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The combined use of vegetation indices and stable isotopes to predict durum wheat grain yield under contrasting water conditions

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ABSTRACT

Improving durum wheat performance to abiotic stresses is often limited by a lack of proper monitoring methods in support of crop management and efficient phenotyping tools for breeding. The objectives of this study were: (1) comparing the performance under contrasting water treatments of different physiological traits, which evaluate plant growth and water status; and (2) understanding how these traits can predict grain yield (GY) performance under contrasting water conditions. Thus, five modern durum wheat genotypes were subjected to rainfed (RF) and supplemental irrigation (SI) treatments. Two categories of physiological traits were tested; (1) the vegetation indices: the Normalized Difference Vegetation Index (NDVI) and the Normalized Green Red Difference Index (NGRDI); and (2) the stable carbon and oxygen isotope compositions ($\delta^{13}C$ and $\delta^{18}O$) of different plant parts. The NGRDI at anthesis and the $\delta^{13}C$ of mature grains were the traits best correlated (positively and negatively, respectively) with GY. Both traits in combination explained at least 50% of variability in GY within each water treatment. The produced path models for RF and SI conditions highlighted the particular role of NGRDI and $\delta^{13}C$ in predicting GY. In addition, the study showed the potential of using vegetation indices derived from digital Red-Green-Blue (RGB) images as a low-cost technique for assessing aerial biomass (AB) and GY under different water availabilities.

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1. Introduction

Durum wheat is one of the most important crops in Mediterranean environments (FAO, 2012), with water stress being the main constraint limiting productivity (Oweis et al., 2000). This limitation is likely to increase in the future because climatic change is expected to decrease precipitation and increase evapotranspiration in the Mediterranean region (Lobell et al., 2008). Crop management and breeding may improve the performance of durum wheat under stress conditions. However, the lack of efficient tools to monitor the performance of agronomical practices or to undertake appropriate phenotyping in breeding programmes limits the efficiency of both avenues.

Information obtained from physiological assays, such as gas exchange and pressure–volume measurements, often provide accurate data on the immediate plant water status, transpiration or photosynthesis (Ferrio et al., 2003). However, it is difficult to upscale the information at the canopy level over a large temporal and/or spatial scale represented by the entire crop cycle (Ferrio et al., 2003; Lambers et al., 1998). Actually, the most successful traits for evaluation integrate crop performance in time (throughout the crop cycle) and space (at the canopy level) in terms of the ability to capture resources (e.g. radiation, water, and nutrients) and how efficiently these resources are used (Araus et al., 2002, 2008). Among the categories of integrative traits it is worth mentioning the analysis of stable isotopes in plant samples, together with the use of proximal (remote) sensing techniques at the field level (Aparicio et al., 2000, 2002, 2004; Araus et al., 2009, 2013; Casadesús et al., 2007; Casadesús and Villegas, 2014; French et al., 2015; Gumma et al., 2015; Hunt et al., 2013; Rahimi et al., 2014; Rorie et al., 2011; Sakamoto et al., 2012a,b; Torbick and Salas, 2015; Usman et al., 2014). A combination of these approaches has been proposed, for example, in breeding (Araus and Cairns, 2014; White et al., 2012), but to our knowledge reports of such studies are scarce. Whereas stable carbon and oxygen isotopes deal with the availability and use of water by the crop, or in other words deal with the water status (Araus et al., 2013), remote sensing-derived indices inform on the radiation uptake and the photosynthetic

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(assimilatory) capacity of the plants (associated with aerial plant growth and biomass) (Araus and Cairns, 2014; Gamon et al., 1995). Therefore, these traits may help to elucidate how crop responses to growing conditions are defined in terms of final yield.

