

DROUGHT STRESS

Is Discrimination of ^{13}C in Potato Leaflets and Tubers an Appropriate Trait to Describe Genotype Responses to Restrictive and Well-Watered Conditions?

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Abstract

Selection for drought tolerance entails prioritizing plant traits that integrate critical physiological processes occurring during crop growth. Discrimination against ^{13}C (Δ) in leaflets (Δ_{leaflet}) and tubers (Δ_{tuber}) was compared under two water regimes in two potato-improved varieties selected to maintain yield under drought conditions (Unica and Sarnav) and one drought susceptible European cultivar (Désirée). In the control treatment, soil water content was kept at field capacity over the whole growth cycle, while in the drought treatment water supply was restricted after tuber initiation (50 % of field capacity). Gas exchange and N content per unit leaf area (N_{area}) as well as Δ were assessed at different stages. Sarnav showed the highest tuber yield in both water conditions, suggesting that yield in the water restriction treatment was largely driven by yield potential in this genotype. Higher stomatal conductance (g_s) and N_{area} and lower Δ_{leaflet} in well-watered Sarnav suggested higher photosynthetic capacity. Under water restriction, Sarnav maintained higher g_s indicating that carbon diffusion was a key factor for biomass accumulation under water restriction. Our results suggest the use of Δ determined after tuber initiation as an indirect selection indicator for tuber yield under both well-watered and restricted soil water availability conditions.

Introduction

Increasing food production for the 9.2 billion people predicted for 2050 will require a 2.5- to 3-fold increase in water use over current consumption (Lal 2013). An increased restriction on agricultural water use is however predicted in tandem with this food demand increment, and limited access to water is becoming a main restrictive factor in agriculture (Cattivelli et al. 2008). In addition, climate change scenarios project the exacerbation of drought in areas where potato cropping is being expanded (Monneveux et al. 2013), highlighting the importance of selecting genetic materials adapted to water restriction. Under mild water stress conditions, it is desirable to prioritize genotypes with a 'risky' or 'opportunistic' behaviour, that is plants with higher carbon assimilation and transpiration and able to take advantage of water pulses, whereas under terminal or severe drought, it is preferable to select water-use efficient plants (Tardieu 2012). However, in

order to mitigate the effects of climatic unpredictability, it is important to select resilient materials showing low variation in yield under different water scenarios (Chapuis et al. 2012, Kahiluoto et al. 2014).

In C_3 crops, discrimination against ^{13}C isotope (Δ) occurs as a part of thermodynamic and kinetic reactions (Farquhar et al. 1989, Dawson et al. 2002) that include carbon diffusion from boundary layer to carboxylation sites and biochemical processes during carbon fixation, conversion and storage (Seibt et al. 2008, Tcherkez et al. 2011). The high heritability of Δ and the low-cost and amenability of its high-throughput screening are considered as advantages for the utilization of this trait as an indirect selection criterion (Easlon et al. 2014). It has been widely utilized in breeding for drought tolerance in cereals (Monneveux et al. 2005), but the potential utilization of Δ to estimate transpiration efficiency, potential yield or drought tolerance in root and tuber crops has been poorly explored. Limited Δ studies in potato

(Jefferies 1995, Jefferies and MacKerron 1997, Deblonde et al. 1999) have been mainly concerned with old European cultivars and have not included new cultivars selected under drought conditions. However, a positive correlation has been noted between leaf Δ and stomatal conductance (g_s) among potato clones by Jefferies (1995). Under moderate water restriction, Deblonde et al. (1999) found a positive association between leaf Δ and drought tolerance (defined as dry weight tuber yield under water limitation relative to dry weight tuber yield under full irrigation). As tuber formation takes longer than leaf development, Jefferies and MacKerron (1997) suggested that the analysis of tuber Δ could provide a more integrated estimation of physiological performance. Moreover, the cereal data indicate that Δ in sink organs could also reflect translocation of carbon products to these organs and predict harvest index (Merah et al. 2001). However, the aforementioned studies concluded that Δ analysed in leaflets (Δ_{leaflet}) is not an appropriate trait for screening tolerance to mild water stress (Deblonde et al. 1999, Wishart et al. 2013), and that differences in Δ in tubers (Δ_{tuber}) do not reflect final yield (Jefferies and MacKerron 1997). We hypothesize that a different result would be observed if improved potato genotypes with a proved high drought tolerance were compared against non-tolerant cultivars. This study was carried out with three varieties (two advanced genotypes and one European cultivar commonly used in carbon isotopes studies in potato) with contrasted levels of drought adaptation, grown under well-watered and restrictive water conditions. Our objectives were: (i) to compare physiological performance and biomass accumulation among genotypes along the growing period and (ii) to test the reliability of Δ_{leaflet} and Δ_{tuber} to determine key mechanisms responsible of the varietal differences in biomass accumulation and tuber yield under different water availability levels.

