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Diallel analysis of salinity tolerance at germination and the early seedling stage in bread wheat (*Triticum aestivum* L.)

Ekmeklik buğdayın (Triticum aestivum L.*) çimlenme ve fide döneminde tuzluluk stresine toleransının diallel analizi*

Ferhat KIZILGEÇİ[1]

[1]Department of Plant and Animal Production, Kızıltepe Vocational School, Mardin Artuklu University, Mardin, Turkey

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Address for Correspondence:
Ferhat KIZILGEÇİ
e-mail:
ferhatkizilgeci@artuklu.edu.tr

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ABSTRACT

Salt stress is one of the most crucial abiotic stress factors and affects about 20 % of the world's irrigated land. The present study was conducted to evaluate the combining effects of salinity stress response at the early seedling stage, (root length, coleoptile length, shoot length, germination rate and germination vigour) using four parents and their 4 x 4 full-diallel crosses of bread wheat. Parents and their F_2 hybrids were assessed in the laboratory under salinity stress induced by sodium chloride (NaCl) with two treatments (0 control, 100 mMol). General Combining Ability (GCA) mean squares were highly significant for all traits studied. The GCA: SCA (Specific Combining Ability) rate was greater than the unit for all the traits studied and additive genes indicated a higher contribution than non-additive genes in the inheritance of these characters. GCA effects showed that '84CZT04' was the desirable general combiner for coleoptile length, root length and shoot length under salinity stress condition. Estimates of the SCA effects of crosses demonstrated that 1 × 4 and 3 × 1 could be regarded as the most desirable cross-combination for root and shoot length at both conditions.

Key Words: Combining ability, Full diallel, Segregation population, Salinity stress, Coleoptile

ÖZ

Tuz stresi en önemli abiyotik stres faktörlerinden biridir ve dünyadaki sulanan alanın yaklaşık olarak % 20'sini etkilemektedir. Bu çalışma, dört ekmeklik buğday ebeveyni ve bunların 4 x 4 tam diallel melezlerinin erken fide aşamasında (kök uzunluğu, koleoptil uzunluğu, sürgün uzunluğu, çimlenme hızı ve çimlenme gücü) tuzluluk stresine tepkisini değerlendirmek amacıyla yürütülmüştür. Ebeveynler ve F2 hibridlerine sodyum klorür (NaCl) uygulanarak (0 kontrol, 100 mMol) laboratuvar koşullarında değerlendirilmiştir. Genel Uyum Yeteneğinin (GUY) kareler ortalaması incelenen tüm özellikler için oldukça yüksek düzeyde olumlu ve önemli bulunmuştur. GUY:ÖUY (Özel Uyum Yeteneği) oranı, incelenen tüm özellikler için bir den büyük bulunmuş bu da eklemeli gen etkisinin eklemeli olmayan gen etkisinden daha yüksek bir katkı sağladığını göstermiştir. Stres koşulları altında '84CZT04' çeşidi GUY etkileri yönünden koleoptil uzunluğu, kök uzunluğu ve sürgün uzunluğu özellikleri için istenen kombiner olduğunu göstermiştir. Melezlerin ÖUY etkilerine ilişkin tahminlerde ise, her iki koşulda 1 × 4 ve 3 × 1'in kök ve sürgün uzunluğu özellikleri için ümitvar melez kombinasyonlar olduğu görülmüştür.

Anahtar Kelimeler: Kombinasyon yeteneği, Tam diallel, Açılma kuşakları, Tuzluluk stresi, Koleoptil

Introduction

Environmental stresses unfavourably influence the growth and production of plants, especially those that are sensitive to salinity. These stresses cause severe changes in plant growth, physiology and metabolism, threatening plant farming around the world (Lunde et al., 2007; Islam et al., 2011). The losses in wheat production are especially due to abiotic stress conditions, such as drought, heat stress and salinity rather than to biotic factors (Abhinandan et al., 2018). Abiotic stress estimated to account for over 50% of yield losses worldwide (Alcázar et al., 2006) Salinity is one of the limiting factors for all plant production. Salty soils are quite common around the world, especially in arid and semi-arid climate regions. Approximately 20 % of the world's irrigable agricultural land is affected by salt (FAO, 2018). However, Wang et al. (2003) predicted that by 2050, 50 % of agricultural land will be affected by salinity. Salt stress is usually caused by sodium salts and NaCl salts in particular.

