RESEARCH

Seeds primed with 5-aminolevulinic acid mitigated temperature and drought stresses of wheat at germination and early seedling growth

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ABSTRACT

Drought and temperature stresses are known as primary factors limiting germination and seedling growth. Seed priming with plant growth regulators is one of the popular approaches to minimize adverse environmental stresses. This study was carried out to examine the role of seed priming with 5-aminolevulinic acid (5-ALA) at different levels (0, 25, 50, and 100 mg L^{-1}) in germination and seedling growth of three wheat (*Triticum aestivum* L.) 'Emam', 'Gomria', and 'Zakia' under high temperature and drought stresses using a range of temperatures (25, 30, and 35 °C) and water potentials (0.0 and -0.5 MPa). Water uptake, germination and early seedling growth attributes were measured. Drought stress and high temperature decreased germination%, germination index, and shoot fresh weight by 39.2%, 35.4%, and 48.6%, respectively, as compared with 0.0 MPa and 25 °C. Seeds of 'Zakia' primed with 50 mg L^{-1} 5-ALA at -0.5 MPa, and 35 °C increased fresh and dry weights of the root by 19.8% and 68.4% relative to 0 mg L^{-1}. For interaction between variety and 5-ALA, the highest germination% (90.3%) was recorded in 'Emam' with 50 mg L^{-1} 5-ALA. Treatment 100 mg L^{-1} 5-ALA decreased mean germination time by 24.5% and 28.9% at 25 and 35 °C, respectively; 50 mg L^{-1} 5-ALA increased seedling vigor index by 16.3% as compared with 0.0 mg L^{-1} at -0.5 MPa and 35 °C. The 5-ALA enhanced water imbibition of the three varieties under drought and temperature stresses. This study suggested that seed priming with 5-ALA is a possible way to mitigate the negative effects produced by drought and temperature stresses on germination and early seedling growth of wheat.

Key words: Abiotic stress, early growth stage, plant growth regulator, *Triticum aestivum*, water imbibition.

INTRODUCTION

The combined effects of environmental stresses severely restricted most growth stages of plants (Hussain et al., 2019; Suliman et al., 2021). Germination is an essential developmental stage change in the life cycle of a plant, which plays an important role in crop establishment and environmental adaptation (Liu et al., 2019). Germination and early stage of growth are most sensitive to environmental stresses, particularly drought and high temperature (Saux et al., 2020; Shahrajabian et al., 2020). Numerous studies have examined the inhibition of seed germination and seedling establishment as a result of heat and drought stress in several crops (Shaban, 2013; Wen, 2015; Saberali and Shirmohamadi-Aliakbarkhani, 2020).

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops for human consumption. Its growth can be affected by drought and temperature stress at any developmental stage (Bharati et al., 2018; Pavia et al., 2019). Wheat germination is an essential factor that contributes to higher grain yield. High temperature stress increased the occurrence of loss of seed quality associated with seed viability and germination (Wen, 2015). Moreover, the effects of increased temperature on wheat include growth retardation by reducing cell division and cell elongation resulting in dwarf plants as well as decreased root growth, number of roots and root diameter (Iqbal et al., 2019; Suliman et al., 2021).

Water deficit is one of the major abiotic stresses that decrease germination, growth, and yield of crops (Saima et al., 2018). Less precipitation in drylands is a primary problem leading to insufficient soil moisture, and thus can inhibit seed germination and limit the establishment and growth of seedlings (Fan et al., 2020). Decreased water potential is a general consequence of drought, which has negative effects on germination and consequent seedling growth (Shahrajabian et al., 2017). The disturbance of water availability during germination may induce erratic and decreased germination, leading to poor stand establishment (Saux et al., 2020). Furthermore, drought stress can delay, reduce, or inhibit germination and seedling vigor of wheat cultivars (Jovović et al., 2018).

Using of plant hormones or plant growth regulators to improve drought and temperature resistance of seeds is an efficient pathway to mitigate the adverse effects of drought and temperature stresses. The 5-aminolevulinic acid (5-ALA) is a kind of non-protein amino acid that supports plant stress tolerance (Anwar et al., 2020). Seed priming with 5-ALA effectively enhanced the performance of rice seeds subjected to accelerated ageing treatment (Kanto et al., 2015). Furthermore, 5-ALA can be combined with other plant growth regulators to alleviate plant stress. In this regard, 5-ALA and citric acid can effectively mitigate chromium stress on sunflower (Farid et al., 2020). It is reported that 5-ALA successfully mitigated the adverse effects of environmental stresses of different plants species including, chilling stress in rice (Sheteiwy et al., 2017), water stress in wheat (Akram et al., 2018), and low temperature and weak light in cucumber seedling (Anwar et al., 2020).

Based on the previous studies, 5-ALA was applied only under an individual stress. There is little knowledge available on the effects of 5-ALA under combined stress of drought and high temperature. The details of the physiological and metabolic mechanisms of 5-ALA in wheat in high temperature and drought stresses still need to be interpreted. We did this study to elucidate the effects of exogenously treated 5-ALA on germination and early seedling growth attributes measurements in wheat seedlings exposed to drought and high temperature stresses.

MATERIALS AND METHODS

A controlled experiment was done in the Joint International Research Laboratory of Agriculture and Agri-Product Safety of the Ministry of Education of China, Yangzhou University, Yangzhou (32°30’ N, 119°43’ E).

