Agència
de Gestió d'Ajuts
Universitaris
i de Recerca

Memòria justificativa de recerca de les convocatòries BE, PIV, BCC, NANOS i BP

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria

BE

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior (BCC)
BE	Beques per a estades per a la recerca fora de Catalunya (BE)
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós (BP)
CTP-AIRE	Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus (CTP). Ajuts de mobilitat de personal investigador.
NANOS	Beques de recerca per a la formació en el camp de les nanotecnologies (NANOS)
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya (PIV)

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.
Relació entre fotosíntesi i respiració en cultius sotmesos a estrés hídric mitjançant isòtops estables

Dades de l'investigador

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Número d'expedient
2007BE-1 00042

Paraules clau: cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.
estrés, cultius, isotopos estables, fotosintesis, respiración,

Data de presentació de la justificació
15/11/2007

Generalitat de Catalunya
Departament d'Innovació,
Universitats i Empresa

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)
La relació entre la fotosíntesis i la respiració va ser estudiada en plantes cultivades utilitzant isòtops estables de carboni i nitrogen, en plantes sotmeses a condicions òptimes de rec i plantes estressades hidricament. Els experiments es van realitzar de Juliol a Octubre 2007. L'anàlisi de la composició isotòpica (δ13C) de la matèria orgànica total (TOM) recentment fixades per les plantes ben regades va demostrar que el carboni nou va ser enviat als teixits apicals i a l'arrel gruixuda. Com que les fulles apicals i les tiges junt amb les inflorescències estaven empobrides en d13C suggereix que aquest teixits van tenir un important paper com ambornals (sumides). En segon lloc, l'anàlisi del δ13C del CO2 respirat immediatament després del marcatge (T=0) demostra que una part important del C respirat per les fulles i els nòduls procedeix del recentment ficat per la fotosíntesis. En les següents collites (T=7 i T=14) aquest percentatge tendeix a disminuir , especialment en les fulles apicals. Es interessant destacar que el d13C de la respiració, encara que T=0 part del CO2 respirat procedeix del CO2 fixat durant la fotosíntesis, aquest percentatge augmenta a T=7. Finalment, el d15N demostra que, de manera similar al mencionat pel 12C, immediatament després del marcatge 15N2 (T=0), les fulles apicals i les tiges, juntament amb l'arrel principal i les nòduls, van ser els teixits amb més força com ambornal (sumides). Cal destacar que el N2 fixats pels nòduls va ser enviat a l'arrel principal on va ser guardat fins que va ser utilitzat pel rebrotament de la part aèria de les plantes.

Resum en anglès (màxim 300 paraules)
The relationship between photosynthesis and respiration were studied in crops using carbon and nitrogen stable isotopes under well watered and water-stressed conditions. The analyses of the 13C isotopic composition (δ13C) of total organic matter (TOM) recently fixed of well-watered plants revealed that it was mainly delivered to apical tissues and tap root. The fact that that the apical leaf and stems together with the inflorescences were d13C depleted, suggests that those tissues were newly formed and had a larger sink strength and metabolic activity. Secondly, the analyses of δ13C of respired CO2 immediately after the labelling (T=0) showed that a significant part of the C respired by leaves and nodules proceeded of the recently fixed CO2. In the following harvests (T=7 and T=14) such percentage tended to decrease, especially in apical leaves. Interestingly, the respiration d13C data also highlighted that even if at T=0 part of the respired proceeded from the CO2 fixed during the labelling, this percentage was even larger at T=7. Finally, the d15N also revealed that, similarly to what described for 12C, immediately after the 15N2 labelling (T=0), apical leaf and stems, together with tap root and in this case the nodules, were the tissues with larger sink strength. It is noteworthy the fact that the largest amount of N2 newly fixed was delivered to the tap roots where it was stored until it was required for the aboveground regrowth period.

Resum en anglès (màxim 300 paraules) – continuació -.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

INTRODUCTION

General circulation models (GCM) predict that in the Mediterranean basin, reduction in precipitation and rising evapotranspiration rates, will exacerbating low water availability problems commonly observed in Mediterranean environments where current annual potential evapotranspiration is often nearly twice the amount of rainfall (Sábate et al., 2002). It has long been known that drought, during the warmest period of the year, is the major stress factor limiting plant species distribution and growth in Mediterranean regions of the world (Mooney, 1983).

