovery and proliferation of putative drought tolerant cell lines. Subsequently, the following traits (a-d) were recorded:

a. CFW : Callus fresh weight (gm)

b. CSR: Callus survival (%)= No. of callus survived / total no. of calli cultured x 100 according to Soliman *et al.*,(2014)

c. CRG: Callus relative growth=callus final weight – callus initial weight / callus initial weight according to Chen *et al.*,(2006)

The callus growth rate under stress or unstressed medium wasmeasured in terms of percent in fresh weight.Micro clump of calli approximately of almost equal size with known weight (callus initial weight) were incubated for 4 weeks on callus induction medium. At the end of the period, random samples of calli were weighted to obtain the callus final weight.

d. CWC: Callus water content (%) = Callus Fresh Weight – Callus Dry weight / Callus Fresh weightx 100 (Soliman and Mohamed, 2013). Callus dry weight was gravimetrically determined after drying at 80 °C for 48 h.

Shoot regeneration

To test the capacity of the genotypes for shoot regeneration in the presence of PEG6000, a group of friable callus was transferred to a previously optimized regeneration medium(M3), representing aM1 medium supplemented with zeatin (ZEA 1 mg L^{-1}), NAA (0.1 mg L^{-1}) and silver nitrate (SN 5 mg L^{-1}) according to (Metwali *et al.,* 2015). The M3 medium was adjusted to pH 5.8 prior to autoclaving. Subsequently, aliquots of 25 mL were dispensed into jars, the selected calli were carefully added, and the cultures were maintained in a growth chamber at 28 ± 2°C and at 16 h photoperiods(13.5 µmol $m^{-2}s^{-1}$)provided by white fluorescent tube lights. A total of 5 explants per jar was cultured, ten jars per treatment were used in a CRD, and the whole experiment was repeated three times. Plant regeneration rate (No. of regenerated explants / No. of planted explants x 100) according to Soliman *et al.*, (2014), shoot length (SL), shoot fresh weight (SFW) and number of leaves (NL)were assessed after 8 weeks.

Root formation

In order to study the plant's ability of root formation, elongated regenerated shoots were cultured on rooting medium M4, being composed of M1 medium supplemented with 1.5 mg L^{-1}indole-

Table 1. Accession number, commercial name, serial code, botanic name and origin of 15 tomato genotypes used for drought resistance evaluation

Accession number	Commercial name	Co.	Botanic name	Origin
LYC3912	Dedication	C1	*Lycopersicon esculentum* Mill.	Russia
LYC4112	Anna Aasa	C2	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum*	Russia
LYC2019	Gelbfruechtig	C3	*Lycopersicon esculentum* Mill. convar. *infiniens*Lehm. var. *cordiforme*	Germany
LYC192	AustralischeFrühe	C4	*Lycopersicon esculentum* Mill. convar. *infiniens* var. *commune*L.H.Bailey	Australia
LYC3152	Australische Rosen	C5	*Lycopersicon esculentum* Mill	Australia
LYC2431	Vencal	C6	*Lycopersicon esculentum* Mill. convar. *fruticosum*Lehm. var. *speciosum*Lehm	Netherlands
LYC2432	Zevat	C7	*Lycopersicon esculentum* Mill. convar. *fruticosum*Lehm. var. *speciosum*Lehm	Netherlands
LYC4242	Petomech	C8	*Lycopersicon esculentum*Mill. convar. *fruticosum*Lehm. var. *speciosum*Lehm	Italy
LYC4079	Sankt Ignatius	C9	*Lycopersicon esculentum*Mill. convar. *infiniens*Lehm. var. *commune*	Italy
LYC1346	Sintesti	C10	*Lycopersicon esculentum*Mill. convar. esculentum var. *esculentum*	Romania
LYC359	Tiganesti	C11	*Lycopersicon esculentum* Mill. convar. *infiniens* Lehm. var. *flammatum* Lehm	Romania
LYC2937	Florida MH-1	C12	*Lycopersicon esculentum*Mill. convar. *fruticosum*Lehm. var. *finiens*Lehm	USA
LYC2493	Sandpoint	C13	*Lycopersicon esculentum*Mill. convar. *fruticosum*Lehm. var. *pygmaeum*Lehm.	USA
LYC2987	California	C14	*Lycopersicon esculentum*Mill.	USA
LYC4113	California Red Cherry	C15	*Lycopersicon esculentum*Mill. convar. *parvibaccatum*Lehm. var. *cerasiforme*(Dunal) Alef	USA

Co. Serial code

3-butyric acid (IBA) and 0.5 mg L^{-1} NAA as described by Metwali *et al.*(2015). For each treatment, 15 shoots were used. They were incubated in a growth chamber at the same condition as described above in the shoot regeneration section. After three weeks, root length (RL) and root fresh weight (RFW) were measured.

Drought tolerance/sensitivity indices

Drought tolerance and sensitive indices were calculated for each genotype based on shoot fresh weight (Table 2).

Protein analyses

Sample preparation and extraction

Leaf samples were collected from 8-week-old plantlets grown under control and drought stress conditions, and stored at -80ºC until protein analysis. Briefly, 0.5 g of leaf tissue was manually ground to a fine powderin a mortar using a pestle and liquid nitrogen. The powder was homogenized with 2 mL extraction buffer containing 1MTris-HCl (pH 8.8) and 0.25 M EDTA. The homogenate was transferred to sterilized Eppendorf tubes, left in a refrigerator overnight, then vortexed for 15 sec and centrifuged at 5000 rpm for 5 min at 0ºC. The supernatants were collected and considered as the soluble leaf protein extract. Protein concentration was estimated using Bradford's method (Bradford, 1976) and expressed as µg/g fresh weight. A standard curve was prepared based on bovine serum albumin.

SDS-PAGE and electrophoresis of protein samples

SDS PAGE of leaf protein was carried out in vertical slab gel using 15% acrylamide according to the methods described by Laemmli (1970).Resolving gels were composed of 1.5M Tris–HCl pH 8.8, 1N HCL, 10% SDS, 0.025% of N,N,N2 ,N2 -tetramethylenediamine (TEMED), 10% ammonium persulfate. Stacking gels contained 1 M Tris–HClpH 6.8, 10% SDS, 0.025% TEMED and 10% ammonium persulfate. A volume of 50 µL of the soluble leaf protein extract was combined with 50 µL of LAN's buffer (10 % SDS, Glycerol, 1 M Tris HCL, pH 8.8, 0.25 M EDTA), and 10 µL 2-mercaptoethanol in an Eppendorf tubeprior to boiling in a water bath for 10 min.Then, 10 ¼Lbromophenol blue was added to each tube before sample loading. Depending on the concentration of protein in the sample, a volume of 15-20 µL was applied to each well.In a separate lane of the gel, the protein ladder ranged from 11-

245 KDa (Geneaid, Company, New Taipei, Taiwan) was applied in order to allow the estimation of the molecular masses of the separated proteins. Electrophoresis was run in a protein II electrophoresis system (Bio-Rad, California, USA) for about one hour in running buffer at 150 V/100 mA. When the bromophenol dye had entered the resolving gel, voltage was increased to 250 V at 80 mAuntil the end of the run. Gels were removed from the plates and shaken in staining solution (455 mL methanol, 90 mL glacial acetic acid, 455 mL distilled water, 1 g Coomassie Brilliant Blue-R250) for 12-18 h. Subsequently, they weretransferred to a distaining solution (140 mL methanol 40 mL glacialacetic acid and 520 mL distilled water) and left while shaking for several hours until the dark background became colorless and blue protein bands appeared. The gels obtained were photographed witha gel documentation system (Syngene, Camridge, UK) .

Molecular weight determination

The molecular weights of the unknown dissociated polypeptides were determined using a standard curve obtained from the R_f-values and molecular weights of protein marker. The molecular weights of the unknown protein bands were calculated by their R_f values using gel analyzer version 3 software program.

Statistical analysis

Analyses of variance and mean comparison of variables were carried out using MStat-C, version 2.10 (Software, MSU, East Lansing, USA). Correlation analyses were performed regarding different selection indices and traits recorded for each salinity level using Microsoft Excel 2007. Ward's minimum variance clustering method was used to classify genotypes into discrete clusters (Romersburg, 1988).