Materials and Methods

Plant material

Three potato genotypes with different responses to water stress (Sarnav, Unica and Désirée) were tested in this study. Sarnav (CIP code No. 397077.16) maintains high yield under water restriction and high evaporative demand conditions (Carli et al. 2014). Unica (CIP code No. 392797.22) is moderately tolerant to drought, in comparison to other *Solanum tuberosum* landraces (Cabello et al. 2012). Désirée is a drought susceptible cultivar (Levy 1986) used in previous carbon isotope studies in potato (Jefferies and MacKerron 1997, Deblonde et al. 1999).

Experimental conditions

Fifty-four tubers per genotype were planted in pots (one tuber per pot) filled with 900 g of a 2 : 1 mixture of sand : peat-based substrate (PRO-MIX, Premier Tech Horticulture, Canada). Plants were grown under glasshouse conditions from May to September 2013 at the International Potato Center (CIP) experimental station in Lima, Peru (12.1°S, 77.0°W, 244 m.a.s.l.). The glasshouse was equipped with two cascade refrigeration systems (60000 British thermal units) to maintain the required temperature for tuber formation. Glasshouse environmental conditions were recorded with a HOBO U12 Outdoor/Industrial Data Logger (Onset Computer Corporation, Bourne, MA, USA). The global average daily temperature, relative humidity, global solar radiation and vapour pressure deficit during the growing period were 12.85 ± 0.1 °C, 79.12 ± 0.39 %, 6.72 ± 0.34 MJ m² day⁻¹ and 0.31 ± 0.01 kPa, respectively (see Table 1 for detailed information).

Each pot was fertilized with 4.6 g, 2.6 ml and 19.2 g of NH₄NO₃, H₃PO₄ and KNO₃, respectively, distributed in 14 weekly applications. Water content (θ) at field capacity (θ_{FC}) was calculated gravimetrically; each pot was weighted (PW), filled with dry substrate and reweighted (DW). Then, each pot was watered until saturation and weighed again when drainage ceased (FCW). Field capacity (θ_{FC}) was calculated as:

$$\theta_{\text{FC}} = \frac{\text{FCW} - \text{DW}}{\text{DW} - \text{PW}} \times 100. \quad (1)$$

The substrate was maintained at θ_{FC} from planting to tuber initiation onset (TIO) defined as the stage when tuberous swelling of the main stolons had twice the stolon diameter (approximately 10 mm) (Jefferies and Lawson 1991, Ewing and Struik 2010) as determined by daily harvest of extra pots. After TIO, at 42, 35 and 31 days after planting (DAP) for Sarnav, Unica and Désirée, respectively, two treatments were applied to each genotype. One treatment was full irrigation (FI), in which θ was maintained at θ_{FC} by replacing the water lost by evapotranspiration three times a week. The other treatment was water restriction (WR) in which the replacement of evapotranspired water was limited to 50 % of θ_{FC} . Water demand was determined gravimetrically. As increasing plant biomass along time lead to θ overestimation at gravimetric measurements, at every harvest the fresh biomass average of the harvested individuals was added to the θ calculations for each treatment. Water availability in the substrate was indicated by its water potential estimated from leaf water potential at dawn (ψ) measured by a pressure chamber (80325 model, Labconco, Kansas City, MO, USA) at different times of the crop cycle. Average ψ in FI and WR treatments were -0.24 ± 0.003 and -0.56 ± 0.01 MPa, respectively.

