

## COMBINING ABILITY OF WATERLOGGING TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.)

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**ABSTRACT.** The mean performance and combining abilities of cross combinations derived from a complete diallel mating and their parents were evaluated under waterlogging conditions. Analysis of variance for combining abilities indicated significant GCA (general combining ability) and SCA (specific combining ability) for single spike yield, SPAD (soil plant analysis development) and leaf area; GCA for NDVI (normalised differences vegetation index) and SCA for Fe and Mn contents in roots and membrane thermal stability. The parents Stendal, Beşköprü and Pamukova 97 were the best combiners in terms of waterlogging tolerance, while Beşköprü × Pamukova 97, Pamukova 97 × Beşköprü, Stendal × Pamukova 97, Stendal × Beşköprü and Beşköprü × Hanlı were identified as the best cross combinations, with high positive specific combining ability effects for most waterlogging related characters.

**Keywords:** Fe and Mn content, flooding, heritability, NDVI, SPAD.

## INTRODUCTION

Waterlogging, caused by heavy rain, typically harms the growth of winter crops such as wheat (Du *et al.*, 2021), maize (Yu *et al.*, 2020) and barley (Setter and Waters, 2003). Wheat is a plant sensitive to flooding in all development periods (Ghobadi *et al.*, 2017; Mfarrej *et al.*, 2022). Anoxic (absence of O<sub>2</sub>) soil conditions inhibited N uptake and disturbed the N balance between roots and shoots; shoot growth was delayed by early leaf senescence occurring in the continuation (Drew and Sisworo, 1977; Zhou *et al.*, 2007; Herzog *et al.*, 2016). Global climatic change can cause excessive precipitation in January and February, especially in the temperate coastal zone, such as in the Aegean and Mediterranean regions (IPCC, 2007; Musgrave, 1994). Higher temperatures in these regions accelerate



crop damage by waterlogging and reduced leaf elongation and grain number per spike, ultimately causing yield losses (Anonymous, 2021). The yield losses can reach 15–25% in wheat depending on waterlogging duration, soil type and genotypes (Setter and Waters, 2003; Yavas *et al.*, 2012).

Anatomical, morphological and physiological responses to mitigate the negative effects of waterlogging have been observed in plants (Gibbs and Greenway, 2003). Early senescence and abscission of leaves (Dong *et al.*, 1983; Morgan and Drew, 1997), decreased plant height (Wu *et al.*, 1992), fewer grains per spikelet and kernel weight (Musgrave, 1994; Olgun *et al.*, 2008) and increasing Fe and Mn absorption under alkaline soil conditions (Stieger and Feller, 1994) have been reported in wheat.

It was emphasised that there are limited cultural measures that can be used to manage waterlogging, although drainage and raised beds have been recommended to be effective (Acuña *et al.*, 2011). Improving waterlogging tolerance in wheat genotypes is one of the major objectives in high-rainfall areas. Collaku and Harrison (2005) emphasised that selection criteria for direct selection in terms of waterlogging were related to many physiological characteristics in wheat. Cai *et al.* (1996), Yavas *et al.* (2012), Özçubukçu *et al.* (2014) and Tiryakioğlu *et al.* (2015) reported variation among genotypes in terms of tolerance to waterlogging. Boru *et al.* (2001) revealed that the additive gene effect was prevalent for waterlogging tolerance when leaf chlorosis was used as the indicator of the tolerance, whereas it was documented that multiple genes

with both additive and non-additive effects managed tolerance of waterlogging in cereals (Ahmed *et al.*, 2013; Tong *et al.*, 2021).

Very few genetic studies have been conducted on waterlogging tolerance, despite the effect of waterlogging on wheat growing both globally and in the Aegean and Mediterranean Region of Turkey. This study was conducted to estimate the waterlogging tolerance of wheat varieties and their reciprocal F<sub>1</sub> crosses from a Griffing I diallel mating design in terms of plant indices such as NDVI (normalised differences vegetation index), CCI (chlorophyll content index), SPAD (soil plant analysis development and chlorophyll concentration), membrane thermal stability and Fe and Mn accumulation in shoots and roots as selection criteria.

