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Near-isogenic wheat lines carrying altered function alleles of the *Rht-1* genes exhibit differential responses to potassium deprivation

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ABSTRACT

Most of the elements involved in the integration of signals of low external K^+-supply into a physiological response pathway remain essentially unknown. The aim of this work was to study the influence exerted by DELLA proteins, which are known to be key components for the control of growth, on plant responses during K^+ deprivation in wheat (*Triticum aestivum*) by using two sets of near-isogenic lines (NILs) in the Maringa and April Bearded cultivars. After K^+ shortage, the NILs of both cultivars containing the *Rht-B1b,Rht-D1b* alleles, which encode altered function DELLA proteins, displayed either a slight or no decrease in chlorophyll content, in contrast to the sharp decrease observed in the NILs having the wild type alleles (*Rht-B1a,Rht-D1a*). That difference was accompanied by a lower relative decrease of biomass accumulation only in the Maringa cultivar. In both cultivars, high chlorophyll retention was coupled with K^+ starvation-induced differences in superoxide dismutase and ascorbate peroxidase activities, which were enhanced in K^+-starved *Rht-B1b,Rht-D1b* NILs. In addition, *Rht-B1b,Rht-D1b* and *Rht-B1a,Rht-D1a* NILs markedly differed in the accumulation of the major cations Ca^{2+}, Na^+ and K^+. These results suggest a major role of the *Rht-1* genes in the control of physiological responses during K^+ deprivation.

1. Introduction

Potassium (K^+) is a major nutrient required by higher plants to complete their life cycle, its concentration surpassed only by that of structural nutrients such as carbon, oxygen, hydrogen and nitrogen [1]. K^+ plays a relevant role as an osmoticum, and contributes to the charge balance across the membranes as well as to the proper activity of more than 50 enzymes [2–6]. Recent evidence has demonstrated that the presence of functional inward K^+ transporters is required for the progression of the cell cycle [7,8] and that a decrease in K^+ concentration is necessary [9–11] and sufficient [12] to induce a cell death program in cell walled eukaryotic organisms. Moreover, K^+ plays an alleviating role when these organisms are faced with several abiotic stresses [13,14]. In agreement with these pivotal functions, long-term K^+ deprivation leads to visible injuries which are preceded by physiological and biochemical changes [15,16]. Not surprisingly, plants are furnished with mechanisms that allow them to cope with K^+ scarcity either by improving K^+ capture and/or K^+ use efficiency. At external K^+ concentrations below 0.5 mM, the uptake of K^+ depends mainly on the AKT1 channel and HAK1-like transporters [17,18], whose activities are finely regulated [19–21]. Although less is known on the molecular basis of K^+ use efficiency, the contribution of the above mentioned, as well as other, K^+ transporters to determining it appears to be evident [22,23]. Transcriptome analyses have suggested that the plant hormones methyl-jasmonate and ethylene play an important function during *Arabidopsis thaliana* acclimation to K^+ scarcity [24,25]. A later study confirmed the role of ethylene and opened major questions on the possible pathways involved in low K^+ signaling [26]. In spite of these important advances, it seems clear that most of the loci underlying the integration of signals of low external K^+ supply into a physiological response pathway remain essentially unknown.

Higher plants live under the influence of numerous environmental factors able to impact on growth and development. Growth results from the integration of several signals into a common response pathway. Research carried out over the last 10 years has revealed a fundamental role of DELLA proteins in controlling the growth of vascular plants [27]. Wild type versions of these nuclear proteins, whose sequences are partially related to the transcriptional regulator SCARECROW, impose a growth restriction that is

Abbreviations: APX, ascorbate peroxidase; *DD*, *Rht-B1b,Rht-D1b*; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance; NILs, near-isogenic lines; SOD, superoxide dismutase; *WT*, *Rht-B1a,Rht-D1a*.

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typically relieved by the action of gibberellins, which leads to DELLAs degradation and subsequently growth [27–29]. While DELLA mutants with loss of function display a slender phenotype, mutants with impaired gibberellin sensitivity (altered function mutants) are responsible for a dwarf phenotype and have been identified in several major crops [30–32]. DELLA proteins have also been proposed to be necessary in the acclimation responses of plants to some stress conditions as well as in light-mediated growth regulation [27]. Interestingly, *Arabidopsis thaliana* plants carrying a disruption of DELLA-encoding genes display a differential root elongation pattern during the response to phosphorus deprivation [33]. This finding indicates that some acclimatory responses to suboptimal concentrations of that mineral nutrient involve DELLA proteins. The possibility that the presence of altered DELLA versions could influence the plant performance during the shortage of other mineral nutrients, such as K^+, has not been explored. This question is particularly relevant for crops, in which the loci that determine improved K^+ use efficiency have been actively searched for [22,23,34–36].

In wheat, the *Rht-B1* and *Rht-D1* genes encode DELLA proteins. Since *Rht-B1b* and *Rht-D1b* are altered function alleles that reduce plant height and increase grain yield, they have been widely used in wheat breeding programs and an extensive literature has been devoted to their characterization [37]. In this work, we used two sets of wheat near-isogenic lines (NILs) to explore the hypothesis that the functional impairment that takes place in bread wheat (*Triticum aestivum*) plants exposed to K^+ deprivation could be influenced by the presence of different *Rht-1* alleles.

