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Interactive effect of static magnetic field and abiotic stressors on growth and biochemical parameters of germinating wheat cultivars

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Abstract

In this study, the effects of the individual and combined application of the static magnetic field and abiotic stressors (60 gL^{-1} PEG6000 (as a drought stress inducer) or 100 mM NaCI (as a salinity stress inducer)) on the cultivars of germinating wheat were investigated by assessing growth, biochemical and antioxidant defence parameters. The seeds of Nina and Flamura-85 species of bread wheat were exposed to 2.9-4.7 mT static magnetic flow density for a period of 0, 2.2 and 19.8 seconds on a band turning with the speed of 1 ms^{-1}. Moreover, this magnetic field application was performed together with abiotic stressors and separately. The growth parameters of both wheat cultivars increased under the static magnetic field applications, while they decreased when subject to the abiotic stress application than the control. The application of the static magnetic field together with abiotic stressors statistically increased the amount of • OH and H_2O_2 in root samples, and the total Chl, Chl a and Chl b amounts in leaf samples. The application of the static magnetic field together with abiotic stressors or separately significantly increased antioxidant enzyme activities (SOD, POD, CAT, APX and GR), and GSH and GSSG content in all experimental groups when compared to the control. Consequently, that the application of the magnetic field triggers the antioxidant defence parameters reduced the negative effects of drought and salinity stresses on the growth parameters in both species.

Key words: Antioxidant defence parameters, biochemical parameters, growth parameters, static magnetic field, salinity, drought, wheat.
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Çimlenmekte olan buğday (*Triticum aestivum* L.) çeşitlerinde büyüme ve biyokimyasal parametreler üzerine statik manyetik alanın ve abiyotik stresörlerin etkileşimleri

Özet

Bu çalışmada, yapay statik manyetik alan ile abiyotik stresörlerin (kuraklık stresi indükleyici (60 gL^{-1} PEG6000) ve tuzluluk stresi indükleyici (100 mM NaCI) tek başına ve beraber uygulanmasının çimlenmekte olan buğday çeşitleri üzerine etkileri büyüme, biyokimyasal ve antioksidan savunma parametreleri değerlendirilerek araştırılmıştır. Nina ve Flamura-85 ekmeklik buğday çeşitlerine ait tohumlar 2.9-4.7 mT statik manyetik akı yoğunluğuna, 1 ms^{-1} hızla dönen bir bant üzerinde 0, 2.2 ve 19.8 saniye süre ile tabi tutulmuşlardır. Ayrıca bu manyetik alan uygulaması abiyotik stresörler ile birlikte ve ayrı ayrı yapılmıştır. Manyetik alan uygulanan her iki buğday çeşidinin büyüme parametreleri kontrol grubuna göre artış gösterirken, abiyotik stres uygulanan deney gruplarında ise kontrole göre düşüş görülmüştür. Manyetik alanın stresörlerle birlikte uygulaması sonucu ise kök örneklerinde • OH ve H_2O_2 miktarlarını, yaprak örneklerinde ise total Chl, Chl a ve Chl b miktarlarını istatistiksel olarak arttırmıştır. Statik manyetik alanın tek başına ve abiyotik stresörler ile kombine uygulaması antioksidan enzim(SOD, POD, CAT, APX ve GR) aktivitelerini ve GSH ve GSSG içeriğini tüm deney gruplarında kontrole göre anlamlı olarak arttırmıştır. Sonuç olarak; manyetik alan uygulaması, antioksidan savunma parametrelerini uyararak her iki buğday çeşidinde, kuraklık ve tuzluluk streslerinin büyüme parametreleri üzerindeki olumsuz etkilerini azaltmıştır.

Anahtar kelimeler: Antioksidan savunma sistemi, biyokimyasal parametreler, büyüme parametreleri, statik manyetik alan, kuraklık, tuzluluk, buğday

Introduction

Increasing the germination performance of seeds is an important aim for plant breeders, especially in cases of low germination percentages due to harsh environmental conditions, dormancy or seed dryness. In recent literature, it has been reported that exposing seeds to magnetic fields (MFs) may accelerate or stimulate seed vigor, growth, and yield (Podleśny et al. 2005; De Souza et al. 2006; Shine et al. 2012; Bilalis et al. 2013). MFs treatments influence photosynthetic pigment contents (Shine et al. 2012) and mineral uptake (Esitken and Turan 2004; Radhakrishnan and Kumari 2012; Bilalis et al. 2013) by altering biochemical processes that involve free radicals and by stimulating the activity of enzymes (Radhakrishnan and Kumari 2012). However, there is not yet a sufficient explanation on exactly how MF affects biological systems via biochemical, biophysical and molecular mechanisms (Harris et al. 2009). Several theories have been proposed, associated with biochemical changes due to the radical-pair mechanism, ion cyclotron resonance mechanisms and ferrimagnetism or enzyme activity (Galland and Pazur 2005).

Wheat (*Triticum aestivum* L.) is a widely cultivated cereal crop worldwide. Abiotic stresses, such as drought, salinity, and low and high temperatures greatly reduce wheat productivity and quality, which is a result of accelerating reactive oxygen species (ROS) accumulations such as hydroxyl radicals ($^{\bullet}OH$), superoxide radicals ($O_2^{\bullet-}$), alkoxyl radicals ($RO^{\bullet}$), hydroperoxide radicals ($HO_2^{\bullet}$) and hydrogen peroxide (H_2O_2). These metabolites are normal products of natural redox reactions in mitochondrial respiratory and photosynthetic electron transport chains, peroxisomes and glyoxysomes. They degrade large macromolecules such as nucleic acid,

proteins, lipids and chloroplast pigments. To avoid the deleterious effects of ROS, plants have evolved antioxidant defence systems. One of these protective mechanisms is the enzymatic antioxidant system, which operates with a sequential and simultaneous action of many enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1. 11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), guaiacol peroxidase (POX, EC 1. 11.1.7) and glutathione reductase (GR, EC 1.8.1.7). There are also non-enzymatic antioxidant defences such as glutathione, ascorbate, α-tocopherol and carotenoid (Gill and Tuteja 2010). Eliminating salinization and drought stress worldwide is not realistic. For this reason, improving environmental-friendly strategies to avoid the negative effects of drought and salinity is important. In light of these facts, this study focused on interacting effect of artificial static magnetic field (SMF) pre-treatments on abiotic stressors (salinity and drought) in the initial growth stage of wheat cultivars based on growth, biochemical and antioxidant defence parameters under individual and combine applications of SMF and abiotic stressors.

Materials and methods

Plant material

Mature wheat (*Triticum aestivum* L.) seeds from Nina and Flamura-85, which are widely cultivated varieties in Thrace Region, were provided by Directorate of Trakya Agricultural Research Institute in Edirne, Turkey. The seeds were harvested during the 2007–2008 season and their water content was measured to be 9.18% (Nina) and 9.86% (Flamura-85).

Experimental design

The experiment included control, two different abiotic stressors (salinity and drought) and artificial SMF pre-treatment (2.2 s and 19.8 s). Each group was designed to test individual and combined effects of abiotic stressors and SMF pre-treatment on growth and several biochemical parameters measured on the 3rd and 5th germination day. Thirty seeds were used for each combination with three repetitions. For SMF pre-treatment, seed surfaces were sterilized with 70% (v/v) ethanol for 5 min and 20% commercial NaOCl bleach (5% active chlorine) solution for 20 min and then thoroughly rinsed four times in sterile distilled water. Then the seeds were placed in petri dishes under aseptic conditions, which contained sterilized agar 10% (w/v). Various SMF flux densities of 2.9–4.7 mT were applied at 0 (as a control), 2.2 and 19.8 s on a moveable belt at a speed of $1ms^{-1}$ under 23±2°C. Afterward, cultures were transferred to clean petri dishes with fresh agar 10% (w/v) with and without 60 gL^{-1} PEG 6000 (as a drought proxy) or 100 mM NaCl (as a salinity inducer). For germinating, all petri dishes were placed in a growth chamber at 25±2°C with a photoperiod of 16 hours light and 8 hours dark for five days.

Measurement of germination percentage, seedling vigour index and growth parameters

Germination percentage was calculated by counting the number of germinated seeds on the 3rd and 5th day. Seedling vigour index was calculated on the 5th day using the following equation: germination percentage ×seedling length (cm), where seedling length= root length + shoot length (Dhanda et al. 2004). Additionally, average weights and lengths for plants and roots were measured on the 5th day.