Table 1 Environmental conditions during the experimental period into the glasshouse

	May	June	July	August	September
Minimum temperature (°C)	7.5 ± 0.3	6.9 ± 0.1	6.9 ± 0.04	7.2 ± 0.7	7.3 ± 0.04
Maximum temperature (°C)	21.6 ± 0.8	19.7 ± 0.6	18.5 ± 0.6	19.5 ± 0.7	21.4 ± 0.9
Average temperature (°C)	13.3 ± 0.2	12.9 ± 0.2	12.4 ± 0.2	12.8 ± 0.2	13.4 ± 0.3
Average relative humidity (%)	76.6 ± 0.5	80.9 ± 0.7	80.9 ± 0.7	78.5 ± 0.7	76.2 ± 0.9
Global solar radiation (MJ m ² day ⁻¹)	8.0 ± 1.0	6.3 ± 0.7	5.5 ± 0.5	6.8 ± 0.7	8.5 ± 1.0
Average vapour pressure deficit (kPa)	0.36 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.32 ± 0.02	0.37 ± 0.02

Average daily values ± S.E.

A complete randomized design was applied. Two harvests were carried out (6 plants per genotype) before irrigation treatments were implemented. After that, four harvests of five plants per irrigation treatment and genotype were carried out. The final harvest was taken when 50 % of plants in each irrigation treatment and genotype reached senescence, which was defined as the stage when 50 % of the upper leaves presented yellowing (Jefferies and Lawson 1991).

Physiological measurements

Stomatal conductance (g_s), net photosynthesis (A) and intrinsic water-use efficiency (A/g_s) were measured for each treatment before every harvest, from the initiation of treatments onwards. Measurements were made on a leaflet of a young, expanded and sun-exposed leaf from 8:00 to 10:00 a.m., using a portable photosynthesis system (LI-6400XT model, LI-COR, Lincoln, NE, USA). The environmental conditions in the chamber were as follows: light saturation (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR), CO₂ concentration of 400 ppm, 20 °C air temperature, boundary layer conductance of 9.29 mol m⁻² s⁻¹ and vapour pressure in the range of 1.1–1.3 kPa.

Tuber dry biomass (TB) was calculated by oven-drying the samples at 60 °C for 3 days. A Gompertz function with TB as the dependent variable and DAP as the independent variable was fitted for every genotype per treatment using the Gauss–Newton algorithm with SAS v.8.02 software (SAS Institute, Cary, NC, USA). The model was used to estimate TB at the usual harvest time (TB_{UHT}): 100 DAP for Désirée and 110 DAP for Sarnav and Unica.

Drought tolerance index (DTI) was estimated to assess genotype response to drought as (Fernandez 1992):

$$\text{DTI} = \frac{\text{TB}_{\text{UHTWR}} \times \text{TB}_{\text{UHTFI}}}{(\text{TB}_{\text{UHTFIA}})^2}, \quad (2)$$

where TB_{UHTWR} is the TB_{UHT} average of a certain genotype under WR, TB_{UHTFI} is the TB_{UHT} average of a certain genotype under FI and TB_{UHTFIA} is the TB_{UHT} average of the three genotypes under FI.

A lineal model between DAP and TB was fitted from TIO through the sequential harvest that provided the highest slope for every genotype and irrigation treatment to estimate a maximum tuber bulking rate. A *t*-Student test between genotypes and irrigation treatment slopes was applied as suggested by Gomez and Gomez (1984).

For every harvested plant, a composed sample of leaflets and tubers was dried (60 °C, 72 h) and ground using a ball mill. The isotopic ratio ¹³C/¹²C of the samples was assessed at the University of California at Davis, through a PDZ Europa ANCA-GSL elemental analyser coupled to PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) operating in continuous flow mode, allowing the determination of

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000], \quad (3)$$

R being ¹³C/¹²C ratio.

The S.E. was 0.1‰. Δ was calculated as (Farquhar et al. 1989):

$$\Delta (\text{‰}) = \left(\frac{\delta a - \delta p}{1 + \delta p} \right) \times 1000, \quad (4)$$

where δp is the carbon isotope composition ($\delta^{13}\text{C}$) of the samples and δa , the $\delta^{13}\text{C}$ of the atmospheric CO₂, -8‰. Leaflet (Δ_{leaflet}) and tuber (Δ_{tuber}) Δ were estimated for every harvested plant. Total nitrogen content in dried leaflets was estimated through the micro-Kjeldahl determination method (Ma and Zuazaga 1942) for every harvest after the irrigation treatments started. Nitrogen content per unit of area (N_{area} , g N m⁻²) was estimated with the specific leaf area values (leaf area/dry leaf weight) of the three genotypes.

