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Rapid method of screening for drought stress tolerance in maize (*Zea mays* L.)

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Abstract

Drought stress is the major production constraint in rainfed maize. Screening for drought tolerance is severely affected by the lack of a simple and reliable phenotyping technique. The objective of this study was to standardize a simple hydroponic based drought screening technique in maize. In this context, one week old uniform seedlings of 55 inbreds and 5 hybrids were transferred to hydroponic solution in the glass house. The seedlings were allowed to acclimatize for next one week in hydroponic solution. The drought stress was imposed by removing seedlings from nutrient solution and exposed to air for 6 and 4 hours daily for a period of 5 and 4 consecutive days in hybrids and inbreds, respectively. Data were recorded on all shoot and root parameters, and based on stress symptoms, a drought tolerance score was given to each genotype. The percent deductions in shoot and root fresh weight from non-stress to stress ranged from 11.7 to 84.4 and 2.1 to 77.5, respectively. Six inbred lines, namely, DQL790-4, CML334, CM140, CML422, CM125 and HKI488 and three hybrids namely DMRH1306, DMRH1410 and PMH4 were found drought tolerant. The effectiveness of this screening technique was compared and confirmed using pots screening as well as by expression profiling of key antioxidant genes (*Sod2, Sod4, Sod9* and *Apx1*) playing role in drought stress tolerance. This phenotyping technique is very short, low cost and simple which can be utilized in preliminary drought screening for large set of maize germplasm and mapping populations.

Keywords: Maize, drought tolerance, hydroponic solution, anti-oxidant genes, expression profiling

Introduction

Maize (*Zea mays* L.) is one of the most versatile crops with wider adaptability in diverse environments. In the last decade, a spectacular increase in maize productivity has been seen due to adoption of single-cross hybrids and expansion of maize cultivation in favorable (winter-season) environments (Yadav et al. 2015; Rakshit et al. 2017). However in India, around 80% of maize is still cultivated as rainfed which is more prone to abiotic and biotic stresses due to their unpredictable and highly variable weather patterns (Yoshida 1977). The drought stress is one of the most detrimental abiotic stresses which adversely affect plant's growth, development and hence yield. To cope up with drought stress, plants alter their metabolism in many ways such as by activating signalling cascades and abscisic acid (ABA) - independent and - dependent regulatory systems, modulating antioxidant defence system to maintain cellular homeostasis, synthesizing and accumulating compatible solutes (such as proline) which assist in osmotic adjustment, and modulating expression of an array of genes encoding for drought responsive transcription factors, heat shock proteins, dehydrins and aquaporins etc. (Shinozaki and Yamaguchi-Shinozaki 2007; Singh and Laxmi 2015; Kaur and Asthir 2017). Compared to drought sensitive genotype, the tolerant genotype exhibited higher root/shoot ratio through increased root length and decreased shoot length, lower reduction in plant growth, relatively higher water content, more photosynthetic pigments, and negligible increment in electrolyte leakage (Sarker and Oba 2018). To minimize yield losses due to drought, development of genotypes that can perform reasonably well in drought-stress environments is an important objective in any maize breeding programme. To

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develop drought tolerant genotypes, the reliable phenotyping technique for large set of diverse germplasm has always remained an important activity of any breeding programme. Various field and glass house based drought screening techniques have been developed on the basis of physiological, biochemical and morphological parameters in maize and other crops (Kumar and Singh 1998; Hura et al. 2009; Kumar et al. 2016a). These techniques have their own de-merits (Nye and Tinker 1997; Passioura et al. 2006; Munns et al. 2010) as they are expensive, time-consuming, and laborious. In the field screening, flowering stage is the most sensitive stage for drought stress. The low moisture stress at this stage may delay the silk emergence leading to prolonged anthesis silking interval (ASI) which affects fertilization and kernels formation (Almeida et al. 2013; Ngugi et al. 2013; Kumar et al. 2016a). However, retaining uniform level of drought stress at this stage across the field is a difficult task. Other than this, the root parameters have been identified as very important traits for survival of plants under any type of abiotic stresses (Smith and De Smet 2012), but the characterization of roots in the field and pot is very difficult. As such there is no user friendly and accurate non-destructive method available for roots characterization under abiotic stress in field and pots experiments. Further, in the era-of climate change, low moisture stress may occur randomly at any stages of crop growth. Therefore, the possibility of identifying drought tolerant genotypes at seedling stage may also be exploited. There are several screening techniques available to identify drought tolerant genotypes at seedling stage (Munns et al. 2010), but they have their own de-merits. For instance, in pot screening it is very difficult to maintain uniform and constant water potential throughout the soil profile in pots, which in turn may affect the nutrient transmission (Nye and Tinker 1977). Similarly, the soil may get easily saturated at the bottom and therefore may lead to variable level of water potentials (Passioura 2006). The pot soil can be replaced with the materials like vermiculite, coco-peat, soil rite but the large particle size of these may further lead to less root contact (Verslues et al. 1998).