It is well know the sensitivity of legume-*Shinorhizobium* symbiosis to ambient stressors like drought (Serraj et al., 1998). Furthermore, some authors (Castellanos et al., 1996; Thomas et al., 2004) suggest that there is a larger effect of water deficit on N accumulation and N_2 fixation, than on biomass accumulation. Alfalfa is a temperate forage frequently exposed to low water availability, N-deficient soils. Photosynthesis supplies organic carbon to nodules where it is used by the nitrogenase enzyme in the bacteroid inside nodules, as a source of energy and reducing power to fix N_2 (Azcón-Bieto et al., 2000). This coupling causes that nitrogenase activity in plants is regulated by photosynthesis (carbon supply), nitrogen availability (N source strength) and N demand (N sink strength). I

One of the difficulties in studies analyzing the processes involved in C and N metabolism (e.g. photosynthesis and respiration) is measuring the different processes in the same experiment. For example, in C metabolism, respiration is active in plants during the light period, thus partially masking photosynthesis, if the latter is estimated using conventional gas exchange methodology. Isotope techniques (used in conjunction with gas exchange and other techniques) stand out prominently among the few tools available to partition, and to quantify allocation and partitioning of C and N compounds (Nogues et al., 2004, 2006). For many years, studies on carbon metabolism in plants used carbon isotopes as tracers. Plants grown in environments with modified isotopic composition will incorporate the tracer in carbon-containing compounds of the plant (Avice *et al*. 1996; Nogués *et al*., 2004) providing essential information about the C sinks to which the recently fixed C is delivered. The C and N labeling methods provides, in a non-invasive and reversible way, information about the role source: sink strength of the different plant tissues. As a consequence, stable isotopes have been used to study the allocation of carbohydrates and aminoacids between different parts of plants, especially in herbaceous species grown in controlled environment conditions (Gebbing *et al*. 1998; Nogués *et al*. 2004; 2006).

Material and methods

Experimental Design and Determinations

Alfalfa seed were sown in 9L white plastic pots filled with sand and grown in a greenhouse at 25/15ºC (day/night) with a photoperiod of 14 hours under natural daylight. During the first month, plants were inoculated three times with *Sinorhizobium meliloti* strain 102F78. Plants were

watered twice a week with Hoagland N-free nutrient solution and once a week with tap water to avoid salt accumulation in pots.

1. ^{12}C and $^{15}N_2$ labeling during regrowht

Plants were grown in optimal growth conditions until the end of second month so to enable the optimum development of root structure. In sixty days old plants the aboveground area was cut in order to study the regrowth capacity of those plants. When plants were 80 days old the plants were transferred to a growth chamber (Conviron E15, Controlled Environments ltd., Winnipeg, Canada). In those chambers and during 10 days we proceeded to the $^{12}CO_2$ and ^{15}N labeling. ^{12}C labeling was carried out by modifying the isotopic composition of the growth chamber. The non-labeled plants were grown in ambient $d^{13}C$ (-10‰) whereas the labeled plants were grown in a more ^{13}C depleted atmosphere (-21.0‰). Parallel to the plant level ^{12}C labeling, those plants were also labeled with $^{15}N_2$ (enriched at 5‰). The double labeling (^{12}C, $^{15}N_2$) of alfalfa plants was conducted for the characterization of both C and N acquisition, storage, remobilization, utilization for growth. The last day of the double labelling (T=0; 90 days old) we proceeded to the leaf, stem root and nodule sampling. Furthermore, we also determined the ^{13}C isotopic composition ($d^{13}C$) on leaf, nodule and root respiration though a specially designed gas exchange chamber. Immediately after those measurements we proceeded to the second defoliation of the 90 days old plants. When the plants were 97 (T=7) and 104 (T=14) days old we proceeded to the leaf, stem root and nodule sampling together with the leaf nodule and root respiration analyses.

2. Physiological characterization of drought effect in C and N_2 removal of alfalfa plants exposed to drought.

When the plants were 100 days old half of the plants (randomly selected) were exposed to drought conditions whereas the others were maintained in optimal water availability conditions. Applied drought consisted on watering suppression. During 7 days, drought plants were grown without any watering, whereas control plants were watered until pot capacity. Water status of control and drought treatments was determined by the leaf relative content (RWC, Watherley, 1950) analyses.