Statistical analysis

The effects of genotype and irrigation treatments on A , g_s , A/g_s , Δ_{leaflet} , Δ_{tuber} and N_{area} , occurring from TIO through the beginning of senescence, were assessed by ANOVA with repeated measures over time using the PROC MIXED procedure (Littell et al. 1998). Each analysis was followed by a Fischer's least significant difference (LSD) test with statistical significance at $P \leq 0.05$. Mann–Whitney *U*-test between

irrigations treatments was applied for Δ_{leaflet} and Δ_{tuber} in each genotype, as well as a Mann–Whitney *U*-test between genotypes for Δ_{leaflet} and Δ_{tuber} data measured before the initiation of irrigation treatments. All the statistical analyses were run with the SAS v.8.02 software (SAS Institute).

Results

All the assessed physiological variables showed highly significant differences ($P \leq 0.01$) between genotypes over time. All the physiological variables, except N_{area} , were significantly affected ($P \leq 0.01$) by the irrigation treatments. Δ_{leaflet} and Δ_{tuber} showed a significant interaction ($P \leq 0.05$) of Genotype \times Irrigation, whilst *A*, g_s and A/g_s did not. The effect of time was highly significant ($P \leq 0.01$) for all the physiological variables, except A/g_s (Table 2).

Water restriction led to a reduction of g_s (44.0 %), *A* (27.0 %), Δ_{leaflet} and Δ_{tuber} (7.8 % and 14.1 %, respectively), as well as an increase (33.0 %) of A/g_s (Fig. 1 and Table 3). Sarnav had a higher DTI (74.6) than Unica (62.1) and Désirée (38.2). Under both FI and WR, Sarnav had the highest final TB, whereas Désirée had the lowest final TB and tuber bulking rate (Table 3). At TIO, Δ_{leaflet} was significantly lower in Sarnav, compared to Unica and Désirée, while Δ_{tuber} was significantly lower in Sarnav and Unica than in Désirée (Fig. 1). At the first harvest after the initiation of treatments (3 weeks after TIO), statistical differences in both Δ_{leaflet} and Δ_{tuber} in response to irrigation treatments (Fig. 1) were noted in the three genotypes.

Under FI, senescence occurred at 136, 119 and 112 DAP in Sarnav, Unica and Désirée, respectively. At this watering regime, from TIO to senescence, Sarnav and Unica presented a higher *A* (3.29 and 4.35 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ more respectively) than Désirée. Also, Sarnav had higher average g_s (0.205 \pm 0.011 $\text{molH}_2\text{O m}^{-2} \text{ s}^{-1}$) than Désirée (0.152 \pm 0.010 $\text{molH}_2\text{O m}^{-2} \text{ s}^{-1}$) and the lowest A/g_s average

value (93.34 \pm 3.64 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) (Table 3). Sarnav also had a significantly lower average Δ_{leaflet} and Δ_{tuber} than the other two genotypes. Sarnav had a significantly higher N_{area} than Unica and Désirée (Table 3).

Under WR, senescence occurred at 99, 94 and 80 DAP for Sarnav, Unica and Désirée, respectively. At this irrigation treatment, there were no significant differences between genotypes in *A* from TIO to senescence (Table 3). Statistically, the highest A/g_s values were noted in Unica, followed by Désirée, and the lowest in Sarnav. Δ_{leaflet} and Δ_{tuber} average values were lower in Sarnav (19.59 \pm 0.13 ‰ and 15.96 \pm 0.21 ‰, respectively) than in Désirée (20.35 \pm 0.14 ‰ and 17.88 \pm 0.22 ‰, respectively). Δ_{tuber} average value was lower in Unica (16.68 \pm 0.21 ‰) than in Désirée (Table 3). Δ_{leaflet} and Δ_{tuber} showed an inverse relation with DTI (Fig. 2).