Seeds of ‘Emam’, ‘Gomria’, and ‘Zakia’, wheat (*Triticum aestivum* L.) obtained from the Ministry of Agriculture of Sudan, were used. Twenty-five seeds of each treatment were soaked with 40 mL of different concentrations of 5-aminolevulinic acid (5-ALA) solution (0, 25, 50, and 100 mg L^{-1}), for 12 h at 25 °C, and then re-dried back for 48 h to near their original weight.

Seeds with uniform size from each treatment were germinated in Petri dishes containing two filter paper moistened with 5 mL distilled water (0.0 MPa) or with polyethylene glycol (PEG) solution (-0.5 MPa). Osmotic potential was created by using PEG 6000 according to the method of Michel and Kaufmann (1973). Among different levels of PEG (-0.1, -0.3, -0.5, -0.7 and -1.2 MPa), -0.5 MPa was selected according to a preliminary experiment. The Petri dishes were separated into three growth chambers (Model PYX-300G-B, Yangzhou Yiwei Automatic Instrument Co. Ltd., Jiangsu, China) set at 25, 30, and 35 °C. All the chambers were set at 55%-60% RH and a photoactive radiation of 500 W m^{-2} (12/12 h day/night). The experiment was designed as a four-factorial experiment arranged in a completely randomized design with three replicates for each treatment. Germination was monitored daily until a constant count was achieved (no more seed can germinate). The seeds were considered germinated when the radicle length reached about 2 mm out of seed surface.

Seed water imbibition test

All the seeds in each Petri dish were weighed before soaking and seed water uptake at 6 and 12 h after the beginning of water imbibition. For each determination, seeds were carefully removed, drained, and blotted quickly with absorbent paper, weighed, and placed again into the Petri dishes. Environment condition for water uptake was maintained the same as in seed germination. Seed water uptake (kg kg^{-1}) was determined as: (final weight - initial weight)/initial weight. The final weight was the seed weight after water uptake and initial weight was the original seed weight before water uptake.

Germination parameters

Germination percentage (GP%) was calculated as:

(Total germinated seeds/Total seed number) ×100

Germination index (GI) was measured according to the following formula (AOSA, 1983):

$$GI = \left[\frac{\text{Number of germinated seeds in the first count}}{\text{Days of first count}}\right] + ... + \left[\frac{\text{Number germinated seeds in the final count}}{\text{Days of final count}}\right]$$

Mean germination time (MGT) was determined according to the method described by Mahmood et al. (2014):

$$MGT (d) = \Sigma niti/\Sigma ni$$

where ti is the number of days after sowing, ni is the number of seeds germinated on the i^{th} day.

Seedling vigor index (SVI) was measured using the formula described by Farahani and Maroufi (2011):

SVI = Germination percentage × Seedling dry weight

Growth attributes

Five seedlings of each replicate of each treatment were sampled to determine plant height, fresh and dry weight of root and shoot. The root and shoot length were measured using a ruler. Root and shoot fresh weights were recorded immediately after harvesting by separating the roots from shoot. The samples were then dried in an oven at 70 °C until constant weight for biomass determination.

Statistical analyses

This study was a four-factorial design arranged in a completely randomized design with three replicates for each treatment. The data collected were subject to ANOVA with the statistical package of MSTAT-C (Gomez and Gomez, 1984). When the F values were significant, means were separated by the LSD test at the 0.05 probability level.

RESULTS

ANOVA showed that drought, variety, temperature, 5-ALA, and their combinations had diverse effects on different parameters of wheat (Tables 1 and 2).

Water uptake

Drought stress significantly decreased seed water uptake. At both time of water imbibition, the highest (0.621 kg kg^{-1}) and lowest (0.157 kg kg^{-1}) values of water uptake were achieved in 'Emam' with 0.0 MPa, 30 °C, 100 mg L^{-1} 5-ALA and 'Gomria' with -0.5 MPa, 35 °C, 50 mg L^{-1} 5-ALA, respectively. Water imbibition was increased gradually with increasing temperature in most treatments. Wheat seeds primed with different levels of 5-ALA showed significant increase in water uptake at 6 and 12 h in all the treatments as compared with unprimed seeds. Water imbibition was increased by 11.4% and 8.4% with 25 mg L^{-1} 5-ALA after 6 h in 'Emam' with 0.0 MPa, 30 °C and -0.5 MPa, 30 °C, respectively, relative to 0.0 mg L^{-1} 5-ALA. After 12 h of imbibition, 50 mg L^{-1} 5-ALA increased water imbibition by 20.8% and 29.9% in 'Zakia' with 0.0 MPa, 35 °C and -0.5 MPa, 35 °C, respectively, as compared with 0 mg L^{-1} 5-ALA (Tables 3 and 4).

Table 1. ANOVA results for germination attributes of wheat plants as influenced by variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) application.

