

Article

Physiological Response of Wheat to Chemical Desiccants Used to Simulate Post-Anthesis Drought Stress

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Abstract: Post-anthesis drought stress is one of the main constraints on the production of wheat (*Triticum aestivum* L.). Because field screening for post-anthesis drought tolerance is difficult, effective and validated methods to simulate drought in order to identify sources of tolerance can facilitate screening of breeding materials. Chemical desiccants are widely used to simulate post-anthesis drought stress. We aimed to identify physiological traits that respond to desiccants as they do to drought. We examined the responses of ‘Norin 61’ to six treatments in a greenhouse: irrigated control, drought after anthesis, and 2% or 4% potassium chlorate (KClO₃) at anthesis (A) or grain filling (GF). We measured $\delta^{13}\text{C}$ in leaves, aboveground fresh biomass, stomatal conductance, chlorophyll content, harvest index, and grain yield. Both 2% and 4% KClO₃ at both A and GF simulated the effect of drought stress. Selection of drought-tolerant genotypes can be aided by chlorophyll content and $\delta^{13}\text{C}$ measurement of leaves when 2% or 4% KClO₃ is used to simulate drought.

Keywords: carbon isotope composition; chemical desiccant; drought; potassium chlorate; wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is a major staple crop, with a global annual production of about 720 million metric tonnes [1]. Recurrent drought associated with climate change is a major constraint to wheat production globally [1]. Although the wheat plant does not demand a lot of water, it is sensitive to water stress [2]. Drought affects many genes and is one of the most important factors limiting crop yields around the world. Researchers use several techniques to mimic natural drought to ease and accelerate evaluation of plants. Polyethylene glycol is frequently used under laboratory conditions to modify osmotic potential and induce plant water deficit [3]. For the selection of cereal cultivars resistant to drought, chemical desiccants can be applied to simulate the effect of stress under field conditions by inhibiting carbon assimilation [4–6]. One advantage of this technique is that water stress can be imposed under irrigated conditions; moreover, this technique can mimic drought effect without the need to expose plants to drought, which is easier, less complicated, helpful and fast. Developing cultivars with superior adaptation to water-limited environments has been impeded by complex interactions between genotype and environment (G × E), leading to changes in the yield rankings of genotypes in different water-limited environments [7]. The spray application of magnesium chlorate or sodium chlorate to the canopy, including the spikes, at 14 days after anthesis, when kernel

growth enters its linear phase, to inhibit photosynthesis reduced the capacity for grain filling from stem reserves by between 5% and 50% [8,9]. Cultivars that translocate more carbohydrate reserves to the grains were better able to maintain a stable kernel weight under desiccation conditions [8]. In addition, the resultant reduction in grain weight is correlated significantly with the reduction in grain weight due to natural drought [5,9].

Under drought, plants reduce their stomatal conductance to conserve water. Under mild to moderate drought stress, stomatal closure (causing reduced leaf internal CO₂ concentration, or C_i) is the major reason for reduced photosynthesis [10]. This leads to less assimilate production and thus lower yields. Severe drought stress further inhibits photosynthesis by decreasing chlorophyll content (mainly the result of damage to chloroplasts caused by reactive oxygen species, affecting chlorophyll components, and by damaging the photosynthetic apparatus [11,12]). Chlorophyll concentration is used as an index of source capacity [13], and so a decrease can indicate a non-stomatal limiting factor. Most previous reports on desiccation did not focus on chlorophyll content and stomatal conductance because of the rapid and strong effect of the desiccant; within 48 h in most cases [8,9]. Most genotypes, especially breeding lines, are sensitive to drought in biomass accumulation [14]. Drought stress can reduce grain yield: an average loss of 17% to 70% was estimated [15]. Conversely, grain yield, biomass, and harvest index indicate tolerance to drought [16–18]. Pre-anthesis assimilates contributed little to grain yield when water supply was abundant, but contributed up to 30% of the final yield when water deficits developed during grain filling [19].

Molecules incorporating heavier isotopes diffuse more slowly and biochemically react more slowly. Plants discriminate against the heavier carbon isotope during photosynthesis and thus the carbon isotope composition ($\delta^{13}\text{C}$; frequently expressed as carbon isotope discrimination, $\Delta^{13}\text{C}$) in plant tissues indicates the photosynthetic performance [20]. In selecting drought-adaptive genotypes for breeding, it is helpful to have physiological indicators of drought-adaptive capabilities, such as the strong relationship between water use efficiency (WUE) and $\Delta^{13}\text{C}$ [21]. Most previous reports investigated the response of yield and its components to drought and desiccation. However, it would be beneficial to compare in more detail the responses to chemical desiccation and to drought [22] to understand to what extent desiccation could be applied as screening tool and which parameters can be useful to confirm such screening.