2. Materials and methods

2.1. Plant material, growth conditions and experimental design

Triticum aestivum NILs of the Maringa [38] and April Bearded [39] cultivars carrying either the wild type alleles *Rht-B1a,Rht-D1a* (hereafter named *WT*) or the altered function alleles *Rht-B1b,Rht-D1b* (hereafter named *DD*) were used. Seeds were surface-sterilized and subsequently germinated in the dark onto filter paper. Seedlings were then transferred to 0.8-L plastic pots filled with a complete nutrient solution with the following composition: 1.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.5 mM H_3PO_4, 0.15 mM NaCl; 50 μM FeEDTA, 50 μM $CaCl_2$, 25 μM H_3BO_3, 2 μM $ZnSO_4$, 2 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM molybdic acid, 2.5 mM 2-(N-morpholino)-ethanesulfonic acid (MES) and 1 mM KCl. The pH was brought to 6.0 ± 0.1 by the addition of $Ca(OH)_2$ and the solution was aerated. Except where indicated, the density of plants was set in four plants per pot. According to the increase in plant size, solutions were renewed either daily or every 2 days, which helped to minimize nutrient depletion in the pots. Temperature in the growth chamber was 22 °C (day/night) and the relative humidity 85%. The photon flux density of photosynthetically active radiation at the plant level was 180 μmol m^{-2} s^{-1}. To attenuate the potential effect of the differences in plant height on light interception, the pots containing dwarf plants were placed 4 cm higher on day 12 after sowing. On that day, half of the pots were randomly selected and received a solution of the same composition as that above described except that KCl was not included, while the other half received the complete solution detailed above. On day 28 after sowing, plants were harvested 3 h after the beginning of the day period. Shoot and root fresh weights were recorded for each individual plant. Individual leaf blades of the third and fourth leaves were used to determine chlorophylls *a* and *b*, while protein carbonylation, lipid peroxidation, and superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were determined for the blade of the fifth leaf.

2.2. Ion accumulation, chlorophyll content and leaf appearance

To determine K^+, sodium (Na^+) and calcium (Ca^{2+}) concentrations, the roots and shoots collected in each experiment were placed in plastic tubes containing HCl to allow the release of free cations [40]. After a week of treatment with HCl 1 N, the concentrations of K^+, Na^+ and Ca^{2+} in the extracts were estimated by Atomic Absorption Spectrometry through the use of a Perkin Elmer AAnalyst 100 spectrometer (emission mode for K^+ and Na^+, absorption mode for Ca^{2+}). To determine the content of chlorophyll, leaves were frozen with liquid nitrogen and stored at −80 °C. Samples were powdered in liquid nitrogen and homogenized. Chlorophyll was extracted with acetone 80% and the amount of chlorophylls *a* and *b* in the extracts determined as described by Inskeep and Bloom [41].

For Maringa NILs a detailed study of leaf appearance was performed. The stage of leaf development estimated through the procedure described by White [42] was periodically recorded 2 h after the start of the light period and plotted against the time elapsed and adjusted to a linear model. The slope of the plot rendered the number of leaves generated by day.

2.3. Electron paramagnetic resonance detection of lipid radicals

Leaves were powdered in liquid nitrogen, homogenized in 100 mM potassium phosphate buffer, pH 7.4, and incubated for 1 h at 30 °C in the presence of the spin trap α-(4-pyridyl-1-oxide)-N-t-butyl nitrone (POBN), 50 mM final concentration. Lipid radicals were detected using EPR. Spectra were recorded at room temperature using a Bruker ECS 106 spectrometer, operating at 9.81 GHz with 50 kHz modulation frequency. EPR instrument settings were as follows: microwave power, 20 mW; modulation amplitude, 1.232 G; time constant, 81.92 ms. The lipid radical adducts were quantified using standard solutions of 4-hydroxy-2,2,6,6-tetramethyl piperidine-N-oxyl (TEMPOL) as previously described [43].

2.4. Carbonyl content in proteins

Carbonyl content was determined as an estimation of oxidatively modified proteins. Leaves were powdered in liquid nitrogen, homogenized and centrifuged at 10,000 × *g* for 20 min. Carbonyl content in the supernatant was detected by reaction with 2,4-dinitrophenylhydrazine for 1 h at room temperature, vortexing every 15 min. After incubation, proteins were precipitated with 10% trichloroacetic acid, the pellet washed several times with ethanol:ethyl acetate (1:1) and resuspended with 6 M guanidine. The absorbance at 360–390 nm was measured and carbonyl content was estimated for blank and samples ($\varepsilon = 22000\ M^{-1}\ cm^{-1}$).

2.5. Enzyme assays

Wheat leaves were powdered with liquid nitrogen, mixed with 50 mM Tris–HCl (pH 7.0), 3 mM $MgCl_2$, 1 mM EDTA, 1% polyvinylpyrrolidone (pH 7.0) and centrifuged at 10,000 × *g* for 20 min. Supernatants were used to measure enzymatic activities. Ascorbate peroxidase (APX) activity was assayed in a reaction mixture containing 50 mM phosphate buffer pH 7.0, 0.5 mM ascorbic acid, 10 mM H_2O_2 and up to 20 μL of samples. The change in absorbance was followed at 290 nm [44]. Parallel measurements in the presence of p-chloromercuribenzoate 50 μM were performed to rule out interference from guaiacol peroxidases. SOD activity was assayed by measuring the inhibition of xanthine oxidase-dependent reduction of nitroblue tetrazolium. The reaction mixture contained 0.1 mM nitroblue tetrazolium, 0.1 mM EDTA, 50 μM xanthine, and xanthine oxidase in 50 mM