## MATERIALS AND METHODS

Five selected varieties from previous studies (Beşkoprü, Hanlı and Pamukova 97 as tolerant; Anapo and Stendal as high yielding and adaptive) were mated in a 5 × 5 complete diallel mating design. The resulting 20 hybrid and their parents were arranged in a randomised complete block design with three replications during the 2012/2013 wheat-growing season in the University of Aydın Adnan Menderes, Faculty of Agriculture, Research and Application Farm Area. Each plot consisted of a plastic tank (0.8 m × 0.38 m × 0.31 m) filled with the soil of a field that suffered a flood in previous years. The experimental soil, with silty clay loam texture, had 7.95 pH, 1.73% organic matter, 13.51% CaCO<sub>3</sub>, high-level P and Fe, intermediate K, Ca, Mg and Na, sufficient Zn, Mn and Cu and very low B. The long-term and last ten years' precipitation and monthly mean temperature were summarised in *Figure 1*. In the

Mediterranean climatic zone, wheat was sown between October and November and harvested in mid-June. When the last 10 years of precipitation were compared to long years of precipitation, it is remarkable that especially January precipitation increased. On the other hand, the precipitation before and after January in the wheat-growing season decreased markedly. Moreover, monthly average temperatures increased during these periods.

The plastic tanks were waterlogged by maintaining irrigation 5 cm above the soil surface and undrained at Zadox Growing Stage 12 and 31 for 10 days according to the method suggested by Setter *et al.* (2009) and Ploschuk *et al.* (2018). Parents and F<sub>1</sub> combinations contained 64 plants in each replication. The doses of fertiliser were 160 kg ha<sup>-1</sup> N, 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 90 kg ha<sup>-1</sup> K<sub>2</sub>O.

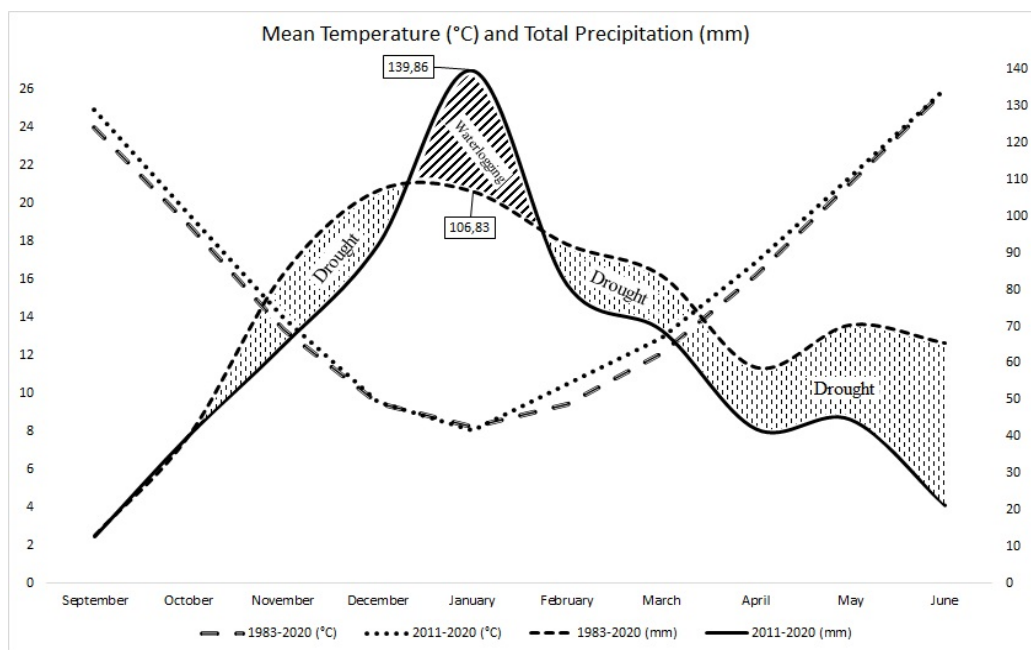
Chlorophyll concentration (SPAD) and chlorophyll content index (CCI) measurements were performed on the uppermost fully expanded leaves of 10 plants per pot ten days a week after the release of the waterlogging stress, using a Minolta SPAD-502 chlorophyll meter and Apogee-CCM 200, respectively. These leaf samples were used for the membrane thermostability index (Blum and Ebercon, 1981). Single leaf area (cm<sup>2</sup>) was determined by a CI-202 Portable Laser Leaf Area Meter. To determine the phytotoxic concentrations of Fe and Mn, contents were analysed in both roots and shoots (Reuter and Robinson, 1997). Single spike yield (g) was determined in 10 plants in the middle rows of pots.