As previously reported [5] compared the effects of magnesium chlorate, sodium chlorate, paraquat, diquat, and the sensing agents potassium iodide (KI) and cresylic acid. When chemicals were applied to only the leaves and stems, no premature senescence of ears and peduncles was observed in the case of KI, suggesting that the other desiccants translocated to the ears. Paraquat, diquat and the two chlorate treatments caused leaf desiccation within 3–4 days of application whereas KI induced rapid senescence of the penultimate and lower leaves in 3–4 days, and more progressive senescence of the flag leaf in 5–7 days [5]. The use of KI or potassium chlorate (KClO₃) was recommended to simulate drought stress in wheat and triticale [5,23,24]. KI reduced the photosynthesis and chlorophyll content but increased sucrose and proline contents of treated wheat plants compared to untreated control plants [25]. However, there are no reports on the physiological effect of the KClO₃ on wheat plants; recently, [26] reported that, in longan trees, KClO₃ treatment reduced photosynthesis rate and stomata conductance but slightly increased CO₂ concentration in the mesophyll. Therefore, they suggested that KClO₃ treatment damaged photosynthetic apparatus.

Although the technique of applying desiccants and then measuring the effect on grain yield has been used widely to evaluate plant response to drought, no suitable indicators have been identified to be used with the desiccation technique to predict or select drought-tolerant plants. Our study aimed at comparing the physiological responses of wheat to chemical desiccation by KClO₃ and drought so as to identify indicators suitable to use with the desiccation technique for predicting the response of wheat to drought stress [8]. Our results reveal the suitability of using potassium chlorate (KClO₃) to simulate drought stress and the measurement of $\delta^{13}\text{C}$ of flag leaves to screen for drought-tolerant wheat genotypes when 4% KClO₃ is used and the measurement of chlorophyll content when 2%

KClO₃ is used. Our study is one of the first to test KClO₃ on wheat and is the first to test physiological responses to desiccation in the selection for drought tolerance.

2. Materials and Methods

This study was conducted during the winter season (November–April) at the Arid Land Research Center, Tottori University, Japan. We used the Japanese standard spring wheat cultivar ‘Norin 61’ which represent the background of most of our breeding materials. The experiment was grown in a randomized complete design with four replications per treatment in a naturally lit glasshouse with a 28/20 °C day/night temperature regime and a relative humidity of 40% to 70%. Four pots per treatment were each filled with 7 kg of organic-peat moss soil. Four seeds were planted in each pot. After 2 weeks, seedlings were thinned to 2 per pot. Irrigation was applied in all treatments (except for drought treatment after anthesis) every 7 to 10 days as needed. Fertilizer was applied as 120 kg N, 60 kg P, and 60 kg K ha⁻¹ as split dose at sowing and before flowering.

2.1. Treatments

Desiccant-treated plants were sprayed with either 2% or 4% (*w/v*) KClO₃ (Wako, Japan) in water [5,9,23,27] at anthesis (A) or during grain filling (GF, 15 days after anthesis) from a 5-L hand-held pressure pump sprayer at about 200 mL per pot. All green parts of the plants were sprayed. The pH was 7 for the 2% concentration and 6.7 for the 4%. Drought-treated plants had their irrigation withheld after anthesis. Control plants were irrigated without spraying.

2.2. Physiological Parameters

We measured chlorophyll content (Chl) in the flag leaves of eight plants in each treatment at 1 to 6 weeks after treatment with a chlorophyll meter (SPAD 502 Plus, Konica Minolta, Japan).

We analyzed gas exchange of eight flag leaves in each treatment. Stomatal conductance was measured at 1, 2 weeks after treatment began with an AP4 leaf porometer (Delta-T Devices, Cambridge, UK).

At maturity, 8 flag leaves were oven-dried at 80 °C for 48 h. Carbon isotope composition was determined with a mass spectrometer (Micromass, Villeurbanne, France) as $\delta^{13}\text{C}$ (‰) = $(R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000$, where R is the ¹³C/¹²C ratio [28].

2.3. Agronomic Traits

Aboveground fresh biomass and grain yield (GY) of the 8 plants in each treatment were recorded at maturity. Harvest index (%) (HI) was calculated as grain yield / aboveground fresh biomass × 100.

