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Published at http://ijate.net/index.php/ijsm http://dergipark.gov.tr/ijsm **Research Article**

Salt, Cold, and Drought Stress on Einkorn and Bread Wheat during Germination

Nusret Zencirci [*,1], Hakan Ulukan [2], Bülent Ordu [3], Didem Aslan [1], Hakan Tahiri Mutlu [3], Mehmet Örgeç [1]

[1] Bolu Abant İzzet Baysal Univ., Sciences and Art Faculty, Biology Dept., 14200, Gölköy, Bolu, Turkey
[2] Ankara Univ., Agricultural Faculty, Field Crops Dept., Dışkapı, Ankara, Turkey
[3] Bolu Abant İzzet Baysal Univ., Economics and Administrative Sciences Faculty, Business Admin. Dept., 14200, Gölköy, Bolu, Turkey

Abstract: Climate changes prompt salt, cold, and drought stresses especially during early crop growth stages. The damages during germination in wheat may even destroy whole crop. Here, 12 bread and 10 einkorn wheats entries were distressed under salt, cold and drought. Germination rate and germination power, coleoptile, shoot length, root length, shoot to root length ratio, root fresh and dry weight and root fresh to dry weight ratio were quantified under six salt, cold, drought stresses and one control. After ANOVA and LSD discriminated the entries, stress tolerance indices differentiated six tolerant and six susceptible entries. MANOVA, Pillai's Trace and Wilks' Lambda tests finalized the stress testing. Shoot and root length, root fresh and dry weight highly differed under salt, cold and drought. Bayraktar-2000 well tolerated salt, drought, salt-drought and salt-cold-drought; Gerek-79 salt, salt-drought, salt-cold-drought; Momtchil salt, cold and salt-cold-drought; İkizce-96 salt, drought and salt-drought. Einkorn Population 14 was susceptible to all stresses except cold and salt-cold; Population 15 to salt, salt-drought and salt-cold-drought; Population 11 to salt, drought, salt-drought and salt-cold-drought. These stresses sharply decreased shoot and root length, root fresh and root dry weight. The higher % decreases under salt, cold and drought were in shoot (59.72, 63.25 and 23.17) and root length (32.91, 51.77 and 34.69), root fresh (44.32, 49.11 and 38.88) and root dry weight (21.63, 42.14 and 41.97). Moreover, Pillai's trace and Wilks' Lambda tests differentiated both characters and entries ($P < 0.01$). In conclusion, Momtchil, Gerek-79, Bayraktar-2000, Populations 5, 6, and 1 are well endorsed against triple seedling stresses.

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Cold,
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1. INTRODUCTION

Wheat has been the main source of food in central - west Asia and Mediterranean basin since the beginning of agriculture [1-4]. It has been cultivated for more than ten thousand years and kept providing staple nutrition for humans since then. Today's global wheat production of

*CONTACT: Nusret ZENCIRCI ✉ nzencirci@yahoo.com Bolu Abant İzzet Baysal Univ., Sciences and Art Faculty, Biology Dept., 14200, Gölköy, Bolu, Turkey

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about 670.8 million tons per year directly influences human survival and life quality by leading the production of various foods, including bread, pasta, noodles, cakes, and biscuits.

Einkorn (*T. monococcum* ssp. *monococcum* L.), the first primary cultivated wheat ancestor emerged around the Karacadağ Mountains in the southeastern Turkey [5]. It has survived as an animal feed [6] in Turkey, Italy, and Bulgaria [2] and has become a popular human food today because of its health supporting characteristics [7].

Stresses like salt, cold or drought applies a force on a unit wheat area as well [8] and restricts its growth and decreases yield [9-12]. Many abiotic factors may affect wheat, reduce productivity and produce stress responses, 31.56% by heat, 26.61% by drought, and 23.38% by salt. Salinity, drought or cold adversely worsens crop yield and quality, limits water absorption, decreases soil osmotic potential (Izadi et al., 2014), induces water deficit, and causes morphological, physiological and biochemical deteriorations, and finally, restricts yield. The salt stress, for example, affects the wheat crop 20-30% [13, 14], cold [15] and drought 100% [16] across the world.

Characterizing genetic resources with efficient screening tests, under *in vivo* and / or *in vitro* salt, cold and drought stresses during germination may identify new salt, cold and drought resistant genotypes [17]. Many studies on single or double biotic or abiotic stresses have been carried out [18-20] but not more than two biotic or abiotic stresses have not yet been fully illuminated. Because combined stresses involve numerous complex physiological, molecular and cellular factors and cause alterations in several plant processes [21].

Statistical analysis for complex characters like we had here require some detailed statistical methods involved. Analysis of variance (ANOVA) was followed by the least significant difference (LSD) comparison (25) in order to test wheat genotypes against salt, cold, and drought in a factorial restricted randomized block design which may serve as the first step in the stress testing procedures (24). Secondly, stress tolerance indices based on the cultivar rankings effectively differentiate tolerant and susceptible entries (24-27). Lastly, multivariate analysis of variance (MANOVA), Wilks' Lambda test, and Pillai's Trace tests, based on the most and the least deteriorated cultivars as well as the most degraded germination characters, comprehensively complete the stress testing through the sample means (26).