Hydroponics with non-ionic osmotica is often used as a way of overcoming the problems of heterogeneity, drainage, and inconstant water potential, but these molecules eventually enter roots and move to shoots through the xylem and affect the normal screening (Hohl and Schopfer 1991; Munns et al. 2010). Besides, high-molecular-weight polyethylene glycol (PEG) has been examined in many studies but its main problem is that it limits O_2 diffusion to roots (Mexal et al. 1975; Verslues et al. 1998).

Keeping this in view, an experiment was framed with objective to standardize a rapid method of screening for drought tolerance in maize. A new phenotyping technique of drought screening has been established in maize using hydroponics without non-ionic/ionic osmotic and PEG to screen large numbers of maize genotypes for low moisture stress at seedling stage. The tolerant and susceptible genotypes identified were also exposed to drought stress in pots. Further, expression profiling of key genes of the antioxidant pathways was done for tolerant and susceptible genotypes using semi-quantitative polymerase chain reaction (PCR).

Materials and methods

Experimental materials and design

In this study, a set of 55 inbred lines along with five hybrids were used as experimental materials for drought stress screening using hydroponic solution in glass house during 2016-17. These genotypes were selected from CIMMYT inbred lines, AICRP maize centers and ICAR-IIMR, Ludhiana materials (Table 1). The five hybrids identified as drought tolerant (DMRH1306, PMH4) and susceptible (DMRH1410, IMH1415, HM5) in field evaluation (Kumar et al. 2016b) were also considered for screening with this protocol. The genotypes identified as highly tolerant and susceptible through this rapid screening method were also exposed to drought stress in pots (dimension: 10×10 cm) filled with soil. Two sets of experiments were constituted; one set was used for screening under stress environment whereas another was kept as control where no stress was given. The inbred lines as well as hybrids were evaluated in completely randomized design using two replications in glass house with each replicate consisted of 8 plants per genotype. The statistical analysis was performed using GENSTAT 17th Edition (VSN International 2014).

Hydroponic solution and growth parameters

The experiment was conducted in tray by growing 7 days old seedlings in hydroponic using modified Hoagland solution (Table 2). A separate set of same genotypes were also grown in readymade Hoagland solution while standardization (Simon et al. 1994). Initially 50 seeds of each genotype were dipped in 1.0% bavistin solution for 3-4 minutes and thereafter

Table 1. Details of inbred lines used in screening and their source, adaptability, percent reduction in fresh shoot as well as in root weight and visual drought score allotted during the evaluation.