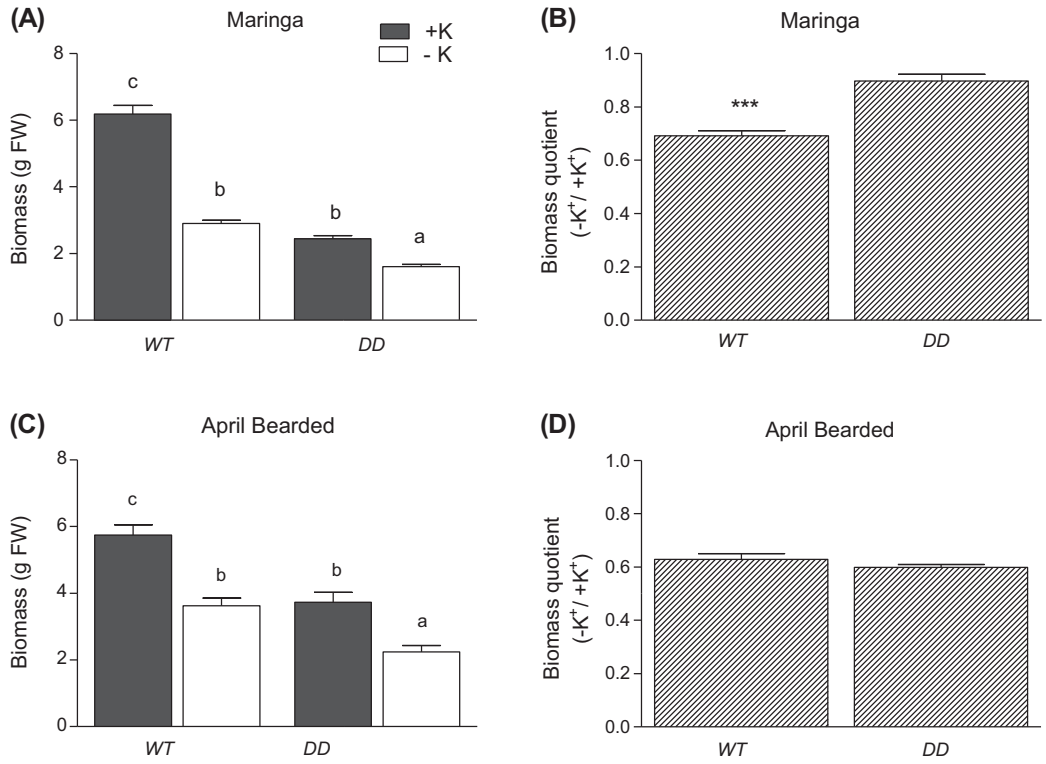

Fig. 1. Effect of long-term K^+ deprivation on biomass accumulation in *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines of *T. aestivum*. Plants were grown from day 12 until day 28 in medium with or without KCl 1 mM. (A and C) Total biomass accumulation in Maringa and April Bearded cultivars, respectively. (B and D) Relative effect of K^+ deprivation, estimated as the quotient between the values recorded in the absence and the presence of K^+ on total biomass accumulation for those cultivars, respectively. Data in (A) and (B) correspond to the mean obtained in four experiments ($n = 14$) and three experiments ($n = 12$) for (C) and (D). Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$). The *** symbol denotes differences between genotypes at $P < 0.001$.

potassium phosphate buffer (pH 7.8). One unit of SOD is considered to be the amount of enzyme that inhibits the control rate by 50% (0.025 units min^{-1} of absorbance at 560 nm).

2.6. Statistical analysis

Values obtained for plants placed in the same pot were averaged and considered as a single replicate. Three or four replicates were performed for each NIL and treatment in each experiment. All experiments were performed at least twice. Results obtained were subjected to ANOVA through the use of the Statistical 7.0 Program (StatSoft, Inc.). Post hoc comparisons were performed with Tukey's test.

3. Results

3.1. Differences between WT and DD NILs in the pattern of biomass accumulation after long-term K^+ deprivation are influenced by the genetic background

Physiological responses to long-term K^+ deprivation were first explored in the Maringa cultivar by comparing the *WT* and *DD* NILs. *DD* plants with a good supply of K^+ showed a significantly lower total biomass than *WT* plants (Fig. 1A). While K^+ deprivation markedly reduced biomass accumulation in *WT* plants, it exerted a less pronounced effect on plants bearing the mutant alleles (Fig. 1A). Since comparisons between genotypes that greatly differ in biomass accumulation can be misleading when only absolute values are taken into account, we next estimated the relative effect of K^+ deprivation in both NILs. The data obtained indicate that K^+ deprivation exerts a much lower relative effect on biomass accumulation in *DD* than in *WT* plants (Fig. 1B). This pattern was found for both the shoot and the root, while no differences between NILs were observed for the distribution of biomass between shoot and root in plants long-term deprived of K^+ (Supplemental Fig. 1). Given that our experiments were performed at a high plant density, we then examined the effect of lowering the number of plants per pot and found that it did not modify the differences found between *WT* and *DD* in the relative effect of K^+ deprivation on biomass accumulation (data not shown).

A detailed study made with this cultivar (Supplemental Fig. 2) confirmed a previous observation that the number of leaves on the main axis generated per day was similar in *WT* and *DD* NILs grown in a non-stressed medium [38]. As early reported for rye [42], long-term K^+ deprivation reduced the rate of leaf appearance, which was slightly higher in the *DD* than in the *WT* Maringa NIL (Supplemental Fig. 2). Furthermore, at the end of the experiment (day 28 after sowing), the sixth leaf became visible only in $15.0 \pm 7.6\%$ of the plants of the *WT* NIL deprived of K^+ and in $95.0 \pm 5.0\%$ of the plants of the *DD* NIL (Supplemental Fig. 2).