RESULTS AND DISCUSSION

The effect of drought stress on callus, shoot and root growth parameters under study wasdetermined for *in vitro* cultures of 15tomato cultivars (*Lycopersicon esculentum* Mill), using different concentrations of PEG6000 for drought stress induction. Callus formation was induced on callus induction medium after 4 weeks**(Fig. 1).**The analysis of variance showed a highly significant two-way interaction (drought level and cultivars) ($P \leq 0.001$) for most of the measured growth parameters **(**Table 3).In addition, comparison of means by Duncan's Multiple Range Test demonstrated that growth parameters under study differed significantly due to drought levels, genotypes and their interaction (Table 4, 5).This significant genotypic variation for all the parameters in either control or both drought treatments suggested that the magnitude of differences was sufficient to provide a reliable basis for the selection of potentiallydrought-tolerant tomato cultivars to be used in field trials being in agreement with the findings previously reported by Manal *et al.* (2013).

Growth parameterssuch as callus fresh weight (CFW), callus relative growth (CRG), callus water content (CWC), and callus survival rate (CSR) of all cultivars investigated were differed significantlyand incrementally decreased as the concentration of PEG was increased (Table 4).On average, with increasing drought stress CFW (90.06% and 77.2%), CRG (90.06% and 65%), CWC

Table 2. Drought tolerance/sensitivity indices and their equations

Drought Tolerance/sensitivity indices	Equation	References
Stress Sensitivity Index (SSI)	SSI= [(1- (Ysi/Ypi)/ SI]	Fischer and Maurer (1978)
Stress Tolerance Index (STI)	STI= [Ypi x Ysi] / (Yp)2	Fernandez (1992)
Tolerance Index (TOL)	TOL = Ypi - Ysi	Hossain et al. (1990)
Geometric Mean Productivity (GMP)	GMP = (Ypi x Ysi)0.5	Fernandez (1992)
Mean Productivity (MP)	MP = (Ypi+ Ysi) / 2	Hossain et al. (1990)
Yield Index (YI)	YI = Ysi / Ys	Gavuzzi et al. (1997)
Yield Stability Index (YSI)	YSI = Ysi / Ypi	Bouslama and Schapaugh (1984)

Where Ypi and Ysi are the shoot fresh weight of a genotype after normal and stressed regeneration, respectively, and SI is stress intensity as calculated as SI = 1-(Ys/Yp); Ys and Yp are the mean shoot fresh weights of all genotypes under stressed and normal conditions, respectively.

(87.67% and 79.9%) and CSR (82.29% and 68.66%) of the tomatoes decreased at 25 and 50 mg L^{-1} PEG, respectively. The responses of all cultivars differed significantly whendrought stress increased, except for CWC of cultivar number C14. Atmaximum stress conditions (50 mg L^{-1} PEG), the maximum growth parameters were reached by the following cultivars:maximum CFW: C3, C10, C11, C14 and C15); maximum CRG: C2, C3, C5, C8, C9, C10, C11 and C15); maximum CWC: C2; C3, C5, C8, C9. C10, C11, C13, C14 and C15); maximum CSR: C3, C5, C10, C11, C12, C13, C14 and C15).Minimum values were recorded for cultivars C6 and C7 regarding CFW, CRG, CWC, and CSR, respectively.

Furthermore,the produced calli were regenerated to shoots after 6-9 weeks of incubation on regeneration mediumto study the effect of drought treatments on other growth parameters, such as plant regeneration rate (PRR), shoot fresh weight, root fresh weight, shoot length, root length, and number of leaves.Regenerated shoots and plantlets are depicted in Figures 2 and 3.As compared to samples grown under normal conditions, a significantlyreduced PRR (72.46% and 57.18%), SFW (55.2% and 34.07%), RFW (77.19% and 46.97%), SL (78.33% and 60.78%), RL (75.8% and 54.84%), and LN (78.6% and 58.04%) were foundfor all cultivars at both drought levels. The lowest valueswere obtained at 50 mg L^{-1} PEG for the cultivars C6, C7, C1, C2 (SFW); C1, C2, C6, and C7 (RFW); C1, C5, C6, C7 (SL); C6, C7, C2, C4, C6, C7 (RL); C6, C7 (PRR); as shown in Table 5.Cultivars C6 and C7 were the only cultivars where a decline for all the attributes was recorded when exposed to drought stress. This reflects the poor performance of these two genotypes under stress. Our study suggests that these genotypes (C6 and C7) might be used as susceptible control in future studies. These genotypic differences observed with respect to the various above-mentioned parameters may be explained by a genetic variability of tolerance to water stress in the plants. The decline in various plant attributes in response to induced stress is a commonly observed phenomenon, which is according to an often genetically-defined tolerance level of theplant (Aazami *et al.*, 2010; Bibi *et al.*, 2012; El-Houssine *et al.*, 2012; Muscolo *et al.*, 2014; Badran *et al.*, 2015).

Selection of drought-resistant cultivars by drought tolerance/sensitivity indices

As shown in Table 6, at 25 mg L^{-1} PEG level, tomato cultivars C9 and C10 exhibited the lowest TOL and SSI values. In contrast, the highest values wererecorded for C2 being followed by C1. Accordingly, at the highest drought level (50 mg L^{-1} PEG), tomato cultivars C1, C2, C5 and C7 showed the highest TOL and SSI values, whereas C3, C9 and C10 yielded the lowest values,possibly indicating their high drought tolerance. The latter was confirmed by the yield parametersYSI and YI at the highest drought level. Highest YSI and YI values were obtained for tomato cultivars C3, C9 and C10. The highest GMP, MP and STI values at 25 mg L^{-1} PEG were recorded for cultivars C3, C7 and C9. At 50 mg L^{-1}, the cultivars C3, C9, C10, C11, C14 and C15 produced the highest values for GMP, MP, and STI. These high rates of YI, MP, GMP and STI indicated the tolerance of the above-mentioned genotypes, particularly C9 and C10, to drought stress and considered for selection under stress conditions (Khayatenezhad *et al.*, 2011). In agreement with our findings, Talebi *et al.* (2009) reportedthe correlation of greater TOL value with an increasing yield reduction under stress conditions.

Correlation analyses between tolerance indices and measured attributes in two separate conditions are presented in **Table 7**. All traits recorded, except for the number of leaves,were negatively correlated ($P<0.05$) with SSI and TOL at both drought levels.This confirms the suitability for these traits for drought screening. Moreover, the positive and significant correlations of MP, GMP, STI, YI and YSI with CFW, CRG, CWC, SFW and SL at low (25 mg L^{-1}) and high (50 mg L^{-1}) PEG stress levels suggest these traits to be reliable indicators for drought resistance screening either at low or high levels of stress. For comparison, the positive correlation among these above mentioned indices with CSR, PRR, RFW and RL was found only at high stress levels (50 mg L^{-1}PEG), implying their suitability as screening parameters only at high levels of stress. Regarding the number of leaves, the association was weak and inconsistent, i.e. either positive or negative correlations were found. However, these results are consistent with those of otherstudies reporting different relationship

Table 3. Analysis of variance for effect of cultivars and drought levels on different callus, shoot, leaves and root parameters of tomato(*Lycopersiconesculentum* Mill)

SOV	df	Callus fresh weight	Callus rate growth	Callus water content	Callus Survival Rate	Plant regen. rate	Shoot fresh weight	Root fresh weight	Shoot length	Root length	No. of leaves
Genotypes	14	0.180**	0.726**	16.80**	248.2**	800.96**	1.05***	0.872***	29.72**	25.33**	5.63**
Treatments	2	2.41***	13.16***	1817.6***	7787.4***	8610.5***	18.01***	3.79***	227.1***	312.5***	82.9***
G. X T.	28	0.126***	0.292***	29.56***	86.79***	189.45***	0.560***	0.128***	7.563***	3.491***	2.46***
Error	88	0.001	0.0120	0.44	0.8175	1.922	0.0051	0.003	0.258	0.249	0.184
CV %		1.49	4.412	0.770	1.093	1.924	6.99	8.844	6.25	5.572	8.55
LSD (0.05)											
Genotypes		0.249	0.105	0.626	0.839	1.288	0.077	0.0675	0.535	0.467	0.408
Treatments		0.011	0.047	0.279	0.375	0.576	0.034	0.0302	0.239	0.209	0.182
G. x T.		0.042	0.179	1.082	1.477	2.264	0.117	0.093	0.814	0.830	0.95

SOV: Source of variance, MS: Mean Square, df: degree of freedom; ** and *** significant at the 0.01 and 0.001 probability levels, respectively.