2.4. Statistical Analysis

One-way ANOVA was performed in Genstat v. 17 software (VSN International, Hemel Hempstead, UK) to assess the effect of treatments on the traits. Means were compared with Fisher’s protected least-significant-difference test at $p = 0.05$. Simple correlation was performed in Genstat to determine the relationship between the trait values across the treatments.

To compare the effects of treatments with the control, we calculated relative performance (RP) as:

$$\text{RP} = (Y_s/Y_p) \times 100 \quad (1)$$

where Y_s = grain yield under treatment condition and Y_p = grain yield under control condition.

3. Results

3.1. Response of 'Norin 61' to Control, Desiccant, and Drought Conditions

Following the application of 2% or 4% KClO₃ at anthesis (A) or during grain filling (GF, 15 days after anthesis), symptoms of desiccation appeared by 5 days, and most of the leaves and glumes were dead within 4 weeks. Leaf blades and glumes showed symptoms first. Peduncles remained greener for about 3 days longer. In comparison, the imposition of drought treatment caused plants to turn completely yellow within 2 weeks after water stoppage (Figure 1).

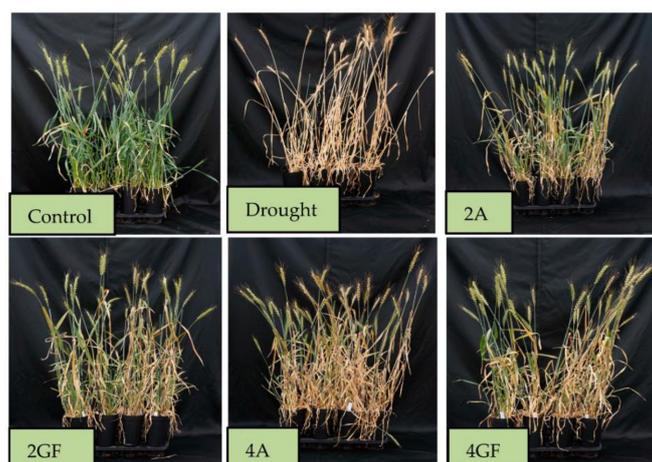


Figure 1. Wheat cultivar 'Norin 61' tested under control, drought stress, and 2% or 4% KClO₃ applied at anthesis (A) or grain filling (GF) stage (these photos were taken 2 weeks after treatments).

3.2. Effects of Drought and Desiccant on Grain Yield, Biomass, and Harvest Index

Significant differences were obtained for the traits measured (Table 1). Grain yield (GY), harvest index (HI), and aboveground fresh biomass were significantly ($p < 0.0001$) lower at both rates of desiccant at both stages (treatments 2A, 4A, 2GF, 4GF) and under drought stress than in the control (Figure 2a–c). Drought stress caused the greatest reduction in GY ($p < 0.0001$) (Figure 2a). 4GF reduced GY significantly more than the other desiccant treatments.

There was no significant difference between desiccation and drought on biomass, and 4A and drought had the lowest biomass (Figure 2b). Drought and desiccation significantly reduced HI (Figure 2c).

Drought reduced relative grain yield (grain yield performance compared to control treatment) significantly more than desiccation did (Figure 2d). 4GF had the greatest effect among desiccation treatments (Figure 2d).

Table 1. Significance of treatment effects on grain yield, relative grain yield, Aboveground fresh biomass, harvest index and leaf carbon isotope composition ($\delta^{13}\text{C}$) at maturity, and chlorophyll content (SPAD unit) and stomata conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) at two weeks after treatments of 'Norin 61'.

Traits	SE \pm	P	l.s.d.
Grain yield	0.14	<0.0001	0.30
Relative grain yield	2.59	<0.0001	7.58
Aboveground fresh biomass	0.12	<0.0001	0.78
Harvest index	2.80	<0.0001	10.64
Chlorophyll content (SPAD unit)	1.72	<0.0001	3.33
Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	6.14	<0.0001	14.52
Carbon isotope composition $\delta^{13}\text{C}$	0.07	0.004	0.47

SE: standard error; P: probability by F test; l.s.d: least significant difference at $p = 0.05$.

