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Molecular characterization of four sorghum cultivars and their ability to germinate under heat, drought and salinity stress

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with 2 figures and 6 tables

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Summary

Hassanein A. M., Salem J. M. & Soltan D. M. 2022. Molecular characterization of four sorghum cultivars and their ability to germinate under heat, drought and salinity stress. - Phyton (Horn, Austria) 61: 117–128, with 2 figures and 6 tables.*

In this work, the ability of four different sorghum cultivars (cv.), 'Dorado' (D), 'Giza 15' (G15), 'Shandaweel 305' (Sh305) and 'Shandaweel 306' (Sh306), to germinate under three abiotic stresses (heat, drought or salinity) was investigated. Although the decrease in seed germination and seedling growth was directly proportional to the increase in stress intensity, the influence of each stress agent was cultivar-dependent. Both cv. G15 and Sh305 tolerated the applied abiotic stresses better than cv. D and Sh306, and the former showed higher percentages of seed germination and seedling growth. In comparison to control, relative extremism in temperature, drought or salinity led to progressive increase of antioxidant enzyme activities (SOD, CAT, APX and POX). The total operating units (Unit g^{-1} FW) of the studied antioxidant enzymes of the stress tolerant cultivars were higher than those of the stress sensitive ones. The study shows that both RAPD and ISSR techniques could be used to characterize the genome of sorghum cultivars, i.e., the two dendrograms obtained by those methods proved to be very similar. The dendrograms did not reflect the ability of the tested cultivars to germinate under the studied stress conditions as one of the two closely related cultivars (cv. Sh305) proved to be tolerant, the other one (cv. Sh306) sensitive to abiotic stresses.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops where it ranks fourth of the world cereal crops after wheat, rice and maize. Sorghum is a plant species which can tolerate and survive under drought and high temperature conditions (Hulse & al. 1980). The ability of sorghum to tolerate drought is attributed to dense root system, conserve stomatal opening even under low water content of the soil, and induction of the right osmotic potential (Wright & al. 1983). Since abiotic stresses represent the major constraints for crop yield and food security (Goswami & Deka 2020), they require intensive human interest to counteract them and provide the appropriate conditions for plants to express their full genetic potential.

Drought, salinity and high temperature adversely affect seed germination as well as plant growth and productivity (Hassanein & al. 2012, Diallo & al. 2019) due to generation of reactive oxygen species (ROS) and other biochemical processes (Sarker & Oba 2018). In plants, ROS are highly reactive, toxic and cause damage to proteins, lipids, carbohydrates and DNA (Hasanuzzaman & al. 2020). ROS are detected in several forms such as superoxide radicals ($O_2 \cdot^-$), hydroxyl radical (OH·), perhydroxy radical ($HO_2 \cdot$) and alkoxy radicals (RO·), as well as molecular forms such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Shao & al. 2008). To minimize the negative effects of ROS, plant cells express a complex antioxidant system, which includes low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) and antioxidant enzymes to scavenge ROS and reduce their oxidative stress. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) are ROS scavengers (Hasanuzzaman & al. 2020).

Stress tolerance varied between plant species and between cultivars of each one, and it is under genetic control (Epstein & Rains 1987, Loutfy & al. 2019). Sorghum cultivars varied significantly in

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growth characteristics, yield, chemical components and stress tolerance (El-Hattab & al. 2000, Younis & al. 2007, Diallo & al. 2019). To assess the genetic relatedness or genome stability, RAPD and ISSR were successfully used (El-Amin & Hamza 2016, Salem & Hassanein 2017, Hassanein & al. 2018).

The ability of sorghum cultivars to tolerate drought and other stress conditions differs from one cultivar to another (Hassanein & al. 2010, El-Kady & al. 2015). There is a lack of information describing the ability of a sorghum cultivar to tolerate different types of abiotic stresses. In addition, there is a lack of information linking between stress tolerance of a particular cultivar and genetic or biochemical factors (Younis & al. 2007). Stress tolerance during seed germination resulted in lowering the reduction of emergence and early seedling growth, which are particularly important stages for a successful establishment of crop plants; these stages affect the overall plant growth and plant yield (Briggs & Aytenfisu 1979). The aim of this work was to detect: (1) the response of four sorghum cultivars to drought, salinity and different temperatures during germination stage, (2) the differences between four sorghum cultivars in production of antioxidant enzymes under the influence of each stress type, and (3) the link between the previous results and the genetic content of these cultivars.

2. Materials and methods

Plant material and seed germination under stress conditions: Grains of four sorghum (*Sorghum bicolor* (L.) Moench) cultivars (cv.), 'Dorado' (D), 'Giza 15' (G15), 'Shandaweel 305' (Sh305) and 'Shandaweel 306' (Sh306), were obtained from Sohag Agricultural Research Center, Egypt. Sorghum seeds were surface sterilized with 5 % (v/v) commercial sodium hypochlorite for 5 min, 0.2 % (w/v) mercuric chloride for 10 min, and 70 % (v/v) ethanol for 8 min. The seeds were rinsed in sterilized deionized water 3 times, 10 min each. Ten healthy seeds were placed in a 9 cm Petri dish (in triplicate) containing a sterile filter paper moistened with 10 mL of 1/10 strength Hoagland solution (Hoagland & Arnon 1950). Without (control) or with stress factor (drought, salinity or heat) for one week. For seed germination, Petri dishes were incubated in the dark for one week at 15, 25, 35 and 40 °C to study the effect of different temperatures, or at 25 °C to study drought and salinity treatments. Five concentrations of polyethylene glycol (PEG) 6000 (0.0, 5, 10, 15 or 20 %) or NaCl (0.0, 50, 100, 150 or 200 mM) were used. Germination percentage (using radicle protrusion as a criterion for germination, Prado & al. 2000), plumule length, radicle length, fresh weight/seedling (without cotyledon) and water content/seedling were determined.

Extraction and assays of antioxidant enzymes: Enzyme extraction was performed by grinding 0.5 g of fresh seedlings in 3 mL extraction buffer at 4 °C. Extraction buffer containing 50 mM phosphate buffer (pH 7), 0.1 mM Na_2EDTA and 1 % (w/v) polyvinylpyrrolidone (PVP) was used. The extract was subjected to centrifugation at 13000 rpm for 15 min at 4 °C, and the obtained supernatants were used to determine enzyme activities.

Superoxide dismutase (SOD) activity: SOD (EC 1.15.1.1) activity of each sorghum cultivar under the applied abiotic stresses was measured according to Giannopolitis & Ries (1977). The ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) was determined to estimate its activity. The reaction mixture (3 mL) containing 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 2 mM riboflavin, 75 mM NBT, 100 mM EDTA and 0.05 mL enzyme extract was prepared. Monitoring of the increase in absorbance at 560 nm was registered following the production of blue color of formazan. SOD activity was defined as the amount of enzyme that inhibits nitroblue tetrazolium photoreduction (Extinction factor E = 10.3 mM cm^{-1}).