All of these abovementioned methods were, in this study, applied during the germination stage in order to investigate germination rate, germination power, coleoptile length, shoot length, root length, shoot to root length ratio, root fresh weight, root dry weight, and root fresh to dry weight ratio of 12 bread and 10 einkorn wheat entries under seven levels of salt, cold and drought stresses.

2. MATERIAL and METHODS

2.1. Plant material

The plant material was 12 bread (*Triticum aestivum* L.) and 10 einkorn wheats (*Triticum monococcum* ssp. *monococcum*) entries. Bread wheat cultivars grown in various wheat growing regions in Turkey were Gerek-79, İkizce-96, Kıraç-66, Kenanbey, Flamura-85, Momtchil, Bayraktar-2000, Tosunbey, Pandas, Pehlivan, Demir-2000 and Gün-91. Einkorn populations from different einkorn growing regions were 1, 2, 4, 5, 6, 9, 10, 11, 14, and 15 (Table 1). The bread wheat cultivar seeds were provided by various agricultural research institutes in Turkey while einkorn population seeds were collected from western Black Sea Region, Turkey (Table 1).

Table 1. Twelve bread and ten einkorn wheat entries tested against seven levels of salt, cold, and drought stresses.

Numbers	Entry	Origin	Stress resistance/ tolerance
1	Gerek-79*	[2]TARI	D / S / C
2	İkizce-96	[1]CRIFC	C
3	Kıraç-66	[2] TARI	D / S / C
4	Kenanbey	[1] CRIFC	C
5	Flamura-85	[3] Thrace ARI	S
6	Momtchil	[3] Thrace ARI	S
7	Bayraktar-2000	[1] CRIFC	D / S / C
8	Tosunbey	[1]CRIFC	C
9	Pandas	[4] EMARI	S
10	Pehlivan	[3]Thrace ARI	S
11	Demir-2000	[1]CRIFC	D / S / C
12	Gün-91	[1]CRIFC	D / S / C
13	Population 1	Bolu, Seben, Haccağız Village	
14	Population 2	Bolu, Seben, Boğaz Region	
15	Population 4	Bolu, Seben, Kavaklı Yazı Village, Field # 1	
16	Population 5	Bolu, Seben, Kavaklı Yazı Village, Field # 2	
17	Population 6	Bolu, Seben, Kavaklı Yazı Village, Field # 3	
18	Population 9	Kastamanonu, İhsangazi, Çatalyazı Village	
19	Population 10	Kastamanonu, İhsangazi, Uzunoğlu District	
20	Population 11	Kastamanonu, İhsangazi, Çay District	
21	Population 14	Kastamanonu, İhsangazi, Center	
22	Population 15	Kastamanonu, İhsangazi, Center	

[1] CRIFC : Central Research Institute for Agricultural Research, Ankara,
[2] TARI : Transitional Zone Agricultural Research Institute, Eskişehir,
[3] Thrace ARI : Thrace Agricultural Research Institute, Edirne,
[4] EMARI : East Meditteranean Agricultural Research Institute, Adana.
* Growth type of bread wheat cultivars; W: Winter; W/F: Winter / Facultative; S: Spring. D: Drought; S: Salt; C: Cold.

2.2. Sterilization

Seeds in all salt, cold and drought tests were first surface-sterilized 30 seconds in 96% ethanol, 15 minutes in 10% NaClO, and rinsed twice in distilled H_2O [22].

2.3. Stress tests

2.3.1. *Salt stress*

Ten seeds in each of three replicates for a given wheat entry were germinated on Whatman number 1 wet filter paper under seven salt (NaCl) levels. The levels were 5 ml doses of 0 (control), 0.05 M, 0.10 M, 0.15 M, 0.20 M 0.25 M and 0.30 M of salt. pH in each petri dish was kept at 5.9 ±1 [22].

2.3.2. *Cold stress*

Three replicates by ten seeds in each wheat entry were germinated on Whatman number 1 wet filter paper under seven cold stress levels of 2, 0, -2, -4, -6 and -8 °C, and the control (23±1 °C). pH was 5.9 ±1 during the tests [22].

2.3.3. *Drought stress*

Ten seeds in all three replicates of each wheat entry were germinated on Whatman number 1 under seven levels of drought stress induced by PEG 6000 of 0.09 M, 0.17 M, 0.25 M, 0.34 M, 0.43 M, 0.51 M and 0.00 M. The pH value was fixed at 5.9 ± 1 [22] during the experiment.

2.4. Germination Tests

The seeds were germinated at 23 ± 1 °C for 8 days (ISTA 2017) and tested *in vitro* under salt, cold, and drought. Each abovementioned stress consisted of seven stress levels. Germination rate, germination power, coleoptile length, shoot length, root length, shoot to root length ratio, root fresh weight, root dry weight, and root fresh to dry weight ratio were measured [23].