Source of variation	Water uptake after 6 h		Water uptake after 12 h		Germination percentage		Germination index		Mean germination time		Seedling vigor index	
	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
Variety (V)	0.004	1.892^{ns}	0.008	3.324*	501.51	4.756*	31.98	4.839*	0.359	4.234*	0.153	6.951*
Drought (D)	0.343	184.3**	1.020	407.7**	13379.6	126.9**	873.1	132.1**	68.670	809.6**	9.012	408.8**
Temperature (T)	0.002	0.905^{ns}	0.002	0.612^{ns}	0.116	0.001**	0.012	0.002**	0.156	1.839**	0.108	4.903**
5-ALA (A)	0.002	0.924*	0.001	0.474**	6817.1	64.65^{ns}	425.5	64.38**	2.464	29.05**	2.812	127.6^{ns}
D×V	0.002	0.923^{ns}	0.008	3.092^{ns}	3436.6	32.59^{ns}	213.5	32.31^{ns}	1.288	15.19^{ns}	0.068	3.079**
D×T	0.002	0.874^{ns}	0.003	1.292**	94.91	0.900**	6.115	0.925**	0.014	0.169**	0.007	0.324**
D×A	0.001	0.664^{ns}	0.001	0.507^{ns}	110.19	1.045^{ns}	6.937	1.049^{ns}	0.029	0.348^{ns}	0.020	0.904^{ns}
V×T	0.006	3.376^{ns}	0.024	9.568^{ns}	83.64	0.793^{ns}	63.82	9.656^{ns}	0.746	8.789^{ns}	0.036	1.628^{ns}
V×A	0.002	1.264**	0.006	2.210^{ns}	104.63	0.992**	6.660	1.008**	0.098	1.154^{ns}	0.009	0.428**
T×A	0.004	2.232^{ns}	0.002	0.634^{ns}	244.41	2.318^{ns}	15.44	2.336^{ns}	0.111	1.307^{ns}	0.057	2.584**
D×V×T	0.003	1.377^{ns}	0.003	1.081^{ns}	76.97	0.730^{ns}	4.789	0.725^{ns}	0.052	0.608^{ns}	0.014	0.653^{ns}
D×V×A	1.8384	0.003^{ns}	0.005	1.873^{ns}	108.64	1.030^{ns}	6.985	1.057^{ns}	0.160	1.883^{ns}	0.051	2.311^{ns}
D×T×A	0.003	1.365^{ns}	0.000	0.185^{ns}	217.59	2.064^{ns}	13.61	2.059^{ns}	0.307	3.619**	0.062	2.826**
V×T×A	0.004	2.047**	0.004	1.489^{ns}	146.14	1.386^{ns}	9.255	1.400^{ns}	0.083	0.984^{ns}	0.045	2.059**
D×V×T×A	0.007	3.638**	0.006	2.598**	119.44	1.133^{ns}	7.633	1.155^{ns}	0.058	0.680^{ns}	0.037	1.697^{ns}

*, ** Significant at the 0.05 and 0.01 probability level, respectively; ^{ns}: nonsignificant.
MS: Mean square.

Table 2. ANOVA results for growth attributes of wheat plants as influenced by variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) application.

Source of variation	Root length		Root fresh weight		Root dry weight		Shoot length		Shoot fresh weight		Shoot dry weight	
	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
Variety (V)	6.885	7.861*	0.010	20.46**	0.0001	42.90**	1.673	2.576^{ns}	0.011	18.40**	0.0004	4.291*
Drought (D)	65.27	74.53**	0.277	575.8**	0.002	179.9**	317.3	488.5**	0.526	845.8**	0.006	575.3**
Temperature (T)	0.114	0.129**	0.002	4.733**	0.0001	7.771**	0.737	1.135**	0.004	6.406**	0.0001	7.867**
5-ALA (A)	278.6	318.1^{ns}	0.229	474.7^{ns}	0.001	147.6*	128.2	197.4^{ns}	0.067	107.4^{ns}	0.001	105.3^{ns}
D×V	26.62	30.39^{ns}	0.029	60.48**	0.0003	33.33**	0.451	0.695^{ns}	0.002	3.773**	0.0001	2.815**
D×T	0.379	0.432**	0.001	1.494**	0.0001	1.903**	0.282	0.433^{ns}	0.001	1.017**	0.0001	0.943^{ns}
D×A	1.052	1.201^{ns}	0.001	2.974^{ns}	0.0001	1.439^{ns}	0.753	1.159^{ns}	0.001	1.001^{ns}	0.0003	1.222^{ns}
V×T	0.623	0.712^{ns}	0.001	2.625^{ns}	0.0003	5.782^{ns}	0.993	1.529^{ns}	0.0005	0.583^{ns}	0.0001	0.734^{ns}
V×A	1.131	1.291^{ns}	0.0005	0.637^{ns}	0.0001	0.379**	0.939	1.446^{ns}	0.001	0.863^{ns}	0.0001	0.760^{ns}
T×A	1.475	1.684**	0.0004	1.020**	0.0005	3.784**	0.589	0.908^{ns}	0.001	0.886^{ns}	0.0001	0.800**
D×V×T	1.616	1.845^{ns}	0.001	1.467**	0.0001	1.157^{ns}	0.797	1.228^{ns}	0.001	1.475^{ns}	0.0001	1.539^{ns}
D×V×A	2.078	2.373^{ns}	0.001	2.693^{ns}	0.0001	3.338^{ns}	0.352	0.543^{ns}	0.001	1.807^{ns}	0.0002	2.422^{ns}
D×T×A	1.274	1.454^{ns}	0.001	1.279^{ns}	0.0001	3.352**	1.734	2.670**	0.002	2.686**	0.0003	2.528**
V×T×A	0.876	1.001^{ns}	0.001	1.428^{ns}	0.0002	2.474**	0.846	1.303^{ns}	0.001	1.684^{ns}	0.0001	1.888**
D×V×T×A	0.423	0.483^{ns}	0.001	1.955*	0.0001	2.951**	0.408	0.628^{ns}	0.0001	0.706^{ns}	0.0001	0.792^{ns}

*, ** Significant at the 0.05 and 0.01 probability level, respectively; ^{ns}: nonsignificant.
MS: Mean square.