Peroxidase (POX) activity: POX (EC 1.11.1.7) activity of each sorghum cultivar under the applied abiotic stresses was determined by mixing supernatants (0.05 mL) with assay mixture (2.950 mL) as described by MacAdam & al. (1992). Assay mixture containing 50 mM potassium phosphate buffer (pH 6.8), 0.1 mM EDTA, 5 mM guaiacol and 0.3 mM H_2O_2 (30 %) was used. Oxidation of guaiacol (E = 26.2 mM cm^{-1}) was measured spectrophotometrically at 470 nm. POX activity was calculated in terms of µM of guaiacol oxidized min^{-1} g^{-1} fresh weight at 25 ± 2 °C (Zhang 1992).

Catalase (CAT) activity: Catalase (EC 1.11.1.6) activity of each sorghum cultivar under the applied abiotic stresses was estimated spectrophotometrically (Aebi 1984). CAT activity of a reaction mixture (3 mL) was estimated by the decrease in absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumed (E = 0.036 mM cm^{-1}). The CAT reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 20 mM H_2O_2 and 0.05 mL enzyme extract. One CAT unit means the amount of enzyme necessary to decompose 1 µmol min^{-1} H_2O_2 under the above-mentioned assay conditions.

Ascorbate peroxidase (APX) activity: APX (EC 1.11.1.11) activity of each sorghum cultivar under the applied abiotic stresses was determined according to Nakano & Asada (1981). APX activity was determined by using 3 mL assay medi-

um. It contained 50 mM phosphate buffer (pH 7.0), 0.5 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.05 mL enzyme extract. The decrease in ascorbate concentration was followed by decline in absorbance at 290 nm for 1 min and activity was calculated using the extinction coefficient (E = 2.8 mM cm^{-1}) for ascorbate.

DNA extraction: Total genomic DNA of each cultivar was extracted from seedlings using the modified CTAB protocol described by Porebski & al. (1997).

RAPD analysis: Twenty-four RAPD primers (OPA-02, OPA-03, OPA-04, OPA-05, OPA-07, OPA-08, OPA-13, OPad-06, OPaf-20, OPar-05, OPat-08, OPav-13, OPaw-10, OPC-02, OPD-01, OPD-18, OPE-02, OPE-03, OPg-05, OPg-09, OPi-05, OPj-15, OPk-02 and OPn-02) were used to amplify the template DNA of the four sorghum cultivars. The reaction mixtures (25 µL each) were composed of 6.5 µL deionized H_2O, 12.5 µL master mix, 3 µL primer and 3 µL template DNA. Amplification process was carried out in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 45 sec, an annealing step at 36 °C for 50 sec, and an elongation step at 72 °C for 1 min. The primer extension was extended to 7 min at 72 °C in the final cycle.

ISSR analysis: Five ISSR primers, ISSR1 (CAG)5G, ISSR2 (CA)8G, ISSR3 (AG)6ACC, ISSR4 (CAG)5T and ISSR5 (GA)7A, were used to amplify the template DNA of the four sorghum cultivars. The ISSR-PCR method was carried out according to Nagaoka & Ogihara (1997). Amplification reactions were carried out in 25 µL volumes containing the same components as for RAPD reactions (see above). The amplification conditions were the same as for RAPD analysis, except for the annealing temperature that was changed to 45 °C for ISSR primers.

Detection of PCR products: Amplification products were resolved by a horizontal gel electrophoresis unit using 1.5 % (w/v) agarose gel containing ethidium bromide (0.5 µg/mL) in 1× TBE (Tris-Borate-EDTA) buffer. Electrophoresis was carried out for 1 h at 70 V. The electrophoretic DNA banding patterns were analyzed by the Multi-Variate Statistical Package (MVSP) computer software program of Nei & Li (1979) and clustered by the unweighted pair group method based on arithmetic mean (UPGMA).

Statistical analysis: Experiments were designed in completely randomized form, and data were statistically analyzed using standard deviation (SD) according to the method described by Snedecor & Cochran (1980). Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16. The level of significance was measured by running a Tukey test at $P < 0.05$ level of significance.

Relative enzyme activity and estimation of the total activity of antioxidant enzymes: To determine the relative enzyme activity, we considered that the activity of each enzyme (Unit g^{-1} FW) under the influence of ideal condition (control) was 100 %. Then, the value obtained under stress conditions was attributed to the value of control. The total activity units were taken as the sum of the units of antioxidant enzymes used (SOD, POX, CAT, APX), and then the obtained results were correlated with that of control.

3. Results

Seed germination under different temperatures: To study the effect of different temperatures on the four sorghum cultivars, their seeds were germinated at 15, 25, 35 and 40 °C. Seed germination was completely inhibited, irrespective of the cultivar type, at 40 °C. The best seed germination was obtained at 25 °C (Table 1). Germination under the influence of 35 °C was dependent on the cultivar where cv. G15 expressed the highest germination percentage (96.67 %). Also, seed germination of cv. Sh305 was high (86.67 %). Both cv. D and Sh306 expressed low germination percentages, 80 % each. At 15 °C, 100 % seed germination was obtained in all studied cultivars, but it exerted a negative effect on plumule or radicle growth. Generally, length of plumule or radicle at low temperature (15 °C) was shorter than at high temperature (35 °C). In comparison to seeds germinated at 25 °C, reduction in length of plumule and radicle in cv. G15 and Sh305 under the influence of relatively high temperature was less pronounced than in cv. D and Sh306. For example, the reduction in plumule and radicle lengths in cv. Sh305 was 35 % and 43 %, but in cv. Sh306 it was 48 % and 50 %, respectively. Water content and seedling fresh weight were only slightly influenced in one week under different temperature treatments.

Seed germination under different PEG concentrations: The tested four sorghum cultivars expressed a decrease in values of seed germination under the influence of PEG, especially at high concentrations (Table 2). Both cv. G15 and Sh305 expressed high germination percentage (93.33 %) under the influence of 15 % PEG, but seed germination decreased in cv. Sh305 (86.67 %) and cv. G15 (83.33 %) when the concentration of PEG was increased up to 20 %. Cultivar D was the most sensitive cultivar as it expressed the lowest value of seed germination and growth parameters

Table 1. Effect of different temperatures on seed germination and seedling growth of four sorghum cultivars (D, G15, Sh305 and Sh306). Values are mean ± SD.