S.No.	Genotype*	Percent reduction in fresh shoot weight	Percent reduction in fresh root weight	Drought score (1-9)
1	CM 125St	43.9	11.8	3.0
2	CM 140St	42.7	11.5	3.0
3	CML 37^{L}	44.1	14.3	5.3
4	CML 40^{L}	53.2	10.0	5.0
5	CML 141^{L}	68.1	45.8	6.5
6	CML 142×150^{L}	71.2	34.4	8.5
7	CML 163^{L}	52.7	24.6	6.0
8	CML 170^{L}	61.8	34.4	5.5
9	CML 171^{L}	84.4	42.4	9.0
10	CML 176St	71.1	72.4	8.2
11	CML 180St	64.1	3.90	7.5
12	CML 186St	56.3	24.6	5.5
13	CML 189St	57.7	25.2	7.5
14	CML 195St	66.4	2.10	8.5
15	CML 206St	57.4	12.6	5.5
16	CML 207St	73.4	68.2	8.2
17	CML 208St	43.4	38.0	5.0
18	CML 220St	49.0	9.70	5.5
19	CML 266^{L}	78.7	77.5	8.1
20	CML 269^{L}	37.4	14.3	5.2
21	CML 271^{L}	68.4	33.8	6.5
22	CML 278^{L}	48.6	43.3	5.4
23	CML 312St	49.5	20.2	6.8
24	CML 317St	78.7	8.00	7.9
25	CML 327St	75.0	69.0	8.5
26	CML 334St	44.3	8.00	3.0
27	CML 409^{L}	56.9	14.3	7.0
28	CML 422^{L}	11.7	7.30	3.0
29	CML 437St	40.5	14.5	6.0
30	CML 446^{L}	77.7	47.3	8.5
31	CML 452^{L}	45.2	55.8	5.0
32	CML 484St	62.2	33.4	7.0
33	CML 493^{L}	42.6	28.6	5.0
34	CML 494^{L}	65.0	30.4	6.5
35	CML 542St	53.8	25.9	7.0
36	CML 550^{L}	49.3	24.6	5.0
37	CML 551^{L}	58.3	10.5	7.0
38	CML 554^{L}	72.6	61.4	8.5
39	CML 556^{L}	72.5	15.1	7.8
40	CML 557^{L}	73.7	46.2	8.5
41	CML 559St	65.3	22.9	9.0
42	DQL 633-1St	15.9	34.6	3.3
43	DQL 790-4St	49.7	10.2	3.0
44	DQL 1019St	69.4	34.2	7.5
45	HKI 193-1St	57.9	39.5	8.5
46	HKI 323St	56.2	19.0	8.0
47	HKI 488-1St	44.5	8.70	3.0
48	HKI 1348St	48.4	17.6	8.0
49	HKI 1378St	61.1	12.3	7.5
50	IC 594467St	73.5	34.9	7.0
51	KDTML-3St	44.3	24.7	6.0
52	KDTML-19St	55.1	21.8	5.0
53	KDTML-66St	63.4	18.6	6.0
54	LM 14St	43.0	28.1	3.2
55	LM 16St	47.3	13.7	5.0
Mean		57.0±1.33	27.6±1.70	6.3±0.16
LSD (*P*=1%)		14.8	9.2	1.9

*****The superscript letters written on genotype name such as 'St' represents the sub-tropical and 'L' Lowland adaptations of the material.

were rinsed with distilled water. The uniform size seeds were put in germinating papers for germination for period of 7days. Thereafter, uniform height seedlings were selected and transferred to plastic trays (dimension: 45 × 33 × 20 cm) containing modified Hoagland nutrients solution and covered by wooden board having small holes for fixing seedlings with support of cotton plug. The trays were filled completely with nutrients solution in such a way that the roots remained dipped in the solution. Nutrients solution was replaced after every three days interval. The nutrient solution was continuously aerated by bubbling air through aquarium pumps in the trays. The growing parameters of 29/ 25^{o}C day/night air temperatures, 16 hrs light, and 60% relative humidity were maintained during the experimentation in the glass house.

Imposing low moisture stress

After shifting of 7 days old uniform seedlings to hydroponic solution under glass house, initially for the first seven days, all seedlings were kept for

Table 2. Detail of macro-micro nutrients concentrations standardized and used for growing of maize seedlings in hydroponic medium

S.No.	Compound	Mol. weight	Stock sol. (μM)	Working sol. (μM)
A.	**Macronutrients**			
1.	KNO_3	101.10	1000	6300
2.	$Ca(NO_3)_2.4H_2O$	236.16	1000	4300
3.	$NH_4H_2PO_4$	115.08	1000	2400
4.	$MgSO_4.7H_2O$	246.48	1000	1200
B.	**Micronutrients**			
1.	KCl	74.55	25.0	57.5
2	H_3BO_3	61.83	12.5	28.75
3	$MnSO_4.H_2O$	169.01	1.0	2.3
4	$ZnSO_4.7H_2O$	287.54	1.0	2.3
5	$CuSO_4.5H_2O$	249.68	0.25	0.575
6	H_2MoO_4(85%MoO_3)	161.97	0.25	0.575
C.	Na Fe EDTA (10% Fe)	558.50	53.7	80.55

acclimatization and establishment in the nutrients solution. There was no stress given in either of the set during acclimatization period. Thereafter, the drought stress was imposed (around two weeks old seedlings) by removing maize seedlings along with tray board from the nutrient solution without blot drying of roots and exposed to air (Fig. 1) for different durations. The experiments were carried out with different durations of drought stress e.g. 6, 7 and 8 hrs stress for 5, 4 and 3 consecutive days, respectively in hybrids and 4, 5, and 6 hrs for 4, 3, 2 days in inbreds, respectively. After stress for specified durations, the seedlings were reverted back to the nutrients solution. In the control set, the seedlings were kept drought free by keeping them in nutrient solution for entire period without any interruptions (Fig. 1). Some of the genotypes noted to be susceptible and tolerant in hydroponic experiment were also validated for low moisture stress response under pots. Stress was imposed by withholding irrigation at 3-5 leaves stage and sustained for 15 days in inbred and 20 days in hybrids which attained 18% soil water content (Fig. 1). The drought score was allotted to each one of them.

