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Use of Carbon Isotope Discrimination (Δ) in Breeding of C_3 Cereals Under Water Deficit Conditions

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Abstract: The aim of this mini review was to present the use of carbon isotope discrimination (Δ) technique in breeding of C_3 cereals. Δ values were related with Water Use Efficiency (WUE) and yield in C_3 species. This method is used, for over 20 years, as an indirect selection criterion of high-yielding genotypes grown under water deficit conditions. Till now, Δ values have been extensively used in selection of high-yielding genotypes of bread or durum wheat, barley and rice in USA, Spain, France, Australia, Greece and numerous other countries. In this study, the advantages and disadvantages of the technique along with its use restrictions are discussed and the use of putative surrogates of Δ is proposed.

Key words: Ash content, barley, drought, grain yield, rice, wheat

INTRODUCTION

Cereals are the basis of human nutrition with increasing demand worldwide. In global level, the annual demand for wheat increases with a rate of 2% while the grain yield increase is restricted to 1% for irrigated and 0.5% for rainfed wheat (Skovmand *et al.*, 2001; Trethowan *et al.*, 2002). Given that, the arable land expansion is not feasible any more; yield increase could be derived from the improvement of agronomic practices and genetic breeding of new cultivars. During 20th century, both factors equally contributed to productivity increase (Turner, 2004; Richards, 2006). Genetic development of cereals is slower in arid and semi-arid environments as Mediterranean due to significant genotype × environment interactions which are ascribed to water availability fluctuations from year to year (Rebetzke *et al.*, 2002). In those environments, water availability is the most limiting factor of cereal productivity (Araus *et al.*, 2002, 2003a). Theoretically, in water limited environments, species or genotypes using soil water more efficiently have an advantage and yield more (Ehleringer, 1993; Tsialtas *et al.*, 2001a).

Grain yield of cereals grown under water deficit conditions is given by an equation first reported by an Australian researcher as follows (Passioura, 1977):

$$\text{Grain yield} = \text{WU} \times \text{WUE} \times \text{HI} \qquad (1)$$

where, WU is the amount of water used by the crop, WUE is the water use efficiency of the water used by the crop (kg dry biomass per kg of water consumed for its production) and HI is the harvest index of the crop (the ratio of grain yield to total biomass). Thus, WUE is a trait contributing to yield increase in water deficit environments under the condition that it is not negatively associated with the other two parameters of Eq. 1. However, under water-limited conditions, a negative relationship between WU and WUE has been reported (Zhang *et al.*, 1998).

Genetic variation of WUE has not been exploited till recently and a main reason of that was the lack of a simple and easy method for WUE estimation especially in field conditions (Udayakumar *et al.*, 1998). The problem has been overcome since Farquhar *et al.* (1989) proved the negative relationship between carbon isotope discrimination (Δ) in tissues of C_3 plant species and WUE. Actually, Δ is an indication of the depletion of plant tissue in ^{13}C compared with atmospheric CO_2. The negative relationship between Δ and WUE was proven for many C_3 crops and species (Turner, 1997). Δ was proposed as an indirect selection criterion for cereal breeding in water limited environments (Hall *et al.*, 1994). Then, in order to determine the relationship between Δ and biomass or grain yield and to select the genotypes with high WUE, systematic efforts must be conducted.

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The aim of this mini review was to resume the recent results of use of stable carbon isotopes in C_3 cereal breeding and to bring up the prospects and the limitations of the technique.

ESTIMATION OF CARBON ISOTOPE DISCRIMINATION VALUES (Δ)

^{13}C natural abundance and Δ estimation: Plant tissues contain a quantity of ^{13}C stable isotope, which is assimilated as $^{13}CO_2$ along with $^{12}CO_2$ during photosynthesis. Plant tissue ^{13}C content is determined on an isotope mass spectrometer, called natural abundance in ^{13}C ($\delta^{13}C$), expressed per mill (‰) and calculated by the following formula:

$$\delta^{13}C\ (‰) = ((R_{sample}/R_{standard})-1) \times 1000 \qquad (2)$$

where, R_{sample} is the ratio $^{13}C/^{12}C$ of the plant tissue sample and $R_{standard}$ is the respective ratio of the standard used. As universally accepted standard, the PDB (Pee Dee Belemnite), a sea fossil limestone derived from an organism called *Belemnitella* is used (Lajtha and Marshall, 1994).

