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Combining carbon-13 and oxygen-18 to unravel triticale grain yield and physiological response to water stress

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ABSTRACT

Water availability in semi-arid regions is increasingly becoming threatened by erratic rains and frequent droughts leading to over-reliance on irrigation to meet food demand. Improving crop water use efficiency (WUE) has become a priority but direct measurements remain a challenge. There is therefore a need to identify reliable proxies and screening traits for WUE. Carbon isotope discrimination ($\Delta^{13}C$) offers potential as a proxy for WUE, but its application is hindered by environmental factors and thus varies greatly among different studies. A two-year study was carried out with four moisture levels, ranging from well-watered (430–450 mm) to severe stress (SS) (220–250 mm), combined with four commercial triticale genotypes grown under field conditions in a hot, arid, steppe climate of Limpopo in South Africa. The study tested the use of $\Delta^{13}C$ as a proxy of intrinsic WUE and grain yield of triticale. Second, $\delta^{13}C$ and $\delta^{18}O$ in combination with measured gas exchanges were used to test the functionality of the dual isotope model to interpret causes of variation in carbon isotope composition. Third, grain filling carbon assimilate sources were inferred from measured flag leaf and grain $\Delta^{13}C$.

The results showed that moisture level significantly influenced grain yield, intrinsic WUE and $\Delta^{13}C$ in triticale. Well-watered conditions increased grain yield, which ranged from 3.5 to 0.8 t ha^{-1} and 4.9–1.8 t ha^{-1} in 2013 and 2014 respectively. $\Delta^{13}C$ was also high under well-watered conditions and decreased with decreasing moisture level while $WUE_{intrinsic}$ increased with decreasing moisture level. The relationship between $\Delta^{13}C$ and grain yield was positive ($P < 0.01$), but only significant under water stressed conditions, indicating dependence of the relationship on moisture level. The relationship between $\Delta^{13}C$ and $WUE_{intrinsic}$ did not depend on the moisture level but showed a negative relationship when data for all moisture levels was combined. $\delta^{13}C$ showed a negative relationship with photosynthetic rate (*A*), while the relationship between stomatal conductance (*gs*) and $\delta^{18}O$ varied with season. Hence, the dual isotope model could only predict that variation observed in $\Delta^{13}C$ and thus intrinsic water use efficiency was due to a concomitant decrease in both *A* and *gs* when transpiration was not limited by evaporative demand. Flag leaf $\Delta^{13}C$ measured under SS at GS71 in the 2014 growing season, was significantly higher (2.2–3.6‰) than grain $\Delta^{13}C$, also measured under SS, suggesting minimal contribution of flag leaf photosynthesis to grain filling. No genotypic differences were observed in $\Delta^{13}C$, grain yield and $WUE_{intrinsic}$, indicating a probable lack of diversity in the studied genotypes.

The results of this study show that carbon isotope discrimination could be useful as a predictor of triticale grain yield in drought prone areas. $\Delta^{13}C$ also offers potential as a proxy for $WUE_{intrinsic}$ and breeding for lower $\Delta^{13}C$ values could result in varieties with higher $WUE_{intrinsic}$ in triticale. Flag leaf photosynthesis and pre-anthesis assimilates contribute much less carbon to grain filling under water stress than previously thought. Lastly, our results show that the dual isotope model is operational, but is not all encompassing but depends on evaporative demand.

1. Introduction

Water availability in semi-arid regions is increasingly becoming threatened by erratic rains, frequent droughts, rising temperatures and evapotranspiration rates leading to over-reliance on irrigation

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to meet food demand. Currently, about 80% of world fresh water is used for irrigation (Morison et al., 2008) and in many dry areas such levels of consumption are unsustainable (Condon et al., 2004) as water resources are also under increased pressure from other users. To feed the projected nine billion people by 2050 (Cleland, 2013), crop management, particularly of cereals, must adapt to climate variability through the use of varieties that use water efficiently (Barnabás et al., 2008). Improving cereal water use efficiency (WUE) has for a long time been one of the main targets of crop research particularly in arid and semi-arid environments, with the aim of finding sustainable ways of increasing crop productivity while reducing water loses (Foley et al., 2011). Crop WUE plays an important role in the exchange of water between the biosphere and the atmosphere and thus has an effect on the global water cycle (Seibt et al., 2008). However, one of the major bottlenecks in cereal breeding to produce "more crop per drop" has been the lack of or the evaluation of appropriate traits (Araus et al., 2008). Direct measurement of WUE under field conditions remain a big challenge due to the large amount of work (Tardieu, 2013) and have stalled the use of the WUE trait in crop improvement programs. There is therefore a need to identify reliable proxies of WUE that can be measured quickly; are correlated to yield and that can also provide the highest repeatability and heritability.

Stable isotope ratios of plant material are a powerful tool in ecological research as they indicate key environmental and physiological processes (Barnard et al., 2012). Carbon isotope composition ($\delta^{13}C$) or the discrimination value ($\Delta^{13}C$) have frequently been used as a time-integrated measure of the intrinsic water-use (Barbour et al., 2011; Farquhar et al., 1989; Cabrera-Bosquet et al., 2009). Intrinsic water use efficiency ($WUE_{intrinsic}$) is the ratio of photosynthetic rate relative to stomatal conductance (*A*/*gs*). Stomatal conductance plays an important role in the trade-off between water conservation and carbon assimilation as it controls both CO_2 uptake and water loss (Araya et al., 2010). Changes in stomatal conductance result in changes in leaf $\delta^{13}C$ and in turn in crop WUE (Farquhar and Richards, 1984). However, according to Seibt et al. (2008), the relationship between $\delta^{13}C$ and WUE is not direct due to the influence of external biotic and abiotic factors on the ratio of intercellular CO_2 to that of the atmosphere (*Ci*/*Ca*) (the primary determinant of discrimination against ^{13}C-CO_2 in leaves). It is also argued that WUE at leaf level depends on evaporative demand, which does not directly affect $\delta^{13}C$ (Seibt et al., 2008). Thus, WUE and $\delta^{13}C$ can vary independently of one another, making the use of $\delta^{13}C$ as a proxy for WUE questionable (Seibt et al., 2008).