Table 4. Mean comparison of CFW, CRG,CWT and CSR of fifteen tomato (*Lycopersiconesculentum* Mill) cultivars after 5 weeks of growing on MS callus induction supplemented with different PEG6000 concentrations

Traits	T.	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
C F W	0	1.97 a	2.24 a	2.07 a	1.92 a	2.11 a	2.02 a	1.88 a	1.95 a	2.21 a	1.91 a	1.95 a	1.98 a	1.92 a	1.93 a	1.97 a
(g.)	25	1.71 b	1.92 b	1.96 b	1.78 b	1.85 b	1.76 b	1.70 b	1.78 b	2.11 b	1.89 a	1.89 b	1.81 b	1.89 b	1.09 b	1.88 b
	50	1.40 c	1.66 c	1.78 c	1.31 c	1.52 c	0.91 c	0.99 c	1.54 c	1.64 c	1.80b	1.76 c	1.63 c	1.50 c	1.86 c	1.80 c
	LSD	0.015	0.054	0.013	0.025	0.015	0.06	0.11	0.067	0.093	0.025	0.032	0.509	0.198	0.004	0.065
CR G	0	2.93 a	3.53 a	3.15 a	2.84 a	3.22 a	3.04 a	2.76 a	2.90 a	3.4 a	2.80 a	2.90 a	2.93 a	2.83 a	2.83 a	2.95 a
	25	2.42 b	2.85 b	2.92 b	2.57 b	2.68 b	2.52 b	2.41 b	2.55 b	2.23 b	2.77a	2.77 b	2.60ab	2.78 b	2.72 b	2.76 b
	50	1.40 c	2.33 c	2.56 c	1.63 c	2.03 c	0.82 c	0.99 c	2.09 c	2.26 c	2.61b	2.53 c	1.90b	2.00 c	1.18 c	2.60 c
	LSD	0.03	0.027	0.027	0.053	0.045	0.12	0.22	0.145	0.197	0.049	0.066	0.783	0.041	0.031	0.097
CW C	0	93.9 a	93.4 a	92.7 a	94.1 a	91.3 a	93.7 a	94.3 a	90.6 a	93.1 a	89.5 a	89.9 a	92.5 a	93.5 a	91.4 a	93.9 a
(%)	25	82.3 b	89.2 b	89.2 b	92.6 b	84.3 b	89.2 b	92.5a	87.6 b	87.9 b	85.1 b	85.4 b	89.5 b	88.2 b	84.1 b	88.1 b
	50	77.9 c	80.4 c	80.3 c	75.7 c	79.9 c	70.3 c	78.6 b	80.1 c	81.6 c	82.9 c	82.6 c	76.6 c	83.4 c	84.3 b	84.1 c
	LSD	1.109	2.2	1.68	0.871	0.562	1.87	2.43	0.798	0.728	0.422	0.748	0.908	0.668	0.528	1.96
CS R	0	94.3 a	95.1 a	96.1 a	94.2 a	93.2 a	95.2 a	95.4 a	95.9 a	94.8 a	97.1 a	96.4 a	94.7 a	94.7 a	93.9 a	94.4 a
(%)	25	78.0 b	77.4 b	88.1 b	80.7 b	79.3 b	76.3 b	72.1 b	80.3 b	78.1 b	91.4 b	86.4 b	85.1 b	85.1 b	86.1 b	84.9 b
	50	68.8 c	61.4 c	72.9 c	60.3 c	70.7 c	44.3 c	54.3 c	67.6 c	67.8 c	86.3 c	76.7 c	73.3 c	73.3 c	74.4 c	70.7 c
	LSD	1.43	5.34	0.502	0.735	2.013	0.453	0.489	1.07	0.837	0.21	0.779	0.787	0.782	1.3	2.57

Table 5. Mean comparison of PRR, SFW, RFW, SL, RL and No. of Leaves of fifteen tomato (*Lycopersiconesculentum* Mill) cultivars after 6-9 weeks of growing on MS regeneration medium supplemented with different PEG6000 concentrations

Traits	T.	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
P R R	0	71.0 a	76.0 a	89.7 a	83.4 a	75.5 a	85.6 a	93.3 a	86.6 a	90.9 a	95.0 a	90.0 a	82.8 a	84.5 a	86.7 a	81.1 a
(%)	25	56.7 b	66.9 b	87.2 b	65.6 b	65.2 b	55.9 b	56.4 b	76.9 b	86.1 b	80.5 b	85.8 b	75.0 b	70.1 b	81.8 b	76.8 b
	50	45.3 c	47.9 c	70.3 c	40.7 c	55.6 c	25.6 c	33.9 c	61.9 c	60.8 c	72.8 c	70.3 c	66.2 c	65.0 c	70.3 c	71.2 c
	LSD	4.62	4.64	1.82	3.66	0.815	1.24	3.22	3.83	1.75	0.642	1.79	1.26	1.45	3.61	1.6
S F W	0	1.80a	3.07a	2.27 a	1.26 a	2.7 a	1.8 a	2.3 a	1.1 a	2.3 a	1.6 a	1.8 a	1.32 a	0.9 a	1.9 a	1.7 a
(g.)	25	0.97b	0.57b	1.4 b	0.6 b	0.5 b	1.4 b	1.7 b	0.7 b	1.9 b	1.20 b	1.09 b	0.60 b	0.5 b	1.1 b	1.2 b
	50	0.35c	0.31c	1.4 b	0.45 b	0.4 c	0.25 c	0.20 c	0.54 c	1.33 c	0.97 c	0.8 b	0.45 c	0.48 b	0.60 c	0.96 c
	LSD	0.94	0.177	0.22	0.155	0.08	0.21	0.23	0.11	0.25	0.08	0.25	0.12	0.04	0.08	0.18
R F W	0	0.80 a	0.85 a	0.56 a	1.0 a	2.33 a	0.82 a	1.6 a	1.26 a	1.03 a	0.98 a	0.80 a	1.15 a	0.86 a	1.25 a	1.13 a
(g.)	25	0.73 b	0.44 b	0.98 b	0.56 b	1.82 b	0.62 b	0.58 b	0.57 b	0.70 b	1.08a	0.79a	1.15a	1.06 b	0.54 b	0.92 b
	50	0.32 c	0.23 c	0.43 b	0.33 c	1.34 c	0.28 c	0.30 c	0.4 c	0.48 c	0.82b	0.47b	0.77b	0.40 c	0.45b	0.65 c
	LSD	0.11	0.09	0.18	0.21	0.12	0.1	0.09	0.17	0.1	0.1	0.19	0.16	0.17	0.15	0.09
S L	0	9.00 a	11.5 a	12.8 a	12.2 a	11.2 a	12.6 a	13.4 a	8.26 a	13.3 a	10.8 a	11.8 a	10.2 a	12.8 a	9.5 a	12.4 a
(cm)	25	4.6 b	7.1 b	11.5 b	9.06 b	3.06 b	10.5 b	7.8 b	7.5 b	11.2 b	9.96 b	10.8 b	9.00 b	12.1 a	10.1a	9.7 b
	50	3.6 c	6.8b	10.6b	8.1 c	3.4b	3.66 c	3.2 c	6.2 c	9.16 c	8.6 c	7.8 c	7.66 c	9.66b	7.6 b	8.2 c
	LSD	0.48	1.7	1.3	0.99	1.15	1.55	0.95	0.64	1	0.8	0.41	1.09	2.02	0.83	0.99
R L	0	9.53 a	12.9 a	11.1 a	13.1 a	16.4 a	9.33 a	12.9 a	11.0 a	10.1 a	9.5 a	11.9 a	12.7 a	9.76 a	12.5 a	11.9 a
(cm)	25	8.16 b	9.3 b	9.00 b	8.9 b	14.2 b	6.23 b	6.56 b	6.33 b	8.2 b	8.7 a	9.1 b	10.9a	9.93 a	7.26 b	9.7 b
	50	5.1 c	6.43 c	7.9 c	5.9 c	10.2 c	3.73 c	3.66 c	4.60b	6.16 c	6.66b	6.46 c	8.00b	6.96b	5.8b	8.16 c
	LSD	0.73	0.66	0.67	2.01	0.57	0.91	0.71	1.04	1.13	0.87	0.54	0.95	0.84	1.54	0.61
No. of L	0	4.33 a	6.06 a	4.33 a	4.83 a	9.5 a	8.16 a	6.83 a	5.5 a	6.16 a	7.33 a	7.33 a	7.66 a	6.00 a	5.83 a	7.16 a
	25	5.00 b	4.66 b	4.66 a	4.33 a	5.33 b	5.5 b	5.16 b	3.83 b	4.16 b	6.50 a	4.83 b	6.83 b	5.83 a	5.16 b	4.5 b
	50	4.66 c	3.16 c	3.83a	2.5b	4.5 b	2.83 c	2.16 c	3.83c	3.30 c	5.50b	3.83 c	4.30 c	4.00b	4.00 c	3.83b
	LSD	0.94	0.66	0.99	0.94	0.88	1.37	0.57	1.11	0.57	0.88	0.82	0.99	0.66	0.47	0.75