2.5. Statistical Analysis

The experiments were set up in a three-replicate (blocks) randomized complete block design (RCBD) with each stress in a factorial restriction [24]. Firstly, Fisher's protected test (F) and the mean separation by the least significant difference [LSD; 25] followed the analyses of variance (ANOVA), which were run in SPSS 21. Secondly, cultivar rank based stress tolerance indices, which were calculated in EXCEL differentiated six tolerant (Gerek-79, Gün-91, İkizce-96, Bayraktar-2000, Pehlivan, and Momtchil; approximately tolerant 25% of all entries) and six susceptible (Population 1, Population-2, Population 4, Population-5, Population 6, and Population 14; approximately susceptible 25% of all entries) wheat entries [24, 26-28] Finally, the sample mean of four the least (Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan) and the most deteriorated (Population 1, Population 4, Population 6, Population 14) wheat entries and germination characters (shoot length, root length, root fresh weight, and root dry weight) were utilized to end up the multivariate analysis of variance (MANOVA), Pillai's Trace, and Wilks' Lambda tests [26].

3. RESULTS and DISCUSSION

Abiotic stresses induced by environmental factors cause serious damages on crop plants including wheat. One or two-way stress studies have mostly been carried out in the literature up to now. In nature, however, stresses influence crop plants in somehow more than two-way combined manners as it we had tested here in this study. Germination is one of the most stress vulnerable growth stages of the crops. Biotic or abiotic factors during germination are so critical since they worsen crop establishment and reduce yield. Salt, drought and cold are the most devastating biotic factors, especially on salt sensitive plants during germination and early seedling stages. The higher the salt concentration in the soil occurs the lower the plants germinates (29). The decreased water intake by osmotic limitations under salt, cold and drought stresses prevents the germination (30).

Here, the most stress destructed germination characters shoot length, root length, root fresh weight, and root dry weight were presented but not the other least degraded ones i.e. germination rate, germination power, coleoptile length, shoot to root length ratio, and root fresh to dry weight ratio. Blocking was effective (Table 2) in this factorially restricted randomized complete block design except for root dry weight under salt and cold stresses. All salt, cold, and drought stress types highly differentiated ($p < 0.01$). Similarly, wheat entries did highly ($p < 0.01$) or just significantly ($p < 0.05$) except root fresh weight under cold and drought. Likewise, stress levels under all three stresses differentiated highly ($p < 0.01$) or just significantly ($p < 0.05$). No stress type by wheat entry interaction was determined.

Table 2. Fisher's protected F value for shoot length, root length, root fresh weight, and root dry weight.

		Shoot length (cm)			Root length (cm)			Root fresh weight (mg)			Root dry weight (mg)		
Sources of variation	D.F.	Salt	Cold	Drought	Salt	Cold	Drought	Salt	Cold	Drought	Salt	Cold	Drought
Blocks	2	6.75 **	12.88*	8.64*	8.87**	4.19**	3.34*	4.08*	4.27**	9.02**	0.42ns	3.02ns	2.97ns
Stress types	153	27.50**	92.16**	56.25**	17.26**	24.69**	44.18**	22.88**	20.86**	56.16**	18.15**	17.34**	39.53**
Entries	21	2.21*	28.89*	1.98*	4.00**	7.16**	5.91**	6.91**	50.72ns	7.68ns	5.22**	5.47**	7.11*
Stress Levels	6	210.13**	602.09*	456.42*	124.36**	159.68**	339.71**	157.65**	138.63**	434.21**	129.64**	219.16**	295.62**
Stress type by entry	44	0.26 ns	1.15ns	0.23 ns	0.16ns	0.45 ns	0.25 ns	0.20s	0.34ns	0.27ns	0.10ns	0.65 ns	0.25 ns
Error	922												

D.F.: Degrees of freedom; * statistically significant at $p > 0.05$; ** statistically significant at $p > 0.05$; ns: non-significant.

3.1. Stress tolerance indices of genotypes under three stress

Stress rank indices differentiated bread and einkorn wheat entries under individual or multi-stresses. Approximately, 25% of all 22 wheat entries were determined tolerant and 25% susceptible under each stress alone or in two-way or three-way combinations [27, 28]. Bread wheat cultivars Bayraktar-2000, Gerek-79, İkizce-96, Demir-2000, Gün-91, Momtchil, and Flamura-85 and einkorn wheat populations 1, 6, 4, 2, 5, and 9 behaved tolerant under various stress combinations. Bread wheat cultivars Momtchil, Gerek-79, Bayraktar-2000 and einkorn populations 5, 6, 1 were the tolerant wheat entries where salt, cold and drought stresses evaluated together (Table 3).

Bread wheat and einkorn entries differed against salt, cold, and drought stresses alone or in combinations. These were einkorn populations 9, 10, 11, 14, 15 and bread wheat cultivars Demir-2000, Pehlivan, İkizce-96, Kıraç-66, Tosunbey, and Bayraktar-2000. Under three stresses, einkorn populations 10, 11, 14, 15 and bread wheat cultivars Tosunbey and Kıraç-66 were susceptible to salt-cold-drought. When two or three stresses were evaluated together Population 10 was the only genotype susceptible to salt-drought and salt-cold-drought. Kıraç-66 was susceptible under the combinations of salt-cold, salt-drought and cold-drought, with stress tolerance indices of 17.17, 14.06, and 14.48, respectively. Population 10 was the most susceptible to salt (20.56), drought (17.11), cold-drought (18.94), and salt-cold-drought (15.07), (Table 3). The most worsened characters were shoot length, root length, root fresh weight, and root dry weight under salt, cold, and drought.