Cultivar	Temperature (°C)	Germination frequency (%)	Plumule length (cm)	Radicle length (cm)	F.W./ seedling (g)	Water content/ seedling (%)
D	**15**	100	4.93 ± 0.23*	5.7 ± 0.26*	0.1197 ± 0.003*	92.1 ± 1.39
	25 (control)	100	10.53 ± 0.05	11.57 ± 1.36	0.1393 ± 0.001	91.2 ± 0.66
	35	80	5.50 ± 0.20*	6.03 ± 0.20*	0.1154 ± 0.005*	89.32 ± 1.25*
G15	**15**	100	6.6 ± 0.36*	6.57 ± 0.20*	0.2352 ± 0.001*	90.21 ± 0.84
	25 (control)	100	13.77 ± 0.20	17.5 ± 0.50	0.2759 ± 0.008	88.51 ± 0.74
	35	96.67	8.53 ± 0.05*	11.1 ± 0.20*	0.2057 ± 0.003*	82.99 ± 4.09
Sh305	**15**	100	5.4 ± 0.2*	5.07 ± 0.37*	0.1268 ± 0.002*	93.36 ± 0.05
	25 (control)	100	9.9 ± 0.17	10.17 ± 0.65	0.1583 ± 0.005	93.77 ± 0.33
	35	86.67	6.47 ± 0.15*	5.83 ± 0.30*	0.1288 ± 0.002*	89.76 ± 0.32
Sh306	**15**	100	4.63 ± 0.15*	3.1 ± 0.1*	0.1160 ± 0.005*	91.99 ± 1.83
	25 (control)	100	8.23 ± 0.46	7.57 ± 0.86	0.1391 ± 0.005	90.89 ± 0.15
	35	80	4.27 ± 0.30*	3.77 ± 0.15*	0.1148 ± 0.012*	89.76 ± 0.32

* Indicates a significant difference between values determined at control and those at the other temperatures (for each cultivar individually) using Tukey test at $P < 0.05$.

Table 2. Effect of different PEG concentrations on seed germination and seedling growth of four sorghum cultivars (D, G15, Sh305 and Sh306). Values are mean ± SD.

Cultivar	PEG conc. (%)	Germination frequency (%)	Plumule length (cm)	Radicle length (cm)	F.W./ seedling (g)	Water content/ seedling (%)
D	**0 (control)**	100	9.5 ± 0.86	7.77 ± 0.35	0.1574 ± 0.014	91.2 ± 0.66
	5	100	9.7 ± 0.35	4.67 ± 0.15*	0.0839 ± 0.002*	88.91 ± 0.71
	10	100	7.27 ± 0.50*	2.57 ± 0.15*	0.0535 ± 0.001*	84.97 ± 0.76*
	15	60	2.33 ± 0.11*	1.43 ± 0.20*	0.0363 ± 0.002*	79.47 ± 2.18*
	20	40	0.00 ± 0.00*	0.1 ± 0.00*	0.0022 ± 0.000*	63.63 ± 0.00*
G15	**0 (control)**	100	16.40 ± 0.85	17.60 ± 1.82	0.2905 ± 0.010	88.51 ± 0.74
	5	100	15.63 ± 1.34	8.40 ± 0.20*	0.1714 ± 0.004*	81.76 ± 3.68*
	10	100	8.93 ± 0.51*	6.20 ± 0.79*	0.0994 ± 0.004*	79.82 ± 0.57*
	15	93.33	4.83 ± 0.41*	4.73 ± 0.40*	0.0813 ± 0.005*	77.42 ± 2.23*
	20	83.33	1.5 ± 0.44*	1.37 ± 0.15*	0.05 ± 0.013*	77.24 ± 2.16*
Sh305	**0 (control)**	100	10.03 ± 0.55	7.33 ± 0.25	0.1453 ± 0.007	93.77 ± 0.33
	5	100	11.57 ± 0.11*	6.77 ± 0.30*	0.1259 ± 0.005*	87.79 ± 0.08*
	10	100	7.37 ± 0.20*	5.03 ± 0.25*	0.0566 ± 0.002*	86.25 ± 2.026*
	15	93.33	4.03 ± 0.25*	3.37 ± 0.15*	0.0466 ± 0.001*	80.15 ± 0.02*
	20	86.67	1.6 ± 0.17*	1.73 ± 0.21*	0.01 ± 0.0005*	78.82 ± 1.97*
Sh306	**0 (control)**	100	13.83 ± 1.55	7.53 ± 0.30	0.1482 ± 0.006	90.89 ± 0.15
	5	100	10.00 ± 0.43*	5.70 ± 0.62*	0.0641 ± 0.003*	88.46 ± 0.25
	10	90	4.50 ± 0.36*	1.80 ± 0.10*	0.0379 ± 0.004*	83.05 ± 2.63*
	15	70	1.77 ± 0.38*	1.37 ± 0.35*	0.0263 ± 0.003*	82.28 ± 0.48*
	20	46.67	0.56 ± 0.12*	0.27 ± 0.06*	0.006 ± 0.001*	67.14 ± 5.13*

* Indicates a significant difference between values of control (0 % PEG) and those of other treatments (for each cultivar individually) using Tukey test at $P < 0.05$.

when the concentration of PEG was increased to more than 10 %. Generally, the estimated growth parameters decreased progressively with the increase in the PEG concentration. Under the influence of 15 % PEG, the reductions in seedling fresh weight were 82 % (cv. Sh306), 77 % (D), 72 % (G15) and 68 % (Sh305). Different values in seedling water content were detected especially under the influence of relatively high PEG concentration (20 %), cv. Sh305 expressed the highest water content

Table 3. Effect of different NaCl concentrations on seed germination and seedling growth of four sorghum cultivars (D, G15, Sh305 and Sh306). Values are mean ± SD.