At early growth stages such as germination and seedlings stand embellishment of major plants is sensitive and finally reduced the final yield under salinity conditions. Therefore, high germination rate and early vigorous growth of plant should be preferred to grow under saline condition (Kizilgeci and Yildirim, 2014). Germination is a significant stage in the life cycle of plants particularly under saline environments as it is determine the degree of crop establishment (Vardar et al., 2014).

Bread wheat (*Triticum aestivum* L.) is the world's leading commonly grown food cereal because of its wider adaptability and nutritional value than other cereals (Yassin et al., 2019). It may be more sensitive to salinity at specific developmental stages, i.e. germination and seedling stage (Yildirim et al., 2015); tilling and stem elongation stage (Mass et al., 1996; Ding et al., 2018); and anthesis and grain filling stage (Francois et al., 1994; Abro et al., 2009; Zheng et al., 2009). Breeders generally unwilling to use of seedling traits for the selection of varieties, given that the selection for yield mostly captures the optimal characteristics of the environment. However, the evaluation of seedling traits may accelerate the selection process for wheat breeding. Early seedling growth characteristics, such as coleoptile, root length and shoot height, could be used as preselection criteria to screening wheat genotypes for salinity tolerance.

The diallel analysis is one of the biometrical techniques that have been used extensively to gain combining abilities information in various crops (Iqbal, 2004). Diallel crosses as mating models are often used to investigate the genetic features of inbred lines in plant breeding studies. Plant breeders often need overall information on the average performance of individual inbreed lines in crosses, known as the general combine ability, for the consequent selection of the best ones for further breeding. Diallel crossing techniques are used for this purpose (Ghosh and Das, 2003) Diallel mating models supply useful genetic information for breeders, such as general combination ability GCA and specific combination ability SCA, to help them develop appropriate breeding and selection strategies (Makumbi, 2005; Zhang et al., 2005; Kizilgeci, 2020). The present study aims to assess the combining effects at early seedling salinity stress response (root length, coleoptile length, shoot length, germination rate and germination vigour) using four parents full-diallel bread wheat crosses.

Materials and Methods

Genetic material, crosses and salinity tolerance evaluation

Four genotypes of bread wheat containing Ballatilla (1), Seri 82 (2), 84CZT04 (3) and HP1744 (4) were used as parents in the current study. Salinity tolerance assessment of four spring wheat cultivars and their full 4 × 4 diallel F2 hybrids was conducted in the laboratory of the department of field crops at Sirnak University in 2017. Sixty seeds of each of the four parents and their F_2 population (total 16 genotypes) were surface sterilized in 10% sodium hypochlorite solution for 10 minutes and then carefully washed three times thoroughly with distilled water to remove the sterilizing extract. Two different concentrations of NaCl, 0 and 100 mMol were used. Twenty seeds of each genotype in 10 ml salt solution were germinated in each Petri-dish (120 mm × 20 mm) with two layers of Whatman no.1 filter paper.

All Petri-dishes were placed in the growing chamber at 24°C for 8 days with 18 hours light/6 hours dark photoperiod. In each of these treatments, a randomized complete design (RCD) with three replications was performed and placed on different shelves in the growth room. The number of germinated seeds on the 4th day was referred to as the germination rate (GR). The percentage of germinated seeds on the 8th day was referred to as the germination vigour (GV). The coleoptile length, root length and shoot height were recorded from ten randomly selected seedlings for each treatment of each replication on 8th day.