Table 3. Six tolerant and six susceptible bread and einkorn wheat entries selected by their stress tolerance rank indices under one-, two-, and three-way salt, cold, and drought stresses.

Tolerance / Susceptibility	Stress types						
	Salt	Cold	Drought	Salt-Cold	Salt-Drought	Cold-Drought	Salt-Cold-Drought
Tolerant	Bayraktar-2000	Population 6	Kenanbey	Population 6	Bayraktar-2000	Gerek-79	Momtchill
	5.44	2.89	6.67	5.39	4.78	5.78	8.30
	Gerek-79	Population 1	Bayraktar-2000	Population 5	İkizce-96	Population 6	Gerek-79
	5.44	5.44	7.33	6.33	6.17	6.06	8.48
	İkizce-96	Population 4	Gün-91	Population 11	Gerek-79	Population 1	Population 5
	5.89	6.11	7.44	7.50	6.78	7.72	8.85
	Gün-91	Population 2	Momtchil	Flamura-85	Gün-91	Flamura-85	Population 6
	6.00	3.13	5.66	7.89	7.11	8.22	8.89
	Demir-2000	Population 5	Population 9	Population 9	Demir-2000	Population 2	Bayraktar-2000
	6.78	6.56	9.00	8.00	8.00	8.44	9.44
	Momtchil	Momtchil	İkizce-96	Population 4	Kenanbey	Population 5	Population 1
	7.44	7.56	9.00	8.22	8.39	8.78	10.26
Susceptible	Population 4	Demir-2000	Pehlivan	Bayraktar-2000	Population 9	Pandas	Population 11
	14.11	16.33	14.00	15.28	14.06	13.78	12.78
	Population 14	Pehlivan	Flamura-85	Kenanbey	Kıraç-66	İkizce-96	Population 15
	14.56	16.78	14.00	15.39	14.06	14.00	14.26
	Population 15	İkizce-96	Population 14	Kıraç-66	Population 15	Population 14	Tosunbey
	17.11	17.78	14.22	17.17	15.67	14.17	14.26
	Population 9	Kıraç-66	Kıraç-66	Pehlivan	Population 14	Bayraktar-2000	Kıraç-66
	18.44	18.44	16.22	17.39	16.06	14.89	14.48
	Population 11	Tosunbey	Population 11	Demir-2000	Population 11	Pehlivan	Population 14
	18.67	19.11	16.56	17.39	18.11	15.44	14.85
	Population 10	Bayraktar-2000	Population 10	İkizce-96	Population 10	Demir-2000	Population 10
	20.56	19.22	17.11	18.22	18.94	15.67	15.07

The shoot length, root length, root fresh weight, and root dry weight were the worst decreased germination characters. The decrease gradually occurred under salt, cold, and drought stresses (Table 4). These four characters with the least worsened Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan bread wheat cultivars and the most worsened einkorn populations 1, 4, 6, 14 were further chosen for multivariate analysis.

Table 4. The most stress responsive shoot length, root length, root fresh weight, and root dry weight.

Stress levels	Stress Types	Shoot length (cm)	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)
Control	Salt	12.35A	7.70A	76.24A	7.11A
	Cold	14.94A	8.36A	77.46A	9.23A
	Drought	14.08A	8.64AB	87.26 A	7.60A-C
Level 1	Salt	10.00B	5.70AB	65.88AB	6.12AB
	Cold	9.72B	5.55AB	52.72AB	6.95AB
	Drought	12.29B	9.01A	86.41AB	9.90 AB
Level 2	Salt	5.47C	4.00A-C	48.34A-C	4.81A-C
	Cold	8.04BC	4.86A-C	45.31A-C	4.49A-C
	Drought	7.37BC	7.48A-C	68.04A-C	9.96A
Level 3	Salt	2.16D	2.39B-D	32.30A-D	3.52A-D
	Cold	6.59CD	4.45A-D	40.67A-D	3.88A-D
	Drought	0.74 D	4.49A-C	36.87A-C	6.73 A-C
Level 4	Salt	0.50E	1.30B-E	19.36B-E	2.37B-E
	Cold	3.03E	2.38B-E	22.03B-E	2.11B-E
	Drought	0.00DE	1.85 C	13.78 C	2.94 A-C
Level 5	Salt	0.07E	0.75C-F	12.03C-F	1.66C-F
	Cold	1.31EF	1.53B-F	13.68B-F	1.34C-E
	Drought	0.00DE	0.38 C	3.53 C	0.90 C
Level 6	Salt	0.00E	0.00E	5.92C-G	1.00C-G
	Cold	0.04FG	0.46C-G	4.05C-G	0.41C-G
	Drought	0.00DE	0.08 C	0.99 C	0.24 C
LSD and CV%	Salt	2.35; 12%	2.19; 19%	10.36; 12%	0.99:13%
	Cold	5.22; 20%	0.36%8%	24.74:20%	2.28;24%
	Drought	1.79; 11%	3,31;22%	1.05; 11%	0.06; 12%
Decrease (%)	Salt	100.00	100.00	92.24	85.94
	Cold	99.73	94.50	94.77	95.56
	Drought	100.00	99.07	98.87	97.60

The highest 100% decrease under salt stress was in the shoot length and root length while root dry weight (85.94%) and root fresh weight (92.24%) decreased the least, respectively. Decrease in shoot length under cold was 99.73%, in root dry weight was 95.56%, in root fresh weight was 94.77%, and in root length was 94.50%. The highest decrease under cold was in shoot length (100%), followed by root length (99.07), root fresh weight (98.87%), and root dry weight (97.60%), respectively. A serious decrease was observed in shoot length at level 3 under salt and drought (i.e., 0.10 M salt and 0.17 M PEG 6000 drought). Level 6 stress (0.25 M, -6 °C, 0.43 M PEG 6000) dramatically decreased all four characters, at least up, to a percentage of 94.50% (Table 4). Further studies to evaluate the effect of stresses in combinations may help to comprehend the consequences of stresses on wheat genotypes.