Cultivar	NaCl conc. (mM)	Germination frequency (%)	Plumule length (cm)	Radicle length (cm)	F.W./ seedling (g)	Water content/ seedling (%)
D	**0 (control)**	100	9.5 ± 0.86	7.77 ± 0.35	0.1574 ± 0.014	91.2 ± 0.66
	50	73.33	5.83 ± 0.35*	5.73 ± 0.45*	0.1200 ± 0.009*	88.23 ± 1.51
	100	70	4.23 ± 0.25*	5.47 ± 0.64*	0.0612 ± 0.001*	86.65 ± 0.36*
	150	46.67	0.62 ± 0.13*	0.62 ± 0.12*	0.0686 ± 0.002*	87.99 ± 1.45
	200	30	0.53 ± 0.06*	0.6 ± 0.26*	0.0272 ± 0.005*	84.79 ± 2.89*
G15	**0 (control)**	100	16.40 ± 0.85	17.60 ± 1.82	0.2905 ± 0.010	88.51 ± 0.74
	50	90	12.97 ± 0.64*	14.20 ± 1.30*	0.2445 ± 0.010*	88.66 ± 0.51
	100	80	9.37 ± 0.32*	10.83 ± 0.57*	0.1766 ± 0.014*	86.78 ± 3.05
	150	76.67	3.37 ± 0.40*	3.67 ± 0.47*	0.086 ± 0.006*	85.78 ± 2.37
	200	56.67	1.13 ± 0.49*	1.50 ± 0.40*	0.048 ± 0.009*	78.33 ± 3.59*
Sh305	**0 (control)**	100	10.03 ± 0.55	7.33 ± 0.25	0.1453 ± 0.007	93.77 ± 0.33
	50	96.67	9.33 ± 0.23	6.57 ± 0.40*	0.1255 ± 0.005*	91.32 ± 0.99
	100	83.33	8.47 ± 0.55*	6.47 ± 0.15*	0.0866 ± 0.003*	89.39 ± 0.02
	150	80	5.93 ± 0.15*	2.27 ± 0.15*	0.0614 ± 0.005*	87.40 ± 1.29*
	200	50	2.17 ± 0.15*	1.13 ± 0.21*	0.0300 ± 0.001*	81.08 ± 0.36*
Sh306	**0 (control)**	100	13.83 ± 1.55	7.53 ± 0.30	0.1482 ± 0.006	90.89 ± 0.15
	50	70	8.83 ± 0.28*	1.17 ± 0.15*	0.0867 ± 0.007*	86.17 ± 0.06
	100	53.33	4.63 ± 0.45*	0.67 ± 0.05*	0.0803 ± 0.004*	85.88 ± 0.62
	150	43.33	1.7 ± 0.72*	1.83 ± 0.71*	0.0486 ± 0.001*	86.91 ± 1.20
	200	20	1.13 ± 0.21*	1.03 ± 0.21*	0.0272 ± 0.006*	81.33 ± 0.81*

* Indicates a significant difference between values of control (0 mM NaCl) and those of other treatments (for each cultivar individually) using Tukey test at $P < 0.05$.

Table 4. Antioxidant enzyme activities (Unit g^{-1} FW) of germinated seeds of four sorghum cultivars (D, G15, Sh305 and Sh306) under the influence of different temperatures for one week. Values are mean ± SD.

Cultivar	Temperature (°C)	SOD		POX		CAT		APX	
		Activity	%	Activity	%	Activity	%	Activity	%
D	**15**	1.21 ± 0.08*	**76.58***	1.91 ± 0.08*	**70.48***	0.19 ± 0.01*	**63.33**	4.67 ± 0.80	**100**
	25 (control)	1.58 ± 0.02	**100**	2.71 ± 0.05	**100**	0.30 ± 0.10	**100**	4.67 ± 0.80	**100**
	35	1.87 ± 0.01*	**118.35***	3.67 ± 0.17*	**135.42***	0.42 ± 0.10*	**140**	27.53 ± 2.91*	**589.5***
G15	**15**	0.90 ± 0.12*	**72.58***	1.06 ± 0.03*	**139.47***	0.36 ± 0.18*	**120**	4.67 ± 0.80*	**142.81***
	25 (control)	1.24 ± 0.05	**100**	0.76 ± 0.08	**100**	0.30 ± 0.10	**100**	3.27 ± 0.80	**100**
	35	2.08 ± 0.06*	**167.74***	1.32 ± 0.04*	**173.68***	0.36 ± 0.18*	**120**	2.33 ± 0.80*	**71.25***
Sh305	**15**	1.89 ± 0.20*	**138.97***	1.09 ± 0.06*	**125.28***	0.60 ± 0.10*	**250***	15.87 ± 0.80*	**178.91***
	25 (control)	1.36 ± 0.06	**100**	0.87 ± 0.04	**100**	0.24 ± 0.10	**100**	8.87 ± 0.80	**100**
	35	1.63 ± 0.11*	**119.85***	1.09 ± 0.06*	**125.28***	0.30 ± 0.10*	**125***	13.07 ± 0.80*	**147.35***
Sh306	**15**	1.54 ± 0.09*	**77.38***	1.17 ± 0.02	**101.73**	0.66 ± 0.10*	**157.14***	16.47 ± 1.92	**98.03**
	25 (control)	1.99 ± 0.01	**100**	1.15 ± 0.10	**100**	0.42 ± 0.10	**100**	16.80 ± 1.4	**100**
	35	2.04 ± 0.004	**102.51**	6.25 ± 0.13*	**534.19***	0.48 ± 0.10	**114.28**	2.33 ± 0.80*	**13.86***

* Indicates a significant difference between values of control (at 25 °C) and those at the other temperatures (for each cultivar individually) using Tukey test at $P < 0.05$.

value (78.82 %), cv. G15 (77.42 %) came at the second position.

Seed germination under different NaCl concentrations: Frequency of seed germination and growth of germinated seeds decreased with the increase of NaCl concentration, but these parameters were cultivar-dependent especially under the influence of high concentrations (Table 3). Cultivars G15 and Sh305 expressed higher values of seed germination than cv. D and Sh306. Seed germi-

Table 5. Antioxidant enzyme activities (Unit g^{-1} FW) of germinated seeds of four sorghum cultivars (D, G15, Sh305 and Sh306) under the influence of different PEG concentrations for one week. Values are mean ± SD.