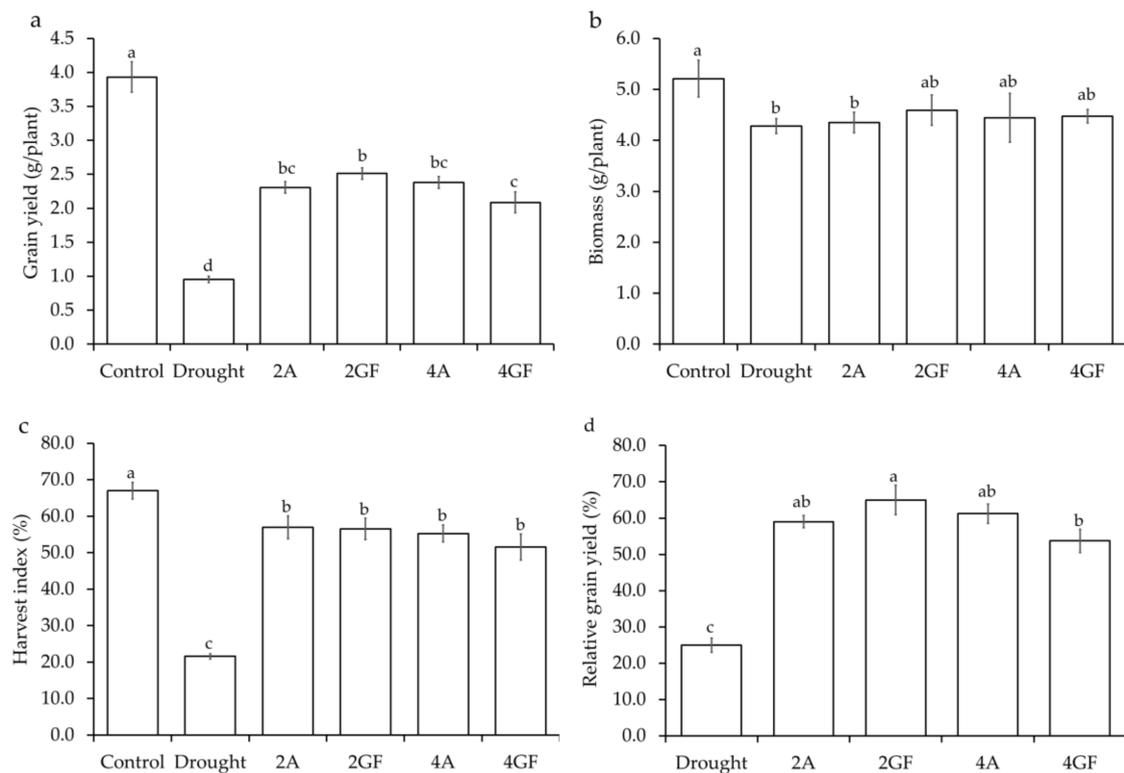


Figure 2. Grain yields per plant (a); aboveground fresh biomass (b); Harvest index (c); HI = grain yield/aboveground fresh biomass \times 100 and relative grain yield (d), relative grain yield = grain yield under stress/grain yield under optimum condition \times 100 of wheat cultivar 'Norin 61' under control, drought stress, and 2% or 4% KClO_3 applied at anthesis (A) or grain filling (GF) stage. Different letters denote significant difference ($p < 0.05$, Fisher's PLSD test).

3.3. Chlorophyll Content and Stomatal Conductance

The chlorophyll content (Chl) began to degrade 1 week after desiccant application and 4 days after water stoppage. Drought treatment caused leaves to become yellowish and dry by about 1 week (Figure 3a). Chl decreased rapidly in 4GF and the decrease was delayed in 2A (although leaves shriveled) before degrading (Figure 3a). By week 4, Chl was degrading in all treatments, and drought and desiccations were significantly less than in the control (Figure 3a). All desiccation treatments were significantly different from the control; drought treatment had the greatest effect (Figure 3a).

Stomatal conductance (g_s) increased relative to the control at 1 week after treatment (Figure 3b). At 2 weeks after treatments, stomata conductance in 2A, 4A and 4GF was comparable to that of the control and higher than that of the drought, whereas in 4A it was higher than the control and drought (Figure 3b).