Interestingly, the above-mentioned differences in the effect of K^+ deprivation on relative biomass accumulation between *WT* and *DD* NILs were not observed in the April Bearded cultivar (Fig. 1, Supplemental Fig. 1).

3.2. Young leaves of Maringa and April Bearded lines containing the DD alleles display a lesser reduction of chlorophyll content when deprived of K^+ than those of the lines containing the WT alleles

Visual inspection of the plants indicated that moderately young leaves displayed acute symptoms of chlorosis in *WT* but not in the *DD* NILs deprived of K^+. Therefore, the chlorophyll content was determined. A decrease in chlorophylls *a* and *b* was observed in

Table 1
Content of chlorophylls *a* and *b* in the 3rd and 4th leaves of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) NILs of the (A) Maringa and (B) April Bearded cultivars at day 28 since sowing. Plants grew for the last 16 d in the presence or the absence of 1 mM KCl.

		3rd leaf		4th leaf	
		Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)
(A) Maringa					
WT	+K	1.374 ± 0.032^{c}	0.320 ± 0.014^{b}	1.198 ± 0.049^{b}	0.281 ± 0.013^{b}
	−K	0.760 ± 0.046^{a}	0.236 ± 0.018^{a}	0.655 ± 0.028^{a}	0.183 ± 0.008^{a}
DD	+K	1.309 ± 0.019^{bc}	0.323 ± 0.010^{b}	1.359 ± 0.016^{c}	0.326 ± 0.008^{c}
	−K	1.241 ± 0.018^{b}	0.339 ± 0.009^{b}	1.293 ± 0.012^{bc}	0.343 ± 0.008^{c}
(B) April Bearded					
WT	+K	1.297 ± 0.036^{c}	0.356 ± 0.008^{b}	1.354 ± 0.035^{b}	0.358 ± 0.006^{b}
	−K	1.056 ± 0.041^{a}	0.310 ± 0.012^{a}	0.949 ± 0.049^{a}	0.261 ± 0.016^{a}
DD	+K	1.422 ± 0.034^{d}	0.389 ± 0.008^{c}	1.500 ± 0.024^{c}	0.399 ± 0.006^{c}
	−K	1.181 ± 0.034^{b}	0.340 ± 0.011^{b}	1.357 ± 0.024^{b}	0.379 ± 0.007^{bc}

Data correspond to 2 experiments ($n = 6$ for Maringa and $n = 8$ for April Bearded, respectively). The errors correspond to SE. For each cultivar, values in the same column labeled with different letters are significantly different ($P < 0.05$).

the third and fourth leaves of Maringa *WT* plants deprived of K^+ (Table 1). Interestingly, no decrease in chlorophylls *a* and *b* was observed in *DD* plants suffering K^+ starvation. In April Bearded, in turn, the content of chlorophylls in the third leaf decreased to a similar extent in both *DD* and *WT* NILs grown in the absence of K^+. However, while the total content of chlorophylls in the fourth leaf sharply decreased for K^+-starved *WT* plants (29.3%), only a slight reduction took place in *DD* plants (8.6%) after a long-term deprivation of K^+ (Table 1). The data on chlorophyll content obtained for both cultivars suggest that *DD* NILs experience delayed senescence when deprived of K^+, which is more clearly observed in Maringa than in April Bearded.

3.3. WT and DD NILs display differences in cation accumulation

The above-mentioned differences between *DD* and *WT* NILs in chlorophyll content could eventually arise from genotypic differences in K^+ accumulation. When K^+ was included in the culture solution, no differences in tissue K^+ concentration were found between Maringa NILs, while a minor difference in the concentration of K^+ in the shoot was detected between April Bearded NILs, being slightly higher in *DD* plants. Values obtained fit well with those formerly reported in Triticeae [42]. When grown in the absence of K^+, a significant difference was observed between *WT* and *DD* NILs of both cultivars for the concentration of K^+ in the shoot, but not in the root, being higher for *DD* NILs (Fig. 2). The absolute differences found between *WT* and *DD* NILs deprived of K^+ were 11.2 and 12.0 μmol g^{-1} fresh weight (which corresponds approximately to 0.40% on a dry weight basis) for Maringa and April Bearded, respectively, which indicate that the concentration of K^+ in shoots was 19.46 and 18.60% higher for the *DD* than for the *WT* NILs of Maringa and April Bearded, respectively.

K^+ deprivation is usually accompanied by an increase in the accumulation of Na^+ [4,36]. Consistently, we found a high Na^+