Spectrophotometric assay of free radicals (•OH and H_2O_2) contents

The formation of H_2O_2 and •OH in the mixture were measured at 550 nm and 412 nm as described by Puntarulo and Cederbaum (1988) and Holland and Story (1981), respectively. The production of •OH or compounds with the oxidizing power of •OH was assayed by the generation of formaldehyde from dimethyl sulfoxide (DMSO). The basic reaction system consisted of 40 mM potassium phosphate buffer (pH 7.4), 120 mM KCl, 0.4 mM NAD(P)H, 30 mM DMSO and 200 µl extracted samples in a final volume of 1 mL. The production of formaldehyde was determined at 412 nm. For spectrophotometric assay of H_2O_2, about 500 µl of homogenate was added to the tubes containing 1.5 mM ferricytochrome. The formation of H_2O_2 in the mixture was measured

at 550 nm by estimating the oxidation product of ferrocytochrome.

Spectrophotometric assay of ferric reducing antioxidant power content

Ferric reducing antioxidant power (FRAP) was measured using Benzie and Strain's (1996) method. An amount of 200 µl extracted samples were mixed with 3 ml FRAP reagent in test tubes and were then incubated in a water bath for 30 minutes at 30ºC. The absorbance of the samples was determined against blank at 593 nm. $FeSO_4.7H_2O$ were used for drawing a standard curve. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of freezed sample.

Spectrophotometric assay of pigment contents

Chlorophyll (Chl) *a+b, a, b* and carotenoids (CAR) content was calculated following Litchtenthaler and Wellburn (1985). 100% acetone was used as a homogenizing solution. Extraction ratio was 1:10. Homogenized mixture is separated by centrifugation at 3000 rpm, for 10 minutes. The analytical determination was performed UV/Visible spectrophotometer at the following wavelengths: 645, 662 and 664 nm, for chlorophyll a and b (according to each extraction solvent) and 470 nm for carotene. Equations used for calculation are presented below.

Chlorophyll a = 11.75 A662 – 2.350 A645

Chlorophyll b = 18.61 A645 – 3.960 A662

Carotene = 1000 A470 – 2.270 Chl a – 81.4 Chl b/227

Enzyme extraction and assay

200 mg frozen leaves and roots samples were homogenized in an ice bath with a 2 ml 100 mM phosphate buffer. Afterward, they were centrifuged at 11.000 *x g* for 25 minutes at 4°C and the supernatant fraction was used to prepare the enzyme assays. All of the procedures in the preparation of enzyme extracts were performed at 4°C. Protein content was determined using Bradford's (1976) method. Superoxide dismutase was assayed by monitoring the superoxide radical-induced NBT reduction at 560 nm (Dhinsa et al. 1981). In order to determine different type of SOD isozymes, either 5 mM KCN (inhibitor of Cu/Zn-SOD) or 5 mM H_2O_2 (inhibitor of Cu/Zn-SOD and Fe-SOD) was added to the spectrophotometric assay buffer (Rao et al. 1996). One unit of SOD activity was defined as the amount of enzyme, which causes a 50% inhibition of the photochemical reduction of nitro blue tetrazolium chloride. Guaiacol peroxidase activity was measured at 470 nm by using H_2O_2 and guaiacol as substrates (Panda et al. 2003). Catalase activity was determined by monitoring the disappearance of H_2O_2 at 240 nm according to Aebi (1984) method. Ascorbate peroxidase activity was determined by measuring the consumption of ascorbate by tracking absorbance at 290 nm (Nakano and Asada 1981). Glutathione reductase activity was determined by measuring the enzyme-dependent oxidation of NADPH by the following absorbance at 340 nm (Foyer and Haliwell 1976).

GSH and GSSG contents

Total and oxidized glutathione contents were measured using the methods of Tietze (1969) and Griffith (1980) with minor modifications. Briefly, 100 mg leaf samples were ground with a mortar and pestle in the presence of 1 ml of 5% (w/v) meta-phosphoric acid. Homogenates were centrifuged at 10,000 × *g* for 20 min at 4°C. Glutathione (as GSH + GSSG) was measured using 1 ml of assay mixture containing 100 mM sodium phosphate buffer (pH 7.5) plus 6.3 mM EDTA, 300 µM NADPH, 6 mM DTNB, 25 µl of homogenate and 0.5 U GR. The change in absorbance at 412 nm was recorded for 2 min. Glutathione concentrations were calculated from a standard curve constructed using commercial GSH over the range 0–0.1 mM. To determine the content of GSSG, 100 µl of the samples were incubated with 2 µl of pure 2-vinylpyridine and 6 µl of 1.5 M triethanolamine for 1 h at 25°C. GSSG concentrations were calculated from a standard curve constructed using commercial GSSG over the range 0–0.01 mM.

Statistical analysis

The significance of the interactions of the three factors (SMFs, abiotic stressors, and cultivars) was analyzed with three-way-

ANOVA in the program SPSS Statistics 20.0 (IBM Corp, Chicago, IL, USA). Prior to analysis, data were checked for normality and homogeneity of variance. Comparisons of mean values in each group between (*SMF x abiotic stressor*, *SMF x cultivar* and *abiotic stressor x cultivar*) and within (*SMFs* (Control, 2.2 s and 19.8 s), *abiotic stressors* (Control, Drought and Salinity) and *cultivars* (Nina and Flamura-85) groups were made using a least significant difference (LSD) post-hoc test at the 95% significance level.

Results

Effects of SMF pre-treatments with and without the combinations of PEG 6000 or NaCl on germination percentage, seedling vigour index and growth parameters

Table 1 showed that separate application of SMFs positively affected seed germination ratios and seedling vigour indexes than the groups of control and combined-applications of SMFs with drought and saline conditions than separate applications of either drought or saline conditions. Additionally, SMF applications increased growth of germinated seeds up to 42% and 46% in Nina and Flamura-85 wheat cultivars, respectively, whereas applications of 60 gL^{-1} PEG 6000 or 100 mM NaCl decreased growth 27.5–46% in Nina and 36–51% in Flamura-85 as compared to the control. Artificial SMF pre-treatment alleviated 60 gL^{-1} PEG6000 or 100 mM NaCl effects on seed growth of both cultivars (Fig. 1). The highest increases in growth parameters were measured to be up to 37% in Nina (as an average plant height on 19.8 s+60 gL^{-1} PEG6000 experimental group) and up to 54% in Flamura-85 (as an average root height on 19.8 s+100 mMNaCl) under SMF pre-treatment with either 60 gL^{-1} PEG6000 or NaCl application as compared to the group without SMF pre-treatment (Table 2).

Table 3 showed three-way ANOVA results of individual and combined applications of SMF (2.2 s and 19.8 s) and abiotic stressors (NaCl and PEG 6000) on Nina and Flamura-85 cultivars. Average plant height (r^2=0.939, all p<0.0001) statistically significant affected between the groups, namely *SMF x stress application*, *SMF x cultivar* and stress *application x cultivar*, but within the groups, namely *2.2s and 19.8 s SMF treatments*, *100 mM NaCl and 60 grL^{-1} PEG 6000 applications,* and *Nina and Flamura-85 cultivars* there was no significant differences in average plant height (all p>0.05). The same effect was observed on average root length (r^2=0.796), average plant fresh weight (r^2=0.969) and average root fresh weight (r^2=0.926) (for most groups, p<0.0001); there were no statistical differences within groups (all p>0.05).

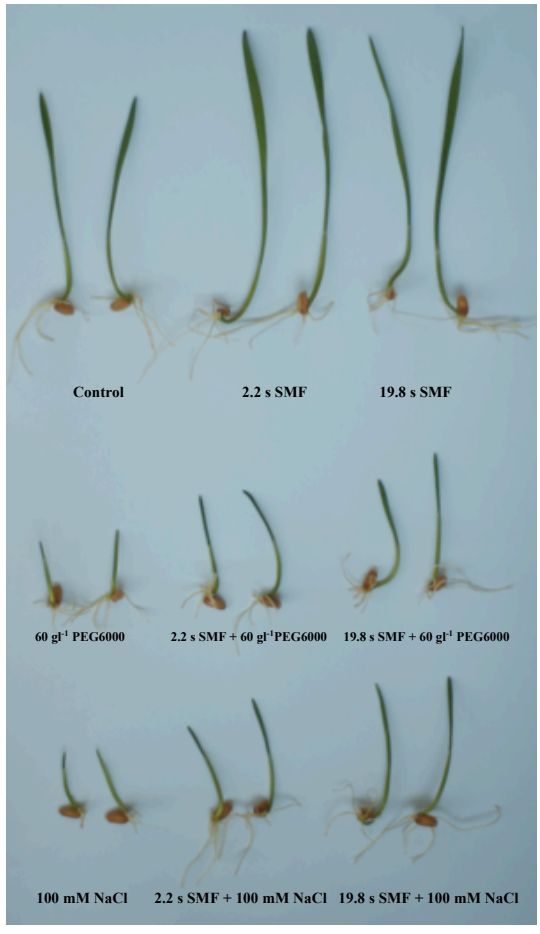

Figure 1. The Flamura-85 cultivar on the 5th day after alone and combined pre-treatments of SMF, 60 gL^{-1} PEG 6000 and 100 mM NaCl.