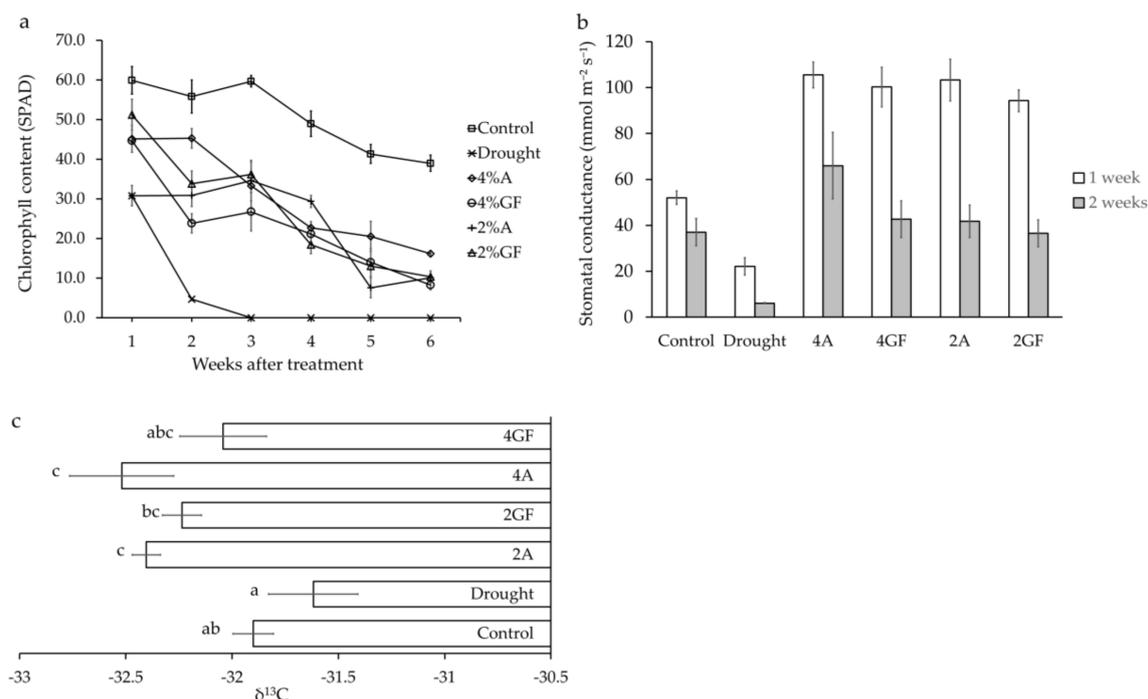


Figure 3. Chlorophyll content (a); stomatal conductance (b) and carbon isotope composition ($\delta^{13}\text{C}$; for 8 flag leaf samples/treatment) (c), for wheat cultivar 'Norin 61' tested under control, drought stress and two concentrations of potassium chlorate (2% and 4%), with each concentration sprayed at one of two stages, anthesis (A) and grain filling (GF). Different letters denote significant difference ($p < 0.05$, Fisher's PLSD test).

3.4. Carbon Isotope Composition ($\delta^{13}\text{C}$)

$\delta^{13}\text{C}$ could discriminate between treatments: $\delta^{13}\text{C}$ was substantially increased by drought and decreased significantly ($p < 0.05$) by desiccation at 2A and 4A relative to the control (Figure 3c). Values varied from -31.61‰ under drought to -31.90‰ in the control to -32.51‰ in 4A. $\delta^{13}\text{C}$ was substantially lower in A than in GF. There was no significant difference between control and 4GF or between drought and 4GF.

3.5. Correlation between Traits

GY was positively correlated with HI ($R = 0.95$, $p < 0.0001$), biomass ($R = 0.87$, $p = 0.02$) and Chl ($R = 0.94$, $p = 0.001$). Chl was positively correlated with HI ($R = 0.92$, $p = 0.005$) and biomass ($R = 0.86$, $p = 0.02$). $\delta^{13}\text{C}$ and g_s correlated negatively ($R = -0.89$, $p = 0.01$) and had no correlation with the other traits.

4. Discussion

Chemical desiccation was effective in simulating post-anthesis drought in wheat, as the GY and physiological responses of 'Norin 61' were affected by the desiccant, although it was not as severe as drought (Figures 2 and 3). This supports the use of chemical desiccation of the canopy after flowering as a means for inhibiting photosynthesis, compelling plants to depend on their capacity for grain filling from stem reserves [8,9].

Although some physiological responses ($\delta^{13}\text{C}$) did not differ significantly between 2% and 4% KClO_3 , they did differ significantly between desiccation and the control (Figure 3c). Differences between drought and desiccant effects could be attributed to their different modes of action, as KClO_3 is an oxidative reagent and is phytotoxic to all green tissues, and has been implicated in reducing the efficiency of photosystem II [29].