Combining ability analysis (GCA and SCA) was performed according to Griffing's Diallel Method 1, Model 1 (fixed effects) (Griffing, 1956) for observed characters using TARPOGEN statistical software (Ozcan and Acikgoz, 1999). The F-test was used for testing the significance of GCA and SCA while GCA and SCA effect was tested by comparing with tabular t values.

## RESULTS AND DISCUSSION

Combining ability analysis showed that both GCA and SCA mean squares were significant for SSY, SPAD and LA (*Table 1*). Notably, these characters were affected by both additive and non-additive gene effects. The significant GCA mean square for NDVI revealed the prevalence of additive gene effect, while significant SCA mean squares for Fe and Mn content in root and MTS indicated the greater importance of the dominant gene effect in controlling the inheritance of these characters. Indeed, waterlogging tolerance is controlled by both additive and non-additive genes (Ahmed *et al.*, 2013; Boru *et al.*, 2001; Tong *et al.*, 2021). The prediction accuracy and evaluations of parents for stress tolerance can be significantly increased by using the GCA effects in wheat and rice (Chen *et al.*, 2016; Longin *et al.*, 2013; Miedaner *et al.*, 2016; Yao *et al.*, 2011). The high GCA values for the number of green leaves (Cao *et al.*, 1994; Zhou *et al.*, 2007) and leaf chlorosis (Boru *et al.*, 2001) were found in terms of waterlogging tolerance.

The present study showed that the inheritance of tolerance indices was very complex and was related to many morphological and physiological processes under waterlogging conditions. It was clear that early generation selection could be efficient for a character under the control of additive genes such as NDVI, whereas selection for SSY, SPAD and LA should be postponed to the later generations in waterlogging tolerance breeding.



**Figure 1** - Monthly precipitation and temperature over the last ten years with long periods (Data were obtained from the Turkish State Meteorological Service)

**Table 1** - ANOVA of combining ability of observed characters under the waterlogging condition

	df	SSY	Fe (shoot)	Fe (root)	Mn (shoot)	Mn (root)	CCI	SPAD	LA	NDVI	MTS
GCA	4	0.016**	124.86	162.73	219.77	1267.73	2.54	9.28**	38.64*	2.25*	22.45
SCA	10	0.014**	76.63	147.37*	137.41	3631.27**	2.39	5.87*	19.18*	0.74	25.32*
REC.	10	0.014**	87.44	99.57	221.54*	598.80	2.83	3.25	9.01	1.12	19.90
Error	48	0.003	66.55	66.68	98.89	592.74	2.15	2.26	9.44	0.85	10.74

\*, \*\*, significant at 5% and 1% probability levels, respectively, REC; reciprocal effect

The positive and significant GCA effects for Beşköprü and Pamukova 97, SCA effects for Pamukova 97 × Beşköprü (2×3) and Beşköprü × Pamukova 97 (3×2) crosses were recorded in single spike yield (Table 2). When the combining ability effects and high yield values were considered together, Beşköprü × Pamukova 97 crosses and their parents could be successfully used in waterlogging tolerance breeding.