Cultivar	PEG (%)	SOD		POX		CAT		APX	
		Activity	%	Activity	%	Activity	%	Activity	%
D	0 (control)	1.19 ± 0.07	100	6.30 ± 0.16	100	0.52 ± 0.075	100	4.67 ± 0.80	100
	5	2.62 ± 0.05*	220.17*	5.82 ± 0.61	92.38	0.80 ± 0.32	152.87	11.20 ± .40*	239.82*
	10	3.52 ± 0.31*	295.79*	6.38 ± 1.61	101.26	0.81 ± 0.08	155.17	13.53 ± .61*	289.72*
	15	3.91 ± 0.45*	328.57*	16.44 ± 3.89*	260.95*	1.5 ± 0.16*	201.14*	13.53 ± .80*	289.72*
	20	3.91 ± 0.31*	328.57*	14.96 ± 0.61*	237.46*	0.95 ± 0.14	181.03	12.18 ± .40*	260.75*
G15	0 (control)	1.18 ± 0.07	100	5.39 ± 0.69	100	0.41 ± 0.14	100	11.67 ± 2.14	100
	5	2.53 ± 0.03*	212.61*	4.52 ± 0.96	83.70	0.51 ± 0.03	124.39	13.60 ± 1.40	116.52
	10	3.31 ± 0.09*	278.15*	11.28 ± 0.80*	208.89*	0.84 ± 0.18*	204.87*	14.46 ± 2.13*	123.90*
	15	2.92 ± 0.01*	245.37*	14.41 ± 1.14*	266.85*	0.92 ± 0.44*	220.97*	16.80 ± 2.80*	143.95*
	20	3.03 ± 0.03*	256.78*	17.69 ± 0.15*	328.20*	1.21 ± 0.07*	294.14*	25.67 ± 1.01*	219.96*
Sh305	0 (control)	1.67 ± 0.45	100	3.53 ± 0.47	100	0.37 ± 0.04	100	6.07 ± 0.81	100
	5	1.86 ± 0.02	111.37	3.85 ± 0.80	109.06	0.59 ± 0.05*	162.29*	10.96 ± 2.25*	180.56*
	10	1.93 ± 0.03*	115.56*	10.26 ± 0.34*	290*	0.84 ± 0.11*	173.77*	11.2 ± 1.4*	184.51*
	15	2.64 ± 0.21*	158.08	15.06 ± 0.38*	441.91*	0.64 ± 0.14*	282.41*	15.41 ± 3.94*	253.82*
	20	3.54 ± 0.66*	211.98*	16.94 ± 0.18*	479.89*	0.3 ± 0.17*	254.10*	29.40 ± 2.80*	484.34*
Sh306	0 (control)	1.25 ± 0.65	100	4.54 ± 0.49	100	0.56 ± 0.03	100	8.57 ± 1.16	100
	5	1.69 ± 0.004*	135.2*	4.76 ± 1.39	104.84	0.81 ± 0.1*	143.61*	17.26 ± 0.80*	201.40*
	10	2.28 ± 0.05*	182.4*	5.78 ± 1.32*	127.31*	0.85 ± 0.26*	151.06*	18.2 ± 2.8*	212.36*
	15	2.80 ± 0.23*	224*	8.47 ± 0.37*	186.56*	1.18 ± 0.3*	208.51*	20.87 ± 4.50*	243.52*
	20	3.66 ± 0.41*	292.8*	11.18 ± 0.39*	246.26*	1.04 ± 0.19*	184.78*	18.93 ± 2.91*	220.88*

* Indicates a significant difference between values of control (0 % PEG) and those of other treatments (for each cultivar individually) using Tukey test at $P < 0.05$.

Table 6. Antioxidant enzyme activities (Unit g^{-1} FW) of germinated seeds of four sorghum cultivars (D, G15, Sh305 and Sh306) under the influence of different NaCl concentrations for one week. Values are mean ± SD.

Cultivar	NaCl conc. (mM)	SOD		POX		CAT		APX	
		Activity	%	Activity	%	Activity	%	Activity	%
D	0 (control)	1.19 ± 0.07	100	5.39 ± 0.46	100	2.76 ± 0.37	100	4.67 ± 0.80	100
	50	1.88 ± 0.12*	157.9*	6.36 ± 0.78*	118.11	2.40 ± 0.27*	86.96*	8.53 ± 0.80*	182.64*
	100	2.80 ± 0.89*	213.4*	6.62 ± 0.29*	122.84*	4.02 ± 0.27*	145.65*	9.93 ± 2.91*	212.63*
	150	2.80 ± 0.89*	235.3*	7.79 ± 0.37*	140.99*	4.88 ± 1.53*	176.81*	8.94 ± 0.92*	181.80*
	200	3.59 ± 0.03*	301.68*	9.19 ± 0.56*	255.99*	5.04 ± 1.26*	182.61*	9.20 ± 2.00*	186.50*
G15	0 (control)	1.50 ± 0.72	100	3.88 ± 0.24	100	0.30 ± 0.10	100	6.53 ± 1.62	100
	50	1.71 ± 0.21*	114*	5.88 ± 0.16*	151.54*	0.24 ± 0.10*	80*	7.00 ± 1.40	107.2
	100	2.29 ± 0.77*	152.7*	9.28 ± 2.60*	239.17*	0.30 ± 0.10	100	7.47 ± 0.81*	114.4*
	150	2.55 ± 0.52*	170*	11.28 ± 0.12*	290.72*	1.20 ± 0.21*	400*	10.27 ± 1.62*	157.3*
	200	2.62 ± 1.03*	174.7*	16.24 ± 5.32*	418.56*	1.44 ± 0.18*	480*	11.67 ± 0.81*	178.7*
Sh305	0 (control)	1.46 ± 0.41	100	3.22 ± 0.140	100	0.24 ± 0.10	100	6.07 ± 0.81	100
	50	2.14 ± 0.03*	146.6*	3.02 ± 0.149	93.06	0.18 ± 0.00*	75*	8.87 ± 0.81*	146.1*
	100	2.33 ± 0.04*	159.6*	6.50 ± 1.13*	200.47*	0.24 ± 0.10	100	9.33 ± 0.81*	153.7*
	150	2.59 ± 0.10*	177.4*	8.86 ± 1.33*	273.26*	0.66 ± 0.10*	275*	9.8 ± 1.42*	161.4*
	200	2.99 ± 0.02*	204.8*	10.46 ± 2.60*	362.74*	0.84 ± 0.10*	350*	10.73 ± 0.81*	176.8*
Sh306	0 (control)	0.44 ± 0.14	100	3.03 ± 0.31	100	0.42 ± 0.10	100	8.57 ± 1.16	100
	50	0.83 ± 0.05*	188.6*	4.91 ± 0.14*	161.08*	0.78 ± 0.10*	185.7*	8.87 ± 0.81	103.5
	100	1.30 ± 0.06*	295.5*	7.88 ± 0.14*	256.16*	1.2 ± 0.68*	285.7*	9.33 ± 0.81	108.9
	150	1.91 ± 0.09*	434.09*	7.83 ± 0.33*	257.15*	1.32 ± 0.45*	314.29*	9.52 ± 6.10*	111.09*
	200	2.78 ± 0.75*	631.82*	7.98 ± 0.44*	262.068*	1.42 ± 0.29*	338.02*	10.20 ± 1.40*	119.02*

* Indicates a significant difference between values of control (0 mM NaCl) and those of other treatments (for each cultivar individually) using Tukey test at $P < 0.05$.

nation of cv. Sh305 was lower than that of G15 under the influence of the highest concentration of NaCl (200 mM). In addition, reduction in seedling fresh weight and length of plumule or radicle in cv. G15 was more pronounced than in cv. Sh305. On the other side, cv. Sh306 was the most salt sensitive sorghum cultivar as it expressed the lowest values of seed germination and seedling growth parameters. Water content of cv. Sh305 was higher than that of cv. G15 when their seeds were germinated for one week in media with or without NaCl.