$\delta^{13}C$ values of the plant tissues are used for the calculation of carbon isotope discrimination values (Δ) using the following formula:

$$\Delta\ (‰) = (\delta_a-\delta_p)/(1+\delta_p/1000) \qquad (3)$$

where, δ_p is $\delta^{13}C$ of the plant sample and δ_a is $\delta^{13}C$ of the ambient CO_2. The current value of δ_a, is ca -8‰ (Guehl *et al*., 1995). From the above-mentioned relationship, it is realized that Δ is a means of comparison of the carbon isotope ratios ($^{13}C/^{12}C$) between the plant tissue and the ambient air. Actually, Δ values reveal how much depleted in ^{13}C, is the plant tissue compared to the air (Guehl *et al*., 1995).

Relation of Δ with WUE: According to Farquhar *et al*. (1989), Δ is related with the ratio of CO_2 concentration in the mesophyll to the CO_2 concentration in the atmosphere (c_i/c_a) as it is indicated by the following formula:

$$\Delta = a + (b-a) \times (c_i/c_a) \qquad (4)$$

where, a is the fractionation against $^{13}CO_2$ during air dilution in the mesophyll (-4.4‰) and b is the fractionation against $^{13}CO_2$ during carboxylation (owing mainly to Rubisco, ca -27‰). Thus, it is easily understandable that Δ is affected by factors that alter the c_i/c_a ratio such as stomatal conductance and leaf photosynthetic capacity. The former factor affects Δ positively and the latter negatively (Richards, 2000).

CARBON ISOTOPE DISCRIMINATION (Δ) AND BREEDING OF C_3 CEREALS

Requirements for a selection criterion: Recently, it is supported that physiological traits could be employed in the exploration of genetic variation in order to select high yielding genotypes (Edmeades *et al*., 2004). The use of an indirect selection criterion in plant breeding should satisfy the following prerequisites: a) the existence of wide genetic variation for that trait, b) close relationship with yield, c) higher heritability than yield and d) rapid, easy and cheap determination of the trait (Araus *et al*., 2002). Δ is a physiological trait, which satisfies the above-mentioned prerequisites and it can be used in breeding studies in order to bridge the gap between genotype and phenotype.

Studies of cereal genetic variation with Δ: The research on employing Δ in C_3 cereal breeding began in mid-'80's when Farquhar and Richards (1984) found that the ratio of stable carbon isotopes ($^{13}C/^{12}C$) in tissues of wheat was negatively related with WUE. Thus, high Δ values are indicative of low WUE. Δ is commonly determined in flag leaf or in mature kernels with the latter determination to be more representative of WUE (Villegas *et al*., 2000; Merah, 2001; Merah *et al*., 2001a, 2002). The existence of genetic variation as regards Δ was reported for bread and durum wheat, barley, triticale and rice (Voltas *et al*., 1998; Royo *et al*., 1999; Peng *et al*., 1998; Merah *et al*., 2001b; Teulat *et al*., 2001; Rebetzke *et al*., 2002; Araus *et al*., 2003b; Çağirgan *et al*., 2005; Impa *et al*., 2005; Misra *et al*., 2006). In addition, genetic variation for Δ values was found in lines of bread wheat derived via intra-cultivar selection (i.e., cv. Nestos) by the honeycomb selection method (Tsialtas *et al*., 2001b; Tokatlidis *et al*., 2004). The present study is not referred to the C_4 cereals like corn and sorghum due to the different photosynthetic pathway they follow, which affects discrimination against $^{13}CO_2$ and thus differentiates the Δ-WUE relationship (Yin and Raven, 1998).