Table 1. Germination percentage on the 3rd and 5th days and seedling vigour index (SVI) on the 3rd and 5th day.

Experimental Groups	Nina			Flamura-85		
	3rd day (%)	5th day (%)	SVI	3rd day (%)	5th day (%)	SVI
Control	30.00	66.67	371.35	30.00	70.00	413.70
2.2 s	50.00	83.33	594.14	53.33	80.00	646.40
19.8 s	53.33	86.67	630.96	53.33	86.67	747.10
60 gL^{-1} PEG6000	26.67	50.00	202.00	26.67	46.67	175.48
2.2 s + 60 gL^{-1} PEG6000	33.33	70.00	379.40	30.00	66.67	336.68
19.8 s + 60 gL^{-1} PEG6000	36.67	76.67	425.52	33.33	63.33	328.68
100 mMNaCl	30.00	43.33	157.72	30.00	50.00	182.50
2.2 s + 100 mMNaCl	36.67	60.00	283.80	33.33	66.67	335.35
19.8 s + 100 mMNaCl	40.00	63.33	309.68	36.67	70.00	362.60

Table 2. Growth parameters of *in vitro* germinated wheat after 5 days (*T. aestivum* L. cvs. Nina and Flamura-85) pre-treated with SMF and with and without 60 gL^{-1} PEG6000 or 100 mM NaCl.

Cultivars	Experimental Groups	Average Plant Height (cm)	Average Root Height (cm)	Average Plant Fresh Weight (mg)	Average Root Fresh Weight (mg)
Nina	**Control**	5.57^{a*}	2.63^{a}	95.50^{a}	33.20^{a}
	2.2 s	7.13^{b}	3.16^{b}	125.50^{b}	46.45^{b}
	19.8 s	7.28^{b}	3.23^{b}	128.50^{b}	47.20^{b}
	60 gL^{-1}PEG6000	4.04^{c}	1.77^{c}	51.55^{c}	22.75^{c}
	2.2 s+60 gL^{-1} PEG6000	5.42^{ac}	2.12^{ac}	63.60^{ac}	27.95^{ac}
	19.8 s+60 gL^{-1} PEG6000	5.55^{a}	2.15^{ac}	65.50^{ac}	27.15^{ac}
	100 mMNaCl	3.64^{c}	1.67^{c}	53.83^{c}	21.15^{c}
	2.2 s+100 mMNaCl	4.73^{ac}	2.02^{ac}	62.65^{ac}	26.45^{ac}
	19.8 s+100 mMNaCl	4.89^{ac}	2.09^{ac}	62.83^{ac}	26.75^{ac}
Flamura-85	**Control**	5.91^{a*}	2.78^{a}	93.50^{a}	37.20^{a}
	2.2 s	8.08^{b}	3.55^{b}	132.30^{b}	52.45^{b}
	19.8 s	8.62^{b}	3.73^{b}	137.00^{b}	52.85^{b}
	60 gL^{-1}PEG6000	3.76^{c}	1.44^{c}	51.05^{c}	22.55^{c}
	2.2 s+60 gL^{-1} PEG6000	5.05^{ac}	2.02^{ac}	69.60^{ac}	30.95^{ac}
	19.8 s+60 gL^{-1} PEG6000	5.19^{ac}	2.09^{ac}	71.00^{ac}	31.25^{ac}
	100 mMNaCl	3.65^{c}	1.37^{c}	53.50^{c}	21.35^{c}
	2.2 s+100 mMNaCl	5.03^{ac}	2.06^{bc}	70.60^{ac}	29.08^{ac}
	19.8 s+100 mMNaCl	5.18^{ac}	2.12^{bc}	71.15^{ac}	30.45^{ac}

***** Letters indicate significant differences ($p<0.05$) among experimental groups according to one-way ANOVA ($n=5$).

Effects of SMF pre-treatments with and without the combinations of PEG 6000 or NaCl on free radicals (•OH and H_2O_2) and ferric reducing antioxidant power contents

Tables 4, 5 and 7 showed that the effects of •OH, H_2O_2 and FRAP contents both leaf and root samples. Between groups in table 7, SMF pre-treatment and abiotic stressors were correlated strongly to •OH contents in leaves and roots (r^2=0.910, all p<0.0001). Individual applications of abiotic stressors and SMF significantly increased •OH content both in leaf (up to 31% and 182% under SMF and abiotic stress, respectively) and root (up to 64% and 126% under SMF and abiotic stress, respectively) compared to control samples (all p<0.0001). There were no statistical significant within the groups (all p>0.005). •OH content increased by up to 83% in leaves and 80% in roots under combined applications of SMFs and abiotic stressors. H_2O_2 content increased in leafs by up to 434% and 104% under abiotic stress and SMF treatments, respectively (r^2=0.848, all p<0.0001). H_2O_2 increases in roots were also significant (r^2=0.898, all p<0.0001). As in other effects, there were no significant within-group differences (all p>0.005). FRAP contents were statistically affected by SMF and abiotic stressors in leaves (r^2=0.373) and roots in (r^2=0.534, p<0.0001).

Table 3. Results of a 3-wayANOVA of growth parameters of *in vitro* germinated wheat after 5 days, showing significant effects of individual and combined effects of static magnetic fields, abiotic stressors and cultivars.

Parameters[a]	**Factor**	**df**	**MS**	**F**
Average Plant Heights (cm)	Abiotic Stressors	2	181.888	1401.955***
	SMFs	2	73.554	566.931***
	Cultivars	1	3.936	30.340***
	Abiotic Stressors x SMFs	4	1.414	10.898***
	Abiotic Stressors x Cultivars	2	8.150	62.824***
	SMFs x Cultivars	2	0.879	6.773**
	Abiotic Stressors x SMFs x Cultivars	4	0.737	5.680***
	Error	504	0.130	
Average Root Heights (cm)	Abiotic Stressors	2	47.821	419.601***
	SMFs	2	10.337	90.700***
	Cultivars	1	0.033	0.292[ns]
	Abiotic Stressors x SMFs	4	0.767	4.461*
	Abiotic Stressors x Cultivars	2	1.744	15.305***
	SMFs x Cultivars	2	0.666	5.847**
	Abiotic Stressors x SMFs x Cultivars	4	0.012	0.104[ns]
	Error	504	0.114	
Average Plant Fresh Weights (mg)	Abiotic Stressors	2	95536.675	3545.758***
	SMFs	2	14675.161	544.655***
	Cultivars	1	1284.195	47.662***
	Abiotic Stressors x SMFs	4	1555.515	57.732***
	Abiotic Stressors x Cultivars	2	14.796	0.549[ns]
	SMFs x Cultivars	2	469.241	17.415***
	Abiotic Stressors x SMFs x Cultivars	4	11.931	0.443[ns]
	Error	504	26.944	
Average Root Fresh Weights (mg)	Abiotic Stressors	2	10149.668	1259.069***
	SMFs	2	2688.700	333.534***
	Cultivars	1	715.473	88.755***
	Abiotic Stressors x SMFs	4	199.699	24.773***
	Abiotic Stressors x Cultivars	2	60.370	7.489**
	SMFs x Cultivars	2	68.317	8.475***
	Abiotic Stressors x SMFs x Cultivars	4	4.604	0.571[ns]
	Error	504	8.061	

[ns]Not Significant;* p<0.05; ** p<0.001; *** p<0.0001; [a]n=30 for growth parameters.

Table 4. ·OH [nmol g^{-1}(fw)], H_2O_2 [µmol g^{-1}(fw)] and FRAP [µmolFe(II) g^{-1}(fw)] content of leaves samples with and without SMF pre-treatment and abiotic stressors.

