



# Could buckwheat and spelt be alternatives to wheat under future environmental conditions? Study of their physiological response to drought

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## ABSTRACT

Drought is one of the most harmful abiotic stresses for plants. Future drought episodes are expected to increase, negatively affecting globally cultivated crops as wheat (*Triticum aestivum*). Together with the crop dependency, future global food security is at risk. Thus, finding alternative crops for wheat capable of resisting drought episodes is essential to ensure food supply. Spelt (*Triticum spelta*) and buckwheat (*Fagopyrum esculentum*) are two underutilized alternative crops, whose drought resistance mechanisms remain unknown. We hypothesize that both species will have drought avoidance characteristics and will have a better drought response than wheat. We grew wheat, spelt and buckwheat in a greenhouse. Drought treatment started 28 days after sowing. When wheat and spelt reached 40 % field capacity (FC), drought was kept for 1-week, while in buckwheat the drought was kept for 4-weeks at 20 % FC. Even if partially inhibited by drought, buckwheat showed an extremely higher total biomass, as well as an extremely higher water use efficiency (WUE) than wheat and spelt. Photosynthetic parameters were extremely reduced in spelt, whereas those were less affected in wheat. The reduction in osmotic potential and the use of osmotic adjustment of spelt in combination with the increases in the antioxidant metabolism indicate the prevalence of drought tolerance mechanisms. The lack of effect on antioxidant metabolism in wheat, along with the reductions in stomatal conductance and water potential indicate the use of drought avoidance mechanisms. Buckwheat showed drought avoidance mechanisms, and its physiological parameters were almost not affected by extreme drought. Overall, spelt showed strong damages under mild drought, whereas buckwheat managed to cope with extreme drought by reducing its water requirements and increasing WUE in order to ensure its photosynthetic activity. Thus, buckwheat appears to be a potential alternative to wheat for extreme drought conditions, while spelt is not.

## 1. Introduction

Due to their nutritional value, cereals are the main source of food for both, humans and livestock (FAOSTAT, 2019). In fact, approximately 40 % of the daily calories consumed worldwide come from rice, wheat and maize, with wheat (*Triticum aestivum*) accounting for the 20 % of global consumption (FAOSTAT, 2019). Nevertheless, the global dependency on such a small number of crops has put global food security at risk, since future drought episodes will negatively impact the production of these cereals (Knox et al., 2012). Not only that, but under the future climatic scenarios, drought episodes are expected to increase, not only in number, but also in intensity (IPCC, 2014). Therefore, it is crucial to find alternative crops to reduce dependency and ensure food supply under the future drought episodes.

Drought is one of the most detrimental and limiting abiotic stresses

for crops, which causes decreases in photosynthesis, vegetative growth, number of flowers and pollen germination (Barnabás et al., 2007; Gray and Brady, 2016). In addition, drought stress contributes to an overproduction and accumulation of reactive oxygen species (ROS), which eventually results in damages to the cell membrane, inhibition of photochemical reactions and damages to the photosynthetic apparatus (Li et al., 2014). Main drought resistance mechanisms are known to be drought avoidance and drought tolerance (Fang and Xiong, 2015). Drought avoidance is defined as the ability of plants to maintain tissue water content regardless of water scarcity (Delfin et al., 2021). Among them, water-saver plants are the ones that avoid drought damage by reducing water loss via stomatal closure, increasing water use efficiency (WUE), inhibiting shoot growth and/or increasing water uptake by increasing root growth (Gilbert et al., 2011; Kooyers, 2015). On the contrary, water-spender varieties use the available water to maximize

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productivity without protecting leaf turgor, while they could also increase root depth and efficiency to maintain high water status (Delfin et al., 2021). On the other hand, drought tolerance is primarily based on the use of osmotic adjustment (OA) and ROS-scavenging systems to reduce oxidative damage (Fang and Xiong, 2015; Kooyers, 2015). A recent meta-analysis published by Li et al. (2021) showed that wild and old cultivated wheat varieties use drought avoidance mechanisms, while modern wheat varieties improved during the last decades mostly rely on drought tolerance strategies. Not only that, but under severe drought stress modern wheat varieties showed lower yields than older varieties (Li et al., 2021). Thus, finding alternative crops for wheat with improved drought avoidance mechanisms seems to have the potential to increase food supply and reduce crop dependency.

Alternative crops could be defined as crops whose cultivation is not widespread through the world, or crops that are widely cultivated in a specific region of the planet. Spelt (*Triticum spelta*) is considered to be a good nourishment source, as well as an ancestral relative of wheat whose cultivation was especially high during the Roman Empire (Campbell, 1997; Salamon et al., 2020). During the last years, spelt has regained interest among the consumers, farmers and breeders due to its high nutritional value and number of ancestral genes (Alvarez, 2021). On the other hand, buckwheat (*Fagopyrum esculentum*) is an underutilized pseudo-cereal with a high nutritional content, whose cultivation is mainly focused on some areas of Asia and Eastern Europe (Farooq et al., 2016). Despite the considerable interest they have been attracting in the recent years, very little is known about the response of spelt and buckwheat to drought stress. In fact, some preliminary work was carried out in the early 1990s analysing drought response and recovery of spelt, but it was focused primarily on tiller production (Cabeza et al., 1993). Similarly, few research have studied in detail the physiological response to drought of buckwheat (Delpérée et al., 2003; Germ et al., 2013; Aubert et al., 2020). Therefore, a huge lack of knowledge exists regarding the drought resistance mechanisms of these alternative crops.

Under future climatic conditions, we will be facing increases in drought intensity and episodes, which will reduce crop production. Using alternative crops with an improved drought avoidance mechanisms as an alternative to wheat, one of the main consumed crops, could have the potential to reduce global crop dependency and ensure food production. Therefore, the aim of this research was to characterize the drought response of the alternative crops spelt and buckwheat to drought, and determine whether they could be a suitable alternative to wheat. Since these species have not been used as much as wheat in breeding programs, we believe that they will conserve drought avoidance mechanisms and will preserve a higher plasticity to cope with drought. Thus, we hypothesize that 1) spelt will have drought avoidance characteristics; 2) buckwheat will show drought avoidance characteristics; and 3) spelt and buckwheat will have a better drought response than wheat, and thus, they will be suitable alternatives to wheat in the future.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Seeds of *T. aestivum* var. Florence Aurora (wheat), *T. spelta* var. Franckenkorn (spelt) and *F. esculentum* var. Kora (buckwheat) were obtained from the Basque Institute for Agricultural Research and Development (NEIKER, Basque Country, Spain). A total of 32 plants per species were grown in 16 pots of 3 L with a 3:1 mixture of perlite: vermiculite. The experiment was conducted in a greenhouse located at 43°19'46.9"N, 2°58'07.6"W under an average temperature of 24.6/21 °C and a relative humidity of 68/77 % for day/night. Natural light was supplemented with 2 VANQ Lamps (VQ-GLTW030) of 30 W each.