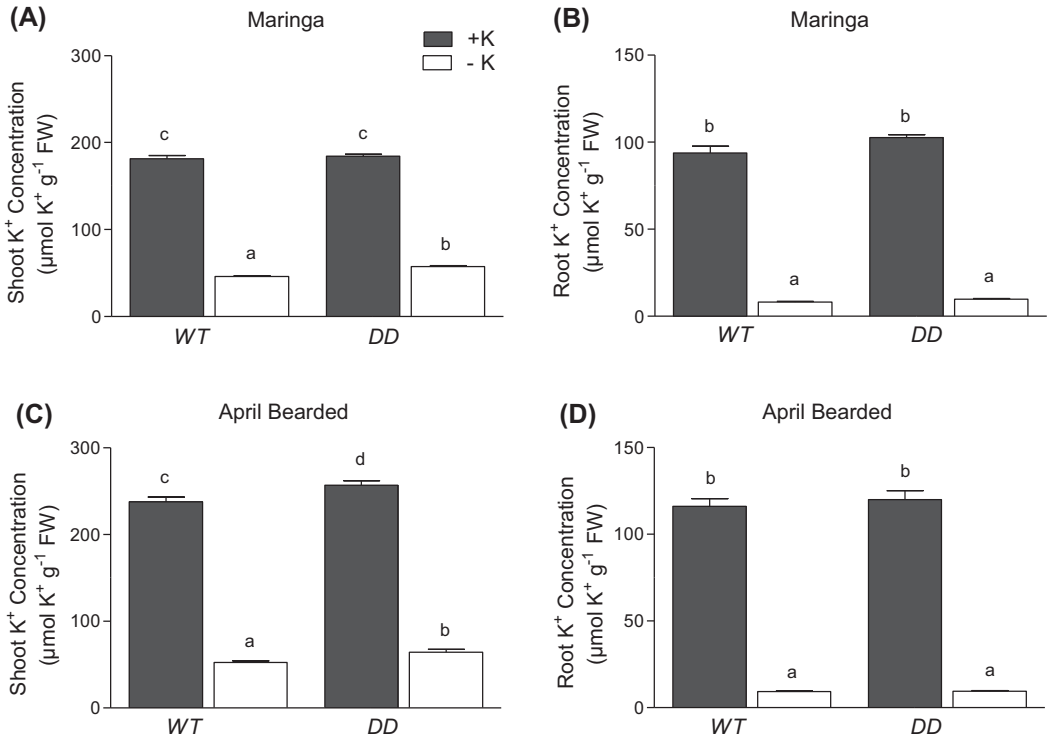

Fig. 2. Concentration of potassium in shoots (A and C) and roots (B and D) of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines grown from day 12 until day 28 in the presence or the absence of KCl 1 mM. Data in (A) and (B) correspond to the mean of three experiments ($n = 10$) for Maringa, while in (C) and (D) correspond to the mean of two experiments ($n = 8$) for April Bearded. Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$).

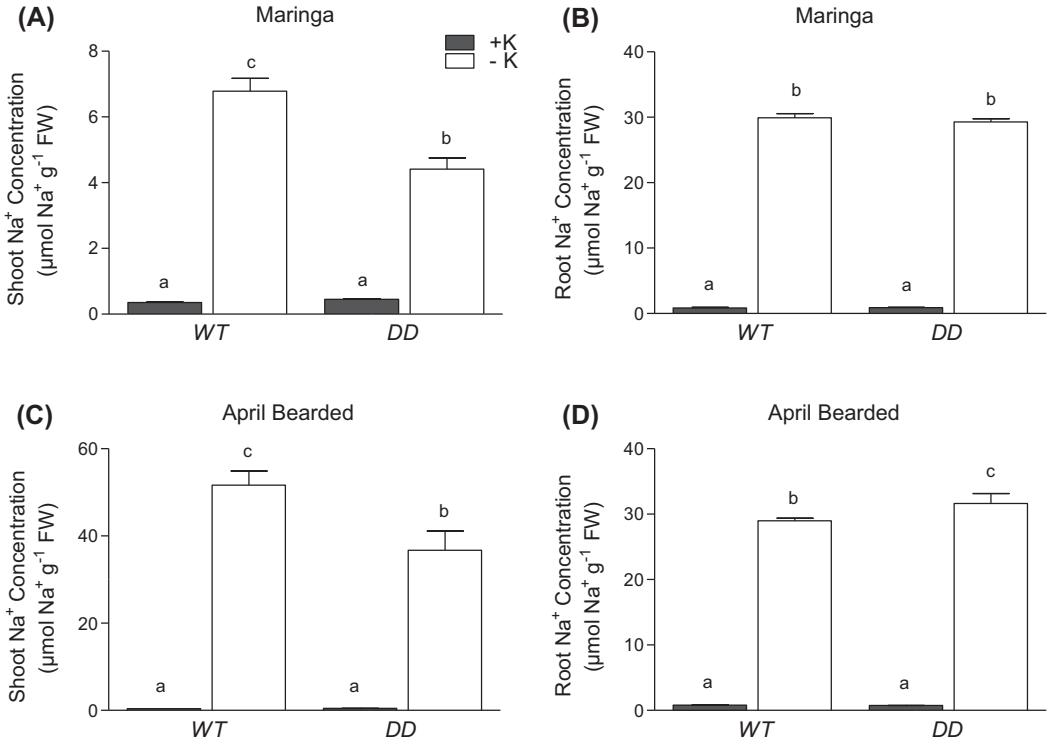

Fig. 3. Concentration of sodium in shoots (A and C) and roots (B and D) of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines grown from day 12 until day 28 in the presence or the absence of KCl 1 mM. Data in (A) and (B) correspond to the mean of three experiments ($n = 10$) for Maringa, while in (C) and (D) correspond to the mean of two experiments ($n = 8$) for April Bearded. Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$).