Fe and Mn concentrations of shoot and root during waterlogging were evaluated to be an indicator of tolerance although the relationships between Fe and Mn accumulation and waterlogging tolerance were poor (Khabaz-Saberi *et al.*, 2012; Setter *et al.*, 2009). The GCA effects of the Stendal variety were negatively significant for Fe (root), Mn (root) and Fe (shoot) while Fe (shoot), Fe (root) and Mn (root) mean values of this variety were generally lower than

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other genotypes (*Table 2*). Significant and positive GCA effects in terms of Mn (root) were observed from Beşköprü and Hanlı with high concentrations (192.3 mg kg<sup>-1</sup> and 259.3 mg kg<sup>-1</sup>, respectively) while the Anapa variety had a significantly positive GCA effect and intermediate mean value (104.0) for Mn (root).

The estimates of GCA effects and their mean performance indicated that Stendal for SPAD and Pamukova 97 for LA were the best combiners (*Table 3*). The SCA effects and corresponding mean performance of crosses were favourable for Fe (root) in Stendal × Anapo (5×1) and Stendal × Pamukova 97 (5×2); for Fe (shoot) in Stendal × Anapo (5×1); for Mn (root) in Anapo × Hanlı (1×4), Pamukova 97 × Beşköprü (2×3), Hanlı × Pamukova 97 (4×2), Beşköprü × Hanlı (3×4), Hanlı × Stendal (4×5); for Mn (shoot) in Pamukova 97 × Anapo (2×1), Beşköprü × Anapo (3×1), Hanlı × Anapo (4×1); for CCI and SPAD in Stendal × Beşköprü (5×3); for LA in Beşköprü × Anapo (3×1), Pamukova 97 × Hanlı (2×4), Hanlı × Pamukova 97 (4×2); for MTS in Pamukova 97 × Stendal (2×5).

The performances (SCA and mean value) of superior crosses, Stendal × Anapo (5×1) and Stendal × Pamukova 97 (5×2), involving Stendal with high GCA effects indicated that cross combinations involved at least one with high GCA effects in terms of Fe and Mn concentrations in both shoot and root (*Table 2*). In addition, the high single spike yield of these combinations under waterlogging conditions revealed that low concentrations of Fe and Mn in the plant could be used as selection criteria

in waterlogging tolerance, whereas Khabaz-Saberi *et al.* (2006), Setter *et al.* (2009) and Khabaz-Saberi and Rengel (2010) stated that high contents of Mn and Fe in the shoot of plants grown in waterlogged acidic soil is not a barrier for the waterlogging-tolerant genotype as it is for intolerant varieties. Moreover, Mn-tolerant genotypes had higher translocation factors in barley (Huang *et al.*, 2015) and maize (Silva *et al.*, 2017). Our study clearly demonstrated that Beşköprü × Pamukova 97 (3×2), Pamukova 97 × Beşköprü (2×3), Stendal × Pamukova 97 (5×2), Stendal × Beşköprü (5×3) and Beşköprü × Hanlı (3×4) are combinations with low Fe and Mn values in roots and shoots rather than translocation factor. Also, Stendal crosses, Stendal × Beşköprü (5×3) and Pamukova 97 × Stendal (2×5), exhibited good performances for CCI, SPAD and MTS when the SCA effect and mean values were evaluated together.

The correlation coefficients between SSY and other characters (rSSY) indicated that SSY with CCI, NDVI and MTS significant positive correlated (*Table 3*), whereas the correlations between SSY and Fe and Mn concentrations of the shoot were significantly positive (*Table 2*). Therefore, these characteristics can be used as indirect selection criteria for improving waterlogging tolerance in wheat. When SCA effects and mean performances of cross combinations were evaluated in the light of these correlations, Pamukova 97 and Beşköprü crosses, Beşköprü × Pamukova 97 (3×2) and Pamukova 97 × Beşköprü (2×3), exhibited high favourable performances in terms of all observed characters.

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These combinations were followed by Stendal × Pamukova 97 (5×2), Stendal × Beşkoprü (5×3) and Beşkoprü × Hanlı

(3×4). It was concluded that these five combinations could be used successfully in breeding tolerance to floods.