Table 3. Effects of variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) on water uptake of wheat seeds at 6 h after the beginning of water uptake.

			5-ALA mg L^{-1}				
Drought	Variety	Temperature	0	25	50	100	Mean
MPa		ºC	kg kg^{-1}				
0.0	Emam	25	0.324	0.366	0.343	0.358	0.348abc
		30	0.351	0.391	0.373	0.306	0.355a
		35	0.361	0.362	0.272	0.378	0.343abc
	Gomria	25	0.326	0.337	0.339	0.400	0.351ab
		30	0.338	0.341	0.358	0.337	0.344abc
		35	0.302	0.403	0.419	0.270	0.349abc
	Zakia	25	0.289	0.330	0.365	0.366	0.338c
		30	0.336	0.339	0.339	0.347	0.340bc
		35	0.266	0.349	0.397	0.370	0.346abc
-0.5	Emam	25	0.249	0.246	0.254	0.289	0.260gh
		30	0.260	0.282	0.284	0.288	0.279ef
		35	0.309	0.352	0.288	0.254	0.301d
	Gomria	25	0.259	0.274	0.260	0.273	0.267fg
		30	0.234	0.231	0.294	0.263	0.256gh
		35	0.293	0.282	0.157	0.283	0.254h
	Zakia	25	0.228	0.264	0.238	0.278	0.252h
		30	0.234	0.258	0.255	0.256	0.251h
		35	0.262	0.246	0.348	0.261	0.279e

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Table 4. Effects of variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) on water uptake of wheat seeds at 12 h after the beginning of water uptake.

			5-ALA mg L^{-1}				
Drought	Variety	Temperature	0	25	50	100	Mean
MPa		ºC	kg kg^{-1}				
0.0	Emam	25	0.433	0.506	0.526	0.503	0.492bc
		30	0.467	0.530	0.533	0.621	0.538a
		35	0.462	0.497	0.453	0.514	0.482cd
	Gomria	25	0.448	0.499	0.487	0.562	0.499b
		30	0.473	0.508	0.518	0.494	0.498b
		35	0.442	0.560	0.550	0.387	0.485bcd
	Zakia	25	0.407	0.479	0.505	0.506	0.474de
		30	0.456	0.485	0.500	0.491	0.483cd
		35	0.409	0.468	0.494	0.496	0.467e
-0.5	Emam	25	0.343	0.307	0.368	0.389	0.352gh
		30	0.344	0.371	0.362	0.367	0.361fg
		35	0.349	0.432	0.379	0.331	0.373f
	Gomria	25	0.339	0.389	0.358	0.385	0.368f
		30	0.310	0.317	0.387	0.352	0.342hi
		35	0.365	0.358	0.294	0.353	0.343hi
	Zakia	25	0.327	0.377	0.331	0.353	0.347ghi
		30	0.322	0.341	0.332	0.351	0.337i
		35	0.334	0.338	0.434	0.335	0.360fg

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Germination percentage

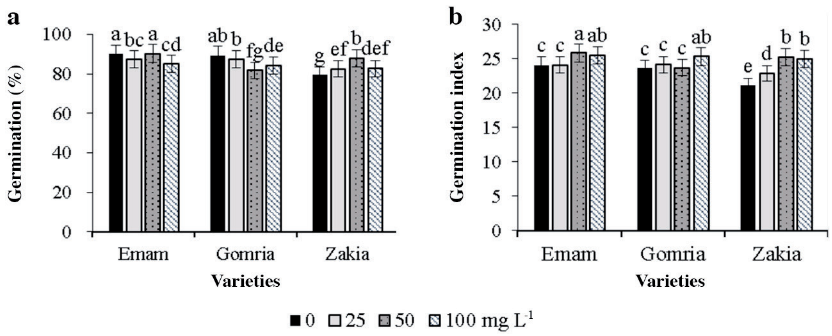
Germination percentage (GP) was gradually decreased with increasing temperature at both levels of drought. Drought stress (-0.5 MPa) and high temperature (35 °C) decreased GP by 39.2% as compared with 0.0 MPa and 25 °C (Table 5). In the interaction of variety and 5-ALA, the highest GP (90.3%) was recorded in 'Emam' and 50 mg L^{-1} 5-ALA. Moreover, 50 mg L^{-1} 5-ALA raised GP of 'Zakia' by 10.5% as compared with 0 mg L^{-1} 5-ALA (Figure 1a).

Table 5. Effects of drought and temperature on germination percentage, germination index, and root length of wheat.

Drought (MPa)	Temperature	Germination	Germination index	Root length
	°C	%		cm
0.0	25	97.08a	27.11a	7.62b
	30	93.06ab	26.11ab	8.33a
	35	90.69b	25.51b	3.56d
-0.5	25	90.14b	25.28b	6.09c
	30	84.44c	23.86c	6.29c
	35	59.03d	17.52d	3.83d

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Figure 1. Effects of variety and 5-aminolevulinic acid (5-ALA) on germination percentage (a) and germination index (b) of wheat seeds.



Bars with the same letters above are not significantly different at the 0.05 probability level.