T., PEG treatment (g L^{-1}). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$).

among the indices and parameters related to drought stress (Anwar *et al.*, 2011; Iker *et al.*, 2011; Manal *et al.*, 2013; Bradar-Jakanovic *et al.*, 2014).

Based on the above-mentionedselection indicesobtained by the low and high stress treatments (25 and 50 mg L^{-1} of PEG, resp.), the 15 tomato cultivars were grouped into three clusters using Ward's minimum variance clustering method (Fig. 4). At 25 mg L^{-1}level, cluster I encompassed the highestnumber of cultivars(12) followed by cluster II (2), and cluster III (1). At 50 mg L^{-1} level, cluster I contained the lowest number of

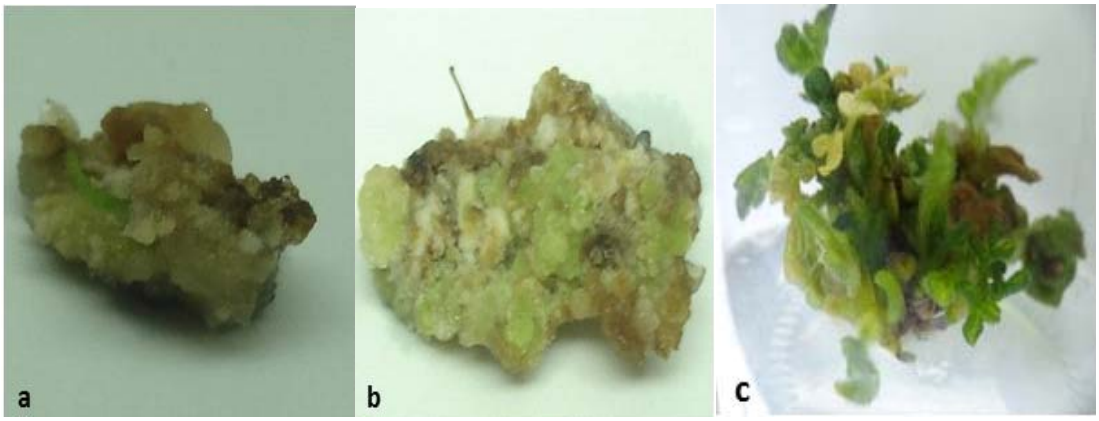

Fig. 1. Callus of cultivar C10 induced from cotyledon explants on MS medium supplemented with 0.4mg L^{-1} NAA, 2 mg L^{-1}kinetin and1 mg L^{-1} 2.4-D after a) 4 weeks.b) callus growthafter 2 weeks on MS regeneration medium. c) axillary shoots from a callus of tomato (*Lycopersicon esculentum* Mill)cultivar C10 after 4 weeks on MS regeneration medium

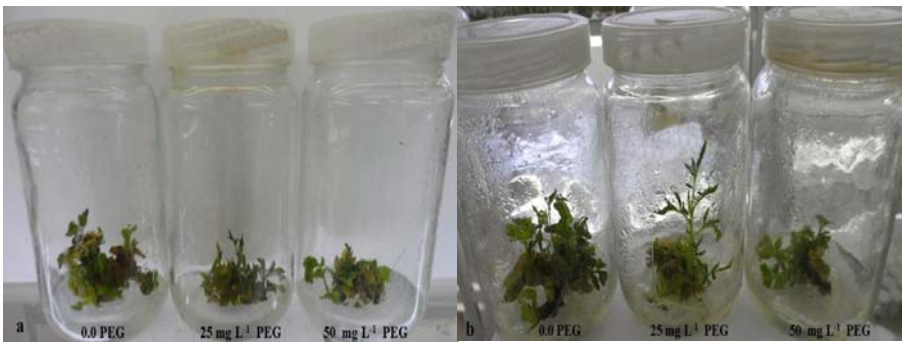

Fig. 2. Sequential growth and development of tomatoshoots(*Lycopersicon esculentum* Mill)cultivar (C3) on MS medium supplemented with zeatin (ZEA 1 mg L^{-1}), NAA (0.1 mg L^{-1}) and silver nitrate (5 mg L^{-1}) after a) 4weeks and b) 6weeks

Fig. 3. Regenerated tomato plants (*Lycopersicon esculentum* Mill)cultivar (C3) on MS medium supplemented with 1.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA ready for explantation

cultivars(4) followed by cluster II (5) and cluster III (6) as shown in Table 8.Genotypes with high GMP, MP and STI indices were considered to be suitable for growing under drought stress or normal environments. In contrast, genotypes with high SSI and TOL values should only be cultivated under non-drought conditions. Cultivars having high YSI, YI and Ys values should be suitable for drought-affected environments. Based on MP, STI, GMP indices and cluster analysis, high rates of these indices indicate endurance of cultivarsto drought stress. Among the 15 cultivars screened, cultivars C3, C9, C10, C11 and C15 were found to possessthe most pronounced drought resistance parameters, irrespective of the growing conditions of our study, including both drought levels(25 and 50 % mg L^{-1}, respectively). The moderate YI, YSI and YS values characterizing cultivars in cluster III confirm their suitability to be cultivated at both drought levels, and these cultivars may be considered as having moderate resistance to drought stress.A selection of six cultivars (C1, C2, C5, C6, C7 and C14) showingthe highest TOL and SSI values and the lowest Ys yieldwas classified as sensitive to drought stress. They should only be suitable for cultivation under non-drought conditions (Fig.4).

Protein analysis

Electrophoretic analyses were carried out on SDS-protein fractions obtained from plantlets of 15 tomato cultivars grown under normal and two levels of drought-induced stress (25 and 50 mg L^{-1} PEG). Densitometer analyses of SDS-PAGE yielded severalprotein bands with different molecular weights ranging from 6.74 kDa to 130 kDa (data not show). Total number of bands ranged from 14 to 29 under control conditions, while 14 to 28 bands were observedunder both level of treatments as shown in Figure 1. Comparing the number of bands at normal condition with the number of bands under at 50 mg L^{-1} PEG among the different tolerant and sensitive cultivars, it was clear that the stress-tolerant cultivars,namely C11, C15 and C10,exhibited more bands at 50 mg L^{-1} PEG(18, 17 and 17, resp.) compared to normal conditions (15, 15, 15, resp.). The stress-sensitive cultivars such as C1, C5, C6 and C14 had higher number of bands (22, 16, 29, and 26, resp.) under normal condition as compared to those under drought treatment (50 mgL^{-1} PEG). Various investigators suggested that the lowernumber of bands in stress-sensitive genotypesas compared tostress-tolerantgenotypes may beassociated with denaturing of the biosynthetic enzymes involved

Table 6. Tolerance indices of 15 tomato (*Lycopersiconesculentum* Mill) cultivars grown under 25 mg L^{-1} (regular) and 50 mg L^{-1} (bold) PEG6000