Statistical analysis

The Diallel analysis was carried on by a SAS program (1998) with genotype and treatment as fixed effects, in accordance with the Griffing (1956) Method 1 (including parents and reciprocals). Multiple comparisons of means of data between among different salinity treatments with genotypes were performed LSD test at 5% significance level.

Results and Discussion

Analysis of variance and mean performance of salinity tolerance traits

Variances estimates for general combining ability (GCA) and specific combining ability (SCA) of the F2 diallel crosses of bread wheat for combined data under non-salinity and salinity stress are given in Table 1. The differences between genotypes were highly significant for all studied characters. The significant differences were observed for all traits, except the coleoptiles length under non-salinity and salinity stress condition. Genotype × Treatment interactions was showed significant differences for root length, shoot length and germination vigour. The CV of the root length was found highly due to increased genetic variation. The effects of GCA were significant for all traits. The variances of SCA were significant for root length, shoot length and germination rate. GCA × T interaction was significant for coleoptile length, root length and germination vigour, while the SCA × T interaction was significant for root length. Reciprocal effect (REC) was significant for the root length, shoot height and germination vigour. Differences for these traits may have occurred due to cytoplasmic effects. REC × T interaction had a highly significant difference for root length, shoot length. The general combining ability (GCA) effect was significantly higher than the specific combining ability (SCA) effects for all features. The GCA:SCA values were determined as 28.1 for coleoptile length, 2.1 for root length, 1.4 for shoot length, 1.8 for germination rate and 5.5 for germination vigour. This confirmed the large contribution of additive gene action in the inheritance of these characters. Muralia and Sastry (2001) reported inherited additive and non-additive gene effects in control of wheat salinity tolerance at the seedling stage.

Table 1. Mean squares due to general (GCA) and specific (SCA) combining ability and their interactions for studied seedling stage traits in F2 diallel crosses under low and high salinity

Source	DF	Coleoptile length	Root Length	Shoot length	GR	GV
Treatment (T)	1	0.131	1116.905***	542.896***	5221.500***	11008.167***
Replication	4	0.032	0.539	0.207	12.500	39.042
Genotype (G)	15	0.172**	17.501***	11.064***	266.300**	114.567**
G×T	15	0.094	12.370***	3.660**	86.211	81.144*
GCA	3	0.594***	31.029***	14.409***	445.667**	271.500**
SCA	6	0.021	14.512***	10.173***	244.694*	49.056
GCA×T	3	0.171*	14.005**	2.373	61.667	214.611**
SCA×T	6	0.136	13.898**	2.823	55.806	47.722
REC	6	0.113	13.726**	10.282***	198.222	101.611*
REC×T	6	0.012	10.024**	5.139**	128.889	47.833
Error	60	0.060	2.451	1.326	96.589	41.931
CV		9.479	23.325	14.138	17.433	7.705
GCA/SCA		28.1	2.1	1.4	1.8	5.5

*, ** and *** Significance at 5%, 1% and 0.1% levels, respectively. GR:germination rate, GV:germination vigour

Evaluation of mean performances of parents and their F_2 hybrids

The mean value of examined characters for parent and F_2 hybrids are shown in Table 2. Mean value for all characteristics under salinity stress were considerably lower than those under non-stressed conditions. When parents' features are examined, coleoptile length ranged from 2.26 cm to 2.90 cm in control and from 2.15 cm to 3.08 cm in salinity stress condition. The root length varied from 8.55 cm to 16.57 cm under control condition and from 2.52 cm to 4.24 cm under salinity stress. The shoot length varied between 10.07-12.19 cm in non-salinity and between 3.6-7.65 cm in salinity treatment. GR ranged from 50.7% to 73.3% in control and from 30.7% to 61.3% in salt condition. GV varied from 86.7% to 94.7% in control and from 57.3% to 80% in salinity treatment.