According to Farquhar et al. (1982), the relationship between $\delta^{13}C$ and WUE exists because isotope discrimination of C_3 plants is linearly linked to *Ci*/*Ca* ratio. A reduction in *Ci*/*Ca* could be the result of either a greater *A* at a constant *gs* or a lower *gs* at a constant *A* or even to changes in both factors (Condon et al., 2004). In order to decipher which of the two (*gs* or *A*) is causing changes in $\delta^{13}C$, oxygen isotope composition ($\delta^{18}O$) is used in a dual isotope model proposed by Scheidegger et al. (2000). Isotopic fractionation of water during transpiration in leaves determines $\delta^{18}O$ (Ripullone et al., 2009) and is therefore a proxy of the evaporative flux and is modified by *gs* and not by *A* (Roden and Siegwolf, 2012). In the dual model, relative humidity is assumed to be a major factor influencing *gs*, whereby lower humidity give rise to lower *gs* (Scheidegger et al., 2000) and thus higher $\delta^{18}O$. Overall, the dual isotope model has potential in evaluating various stress factors in plants (Scheidegger et al., 2000).Therefore, by measuring $\delta^{13}C$ and $\delta^{18}O$ in the same material, $\delta^{18}O$ enables the assessment of the factor (*A* or *gs*) which drives the variation in $WUE_{intrinsic}$ (i.e. variation in $\delta^{13}C$) under varying growing conditions (e.g. drought). $\delta^{13}C$ would be useful information for cultivar improvement and breeders could then use this trait screening approach in breeding programs.

$\delta^{18}O$ has been used in several small grain crop studies (Cabrera-Bosquet et al., 2011; Cabrera-Bosquet et al., 2009; Ferrio et al., 2007). It has been used to assess long-term transpiration performance of genotypes (Cabrera-Bosquet et al., 2009; Sheshshayee et al., 2010) as well as a grain yield predictor (Ferrio et al., 2007), but to our knowledge, less is known about the applicability of the dual isotope model in annual crops. The dual isotope model has mainly been tested for tree species (Barnard et al., 2012; Ripullone et al., 2009; Roden and Farquhar, 2012).

The measurement of carbon isotope discrimination in plant material offers a powerful means of evaluating WUE at leaf level as it can provide repeatability and heritability required for a selecting trait (Condon and Richards, 1992). Plants are sensitive to changes in soil moisture (Davies and Gowing, 1999) and $\delta^{13}C$ measured in plant material is capable of detecting subtle changes in *Ci*/*Ca* resulting from small soil moisture fluctuations (Farquhar et al., 1989). Even though, ^{13}C shows potential, not many small grain cultivars have been selected for high WUE using this tool. We are only aware of a single study by Rebetzke et al. (2002) which reported the selection of wheat cultivars using $\Delta^{13}C$ as a selecting trait and there are currently no reports on triticale selection via $\Delta^{13}C$. There is also very limited information on $\delta^{13}C$ variation in triticale genotypes. The available literature are mainly comparison studies between triticale and other small grain cereals (e.g. Motzo et al. (2013) and Yousfi et al. (2010)). Triticale was selected for this study as it out yields wheat in both favourable and unfavourable conditions (Bassu et al., 2011; Estrada-Campuzano et al., 2012). It is also believed that triticale will become more important in the future than wheat if grain quality is improved (Blum, 2014). Its importance will arise due to: climate change; the spreading of agriculture in marginal lands and the need to feed the ever increasing population under harsh conditions.

The main purpose of the study was to test if stable isotopes of carbon and oxygen can be used for screening drought stressed triticale genotypes. Specifically, we aimed to: (1) test the use of carbon isotope discrimination as a proxy of intrinsic WUE and grain yield in field grown triticale; (2) test if ^{18}O and ^{13}C data can be used to assess whether changes in $\delta^{13}C$ are due to changes *A* and/or *gs* of field grown triticale and (3) to explore the use of carbon isotope discrimination to infer sources of carbon assimilates to grain filling.

2. Materials and methods

2.1. Study site and experimental design

The study was carried out at the University of Limpopo experimental farm, Syferkuil (23°50′S; 029°41′E), Limpopo Province, South Africa during two winter seasons; June to October in 2013 and July to November in 2014. According to the Koppen-Geiger climate classification, the local climate falls under BSh (arid, steppe, hot) (Kottek et al., 2006). The area receives rainfall, ranging from 400 to 600 mm per annum (Benhin, 2006), of which 85% falls in summer; i.e. between November and March. Average minimum and maximum temperatures are 4–20 °C in winter and 17–27 °C in summer. The weather conditions experienced in the two growing seasons are shown in Fig. 1. Fig. 1A shows the daily maximum and minimum temperatures recorded on station at Syferkuil in 2013 and 2014 growing seasons. Daily maximum temperatures during the reproductive months (September to November) of triticale reached as high as 35 °C in both years (Fig. 1A, double arrow). The same Fig. 1A also shows optimum temperature range for triticale (12–25 °C), represented by lines at 25 and 12 °C. Mean temperatures for both years are shown on Fig. 1B together with total monthly rainfall for the two seasons.