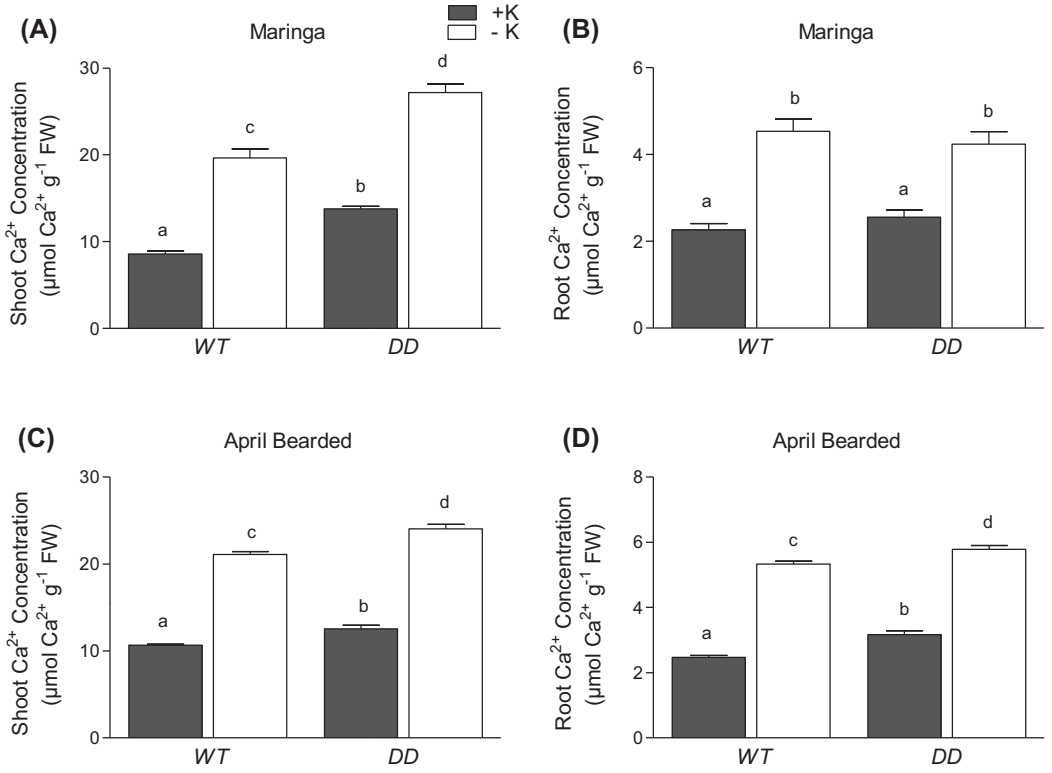

Fig. 4. Concentration of calcium in shoots (A and C) and roots (B and D) of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines grown from day 12 until day 28 in the presence or absence of KCl 1 mM. Data in (A) and (B) correspond to the mean of three experiments ($n = 10$) for Maringa, while in (C) and (D) correspond to the mean of two experiments ($n = 8$) for April Bearded. Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$).

concentration in shoots and roots of plants grown in a medium without KCl addition (Fig. 3). The concentration of Na^+ in the aerial part was similar in both NILs grown in the presence of K^+, while in the absence of KCl it was significantly higher in *WT* than in *DD* NILs for both Maringa and April Bearded (Fig. 3). This enhancement of shoot Na^+ concentration in *WT* NILs was sufficient in April Bearded, but not in Maringa, to generate a similar concentration of alkali cations ($K^+ + Na^+$) in both *WT* and *DD* NILs grown in the absence of K^+ (Supplemental Fig. 3). A comparison of the values obtained for the accumulation of Na^+ in the shoots of plants deprived of K^+ between the experiments performed with the April Bearded and Maringa cultivars indicated that it was considerably higher in the former, suggesting that Maringa behaves as a shoot Na^+ excluder when grown under this stress condition (Fig. 3).

Model cell walled eukaryotic organisms, such as Arabidopsis [16] and *Saccharomyces cerevisiae* [12], experience an enhanced Ca^{2+} accumulation when exposed to K^+ deprivation. Here, we found a clear enhancement of Ca^{2+} in the shoots and roots of Maringa and April Bearded plants deprived of K^+. Interestingly, *DD* NILs of both cultivars accumulate Ca^{2+} in shoots at a higher concentration than *WT* NILs when grown both in the presence and the absence of 1 mM KCl (Fig. 4).

3.4. WT and DD NILs differ in their antioxidant response after long-term K^+ deprivation

It has been reported that DELLA proteins could play a role in the antioxidant response of plants suffering specific stress conditions [45]. Therefore, we next explored the possibility that the differences in the decrease in chlorophyll content between *DD* and *WT* NILs when deprived of K^+ are associated with a difference in the oxidative modifications suffered by plants. In this regard, the presence of oxidatively modified proteins and the extent of lipid peroxidation in the fifth leaf were evaluated in the Maringa cultivar. As shown in Fig. 5, no significant enhancement of protein carbonylation was detected after a long-term K^+ deprivation. In turn, the content of lipid radicals sharply increased for plants long-term exposed to K^+ deprivation (Fig. 5). No significant differences between *DD* and *WT* NILs were detected for these indicators of oxidative damage (Fig. 5).

Total SOD and APX activities were also measured in the fifth leaf of Maringa and April Bearded plants (Fig. 6). No differences were found in SOD activity in either cultivar between *WT* and *DD* NILs grown in the presence of 1 mM KCl. For APX activity, in turn, a basal difference between *DD* and *WT* NILs was observed in Maringa but not in April Bearded. The activity of both enzymes increased in plants long-term deprived of K^+. In this growth condition, SOD and APX activities were significantly higher in *DD* than in *WT* NILs, the differences being more pronounced for SOD.