3.2. Four the best bread wheat and four the worst einkorn wheat genotypes under three stress

Bread wheat cultivars Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan were destructed the least, tolerant in other words, under salt, cold and drought stresses. Einkorn populations 1, 4, 6 and 14 worsened the most. These eight entries were selected for multivariate analysis as mentioned below. Bread wheat cultivars had, in general, higher values for shoot length, root length, root fresh weight, and root dry weight than einkorn populations (Table 5; Figure 2).

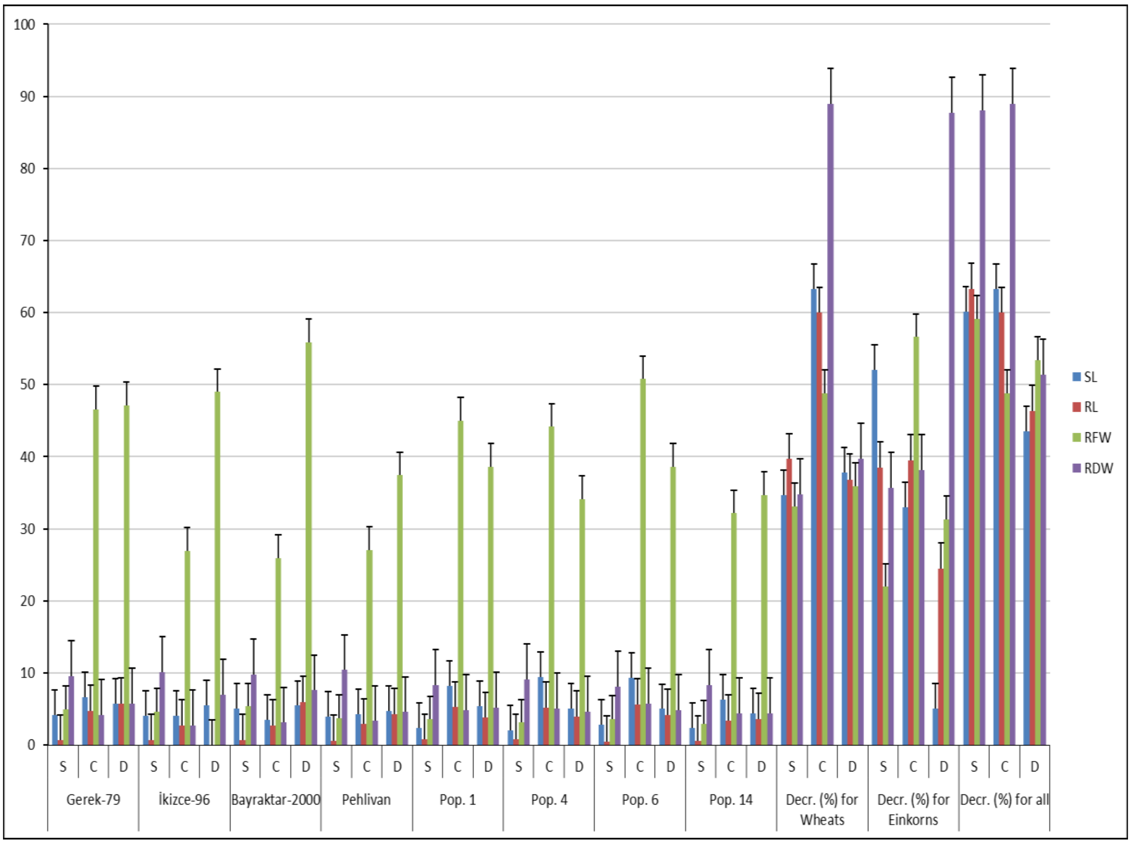

Figure 1. Four most worsened germination characters (shoot length, root length, root fresh weight, root dry weight), four einkorn populations (1, 4, 6 and 14), and four least worsened bread wheat cultivars (Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan) under salt, cold, and drought together.

Shoot length, root length, root fresh weight, and root dry weight in four bread wheat cultivars under salt stress decreased by 22.20%, 20.00%, 29.98% and 8.07%, respectively. The percentage decreases in the same characters were 40.80%, 43.09%, 42.15%, and 25.59%, under cold and 17.42%; 25.04%, 23.51% and 39.73%, under drought stress, respectively (Table 5). The same characters were also worsened in four einkorn populations, 27.30%, 32.91%, 16.24% and 8.56%, under salt stress; 32.94%, 39.85%, 36.68% and 24.19%, under cold stress; and 19.23%, 12.41%, 11.65% and 15.35%, under drought stress, respectively. The highest decreases in entries were 59.72%, 32.91%, 44.32%, and 21.63% under salt; 63.25%, 51.77%, 49.11% and 42.14% under cold stress; and 23.17%, 34.69%, 38.88% and 41.97% under drought stress, respectively (Figure 1).