The hybrid values ranged from 2.37 cm (2 × 1) to 3.03 cm (3 × 1) in control and 2.29 cm (2 × 4) to 2.86 cm (4 × 3) for coleoptiles length in salinity stress, 3.87 cm (3 × 1) to 13.40 cm (3 × 2) in control and 2.12cm (2 × 3) to 4.77 cm (4 × 1) in salinity stress for root length, 6.26 cm (3 × 1) to 12.36 cm (3 × 4) in control and 3.21cm (1 × 2) to 7.79 cm (4 × 1) in salt condition for shoot length, 50.7% (3 × 1) to 77.3 (1 × 2) in control and 44.0 % (3 × 1) to 56.0 % (1 × 2) in salinity stress for GR, 92% (1 × 3) to 100% (2 × 4) in control and 66.6% (1 × 3) to 85.4% cm (2 × 4) in salinity stress for GV.

Coleoptile length was increased under stress conditions in some genotypes. The highest increase was seen in '84CZT04' (15.8 %). Similar to our research, Kizilgeci et al. (2010) determined the highest coleoptile length in 100 mMol salt treatment. 'HP1744' was the most affected genotype in terms of root length (81%) and the shoot length (65%) in salinity treatment compared to control, while 3 × 1 (11%) was less affected. Growth parameters that were analyzed (root and shoot length) showed that 100 mMol NaCl treatment caused stress at genotypes examined. However, based on our data, 3 x 1 was also found to be less affected than other genotypes, indicating that 3 x 1 is more salinity tolerant than other genotypes. Many researchers reported that in the early seedling period, salinity stress has negative effects on root length, seedling length, germination rate and germination vigour (Kizilgeci et al., 2010; Bouthour et al., 2015; Yildirim et al., 2015; Oral et al., 2019; Kizilgeci et al., 2020).

Ekmekci et al. (2005), with an increased salt concentration in the growing medium, plant water intake becomes difficult. An increase in osmotic pressure prevents plant roots from getting water (Ayyıldız, 1990). The first symptom of plants not getting enough water is leaf area decrease.

Estimates of general and specific combining ability effects

The effects of general combining ability for parents, specific combining ability for diallel crosses and reciprocal combining ability for reciprocal hybrids were estimated and represented in Table 3.

'84CZT04' gave maximum positive GCA effect values for coleoptile length, root length and shoot height at both conditions. '84CZT04' was found to be best combiner for coleoptile length, root length and shoot length. 'HP1744' recorded the highest positive for germination vigour under salinity stress. 'Ballatilla' recorded the highest negative GCA effect value for root length; shoot height, germination rate and germination vigour.

The cross 1 × 4 gave maximum positive SCA effect values for root length and shoot length at both conditions, while the cross 3 × 4 maximum positive SCA effect values for root length at salinity stress. The cross 1 × 3 exhibited maximum negative effect for root and shoot length at both conditions, while the cross 3 × 1 showed maximum positive effect.

The reciprocal cross 3 x 1 recorded maximum positive effect for root length, shoot height and germination rate at non-salinity stress. The cross 4 x 2 recorded maximum negative effect value root length and shoot height and maximum positive value for germination vigour under both conditions. Griffing (1956) suggested that reciprocal effects can be used to identify variability due to maternal effects and sex-linked genes.

The previous studies were presented signified to positive and negative reciprocal effect, indicating the presence of maternal effect (Mahmood, 2010; Mohammad, 2012). Rebetzke et al. (2004) noted that coleoptile length had high heritability and additive gene action. Varieties with longer coleoptiles commonly have improved rates and vigour and produce more plants (Rebetzke et al., 2007)

Table 2. The average value of all characteristics for parents, F2 diallel crosses and reciprocal crosses of bread wheat under salinity and non salinity stress conditions