Defining rating scale and recording of observations

After giving drought stress for specified duration in the screening experiment, all the genotypes were rated for drought response using 1.0 to 9.0 scale (Fig. 2). The disease rating scale of 1.0 to 9.0 for foliar leaf diseases of maize defined by Balint-Kurti et al. (2006) was considered as base line for defining the various classes. The 1.0 to 9.0 scale used for drought tolerance scoring was defined as $\leq$3.0 = green plants with slight wilting (tolerant), 3.1 to 5.0 = green plants with moderate wilting (moderately tolerant), 5.1 to 7.0 = leaves turning yellowish green with moderate to high wilting (moderately susceptible), and 7.1 to 9.0 = leaves yellow-brown to completely dried leaves and/ or stems (susceptible). The symptoms were recommended to each class based on visual observations for maximum and minimum responses observed during the drought stress screening. Genotypes with the lowest (1.0) and highest scores (9.0) on 1.0 to 9.0 rating scale were considered as highly tolerant and highly susceptible to drought stress, respectively. After the completion of specified durations in stress as well as controlled experiments, data on shoot and root weight (gm) on wet and dry bases and root length (cm) on fresh bases was recorded for five plants of each genotype per replication. For dry bases, the samples were dried in oven for 72 hrs at 65^{o}C temperature. The percent reduction in shoot and root fresh and dry weight and root length on fresh bases for control versus drought stress treatment was calculated. The drought score given based on visual observations of drought symptoms to each genotype was correlated with the percent reduction of shoot and root weight and root length.

Expression profiling of antioxidant genes

The total RNA was isolated from leaf samples of visually scored highly tolerant and susceptible genotypes for stress as well as non-stress conditions using Ambion Pure Link™ Plant RNA kit (Invitrogen). The quality and quantity of total RNA was assessed by Nano-Drop 1000 spectrophotometer. The RNA samples with A260/A280 ratio of ~ 2.0 and A260/A230 ratio of 2.0-2.2 were considered suitable for expression studies. The isolated, purified and quantified RNA was stored at -80^{o}C for further studies. First strand cDNA was synthesized using Super Script III® first strand cDNA synthesis system (Invitrogen, USA). The synthesized cDNA was stored at –20^{o}C and further used for semi-quantitative RT-PCR analyses. Semi-quantitative analysis was performed with gene-specific primers of four antioxidant genes encoding for superoxide dismutase (*Sod2*, *Sod4* and *Sod9*: NCBI accession no. KR136339.1, U34727.2 and

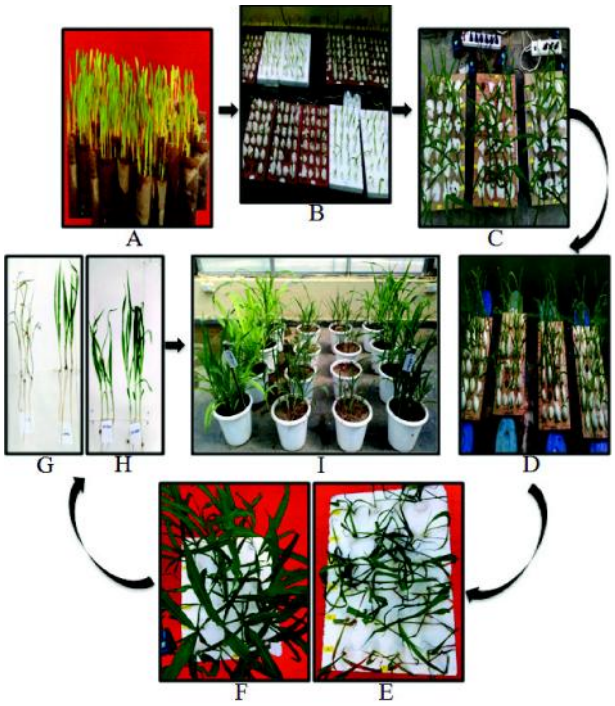

Fig. 1. Detail steps of screening for drought stress tolerance in maize using hydroponic based method. A: Seven days old seedling in germination paper, B&C: Acclimatization in hydroponic solution, D: Seedling exposed to air for drought stress, E: Genotypes showing stress symptoms, F: Seedlings under non-stress (control), G&H: Susceptible and tolerant genotypes with their control, I: Confirmation under pots screening