Table 5. Four most worsened germination characters (shoot length, root length, root fresh weight, root dry weight), four einkorn populations (1, 4, 6 and 14), and four least worsened bread wheat cultivars (Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan) under salt, cold, and drought together, which were used in multivariate analyses.

Genotypes	Stress type	Shoot length (cm)	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)
Gerek-79	Salt	4.20ab	0.66a-c	5.00ab	9.56b-l
	Cold	6.69a-k	4.78a-g	4.65a-d	4.18b-l
	Drought	5.74a-c	5.79ab	4.71a-h	5.79c-h
İkizce-96	Salt	4.11a-c	0.75a-e	4.65a-d	10.10b-h
	Cold	4.02g-s	2.71j-t	2.69i-t	2.70m-u
	Drought	5.56a-d	5.26a-I	4.89a-g	6.99a-c
Bayraktar-2000	Salt	5.09a	0.74a-e	5.37a	9.78b-I
	Cold	3.48g-u	2.72j-t	2.58j-u	3.11g-t
	Drought	5.47a-f	5.97 a	5.58ab	7.60 ab
Pehlivan	Salt	3.96a-f	0.60a-f	3.76c-l	10.40a-e
	Cold	4.25g-r	2.90j-r	2.70i-s	3.35e-o
	Drought	4.74a-o	4.34c-k	3.74f-p	4.58f-q
Population 1	Salt	2.40h-q	0.78a-l	3.57c-n	8.30i-s
	Cold	8.20a-e	5.28a-b	4.5.0a-e	4.88a-d
	Drought	5.46a-f	3.82h-p	3.85d-m	5.21d-l
Population 4	Salt	2.05j-s	0.79ao	3.11g-p	9.11b-m
	Cold	9.47a	5.17a-c	4.41a-h	5.07ab
	Drought	5.12a-k	3.96f-n	3.41h-r	4.66e-p
Population 6	Salt	2.82c-n	0.53a-m	3.61c-m	8.15i-u
	Cold	9.33a-b	5.62a	5.07a	5.79a
	Drought	5.02a-l	4.19e-m	3.86d-e	4.88d-n
Population 14	Salt	2.36h-r	0.54a-k	2.99h-q	8.33i-q
	Cold	6.35a-l	3.38g-p	3.21e-n	4.38b-g
	Drought	4.41a-q	3.67j-s	3.46h-q	4.41g-r
LSD and CV%	Salt	0.85; 12%	0.06:11%	0.37; 11%	0.84; 16%
	Cold	0.14; 18%	0.09%12%	0.42;14%	0.89%18%
	Drought	0.27; 9%	0.18;14%	0.58: 15%	0.61
Decrease in wheat (%)	Salt	22.20	20.00	29.98	8.07
	Cold	40.80	43.09	42.15	25.59
	Drought	17.42	25.04	23.51	39.73
Decrease in einkorn (%)	Salt	27.30	32.91	16.24	8.56
	Cold	32.94	39.85	36.68	24.35
	Drought	19.23	12.41	11.65	15.35
Decrease in all (%)	Salt	59.72	32.91	44.32	21.63
	Cold	63.25	51.77	49.11	42.14
	Drought	23.17	34.69	38.88	41.97