Pots were watered three times per week to keep field capacity (FC) with Hoagland's solution (Hoagland and Arnon, 1938) (well-watered treatment, WW), as well as rotated three times per week to avoid

intra-greenhouse environmental gradients. 28 days after sowing (DAS), drought was imposed by withholding irrigation in half of the pots. Plant health was monitored measuring chlorophyll content with a Minolta SPAD 502 Plus (Konica Minolta Optics, Japan) and chlorophyll fluorescence using a FluorPen FP 110 (Photon Systems Instruments, The Czech Republic). When wheat and spelt reached 40 % ± 5 FC, this stress was kept for 1-week (mild drought treatment, MD). However, unlike in wheat and spelt, when buckwheat reached 40 % FC, we observed little or no effect of drought on the measured parameters, as well as a lack of damages in the aerial tissues of the drought treatment plants. Therefore, we decided to increase drought intensity in buckwheat: FC was reduced to 20 % ± 5, and kept for 4-weeks (extreme drought treatment, ED).

All the in vivo measurements and plant material harvesting were performed at the end of the drought treatment: 59 DAS in wheat and spelt (31 days of drought) and 68 DAS in buckwheat (40 days of drought).

### 2.2. Soil and leaf water parameters

Cumulative transpiration of eight pots per species and treatment was calculated by weighing pots three times per week before and after watering. Relative soil water content (RSWC) was determined as  $RSWC = 100 (SFW - SDW) / (SFW_i - SDW)$ , where SFW, SDW and SFW<sub>i</sub> were the soil fresh weight, soil dry weight and the initial soil fresh weight, respectively.

Leaf water potential ( $\Psi_w$ ) of four leaves per species and treatment was measured using the Scholander pressure-equilibration technique (Scholander et al., 1965) six h after dawn. Due to its high  $\Psi_w$  value, it was not possible to quantify the  $\Psi_w$  using pressure chambers method in buckwheat, as previously reported by Delpérée et al. (2003). Leaf osmotic potential ( $\Psi_o$ ) of the same four leaves was measured by analysing the freezing point of sap of leaf segments using an OSMOMAT 030 cryoscopic osmometer (Gonotec GMBH, Berlin, Germany) and calculated as  $\Psi_o = M \times T \times R$ , where M was the concentration (osmol·kg<sup>-1</sup>), T was the temperature of the sample (298 K) and R was the molar gas constant (0.00832 L·MPa·K<sup>-1</sup>·mol<sup>-1</sup>). The osmotic potential at full turgor ( $\Psi_o^{100}$ ) was measured similar to  $\Psi_o$ . Full turgor of leaves was obtained by cutting four leaves per species and treatments and incubating them in deionized water at 4 °C in dark for 24 h. Pressure potential ( $\Psi_p$ ) was calculated as the difference between  $\Psi_w$  and  $\Psi_o$ . Cell wall elasticity ( $\epsilon$ ) was calculated as  $\epsilon = (\Psi_p^{100} - \Psi_p) / (100 - RLWC) \times 100$ .

Relative leaf water content (RLWC) was calculated as the  $RLWC = (FW - DW) / (TW - FW) \times 100$ , where FW, DW and TW were the leaf fresh weight, dry weight and turgid weight, respectively. Four leaves per species and treatment were used in wheat and spelt, and eight leaves per treatment in buckwheat. Dehydration (DH) of plants under drought treatments was calculated as the difference of  $\Psi_o$  and  $\Psi_o^{100}$  between treatments. Osmotic adjustment (OA) was calculated as the difference of  $\Psi_o^{100}$  of plants under drought and control conditions.

### 2.3. Gas exchange and photochemical parameters

Gas exchange parameters of six fully developed leaves per species and treatment were measured using a Li-Cor 6400 (Li-Cor Inc., Lincoln, NE, USA). Measurements for wheat and spelt were made on the leaf preceding the Flag Leaf, while in buckwheat the measurement was made on the fifth fully developed leaf counting from the top. The temperature of the cuvette was held at 24 °C at a relative humidity of 60 %. Measurements were made three h after dawn under a photosynthetic flux density of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , provided by a red/blue LED light source (model LI 6400-40, Li-Cor Inc.). Intercellular CO<sub>2</sub> concentration (Ci), stomatal conductance (gs), net photosynthetic CO<sub>2</sub> assimilation (A) and instantaneous transpiration (E) were calculated according to von Caemmerer and Farquhar (1981). The carboxylation capacity (A/Ci) was calculated as the ratio between A and Ci.

Quantum yield of PSII ( $\Phi_{PSII} = (F_m' - F_s) / F_m'$ ) was determined with

an integrated leaf fluorescence chamber (Li-6400–40; Li-Cor Inc.) by measuring  $F_s$ , the variable fluorescence at steady state, and  $F_m'$ , the maximum light-adapted fluorescence (Schindler and Lichtenthaler, 1996). Electron transport rate ( $ETR$ ) was determined as  $ETR = \Phi_{PSII} \times PPFD \times 0.85 \times 0.5$ , where  $PPFD$  refers to the Photosynthetic Photon Flux Density, and the ratio of electron transport rate to net photosynthetic  $CO_2$  assimilation ( $ETR/A$ ) was calculated as the ratio between  $ETR$  and  $A$ .

#### 2.4. Antioxidant parameters

Leaves of two plants per pot were pooled as one biological replicate for antioxidant enzyme activity measurements. Four replicates per species and treatment were used. Activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) were measured as described in Pérez-López et al. (2009) with little modifications.

Briefly, SOD, CAT and GR were extracted in a buffer composed by 50 mM Tris-HCl (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2 % Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 2 mM dithiothreitol. Supernatant from samples were filtered over Sephadex G-25 columns equilibrated with 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA and 0.2 % Triton X-100. CAT was measured at 240 nm as described by Aebi (1984). GR activity was measured at 340 nm according to Edwards et al. (1990). Activity of SOD was measured at 550 nm as described by McCord and Fridovich (1969). APX was extracted in a mixture of 50 mM  $KH_2PO_4$  and  $K_2HPO_4$  (pH 7.8) containing 0.1 mM EDTA, 0.2 % Triton X-100, 5 mM cysteine, 2 mM ascorbate (AsA) and 0.1 mM PMSF. APX activity was measured at 290 nm in accordance with Hossain and Asada (1984). All enzyme activities were expressed on a protein basis, which was measured according to the Bradford (1976) method.

#### 2.5. Growth parameters and informatics measurements

Leaves, stems and roots of four plants per species and treatment were harvested in wheat and spelt, and eight plants per treatment in buckwheat. Plant organs were oven-dried for 72 h for dry-weight (DW) measurements.  $WUE$  was calculated by dividing plant DW and cumulative transpiration.