4.1. Use of Chemical Desiccants to Simulate Drought

The development of drought-tolerant genotypes is a goal of wheat breeders. Under post-anthesis drought stress, the ability to remobilize stem reserves is a potentially useful trait [30]. The selection of genotypes on the basis of their grain filling capacity is complicated by the erratic occurrence of drought stress and the lack of inexpensive and fast methods for screening [8]. The application of 4% KClO₃ was successfully used to simulate drought 15 days after anthesis in triticale [23]. Using KClO₃ enabled us to evaluate plants under optimal conditions, and we found that desiccation had less effect on GY of 'Norin 61' compared to drought (Figure 2a,d). Desiccation has been used in many cereal crops, and reductions in grain yield were comparable to those under drought, making it suitable for evaluation of drought tolerance [4,22,31,32]. Our results show that the photosynthetic source was destroyed within 2 weeks after the imposition of drought and 4 weeks after the application of desiccant. As KClO₃ killed tissues slowly, its use allowed us to evaluate the gradual deterioration of agronomic and physiological traits (Figures 2 and 3). Other reports suggested that this technique inhibits photosynthesis without a measurable effect on the translocation of assimilates to developing grains: green tissues of triticale dried completely within 48 h after application [23]; and in a study of sodium chlorate treatments, wheat leaves became desiccated 3–4 days after whole-plant application, but ears became desiccated 7–14 days after application [5], whereas in our results it took 1–4 weeks for leaf and 14–30 days for ears. These results suggest different responses of plants to different desiccants at different stages.

The use of chemical desiccation to assess drought tolerance [8] has obvious advantages. However, a critical evaluation is first needed to assess its applicability (concentration and stages). Selection needs to be done under irrigated conditions, in which leaf diseases may occur [8].

4.2. Effects of Desiccation and Drought on Stomatal Conductance, Chlorophyll Content, and $\delta^{13}\text{C}$

4.2.1. Effect on Stomatal Conductance and $\delta^{13}\text{C}$

The g_s was significantly greater in desiccant treatments or comparable to the control at 1 and 2 weeks (Figure 3b). This shows that desiccation increases stomatal conductance, although other studies reported that g_s and photosynthesis rate were lower in desiccant treatments than in the control [25,26]. Interestingly, longan tree [33] showed an increase in photosynthesis and efficacy of photosystem II although g_s was not changed. These contradicting reports and our results reveal that the effect of chlorate on photosynthesis is still equivocal [26]. Following desiccation treatments, proline and soluble sugars increased dramatically in the treated plants compared to the control [25,26], similar to the plant response under drought [34]. In this study, we observed a gradual onset of leaf yellowing following the desiccant treatments, and thus gradual damage to the leaf tissue is expected. The increased g_s of the desiccated plants could be explained by assuming that the desiccant increased the proline and soluble sugars in the leaf which in turn increased the water uptake in the desiccated plants. As water was available and the leaf damage started at relatively the same time as when the g_s was measured (1 week) after the treatment, it is possible to expect increased g_s in the desiccated plants than in the control or drought.

A decrease in photosynthesis could not be entirely attributed to loss of chlorophyll or impediment in stomatal conductance. The increased internal carbon dioxide concentration suggests a more direct damage to photosynthetic mechanism [25]. A high level of sucrose is known to depress photosynthesis rate by inhibition of Rubisco [25,35]. At 2 weeks after desiccation, the g_s decreased and was higher in 4A than in the control (Figure 3b). This decrease in g_s could be explained by the fact that the damage of the desiccant to the chlorophyll and the high concentration of sugars in the leaves of the desiccated plants decreased the photosynthetic rate and thus increased the internal CO₂ that led to stomatal closure.

Plants discriminate against the heavier carbon isotope ($\delta^{13}\text{C}$) during photosynthesis, and the extent of this discrimination depends on the ratio of intercellular versus external CO₂ concentration (C_i/C_a) in photosynthetic organs [36–38]. $\delta^{13}\text{C}$ is negatively related to C_i/C_a [37], and thus to the