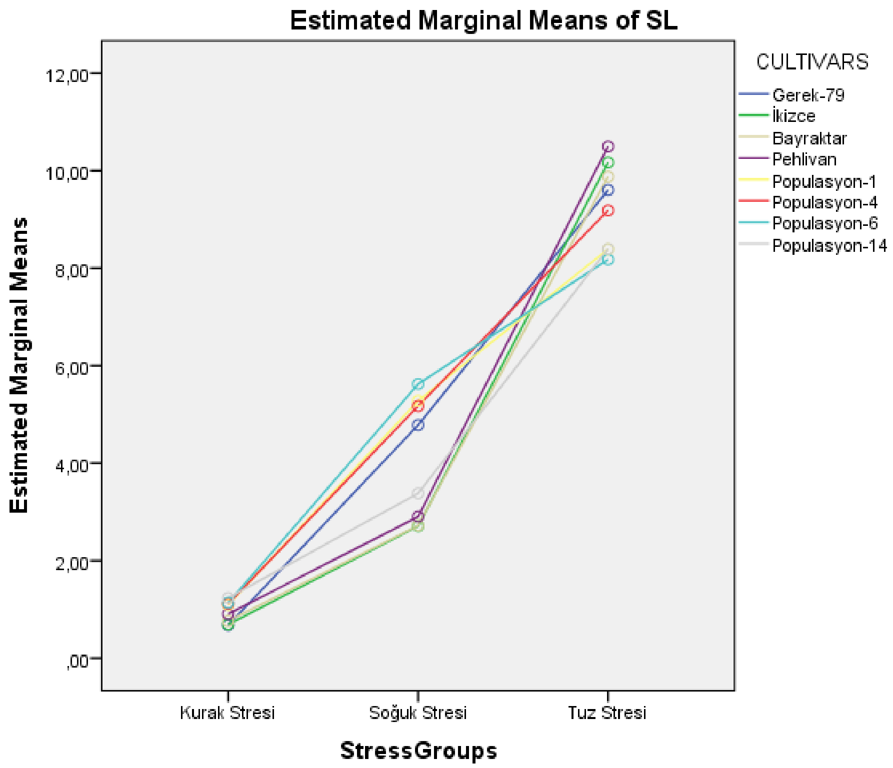

Figure 2. The shoot lengths of four most deteriorated einkorn populations (1, 4, 6 and 14), and four least worsened bread wheat cultivars (Gerek-79, İkizce-96, Bayraktar-2000 and Pehlivan) under salt, cold, and drought together, which were all three used in multivariate analyses.

3.3. Shoot length, root length, root fresh weight, and root dry weight of the least responsive four bread wheat and the most responsive four einkorn genotypes under salt, cold, and drought

A multivariate analysis of variance (MANOVA) was performed on germination characters, bread wheat cultivars, einkorn populations, and salt, cold, and drought stresses. Furthermore, Pillai's Trace and Wilks' Lambda tests were applied on data [26].

All salt, cold and drought stresses, wheat entries, and stress by wheat entry interaction(s) significantly ($P < 0.01$) affected the independent variables (Table 7). Partial Eta squared indicated a strong effect on all three stresses and a weak effect on wheat entries and stress by wheat entry interactions. Pillai's Trace and Wilks' Lambda were highly significant ($p < 0.01$) for stress types of salt, cold, drought, wheat entries, and stress type by entry interaction. The between-subject effects were also significant for all stresses and stress by wheat entry interactions ($P<0.05$), but insignificant for wheat entries. Partial Eta squared had the highest effect on the shoot length and the lowest on the root dry weight. Stress by wheat entry interactions affected shoot length the most (Table 6).

Table 6. MANOVA for four most worsened germination characters (shoot length, root length, root fresh weight, root dry weight), four einkorn populations (1, 4, 6 and 14), and four least worsened bread wheat cultivars (Gerek-79, İkizce-96, Bayraktar-2000, and Pehlivan) under salt, cold, and drought together.

Source	Dependent Variable	df	F	Partial Eta Squared
Stress types	SL	2	325.094**	0.639
	RL	2	21.512**	0.105
	RDW	2	10.890**	0.056
	RFW	2	42.442**	0.187
Cultivars	SL	7	0.571	0.011
	RL	7	0.816	0.015
	RDW	7	2.008	0.037
	RFW	7	0.919	0.017
Stress types* Cultivars	SL	14	3.633**	0.121
	RL	14	2.892**	0.099
	RDW	14	2.279**	0.080
	RFW	14	2.784**	.096
Error	SL	368		
	RL	368		
	RDW	368		
	RFW	368		

Table 7. Pillai’s trace and Wilks’ lambda multivariate tests for intercept + stress types + cultivars + Stress types * cultivars.

Effect	
Intercept	Pillai's Trace**
	Wilks' Lambda**
Stress types	Pillai's Trace**
	Wilks' Lambda**
Cultivars	Pillai's Trace**
	Wilks' Lambda**
Stress types * Cultivars	Pillai's Trace**
	Wilks' Lambda**

**The tests are significant at the 0.01 level.
**The mean difference is significant at the 0.01 level.

All shoot length, root length, root dry weight, and root fresh weight differed between drought, cold, and salt pairs except root dry weight and root fresh weight between cold-salt stress pairs (Table 8). Pillai’s Trace and Wilks’ lambda were highly significant for shoot length, root length, root dry weight under slat, cold, and drought (Table 9).

Table 8. Multiple comparisons of shoot length, root length, root dry weight, and root fresh weight under salt, cold, and drought.

Dependent Variable	Stress types (I)	Stress types(J)	Mean Difference (I-J)	Std. Error
SL	Drought	Cold	-3.1199**	0.37594
		Salt	-8.3353**	0.37594
	Cold	Salt	-5.2155**	0.26583
RL	Drought	Cold	2.2706**	0.45295
		Salt	2.9703**	0.45295
	Cold	Salt	0.6997	0.32028
RDW	Drought	Cold	1.9190**	0.41178
		Salt	1.5070**	0.41178
	Cold	Salt	-0.4120	0.29117
RFW	Drought	Cold	32.7163**	3.82036
		Salt	33.1244**	3.82036
	Cold	Salt	-0.4081	2.70140

Fresh Weight. Based on observed means. The error term is Mean Square (Error) = 612,996.
*The mean difference is significant at 0.05 level and ** at the 0.01 level.

Table 9. Pillai's trace and Wilks' lambda multivariate tests for shoot length, root length, root dry weight, and root fresh weight under salt, cold, and drought.

	Value	F
Pillai's trace	1.451	241.710**
Wilks' lambda	0.044	343.948**

**The mean difference is significant at the 0.01 level.

Among four bread and einkorn multi-trait analyzed wheat entries, Gerek-79 and Population 14 significantly differed ($P < 0.05$) for root fresh weight. Gerek-79 - Population 14 and Pehlivan – Gerek-79 highly but not significantly differed (Table 10). Pillai's trace and Wilks' lambda tests also highly significant for these cultivar pairs in shoot length, root length, root dry weight, and root fresh weight (Table 11).