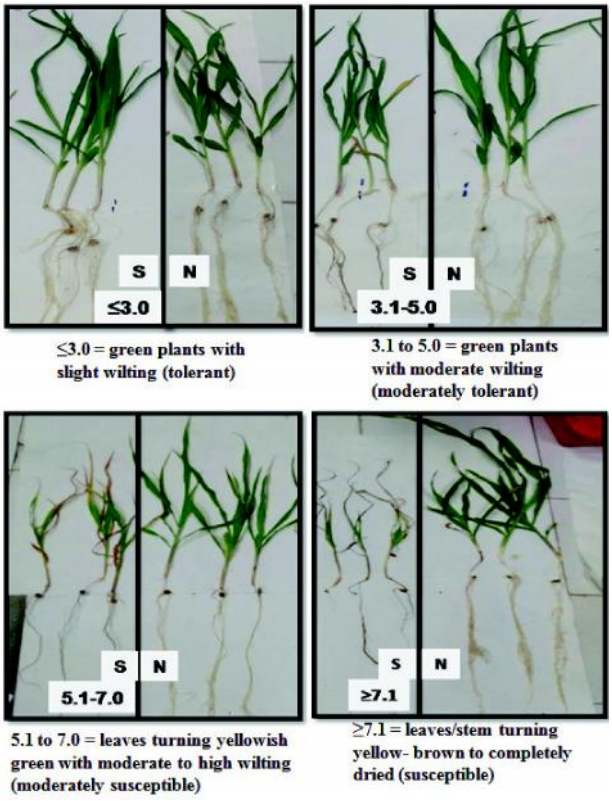

Fig. 2. The 1.0 to 9.0 rating scale designed on the bases of visual symptoms observed during hydroponic screening. The scale can be used for giving drought tolerant score to any genotypes during hydroponic based drought screening. The S&N referred for plant seedlings under drought stress and non-stress, respectively

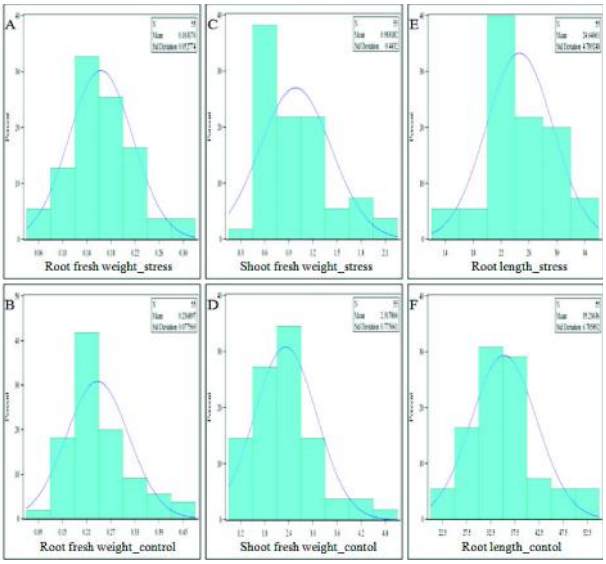

Fig. 3. Phenotypic variations for shoot and root fresh and dry weight and root length parameters under drought stress and control conditions

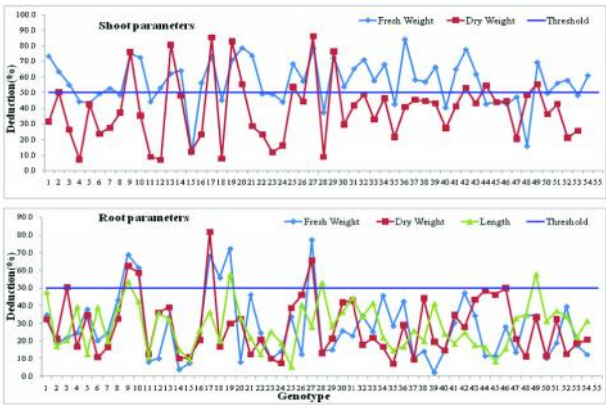

Fig. 4. Percent reduction in shoot and root fresh and dry weight and root length parameters from non-stress to stress. The percent deductions in shoot parameters were found higher and more above the threshold as compared to the root parameters. The 1-55 serial numbers correspond to the genotypes name given in Table 1