Photos of the harvested leaves were taken for measuring leaf area (LA) with the open-source software Fiji (Fiji Is Just ImageJ; Schindelin et al., 2012) prior to oven-drying them. Stomatal density (SD) was measured by obtaining leaf imprints from adaxial and abaxial surfaces as explained by Casado-García et al. (2020). Photos of stomata were taken using a Nikon ECLIPSE 50i fluorescence microscope (Nikon corporation, Japan) with a Leica DFC 420 C camera (Leica Microsystems, Germany). Three photos of the adaxial and three photos of the abaxial surfaces of four plants per species and treatment were used. Measurements were made with LabelStoma tool (Casado-García et al., 2020).

Briefly explained, photos were loaded into LabelStoma and the scale and area to be analysed were established. Automatic stomata detection was performed using the default parameters. Each photo was reviewed individually and, if necessary, undetected stomata were added manually.

#### 2.6. Statistical analysis and figures

All figures were performed using GraphPad Prism version 8.2.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Statistical analysis was done using SPSS version 27.0 for Windows (IBM Corp, Armonk, NY). The effect of water treatment (WW, MD and ED), species (wheat, spelt and buckwheat) and their interaction were determined by two-way ANOVA. Afterwards, data was compared using Duncan's Post-Hoc test.  $P$  values < 0.05 were considered statistically significant.

### 3. Results

#### 3.1. Growth parameters

Buckwheat showed increased growth under WW, especially for aboveground organs, which were 12 times bigger than in wheat and spelt (Fig. 1A–D). Likewise, the biomass accumulation of buckwheat under ED was significantly reduced by approximately 45 % for all organs, while among wheat and spelt we only found leaf DW to be significantly reduced in spelt (Fig. 1A). As occurred with the growth, buckwheat showed significantly higher shoot/root ratio and LA (Fig. 1E–F), which were significantly reduced under ED. On the contrary, among wheat and spelt, we only found LA to be significantly reduced by drought in spelt (Fig. 1F).

Buckwheat had a  $WUE$  of 4.3 g DW  $kg^{-1}$   $H_2O$  under control conditions, while for wheat and spelt these values were of 1.76 and 1.64 g DW  $kg^{-1}$   $H_2O$ , significantly smaller (Fig. 2). Neither wheat nor spelt  $WUE$  was affected by MD, whereas buckwheat showed a significantly higher  $WUE$ , with values 24 % higher than under WW and 2.7 times bigger than in wheat and spelt (Fig. 2).

#### 3.2. Water relations

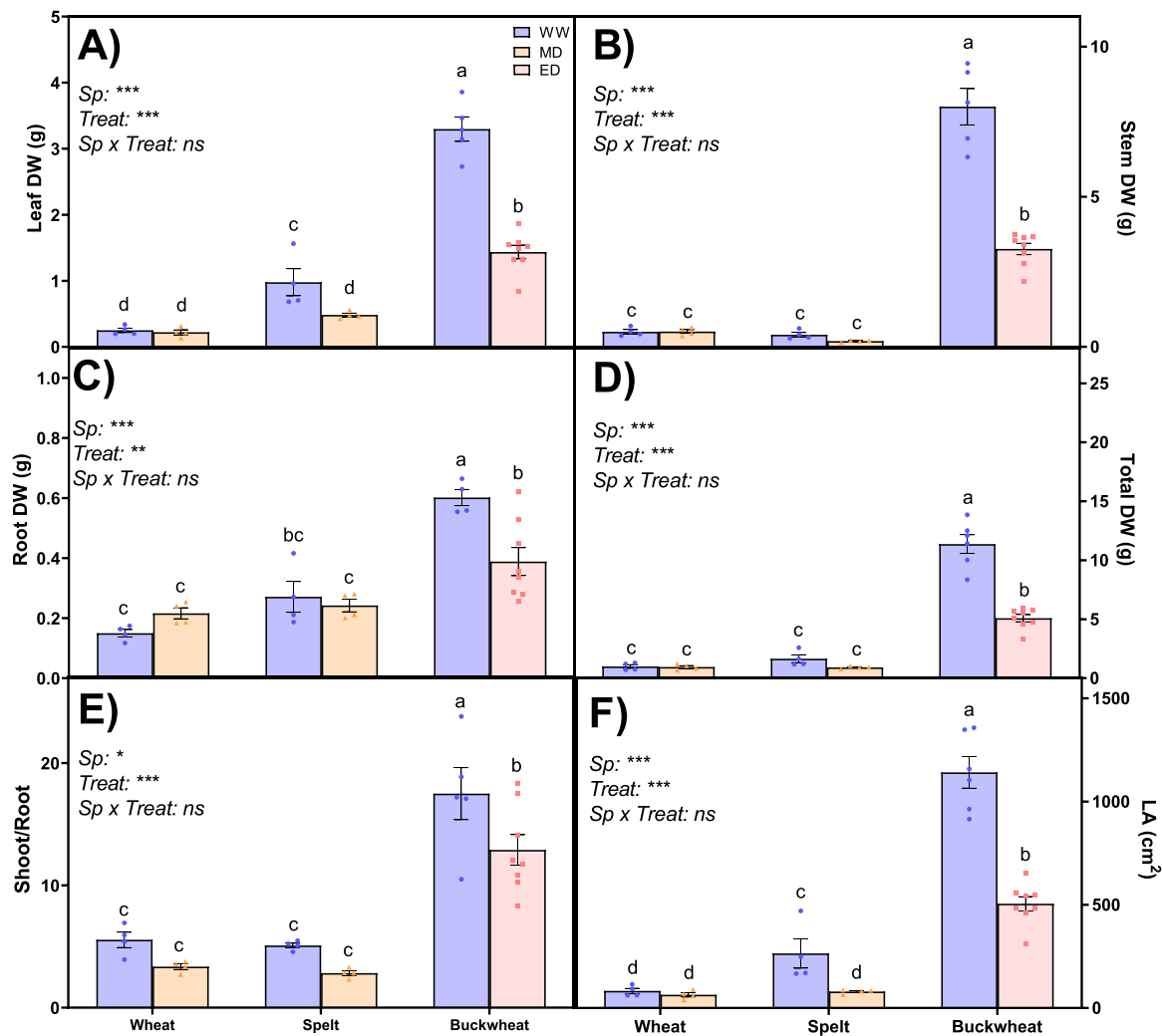
We observed a progressive decrease in  $RSWC$  for wheat and spelt, reaching 40 %  $\pm$  5 after 47–49 DAS, while this value was reached at 35–36 DAS in buckwheat (Fig. 3). Similarly, 20 %  $\pm$  5  $RSWC$  was reached in buckwheat at 39–40 DAS.