Antioxidant enzyme activities under different temperatures: Activities of antioxidant enzymes under the influence of the applied temperatures were estimated (Table 4). In seedling tissues of cv. D, SOD activity as well as activities of the other studied enzymes decreased at relatively low temperature (15 °C), but it increased at relatively high temperature (35 °C). Decrease of SOD activity under the influence of low temperature was also registered in cv. G15. In the other two cultivars (Sh305 and Sh306), SOD activity increased under low and high temperatures. The activity of the other three enzymes (POX, CAT and APX) increased under low and high temperatures when cv. G15, Sh305 and Sh306 were used. Under low and high temperatures, the total operating units of all four enzymes in seedling tissues of cv. Sh305 and Sh306 were higher than in those of cv. D and G15. At relatively high temperatures, the relative increase in SOD, POX and APX activities of cv. G15 and Sh305 seedlings was higher than that of cv. D and Sh306. On the other side, under relatively low temperature, cv. Sh306 expressed the highest value of CAT, cv. Sh305 came at the second position.

Antioxidant enzyme activities under different PEG concentrations: Activities of all studied enzymes increased with increase of PEG concentrations in all cultivars (Table 5). The activity of SOD in seedlings of cv. D and Sh306 was higher than in those of cv. G15 and Sh305. On the other side, POX and APX activities of cv. G15 and Sh305 seedlings were higher than those of cv. D and Sh306, especially under relatively high concentrations of PEG (15 and 20 g/L). Also, total operating units of SOD, POX, CAT and APX expressed per gram of cv. G15 and Sh305 seedling were higher than those of cv. D and Sh306, especially under the influence of high PEG concentrations (15 and 20 g/L). In comparison to seedlings germinated in PEG-free medium, the relative increase in POX, CAT and APX of cv. G15 and Sh305 was higher than that of cv. D and Sh306.

Antioxidant enzyme activities under different NaCl concentrations: The activities of all investigated enzymes progressively increased with the increase of NaCl concentrations (Table 6). Under the highest concentration of NaCl (200 mM), cv. D expressed the highest SOD and CAT activities. As well as under relatively high concentrations of PEG, both cv. G15 and Sh305 expressed higher POX and APX activities than cv. D and Sh306 under relatively high concentrations of NaCl (150 and 200 mM). The relative increase in SOD activity in cv. D and Sh306 seedlings under the influence of high NaCl concentrations (150 and 200 mM) was higher than in cv. G15 and Sh306 seedlings. On the other side, relative increase in POX and APX in seedlings of cv. G15 and Sh305 was higher than in those of cv. D and Sh306. In addition, total operating units of SOD, POX, CAT and APX per gram of seedling of cv. G15 and Sh305 were higher than those of cv. D and Sh306.

Molecular characterization: In total, 24 RAPD primers were used for amplification of genomic DNA of the four sorghum cultivars. In this work, 19 primers (79.17 %) were successfully analyzed where they resulted in 86 polymorphic out of 196 fragments (43.88 %). Two primers (OPC-02 and OPD-01) showed a monomorphic pattern as no polymorphic PCR products were obtained. The number of amplified fragments ranged from one (OPat-08) to 17 bands (OPA-07). Six primers showed more than 60 % polymorphism (OPA-05, OPA-07, OPA-13, OPg-09, OPad-06 and OPK-02). Percentage of polymorphism ranged from 0 % (OPC-02, OPD-01, OPi-05 and OPar-05) to a maximum of 100 % (OPA-13, OPad-06 and OPk-02), with an average of 44.91 %. Two primers (OPg-09 and OPA-07) expressed the highest number of polymorphic RAPD markers (Fig. 1A). The average number of bands per polymorphic primers was 10.32 and average number of polymorphic bands per polymorphic primers was 4.53. The size of fragments varied between 200 bp and 1800 bp. In the dendrogram based on UPGMA analysis (Fig. 1B), cv. G15 appeared in an isolated position, the other three cultivars grouped into one cluster, and cv. Sh305 and Sh306 were closest to each other.

For comparison, five ISSR primers were used and produced 40 bands of which 12 were polymorphic (30 %). Number of bands varied from 3 (ISSR-2) to 10 (ISSR-3), and the size of fragments ranged from 150 bp to 1000 bp (Fig. 2A). Average numbers of bands and polymorphic bands per primer were 8 and 2.4, respectively. Percentage of polymorphism ranged from 22.22 % (ISSR-1, ISSR-4) to 45.45 % (ISSR-3), with an average polymorphism of 33.84 % across all cultivars. The primer ISSR-3 gave the highest number of polymorphic ISSR markers. UPGMA analysis of five ISSR primers for the four cultivars resulted in the same dendrogram as was constructed by RAPD markers (Fig. 2B).