Cultivars	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
Ypi	1.8	3.07	2.28	1.26	2.75	2.05	2.39	1.04	2.25	1.56	1.86	1.32	0.87	1.99	1.72
Ysi	0.98	0.58	1.44	0.6	0.54	1.44	1.74	0.96	1.87	1.21	1.1	0.6	0.5	1.14	1.17
Ysi	**0.36**	**0.32**	**1.4**	**0.45**	**0.4**	**0.26**	**0.21**	**0.54**	**1.34**	**0.97**	**0.87**	**0.45**	**0.48**	**0.6**	**0.96**
SSI	1.03	1.82	0.82	1.17	1.79	0.67	0.61	0.76	0.38	0.51	0.92	1.22	0.97	0.96	0.72
SSI	**1.22**	**1.36**	**0.58**	**0.97**	**1.29**	**1.32**	**1.38**	**1.06**	**0.615**	**0.57**	**0.81**	**0.99**	**0.68**	**1.05**	**0.66**
STI	0.49	0.5	0.929	0.215	0.418	0.834	1.18	0.21	1.19	0.54	0.58	0.23	0.13	0.64	0.57
STI	**0.18**	**0.27**	**0.91**	**0.16**	**0.31**	**0.14**	**0.13**	**0.16**	**0.85**	**0.43**	**0.46**	**0.17**	**0.12**	**0.34**	**0.46**
TOL	0.83	2.49	0.83	0.66	2.21	0.62	0.65	0.35	0.38	0.35	0.77	0.72	0.38	0.85	0.55
TOL	**1.44**	**2.75**	**0.87**	**0.8**	**2.34**	**1.79**	**2.18**	**0.49**	**0.92**	**0.58**	**0.99**	**0.86**	**0.39**	**1.39**	**0.75**
MP	1.39	1.83	1.87	0.94	1.65	1.75	2.07	0.87	2.06	1.39	1.49	0.97	0.69	1.57	1.45
MP	**1.08**	**1.67**	**1.84**	**0.86**	**1.58**	**1.16**	**1.23**	**0.79**	**1.79**	**1.27**	**1.37**	**0.88**	**0.67**	**1.23**	**1.34**
GMP	1.33	1.33	1.82	0.87	1.22	1.72	2.04	0.85	2.05	1.38	1.43	0.89	0.66	1.51	1.42
GMP	**0.8**	**0.99**	**1.78**	**0.75**	**1.05**	**0.73**	**0.71**	**0.75**	**1.74**	**1.23**	**1.27**	**0.77**	**0.65**	**1.09**	**1.29**
YSI	0.54	0.18	0.63	0.47	0.19	0.7	0.73	0.66	0.83	0.77	0.58	0.46	0.57	0.58	0.68
YSI	**0.197**	**0.103**	**0.615**	**0.36**	**0.147**	**0.125**	**0.086**	**0.522**	**0.594**	**0.624**	**0.46**	**0.34**	**0.55**	**0.31**	**0.56**
YI	0.94	0.55	1.39	0.58	0.52	1.38	1.68	0.66	1.79	1.16	1.05	0.58	0.51	1.09	1.13
YI	**0.55**	**0.49**	**2.18**	**0.7**	**0.63**	**0.4**	**0.32**	**0.85**	**2.08**	**1.52**	**1.36**	**0.7**	**0.75**	**0.94**	**1.49**

in amino acids and protein synthesis under abiotic stress (Dubbey and Ranu, 1989). Rashed *et al*.,(2004) concluded that protein biosynthesis might be affected more intensely by stress-specific regulations in the less tolerant genotypes. Also, the results showed that the number of polymorphic protein bands differed among the individualcultivars under different treatments of PEG,indicating that the cultivars possess a variable ability in their adaption to drought stress via expression of stress resistance gene.This result supports the previous findings ofUllah *et al.,* (2014) and Abu Hena *et al.,* (2010),indicating that drought adaptive changes largely rely on alterations in gene expression and some transcription factor function as transcriptional activators in the expression of stress inducible gene. In our study, a total of 47 polymorphic bands with an average 42.73 % of polymorphism were recorded. The highest percentage of polymorphism (35%) was recorded for the suggested drought-resistant cultivar C11, revealing 7 polymorphic bands of 20 total bands under 25 mg $^{L-1}$ PEG. In contrast, the sensitive cultivar C5displayed the lowest relative polymorphism (12.5%)at the highest level of PEG (50 mg L^{-1}) as shown in Table (9).

Table 7. Correlation coefficient between stress resistance indices and measured traits under 25 mg L^{-1} (regular) and 50 mg L^{-1} (bold) PEG6000

Triat	SSI	STI	TOL	MP	GMP	YSI	YI
CFW	-0.059	0.084	0.02	0.078	0.044	0.059	0.122
CFW	**-0.625***	**0.552***	**-0.367**	**0.31**	**0.616***	**0.614***	**0.646****
CRG	-0.131	0.295	0.046	0.302	0.278	0.131	0.139
CRG	**-0.664****	**0.568***	**-0.374**	**0.328**	**0.604***	**0.671****	**0.669****
CWC	-0.096	0.144	-0.131	0.013	0.053	0.096	0.069
CWC	-0.546*	0.39	-0.288	0.226	0.445*	0.538*	0.49
CSR	-0.12	-0.327	-0.26	-0.366	-0.28	0.12	0.116
CSR	**-0.647****	**0.342**	**-0.513***	**0.027**	**0.398**	**0.628***	**0.515***
PRR	-0.288	0.072	-0.25	-0.007	0.079	0.288	-0.037
PRR	**-0.747****	**0.497**	**-0.580****	**0.328**	**0.541***	**0.759****	**0.668****
SFW	-0.751**	0.940**	-0.404	0.738**	0.922**	0.751**	-0.203
SFW	**-0.857****	**0.934****	**-0.525**	**0.528***	**0.925****	**0.821****	**1.00****
RFW	-0.12	-0.327	-0.26	-0.366	-0.28	0.12	0.116
RFW	**-0.647****	**0.342**	**-0.513***	**0.027**	**0.398**	**0.628***	**0.515***
SL	-0.590*	0.191	-0.611*	-0.079	0.136	0.590*	0.401
SL	**-0.872****	**0.572***	**-0.674****	**0.098**	**0.553***	**0.832****	**0.736****
RL	0.651**	-0.385	0.535*	-0.151	-0.356	-0.651**	0.082
RL	**-0.356**	**0.354**	**-0.064**	**0.297**	**0.398**	**0.269**	**0.344**
LN	0.073	-0.211	-0.047	-0.151	-0.201	-0.073	0.234
LN	**0.059**	**-0.376**	**-0.017**	**0.297**	**-0.309**	**-0.059**	**0.042**

Table 8. Comparison profile of the tomato (*Lycopersiconesculentum* Mill)cultivars group classified by Ward's minimum variance clustering method based on selection indices

Cluster groups	**Drought level (25 mg L^{-1}) PEG6000**								
	YPi	Ysi	TOL	MP	YI	SSI	YSI	STI	GMP
Cluster I (12)	1.79	1.17	0.63	1.48	1.12	0.81	0.64	0.63	1.44
Cluster II (2)	2.91	0.56	2.35	1.73	0.54	1.81	0.19	0.46	1.27
Cluster III (1)	0.87	0.50	0.378	0.68	0.50	0.968	0.567	0.122	0.658
	Drought level (50 mg L^{-1}) PEG6000								
Cluster I (4)	2.34	0.36	1.98	1.35	0.56	1.27	0.16	0.23	0.89
Cluster II (5)	1.93	1.11	0.82	1.52	1.73	0.65	0.57	0.62	1.46
Cluster III (6)	1.12	0.46	0.64	0.80	0.75	0.93	0.44	0.15	0.73

Table 9. Monomorphic bands, polymorphic bands, total polypeptides bands and polymorphism percentage for the 15 different tomato (*Lycopersiconesculentum* Mill)cultivars growing under 0.0, 25 and 50 mg L-1 PEG analyzed using SDS-PAGE