Spelt showed a significantly higher cumulative transpiration under control conditions (Fig. 4), which was significantly reduced in a 41 % after the MD treatment. Buckwheat had the lowest value of cumulative transpiration, and was significantly reduced by 17 %. Under 20 % FC, buckwheat managed to keep extremely lower cumulative transpiration rates per week, showing a 50–60 % reduction from start to end of the drought treatment.

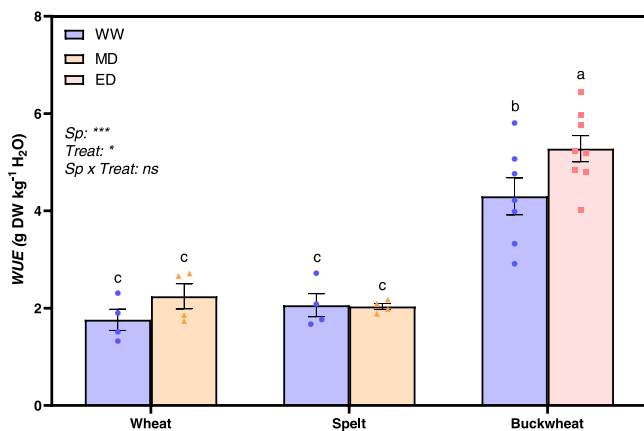
Both, wheat and spelt, showed significantly lower  $\Psi_w$  under MD treatment (Table 1). Not only that, but spelt showed a significant reduction in  $\Psi_o$ , reaching a value of  $-2.607$  MPa, while  $\Psi_o$  was not affected in wheat nor in buckwheat.  $\varepsilon$  showed an opposite trend in wheat and spelt when exposed to drought: it increased from 8.667 MPa to 14.705 MPa in wheat, whereas it decreased from 37.520 MPa to 5.348 MPa in spelt. The  $RLWC$  was significantly reduced by 10.5 % in spelt, while it remained similar to control conditions in wheat and buckwheat. Also, spelt showed a  $DH$  level of 0.937 MPa, which was significantly higher than the  $DH$  values of wheat (0.491 MPa) and buckwheat (0 MPa). In the same way, spelt showed an  $OA$  three times higher than the rest of the species. Thus, it appears that the water parameters of buckwheat were not affected by ED, while under MD the same parameters for spelt and wheat were highly and slightly affected, respectively.

#### 3.3. Gas exchange parameters

Spelt showed significantly higher values of  $g_s$  and  $E$  under control conditions than wheat and buckwheat (Fig. 5A–B), and thus, was the most affected by the drought treatment. MD induced an extreme reduction of 75 %  $g_s$  in spelt, which was turned into a 72 % reduction of  $E$ . In wheat, drought significantly reduced  $g_s$  and  $E$  by 58 % and 56 %. On the contrary, although significant, the reductions in buckwheat were only of 39 % and 36 %. Also, although the values of  $\Phi_{PSII}$  were similar for the three species, spelt was the only species to have it significantly reduced (Fig. 5C). Drought significantly reduced  $A$  by half in wheat and by 92 % in spelt, while it had not significant effect on buckwheat (Fig. 5D).  $C_i$  was not affected in wheat by the drought treatment, while in spelt it was significantly increased (Fig. 5E). Nevertheless, despite the lack of effect in  $C_i$  in wheat and its accumulation in spelt,  $A/C_i$  was significantly reduced in both species, with this reduction being caused



**Fig. 1.** Effect of well-watered (WW), mild drought (MD) and extreme drought (ED) conditions in wheat, spelt and buckwheat in **A)** leaf dry-weight (DW), **B)** stem DW, **C)** root DW, **D)** total DW, **E)** shoot/root ratio and **F)** leaf area (LA). Each bar represents mean  $\pm$  standard error (S.E.) of at least 4 replicates. The applied drought treatments are represented as well-watered (WW, light blue bars), mild drought (MD, light orange bars) and extreme drought (ED, light red bars). Blue circles, orange triangles and red squares are used to represent single replicates under WW, MD and ED, respectively. Different letters are used to represent values significantly different between treatments and species ( $P < 0.05$ ). Results for a two-way ANOVA for species (*Sp*), treatment (*Treat*) and their interaction are represented as ns (non-significant), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ).



**Fig. 2.** Effect of WW, MD and ED conditions in water use efficiency (WUE) in wheat, spelt and buckwheat. Each bar represents mean  $\pm$  S.E. of at least 4 replicates. Growth conditions and statistical analysis are explained in Fig. 1.

by A (Fig. 5D-F). In buckwheat, although *C<sub>i</sub>* was slightly decreased, *A/C<sub>i</sub>* remained unchanged under the ED treatment (Fig. 5E-F). Even though the three species had similar values of *ETR* under control conditions, *ETR* of spelt was significantly reduced by 73 % and *ETR/A* was significantly increased by 254 % under MD (Fig. 5G). Similarly, *ETR/A* values were significantly increased in wheat by 110 %, mainly driven by *A* decrease (Fig. 5G-H). On the contrary, ED showed no influence on *ETR* nor *ETR/A* in buckwheat. Overall, it seems that the photosynthetic parameters of spelt are extremely damaged by MD, while the same parameters are less affected in wheat and not affected at all in buckwheat under ED.

Buckwheat showed significantly higher abaxial *SD* than wheat and spelt under WW, while this value was significantly lower for the adaxial *SD* (Fig. 6). However, the sum of the abaxial and the adaxial *SD* did not differ among the three species under control conditions. On the contrary, under the drought treatments, wheat and spelt showed no differences neither in the *SD* of both sides of the leaves nor in the total *SD*, while in buckwheat there was a significant increase in the adaxial, abaxial and total *SD* (Fig. 6).

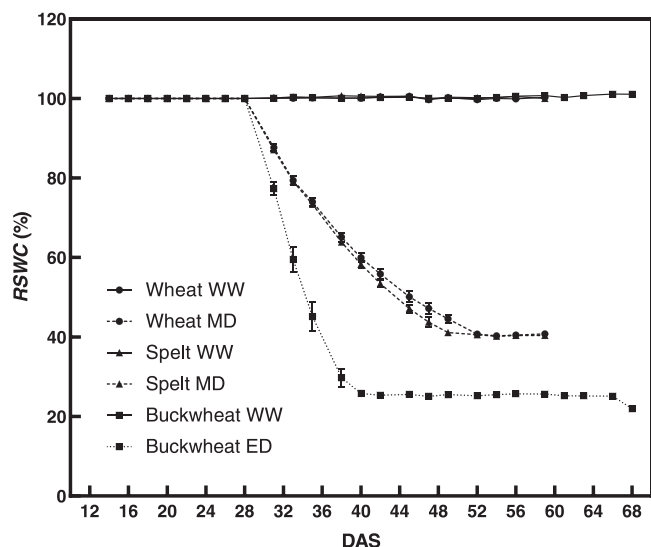


Fig. 3. RSWC over time for well-watered (WW), mild drought (MD) and extreme drought (ED) treatments in wheat, spelt and buckwheat. Solid lines are used to represent WW treatments, dashed lines represent MD treatments and dotted lines represent ED. Circles, triangles and squares represent results for wheat, spelt and buckwheat, respectively. Each point represents the mean  $\pm$  S.E. of 8 replicates.