transpiration efficiency at the stoma (CO_2 assimilation/transpiration). Conditions that induce stomatal closure, such as water deficit, restrict the CO_2 supply to carboxylation sites, which then increases the $\delta^{13}\text{C}$ (or decreases $\Delta^{13}\text{C}$) of plant matter [28,38]. Interestingly in this study when we measured the $\delta^{13}\text{C}$ in the flag leaves, we found that the desiccated plants had lower $\delta^{13}\text{C}$ values than the control and the drought, although the differences were not significant in some cases (Figure 3c). This result further supports the observed increase in the g_s of the desiccated plants. The general trend of increasing ^{13}C in the flag leaf with drought stress found in our present study (Figure 3c) agrees with the results of previous studies on rice [39,40] and wheat [41,42]. As long as the $\delta^{13}\text{C}$ and g_s are closely related and the level of $\delta^{13}\text{C}$ is determined by the stomata behavior [28,38,40], the decreased levels of $\delta^{13}\text{C}$ of the desiccated plants could be explained in the same manner described above for the g_s . The higher g_s of the desiccated plants induced by the accumulation of proline and soluble sugars led to the lower values of $\delta^{13}\text{C}$ observed for the desiccated plants. Interestingly, the $\delta^{13}\text{C}$ values agreed well with the g_s values at 2 weeks after the desiccation treatment (Figure 3b,c). We could not detect high significant differences between the $\delta^{13}\text{C}$ of the desiccated plants and that of the control or drought. In rice, it is reported that the variation in CID values in response to water regimes was less compared to that between the genotypes [43]. As long as the impact of the desiccation may vary among different genotypes, special attention is required to improve the understanding of genetic response of $\delta^{13}\text{C}$ under desiccation.

There was no correlation between both g_s and $\delta^{13}\text{C}$, and grain yield; possibly due to the use of only one genotype in this study, since that the cultivar effects on $\delta^{13}\text{C}$ values depends on drought stress intensity, genotype and soil moisture content [40]. Therefore, the differences in $\delta^{13}\text{C}$ value between drought stress and non-stressed conditions are critical. [44] Reported in their study of genetic relationships between wheat stomatal traits and yield in response to drought, no significant correlations observed between yield and stomatal traits under field conditions. However, they found some overlaps between quantitative trait loci (QTLs) for stomatal traits and yield across environments and they suggested that stomatal traits could be an underlying mechanism increasing yield at specific loci.

4.2.2. Effect on Chlorophyll Content

Drought stress reduced chlorophyll (Chl) the most (Figure 3a), as previously reported [45]. Desiccation initially increased the Chl content before decreasing it (Figure 3a). A previous study reported that drought initially increased Chl before decreasing it [25]. This is not unusual because, at the beginning of drought, the plant tissues may have shriveled and the cells condensed and before Chl degradation onset this cell condensation was reflected in higher SPAD values, and when the Chl degradation took place with the progress of the stress the SPAD values decreased. Our results reveal that Chl in all treatments showed changes with time, and 4GF caused the highest damage in desiccation treatments (Figure 3a). These results show that the effect of desiccant on Chl depends on both time and concentration, as previously reported [25,45]. A similar significant decrease of Chl-*a* and -*b* under drought in six wheat cultivars was reported [46]. The effect of desiccation varied with plant stage (Figure 3a). Decreased or unchanged Chl under drought stress has been reported in other species, depending on the duration and severity of drought [47]. The decrease in Chl under drought stress is mainly the result of damage to chloroplasts caused by reactive oxygen species [12]. Chl was correlated significantly with GY, HI and biomass. This result suggests that Chl can be an indirect indicator of drought tolerance when a desiccant is applied, since a strong correlation between high Chl and grain yield under drought was reported [22].

4.3. Effect of Desiccation on Aboveground Fresh Biomass, Harvest Index, and Grain Yield

4.3.1. Analysis of Yield Reduction under Desiccation Treatments

Drought reduced grain yield by about 70% relative to the control (Figure 2a,d), as previously reported [48,49]. We found significant differences in relative grain yield between desiccation and

drought. These differences can be explained by the fact that it took 3 to 4 weeks for the desiccant to work, and therefore it affected only GF, whereas severe drought throughout the post-anthesis period probably affected early grain growth and related traits, as previously reported [50,51]. Desiccation reduced grain yield by 35% to 45% (Figure 2a,d), as similarly reported [4,8]. The application of 4% magnesium chlorate reduced grain yield by 15% to 32% in four wheat cultivars [8]. Similarly, 2% sodium chlorate reduced grain yield by 19% to 38% in 28 wheat cultivars [4]. However, the application of 4% sodium chlorate 10 days after anthesis reduced wheat kernel weight by 77% [5]. This apparent smaller decrease in grain yield at 2A and 2GF could be explained by the lower toxicity of the solution to the growing endosperm, but a higher contribution from current photosynthesis cannot be ruled out, since senescence took 3 to 4 weeks to manifest. Pre-anthesis assimilates contribute little to grain yield in cereals when water supply is abundant, but could contribute up to 30% of the final yield when water deficit develops during grain filling [19]. Small reductions in kernel weight and yield caused by chemical desiccation were associated with small kernel size [9]. The effectiveness of 4GF may be due to a direct effect of the chemical on the grains of cultivars with large yield potential [33]. Cultivars with historically stable kernel weights or yields across environments tend to have less chemical desiccation injury [4].