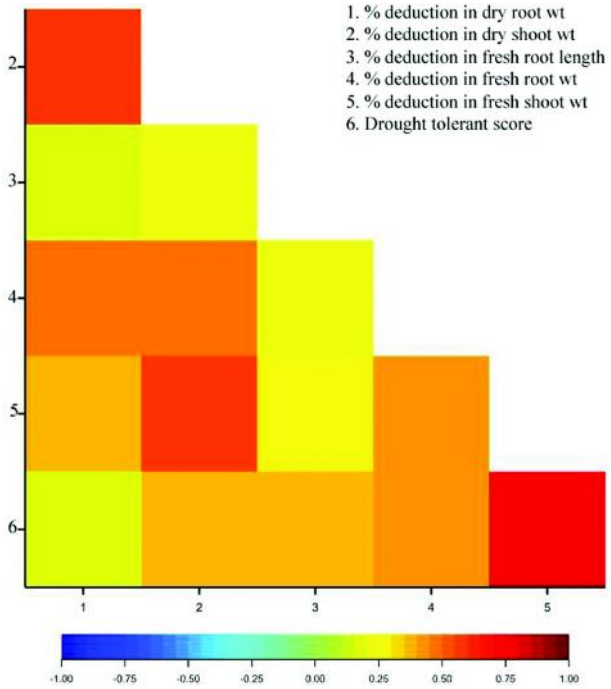

Fig. 5. Correlation between the percent reduction in shoot and root parameters and drought tolerant score. The positive correlation was observed for all reductions with drought tolerant score. There was very high positive significant correlation (r =0.76) observed between drought tolerant score and percent deduction in shoot fresh weight

KR136341.1, respectively) and ascorbate peroxidase (*Apx1*: NCBI accession no. KR136342.1). The primers were designed by using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/) and their specificity was checked by Primer-Blast software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR amplifications were performed in 20 µl of reaction volume containing 100 ng template cDNA, 1x *Taq* polymerase buffer, 0.4 µL of 50mM $MgCl_2$, 0.4 ìL of 10mM dNTP mix, 0.4 µM of each forward and reverse primer and 1U of *Taq* DNA polymerase. The PCR amplification was carried out in a BioMetra thermal cycler programmed as follow: initial denaturation for 3 min at 94°C, following 28 cycles of denaturation at 94°C for 30 s; annealing 58°-60°C for 30 s; and extensions at 72°C for 30 s. After completion of semi quantitative PCR, amplicons were analyzed by agarose gel electrophoresis and documented by using Alpha Innotech gel documentation system.

Results and discussion

Hydroponic medium and duration of stress

The Hoagland solution (Simon et al. 1994) was standardized with certain modifications in micro and macro nutrients concentration and the best one of these was worked out for normal maize seedling growth in the glass house (Table 2). This protocol may be utilized for growing maize in hydroponic solution under control environments. Based on visual observations of seedlings in stress as well as in control, the stress for 6 hours for a period of 5 days for hybrids and 4 hours daily for a period of 4 consecutive days for inbreds was found suitable for distinguishing the tolerant and susceptible genotypes (Fig. 2). The analysis of variance revealed sufficient genetic variability for root and shoot parameters. The drought stress of 7 and 8 hrs for 4 and 3 consecutive days in