Experimental Groups	Nina			Flamura-85		
	·OH	H_2O_2	FRAP	·OH	H_2O_2	FRAP
Control	12.84^{a*}	1.93^{a}	21.38^{a}	14.62^{a}	3.42^{a}	19.24^{a}
2.2 s	14.93^{a}	3.22^{b}	28.62^{b}	17.85^{b}	5.12^{b}	25.76^{b}
19.8 s	15.69^{b}	3.66^{b}	29.34^{b}	18.31^{b}	5.84^{b}	26.62^{b}
60 gL^{-1} PEG6000	31.13^{b}	10.07^{c}	28.66^{b}	32.69^{c}	14.06^{c}	29.38^{c}
2.2 s+60 gL^{-1} PEG6000	23.33^{c}	7.98^{c}	25.5^{ab}	24.49^{bc}	9.40^{bc}	23.36^{b}
19.8 s+60 gL^{-1} PEG6000	25.01^{c}	8.62^{c}	25.7^{ab}	25.22^{bc}	9.98^{bc}	23.96^{b}
100 mMNaCl	26.67^{c}	8.54^{c}	30.36^{b}	29.73^{c}	12.90^{c}	29.18^{c}
2.2 s+100 mMNaCl	19.65^{b}	5.20^{bc}	27.22^{b}	22.06^{bc}	8.98^{bc}	23.56^{b}
19.8 s+100 mMNaCl	21.01^{b}	5.68^{bc}	27.66^{b}	22.11^{bc}	8.55^{bc}	23.69^{b}

* Letters indicate significant differences ($p<0.05$) among experimental groups according to one-way ANOVA ($n=5$).

Table 5. ·OH [nmol g^{-1}(fw)], H_2O_2 [µmol g^{-1}(fw)] and FRAP [µmolFe(II) g^{-1}(fw)] content of root samples with and without SMF pre-treatment and abiotic stressors.

Experimental Groups	Nina			Flamura-85		
	·OH	H_2O_2	FRAP	·OH	H_2O_2	FRAP
Control	16.15^{a*}	2.82^{a}	23.38^{a}	17.28^{a}	4.92^{a}	22.88^{a}
2.2 s	22.31^{b}	4.28^{b}	30.26^{b}	20.16^{a}	6.52^{b}	27.36^{b}
19.8 s	20.67^{b}	4.59^{b}	30.82^{b}	20.95^{b}	6.72^{b}	27.58^{b}
60 gL^{-1}PEG6000	34.97^{c}	10.58^{c}	30.08^{a}	34.03^{c}	17.71^{c}	31.58^{c}
2.2 s+60 gL^{-1} PEG6000	25.27^{b}	8.36^{c}	27.10^{ab}	26.17^{bc}	10.88^{bd}	25.64^{a}
19.8 s+60 gL^{-1} PEG6000	26.65^{b}	8.10^{c}	27.88^{ab}	26.46^{bc}	11.12^{d}	25.28^{a}
100 mMNaCl	29.79^{c}	9.71^{c}	31.08^{b}	29.99^{c}	15.98^{c}	30.92^{c}
2.2 s+100 mMNaCl	23.27^{b}	6.88^{bc}	26.84^{a}	23.72^{bc}	9.14^{ad}	25.68^{a}
19.8 s+100 mMNaCl	24.85^{b}	6.94^{bc}	26.72^{a}	23.43^{bc}	9.90^{d}	25.78^{a}

* Letters indicate significant differences ($p<0.05$) among experimental groups according to one-way ANOVA ($n=5$).

Table 6. Pigment content of 5-old-day *in vitro* germinated wheat (*T. aestivum* L. cvs. Nina and Flamura-85) with and without SMF pre-treatment and abiotic stressors.

Varieties	Experimental Groups	Chl*a+b*	Chl*a*	Chl*b*	CAR
		[mg(gfw)$^{-1}$]			
Nina	**Control**	0.097^{a*}	0.063^{a}	0.034^{a}	0.022^{a}
	2.2 s	0.127^{b}	0.083^{b}	0.044^{b}	0.029^{b}
	19.8 s	0.123^{b}	0.083^{b}	0.040^{a}	0.028^{b}
	60 gL^{-1}PEG6000	0.068^{c}	0.041^{c}	0.027^{c}	0.025^{a}
	2.2 s+60 gL^{-1} PEG6000	0.092^{a}	0.061^{a}	0.031^{a}	0.029^{b}
	19.8 s+60 gL^{-1} PEG6000	0.087^{ac}	0.059^{a}	0.028^{c}	0.030^{b}
	100 mMNaCl	0.063^{c}	0.035^{c}	0.028^{c}	0.026^{a}
	2.2 s+100 mMNaCl	0.082^{ac}	0.053^{ac}	0.029^{c}	0.030^{b}
	19.8 s+100 mMNaCl	0.079^{ac}	0.051^{ac}	0.028^{ac}	0.030^{b}
Flamura-85	**Control**	0.107^{a*}	0.071^{a}	0.036^{a}	0.025^{a}
	2.2 s	0.131^{b}	0.088^{b}	0.043^{b}	0.031^{b}
	19.8 s	0.128^{b}	0.085^{b}	0.043^{b}	0.033^{b}
	60 gL^{-1}PEG6000	0.073^{c}	0.045^{c}	0.028^{c}	0.027^{a}
	2.2 s+60 gL^{-1} PEG6000	0.097^{a}	0.063^{a}	0.034^{a}	0.033^{b}
	19.8 s+60 gL^{-1} PEG6000	0.095^{ac}	0.060^{a}	0.035^{a}	0.032^{b}
	100 mMNaCl	0.069^{c}	0.045^{c}	0.024^{c}	0.025^{a}
	2.2 s+100 mMNaCl	0.092^{a}	0.059^{a}	0.033^{a}	0.032^{b}
	19.8 s+100 mMNaCl	0.088^{ac}	0.057^{a}	0.031^{a}	0.033^{b}

***** Letters indicate significant differences ($p<0.05$) among experimental groups according to one-way ANOVA ($n=5$).

Effects of SMF pre-treatments with and without the combinations of PEG 6000 or NaCl on pigment contents

In our study, Chl*a*+*b*, Chl*a* and Chl*b* content increased significantly in leaf samples under individual and combined interactions between abiotic stress and SMF (r^2=0.693 in Chl*a*+*b*,p<0.001; r^2=0.701in Chl*a*,p<0.0001; r^2=0.399 in Chl*b*, p<0.005). With SMF pre-treatment, there increases were significant compared to the control (p<0.0001 in Chl*a*+*b*andChl*a*, p<0.005 in Chl*b*). Under abiotic stress, these effects decreased significantly (all p<0.0001). The combined effects of SMFs and abiotic stressors statistically increased Chl*a*+*b*, Chl*a* and Chl*b* content (all p<0.0001) compared to individual applications of salinity and drought. Again, there were no statistically significant differences within groups (all p>0.005). Abiotic stress and combinations of abiotic stress treatments with SMFs statistically increased leaf CAR content compared to the control (p<0.005; Tables 6 and 7).

Table 7. Statistical significance of F value (three-way ANOVA) for individual and combined effects of SMF pre-treatments and abiotic stressors on biochemical parameters in leaves and roots.

Parameters[a]	Abiotic Stressors	SMFs	Cultivars	Abiotic Stressors x SMFs	Abiotic Stressors x Cultivars	Cultivars x SMFs	Abiotic Stressors x SMF x Cultivars
Leaf Samples							
•OH	269.384***	76.557***	8.127^{ns}	52.878***	2.796^{ns}	0.723^{ns}	0.758^{ns}
H_2O_2	162.147***	25.605***	20.262***	28.576***	0.504^{ns}	0.184^{ns}	0.330^{ns}
FRAP	2.317^{ns}	2.697^{ns}	2.078^{ns}	11.232***	1.858^{ns}	2.285^{ns}	1.144^{ns}
Chl*a+b*	80.930***	21.335***	1.061^{ns}	3.785*	0.416^{ns}	0.103^{ns}	0.043^{ns}
Chl*a*	83.471***	24.804***	1.406^{ns}	3.505*	0.704^{ns}	0.084^{ns}	0.063^{ns}
Chl*b*	26.620***	4.972**	0.213^{ns}	3.634*	0.137^{ns}	0.531^{ns}	0.206^{ns}
CAR	6.972**	0.080^{ns}	0.217^{ns}	4.535**	0.750^{ns}	0.534^{ns}	0.491^{ns}
Root Samples							
•OH	181.663***	41.456***	0.037^{ns}	83.892***	0.102^{ns}	2.102^{ns}	2.265^{ns}
H_2O_2	212.429***	46.441***	70.635***	35.048***	10.257***	9.892***	8.387***
FRAP	2.397^{ns}	1.762^{ns}	0.813^{ns}	23.577***	4.739^{ns}	2.491^{ns}	0.290^{ns}

^{ns}Not Significant;* $p<0.05$; ** $p<0.001$; *** $p<0.0001$.