Germination index

Temperature and drought stress gradually decreased germination index (GI). At 35 °C and -0.5 MPa, GI was decreased by 35.4% as compared with 25 °C and 0.0 MPa (Table 5); GI was increased with increasing 5-ALA concentration within the three varieties. 'Emam' and 'Zakia' with 50 mg L^{-1} 5-ALA achieved higher GI (25.9% and 25.3%) respectively. In addition, 100 mg L^{-1} 5-ALA raised GI of 'Gomria' by 7.1% relative to 0 mg L^{-1} (Figure 1b).

Mean germination time

Drought and high temperature increased mean germination time (MGT) of all the treatments. For example, at 25 mg L^{-1} 5-ALA, high temperature of 35 °C increased MGT by 57.9% and 30.3% at 0.0 MPa and -0.5 MPa, respectively, as compared with 25 °C. -0.5 MPa raised MGT under all levels of temperature and 5-ALA as compared with 0.0 MPa. Lower MGT (1.10 d) was recorded at 0.0 MPa, 30 °C, and 25 °C and 50 mg L^{-1} 5-ALA. In 0.0 MPa, 100 mg L^{-1} 5-ALA decreased MGT by 24.5% and 28.9% at 25 and 35 °C, respectively, as compared with 0.0 mg L^{-1} 5-ALA (Table 6). MGT was significantly different among the three varieties, the lowest MGT (1.72) was recorded in 'Gomria' (Figure 2a).

Seedling vigor index

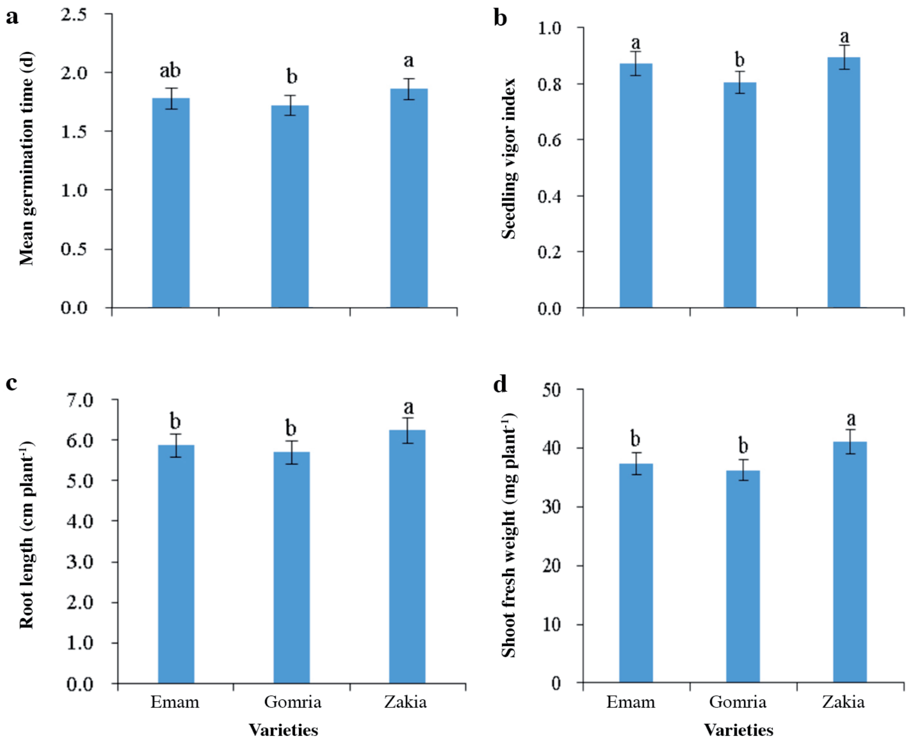
The interaction of drought, temperature, and 5-ALA had significant effects on seedling vigor index (SVI). At 0 mg L^{-1} 5-ALA and 25 °C, drought stress decreased SVI by 43.8% as compared with 0.0 MPa. At 0.0 mg L^{-1} 5-ALA temperature of 35 °C decreased SVI by 34.8% and 22.2% in 0.0 and -0.5 MPa, respectively, relative to 25 °C. Application of 5-ALA increased SVI, at 0.0 MPa and 35 °C, 100 mg L^{-1} 5-ALA raised SVI by 20.5% as compared with 0.0 mg L^{-1}. Moreover, 50 mg L^{-1} 5-ALA increased SVI by 16.3% compared with 0.0 mg L^{-1} at -0.5 MPa and 35 °C (Table 6). Of the three varieties, 'Zakia' had the greatest SVI (0.895), followed by 'Emam' (Figure 2b).

Table 6. Effects of drought, temperature, and 5-aminolevulinic acid (5-ALA) on mean germination time and seedling vigor index of wheat.

Parameters		Mean germination time				Seedling vigor index			
		5-ALA mg L^{-1}				5-ALA mg L^{-1}			
Drought	Temperature	0	25	50	100	0	25	50	100
MPa	ºC	d							
0.0	25	1.47k	1.14mno	1.12mno	1.11no	1.12e	1.24a	1.21ab	1.21ab
	30	1.30l	1.10o	1.10o	1.18mn	1.17cd	1.15de	1.20bc	1.16d
	35	1.59j	1.80m	1.20m	1.13mno	0.73l	0.82ghi	0.86fg	0.88f
-0.5	25	2.43d	2.05gh	2.42d	2.08g	0.63m	0.77k	0.66m	0.81hij
	30	2.32e	2.20f	1.87i	1.99h	0.79jk	0.80ijk	0.79ijk	0.84gh
	35	2.64bc	2.67b	2.57c	2.94a	0.49o	0.41p	0.57n	0.29q

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Figure 2. Effects of variety on mean germination time (a), seedling vigor index (b), root length (c) and shoot fresh weight (d) of wheat seedlings.