**Table 2** - Mean performances and combining abilities of parents and crosses for single spike yield (SSY), Fe and Mn contents in roots and shoots

	Spike Yield (g)		Fe (root) (mg kg <sup>-1</sup> )		Fe (shoot) (mg kg <sup>-1</sup> )		Mn (root) (mg kg <sup>-1</sup> )		Mn (shoot) (mg kg <sup>-1</sup> )	
	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA
Anapo (1)	1.43	-0,02	1875.7	-38.2	546.7	78.3	104.0	-8.4*	49.7	5.7*
Pamukova 97 (2)	1.28	0,03*	2311.0	40.7	841.0	-38.1	194.3	0.1	47.3	1.3
Beşkoprü (3)	1.31	0,06**	2825.3	135.8	1164.7	-6.7	192.3	13.5*	58.3	7.2**
Hanlı (4)	1.10	-0,04	2488.7	61.1	1120.7	127.7	259.3	8.4*	77.0	1.4
Stendal (5)	1.11	-0,01	1303.7	-199.6*	430.3	-161.3*	128.0	13.5*	56.7	-1.2
1×2	1.13	-0,10*	1624.3	-164,2	545.7	-101,3	135.1	3,7	80.0	7,7
2×1	1.13	0,01	1949.5	162,3	791.1	122,7	107.2	-14.0	58.33	-10,8*
1×3	1.14	-0,10	1929.8	165,7	1066.3	18,4	160.3	36,6**	67.33	-8,0
3×1	1.17	0,02	2494.3	282,7*	572.3	-247.0	174.3	7.0	42.33	-12,5**
1×4	1.11	0,01	1722.7	-217,0	1515.3	224,6	81.4	-36,1**	76.67	-6,8
4×1	0.97	-0,19**	1786.4	31,7	1250.3	-355,3*	98.3	8,5	33.2	-21,8**
1×5	1.13	-0,02	2269.3	212,2	1206.3	197,6	114.2	0,5	72.33	3,7
5×1	1.21	0,03	1576.3	-346,5**	481.7	-362,3*	94.3	-9,8	53.2	-9,7
2×3	1.27	0,12**	1862.3	-291,4*	723.7	-115,9	86.3	-50,9**	28.33	-8,0
3×2	1.32	0,15**	1804.7	-28,7	413.7	-155.0	90.3	2.0	33.5	-7,7
2×4	1.30	0,03	2433.7	92,1	573.3	-10,5	158.1	-15,1	28.33	6,1
4×2	1.15	-0,07*	1850.7	-291,5*	594.4	-214,5	80.3	-39.0**	58.33	-5,0
2×5	1.24	0,03	2282.9	82,1	568.3	39,8	120.3	-6,2	25.33	4,0
5×2	1.31	0,02	1461.2	-410,5**	571.3	1,5	91.7	-14,3	22.6	-6,7
3×4	1.33	0,02	1850.5	-362,1**	541.7	-220,2	90.3	-33,7**	37.33	-6,3
4×3	1.19	-0,07*	1716.7	-67.0	718.7	88,5	137.3	23,5	47.67	5,2
3×5	1.26	0,01	1816.3	-117,7	380.3	-131,0	149.7	8,2	39.33	-5,4
5×3	1.31	0,03	1717.7	-49,2	480.3	50.0	118.2	-15,8	22.33	1,5
4×5	1.17	0,05	1975.3	68,9	413.3	-129,9	95.3	-32,0**	37.67	-6,7
5×4	1.27	0,04	1782.6	-96,7	518.3	152,5	81.7	-6,8	58.33	10,3*
LSD	0.13		730.4		Ns		68.9		28.1	
SE GCA		0.03		73.0		72.96		6.9		2.8
SE SCA		0.07		131.7		131.54		12.4		5.0
r (SSY)			0.004		-0.532**		-0.257		-0.521**	

\*, \*\*: significant at 5% and 1% probability levels, respectively

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**Table 3** - Mean performances and combining abilities of parents and crosses for chlorophyll content index (CCI), SPAD, leaf area (LA), NDVI and membrane thermal stability (MTS)