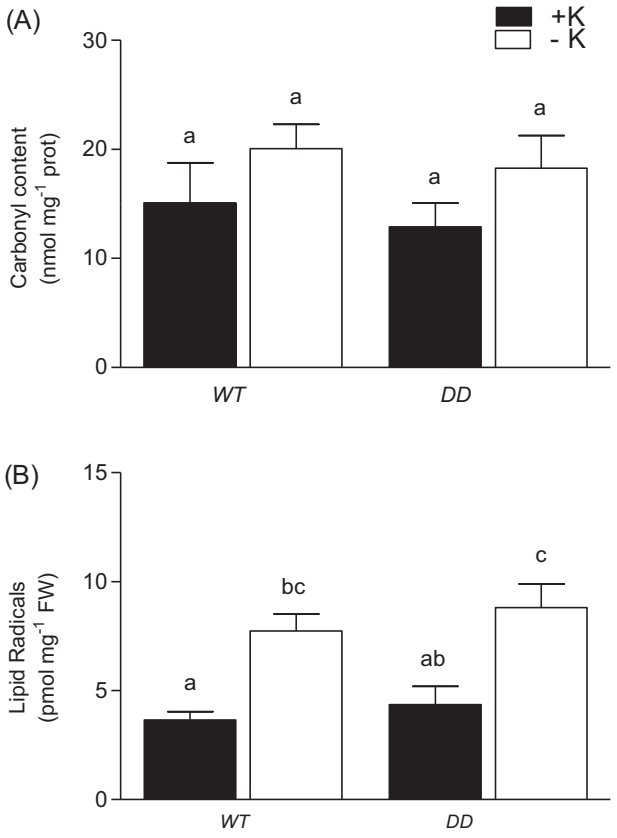

Fig. 5. Effect of long-term potassium deprivation on indicators of oxidative modifications in the fifth leaf of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines of *T. aestivum* cv Maringa grown from day 12 until day 28 in the presence or the absence of KCl 1 mM: (A) carbonyl content in total protein extracts from leaves; (B) carbon-centered radicals evaluated in leaves in the presence of POBN as spin trap. Data shown in (A) and (B) correspond to three ($n = 10$) and two ($n = 6$) experiments, respectively. Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$).

4. Discussion

Altered mutant versions of the wheat DELLA encoding genes *Rht-1* play a major role in agriculture [37]. After the cloning and subsequent characterization of the Arabidopsis *Rht-1* counterparts, it has been proposed that DELLA proteins may be necessary for acclimation of plants to some stress conditions [45,46], such as low phosphate availability [33]. Thus, an important question is whether or not DELLAs contribute to determining the physiological responses of plants, particularly crops, exposed to other conditions of nutrient scarcity. Here we found that, when exposed to K^+ deprivation, wheat NILs that carry the altered function *DD* alleles display an array of physiological responses – including a delayed decrease in chlorophyll content – qualitatively different from that displayed by lines carrying the *WT* alleles. Although a contribution of loci closely linked to the *Rht-B1,Rht-D1* genes to the pattern here reported cannot be entirely ruled out, the similar data obtained for both sets of NILs, which result from at least six backcrosses [38,39], suggest a role of these genes in the acclimation of wheat plants to K^+ starvation. In turn, the differences between Maringa and April Bearded spring cultivars in the pattern of relative biomass accumulation and to a lesser extent in chlorophyll retention indicate that the degree of influence exerted by the presence of altered function alleles on growth during the course of K^+ deprivation partially depends on the variety as formerly shown for other traits conferred by the *Rht-1* genes [39]. The precise determinants of this difference remain unknown.

It has been early observed that bean plants deprived of K^+ display an increased antioxidant response, and that such a response confers some degree of protection against the degradation of chlorophyll driven by the herbicide paraquat [47]. Later studies also showed that, in mulberry and soybean [48,49] as well as in maize and *Hordeum maritimun* [50,51], K^+ deprivation leads to an enhancement of antioxidant defenses, particularly of SOD activity. Interestingly, the antioxidant defense of Arabidopsis plants, remarkably SOD activity, has been recently reported to be modulated by DELLA proteins [45]. According to those previous reports, we found that the chlorophyll retention observed in *DD* NILs suffering K^+ deprivation paralleled a further enhancement of SOD and, to a lesser extent, of APX activities. Besides, as recently shown for *Hordeum maritimum*, another Triticeae [51], our data point out that, in wheat, lipid peroxidation is a primary modification induced by reactive oxygen species generation in K^+-deprived plants. The fact