Cultivars		C1			C2			C3			C4			C5	
Treatments	a	b	c	a	b	c	a	b	c	a	b	c	a	B	c
Monomorphic bands	16	16	16	16	16	16	16	16	16	21	21	21	21	21	21
Polymorphic bands	3	4	6	4	5	2	4	7	4	7	6	7	8	8	3
Unique bands	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0
Poly. + Uniq. bands	6	4	6	4	5	3	4	7	5	7	6	7	8	8	3
Total number of bands	22	20	22	20	21	19	20	23	21	28	27	28	29	29	24
Polymorphism %	27.3	20	27.3	20	23.8	15.8	20	30.5	23.8	25	22.3	25	27.6	27.6	12.5
‘Cultivars		C6			C7			C8			C9			C10	
Treatments	a	b	c	a	b	c	a	b	c	a	b	c	a	B	C
Monomorphic bands	21	21	21	21	21	21	13	13	13	13	13	13	13	13	13
Polymorphic bands	5	5	4	4	6	7	6	4	3	1	2	1	2	4	4
Unique bands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poly. + Uniq. bands	5	5	4	6	7	7	6	4	3	1	2	1	2	4	4
Total number of bands	26	26	25	27	28	28	19	17	16	14	15	14	15	17	17
Polymorphism %	19.3	19.3	16	22.3	25	25	31.6	23.6	18.8	7.2	13.4	7.2	13.4	23.6	23.6
Cultivars		C11			C12			C13			C14			C15	
Treatments	a	b	c	a	b	c	a	b	c	a	b	c	a	b	C
Monomorphic bands	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Polymorphic bands	4	2	7	3	4	3	2	1	1	2	2	1	2	3	4
Unique bands	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0
Poly. + Uniq. bands	2	7	5	3	4	3	2	1	1	3	2	1	2	6	4
Total number of bands	15	20	18	16	17	16	15	14	14	16	15	14	15	19	17
Polymorphism %	13.4	35	27.8	18.8	23.6	18.8	13.4	7.2	7.2	18.8	13.4	7.2	13.4	31.6	23.6

a) Control (0.0) b) 25 mg L^{-1} c) 50 mg L^{-1}

Based on our SDS-PAGE analysis, newly synthesized protein bands of drought treated cultivarswere observed at molecular weights of 61.900, 15.263, 110.115, 16.456,16.130, 15.316, 46.922, 54.003, 104.321, and 107.190kDa as shown in Figure5. The stress-tolerant cultivarsC3exhibited two new bands(No. 11 and 25) at molecular weights of 61.900 and 15.263kDa, resp., which were only present at the high level of stress (50 mg L^{-1}PEG). A band at a molecular weight of 110.115 kDa was present in the moderatelyresistant cultivar C4, while it was missing in sensitive cultivars C5, C6 and C7 in the last treatment (50 mg L^{-1} PEG) and all the treatments, respectively. Moreover, the tolerant cultivars C9, C10 and C11 were characterized by a band at 16.456 kDa underdrought stress only (25 and 50 mg L^{-1}PEG). The bands number 2, 20 and 21 (107.190, 16.130 and 15.316 andKDa, resp.) were present only under 25 mg L^{-1}PEG in cultivar C15,while they were absent in cultivars C12, C13 and C14 under all treatments. In addition to these bands, a band at 46.922 kDa was found in C12, C13, C15 and C14 under normal condition and 25 mg L^{-1} PEG, however,being absent at the higher drought level (50 mg L^{-1}PEG). In additioan, a further band (54.003 kDa) was observed in moderate (C12) and tolerant (C15) cultivars following all treatments, while it wasabsent in moderate (C13) and sensitive (C14) cultivars under drought stress at both PEG levels (25 and 50 mg L^{-1}). One band was detected at molecular weight (104.321 kDa) in C12 and C15 under 25 and 50 mg L^{-1}PEG,

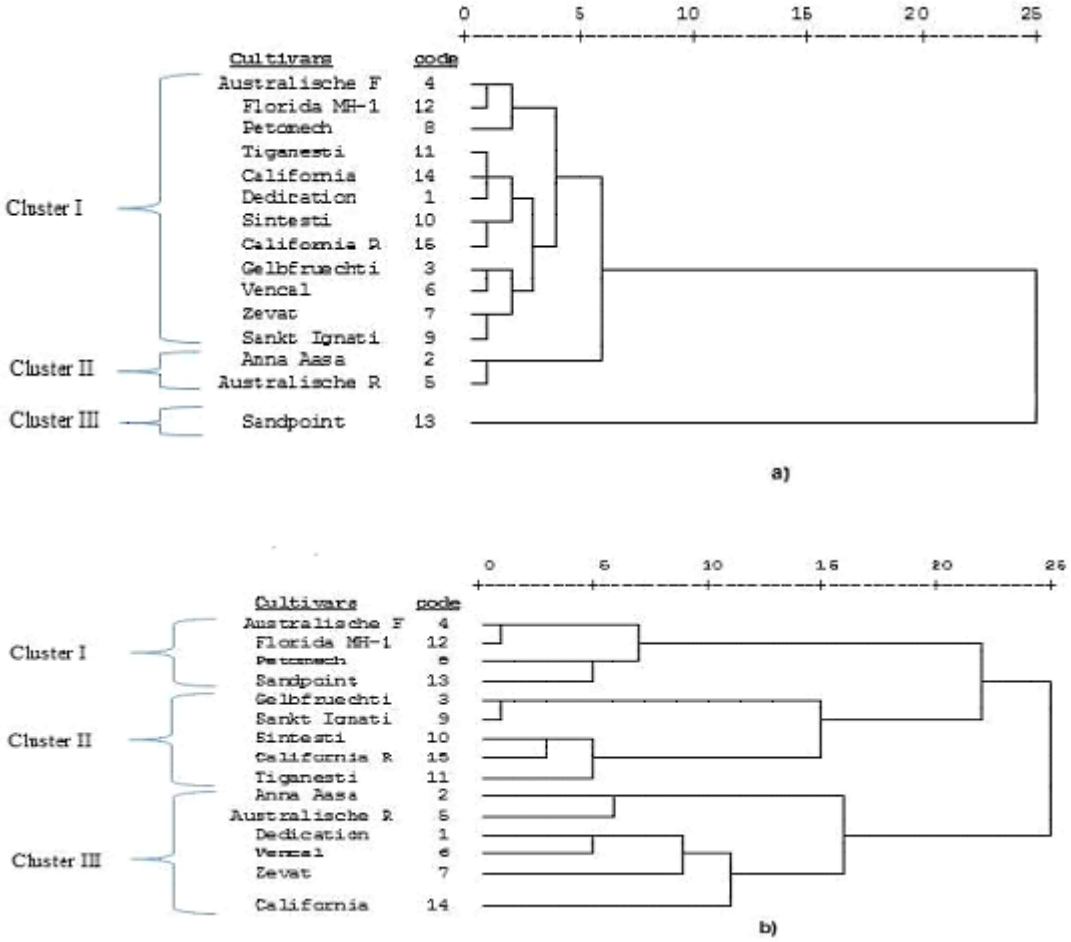

Fig. 4. Diagram of 15 tomato(*Lycopersiconesculentum* Mill) cultivars for 7 selection indices using hierarchical cluster analysis (ward's method and squared Euclidean distance) under a) 25 mg L^{-1} PEG and b) 50 mg L^{-1} PEG