Many studies about the effect of stress on wheat are conducted individually; however, biotic and/or abiotic stresses attack wheat together. Recently, some studies have been undertaken by a two- or two-way biotic-biotic, biotic-abiotic stress study approaches. Thus, it seemed appropriate to engage in this current study on the individual effects of salt, cold, and drought stresses in a combined manner on wheat.

Table 10. Multiple comparisons of shoot length, root length, root dry weight, and root fresh weight observed with bread and einkorn wheats under salt, cold, and drought.

Dependent Variable	(I) CULTIVARS	(J) CULTIVARS	Mean Difference (I-J)	Std. Error	Sig.
RL	Gerek-79	Populasyon 14	1.5549	0.59305	0.151
RDW	Pehlivan	Gerek-79	-1.4550	0.53914	0.126
RFW	Gerek-79	Populasyon 14	15.9906*	5.00202	0.032

Based on observed means.

Table 11. Pillai's trace and Wilks' lambda multivariate tests for shoot length, root length, root dry weight, and root fresh weight under salt, cold, and drought.

	Value	F
Pillai's trace	0.143	1.956**
Wilks' lambda	0.861	1.995**

**The mean difference is significant at the 0.01 level.

Plant germination decreases when salt concentration in the soil increases [29]. Reduced intake of water due to osmotic limitations and Na and Cl ion toxicity worsen germination characteristics [30]. Species and genotypes differ in their reactions to individual and most likely combined biotic stresses; thus, a detailed exploration is needed. Einkorn seemed more tolerant environmental stresses than bread wheats in the previous studies [19, 20, 31]. Mahmoodabad [32] reported the lowest germination rate at 2°C, with some differences among bread wheat cultivars of Gaspard, Sardari, Cascogen, Bezostaja-1, and MV-17. Similarly, the root length was the same in Gaspard, Bezostaya-1 and Cascogen as Bezostaya 1, which has been frequently reported to have the longest shoot and root lengths at the lower temperatures. The characteristics of cultivars under different stresses differed during the germination and early seedling stages in some previous studies. Chilling temperatures between 0-12°C significantly delayed the onset and reduced the germination rate in the cultivated plants [33, 34]. Cold also led to poor seed germination, uneven stand establishment, and poor crop performance [33]. Most commercial crop cultivars have been highly sensitive to cold during seed germination despite available genetic variation within and between related wild species [33, 34].

Among abiotic stresses, drought widely spreads around the world, upsetting seed germination and seedling growth, and resulting in a poor establishment and a decreased seedling growth. Poor establishment, in turn, decreases weed competitiveness, shades soil surface, restricts light interception, declines early season growth, and reduces yield. Similarly, shoot length, root length, root fresh weight, and root dry weight gradually decreased under salt, cold and drought stresses in this study (Table 4). This was parallel with the results of Mahmoodzadeh [35] who obtained significant shoot and root length differences in bread wheat cultivars. All four: shoot length, root length, root fresh weight and root dry weight characters similarly followed the same trend against salt, cold, and drought stresses in the present study.

Stress tolerance indices [24, 27, 28] successfully differentiated bread wheat cultivars and einkorn populations under individual or combined salt, cold, and drought stresses (Table 3). Bayraktar-2000, Gerek-79, İkizce-96, Demir-2000, Gün-91, Momtchil, and Flamura-85 bread wheat cultivars and 1, 6, 4, 2, 5, and 9 einkorn wheat populations were tolerant under various stress combinations. Bread wheat cultivars Momtchil, Gerek-79, and Bayraktar-2000 and einkorn populations 5, 6, and 1 were the tolerant wheat entries when salt, cold and drought stresses evaluated together (Table 3). The values by Ali and El-Sadak [36] who compared the stress susceptibility and stress tolerance indices, mean and geometric mean productivity were not parallel with the stress indices here. When multivariate analysis variance, partial Eta squared values, and Pillai's trace and Wilks' Lambda tests were considered partial Eta revealed a strong effect for three stresses and a weak effect for wheat entries and stress by wheat interaction (Table 7) similarly to the stress tolerance indices in this study.

4. CONCLUSION

Previous studies have mostly investigated individual stresses and their effects on plants because of an easy testing and a statistical analysis. However, plants are simultaneously exposed to more than one stress in their real-life cycles. Therefore, studying stresses in a combined approach by multivariate analysis methods is expected to provide a better understanding of the stresses on wheat. This study, therefore, presented a novel approach and produced valuable results about the effects of three stresses on wheat and einkorn entries in a combined understanding.

Orcid

Nusret ZENCIRCİ https://orcid.org/0000-0003-3460-7575
Hakan ULUKAN https://orcid.org/0000-0003-0203-6851
Bülent ORDU https://orcid.org/0000-0002-3103-9325
Didem ASLAN https://orcid.org/0000-0001-6747-2852
Hakan Tahiri MUTLU https://orcid.org/0000-0002-8964-2696
Mehmet ÖRGEÇ https://orcid.org/0000-0002-9446-7538

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