After the above described 7 days of water suppression, when the plants were 108 days (T=30) old, in order to determine the drought effect on the gas exchange (photosynthesis A, respiration Resp., conductance g, water use efficienciy, WUE) parameters fully expanded apical leaves were enclosed in a gas exchange leaf chamber (LiCor 6400, Li-Cor, Inc., Lincoln, USA) equipped with a leaf chamber fluorometer 6400-40 (LiCor 6400, Li-Cor, Inc., Lincoln, USA) for the chlorophyll fluorescence analyses. Photosynthetic assimilation (A) was estimated at a saturating PPFD of 1200 μmol $m^{-2}s^{-1}$ using equations developed by von Caemmerer and Farquhar (1982). The gas exchange response to CO_2 was measured from 0 to 1000 μmol

mol^{-1} CO_2. Measurements started at 400 μmolmol^{-1} of CO_2, decreased stepwise until 250, 100, 0 μmol mol^{-1} and restarted at 400 and increased stepwise until 700, 850, and 1000 μmol mol^{-1}. Estimation of the maximum carboxylation velocity of Rubisco (V_{cmax}) were made by fitting a maximum likelihood regression below and above inflexion of the A/Ci response using the method of Ethier and Livingston (2004). The leaf internal CO_2 concentration (C_i) was estimated as described by Farquhar and Sharkey (1982). Stomatal conductance was calculated as described by von Caemmerer and Farquhar (1981). Electron transport rate (ETR) values were recalculated as described by Ghashghaie & Cornic (1994).Similarly, in order to analyse drought effect on the ^{12}C, $^{15}N_2$ delivery and mobilization, we proceeded to the leaf and nodule sampling together with respiration analyses (equally to what described for the 90 days old plants). Finally so to test the effect of drought on nodule activity, and consequently on N_2 fixation, we proceeded to the N_2 activity determination according to what described by (Arrese-Igor et al., 1998).

Results and discussion

1. ^{12}C and $^{15}N_2$ labeling during regrowht

The analyses of the ^{13}C isotopic composition ($\delta^{13}C$) of total organic matter (TOM) recently fixed (Figure 1) revealed was mainly maintained/delivered to apical tissues and tap root. The fact that that the apical leaf and stems together with the inflorescences were d^{13}C depleted, suggests that those tissues were newly formed and had a larger sink strength and metabolic activity.

The analyses of $\delta^{13}C$ of respired CO_2 (Figure 2) immediately after the labeling (T=0) showed that a significant part of the C respired by leaves and nodules proceeded of the recently fixed CO_2. In the following harvests (T=7 and T=14) such percentage tended to decrease, especially in apical leaves. Interestingly, the respiration d^{13}C data also highlighted that even if at T=0 part of the respired proceeded from the CO_2 fixed during the labeling, this percentage was even larger at T=7.

In the other hand, d^{15}N also revealed (Figure 3) that, similarly to what described for ^{12}C, immediately after the $^{15}N_2$ labeling (T=0), apical leaf and stems, together with tap root and in this case the nodules, were the tissues with larger sink strength. It is noteworthy the fact that the largest amount of N_2 newly fixed was delivered to the tap roots where it was stored until it was required for the aboveground regrowth period.

2. Physiological characterization of drought effect in C and N_2 removal of alfalfa plants exposed to drought.

The water status analyses confirmed that suppression of irrigation during 7 days affected negatively relative water content (RWC, Table 1) of alfalfa plants subjected to drought. Gas exchange parameters revealed that photosynthesis (A) decreased dramatically (Table 1) in droughted plants, as a consequence of stomatal closure (*gs*) and the reduction in carboxylation capacity of rubisco (Vcmax). Ci (Table 1) data confirmed that droughted plants had lower intercellular CO_2 available. Table 1 also highlighted the fact that although droughted plants had lower photosynthetic rates they were capable to increase WUE due to their lower stomatal opening.

The chlorophyll fluorescence data showed that even if photosystem II maximal photochemical efficiency (Fv/Fm) was not affected by water availability (Table 2), the lower electron transport rate (ETR) detected in droughted plants could have contributed to reduce the photosynthetic capacity of those plants. ETRc confirmed that in droughted plants less energy was delivered to photosynthetic carboxylation processes.

In the other hand, the isotopic composition analyses revealed that regardless of water availability, after 30 days of labeling (Figure 4) the amount of remaining labeled C decreased. Furthermore that suppression of watering tended to increase its values, probably as a consequence of the previously described stomatal closure. It is also remarkable the fact that, in both control and droughted treatments, the labeling degree diminished in apical leaves and especially in nodules were no statistical differences on C labeling were observed.