### 3.4. Antioxidant metabolism

We found no effect of drought treatments on the antioxidant metabolism enzymes in wheat nor buckwheat (Fig. 7A–D). APX activity was significantly increased 160 % in spelt under MD, while we did not find any remarkable effect on SOD, CAT and GR (Fig. 7A–D).

## 4. Discussion

The global dependency to a few number of crops, along with the

increases in number and intensity of the drought episodes threatens global food security (IPCC, 2014). Modern wheat varieties have shown decreases in production when exposed to severe drought stress (Li et al., 2021), and thus, it is essential to find alternative crops to wheat with the ability to resist drought stress. Here, we have characterized the drought response of wheat and two alternative species: spelt and buckwheat. Our research provides data which demonstrates that 1) spelt activates drought tolerance mechanisms in the presence of a mild intensity drought, even though these mechanisms are not enough to withstand a mild drought treatment; 2) buckwheat activates drought avoidance mechanisms and manages to cope with an extreme drought treatment better than wheat does with a mild drought; and 3) buckwheat may have the potential to be used as an alternative crop to wheat under extreme drought conditions.

Among all the crops, buckwheat stood out in terms of growth, since under control conditions it showed an average biomass accumulation 7 and 12 times higher than spelt and wheat, respectively (Fig. 1A–F). In order to test whether the differences in total biomass among species were explained due to the differences in growth days, we analysed the relative growth rate (RGR, Sup. Fig. 1), which showed that there were barely any differences. Given the exceptionally higher biomass accumulation of buckwheat, the reported growth reduction for this species under extreme drought was not particularly surprising. Even though the overall growth of buckwheat was inhibited, we observed a decrease in the shoot/root ratio when exposed to drought, supporting previous hypothesis on buckwheat growth response to drought (Aubert et al., 2020). In spite of everything, buckwheat was the species with higher biomass under both water treatments, suggesting an improved use of water resources.

As a matter of fact, we found buckwheat to have higher levels of WUE under well-watered conditions, as well as maintaining higher levels of WUE under drought. This is a highly valued trait in crop breeding programs (Richards et al., 2002; Condon et al., 2004), and thus, exploitation of the high WUE of buckwheat to get improved drought-resistant crops could be worthwhile. In addition to having a lower WUE, wheat and spelt showed no variation in this parameter when exposed to drought stress, which indicates the ability of buckwheat to control water

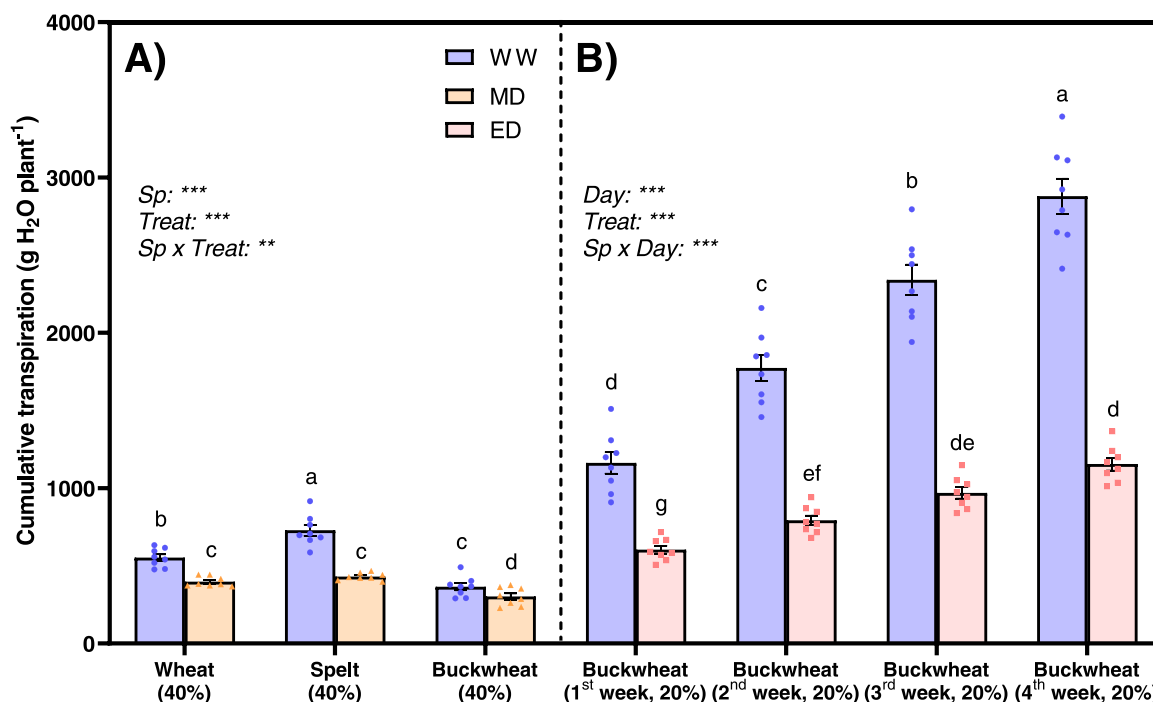


Fig. 4. Effect of drought in cumulative transpiration when A) achieving 40 % FC in wheat, spelt and buckwheat and B) achieving 20 % FC and its evolution over 4-weeks in buckwheat. Each bar represents mean  $\pm$  S.E. of 8 replicates. Growth conditions and statistical analysis are explained in Fig. 1.



**Table 1**

Effect of WW, MD and ED conditions in water potential ( $\Psi_w$ ), osmotic potential ( $\Psi_o$ ), pressure potential ( $\Psi_p$ ), cell wall elasticity ( $\epsilon$ ), relative leaf water content (RLWC), dehydration (DH) and osmotic adjustment (OA). Growth conditions and statistical analysis are explained in Fig. 1. For each species and treatment, mean  $\pm$  S.E. of at least 4 replicates are showed. Different letters are used to represent values significantly different among species and treatment ( $P < 0.05$ ).