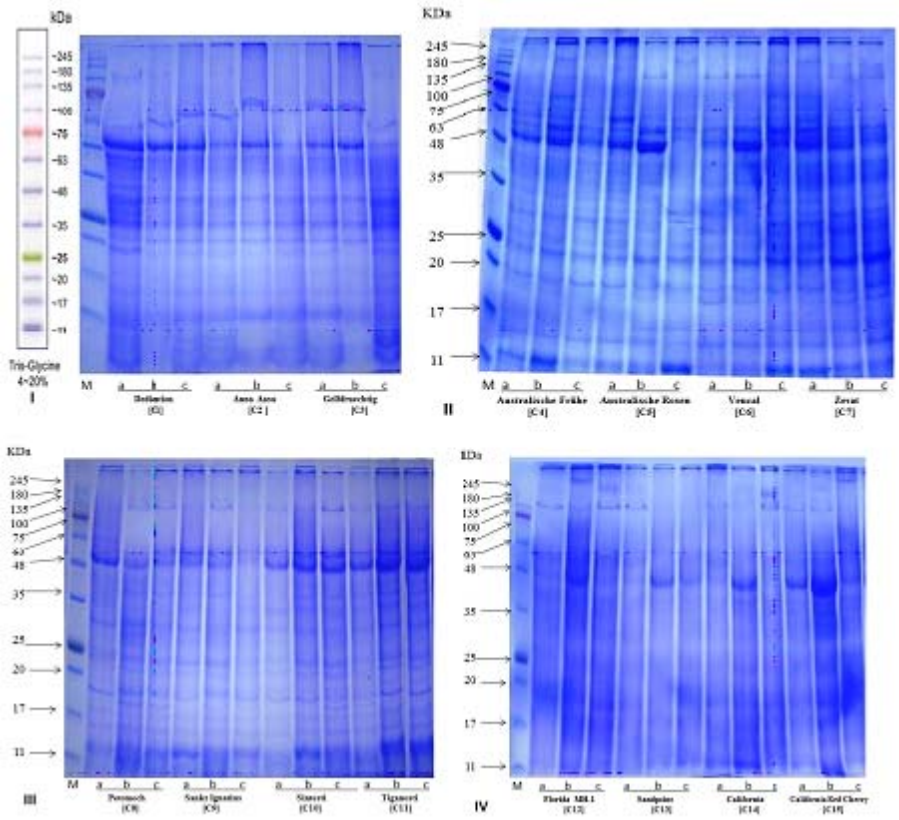

Fig. 5. SDS-PAGE protein patterns of 15 tomato (*Lycopersiconesculentum* Mill)cultivars in response to drought stress. M) Protein marker; lane a) control; Lane b) 25 mg L^{-1} PEG and Lane C) 50 mg L^{-1} PEG

respectively, while it was absent in C13 and C14. Those bandsmight be used as a molecular marker for the identification of drought tolerance in tomato, as they were present in stress-tolerant cultivarsunder treatment only, while stress-sensitive cultivars did not exhibit those bands especially under drought stress (25 and 50 mg L^{-1} PEG). On the other hand, no negative molecular marker associated with salt tolerance in tomatocultivarshas been detected in this experiment. There are quantitative (band intensity) differences for proteins among the cultivars. In various studies, many drought stress proteins having different molecular weights were found to be expressedwhen exposed to drought stress(Kamal *et al.*, 2010, Nayer and Reza 2008, Nayyer *et al.*, 2006, Bibi *et al.*, 2009 and Shihai *et al.*, 2015). The synthesis of stress proteins and the observed distinctions in protein synthesis patterns suggested may be useful biomarkers of different ecological strategies (DeBriito and Benjamin,2011). Suchvariations in intensity of protein bands have beendetected in a previous study by Hurkman and Tanka (1988) and Diana *et al.*, (2002), considering that the band intensity is directly related to protein concentration. Usually,theconcentration of a protein is modulatedby genetic and environmentalfactors. Higher plants exposed to abiotic stress such as drought exhibit a characteristic set of cellular and metabolic responses, including a decrease or increase in the synthesis of proteins(Bayoumi *et al.*,2008).

ACKNOWLEDGMENTS

The project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz

University, under grant number 5-965-35-HiCi. The authors, therefore, acknowledge with thanks DSR technical and financial support .

REFERENCES

1. Aazami M A, Torabi M and Jalili E., In vitro response of promising tomato genotypes for tolerance to osmotic stress. *African Journal of Biotechnology,* 2010; **9**(26): 4014-4017.
2. Abu Hena M, Ki-Hyun K, Kwang H, Jong-Soon C, Byung-Kee B,HisashiT, Hwa Young H, Chul–Soo P, Sun–Hee W., Abiotic stress responsive proteins of wheat grain determined using proteomics technique. *AJCS* 2010; **4**(3):196-208
3. Adachi Y, Kimura K, Saigusa M, Ohyama T, Takahashi Y, Watanabe H. Growth promoting effect of L-β-phenyllactic acid on rice (*Oryza sativa* L.) seedling grown under polyethylene glycol (PEG)-induced water deficit condition. *Plant Root*, 2014; **8**: 64-71.
5. Adele M, Maria S, Umberto A, Carmelo S, Albino M., Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *Journal of Plant Interactions*, 2014; **9**(1):354-363
6. Ania W., Use of Biotechnology in Agriculture—Benefits and Risks. *Biotechnology*, 2003; **3**: 1-6.
7. Anwar J, Subhani M, Hussain M, Ahmed J Munir M., Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Pakistan Journal of Botany,* 2011; **43**: 1527-1530.
8. Azeez M A, Morakinyo J A., Electrophoretic characterization of crude leaf proteins in Lycopersicon and Trichosanthes cultivars. *African Journal of Biotechnology*, 2004; **3**(11): 585-587.
9. Badran A, AbdAlhady M, Hassan W., *In Vitro* Evaluation of Some Traits in *Stevia rebaudiana*(Bertoni) under Drought Stress and Their Relationship on Stevioside Content. *American Journal of Plant Sciences*, 2015; **6**: 746-752
10. Bayoumi T, Manal E, Metwali E., Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *African Journal of Biotechnology*, 2008; **7**(14):2341-2352.
11. Bibi H, Sadaqat A, Tahir M, Akram M., Screening of sorghum (*Sorghum bicolor* VarMoench) for drought tolerance at seedling stage in polyethylene glycol. *The Journal of Animal and Plant Sciences*, 2012; **22**(3): 671-678
12. Bibi N, Hameed A, Ali H, Iqbal N, Haq M, Atta B, Shah T, Alam., As water stress induced variations in protein profiles of germination cotyledon from seedlings of chickpea genotypes *Pak. J. Bot*., 2009; **41**(2): 731-736.
13. Bouslama M, Schapaugh WT., Stress tolerance in soybean. Part. 1: Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci* 1984; **24**: 933-937.
14. Boyer J S., Plant productivity and environment. *Science*, 1982; **218**: 443–448.
15. Bradford M M., A rapid and sensitive method for thequantitation of microgram quantities of protein utilizing theprinciple of protein-dye) binding. *Annals of Biochemistry*, 1976; **72**: 248-254.
16. Chen RD, Tabaeizadeh Z., Alteration of gene expression in tomato plants (*Lycopersicone sculentum*) by drought and salt stress. *Genome* 1992; **35**: 385-391.
17. De Britto A, Benjamin J., Drought Stress and Its Impact on Protein in Three Species of Vitex. *Journal of Stress Physiology and Biochemistry*, 2011; **7**(3):152-158
18. Diana J, Romero-Garcia J, Rodriguez R, Sanchez J., Tends in new crops and new uses. In: JanickJW(eds.) *ASHS press*. 2002; 143-149.
19. Dubey R, Ranu M., Influence of NaCl salinity on growth and metabolic status of proteins and amino acids in Rice seedlings. *Journal Agronomy Crop Science*, 1989; **162**:97-106.
20. Ehab M R Metwali, Hemaid I A Soliman, Howladar S M, Al-Zahrani H S, Fuller M P., In vitro Selection and Characterization of Drought-tolerant of some Fig (*Ficus carica* L.) Plants using DNA Markers. *Journal of Pure and Applied Microbiology*, 2014; **8**(6): 4373-4384
21. El Houssine B, Mohammed D., Response of durum wheat (*Triticum durum* Desf.) callus culture to osmosis induced drought stress caused by polyethylene glycol (PEG). *Annals of Biological Research*, 2012; **3**(9):4555-4563
22. Elbeshehy E, Ehab M R Metwali, Omar A Almaghrabi. Antiviral activity of *Thujaorientalis* extract against watermelon mosaic virus (MWV) on *Citrulluslanatus*. *Saudi Journal of Biological Science*, 2015; **22**: 211-219
23. Elizabeta M, Blagica D, Koo P, Zoran T., Differences in tomato seed protein profiles obtained by SDS-PAGE analysis. *Journal of Agricultural Sciences* 2008; **53**(1):13-23.
24. El-sayed A, Mahdia G, Fouad H, Mona Ismail., Responses to NaCl Salinity of Tomato Cultivated and BreedingLines Differing in Salt Tolerance in Callus Cultures. *International*