Table 8. Antioxidant enzyme activities of 5-old-day *in vitro* germinated wheat leaf samples (*T. aestivum* L. cv. Nina) pre-treated to non-uniform SMF in various times with and without 60g L^{-1}PEG6000 or 100 mMNaCl.

Experimental Groups	Total-SOD (U mg^{-1} protein)	Fe-SOD (U mg^{-1} protein)	Mn-SOD (U mg^{-1} protein)	Cu/Zn-SOD(U mg^{-1} protein)	POX (ΔA_{470} mg^{-1} protein)	CAT (ΔA_{240} mg^{-1} protein)	APX (ΔA_{290} mg^{-1} protein)	GR (U mg^{-1} protein)
Control	164.33^{a*}	32.58^{a}	54.84^{a}	76.91^{a}	104.78^{a}	3.37^{a}	30.71^{a}	156.85^{a}
2.2 s	206.16^{b}	55.59^{b}	55.91^{a}	94.66^{b}	195.25^{b}	5.54^{b}	52.88^{b}	225.30^{b}
19.8 s	207.37^{b}	57.26^{b}	54.86^{a}	95.25^{b}	205.89^{b}	5.68^{b}	53.91^{b}	235.14^{b}
60 gL^{-1} PEG6000	283.16^{b}	87.46^{c}	74.59^{b}	121.11^{c}	362.76^{c}	8.44^{c}	65.94^{c}	575.64^{c}
2.2 s + 60 gL^{-1} PEG6000	213.50^{b}	65.46^{bc}	52.68^{a}	95.36^{b}	252.29^{bc}	5.93^{b}	49.94^{b}	355.49^{bc}
19.8 s +60 gL^{-1} PEG6000	219.58^{b}	67.03^{bc}	55.98^{a}	96.57^{b}	260.60^{bc}	5.31^{b}	51.67^{b}	390.90^{bc}
100 mMNaCl	305.58^{c}	91.22^{c}	83.98^{b}	130.38^{c}	256.77^{c}	9.02^{c}	67.23^{c}	606.06^{c}
2.2 s + 100 mMNaCl	234.12^{bc}	71.29^{bc}	61.09^{a}	101.74^{b}	205.60^{b}	6.74^{bc}	52.58^{b}	364.94^{bc}
19.8 s + 100 mMNaCl	240.14^{bc}	69.60^{bc}	61.22^{a}	109.32^{bc}	222.68^{b}	6.79^{bc}	55.59^{b}	374.79^{bc}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests.

Effects of SMF pre-treatments with and without the combinations of PEG 6000 or NaCl on antioxidant enzymes

Activities of antioxidant enzymes in leaf and root samples of both wheat cultivars under the pre-treatments with and without the combination of PEG6000 or NaCl are presented in Table 8-11. Antioxidant enzyme activities increases under SMF pre-treatments were statistically significant ($p<0.005$). For Nina, SMF pre-treatment resulted in maximum increases in total-SOD, POX, APX, CAT and GR activities were observed in the leaf samples of 26.2, 96.5%, 75.6%, 68.6% and 49.9% relative to the control; in root samples they also increased by 34.2%, 41.7%, 40.5%, 67.7% and 70.5%, respectively. Similar increases were observed in Flamura-85. Among the three SOD isozymes (Fe-SOD, Mn-SOD and Zn/Cu-SOD), Fe-SOD was affected more than the others by SMF pre-treatment.

Cultivars exposed to 60 gL^{-1}PEG6000 or 100 mMNaCl were statistically different from the control (at $p<0.05$ level). As for the combined applications of SMF and 60 gL^{-1} PEG6000 or 100 mMNaCl, there were statistical differences in stimulating antioxidant enzyme activity in leaf and root samples of both wheat cultivars except Mn-SOD ($p<0.05$). SMF pre-treatment of Nina with 60 gL^{-1} PEG6000 had maximum increases in total-SOD, POX, APX, CAT and GR activities in leaf samples of 33.6%, 148.7%, 68.3%, 76% and 149.2%; in the root samples increases were 161.5%, 41.4%, 54.7%, 86.8% and 146.8%, respectively. Similar increases were observed under the combination of SMF pre-treatment with 100 mMNaCl. Exposed to SMF and 60 gL^{-1} PEG6000, antioxidant enzyme activity increased in Flamura-85 leaf and root samples. Compared to the control, maximum increases for total-SOD, POX, APX, CAT and GR were 36.8%, 39.5%, 43.4%, 51.5% and 86.30% in leaf samples and 28.98%, 72.15%, 16.08%, 98.15% and 60.34% in root samples, respectively. Similar increases were observed under the combination of pre-treatment and 100 mMNaCl in both wheat cultivars.

A three-way ANOVA showed statistically significant effects on antioxidant enzyme activities in leaf samples for individual effects of SMF pre-treatment and abiotic stress, except for Mn-SOD activity, and under individual and combined interaction effects SMF pre-treatments, abiotic stresses, and cultivars (Table 14). As for root samples, a three-way ANOVA indicated statistically significant effects on the enzyme activities of SOD isozymes and POX under individual SMF pre-treatment, on the all antioxidant enzyme activities under individual effects of abiotic stress, on the enzyme activities of SOD isozymes, POX, CAT and APX under interactions of SMF and abiotic stresses and on the activity of APX under combined effects of abiotic stresses and cultivars and SMF, abiotic stress and cultivars (Table 15). There was no statistically significant differences between the two cultivars, in both leaf and root samples, except for APX activity in root samples.

Effects of SMF pre-treatments with and without the combinations of PEG 6000 or NaCl on the contents of GSH and GSSG and the ratios of GSH/GSSG

The effects of pre-treatment with and without 60 gL^{-1} PEG6000 or 100 mM NaCl on GSH, GSSG contents and GSH/GSSG ratios are presented in Tables 12 and 13. According to these results, GSH and GSSG were stimulated in all groups under SMF pre-treatment alone and combined with NaCl or PEG6000. These increases were remarkable with 60 gL^{-1} PEG6000 or 100 mM NaCl. The applications of SMF with NaCl or PEG 6000 also changed GSH and GSSG content. GSH/GSSG ratios decreased under the combined application of SMF with and without 60 gL^{-1} PEG6000 or 100 mMNaCl. These reductions ratios were greater under PEG6000 or NaCl treatment more than individual SMF pre-treatment or combined with PEG6000 or NaCl.

A three-way analysis of variance showed that statistical significance ($p<0.005$) on the contents of GSH, GSSG and GSH/GSSG ratios in leaves and roots under individual effects of SMF and abiotic stresses. There was no statistically significant difference between the two cultivars (Tables 14-15).

Table 9. Antioxidant enzyme activities of 5-old-day in vitro germinated wheat root samples (T. aestivum L. cv. Nina) pre-treated to non-uniform SMF in various times with and without 60 gL^{-1}PEG6000 or 100 mMNaCl.