The analyses of respired CO_2 isotopic composition (Figure 5) showed that, 30 days after the labeling in optimal water availability conditions no differences were observer on $d^{13}C$. However in droughted plants respiration of labeled plants was more depleted, which means that part of the carbohydrates that those plants were respiring proceeded from CO_2 fixed during the labeling. We would also like to remark that regardless of the labeling, respiration of droughted plants was ^{13}C enriched compared with the fully watered plants.

Finally and as it is shown in figure 6, days after the labeling almost all the labeled N disappeared in control and droughted plants. We would also like to highlight the fact that nodules of droughted plants were little bit enriched in ^{15}N when compared with fully watered plants. Such results suggest that since droughted plants had a lower nitrogenase (N_{ase}) activity (Table 1), the nodules of those plants decreased ^{15}N discrimination in order to increase, its N resources as much as

possible. Reduction in Nase activity could be related with the above described reduction in photosynthetic capacity of droughted plants.

Figures and Tables

Figure 1. ^{13}C isotopic composition (‰) of total organic matter (TOM) on alfalfa infloresce, apical leaf, basal leaf, apical stem, basal stem, nodule and tap root immediately after the ^{12}C labeling (T=0).

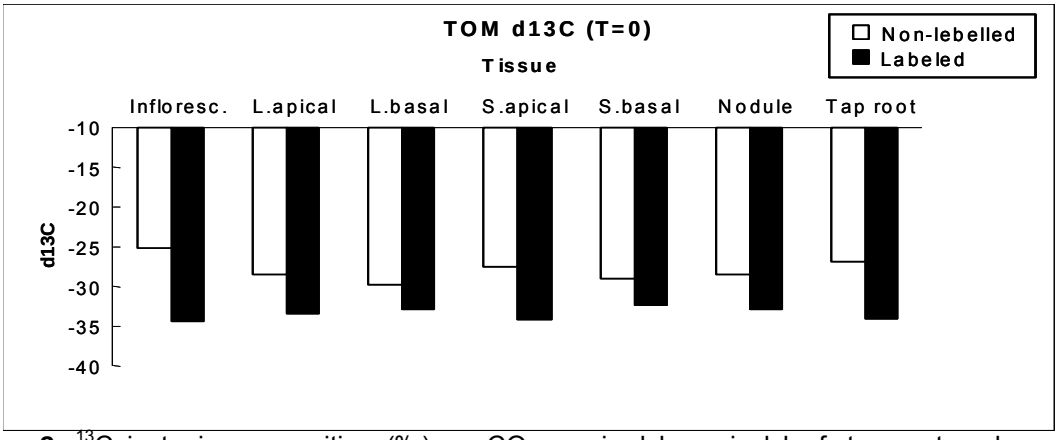

Figure 2. ^{13}C isotopic composition (‰) on CO_2 respired by apical leaf, tap root and nodule respiration immediately (T=0) after the ^{12}C and $^{15}N_2$ labeling, 7 days after the labeling (T=7) and 14 days after the labeling (T=14).

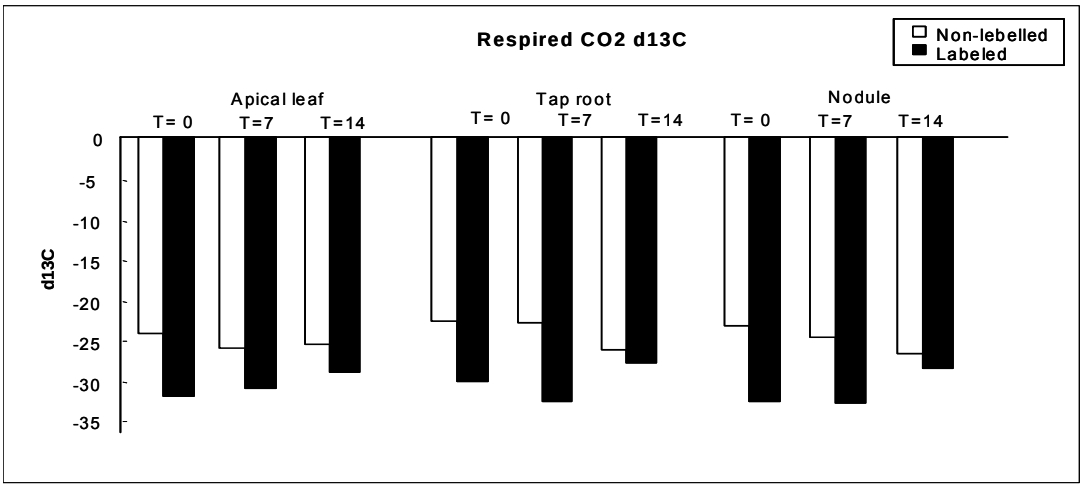

Figure 3. $^{15}N_2$ isotopic composition (‰) on total organic matter (TOM) on inflorescence, apical leaf, basal leaf, apical stem, basal stem, nodule and tap root immediately after the labeling (T=0).