Journal of Agriculture and Biology, 2004; **6**(1):19-26

25. FAOSTAT., Food and Agricultural Organization of the United Nations. Statistical Databases, at http://www.fao.org, 2012.
26. Fernandez GCJ., Effective selection criteria for assessing stress tolerance. In: Kuo CG (ed) Proceedings of the International Symposium on Adaptation of vegetables and other food crops in temperature and water stress, Tainan, Taiwan, 1992.
27. Fischer RA, Maurer R., Drought resistance in spring wheat cultivars.I. Grain yield response. *Aust J Agric Res* 1978; **29**: 897–907
28. Furdi Florina., Assessment of genetic diversity in a collection of local tomatoes by SDS-PAGE method. *Journal of Horticulture, Forestry and Biotechnology*, 2012; **16**(3): 133-136.
29. Gavuzzi P, Rizza F, Palumbo M, Campaline R G, Ricciardi G L, Borghi B., Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Plant Science*, 1997; **77**: 523-531
30. Gavuzzi P, Rizza F, Palumbo M, Campaline RG, Ricciardi GL, Borghi B., Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Plant Sci* 1997; **77**: 523-531
31. Hassan M, Serag M, El-Feky F., Changes in nitrogen content and protein profiles following *in vitro* selection of NaCl resistant mung bean and tomato. *Acta Physiologiae Plantarum*, 2004; **26**: 165-175.
32. Hemaid I A Soliman and Mohamed H Hendawy., Selection for Drought Tolerance Genotypes in Durum Wheat(*Triticum durum*Desf.) Under *In vitro* Conditions. Middle-*East Journal of Scientific Research*, 2013; **14** (1): 69-78
33. Hemaid I E Soliman, Ehab M R Metwali ,Omar A Almagrhabi., Micropropagation of *Stevia rebaudiana*Betroni and assessment of genetic stability of in vitro regenerated plants using inter simple sequence repeat (ISSR) marker. *Plant biotechnology*, 2014; **31**(3):249-256.
34. Hossain ABS, Sears AG, Cox TS, Paulsen GM., Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci* 1990; **30**: 622-627
35. Hurkman W, Tanaka C., Polypeptide change induced by salt stress, water deficit and osmotic changes stress in barley roots: accumulation using twodimensional gel electrophoresis. 1988; **9**: 781-787
36. Iker E, Tatar O, Aykut T, Tousun M., Determination of tolerance level of some wheat genotypes to post-anthesis drought. *Turkish Journal of Field Crops*, 2011; **16**: 59-63.
37. Imran M, Abdul R, Ishfaq A, Shuaib K., Interaction of callus selection media and stressduration for in vitro selection of drought tolerant callusof wheat. *African Journal of Biotechnology*, 2012; **11**(17): 4000-4006
38. Kamal A, Kim K, Shin K, Choi J, Baik B, Tsujimoto H, Heo H, Park C, Woo S., Abiotic stress responsive proteins of wheat grain determined using proteomics technique. *Aust. J. Crop Sci.* 2010; **4**(3):196-208
39. Khayatnezhad M, Gholamin R, Jamaati S, Zabihie R., Investigation and selection drought indexes stress for corn genotypes. *AmerEur J Agric Environ Sci* 2011; **8**: 822–826.
40. Laemmli U K., Cleavage of structural proteins during assembly of head bacteriophage T4. *Nature*, 1970; **227**: 680-685.
41. Manaj K Raia, Rajwant K Kaliaa, RohtasSingha, Manu P Gangolaa, Dhawan A K., Developing stress tolerant plants through in vitro selection—An overview of the recent progress. *Environmental and Experimental Botany*, 2011; **71**: 89-98
42. Manal M, Ehab M, Ahmed I., Assessement of genetic diversity of sorghum (*Sorghum bicolour* L. Mench) genotypes under saline irrigation water based on some selection indices. *Australian Journal of Crop Science*, 2013; **7**(12): 1935-1945.
43. Mennella G, Onofaro S V, D'alessandro A, Coppola R, Poma I., Tomato Ecotype Characterization by Anionic Exchange High Performance Liquid Chromatography Analysis of Endosperm Seed Proteins. *Acta Horticulture*, 2001; **546**: 453- 457.
44. Metwali M R Ehab, Hemaid I E Soliman, Fuller M P, Omar A A., Improving fruit quality in tomato (*Lycopersicumesculantum* L.) under heat stress by silencing the Vir 1 gene using small interfering RNA technology. *Plant Cell, Tissue and Organ Culture*, 2015; **121**:153-166.
45. Milka B, Zdenka G, Suzana P, Milan U, Jasmina Z., Shoot and root dry weight in drought exposed tomato populations. *Genetika*, 2014 ; **46**(2): 495-504
46. Mohammad A, Saeedreza V and Abdolreza B., Biochemical Changes and SDS-PAGE Analyses of Chickpea (*Cicerarietinum* L.) Genotypes in Response to Salinity During the Early Stages of Seedling Growth. *J. Biol. Environ. Sci* 2014; **8**(23): 99-109
47. Murashige T, Skoog F., A revised medium for rapid growth andbioassays with tobacco tissue cultures. *Physiol. Plant.* 1962; **15**: 473–497.

48. Nayer M, Reza H., Effects of Drought Stress on Soluble Proteins in two Maize Varieties. *Turkish Journal of Biology*, 2008; **32**:23-30**.**
49. Nayyer I, Hina A, Muhammad K, Riaz K, Muhammad A., Effect of Water Deficit on Protein Reserves in Cotyledons of Germinating Cotton Seeds. *ScienceAsia*,2006; **32**: 329-331
50. Noaman S H, Lamis D S, El-Sayed Ah and Eman E S., In vitro selection for water stress tolerant callus line of Helianthus annus L. Cv. Myak. *Int. J. Agric. Biol*. 2004; **6**(1): 13-18.
51. Pena R D L and Hughes J., Improving vegetable productivity in a variable and changing climate. *SAT eJournal*, 2007; **4**: 1-22
52. Ragab AR, Abdel-Raheem A T, Kasem Z A, Omar F D, Samera A M., Evaluation of R1 tomato somaclone plants selected under poly ethylene glycol (PEG) treatments. *Afr. Crop Sci. Soc*., 2007; **8**: 2017-2025.
53. Rai M K, Kalia R K, Singh R, Gangola M P, Dhawan A K., Developing stress tolerant plants through in vitro selection—An overview of the recent progress. *Environmental and Experimental Botany*, 2011; **71**: 89-98
54. Rashed M, El-Seoudy A, Fahmy E, Salam T, Abdel-Aziz S, Abdel-Bary A., Molecular genetic markers for salt stress tolerance in wheat. *Egypt Journal of Genetic and Cytology*, 2004; **33**(1): 163-183
55. Romersburg H C., Cluster analysis for researchers. Lifetime Learning Publications Belmont, California, 1988.
56. Shatnawi M, Faouri A, Al-Mazraawi M, Shibli R, Makhadmeh I., Tissue culture and salt stress in *Chrysanthemum morifolium*. *Acta Hortic*, 2009; **829**:189-196.
57. Shihai Y, Xiong L, Yushou M, Xudong S, Yungiang Y, Yonqping Y., Proteome response of wild wheat relative Kengyiliathoroldiana to drought stress. *Canadian Journal of Plant Science*, 2015; **95**(2): 237-249
58. Sonia G, Shakeel A J, Sadar U S., Genotypic differences against PEG simulated drought stress in Tomato. *Pak. J. Bot.*, 2013; **45**(5): 1551-1556.
59. Talebi R, Fayaz F, Naji N., Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* Desf.). *Gen Appl Plant Physiol* 2009; **5**: 64-74
60. Ullah M, Akhtar N, Mehmood N, Shah I., Effect of mannitol induced drought stress on seedling traits and protein profile of two wheat cultivars. *The Journal of Animal and Plant Sciences* 2014; **24**(4): 1246-1251
61. Waraich E A, Ahmad R, Ashraf M Y, Saifullah., Role of mineral nutrition in alleviation of drought stress in plants. *Aust. J. Crop Sci.*, 2011; **5**: 764-777.