Experimental Groups	Total-SOD (U mg^{-1} protein)	Fe-SOD (U mg^{-1} protein)	Mn-SOD (U mg^{-1} protein)	Cu/Zn-SOD (U mg^{-1} protein)	POX (ΔA_{470} mg^{-1}protein)	CAT (ΔA_{240} mg^{-1}protein)	APX (ΔA_{290} mg^{-1}protein)	GR (U mg^{-1} protein)
Control	224.93^{a*}	59.12^{a}	74.16^{a}	91.65^{a}	203.63^{a}	8.11^{a}	18.04^{a}	200.58^{a}
2.2 s	293.33^{b}	91.04^{b}	76.22^{a}	126.07^{b}	277.14^{b}	13.51^{b}	25.03^{b}	321.21^{b}
19.8 s	301.81^{b}	95.93^{b}	75.30^{a}	130.58^{b}	288.45^{b}	13.60^{b}	25.35^{b}	341.96^{b}
60 gL^{-1} PEG6000	464.77^{c}	164.99^{c}	97.49^{b}	202.29^{c}	445.19^{b}	17.28^{c}	32.43^{c}	736.70^{c}
2.2 s +60 gL^{-1} PEG6000	347.32^{bc}	118.51^{bc}	81.58^{a}	147.23^{bc}	278.98^{ab}	15.15^{bc}	27.61^{bc}	491.19^{bc}
19.8 s + 60 gL^{-1} PEG6000	363.05^{bc}	128.36^{bc}	84.28^{a}	150.41^{bc}	287.96^{b}	15.02^{bc}	27.91^{bc}	495.02^{bc}
100 mMNaCl	518.57^{c}	188.75^{c}	111.20^{b}	218.62^{c}	463.43^{ab}	18.93^{c}	34.95^{c}	739.85^{c}
2.2 s + 100 mMNaCl	377.03^{bc}	129.72^{bc}	88.25^{ab}	159.06^{bc}	235.29^{a}	16.80^{bc}	27.65^{bc}	471.70^{bc}
19.8 s + 100 mMNaCl	390.80^{bc}	133.93^{bc}	91.73^{ab}	165.14^{bc}	231.51^{a}	16.39^{bc}	27.25^{bc}	474.78^{bc}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests.

Table 10. Antioxidant enzyme activities of 5-old-day *in vitro* germinated wheat leaf samples (*T. aestivum* L. cv. Flamura-85) pre-treated to non-uniform SMF in various times with and without 60 gL^{-1} PEG6000 or 100 mM NaCl.

Experimental Groups	Total-SOD (U mg^{-1} protein)	Fe-SOD (U mg^{-1} protein)	Mn-SOD (U mg^{-1} protein)	Cu/Zn-SOD (Umg^{-1} protein)	POX (ΔA_{470} mg^{-1}protein)	CAT (ΔA_{240} mg^{-1}protein)	APX (ΔA_{290} mg^{-1}protein)	GR (U mg^{-1} protein)
Control	140.59^{a*}	33.28^{a}	47.69^{a}	59.62^{a}	147.72^{a}	3.90^{a}	22.37^{a}	146.57^{a}
2.2 s	180.05^{b}	55.12^{b}	49.37^{a}	75.56^{b}	190.82^{b}	7.51^{b}	33.07^{b}	166.04^{a}
19.8 s	183.06^{b}	58.30^{b}	47.87^{a}	76.89^{b}	190.74^{b}	7.58^{b}	34.91^{b}	165.56^{a}
60 gL^{-1} PEG6000	242.84^{c}	78.27^{c}	73.03^{b}	91.54^{c}	238.57^{c}	6.91^{c}	37.79^{c}	482.71^{b}
2.2 s + 60 gL^{-1} PEG6000	192.33^{ab}	69.92^{bc}	44.54^{a}	77.87^{a}	204.98^{bc}	5.69^{bc}	31.87^{b}	270.04^{c}
19.8 s + 60 gL^{-1} PEG6000	171.37^{b}	68.55^{bc}	41.03^{b}	81.79^{bc}	206.07^{bc}	5.91^{bc}	32.07^{b}	273.06^{c}
100 mMNaCl	270.95^{c}	85.72^{c}	82.92^{c}	102.31^{c}	253.92^{c}	8.29^{b}	38.45^{c}	547.29^{d}
2.2 s + 100 mM NaCl	201.06^{bc}	74.16^{c}	45.46^{a}	81.44^{bc}	204.14^{bc}	6.54^{c}	30.46^{b}	267.17^{c}
19.8 s + 100 mMNaCl	202.27^{bc}	71.72^{bc}	48.43^{a}	82.12^{bc}	208.54^{bc}	6.14^{c}	31.29^{b}	254.94^{c}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests.

Table 11. Antioxidant enzyme activities of 5-old-day *in vitro* germinated wheat root samples (*T. aestivum* L. cv. Flamura-85) pre-treated to non-uniform SMF in various times with and without 60 gL^{-1} PEG6000 or 100 mM NaCl.

Experimental Groups	Total-SOD (Umg^{-1} protein)	Fe-SOD (Umg^{-1} protein)	Mn-SOD (Umg^{-1} protein)	Cu/Zn-SOD (Umg^{-1} protein)	POX (ΔA_{470} mg^{-1}protein)	CAT (ΔA_{240} mg^{-1}protein)	APX (ΔA_{290} mg^{-1}protein)	GR (Umg^{-1} protein)
Control	176.57^{a*}	49.55^{a}	57.71^{a}	69.31^{a}	246.11^{a}	6.48^{a}	7.90^{a}	168.49^{a}
2.2 s	251.65^{b}	97.55^{b}	65.32^{a}	88.78^{b}	314.85^{b}	10.01^{b}	10.59^{b}	215.23^{b}
19.8 s	251.92^{b}	93.33^{b}	68.66^{a}	89.93^{b}	319.64^{b}	10.09^{b}	10.95^{b}	245.43^{b}
60 gL^{-1} PEG6000	271.42^{c}	91.10^{b}	78.22^{a}	102.10^{c}	533.09^{c}	15.58^{c}	13.03^{c}	559.56^{c}
2.2 s + 60 gL^{-1} PEG6000	227.73^{ab}	80.72^{b}	60.03^{a}	86.98^{b}	418.12^{bc}	12.31^{bc}	9.04^{a}	270.15^{b}
19.8 s + 60 gL^{-1} PEG6000	225.82^{ab}	79.35^{b}	59.25^{a}	87.22^{a}	423.67^{bc}	12.84^{bc}	9.17^{b}	268.27^{b}
100 mMNaCl	277.55^{c}	87.54^{b}	71.73^{a}	118.28^{c}	570.94^{c}	16.07^{c}	14.59^{c}	555.24^{c}
2.2 s + 100 mMNaCl	210.11^{ab}	69.27^{b}	55.97^{a}	84.87^{b}	435.50^{bc}	13.26^{bc}	10.19^{b}	251.00^{b}
19.8 s + 100 mMNaCl	212.05^{ab}	70.64^{b}	55.84^{a}	85.57^{b}	446.80^{bc}	13.03^{bc}	10.24^{b}	251.93^{b}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests.

Table 12. GSH and GSSG content and GSH/GSSG ratios in leaf and root samples of 5-old-day *in vitro* germinated Nina wheat, pre-treated to non-uniform SMF in various times with and without 60 gL^{-1} PEG6000 or 100 mMNaCl

The Part of Organs	Experimental Groups	GSH nmolmg^{-1}protein	GSSG nmolmg^{-1}protein	GSH/GSSG
Leaves	**Control**	131.57^{a*}	35.57^{a}	3.70^{a}
	2.2 s	150.53^{a}	47.88^{b}	3.14^{a}
	19.8 s	153.33^{a}	49.76^{b}	3.08^{a}
	60 gL^{-1}PEG6000	289.22^{b}	114.97^{c}	2.52^{b}
	2.2 s+60 gL^{-1} PEG6000	207.65^{c}	75.88^{bc}	2.73^{b}
	19.8 s+60 gL^{-1} PEG6000	204.06^{c}	75.55^{bc}	2.70^{b}
	100 mMNaCl	272.75^{b}	109.12^{c}	2.49^{b}
	2.2 s+100 mMNaCl	199.74^{c}	75.15^{bc}	2.66^{b}
	19.8 s+100 mMNaCl	195.75^{c}	75.43^{bc}	2.60^{b}
Roots	**Control**	163.77^{a*}	42.22^{a}	3.88^{a}
	2.2 s	211.09^{b}	66.38^{b}	3.18^{a}
	19.8 s	219.65^{b}	69.53^{b}	3.16^{a}
	60 gL^{-1}PEG6000	354.99^{c}	174.23^{c}	2.04^{b}
	2.2 s+60 gL^{-1} PEG6000	284.43^{bc}	132.69^{d}	2.14^{b}
	19.8 s+60 gL^{-1} PEG6000	293.94^{bc}	135.68^{d}	2.17^{b}
	100 mMNaCl	387.67^{c}	195.77^{c}	1.98^{b}
	2.2 s+100 mMNaCl	286.75^{bc}	129.71^{d}	2.21^{b}
	19.8 s+100 mMNaCl	289.77^{bc}	127.99^{d}	2.26^{b}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests

Table 13. GSH and GSSG content and GSH/GSSG ratios in leaf and root samples of 5-old-day *in vitro* germinated Flamura-85 wheat, pre-treated to non-uniform SMF in various times with and without 60 gL^{-1} PEG6000 or 100 mM NaCl.