Species	Treatment	$\Psi_w$ (MPa)	$\Psi_o$ (MPa)	$\Psi_p$ (MPa)	$\epsilon$ (MPa)	RLWC (%)	DH (MPa)	OA (MPa)
Wheat	WW	-0.805 $\pm$ 0.125 <sup>a</sup>	-1.832 $\pm$ 0.123 <sup>b</sup>	1.027 $\pm$ 0.172 <sup>a</sup>	8.667	96.84 $\pm$ 0.807 <sup>ab</sup>	0.491 $\pm$ 0.102 <sup>b</sup>	0.099
	MD	-1.530 $\pm$ 0.107 <sup>b</sup>	-1.890 $\pm$ 0.153 <sup>b</sup>	0.360 $\pm$ 0.195 <sup>b</sup>	14.705	92.93 $\pm$ 0.828 <sup>b</sup>		
Spelt	WW	-1.030 $\pm$ 0.150 <sup>a</sup>	-1.336 $\pm$ 0.037 <sup>c</sup>	0.306 $\pm$ 0.156 <sup>b</sup>	37.520	97.17 $\pm$ 0.768 <sup>ab</sup>	0.937 $\pm$ 0.045 <sup>a</sup>	0.304
	MD	-1.637 $\pm$ 0.068 <sup>b</sup>	-2.607 $\pm$ 0.143 <sup>a</sup>	0.971 $\pm$ 0.211 <sup>a</sup>	5.348	86.92 $\pm$ 5.931 <sup>c</sup>		
Buckwheat	WW	–	-0.782 $\pm$ 0.011 <sup>d</sup>	–	–	98.34 $\pm$ 1.074 <sup>ab</sup>	-0.008 $\pm$ 0.015 <sup>c</sup>	0.116
	ED	–	-0.869 $\pm$ 0.079 <sup>d</sup>	–	–	99.778 $\pm$ 0.119 <sup>a</sup>		

ANOVA	$\Psi_w$	$\Psi_o$	$\Psi_p$	RLWC
Species	ns	***	ns	ns
Treatment	***	***	ns	**
Interaction	ns	***	***	ns

resources more efficiently than wheat and spelt. The better use of water resources in buckwheat could be explained by increases in water uptake and/or reductions in water loss, which are two adaptive strategies that mitigate drought impact (Blum, 2005). In this case, it was essentially explained by a strict regulation of cumulative transpiration, since buckwheat managed to restrict transpiration over time when exposed to extreme drought. The higher proportion of abaxial stomata in relation to adaxial stomata in buckwheat could be restricting cumulative transpiration, reducing the evaporative demand and thus, causing reductions in total transpiration. Besides, buckwheat showed a significant decrease in shoot/root ratio, which contributes to the better use of water resources by increasing water uptake under drought.

The decrease of  $\Psi_w$  under water scarcity is also a mechanism that plants activate to increase water uptake, and thus, it has been associated with an improved drought response (Robredo et al., 2007). Even though we were unable to quantify  $\Psi_w$  of buckwheat (see 2. Materials and Methods), we found that wheat and spelt had similar reductions in  $\Psi_w$  under mild drought. Although dehydration (passive mechanism) was the main driving force in response to drought in wheat and spelt, the degree of dehydration in spelt was double than in wheat. On the contrary, buckwheat showed no signs of dehydration, which together with the RLWC results, indicate that it was capable of maintaining an optimal water status inside the plant even if it was under an extreme drought treatment. Water scarcity promotes water flow outside the cells, and thus, dehydration commonly causes decreases in  $\Psi_p$  (Chen et al., 2020), as occurred in wheat. The accumulation of different osmolytes inside the cells (OA) could also promote decreases in  $\Psi_w$ , which increases the turgor of the cells (Nemeskéri and Helyes, 2019). This would explain the observed reductions in  $\Psi_o$  and increases in  $\Psi_p$  for spelt. Nevertheless, the activation of these mechanisms were not enough for spelt to maintain RLWC and overcome the impact of mild drought stress on the photosynthetic parameters.

Actually, the photosynthetic processes of spelt were the most damaged ones by the drought treatment. Mild drought induced an extreme stomatal closure and transpiration decrease on this species, while in wheat these reductions were more conservative. Stomatal closure is a short-term strategy to prevent water loss in most plant species, but could result in limitations of photosynthesis under water scarcity if it is maintained for long periods (Diaz-Espejo et al., 2007; Silim et al., 2009). However, in buckwheat we only found a slight closure of stomata and decrease in transpiration under extreme drought, with no significant effect on  $A$ . These results are in contradiction with previous results, which reported reductions higher than 80 % in  $E$ ,  $g_s$  and/or  $A$  under water stress (Delpérée et al., 2003; Germ et al., 2013; Aubert et al., 2020). Nevertheless, it should be noted that, as well as using distinct varieties and drought intensities, the drought treatments were combined with different levels of light (Delpérée et al., 2003) or UV-B radiation (Germ et al., 2013), which could explain the reported differences (Masojádek et al., 1991; Itam et al., 2020; Wasaya et al.,

2021). Despite the reduction in  $g_s$ , neither wheat nor spelt showed variations in  $SD$  in response to drought, while buckwheat increased it in both sides of the leaves. However, the increase in  $SD$  could be the result of a concentrating effect of stomata due to the reduction in  $LA$ . Unlike in buckwheat, photosynthesis in spelt and wheat was inhibited when exposed to drought. This inhibition of  $A$  induced decreases in  $A/C_i$  in wheat and spelt, suggesting non-stomatal limitations (Miranda-Apodaca et al., 2018). However, while we found spelt to have an extremely reduced  $\Phi_{PSII}$ , indicating a down-regulation or structural alteration of the photosynthetic apparatus (Foyer et al., 2017), we did not observe the same trend in wheat. This different photochemical sensitivity between both *Triticum* species under drought could explain the better behaviour of  $A$  in wheat. In addition, the decreases in  $A$  induced  $ETR/A$  to be significantly increased in wheat and spelt, suggesting a deviation of electrons to other processes that are not the photosynthesis.