Discussion

Varietal physiological responses under well-watered conditions

Leaf Δ reflects integral photosynthetic activity during the growing period (Dawson et al. 2002, Easlon et al. 2014). Diffusive exchange (stomata and mesophyll conductance) and photosynthetic biochemistry determine the leaf Δ (Wong et al. 1979, Farquhar et al. 1989, Seibt et al. 2008). Under non-limitation of carbon diffusion, a higher Δ is expected because of Rubisco discrimination against the heavier isotope caused by the ‘normal’ kinetic effect (*sensu* Farquhar et al. 1989). For this reason, a higher Δ is associated to a higher g_s or lower intrinsic water-use efficiency (A/g_s) (Farquhar et al. 1982). However, contrary to this expectation, under no-water restriction, the genotype with highest g_s (Sarnav) and lower A/g_s showed the lowest Δ , suggesting a higher photosynthetic capacity and Rubisco activity (Condon et al. 2004). Sarnav also showed higher N_{area} , a trait closely related with Rubisco content and activity (Hanba et al. 1999, Wright et al. 2001) suggesting that improved photosynthetic machinery allowed Sarnav to fix more carbon than Désirée and Unica (Table 3).

Condon et al. (2004) suggested assessing leaf Δ in early formed tissue of well-watered plants as a way to predict yield in wheat. In addition, measuring Δ in such conditions ensures high heritability of the trait, and allows higher repeatability of measurements and saves time in the screening of germplasm in breeding programmes. In the present study, the differences in Δ_{leaflet} among genotypes at early stages (approximately 20 DAP before TIO) were more pronounced (Fig. 1) with Sarnav showing the lowest Δ_{leaflet} . This result was negatively related with the tuber dry biomass at final harvest under well and restrictive water

Table 2 *F*-values of ANOVA with repeated measurements in time comparing genotypes (G), irrigation treatments (IT) and G \times IT interaction for net photosynthesis (*A*), intrinsic water-use efficiency (A/g_s), stomatal conductance (g_s), leaflet and tuber discrimination against ¹³C (Δ_{leaflet} and Δ_{tuber} , respectively) and nitrogen content per area (N_{area}) factors from tuber initiation onset through senescence

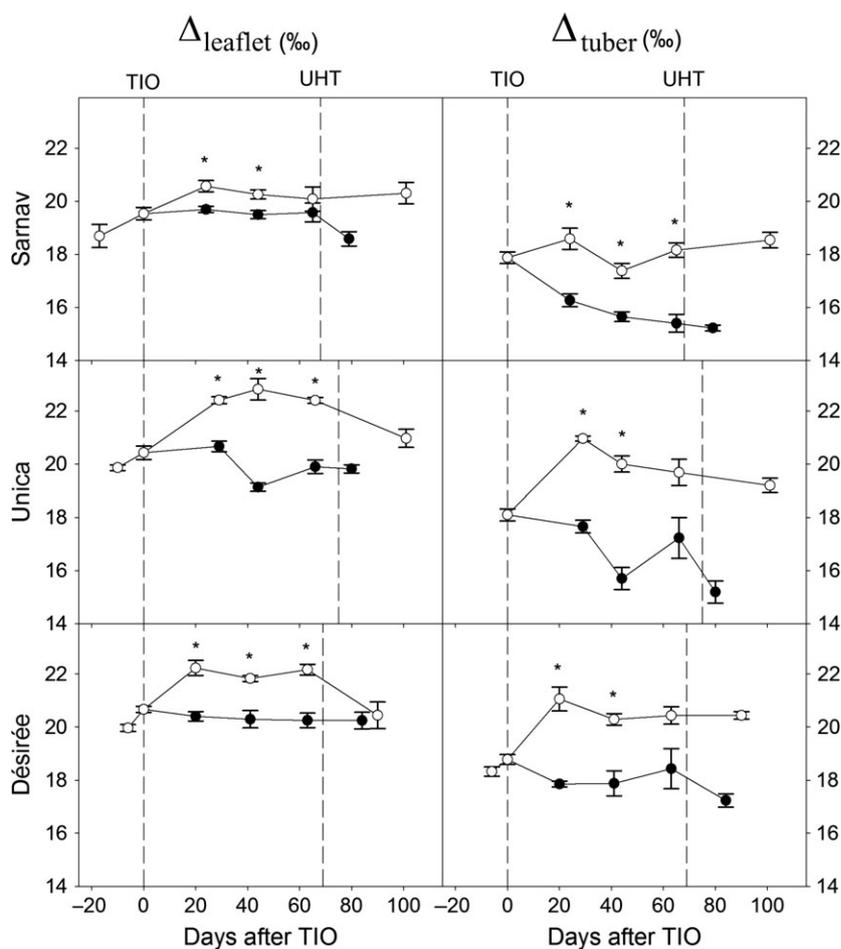
	Genotype	Irrigation treatment	G \times IT	Time
<i>A</i>	10.52**	131.86**	2.28 ^{ns}	51.75 **
g_s	7.27 **	146.17 **	0.59 ^{ns}	30.21 **
A/g_s	13.94**	99.86**	2.17 ^{ns}	0.69 ^{ns}
Δ_{leaflet}	63.22**	237.13 **	26.35**	6.60**
Δ_{tuber}	73.95**	253.43**	4.13*	16.43**
N_{area}	8.09**	0.9 ^{ns}	12.20**	76.29**