The Part of Organs	Experimental Groups	GSH nmolmg^{-1}protein	GSSG nmolmg^{-1}protein	GSH/GSSG
Leaves	**Control**	111.78^{a*}	29.57^{a}	3.78^{a}
	2.2 s	167.33^{b}	51.65^{b}	3.24^{a}
	19.8 s	174.31^{b}	54.30^{b}	3.31^{a}
	60 gL^{-1}PEG6000	329.25^{c}	127.86^{c}	2.57^{b}
	2.2 s+60 gL^{-1} PEG6000	269.05^{d}	97.13^{bc}	2.76^{b}
	19.8 s+60 gL^{-1} PEG6000	264.67^{d}	95.66^{bc}	2.77^{b}
	100 mMNaCl	352.75^{c}	142.81^{c}	2.47^{b}
	2.2 s+100 mMNaCl	259.79^{d}	93.78^{bc}	2.77^{b}
	19.8 s+100 mM NaCl	255.88^{d}	93.39^{bc}	2.74^{b}
Roots	**Control**	143.58^{a*}	37.59^{a}	3.82^{a}
	2.2 s	185.21^{b}	57.52^{b}	3.22^{a}
	19.8 s	192.22^{b}	59.14^{b}	3.25^{a}
	60 gL^{-1}PEG6000	374.11^{c}	178.15^{c}	2.10^{b}
	2.2 s+60 gL^{-1} PEG6000	297.43^{d}	127.63^{bc}	2.33^{b}
	19.8 s+60 gL^{-1} PEG6000	301.09^{d}	126.64^{bc}	2.38^{b}
	100 mMNaCl	393.91^{c}	192.15^{c}	2.05^{b}
	2.2 s+100 mMNaCl	306.55^{d}	131.00^{bc}	2.34^{b}
	19.8 s+100 mMNaCl	309.11^{d}	133.26^{bc}	2.32^{b}

* Different letters indicate significant differences ($p<0.05$) among the experimental groups according to one-way ANOVA, post hoc LSD tests.

Table 14. Statistical significance of F value (three-way ANOVA) for individual and combined effect of static magnetic field, abiotic stress and cultivar for antioxidant defence parameters of leaf samples from 5-day-old *in vitro* germinated wheat.

Treatments	df	T-SOD	Fe-SOD	Mn-SOD	Cu/Zn-SOD	POX	CAT	APX	GR	GSH	GSSG	GSH/GSSG
SMFs	2	8.263^{**}	18.372^{***}	4.841^{ns}	6.236^{*}	5.642^{*}	15.159^{***}	8.360^{*}	62.291^{***}	18.742^{***}	60.082^{***}	1.077^{ns}
Abiotic Stressors	2	23.820^{***}	86.621^{***}	0.466^{ns}	13.164^{***}	154.049^{***}	16.426^{***}	35.936^{***}	617.324^{***}	345.666^{***}	367.473^{***}	86.140^{***}
Cultivars	1	0.755^{ns}	0.720^{ns}	0.296^{ns}	0.660^{ns}	2.402^{ns}	0.022^{ns}	1.781^{ns}	0.513^{ns}	0.392^{ns}	0.109^{ns}	0.070^{ns}
SMFs x Abiotic Stresseors	4	66.461^{***}	33.512^{***}	1.574^{ns}	33.568^{***}	62.272^{***}	43.698^{***}	13.834^{***}	50.232^{***}	33.297^{***}	52.872^{***}	16.126^{***}
SMFs x Cultivars	2	0.514^{ns}	0.066^{ns}	0.055^{ns}	0.941^{ns}	0.068^{ns}	0.311^{ns}	0.125^{ns}	0.065^{ns}	0.332^{ns}	0.193^{ns}	0.298^{ns}
Abiotic Stressors x Cultivars	2	0.711^{ns}	0.402^{ns}	0.048^{ns}	0.399^{ns}	0.098^{ns}	0.039^{ns}	0.252^{ns}	0.046^{ns}	0.147^{ns}	0.825^{ns}	1.015^{ns}
SMFs x Abiotic Stressors x Cultivars	4	0.098^{ns}	0.137^{ns}	0.020^{ns}	0.103^{ns}	0.163^{ns}	0.165^{ns}	0.160^{ns}	0.031^{ns}	0.037^{ns}	0.512^{ns}	0.235^{ns}

^{ns}Not Significant;* $p<0.05$; ** $p<0.001$; *** $p<0.0001$.

Table 15. Statistical significance of F value (three-way ANOVA) for individual and combined effect of static magnetic field, abiotic stress and cultivar for antioxidant defence parameters of root samples from 5-day-old *in vitro* germinated wheat.

Treatments	df	T-SOD	Fe-SOD	Mn-SOD	Cu/Zn-SOD	POX	CAT	APX	GR	GSH	GSSG	GSH/GSSG
SMFs	2	60.495^{*}	27.543^{*}	19.948^{*}	10.729^{*}	201.380^{*}	33.912^{*}	39.661^{*}	1.129^{ns}	49.866^{*}	66.830^{*}	1.971^{ns}
Abiotic Stressors	2	614.390^{*}	307.754^{*}	75.676^{*}	235.072^{*}	107.813^{*}	125.888^{*}	148.822^{*}	13.340^{*}	437.658^{*}	988.932^{*}	662.576^{*}
Cultivars	1	0.561^{ns}	1.617^{ns}	1.597^{ns}	0.078^{ns}	1.441^{ns}	0.420^{ns}	13.054^{*}	0.554^{ns}	0.023^{ns}	1.259^{ns}	4.067^{ns}
SMFs x Abiotic Stresseors	4	99.145^{*}	23.786^{*}	37.293^{*}	61.005^{*}	145.196^{*}	18.124^{*}	90.169^{*}	1.632^{ns}	45.340^{*}	50.720^{*}	7.847^{*}
SMFs x Cultivars	2	0.058^{ns}	0.148^{ns}	0.239^{ns}	0.424^{ns}	0.060^{ns}	0.129^{ns}	4.387^{ns}	0.067^{ns}	0.158^{ns}	0.084^{ns}	0.554^{ns}
Abiotic Stressors x Cultivars	2	0.044^{ns}	0.494^{ns}	0.393^{ns}	0.100^{ns}	0.024^{ns}	0.225^{ns}	13.697^{*}	0.009^{ns}	0.058^{ns}	0.115^{ns}	1.468^{ns}
SMFs x Abiotic Stressors x Cultivars	4	0.009^{ns}	0.135^{ns}	0.128^{ns}	0.215^{ns}	0.302^{ns}	0.166^{ns}	5.879^{*}	0.208^{ns}	0.024^{ns}	0.153^{ns}	0.368^{ns}

^{ns}Not Significant; * $p<0.001$

Discussion

In this study, we investigated interactive effect of artificial SMF pre-treatments and abiotic stressors on the germination stages of two different wheat cultivars with assessing growth parameters (germination rate, seedling vigour index, average plant and root fresh weights and average plant and root heights) and some biochemical parameters (chloroplast pigments (Chl*a+b,a, b* and CAR), free radicals ($^{\bullet}OH$ and H_2O_2), FRAP content and antioxidant defence parameters including activities of SOD isozymes, CAT, POX, APX and GR and GSH and GSSG contents).

According to previous reports, SMF pre-treatment increased germination performance or growth in tomatoes (De Souza et al. 2006), soybeans (Shine et al. 2012) and cotton (Bilalis et al. 2013) with different combinations of MF density, frequency and exposure time. For this reason, some researchers suggested that MF pre-treatment under appropriate conditions is a more environmentally-friendly approach than fertilizer to increase yield performance (Bilalis et al. 2013). MF affects radical-pair mechanisms in the cell (Galland and Pazur 2005) and influences cell membrane structures by changing cell membrane permeability, ion transportation and mineral uptake (Shine et al. 2012). Common opinion among the scientist, these effects could be biological responses in cells (Podleśny et al. 2005; De Souza et al. 2006; Wang et al. 2008; Shine et al. 2012; Bilalis et al. 2013), and in another general view harsh environmental conditions (salinity and drought) decrease growth parameters in plants (Wang et al. 2009; Bhardwaj et al. 2012). Our study was consistent with previous ones in terms of seed germination ratios, seedling vigour indexes, average plant and root fresh weights and average plant and root heights. These measures of growth decreased with salinity and drought compared to the control, but increased with individual and combined applications of SMF for both abiotic stressors.