Parents/ Hybrids	Coleoptile length (cm)			Root length(cm)			Shoot length(cm)			Germination Rate (%)			Germination vigour (%)		
	C	S	M	C	S	M	C	S	M	C	S	M	C	S	M
1	2.58	2.31	2.45	9.54	2.81	6.17	10.07	5.12	7.59	50.7	30.7	40.7	94.7	57.3	76.0
2	2.26	2.40	2.33	8.55	2.52	5.53	10.66	6.71	8.68	73.3	61.3	67.3	96.0	77.3	86.7
3	2.66	3.08	2.87	16.57	4.24	10.40	12.19	7.65	9.92	61.3	49.3	55.3	86.7	72.0	79.3
4	2.90	2.15	2.53	11.68	2.16	6.92	10.24	3.60	6.92	60.0	60.0	60.0	94.0	80.0	87.0
1x2	2.53	2.57	2.55	6.11	2.92	4.51	8.41	3.21	5.81	77.3	56.0	66.7	94.0	66.7	80.3
1x3	2.66	2.59	2.62	9.43	2.82	6.13	10.52	6.04	8.28	69.3	46.7	58.0	92.0	66.6	79.3
1x4	2.56	2.45	2.50	11.01	2.57	6.79	11.74	4.30	8.02	72.0	46.7	59.3	96.0	70.7	83.3
2x1	2.37	2.41	2.39	8.97	3.13	6.05	10.29	4.13	7.21	64.0	49.3	56.7	94.7	73.3	84.0
2x3	2.74	2.51	2.62	11.11	2.12	6.61	10.55	4.39	7.47	66.7	49.3	58.0	94.7	70.7	82.7
2x4	2.52	2.29	2.40	7.34	2.57	4.95	9.48	4.55	7.02	68.0	53.3	60.7	100.0	85.4	92.7
3x1	3.03	2.80	2.91	3.87	3.44	3.66	6.26	5.56	5.91	50.7	44.0	47.3	96.0	72.0	84.0
3x2	2.72	2.70	2.71	13.40	3.76	8.58	11.37	7.35	9.36	62.7	46.7	54.7	97.3	69.3	83.3
3x4	2.51	2.60	2.56	12.98	4.51	8.75	12.36	7.23	9.79	58.7	48.0	53.3	96.0	80.0	88.0
4x1	2.59	2.58	2.58	10.25	4.77	7.51	10.68	7.79	9.24	57.3	52.0	54.7	96.0	80.0	88.0
4x2	2.63	2.53	2.58	12.20	4.12	8.16	11.94	7.63	9.78	54.7	45.3	50.0	94.7	66.7	80.7
4x3	2.79	2.86	2.83	8.97	4.37	6.67	11.58	6.98	9.28	73.3	45.3	59.3	93.3	85.3	89.3
Mean Parent	2.60	2.49	2.54	11.58	2.93	7.26	10.79	5.77	8.28	61.3	50.3	55.8	92.8	71.7	82.3
Mean Hybrid	2.64	2.58	2.61	9.64	3.42	6.53	10.43	5.76	8.10	64.6	48.6	56.6	95.4	73.9	84.6
LSD (5%)	ns	0.30	0.28	3.97	1.76	2.10	1.94	1.89	1.31	ns	ns	11.2	ns	12.9	7.5

ns: non-significant, Ballatilla (1), Seri 82 (2), 84CZT04 (3), HP1744 (4), C:Control, S:Salt, M: Means

Table 3. Effects of general (GCA) and specific combining ability (SCA) for parents, F2 diallel crosses and reciprocal crosses (REC)