Significant at ** $P < 0.01$, * $P < 0.05$.
ns, no significant ($P > 0.05$).

Table 3 Maximum tuber bulking rate (MTBR), final tuber dry biomass (final TB), net photosynthesis (A), intrinsic water-use efficiency (A/g_s), stomatal conductance (g_s), leaflet and tuber discrimination against ^{13}C (Δ_{leaflet} and Δ_{tuber} , respectively) and nitrogen content per area (N_{area}) average values from tuber initiation onset through senescence for each genotype and irrigation treatments

	Full Irrigation			Water restriction		
	Sarnav	Unica	Désirée	Sarnav	Unica	Désirée
MTBR (g day^{-1})	$1.66 \pm 0.05\text{A}$	$1.57 \pm 0.06\text{A}$	$1.34 \pm 0.06\text{B}$	$1.30 \pm 0.06\text{a}$	$1.16 \pm 0.05\text{a}$	$1.04 \pm 0.15\text{a}$
Final TB (g plant^{-1})	$170.1 \pm 6.2\text{A}$	$147.6 \pm 5.5\text{B}$	$112.8 \pm 4.3\text{C}$	$76.0 \pm 2.0\text{a}$	$54.3 \pm 4.5\text{b}$	$45.9 \pm 3.1\text{c}$
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$17.85 \pm 0.93\text{A}$	$18.91 \pm 0.60\text{A}$	$14.56 \pm 0.69\text{B}$	$13.61 \pm 1.42\text{a}$	$12.44 \pm 0.94\text{a}$	$11.43 \pm 0.63\text{a}$
g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	$0.205 \pm 0.015\text{A}$	$0.179 \pm 0.008\text{AB}$	$0.152 \pm 0.013\text{B}$	$0.128 \pm 0.018\text{a}$	$0.088 \pm 0.010\text{b}$	$0.084 \pm 0.005\text{b}$
A/g_s ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	$93.34 \pm 3.76\text{B}$	$107.57 \pm 2.83\text{A}$	$104.16 \pm 3.80\text{A}$	$117.43 \pm 4.53\text{c}$	$150.17 \pm 5.29\text{a}$	$138.05 \pm 3.30\text{b}$
Δ_{leaflet} (‰)	$20.31 \pm 0.22\text{C}$	$22.54 \pm 0.23\text{A}$	$22.08 \pm 0.21\text{B}$	$19.59 \pm 0.18\text{b}$	$19.92 \pm 0.40\text{b}$	$20.35 \pm 0.20\text{a}$
Δ_{tuber} (‰)	$18.03 \pm 0.17\text{B}$	$20.21 \pm 0.13\text{A}$	$20.59 \pm 0.13\text{A}$	$15.96 \pm 0.10\text{c}$	$16.68 \pm 0.30\text{b}$	$17.88 \pm 0.17\text{a}$
N_{area} (g m^{-2})	$1.093 \pm 0.048\text{A}$	$0.929 \pm 0.026\text{B}$	$0.943 \pm 0.030\text{B}$	$1.046 \pm 0.025\text{a}$	$1.043 \pm 0.029\text{a}$	$1.110 \pm 0.024\text{a}$

MTBR statistical differences were proved with a t -Student slope analysis ($P < 0.05$), and all the other variables with a LSD mean comparison ($P < 0.05$). Different letters compare the genotype effect within the irrigation treatments.

**Fig. 1** Leaflet (Δ_{leaflet}) and tuber (Δ_{tuber}) discrimination against ^{13}C (average values \pm S.E.) temporal trend for each genotype assessed. Black bullets represent water restriction treatment and white bullets full irrigation. * $P < 0.05$.