Metabolically active cells produce ROS due to biological functions, such as mitochondrial respiration, chloroplast electron transport chain and peroxisome/glyoxisome activity. Bailly (2004) and El-Maarouf et al. (2008) reported that cell-controlled ROS accumulation could play a beneficial role in germination and growth by changing gene expression during seed development, dormancy and germination, protecting against pathogen-attacks, elongating cell walls, regulating of redox signalling and interacting with abscisic acid and gibberellins transduction pathways. Drought and salinity strongly induce ROS accumulation in germinated seeds. If these accumulations exceed a controlled amount, ROS restrict plant development and reduce crop yields (Wang et al. 2009; Pratap and Sharma 2010). The energy level of MF is not enough to break molecular or chemical bonds for creating ROS in the cell, but it can influence nuclear and electron spins of ROS via Zeeman splitting, hyperfine interaction, electron exchange and dipole-dipole interaction (Galland and Pazur 2005). As a result, the kinetics and yield of chemical reactions are affected due to spin precession rates of unpaired electrons and consequent effects on the lifetime or concentration of free radicals (Timmel et al. 1998). Like us, Podleśny et al. (2005), Shine et al. (2012) and Bhardwaj et al. (2012) measured increasing ROS concentrations in pea, soybean and cucumber seedlings under MF pre-treatment. Additionally, Hajnorouzi et al. (2011) detected an increase in total antioxidant capacity via the DPPH method in maize under MF treatment.

Increase in photosynthetic capacity is another important factor for biomass increase during plant growth (Shine et al. 2012). Thylakoid membranes of chloroplasts are the major source of singlet oxygen ($^1O_2$) as a result of photo-excitation of chlorophyll-to-chlorophyll triplet state, which then reacts with O_2. An increased-chlorophyll-triplet-state in photosynthetic machinery reduces chlorophyll content in plant leaf because of the degradation in chlorophyll molecules. Hakala-Yatkin et al. (2011) reported that external MF application protects plants against strong light by slowing oxygen production. Carotenoid, which are located within the thylakoid membranes of the chloroplast, are vital for detoxifying $^1O_2$ and

triplet chlorophyll, besides absorbing light in appropriate wavelengths and transferring it to the chlorophyll. There was an increase in ratios of Chl*a*+*b*, Chl*a* and *b* content for potato, mung bean and maize leaves under MF conditions (Chen et al. 2011; Rakosy-Tican et al. 2005; Shine and Guruprasad 2012). Additionally, Rakosy-Tican et al. (2005); Shine and Guruprasad (2012), reported that MF treatment increased CAR content in the cell. In the presented study, we have found similar results about Chlorophylls and carotenoids contents.

As we mentioned above, ROS play a dual role during plant development. Lower concentrations of ROS are involved in cell signaling, acclimation and cross-tolerance while their higher concentrations are extremely harmful to cellular components (Reddy and Raghavendra 2006). For example, H_2O_2 is involved in programing cell death, somatic embryogenesis, response to wounding, root gravitropism and ABA-mediated stomata closure, besides damaging of cellular macromolecules. It can be formed from $O_2^{\bullet-}$ by SOD as well as by spontaneous dismutation in the cell.

In our study, total-SOD enzyme activities increased under 2.2 and 19.8 s SMF pre-treatment in both wheat cultivars. The activity of Fe-SOD, Zn/Cu-SOD and Mn-SOD changed, and Fe-SOD was the most affected. POX is one of the key enzymes in plants and has a dual-function depending on the site of production and developmental stage of the plant, such as cytosolic POX (Compound I) detoxified H_2O_2, whereas the Compound III of POX catalyses the generation of $^{\bullet}OH$ from $O_2^{\bullet-}$ derived H_2O_2 via the *hydroxylic cycle* (Liszkay et al. 2003). POX is also involved in cell wall construction, differentiation and plant response to biotic and abiotic stress (Ghamsari et al. 2007). CAT is another important ion contained in the H_2O_2-scavenging enzyme within the cell (Scandalios et al. 1997). The combination of APX and GR can also remove H_2O_2 via the Ascorbate-Glutathione (AsA–GSH) cycle. GSH and GSSG are other important components, which are involved in the AsA–GSH cycle, an H_2O_2-scavenging pathway. GSH can react chemically with $O_2^{\bullet-}$, $^{\bullet}OH$ instead of H_2O_2 and therefore plays an important role in intracellular defence against ROS. GSH–GSSG is one of the crucial redox pairs in the cell, and the balance between them is central to maintaining a state of cellular redox. This ratio is maintained by GR (Foyer and Noctor 2011). Sumugat (2004) reported lower activities of SOD POX, APX and GR decreased seed germinability in the stored seed; the post-harvesting activity of priming increased these enzyme activities. This result showed that seed vigour is highly correlated with these antioxidant enzymes activities.

In our study, the enzyme activities of POX, CAT, APX and GR and the content of GSH and GSSG were enhanced while the ratios of GSH/GSSG decreased under SMF pre-treatment in all groups. MF treatment increased CAT activity by up to 95% in soybean seedlings (Shine et al. 2012) and 69% in *Chlorella vulgaris* (Wang et al. 2008), SOD activity up to 87% in tobacco suspension-culture (Sahebjamei et al. 2007) and 124% in *Chlorella vulgaris* (Wang et al. 2008), cytosolic POX activity less than 50% in *Chlorella vulgaris* (Wang et al. 2008) and up to 27% in soybean (Shine et al. 2012), but decreased CAT and APX activities in tobacco suspension-culture (Sahebjamei et al. 2007) and SOD and APX activities in soybean (Shine et al. 2012).

The general assumption is that tolerance or sensitivity to drought or salinity in plants is well correlated with inherent antioxidant responses. In this study, the activity of antioxidant enzymes (total-SOD, Fe-SOD, Mn-SOD, Cu/Zn-SOD, CAT and POX), which are responsible for ROS detoxification, increased in leaf and root samples of both wheat cultivars under PEG 6000 and NaCl-treatment. Under stress conditions, the activities of APX and GR and the contents of GSH and GSSG increased while GSH/GSSG dramatically decreased. APX, GR, GSH and GSSG are important components of AsA–GSH cycles in the intracellular compartment. Wang et al. (2009) detected that a treatment of 35% PEG or 200 mM NaCl, induced H_2O_2 content and SOD and APX activity in Xinmu No. 1 and Northstar alfalfa varieties. In black gram, the highest rate of PEG 6000 treatment

significantly decreased germination percentage, seedling growth parameters, amylase activity, chlorophyll and carotenoid contents but increased the activities of SOD, CAT and POX (Pratap and Sharma 2010).

In our study, the combined effect of 2.2 and 19.8 s SMF pre-treatment with 60 gL^{-1} PEG6000 or 100 mMNaCl significantly stimulated the activity of total-SOD, Fe-SOD, Cu/Zn-SOD, CAT and POX in all groups. As for AsA–GSH cycles' components (APX, GR, GSH and GSSG), their activities and contents increased remarkably with 2.2 and 19.8 s SMF and 60 gL^{-1} PEG6000 or 100 mM NaCl. The ratio of GSH/GSSG increased slightly in all groups, between 6.37–14.15%, compared to the application of only 60 gL^{-1} PEG6000 or 100 mMNaCl. In *Cucumis sativus* L., Piacentini et al. (2001) detected that EMF treatment enhanced growth parameters and the activities of SOD, CAT and GR and also senescence-delay effects.

Conclusion

This study addressed effects of artificial SMF pre-treatments on abiotic stress in the germination stages of two different wheat cultivars depending on assessing the physiological (germination rate, seedling vigour index, average plant and root fresh weights and average plant and root lengths) and biochemical (chloroplast pigments (Chl*a+b,a, b* and CAR), free radicals ($^{\bullet}OH$ and H_2O_2), FRAP content and antioxidant defence parameters including activities of SOD isozymes, CAT, POX, APX and GR and GSH and GSSG contents). Appropriate SMF pre-treatment times affected changes in the intercellular redox in the cell. Perturbed antioxidant defence systems are important to combat abiotic and biotic stress factors during plant development and improving crops against harsh environmental conditions is a top priority for breeders. But the breeding process takes quite a long time, and sometimes breeders need temporary and quick solutions to combat harsh environmental conditions. These results show that static magnetic field pretreatment compensated for the negative effects of drought and salinity stresses on the growth parameters in both cultivars duo to stimulating the antioxidant defence system, especially in an agriculturally important crop, in order to reduce yield losses and to provide breeders with temporary and quick solutions.

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