In the present study, we found spelt to significantly increase the activity of the APX antioxidant enzyme, indicating a deviation of electrons from photosynthesis to Mehler reaction (Cruz de Carvalho, 2008). On the contrary, we found no effect of drought treatments on antioxidant metabolism of wheat and buckwheat. Upregulation of antioxidant metabolism, along with the use of OA are two key mechanisms used by the drought tolerant species to cope with drought stress (Kooyers, 2015). On the contrary, drought avoidance species mainly focus on maintaining normal physiological processes by increasing  $WUE$  and/or modifying growth rates to avoid the harmful effects caused by drought (Fang and Xiong, 2015; Delfin et al., 2021). Wild wheat and old cultivars have been reported to use primarily drought avoidance mechanisms, while modern varieties mainly rely on drought tolerance mechanisms (Li et al., 2021). Because of the few breeding programs conducted with spelt and its close relation with wheat, we initially hypothesized that it would mainly use drought avoidance strategies. Unexpectedly, spelt significantly increased OA and the ROS-scavenging enzyme APX, indicating the prevalence of drought tolerance mechanisms (Kooyers, 2015). On the contrary, our results showed that wheat based its response to mild drought on drought avoidance mechanisms. These contradictory results are not necessarily incompatible, considering that drought resistance strategies are not mutually exclusive and could alternately occur in the same species depending on the intensity of the drought (Shavrukov et al., 2017). In fact, wheat has been reported to use drought avoidance, drought tolerance and even drought escape mechanisms (Izanloo et al., 2008; Dolferus, 2014; Fang et al., 2017; Shavrukov et al., 2017). Concerning buckwheat, although we found its growth to be inhibited by extreme drought, most of the analysed parameters were little or not affected. Buckwheat showed low values of  $g_s$  and  $E$  under control conditions, which were slightly reduced under extreme drought. Low  $g_s$  and  $E$  values have been related with less water loss during drought, and are interesting traits to be taken into consideration when searching drought resistant crops (Medrano et al., 2002). This would explain the observed reductions in cumulative transpiration over time and consequent

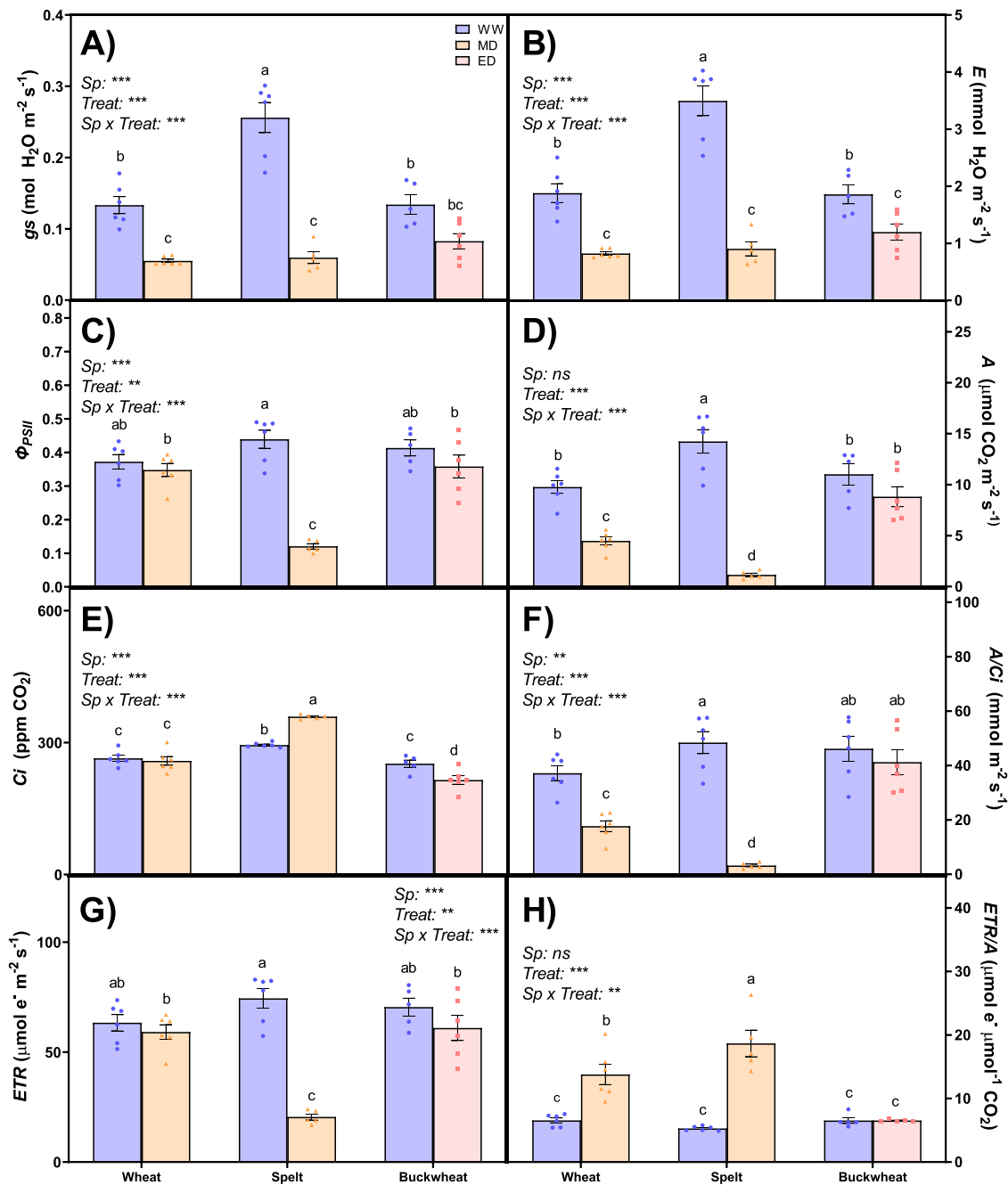
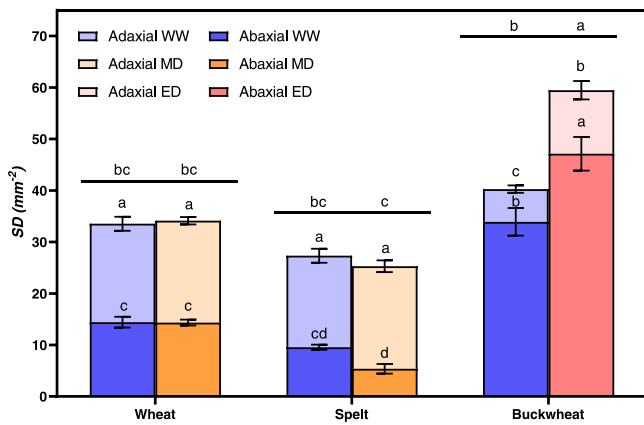


Fig. 5. Effect of WW, MD and ED conditions in wheat, spelt and buckwheat for A) stomatal conductance ( $g_s$ ), B) transpiration rate ( $E$ ), C) quantum yield of PSII ( $\Phi_{PSII}$ ), D) net photosynthetic  $\text{CO}_2$  assimilation ( $A$ ), E) intercellular  $\text{CO}_2$  concentration ( $C_i$ ), F) carboxylation capacity ( $A/C_i$ ), G) electron transport rate ( $ETR$ ) and H) the ratio of electron transport rate to net  $\text{CO}_2$  photosynthetic assimilation ( $ETR/A$ ). Each bar represents mean  $\pm$  S.E. of 6 replicates. Growth conditions and statistical analysis are explained in Fig. 1.

increases in  $WUE$  by the end of the extreme drought treatment. Thus, buckwheat appears to be a drought-avoiding species, as previous research have also suggested (Aubert et al., 2020), and more specifically, a water-saving species.