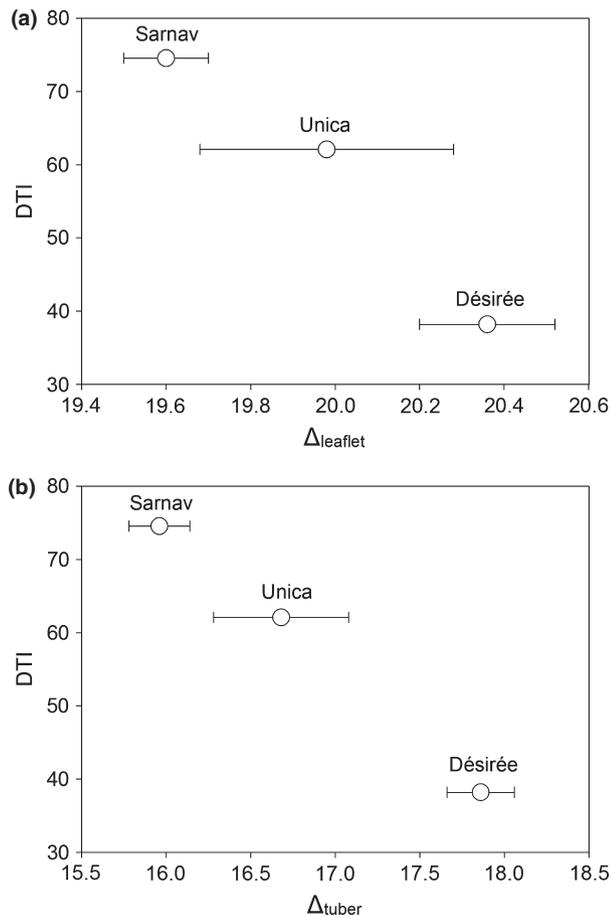


Fig. 2 The three genotypes under water restriction leaflet (Δ_{leaflet}) and tuber (Δ_{tuber}) discrimination against ^{13}C from tuber initiation onset through senescence average values vs. drought tolerance index at the usual agronomical harvest time (DTI) plots.

conditions, where Sarnav showed the highest values (Table 3). The negative relationship between final yield and Δ of early formed foliar tissue was also found in cereals (Condon and Richards 1993). Ramírez, Rolando and Yactayo found a similar response of potato genotypes for Δ at early stage and final tuber yield [*Désirée* \approx *Unica* (19.9 ± 0.2 and 19.7 ± 0.1 ‰, respectively) > *Sarnav* (18.9 ± 0.2 ‰)] in a trial carried out in the conditions of the southern coast of the Peruvian desert (Arequipa, $16^{\circ}29.6'S$, $72^{\circ}05.7'W$, 1292 m.a.s.l.) characterized by high vapour pressure deficit and global radiation (1.17 ± 0.04 kPa and 25.01 ± 0.35 MJ $\text{m}^2 \text{day}^{-1}$ of average daily values, respectively) during the growing period. Contrary to Wishart et al. (2014) who measured Δ_{leaflet} in potato during tuberization in optimum water conditions, our results highlight the relevance of conducting Δ_{leaflet} assessments under well-watered conditions at early growth stages (before TIO) to predict potato potential yield under

a large range of environmental conditions including water restriction.