	CCI		SPAD		Leaf Area (cm <sup>2</sup> )		NDVI		MTS (%)	
	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA	$\bar{x}$	GCA/SCA
Anapo (1)	18.7	-0.21	32.3	-0.48	32.2	-1.66	55.8	0.45	77.6	-0.41
Pamukova 97 (2)	15.4	0.34	32.7	0.73	41.6	6.07**	55.1	0.46	63.8	-1.16
Beşköprü (3)	15.4	0.52	33.2	-1.17**	32.8	0.81	54.5	0.04	75.3	-1.09
Hanlı (4)	10.4	-0.47	32.8	-0.31	28.6	-1.51	54.5	-0.48	61.1	2.50**
Stendal (5)	16.4	-0.10	34.5	1.22**	35.2	-3.71*	53.5	-0.48	72.3	0.16
1×2	11.2	-0.62	29.1	-1.90*	49.2	1.86	51.9	0.23	69.4	0.17
2×1	10.2	0.87	31.2	1.02	37.8	-5.70**	51.7	-0.09	76.4	3.48*
1×3	12.2	-1.13	28.6	-1.59	35.9	4.92**	52.6	0.16	71.1	1.85
3×1	13	0.38	28.5	-0.04	46.7	5.37**	51.3	-0.33	78.2	3.58*
1×4	13.3	-0.49	33.7	1.58*	40.1	-0.09	52.4	-1.15**	67.5	-2.34
4×1	10.3	-0.13	31.5	-1.07	27.8	-6.17**	50.1	-1.14**	60.6	6.57**
1×5	18.4	1.36	33.3	-0.04	26.8	-4.99**	55.4	0.53	78.9	3.15
5×1	13.4	-2.48**	31.6	-0.85	26.9	0.03	54.9	-0.27	75.4	-1.76
2×3	14.6	1.082	34.4	0.36	48.7	1.19	55.7	0.39	72.7	0.47
3×2	17.0	1.217	29.1	-2.73**	41.9	-3.37*	55.4	-0.15	78.3	-0.18
2×4	15.1	-0.091	33.5	0.28	44.6	7.36**	55.7	-0.41	80.2	3.58*
4×2	13.9	-0.583	31.4	-1.06	53.7	4.52**	52.7	-1.51**	78.2	-0.99
2×5	16.2	-0.145	35.5	1.86*	34.0	-2.65	55	0.27	81.1	3.90*
5×2	16.6	-0.8	35.7	0.10	39.9	2.97	53.9	-0.98*	78.2	-3.95*
3×4	15.1	-0.328	27.7	-1.93**	38.6	-2.10	54.7	0.48	74.4	0.083
4×3	12.5	-0.85	29.1	0.68	30.3	-4.13**	54.6	-0.02	77.1	1.362
3×5	11.3	-1.315	29.2	-0.57	40.3	2.07	54	-0.87	70.3	-5.62**
5×3	15.2	1.97**	33.3	2.07**	32.5	-3.93**	52.6	-0.69	75.1	-2.59
4×5	13.5	-0.971	31.3	-1.58*	33.9	0.40	54.5	0.21	69.5	0.84
5×4	13.4	-0.05	30.9	-0.17	30.9	-1.53	53.2	-0.63	76.0	-1.76
LSD	ns		4.27		16.7		2.60		9.26	
SE GCA		0.41		0.42		0.87		0.25		0.92
SE SCA		0.75		0.77		1.56		0.46		1.67
r (SSY)	0.621**		0.031		0.158		0.567**		0.625**	

\*, \*\*: significant at 5% and 1% probability levels, respectively

## CONCLUSIONS

Beşköprü (3), Pamukova 97 (2) and Stendal (5) were good general combiners and 3×2, 2×3, 5×2, 5×3 and 3×4 were the most promising combinations that had better agronomic characters to withstand waterlogging tolerance based on the current finding. The effect of additive genes for traits such as SPAD and NDVI and non-additive genes for traits such as Fe and Mn content in the plant indicated that modified bulk selection could be beneficial to improve plant ideotypes for waterlogging tolerance breeding.

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