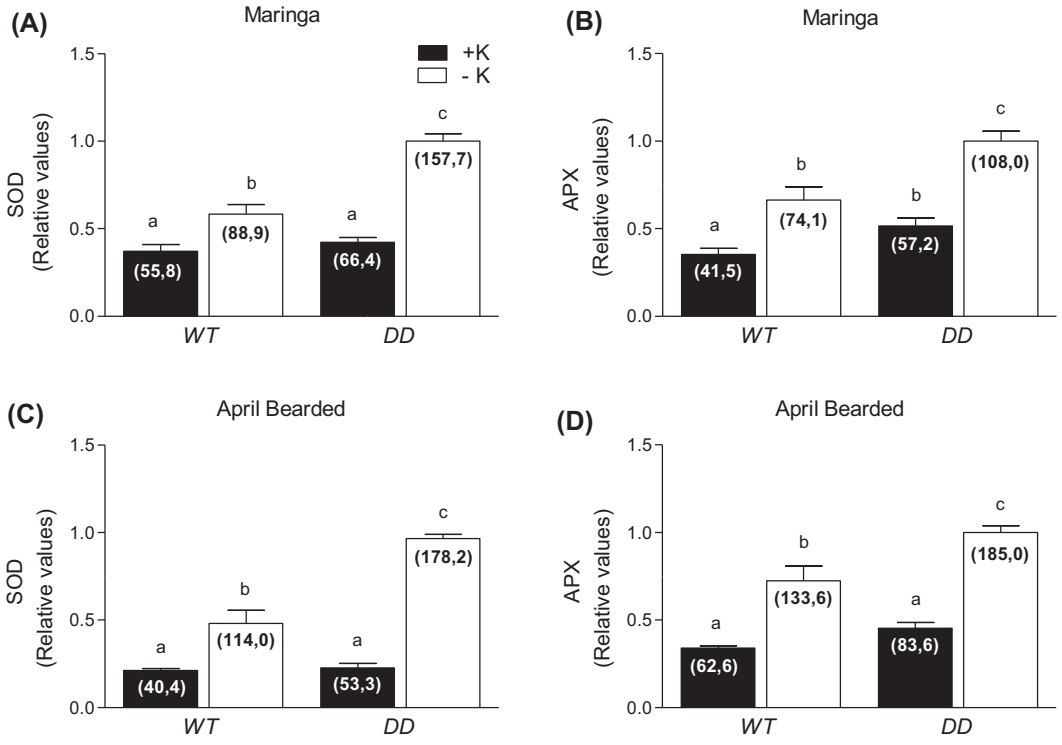

Fig. 6. Effect of long-term K^+-deprivation on the antioxidant enzyme activities in the fifth leaf of *Rht-B1a,Rht-D1a* (*WT*) and *Rht-B1b,Rht-D1b* (*DD*) near-isogenic lines. The trend of enzyme activity was expressed on a relative base. (A) and (C) correspond to total superoxide dismutase (SOD) activity, while (B) and (D) correspond to total ascorbate peroxidase (APX) activity. Absolute mean values, in μmol ascorbate h^{-1} mg^{-1} prot for APX and in U mg^{-1} prot for SOD are given between parentheses within each bar. Results shown in (A) and (B) for Maringa were obtained in four experiments ($n = 12$). Results shown in (C) and (D) for April Bearded correspond to two experiments ($n = 8$). Error bars correspond to SE. Different letters indicate significant different values ($P < 0.05$).

that the differences in the enzymatic antioxidant defense between *WT* and *DD* NILs were not accompanied by a difference in the indicators of oxidative modifications but paralleled an important difference in chlorophyll retention after a long starvation of K^+ is consistent with the hypothesis that the presence of altered versions of DELLA proteins influences the response of wheat plants to K^+ deprivation through an antioxidant signaling pathway. The possibility that other traits associated with a slow growth habit contribute to determining the differences here observed between *WT* and *DD* NILs in chorophyll retention must not be excluded.

A previous study has shown that, in Arabidopsis, DELLAs do not influence the accumulation of phosphorus and that the application of gibberellins does not affect the accumulation of several cations, including Ca^{2+} [33]. Our findings indicate that *WT* and *DD* NILs markedly differ in the accumulation of Ca^{2+}, Na^+ and K^+, thus suggesting that, in wheat, altered versions of DELLAs exert a major role in the dynamics of accumulation of these major cations. The relative differences found between NILs in shoot K^+ concentration after a long-term K^+ deprivation were close to 20% and occurred in the suboptimal range, where growth increases almost linearly with tissue K^+ concentration [42,52]. Thus, these differences could contribute to the improved performance displayed by plants carrying the *DD* alleles when deprived of K^+. In turn, differences in shoot Na^+ concentration between *WT* and *DD* NILs advocate for a role of the *Rht-1* genes in the control of Na^+ distribution within the plant, a process that involves transporters acting in the loading and unloading of both xylem and phloem elements [53–55]. Since the accumulation of transcripts coding for some of those transporters is influenced by the status of K^+ in plant tissues, the possibility that the enhanced accumulation of Na^+ in the shoot of *WT* NILs could be, at least partially, an indirect effect of their lower concentration of K^+ should not be excluded. The higher enhancement of Na^+ accumulation observed in the April Bearded *WT* NIL allowed keeping a high concentration of alkali cations and matching it with that found in *DD* plants. Because Na^+ is able to partially substitute for K^+ [1], maintenance of a high alkali cation concentration could contribute to reducing differences in plant performance between NILs as observed for this cultivar when deprived of K^+. An intrinsic difference between *WT* and *DD* NILs was observed for Ca^{2+}, which was preferentially accumulated by *DD* NILs grown both in the presence and absence of K^+. Ca^{2+} is a major element in plant nutrition, where it plays an important role in membrane integrity and in the maintenance of ion selectivity [1]. The extent to which the enhanced accumulation of Ca^{2+} could contribute to determining the retarded execution of the senescence program that takes place in plants with altered function alleles suffering K^+ deficiency deserves to be further examined.

Research on DELLA proteins uncovered that they are usually destined for degradation at the proteasome, after binding to the complex formed between GA and its receptor, a process that is impaired in plants with altered function alleles. Early evidence obtained in sunflower plants, which is consistent with the findings reported here, supports a role of gibberellins during the course of K^+ deprivation [56]. However, it has also been shown that non-gibberellin routes can modulate DELLAs activity [27]. Therefore, the precise role of gibberellins and their potential integration with other hormonal signals [24–26] under this particular stress condition are still major questions to be resolved.

In summary, data shown here support the hypothesis that, in wheat, the presence of altered function DELLA proteins confers a differential acclimatory response during K^+ deprivation, which involves a modification of the antioxidant defense as well as of the dynamics of accumulation of major cations. Besides, differences found between *WT* and *DD* NILs could be of interest for the design of strategies aimed to improve crop performance at K^+-limiting supplies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.plantsci.2011.10.011.

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