In C_3 plants the carbon isotope composition ($\delta^{13}C$; frequently expressed as a discrimination against surrounding air, $\Delta^{13}C$) measured in plant tissues was determined as negatively correlated with the ratio of intercellular CO_2 leaf concentration to ambient CO_2 (C_i/C_a) and positively correlated with the ratio of net assimilation to water transpired (A/E). Therefore, $\delta^{13}C$ is positively related to water use efficiency (*WUE*) (Araus et al., 2013; Farquhar and Richards, 1984; Monneveux et al., 2006). Genetic variability for $\delta^{13}C$ has been reported in wheat (e.g. Araus et al., 2003a,b, 2013; Condon et al., 1987, 1993, 2002, 2004; Rebetzke et al., 2002). Further, $\delta^{13}C$ is a highly heritable trait that is relatively easy to manipulate in breeding populations (Condon and Richards, 1992; Rebetzke et al., 2002). Correlations between $\delta^{13}C$ and grain yield (GY) are normally high, and either negative or positive according to the plant tissue sampled and environmental conditions tested (Araus et al., 2003a,b; Condon and Richards, 1992; Voltas et al., 1999). High $\delta^{13}C$ has been used as a selection trait for high *WUE* at the seedling stage in commercial wheat varieties for the summer dominant rainfall environments of Australia where crop yield relies on the water accumulated before planting (Condon et al., 2004; Rebetzke et al., 2002; Richards et al., 2011). However, in the Mediterranean basin environments characterized by precipitation after planting and moderate to medium drought during the reproductive part of the crop, the efficient use of water (*EUW*) by the crop rather than the *WUE* is the factor affecting productivity (Blum, 2009). Since genotypes exhibiting a higher crop water status, and thus a higher *EUW*, are those with a lower *WUE* (therefore lower $\delta^{13}C$), these genotypes with a lower $\delta^{13}C$ of mature grains (or of any other plant part developed at the end of the crop) may reflect better growing conditions and therefore exhibit higher GY (Araus et al., 2013). In fact, negative phenotypic and genotypic correlations between $\delta^{13}C$ and both GY and aerial biomass (AB) have been reported (Araus et al., 1998, 2003a, 2013; Merah et al., 2001; Monneveux et al., 2006), meaning that genotypes that are able to maintain higher water use (even if it is at the expense of a lower *WUE*) are the most productive (Araus et al., 2008, 2013). In the case of Australia, the high $\delta^{13}C$ genotypes, which were selected for the summer dominant rainfall environments of Australia, have almost no increased GY in either the winter dominant rainfall environments (Mediterranean environments) or in the environments with highly variable rainfall of Australia (Condon et al., 2004). Overall, high $\delta^{13}C$ (and therefore *WUE*) is a ‘conservative’ trait in terms of water use and crop growth rate, and thus in the absence of soil water deficit, high $\delta^{13}C$ genotypes tend to grow slower than low $\delta^{13}C$ genotypes, resulting in lower AB and GY (Condon and Richards, 1992; Condon et al., 2004). However, the contradictory results of the relationship between $\delta^{13}C$ and GY may also be due to the fact that frequently these studies have used sets of genotypes that have variations not only in $\delta^{13}C$, but in heading and/or anthesis dates as well. Phenology could strongly influence GY and $\delta^{13}C$, being responsible for the negative relationships between $\delta^{13}C$ and GY (Araus et al., 2002, 2003a; Condon et al., 2004). Therefore, when studying the relationship between $\delta^{13}C$ and GY it is necessary to test genotypes with a similar phenology (Araus et al., 1998, 2003a; Condon et al., 2004; Rebetzke et al., 2002; Richards et al., 2011).