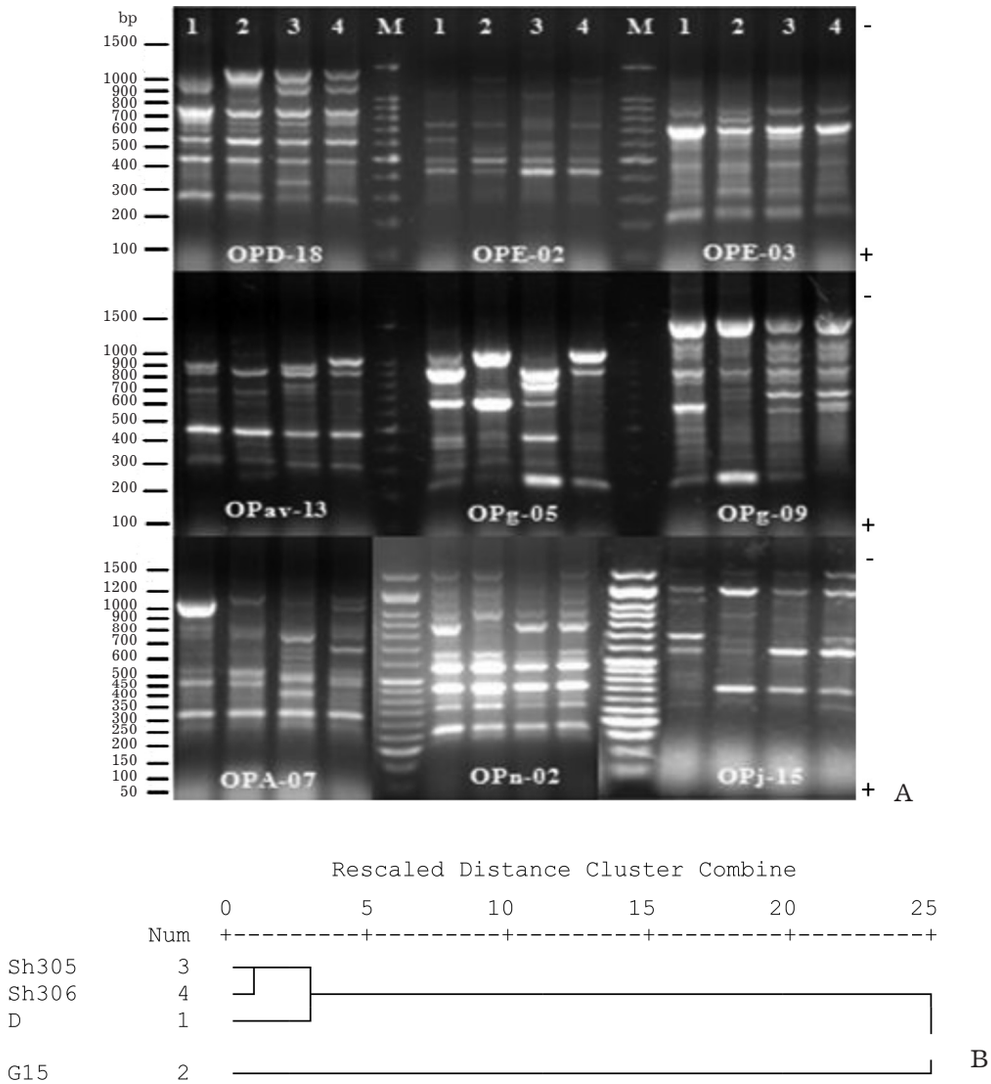

Fig. 1. (A) RAPD-PCR profiles generated by primers OPA-07, OPD-18, OPE-02, OPE-03, OPav-13, OPg-05, OPg-09, OPn-02 and OPj-15. (Lane 1: cv. D, lane 2: cv. G15, lane 3: cv. Sh305 and lane 4: cv. Sh306). M: DNA ladder. (B) UPGMA-based cluster tree of the 4 sorghum cultivars with 24 RAPD primers.

4. Discussion

In this work, four sorghum cultivars widely cultivated in Egypt were used to investigate their ability to germinate efficiently under the influence of three different environmental stress conditions (temperature, drought and salinity).

While best seed germination was obtained at 25 °C, it was completely inhibited at 40 °C, irrespective of the cultivar type. Percentage of seed germination as well as plumule and radicle lengths under the influence of 35 °C were cultivar-dependent, and cv. G15 and Sh305 expressed high values of the determined parameters. On the other side, lower germination percentages were expressed by cv. D and Sh306. Literature confirmed that, while a soil temperature ranging from 21–35 °C was recommended for germination of sorghum seeds (Bajay & Papp 1969), soil temperature ranging from 40–48 °C was lethal (Kailasanathan & al. 1976). Germination of sorghum seeds at 15 °C, below the recommended temperature, resulted in 100 % seed germination in all studied cultivars but it exerted a negative effect on plumule or radicle growth, which may be due to slow emergence, growth rate after emergence, and molecular aspects (Hassan & al. 2020). In semi-arid regions, sorghum is able to grow at air temperature exceeding 40 °C (Yan & al. 2011), where 55 °C were measured for leaves and 60 °C for the soil surface (Peacock & Ntshole 1976). The results obtained from the present research were consistent with the

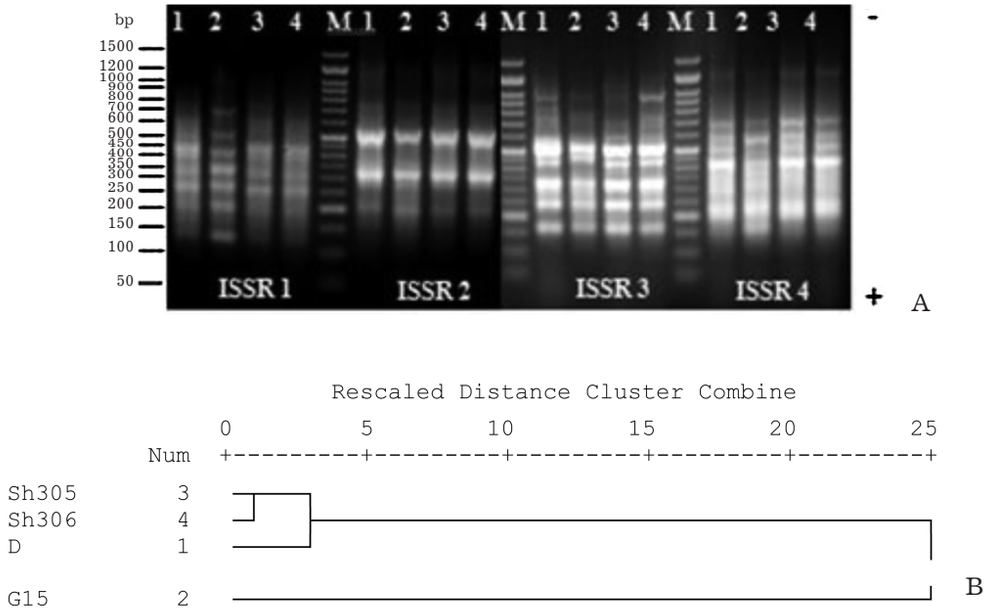

Fig. 2. (A) ISSR-PCR profiles generated by primers ISSR1, ISSR2, ISSR3 and ISSR4. (Lane 1: cv. D, lane 2: cv. G15, lane 3: cv. Sh305 and lane 4: cv. Sh306). M: DNA ladder. (B) UPGMA-based cluster tree of the 4 sorghum cultivars with 5 ISSR primers.

conclusions of Peacock & Ntshole (1976) as that the germination of sorghum seeds is more sensitive to high temperatures than the vegetative growth of the plant. Cultivars G15 and Sh305 were recommended to sow under relatively high temperature (up to 35 °C).

Germination and seedling growth parameters under the influence of PEG concentrations higher than 10 % were cultivar-dependent. Two cultivars (G15 and Sh305) expressed higher values of seed germination percentage and seedling growth parameters than the other two cultivars (D and Sh306). The data of our work was consistent with several other works (Ibrahim & al. 2013, Abdo & al. 2014, El-Kady & al. 2015). Under drought stress, while significant increase in grain biomass, shoot dry weight, straw weight, length of lateral branches and panicles was detected in cv. G15, these parameters were reduced or unchanged in cv. D (Ibrahim & al. 2013). Abdo & al. (2014) reported that Sh305 expressed higher grain yield and water use efficiency under all irrigation treatments than cv. D. El-Kady & al. (2015) evaluated 40 sorghum crosses under different irrigation levels; all the tested crosses were drought-tolerant and significantly outyielded the hybrid cv. Sh306. During seedling stage, the detected decrease in the measured growth parameters under drought stress of the four sorghum cultivars may be due to the loss in cell turgor leading to decrease of cell expansion and cell size (Hale & Orcutt 1987). Data indicated that cv. Sh305 and G15 were recommended to sow where the soil has low water content.