Carbon discrimination for assessing drought tolerance in potato

The capacity of Sarnav to maintain yield under water restriction (Carli et al. 2014) was confirmed in the present experiment. Fischer et al. (2011) pointed out that potential yield, earliness and drought tolerance are the three broad factors that influence yield in proportions depending on drought severity. These three factors have contributed to the higher tuber yield of water-restricted Sarnav, compared to the other genotypes under similar conditions. When drought is absent or mild, yield potential determines production, conversely, when drought becomes more severe, drought escape or drought tolerance becomes more important. The high yield potential noted in Sarnav, associated to its high intrinsic photosynthetic capacity, could have contributed in our conditions to maintain the highest yield under water restriction conditions. The longer period of biomass accumulation driven by its long growing cycle with a concomitant delayed senescence, that is low rate of chlorophyll reduction and a late senescence onset, has been identified as an important factor that allows Sarnav to have a high tuber yield under mild water stress (Rolando et al. 2015). Thus, drought tolerance in Sarnav could be the result of various physiological mechanisms, that is osmotic adjustment, better access to water, protection against reactive oxygen species through detoxication-related enzymes synthesis and maintenance of protein folding (Legay et al. 2011). In this experiment, Sarnav showed higher g_s , bulking rate, final tuber yield and DTI than Unica and *Désirée* under water restriction conditions. The higher g_s in Sarnav under water restriction, associated to a lower intrinsic water-use efficiency (A/g_s), allowed it to take advantage of water pulses and make a more effective use of water (Blum 2009) reflecting an opportunistic strategy highly profitable under mild drought scenarios (Tardieu 2012). In agreement with Levy (1986), *Désirée* was the most drought susceptible genotype. In contrary to the response observed under well-watered conditions, the lack of difference between Sarnav and Unica for Δ_{leaflet} , and among all genotypes for N_{area} suggested that under water restriction diffusion of CO_2 (through stomata and mesophyll) was the main driver of Δ rather than photosynthetic capacity. Because the higher g_s in Sarnav than Unica, we hypothesize that the low Δ_{leaflet} in the latter could be promoted by an increase in mesophyll conductance (g_m) which is an important driver of Δ and intrinsic water use efficiency (Barbour et al. 2010, Flexas et al. 2013). Because leaf morphological traits (leaf mass area, thickness, leaf density among others) influence g_m under drought conditions (Flexas et al. 2008,

2012), more studies are necessary to assess the effects of water restriction on leaf structural change and concomitant changes in g_m in potato.

The lower average value of Δ_{tuber} compared to Δ_{leaflet} is likely to be due to isotope fractionation of enzymatic reactions that lead to C-C bonds formation causing ^{13}C -enrichment in sink and storage tissues (Tcherkez et al. 2011). Basu et al. (1999) found a reduction of photosynthesis and quantum yield of photosystem II in plants with detached tuber, revealing a dynamic communication between sources (photosynthetic tissue) and sink organs (tubers) in potato. Enrichment in ^{13}C reflected by lower Δ in sink organs in potato has been attributed to respiratory consumption of carbohydrates (Maunoury-Danger et al. 2009). The low value of Δ_{tuber} in Sarnav could reflect the high maintenance respiration activity that is needed for supporting the biochemical mechanisms at work in this genotype to endure water restriction (Legay et al. 2011). Sarnav and Unica are regarded as improved varieties (Cabello et al. 2012) with high genetic variability caused by their breeding out of different Andean potatoes (see Gutiérrez et al. 2007, Legay et al. 2011, Carli et al. 2014 for more details on their pedigree and origin). Contrary to the findings of Jefferies and McKerron (1997), who worked with non-Andean *S. tuberosum* genotypes, average Δ_{tuber} from TIO to senescence in Sarnav and Unica was negatively associated with final yield, average g_s (Table 3) and drought tolerance index (Fig. 2). However, more studies assessing Δ_{tuber} in an appropriate developmental stage and incorporating improved varieties, genetic stocks and landraces are necessary to analyse the usefulness of this integrative trait in drought tolerance selection programmes.

Conclusion

Carbon isotope discrimination is an integrative physiological trait which in this work has provided a better understanding of the behaviour of some potato varieties under contrasted watering regimes. Our study suggests that under well-irrigated conditions, growth and tuber yield in potato are positively associated with photosynthetic capacity. The assessment of Δ_{leaflet} during early growth (before TIO) under well-irrigated condition can be used to assess intrinsic photosynthetic capacity in different genotypes. In conditions of water restriction after tuber initiation, tuber yield appears to be driven by both yield potential (related to carbon diffusion) and drought tolerance mechanisms. Δ_{tuber} appears to be a suitable trait to estimate drought tolerance, but studies with more contrasted genotypes are still required. Determining Δ_{tuber} before senescence, that is between 30 and 60 days after TIO, or 350 and 700 °C days of accumulated thermal time after TIO is recommended. Further studies are however necessary to assess the influ-

ence of g_m and leaf morphological traits on Δ_{leaflet} . The participation of both yield potential and drought tolerance mechanisms in the maintenance of yield and in proportions that vary with the intensity of water restriction gives genotypes like Sarnav a high level of resilience under different soil water availability levels.

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