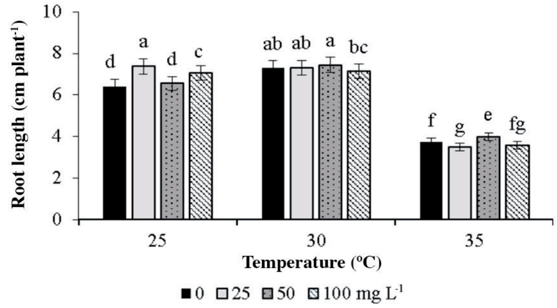


Bars with the same letters above are not statistically different at the 0.05 probability level.

Root length

Drought stress at 25 °C declined root length by 20.1%, in contrast at 35 °C root length was raised by 7.6% as compared with 0.0 MPa. High temperature decreased root length by 53.3% and 37.1% at 0.0 and -0.5 MPa relative to 25 °C. Moreover, 30 °C had greatest root length compared with 25 and 35 °C in two levels of drought (Table 5). Application of 50 mg L^{-1} 5-ALA increased root length by 1.9% and 7.0% at 30 and 35 °C respectively compared with 0 mg L^{-1} 5-ALA (Figure 3). Root length was affected significantly by varieties, 'Zakia' achieved the longest root length (6.23 cm $plant^{-1}$) (Figure 2c).

Figure 3. Effects of temperature and 5-aminolevulinic acid (5-ALA) on root length of five wheat seedlings.



Bars with the same letters above are not significantly different at the 0.05 probability level.

Root weight

Four factors affected significantly on root fresh weight. It was decreased by -0.5 MPa in almost all treatments as compared with 0.0 MPa, except in 'Zakia', 35 °C and 0 mg L^{-1} 5-ALA. Root fresh weight also was reduced with increased temperature, e.g., at 0.0 MPa, 'Emam' and 0 mg L^{-1} 5-ALA, high temperature decreased root fresh weight by 60.8% relative to 25 °C. Moreover, at -0.5 MPa and 100 mg L^{-1} 5-ALA, high temperature decreased root fresh weight of 'Zakia' by 54.8% as compared with 25 °C. Application of 25 mg L^{-1} 5-ALA at 0.0 MPa, and 35 °C increased root fresh weight of 'Zakia' by 73.6% relative to 0 mg L^{-1}. In addition, 50 mg L^{-1} 5-ALA at -0.5 MPa, and 35 °C increased root fresh weight of 'Zakia' by 19.8% relative to 0 mg L^{-1}. Effect of varieties on this trait was significant, where the higher (56.7 mg) root fresh weight was obtained by 'Emam' at 0.0 MPa, 25 °C and 25 mg L^{-1} 5-ALA. Moreover, at 50 and 100 mg L^{-1} 5-ALA 'Zakia' performed better than 'Emam' and 'Gomria' (Table 7).

Within all the treatments, the maximum root dry weight (7.20 mg $plant^{-1}$) was recorded in 'Zakia' with 0.0 MPa, 25 °C and 50 mg L^{-1} 5-ALA. Drought stress reduced root dry weight by 45.2% compared with 0.0 MPa in 'Emam' with 25 °C and 0 mg L^{-1} 5-ALA. Root dry weight was decreased gradually with increasing temperature at 0.0 MPa. Although at -0.5 MPa, 30 °C had the greatest root dry weight. Treatment 100 mg L^{-1} 5-ALA raised root dry weight of 'Zakia' by 54.8% relative to 0 mg L^{-1} 5-ALA at 0.0 MPa and 35 °C. Furthermore, 100 mg L^{-1} 5-ALA raised root dry weight of 'Gomria' by 12.7% relative to 0 mg L^{-1} 5-ALA at -0.5 MPa and 30 °C (Table 8).

Table 7. Effects of variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) on root fresh weight of wheat plants.

			5-ALA mg L^{-1}				
Drought	Variety	Temperature	0	25	50	100	Mean
MPa		°C	kg kg^{-1}				
0.0	Emam	25	51.9	56.7	50.6	52.4	52.9a
		30	46.6	47.4	46.4	48.3	47.2d
		35	20.3	18.9	20.0	21.2	20.1m
	Gomria	25	40.8	49.8	42.6	43.4	44.2e
		30	40.8	47.6	43.8	43.0	43.8e
		35	21.0	20.2	18.4	23.8	20.9l
	Zakia	25	44.0	51.0	54.2	54.0	50.8b
		30	55.2	46.0	50.8	46.2	49.6c
		35	18.2	31.6	28.4	20.4	24.7k
-0.5	Emam	25	25.8	30.4	30.8	29.4	29.1i
		30	26.2	27.8	26.6	24.2	26.2j
		35	14.8	14.0	19.4	12.2	15.1p
	Gomria	25	27.8	34.2	28.8	34.4	31.3g
		30	24.4	25.0	24.4	24.8	24.7k
		35	17.4	17.2	13.4	15.6	15.9o
	Zakia	25	32.6	35.6	28.4	39.4	34.0f
		30	28.6	30.8	29.0	31.4	30.0h
		35	20.2	14.8	24.2	17.8	19.3n

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Table 8. Effects of variety, drought, temperature, and 5-aminolevulinic acid (5-ALA) on root dry weight of wheat plants.