Under salt stress, two sorghum cultivars (Sh305 and G15) showed better seed germination than the other two (Sh306 and D). Hassanein & al. (2010) reported that cv. D was not recommended to cultivate in saline soil. Seed germination of cv. G15 was higher than that of cv. Sh305 under the influence of the highest concentration of NaCl (200 mM), while cv. Sh306 expressed the lowest value of seed germination. The estimated growth parameters decreased with the increase of NaCl concentrations, but they did not produce clear signs of the differential response of the tested cultivars to the applied concentration of NaCl. Ability of sorghum cultivars to grow under salt stress may be due to reduction of Na^+ and Cl^- transport from the roots to the shoot, compartmentalization of part of these ions in specific places in plant organs (Lacerda & al. 2001), accumulation of organic solutes (Lacerda & al. 2003), detoxification of reactive oxygen species (Türkan & al. 2005), and regulation of gene expression (Hassanein 1999, Zhao & al. 2020). Since stress tolerance during seed germination resulted in reduction of early seedling growth leading to reduction of vegetative plant growth and plant yield (Briggs & Aytenfisu 1979), cultivars expressing high germination (such as cv. G15 and Sh305) and emergence under relatively high concentration of NaCl can be recommended to be cultivated economically in saline soil.

Temperature ranging from 21–35 °C was recommended for germination of sorghum seeds (Bajay & Papp 1969). Consequently, the used temperature (15–35 °C) did not exert profound changes in the activities of the investigated enzymes. In comparison to seeds germinated at 25 °C, seedling tissues of the four cultivars showed increase of SOD activity under the influence of relatively high temperature (35 °C). Increase of SOD activity may be correlated with the levels of H_2O_2 in stressed tissues (Naji & Devaraj 2011, Hasanuzzaman & al. 2020). The activity of the other three enzymes (POX, CAT and APX) in cv. G15, Sh305 and Sh306 increased under low and high temperatures, to control the toxic effect of H_2O_2 (Agarwal & Pandey 2004). Total operating units (Unit g^{-1} FW) of the investigated four enzymes in cv. Sh305 and Sh306 seedlings were higher than those in cv. D and G15. On the other hand, in comparison to that of control, the relative increase of SOD, POX and APX activities in cv. G15 and Sh305 seedlings was higher than in cv. D and Sh306 under high temperature (35 °C). Consequently, cv. G15 and Sh305 expressed high percentage of seed germination and less reduction in plumule and radicle lengths than cv. D and Sh306 under the influence of relatively high temperature.

While the activities of all studied enzymes increased with the increase of PEG concentrations in all cultivars, POX and APX activities, relative increase in POX, CAT and APX activities, and SOD, POX, CAT and APX total operating units (Unit g^{-1} FW) in cv. G15 and Sh305 seedlings were higher than in cv. D and Sh306, especially under relatively high concentrations of PEG (15 and 20 g/L). Maybe this situation creates a sufficient scavenging system to control the ROS emerging under drought stress (Singh & Sharma 2013), which leads to an increase of seed germination and growth parameters of seedlings. CAT and SOD activities of drought tolerant cultivars were higher than those of sensitive ones (Goswami & Deka 2020). On the other side, the values of SOD activities in cv. D and Sh306 seedlings were higher than those in cv. G15 and Sh305. Increase in SOD activity under drought condition was followed by decrease in its activity after prolonged drought period or under severe drought stress, which may be due to a disturbed balance between production of ROS and scavenging tools (Van Breusegem & al. 1998).

Expression of the studied enzymes under salt stress was the same as under drought stress where activities of all investigated enzymes progressively increased with the increase of NaCl concentrations. Both cv. G15 and Sh305 expressed higher POX and APX activities, higher relative increase in POX and APX, and higher total operating units (Unit g^{-1} FW) of SOD, POX, CAT and APX than cv. D and Sh306. In this concern, the ability to control the oxidative stress associated with salinity stress of the stress tolerant cv. Sh305 and G15 seedlings was higher than that of the sensitive ones (cv. D and Sh306), and this was also reported in other works (Blokhina & al. 2003, Caverzan & al. 2012). Goswami & Deka (2020) found that an *Amaranthus tricolor* cultivar does not require simultaneous stimulation of all antioxidant enzymes for drought tolerance. Only some of them (SOD, CAT and ASA-GSH cycle enzymes) are sufficient to establish ROS detoxification in the tolerant cultivar. Consequently, cv. Sh305 and G15 expressed higher seed germination under the highest concentration of NaCl.

Two DNA-based markers, RAPD and ISSR were used to assess the genetic diversity in sorghum and other plant species (Hassanein & al. 2018, Bellah & al. 2020). The number of polymorphic bands obtained from each technique indicated that both RAPD and ISSR could be used successfully to characterize sorghum cultivars. In this work, RAPD was sufficient to characterize sorghum cultivars as was reported by Akhare & al. (2008). In addition, our results revealed that similar groupings were obtained when RAPD or ISSR were used as was reported by Karaca & Izbirak (2008), but they also reported that ISSR technique was more efficient than RAPD. In our work, no difference could be detected between the RAPD and ISSR dendrograms. The dendrograms obtained by RAPD or ISSR isolated one branch (cv. G15) from a cluster. The cluster was divided into the cv. D lateral branch and the closest cultivars Sh305 and Sh306 in a subcluster. The dendrograms did not reflect the ability of seeds of the four cultivars to germinate under the influence of environmental stresses; cv. Sh305 and Sh306 appeared in the same branch, but they expressed clear differences in stress tolerance. However, there is an interesting correlation with plant height. While the first cultivar (cv. G15) is the tallest (Abdel-Motagally 2010), the second one (cv. D) is the shortest (Younis & al. 2000). The other two cultivars (Sh305 and Sh306) are local hybrids, and they are intermediate in height between the two others (Ahmed & al. 2013).

5. Conclusion

Our data indicated that stress-tolerant sorghum cultivars showed higher protection against oxidative damage during seed germination through enhancement of the production of antioxidant enzymes than sensitive cultivars. Consequently, cv. Sh305 and G15 are recommended to sow where the temperature, water scarcity and soil salinity are relatively high. RAPD or ISSR were efficiently used to characterize sorghum cultivars genetically. The

data from this work and others (Peacock & Ntshole 1976, Hassanein & al. 2010, Ibrahim & al. 2013, Abdo & al. 2014, El-Kady & al. 2015) on sorghum cultivars confirm the necessity of conducting more studies taking into account the influence of various stress factors during germination and other growth stages before recommendation of certain cultivars for economic cultivation under the influence of several abiotic stresses.

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