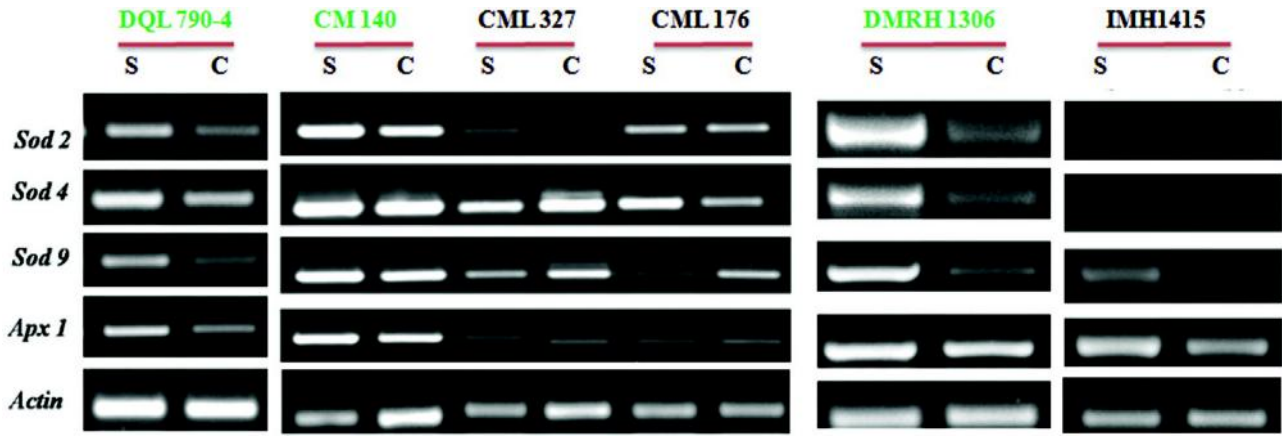

Fig. 6. Expression profiling of antioxidant genes for drought tolerant (DQL790-4, CM140, DMRH1306) and susceptible (CML327, CML176, IMH1415) genotypes. The *Actin* gene was used as an internal control. The S and C represent the samples under stress and control conditions, respectively. The relatively higher expression for antioxidant genes was observed in tolerant genotypes compared to susceptible and their control

hybrids gave total sum of 28 and 24 hrs stress, respectively, throughout the protocol, which is lower than the standardized duration of total sum of 30 hrs (@6 hrs for 5 days). But we observed very less recovery of seedlings at 7 and 8 hrs stresses for 4 and 3 consecutive days, which have indicated that more than 6 hrs stresses in a day is beyond the capability of even any tolerant genotypes to survive and recover back. The total duration of stress was almost half in the inbred lines than that of hybrids; this is because of weak and homozygous nature of the inbred as compared to hybrids which exploited heterosis phenomenon. The standardized duration of drought stress has resulted in significant changes in shoot as well as in root parameters of tolerant and susceptible genotypes (Table 1), hence it may be utilized while drought screening using hydroponic.

Variation for drought stress tolerance and growth parameters

There was sufficient genetic variation observed for drought tolerance score, root and shoot dry and fresh weight and root length under stress as well as non-stress conditions (Table 1, Fig. 3). The percent reductions in shoot and root fresh weights from non-stress to stress were ranges from 11.7 to 84.4% and 2.1 to 77.55%, respectively. The high ranges for these traits have witnessed the effectiveness of stress screening technique. Plant recovery after rehydration is an essential trait for plant survival (Moreira et al. 1990; Singh et al. 2013). Out of 55 maize inbreds, only 6 lines, *viz*., DQL790-4, CML334, CM140, CML422, CM125 and HKI488 and of five hybrids, three namely, DMRH1306, DMRH1410 and PMH4 were found tolerant with drought score ~3.0 (Table 1). Further, two hybrids, *viz*., HM5 and IMH1415 were found highly susceptible to drought stress with drought score of >8.0. The DMRH1306 and PHM4 were also found drought tolerant under field evaluation, but DMRH1410 has shown drought susceptibility (Kumar et al. 2016b). One of the possible reasons for this deviation in the response may be that the hybrids in the field were evaluated for low moisture stress tolerance at flowering and grain filling stage, hence, these findings may not corroborate with the seedling stage response in the hydroponic. Further, three inbred lines, *viz.,* CM140, LM14 and DQL 633-1 were found moderately tolerant to drought with drought score ranges from 3.2-3.3 (Table 1). The overall percentage shoot and root fresh weight reduction from non-stress to stress for all these tolerant to moderately tolerant genotypes ranged from 11.7% (CML422) to 44.5% (HKI488-1), and 7.3% (CML422) to 28.1% (LM14), respectively (Table 1). Inbred line CML422 had minimum drought score of 3.0 and lowest reduction in shoot (11.7%) as well as root (7.3%) fresh weight from non-stress to stress amongst all genotypes.

The identified six tolerant inbreds and three hybrids along-with four susceptible inbreds were validated by imposing stress in pots filled with soil under glass house conditions (Fig. 1). Of the six inbred lines, two CML334 and CML422 showed average drought score of 3.0 and 3.5, respectively. However, the inbread lines HKI488, DQL790-4, CM140 and CM125 have shown the score between 4.0 to 5.0. In case of hybrids, the PMH4, DMRH1306 and DMRH1410 showed average drought score of 3.0, 3.2 and 4.4, respectively. Most of the genotypes found tolerant and susceptible under hydroponic experiment have retained their response to drought stress under pots screening with slight changes in score. The correlation between drought score of hydroponic and pot screening was 0.77 ($P < 0.01$). Generally, it is very difficult to take root observations in pots as well as in field screening. However, the hydroponic based method is very easy to record all roots parameters without their destruction.