Drought	Variety	Temperature	5-ALA mg L^{-1} 0	25	50	100	Mean
MPa		°C	kg kg^{-1}				
0.0	Emam	25	6.46	6.80	6.40	7.14	6.70a
		30	5.60	6.00	6.00	6.20	5.95b
		35	3.60	3.40	3.94	4.20	3.79fgh
	Gomria	25	5.46	6.00	5.40	5.74	5.65bc
		30	5.14	5.94	5.26	5.14	5.37c
		35	3.74	3.34	3.14	3.74	3.49gh
	Zakia	25	5.94	6.80	7.20	7.06	6.75a
		30	7.07	6.26	7.14	6.46	6.73a
		35	3.14	5.40	5.46	4.86	4.72d
-0.5	Emam	25	3.54	4.00	4.06	4.26	3.97efg
		30	4.54	4.86	4.94	4.46	4.70d
		35	3.00	3.20	4.40	2.60	3.30h
	Gomria	25	4.00	4.66	4.26	4.74	4.42de
		30	4.26	4.34	4.54	4.80	4.49de
		35	4.26	3.94	2.80	3.14	3.54gh
	Zakia	25	4.20	4.94	3.94	5.54	4.66d
		30	4.66	5.94	5.26	6.06	5.48bc
		35	3.80	3.26	6.40	3.46	4.23def

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Shoot length

Shoot length was influenced significantly by the interactions between drought, temperature and 5-ALA. As compared with 0.0 MPa, -0.5 MPa reduced shoot length. Treatment 30 °C had the longest shoot length as compared with the high and control temperatures among all the combinations between three factors. Moreover, 35 °C decreased shoot length by 52.8% at -0.5 and 100 mg L^{-1} 5-ALA relative to 25 °C. At 25 °C, 50 mg L^{-1} 5-ALA increased shoot length by 10.5% and 5.8% in 0.0 and -0.5 MPa, respectively, as compared with 0.0 mg L^{-1} 5-ALA (Table 9).

Shoot weight

The interactions between drought, temperature and 5-ALA were significant on shoot fresh weight; 30 °C had higher shoot fresh weight than 25 and 35 °C. Treatments -0.5 MPa and 35 °C decreased shoot fresh weight by 48.6% in relative to 0.0 MPa and 25 °C, at 0 mg L^{-1} 5-ALA. Application of 25 mg L^{-1} 5-ALA at 0.0 MPa and 35 °C increased shoot fresh weight by 10.1% as compared with 0 mg L^{-1} (Table 9). Data in (Figure 2d) indicated that 'Zakia' was 9.6% and 13.3% higher in shoot fresh weight than 'Emam' and 'Gomria', respectively.

Table 9. Effects of drought, temperature, and 5-aminolevulinic acid (5-ALA) on shoot length and shoot fresh weight of wheat plants.

Parameters		Shoot length cm $plant^{-1}$				Shoot fresh weight mg $plant^{-1}$			
		5-ALA mg L^{-1}				5-ALA mg L^{-1}			
Drought	Temperature	0	25	50	100	0	25	50	100
MPa	°C								
0.0	25	6.19c	6.90b	6.84b	6.30b	46.0cd	49.3b	49.4b	47.1c
	30	7.72a	7.85a	7.86a	7.76a	54.2a	54.2a	53.2a	53.9a
	35	4.38g	5.43d	4.91e	5.28d	40.9e	45.0d	41.7e	41.7e
-0.5	25	3.81i	4.37g	4.03h	4.64f	28.6h	32.8g	27.1h	34.6f
	30	5.08e	5.08e	5.32d	5.23d	33.9fg	33.0g	32.8g	33.5fg
	35	3.10j	2.49k	2.94j	2.19l	23.6i	19.3j	23.2i	17.2k

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Drought stress reduced shoot dry weight by 34.5% as compared with 0.0 MPa (Figure 4). Shoot dry weight was higher at 30 °C than at 25 and 35 °C. The maximum shoot dry weight (6.14 mg $plant^{-1}$) was recorded in 'Zakia' with 30 °C and 100 mg L^{-1} 5-ALA. Treatment 100 mg L^{-1} 5-ALA increased shoot dry weight by 14.0%, 3.9%, and 2.6% in 'Emam' with 25 °C, 'Gomria' with 30 °C and 'Zakia' with 35 °C, respectively, relative to 0 mg L^{-1} 5-ALA. In terms of the performance of varieties at 35 °C and (0 and 25) mg L^{-1} 5-ALA, 'Gomria' was better than 'Emam' and 'Zakia'. While at 35 °C and (50 and 100) mg L^{-1} 5-ALA, 'Zakia' was the best (Table 10).

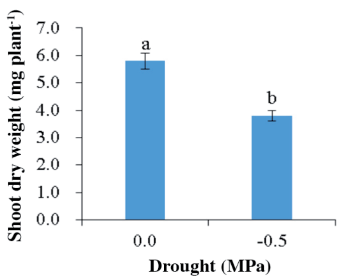
DISCUSSION

High-quality germination and good seedling vigor are critical to achieve a reasonable plant population (Nimir et al., 2014). Germination and early seedling growth are the most vulnerable stages to environmental stress. In the present study, individual drought and heat stress separately reduced GP, GI and SVI. Similar results were recorded in *Tanacetum cineraiifolium* (Shahrajabian et al., 2020) and *Stipagrostis ciliata* (Fakhfakh et al., 2018). This study also indicated that temperature or drought stress during germination and early seedling growth adversely affected the attributes of germination and seedling growth in wheat varieties. The effects on these attributes became more severe when both stresses were present at the same time.