Relationships of Δ with WUE and yield: Till now, extended research has been conducted on establishing relationships between Δ and total biomass production or grain yield of cereals grown on various growing conditions (glasshouse or filed experiments, under dry-land or irrigated conditions). Under well-watered or moderate drought conditions, a positive relationship between Δ determined in leaf or grain and yield was found for bread and durum wheat (Condon *et al*., 1987, 1993; Morgan *et al*., 1993; Sayre *et al*., 1995; Araus *et al*., 1998; Merah *et al*., 1999, 2000, 2001c, d) and barley (Craufurd *et al*., 1991; Voltas *et al*., 1998). Thus, the highest yielding genotypes were those that had of the highest Δ values and consequently the lowest WUE. This finding sounds weird since based on Eq. 1, genotypes with high WUE are expected to be the most productive. However, negative relationships between grain yield or aboveground biomass and Δ have been found when water availability (precipitation or soil water) is very limited (Al Hakimi *et al*., 1996; Ehdaie and Waines, 1996; Richards, 1996; Reynolds *et al*., 2007). The positive relationship between Δ and yield could be ascribed to the fact that genotypes with high Δ keep more their stomata open and thus transpire more water (higher WU in Eq. 1) increasing yield (Fischer *et al*., 1998; Monneveux *et al*., 2004a). The total WU of a crop is a function of the genotype and the soil moisture conditions. High yields are related with high total WU (Cabuslay *et al*., 2002; Hu *et al*., 2006; Cabrera-Bosquet *et al*., 2007) but genotypes or growing conditions which contribute to high WU decrease WUE (Zhang *et al*., 1998) meaning that increase Δ values. Obviously, high yielding genotypes which show high Δ values exploit greater amounts of water from deeper soil layers through the development of a more extensive and intensive root system (Araus *et al*., 2002; Slafer *et al*., 2005). On the contrary, when genotypes can not access more water as it happens in extremely dry environments or in pot experiments, genotypes with high WUE (low Δ values) seem to have an advantage since traits contributing to persistence but not to productivity are favoured (Araus *et al*., 2002, 2003a). In general, selection for low Δ contributes to grain yield increases when conducted in low-rainfall (< 350 mm year^{-1}) (Slafer *et al*., 2005) or in low-productive environments (< 2000 kg grain ha^{-1}) (Araus *et al*., 2003b). Thus, Rebetzke *et al*. (2002) crossing an elite wheat cultivar (Hartog) with genotypes exhibiting low Δ bred a new cultivar with low Δ and higher grain yields, adapted to the semi-arid environment of NW Australia. In conclusion, it is realized that Δ values is a useful criterion for genotype selection in environments with high genotype × environment interactions as regards grain yield (Voltas *et al*., 1999; Condon *et al*., 2004).

Studies on wheat and barley cultivars released from 19th century till recently showed that Δ values increased through the time (Muñoz *et al*., 1998; Zhao *et al*., 2001; Xue *et al*., 2002). On parallel, in durum wheat (*Triticum turgidum* L.) and barley (*Hordeum vulgare* L.), Δ was positively related with HI (Merah *et al*., 2001c; Teulat *et al*., 2001) and thus selection for higher biomass/yield contributed to selection for higher HI. This means that cultivars are selected for their ability to exploit more available resources and thus, to have high productive potential (Jiang *et al*., 2003). Based on the latter, Tambussi *et al*. (2005) and Slafer *et al*. (2005) proposed that genotypic selection for high productivity could contribute to increases of cereal yields under the Mediterranean conditions.

The semi-arid environments, especially those where supplemental irrigation is a standard practice, are prone to soil salinization due to NaCl accumulation at the surface layer. In such environments, plants have to cope with a combination of salinity, water and osmotic stresses, which falsely considered as drought stress (Munns, 2002). Δ values have been proposed as a selection criterion for high grain yield under stressful conditions, especially for barley. While Isla *et al*. (1998) stated that grain yield but not Δ is a suitable selection criterion for higher yield under salinity, other researchers reported a positive relationship between grain yield and Δ and thus proposed Δ values as an indirect selection criterion under salinity conditions (Ansari *et al*., 1998; Shahhen and Hood-Novotny, 2005).

Heritability of Δ: The higher heritability compared to yield is the third criterion a physiological trait should fulfil in order to be used as a selection tool in breeding studies. In many works, Δ had higher heritability compared to yield as it was reported in wheat (Ehdaie *et al*., 1991; Condon and Richards, 1992; Rebetzke *et al*., 2006) and other C_3 crops such as cotton (Stiller *et al*., 2005) and beans (Zacharisen *et al*., 1999). Moreover, since the chromosomal and gene sites controlling Δ have been identified in wheat, barley and rice (Handley *et al*., 1994; Ehdaie and Waines, 1997; Laza *et al*., 2006; Mohammady-D, 2005; Mohammady-D *et al*., 2005), Δ values can be used in biotechnological research in the direction of the bridging the gap between genotype and phenotype (Araus *et al*., 2003a; Wilson *et al*., 2003).