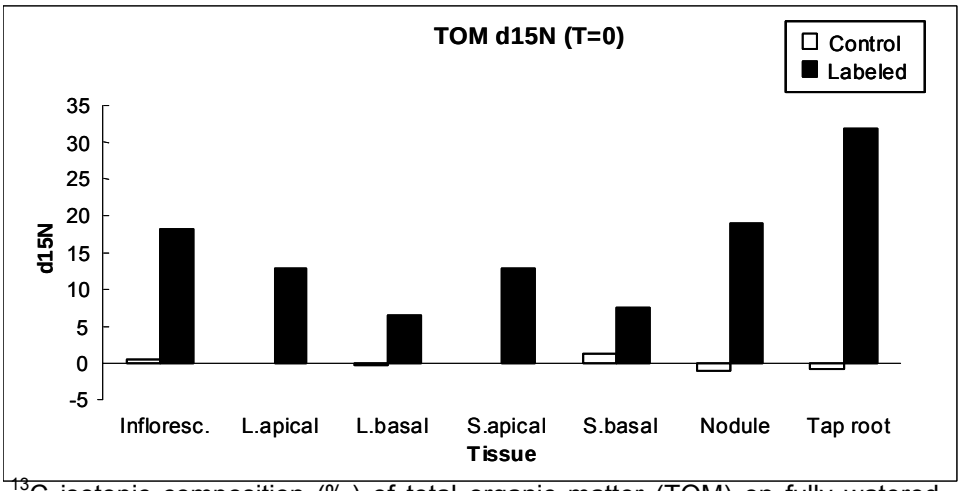

Figure 4. ^{13}C isotopic composition (‰) of total organic matter (TOM) on fully watered (CONTROL) and partially watered (DROUGHT) alfalfa apical leaf, and nodule 30 days after the ^{12}C (T=30).

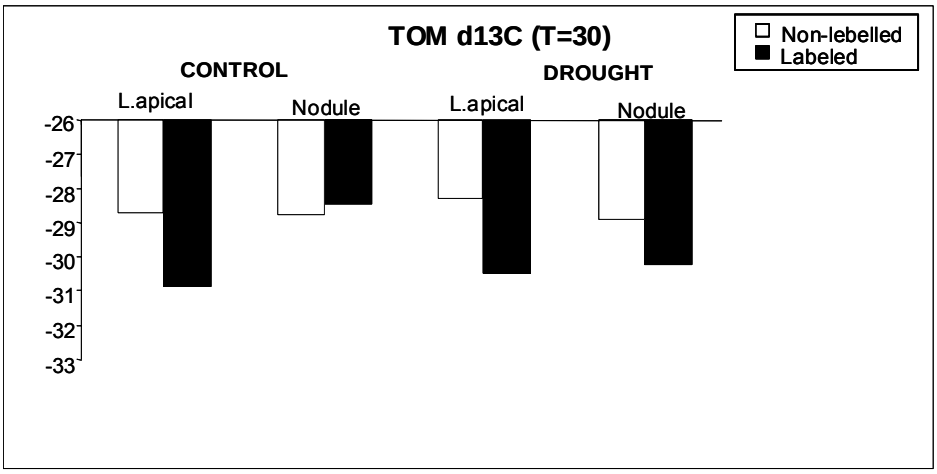

Figure 5. ^{13}C isotopic composition (‰) of respired CO_2 on fully watered (CONTROL) and partially watered (DROUGHT) alfalfa apical leaf, and nodule 30 days after the ^{12}C (T=30).