Germination is a process dependent on a series of physical and metabolic events. And it is controlled by multiple external factors, such as temperature and water. Germination is accelerated by increasing temperature within a certain limit, but extreme temperature inhibits germination (Cui et al., 2014). In the present study, we noticed that germination of wheat seeds was increased at temperature of 30 °C, but decreased at 35 °C. Buriro et al. (2011) reported that high GP and GI occurred at a temperature range from 20 to 30 °C.

In the present study, seed water imbibition was significantly decreased under water deficit conditions created by PEG (Tables 3 and 4). Low water uptake by PEG can influence the processes of hydrolysis of seed reserve, initiation of enzyme activities and cell elongation, leading to reduced embryo growth followed by poor emergence (Soleymani and Shahrajabian, 2018; Hasanuzzaman and Fotopoulos, 2019).

Figure 4. Effects of drought on shoot dry weight of five wheat seedlings.



Bars with the same letters above are not significantly different at the 0.05 probability level.

Table 10. Effects of variety, temperature, and 5-aminolevulinic acid (5-ALA) on shoot dry weight of wheat plants.

		5-ALA mg L^{-1}			
Variety	Temperature (ºC)	0	25	50	100
Emam	25	4.30lmno	5.00fghi	4.86ghij	4.90ghij
	30	5.64cd	5.56cde	5.26defg	5.66bcd
	35	4.16mnop	3.74qr	4.56jklm	3.90opq
Gomria	25	4.14nopq	5.00fghi	4.70ijkl	4.90ghij
	30	5.16efgh	5.36cdef	5.54cde	5.36cdef
	35	4.40klmn	4.40klmn	3.44r	3.76pqr
Zakia	25	4.74ijk	5.04fghi	4.74ijk	5.26defg
	30	6.06ab	5.74abc	5.76abc	6.14a
	35	3.80pqr	3.90opq	4.80hijk	3.90opq

Means followed by different letters in the same column are significantly different at the 0.05 probability level.

Wheat seeds primed with 5-ALA enhanced water imbibition under normal and stress conditions of drought and temperature (Tables 3 and 4). Root length at drought stress and high level of temperature was also increased. Root elongation is one of the adaptation mechanisms of the plants to acquire water under adverse conditions. Our finding was in accordance with those of Henry et al. (2011), who reported that a deep root system of the plant grow in limited water conditions is found to be useful for acquiring moisture from soil profiles.

Seeds treated with 5-ALA has been reported to improve germination of different crops under adverse environmental conditions (Kanto et al., 2015). In this study, 5-ALA decreased MGT and enhanced SVI under drought and temperature stress (Table 7). Similarly, Han et al. (2018) demonstrated that 5-ALA decreased MGT under drought stress. Furthermore, 5-ALA improved the cold stress tolerance of pepper by enhancing the final GP and germination rate as a seed treatment (Korkmaz and Korkmaz, 2009). 5-Aminolevulinic acid could increase seed respiratory rate and provide more energy for seed germination under adverse conditions (Fu et al., 2014).

Drought and heat stress significantly decreased shoot length, root length, root fresh and dry weights, shoot fresh and dry weights. Our finding is consistent with those obtained by Liu et al. (2019), who reported that high temperature stress suppressed plumule and radical growth in germinated seedlings during seed imbibition in rice. Application of 5-ALA as seed pre-sowing treatment enhanced length, fresh and dry weight of root and shoot under drought and temperature stress. Similar results were obtained by Kanto et al. (2015), who reported that 5-ALA increased root and shoot length and seedling growth of rice. The increase in seedling length and growth could be due to the improvement of SVI that occurred by 5-ALA (Table 6). Furthermore, application of 5-ALA to the soil significantly increased plant height, fresh and dry weight (Anwar et al., 2020; Suliman et al., 2021). The physiological mechanism behind 5-ALA in mitigation temperature and drought stress still need to be interpreted.

The three varieties differed in their responses to treatments at germination and seedling growth. At germination, 'Emam' outperformed other varieties in GP and GI (Figure 1). The success of seed germination was associated with water absorption. 'Emam' exhibited higher water uptake at two time of water determination. For seedling growth, 'Zakia' surpassed other varieties in root length, shoot fresh and dry weight (Table 9, Figures 2c and 2d). The variations between varieties could be attributed to their different inherited genotypes. Varieties responded differently to 5-ALA and seeds primed with 5-ALA enhanced the GP and GI of 'Emam' and 'Zakia', but reduced the GP of 'Gomria' (Figure 1).

CONCLUSIONS

Our experiment studied the changes in germination characteristics and subsequent growth of three varieties of wheat exposed to drought and high temperature stresses and attempted to alleviate these stresses by seed priming with exogenous 5-aminolevulinic acid (5-ALA). Drought and high-temperature stress considerably suppressed germination and seedling growth. Seeds priming with 5-ALA significantly enhanced seeds water uptake, germination attributes, and growth of root and shoot. 5-ALA could help seeds to mitigate drought and temperature stress during seed germination and early seedling growth.

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