Advantages and disadvantages of Δ: The main advantage of Δ values in WUE estimation is that Δ is not an instantaneous measurement of WUE, which could be affected by environmental changes but it is an estimation of WUE for all the term during which ^{13}C was assimilated (Adams and Grierson, 2001). Also, repeated, non-destructive measurements of Δ could be conducted under field conditions using small plant samples, which are analyzed immediately or after storage. It is clear that the use of stable carbon isotopes is a quick and easily applied method for WUE estimation in large numbers of samples. Its disadvantage is the need for modern, specialized and expensive equipment (mass spectrometers) the use of which, however, has recently spread worldwide. Finally, the relatively high cost of stable isotope analysis (~ € 6-10 sample^{-1}) restricts the wider use of Δ values in extensive breeding programs in which a large number of genotypes should be tested (Voltas *et al*., 1998). For these reasons, cheap and easily determined surrogates of Δ should be found in order to use Δ during the first steps of selection programs aiming to restrict the cost of isotope analyses.

SURROGATES OF Δ VALUES

The most commonly used surrogates of Δ values are the ash content and specific elements (K and Si) of plant tissues (Masle *et al*., 1992; Merah *et al*., 1999; Araus *et al*., 2001; Monneveux *et al*., 2004b). The hypothesis is that these surrogates are passively accumulated in plant tissues via the transpiration stream and thus, high Δ values (i.e., increased transpiration) would be accompanied by high accumulation of ash and inorganic elements in plant tissues. The ash content in plant tissues (flag leaf, kernel) was related (positively and negatively, respectively) with Δ values and grain yield (Merah *et al*., 2001e). Masle *et al*. (1992) reported a positive correlation between Δ and K concentration while Merah *et al*. (2001e) found no relationship between the two parameters in the flag leaves of durum wheat. The latter could be attributed to the fact that the accumulation of the inorganic elements in plant tissues is not just a passive procedure but it is significantly affected by genotypic variation in element acquisition (Brown and Byrd, 1997). Indeed, Zhao *et al*. (2007) reported a positive or negative relationship between Δ and K concentration in coexisting species in a semi-arid environment of China. Also, no significant relationships between Δ and Si concentration in leaves were reported by Masle *et al*. (1992) and Merah *et al*. (1999). In those species or environments where the relationship between Δ and surrogates is positive, the latter could substitute for the former. Moreover, Merah *et al*. (2001e) found that ash content is related not only with Δ values and grain yield but also with HI. Recently, Misra *et al*. (2006), working in various microenvironments of India peninsula, found that ash content was significantly related with wheat yield even when no significant relationship between Δ and yield was evident. Tsialtas *et al*. (2005) reported analogous results in lines selected in a bread wheat cultivar. Alike Δ values, surrogates of Δ (ash content and K) have high heritability (Tsialtas *et al*., 2002; Condon and Richards, 1992; Rebetzke *et al*., 2006) and consequently they fulfil an important prerequisite for their use as a selection criterion.

Finally, Ferrio *et al*. (2001) proposed the use of Near Infrared Reflectance Spectroscopy (NIRS) as a quick and easy indirect estimation of Δ values since a positive relationship between Δ values measured in grains of durum wheat and Δ estimates using NIRS was found.

CONCLUSIONS

Δ, determined in flag leaf or grain, is considered as an indirect assessment of long-term WUE in C_3 cereals. For over 20 years, Δ is widely used in grain yield variation studies to indirectly select high yielding genotypes. Already, a commercial wheat cultivar created using Δ technique, has been released in Australia and it is adapted in severe water-limited environments. Climatic change (higher temperature, lower or unpredictably distributed precipitation) will intensify water stress on C_3 cereals now growing under less stressful conditions. Thus, Δ technique would be useful for screening genotypes for their yielding ability under very stressful conditions (grain yield <2000 kg ha^{-1}) under which WUE could significantly contribute to final grain yield. The high cost of Δ determination is a main limitation of the widespread adoption of the technique. However, this disadvantage could be partially overcome by finding reliable putative surrogated of Δ, which could be used at the first stages of a selection program. Ash content, determined mainly in grain, is an effective and cheap surrogate of Δ and it is more related with grain yield, compared to Δ, under certain conditions.

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