4.3.2. Biomass and Harvest Index

The low biomass reduction under desiccation (Figure 2b) may be associated with high WUE, as a basic unit of production is the quantity of carbon gained by photosynthesis in exchange for water used in transpiration [52]. Our results explain that stem reserves contributed better to grain yield (harvest index) under desiccation (40% to 60%) than under drought (20%) (Figure 2c). This contribution may have been inflated, since photosynthesis continued for up to 4 weeks under desiccation and 2 weeks under drought. These results also suggest that this technique could be used to estimate the capacity for the remobilization of stored assimilates under desiccation, since there were significant differences between control and desiccation treatments (Figures 2c and 3a). On the other hand, the reduction in leaf chlorophyll content limits photosynthesis and further decreases biomass production, consistent with the positive correlation between total Chl and aboveground fresh biomass ($R > 0.80$, $p < 0.05$). Cultivars that translocate more carbohydrate reserves to the grains are better able to maintain a stable kernel weight under desiccation conditions [8].

4.4. Association between Chemical Desiccant and Drought Stress in Effects on Yield Traits

Significant positive correlations between grain yield and physiological traits under both stress and optimum conditions imply the direct contribution of these yield components (above fresh biomass and harvest index) to yield and should be considered as important targets during selection [27,53]. Reductions in grain yield under chemical desiccation and under control conditions were not significantly associated. Similarly, there was no reported association between reductions in kernel weight due to chemical desiccation and reductions in kernel weight under control conditions [5]. Associations between kernel weight and injury/loss to kernel weight were not consistent with the environment, so potential kernel weight may be affected at anthesis, and thus final kernel weight may vary according to environment [54]. This in turn may greatly influence the final grain yield results, since tolerance to desiccation is measured with respect to potential grain yield. Correlations of yield reductions between desiccant and drought were not always strong if drought occurred earlier than grain filling [33]. The extent of injury to kernel weight by drought stress was positively correlated with injury to kernel weight by chemical desiccation [4,9].

4.5. Relationship between Response to Chemical Desiccation and Drought Resistance

The smaller reduction of grain yield in 2A, 2GF, and 4A than under drought was similar to previous results of low reductions in kernel weight and grain yield of chemically desiccated plants [4,9], which can be explained by translocation of most of the soluble assimilates to the grains.

Furthermore, if grain filling in non-desiccated controls were subject to stress, estimates of grain yield injury would be inaccurate [9]. The moderate association between potential contributions and grain yield reductions may have been caused by the desiccant itself through some phytotoxic effect on kernel growth. Low reductions in grain yield may be linked more to the lower stress encountered during grain filling than to the potential contributions to yield from stem reserves. Other studies also found that the reduction in grain weight resulting from chemical desiccation was correlated significantly with the reduction in grain weight due to natural drought [4,9].

4.6. Relationship between $\delta^{13}\text{C}$ and Yield Characters

$\delta^{13}\text{C}$ was not correlated with grain yield as previously reported [33]. Other previous studies found a weak correlation between grain yield and carbon isotope discrimination (CID) under drought conditions [55,56] and a strong correlation only under severe drought [57]. Thus, the higher grain yield of a genotype with a high CID (low $\delta^{13}\text{C}$) is probably explained by a faster growth rate, as confirmed in cotton and wheat [58,59]. A negative correlation of CID with grain yield and biomass in the absence of water stress was reported [60]. Under water replete conditions, stomatal conductance is likely to be high, so higher photosynthetic capacity would reduce C_i , leading to lower CID values (higher $\delta^{13}\text{C}$) However, there are some doubts about using CID for selecting genotypes for high yield under drought [61,62]. A positive relationship between CID and harvest index in durum wheat [63] suggests that higher WUE may result in reduced dry matter partitioning to grain. GY was negatively correlated with $\delta^{13}\text{C}$ mainly under early drought conditions [63].

5. Conclusions

Potassium chlorate induced plant desiccation that mimicked the effects of water stress. Future work should compare the physiological responses to KClO_3 and those occurring in managed drought using more wheat germplasms. chlorophyll content and $\delta^{13}\text{C}$ measurement of leaves can be used to aid and complement the selection of drought-tolerant genotypes when KClO_3 is applied.

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