Overall, buckwheat showed a higher biomass production as well as increased levels of  $WUE$  under control conditions. In addition, it managed to cope with the imposed extreme drought treatment by increasing even more its  $WUE$  and maintaining its photosynthetic parameters. Given the perspective of the increases in the number of drought events, high  $WUE$  is considered to be a desirable trait for crops in order to reduce the use of water resources. Likewise, although we found the growth of wheat and spelt to be little or no affected by the mild drought, its negative impact on the rest of the analysed parameters

makes us believe that a drought treatment as extreme as the one applied to buckwheat would have resulted in a higher reduction of growth –or even death–. Therefore, our results show the clear potential of buckwheat over spelt to be an alternative crop for wheat under the future climatic conditions. However, it should be noted that this research has analysed the effect of drought on the vegetative growth, whose effects do not necessarily have to be similar through the reproductive period. It has previously been reported that drought has a significant impact on grain yield when imposed at vegetative stage of wheat, although this impact could be greater at reproductive stage (Tatar et al., 2015; Abid et al., 2017; Li et al., 2019; Ulfat et al., 2021). Therefore, future research should also focus on the analysis of the reproductive period, to verify whether the potential of buckwheat to be an alternative crop to wheat is



**Fig. 6.** Effect of WW, MD and ED conditions in the stomatal density (SD) of wheat, spelt and buckwheat. Blue, orange and red bars represent adaxial stomata, while light blue, light orange and light red bars represent abaxial stomata for WW, MD and ED treatments, respectively. The letters above the horizontal lines represent the statistical comparison for total stomata among species as explained in Fig. 1. Similarly, the letters above S.E. bars represent the statistical comparison for adaxial stomata among species as well as the abaxial stomata among species under drought.

not limited to the better response at vegetative growth, but also to an improved grain yield.

**5. Conclusions**

Increases in drought episodes and their intensity will be one of the main factors to cause a reduction in the production of conventional crops, including wheat (*Triticum aestivum*). Therefore, it is essential to find alternative crops that are capable of withstanding future drought episodes in order to ensure food supply. In this research we have grown wheat, spelt and buckwheat in a greenhouse and evaluated the response

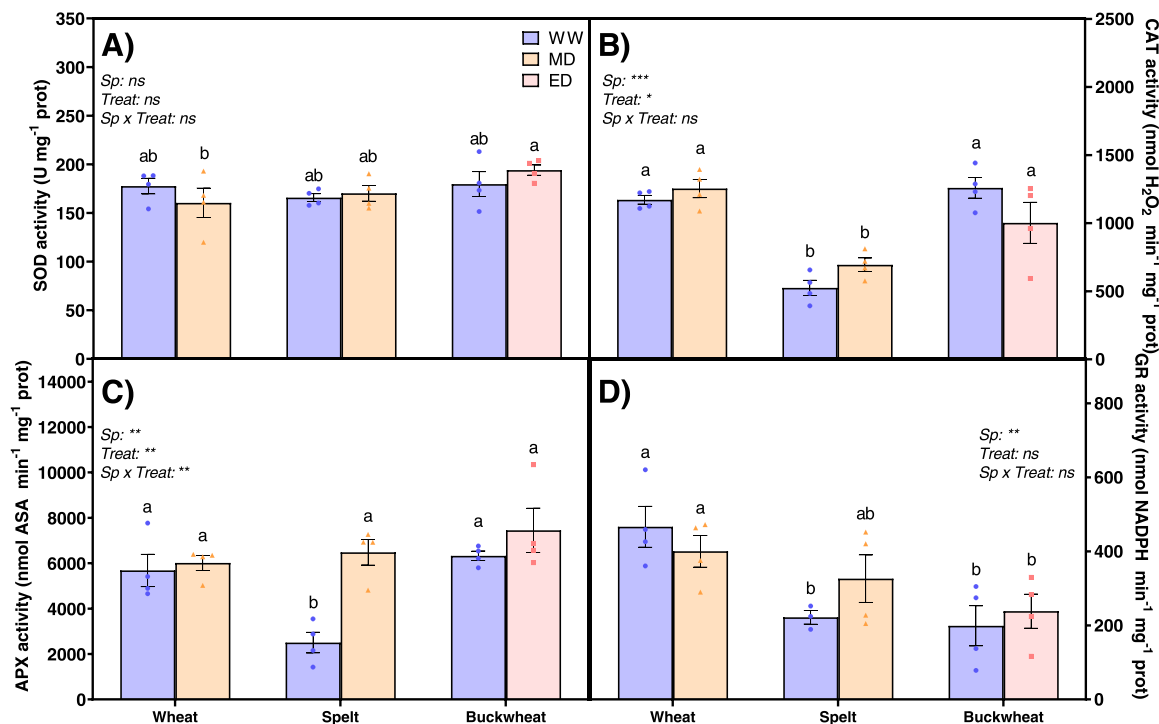
of wheat and spelt (*Triticum spelta*) to mild drought (MD), and of buckwheat (*Fagopyrum esculentum*) to extreme drought (ED). Under well-watered conditions, we found no significant differences among wheat and spelt, although buckwheat showed significantly higher biomass accumulation and water use efficiency (WUE). Under MD, we found spelt to rely on drought tolerant mechanisms, increasing antioxidant metabolism and osmotic adjustment, while wheat relied on drought avoidance mechanisms. Nevertheless, spelt was not able to overcome the MD, showing extreme reductions on the quantum yield of PSII ( $\Phi_{PSII}$ ) and net photosynthetic assimilation (A). Thus, it showed damages on its photosynthetic machinery. On the contrary, even though the stomatal conductance ( $g_s$ ), transpiration (E) and A were reduced in wheat, this reduction was not as extreme as in spelt, and it showed no damage to the photosynthetic machinery nor increases in the antioxidant metabolism. On the other hand, although its dry-weight (DW) was reduced, buckwheat was capable of coping with the ED by increasing even more its WUE and maintaining its photosynthetic parameters. Taking into consideration that buckwheat was able to withstand ED, while under MD wheat was slightly damaged and spelt was extremely damaged, our results show that buckwheat may have the potential to be an alternative crop to wheat in the future.

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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



**Fig. 7.** Effect of WW, MD and ED conditions in wheat, spelt and buckwheat for A) superoxide dismutase (SOD), B) catalase (CAT), C) ascorbate peroxidase (APX) activity and D) glutathione reductase (GR). Each bar represents mean ± S.E. of at least 3 replicates. Growth conditions and statistical analysis are explained in Fig. 1.



## Data Availability

Data will be made available on request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agwat.2023.108176](https://doi.org/10.1016/j.agwat.2023.108176).

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