	Coleoptile length			Root length			Shoot length			Germination rate			Germination vigour		
	C	S	M	C	S	M	C	S	M	C	S	M	C	S	M
								GCA							
1	-0.02	-0.05	-0.03	-1.53**	-0.14	-0.84***	-0.77*	-0.61**	-0.69***	-2.25	-4.50**	-3.38**	0.00	-5.33**	-2.67**
2	-0.13*	-0.07**	-0.10*	-0.60	-0.34*	-0.47**	-0.10	-0.18	-0.14	3.75	3.83*	3.79**	1.17	0.00	0.58
3	0.09	0.22***	0.16***	1.49**	0.39**	0.94**	0.35	0.84**	0.60**	-0.75	-1.67	-1.21	-1.92*	0.17	-0.88
4	0.05	-0.10**	-0.03	0.64	0.10	0.37	0.51*	-0.06	0.23	-0.75	2.33	0.79	0.75	5.17***	2.96**
								SCA							
1×2	-0.04	0.06	0.01	-0.46	0.21	-0.12	-0.30**	-1.31	-0.80**	5.42	4.33	4.88*	-1.58	2.00	0.21
1×3	0.14	-0.04	0.05	-3.43***	-0.41	-1.92***	-1.72***	-0.20	-0.96**	-0.75	2.50	0.88	1.17	1.17	1.17
2×3	0.13	-0.10	0.02	1.24	-0.40	0.42	0.18	-0.56	-0.19	-2.08	-3.17	-2.63	2.00	-3.50	-0.75
1×4	-0.09	0.11	0.01	1.40*	0.41	0.91*	0.94*	0.94*	0.94**	3.92	2.50	3.21	0.50	2.17	1.33
2×4	0.03	0.03	0.03	-0.40	0.28	-0.06	-0.22	0.56	0.17	-5.42	-5.83	-5.63*	0.67	-2.50	-0.92
3×4	-0.12	0.06	-0.03	-1.28	0.65*	-0.31	0.58	0.55	0.57*	3.75	-3.00	0.38	1.08	4.00	2.54
								REC							
2×1	0.08	0.08	0.08	-1.43	-0.11	-0.77	-0.94	-0.46	-0.70*	6.67	3.33	5.00	-0.33	-3.33	-1.83
3×1	-0.18	-0.11	-0.15*	2.78**	0.31	1.24**	2.13**	0.24	1.19**	9.33*	1.33	5.33	-2.00	-2.67	-2.33
4×1	-0.01	-0.07	-0.04	0.38	-1.10**	-0.36	0.53	-1.75**	-0.61	7.33	-2.67	2.33	0.00	-4.67	-2.33
3×2	0.01	-0.09	-0.04	-1.14	-0.82*	-0.98*	-0.41	-1.48**	-0.94**	2.00	1.33	1.67	-1.33	0.67	-0.33
4×2	-0.06	-0.12	-0.09	-2.43**	-0.78*	-1.60**	-1.23*	-1.54**	-1.38**	6.67	4.00	5.33	2.67	9.33**	6.00**
4×3	-0.14	-0.13	-0.13	2.01*	0.07	1.04	0.39	0.13	0.26	-7.33	1.33	-3.00	1.33	-2.67	-0.67

*, ** and *** Significance at 5%, 1% and 0.1% levels, respectively. Ballatilla (1), Seri 82 (2), 84CZT04 (3), HP1744 (4); C: Control, S:Salt, M: Means

Conclusion

This research showed a greater ratio of the additive and additive x additive than the non-additive genetic variance in controlling the inheritance of all the characteristics studied under non-stress and salinity stress level. The best parent(s) may be chosen for a breeding program by predicting parents' genetic merit based on the breeding priority of main genetic effects. GCA effects showed that ‘84CZT04’ was the desirable general combiner for coleoptile length, root length and shoot length, whereas HP1744 was a good general combiner for root length, germination rate and germination force under salinity stress. Estimates of the SCA effects of crosses demonstrated that 1 x 4 and 3 x 1 could be regarded as the most desirable cross-combination for root length and shoot length, respectively. These findings could be beneficial for plant breeders and diallel analysis could also be used as a useful method for testing future or existing varieties at early stage for salt stress resistance.

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Authors’ Contributions: FK designed, conducted the research, as well as prepared the manuscript.

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