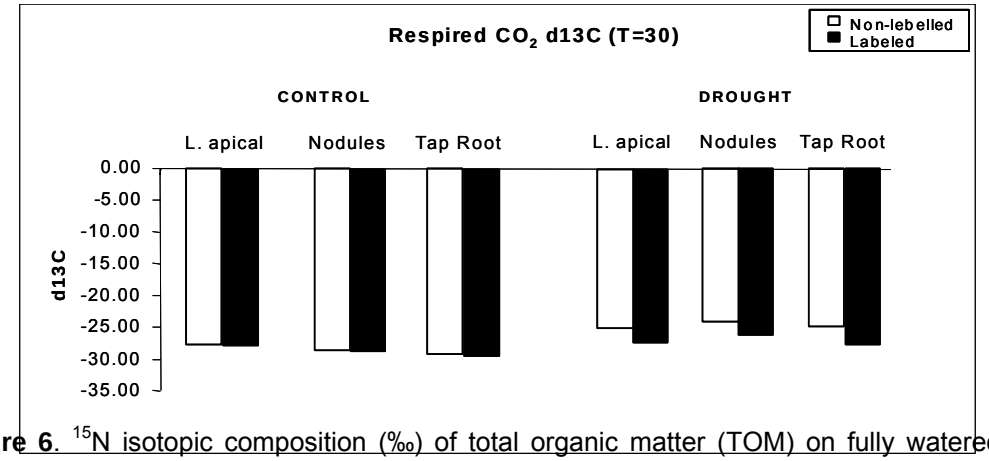

Figure 6. ^{15}N isotopic composition (‰) of total organic matter (TOM) on fully watered (CONTROL) and partially watered (DROUGHT) alfalfa apical leaf, and nodule 30 days after the labeling (T=30).

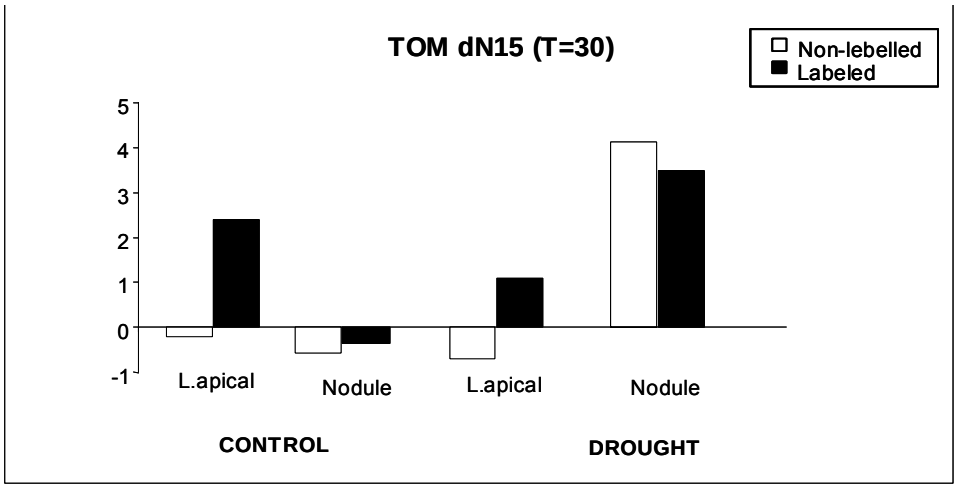

Table 1. Leaf relative water content (RWC), photosynthesis (A), rubisco maximum carboxilation rate (Vcmax), stomtatal conductance (gs), , photosynthetic water use efficiency (WUEph), intecerlullar CO_2 concentration (Ci) an nitrogenase (Nase) activity on fully watered (CONTROL) and partially watered (DROUGHT) alfalfa apical leaf, and nodule 30 days after the ^{12}C (T=30).

Parameters	Control	Drought
RWC (%)	91.30	35.91
A (μmol $mol^{-1}m^{-2}s^{-1}$)	23.75	13.52
Vcmax (μmol CO_2 $m^{-2}s^{-1}$)	127.15	106.11
gs (mmol $m^{-2}s^{-1}$)	54.22	14.18
WUEph (mg MS g^{-1} H_2O**)**	4.76	9.71
Ci (μmol mol^{-1})	300.83	224.25
Nase (μmol h^{-1} g^{-1} DM)	41.80	10.05

Table 2. Photosystem II maximal photochemical efficiency (Fv/Fm), electron transport rate (ETR), electron transport through photosynthetic carbon reduction (ETRc) and electron transport through photorespiratory carbon oxidation

(ETRo) on fully watered (CONTROL) and partially watered (DROUGHT) alfalfa apical leaf, and nodule 30 days after the ^{12}C (T=30).

Parameters	Control	Drought
Fv/Fm	0.77	0.78
ETR	149.30	127.44
ETRc	115.48	79.00
ETRo	33.82	48.44

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