Further, it was observed that per cent reduction in root parameters of tolerant as well as susceptible genotypes from non-stress to stress was not as high as it was for shoot parameters (Table 1, Fig. 4). Out of 55, only in 6 genotypes, the per cent reduction in roots parameters was found above the threshold level (50%), however, in 34 genotypes, the per cent reductions in shoot parameters were above the threshold level (Fig. 4). In fact, roots are the important part of plant system which acts as their life lines during survival in either of stress or non-stress environments, therefore, plants have general tendency to retain their root parameters for their better survival. Further, root systems have extra advantages of less weight and biomass than shoot and they remained in direct contact of nutrients medium which help them for quick recovery under stress. The drought score recorded based on the visible stress symptoms on plants was positively correlated with all the percent reductions from non-stress to stress for shoot as well as root dry and fresh weight and length but correlation coefficient was highest for per cent reduction in shoot fresh weight ($r = 0.76$; $p < 0.001$) (Fig. 5). The drought score is given based on the 1.0-9.0 scale, which have been defined based on the shoot parameters. Hence, it is obvious to have strong correlation with the per cent

reduction of shoot fresh weight from non-stress to stress. Further, it also corroborate the effectiveness of 1.0-9.0 rating scale for assigning the drought tolerance score to each genotypes while screening.

Expression analysis for selected antioxidant genes

Most of the abiotic stresses, like drought, salinity, cold, heat, water logging etc., lead to over synthesis of Reactive Oxygen Species (ROS). These ROS causes damage to cellular macromolecules such as carbohydrates, proteins, DNA and lipids due to oxidative stress. Therefore, generation of ROS is detrimental to plant growth and development (Mittler 2002; Gill and Tuteja 2010). To cope up the oxidative stress, plants have inbuilt antioxidant defense systems/ROS scavenging pathways which protect plants against damages caused by oxidative stress. The cellular antioxidant defense system includes many enzymes, *viz*., superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) etc., playing important role in stress tolerance and cellular survival (Mittler 2002; Das and Roychoudhury 2014). In the present study, the expression pattern of four genes (*Sod2, Sod4, Sod9* and *Apx1*) coding for antioxidant defense system enzymes in hydroponically grown genotypes were analyzed. Under drought stress, expression of all four genes was significantly higher in selected tolerant genotypes (DQL790-4, CML206 and DMRH1306) compared to their respective genotypes grown under non-stressed environment (Fig. 6). However, in susceptible genotypes (CML327, CML176 and IMH1415), the level of expression of most of anti-oxidant genes was low (Fig. 6). Since these genes are involved in alleviating oxidative stress in plants via reactive oxygen species (ROS) scavenging pathway, it is expected that their increased expression helps in protecting vital macromolecules and sustaining plants survival under stress situations. Previous studies have also shown increased activity of SOD, APX, and CAT in response to drought stress (Sairam et al. 1998; Sharma and Dubey 2005a, 2005b; Zlatev et al. 2006; Eyidogan and Öz 2007). Many studies have shown that over-expression of antioxidant gene(s) singly or in combinations resulted into effective detoxification of ROS which in turn enhance tolerance to various abiotic stresses (Wang et al. 2010, Gill and Tuteja 2010). Higher expression of these genes in any genotype can be correlated with its stress tolerance than others since they can maintain equilibrium between level of ROS generation and scavenging. Therefore, the higher expression of these genes in tolerant genotypes than that of susceptible one during drought stress is definitely supporting the effectiveness of this screening methodology for identifying drought tolerant genotypes.

The standardized hydroponic based drought stress screening technique is a rapid, low cost and simple as the whole process takes only around three weeks to identify tolerant and susceptible maize genotypes. Roots as well as shoots parameters of each plant under stress as well as non-stress conditions can be recorded easily. The identified drought tolerant and susceptible genotypes through this method have retained their almost similar responses to drought stress while pots screening. Besides, expression patterns of key antioxidant genes have again confirmed the tolerance as well as susceptibility of identified genotypes at the molecular level. Since, the screening is under control conditions; other growth parameters can be easily regulated for preliminary phenotyping of large set of maize germplasm and mapping populations. The identification of drought tolerant genotypes and genomic regions may further contribute for remarkable achievements in development of drought tolerant hybrids for rainfed ecology.

Author Contribution

Conceptualization of research (BK); Designing of the experiments (BK); Contribution of experimental materials (BK); Execution of field/lab experiments and data collection (SS, TT, SK, M, HRP, BK, GC, and AKJ); Analysis of data and interpretation (BK, KK, SLJ); Preparation of manuscript (BK, KK, SLJ, SR).

Declaration

The authors declare no conflict of interest.

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