The oxygen isotope composition ($\delta^{18}O$) of plant tissues is known to reflect the evaporative conditions throughout the crop cycle (Barbour et al., 2000) and thus it has been proposed as a proxy method for measuring transpiration as well as an indicator of genotypic differences in stomatal conductance (g_s) (Araus et al., 2013; Barbour et al., 2000; Cabrera-Bosquet et al., 2011; Elazab et al., 2012; Ferrio et al., 2007). Leaf $\delta^{18}O$ has been negatively correlated with the transpiration rate (T) (Barbour et al., 2000; Barbour and Farquhar, 2000; Cabrera-Bosquet et al., 2009a,b; Ferrio et al., 2007) and under well-watered conditions in wheat the $\delta^{18}O$ of flag leaves correlates negatively with GY and g_s (Barbour et al., 2000). Cabrera-Bosquet et al. (2009b) found negative correlations in maize between the $\delta^{18}O$ of grains and GY under well-watered and moderate water stress conditions, whereas under severe water stressed conditions the correlations were positive. However, the correlation of $\delta^{18}O$ analyzed in mature grains of wheat with GY is frequently weak or absent (Araus et al., 2013; Ferrio et al., 2007). Nevertheless, the combined measurements of $\delta^{13}C$ and $\delta^{18}O$ in the same plant tissue may help in separating the independent effects of photosynthetic capacity (A) and g_s on $\delta^{13}C$ because $\delta^{18}O$ is not affected by photosynthesis (Araus et al., 2013; Barbour and Farquhar, 2000; Elazab et al., 2012).

The assessment of AB is important for monitoring crop growth because it could reflect the effect of stresses on crop growth and senescence (Araus et al., 2008; Royo and Villegas, 2011). Larger green AB represents higher potential canopy photosynthesis and thus more yield. Therefore, the effect of water stress in limiting plant growth has a subsequent impact on reducing the photosynthetic potential at the crop level and thus GY. In that sense a number of studies have revealed that spectral reflectance or/and digital imaging by ground-based remote sensing has the potential to provide precise, non-destructive instantaneous quantitative estimates of AB and GY (Aparicio et al., 2004; Raun et al., 2001). The Normalized Difference Vegetation Index (NDVI) is among the most usual of spectral reflectance indices, and it is related to the photosynthetically active AB (Ferrio et al., 2005). The NDVI has been used as an indicator of AB and GY in durum wheat (Aparicio et al., 2000, 2002, 2004; Casadesús et al., 2007; Ferrio et al., 2005) and bread wheat (Gutiérrez-Rodríguez et al., 2004; Lobos et al., 2014). The introduction of low-cost portable and easy to handle active spectroradiometers (such as GreenSeeker and Crop Circle) has become a very useful alternative as they can measure the NDVI directly. Moreover, these spectroradiometers are active in the sense that they have their own light source, and thus can be used under diverse atmospheric conditions (Araus et al., 2009; Marti et al., 2007). As a limitation, NDVI values saturate at high green biomass densities (i.e. high leaf area index), which means that this technique may have a low precision at key moments of the crop cycle (e.g. anthesis), especially at high plant densities under favourable agronomic conditions.

In recent years the use of digital Red-Green-Blue (RGB) images has been proposed as an alternative to develop vegetation indices that may replace spectroradiometrical based NDVI (Casadesús et al., 2007; Casadesús and Villegas, 2014; Hunt et al., 2013; Sakamoto et al., 2012a). The price, size, and the easy use of conventional digital cameras make them viable alternatives to assess AB and GY in cereals (Casadesús et al., 2007; Casadesús and Villegas, 2014; Mullan and Reynolds, 2010). A number of studies have used digital RGB imaging to measure different colour parameters such as: greenness; intensity of green, red and blue; and derived normalized indices from the green, red and blue bands (Casadesús et al., 2007; Gitelson et al., 2002; Kipp et al., 2014; Mullan and Reynolds, 2010). Such information has allowed estimation of a wide range of crop traits in durum and bread wheat such as early vigour, leaf area index, leaf senescence, AB and GY (Casadesús et al., 2007; Casadesús and Villegas, 2014; Gitelson et al., 2002; Mullan and Reynolds, 2010). Moreover, a large number of digital RGB images (i.e. photos) can be obtained with a minimum effort (Casadesús and Villegas, 2014) and processed with suitable (either open-access or commercial photo editing) software to extract parameters related to green AB (Casadesús et al., 2007). Among the different indices derived from RGB images, the Normalized Green Red Differences Index (NGRDI), which uses the Green and Red regions of the

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