13C-labelling of leaf photoassimilates to study the source–sink relationship in two Iranian melon cultivars

Taher Barzegar a, *, Franz-W. Badeck b, Mojtaba Delshad a, Abdol-Karim Kashi a, Daniel Berveiller c, Jaleh Ghashghaie d

a Department of Horticultural Sciences, University College of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran
b CRA-GPC Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Genomics Research Centre, Via San Protaso, 302, 29017 Firenzezola d’Arda, Piacenza, Italy
c Laboratoire d’Ecologie, Systématique et Evolution (ESE), CNRS AgroParisTech-UMR 8079, Bâtiment 362, Université de Paris-Sud XI, F-91405 Orsay Cedex, France

A R T I C L E I N F O

Article history:
Received 7 September 2011
Received in revised form 5 December 2012
Accepted 12 December 2012

Keywords:
Iranian melon Source–sink Photosynthesis 13C-labelling Thinning

A B S T R A C T

To study the effect of fruit position on the stem on photoassimilate partitioning in two Iranian melon cultivars (Suksi-Sabz and Jalali-Zard), a greenhouse experiment was conducted. Three fruit positions (retaining one fruit on 3rd, 7th or 11th node of two lateral branches) and three leaf positions for 13C-labelling (3rd, 6th or 12th leaf from the base of one branch) were compared during the experiment. Results showed that the time at which the maximum photosynthesis rate occurred in selected leaves was different according to leaf rank and this can affect the role and share of a given leaf in fruit feeding. Labelling data indicated that fruits retained on the 7th node import higher photoassimilates (13C-labelled) than the others and that the adjacent or nearer leaves have more important role in a given fruit feeding. These results could explain why experienced Iranian melon producers believe thinning (removing all female flowers before and after 6th–8th nodes of each lateral branch) results in largest fruits and the best quality.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Iran is one of the main producers of melons after China and Turkey (FAOSTAT, 2005) with great potential to produce and export high quality melons. Melon (Cucumis melo L.) is generally cultivated in arid and semi-arid regions of Iran. The geographical origin of C. melo L. is unclear, but it is traditionally considered to be a desert plant and is thought to originate from Africa and first was domesticated as a food source in Egypt and Iran around 3000 years BC (Robinson and Decker-Walkers, 1999).

The regulation of fruit size is a major issue in melon production. The growth and the yield of crops are limited by the size and activity of either carbon source or sinks (Ho et al., 1989). It is understood that sink size is determined by physical and physiological restrictions of an organ’s assimilate import capacity and the source–sink relationship is regarded as an important factor in the determination of fruit size (Ho, 1988; Brummell et al., 1997). When no pruning or thinning practices are conducted, melon plants usually are able to produce 4–5 low quality small fruits during the growing season. Valantin et al. (1998) reported that while single leaf photosynthesis remained unchanged under high fruit loading, net photosynthesis of the whole plant was reduced by 30% due to decrease of total leaf area (from 2.5 to 1.7 m2). When some fruits are removed from a melon plant, the plant reinvests the available photosynthates into the remaining fruit or into vegetative growth. de Queiroga et al. (2008) showed that the number of fruits per plant can affect plant total leaf area and commercial yield, i.e. an increase in total plant leaf area and commercial yield in plants bearing 2 fruits compared to those bearing 1 fruit. Hughes et al. (1983) fed individual muskmelon leaves with 13C02 and reported that when a leaf at the 3rd node acropetal to a fruit was labelled it exported 65% of the labelled carbon within 6 h, while leaves at greater distance from the fruit retained the label for longer. They also observed that the 14C level in the fruit was the highest when the nearest leaf was labelled, and that the label in fruit (and in the stem) decreased with increasing distance between labelled leaf and fruit. They concluded that the influence of the melon fruit as a sink on the gradient of 14C from source leaves along the branch was limited to a few nearest internodes along the branch.

To our knowledge, no research has been carried out on pruning–thinning effect on carbon allocation to fruits in Iranian melons. Experienced Iranian farmers, using pruning practices (cutting the main stem and maintaining two lateral branches) and thinning (removing all female flowers produced before and after 6th–8th node from the base of each lateral branch), traditionally keep only one fruit on one of 6th, 7th
Table 1
Description of growth stages of different leaves at different days after pollination (DAP).

<table>
<thead>
<tr>
<th>Leaf rank</th>
<th>Time (DAP)</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>Mature</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Senescent</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>Mature</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>Fully expanded</td>
</tr>
</tbody>
</table>

Young, still growing leaf; fully expanded, just finished growing; mature, already finished growing with no senescence symptoms; senescent, having some yellow patches on blade.

or 8th node of each lateral branch, i.e. keep two fruits per plant. They believe that these practices are necessary to increase average fruit weight, yield and quality of melon.

If reducing the number of fruits per plant is accepted, since melon plants are grapevine type and capable to bear fruits at different nodes along the main stem or any lateral branches, the question arises which node is the best position for fruit set and how fruit position on the stem affects the carbohydrate partitioning pattern and source–sink relations in Iranian melon plants? We conducted 13C-labelling experiments on two Iranian melon cultivars (Suski-Sabz and Jalali-Zard) to study the effect of fruit position on its potential to absorb assimilates and also the role of different leaves in supplying assimilates to the fruit. So, the objectives of this work were to examine: (i) whether the proximity of a leaf to the sink fruit significantly affects its role in fruit feeding and (ii) which fruit position on the stem (i.e. maintaining of fruit on which node) has the highest potential to absorb assimilates from source leaves.

2. Materials and methods

2.1. Plant material and growth conditions

The experiments were conducted in the greenhouse at the Université de Paris-Sud XI (Orsay, France). Two commercial Iranian melon cultivars, “Suski-Sabz” and “Jalali-Zard” (C. melo L.), with dark green yellow fruits respectively, were used for the experiments. These cultivars are under cultivation for hundreds of years and have genetic stability in most traits. They have large-sized oval fruits weighing approximately 4 kg per fruit at harvest time.

The seeds were sown on 26th May 2010 in pots filled with a peat soil mixed with sand (8:1) and with slow-releasing NPK complex fertilizer. When the first leaf had expanded, seedlings were transplanted to the soil (fertilizer had been pre-applied) at 70 cm spacing in 9 rows, each 5 m in length, with 2 m between rows. The experimental plots of about 90 m² in the greenhouse were under natural light conditions.

After pruning (removing the apex of the main stem at a very early stage), plants were trained to have two lateral branches and only 2 fruits per plant were allowed to set (i.e. one fruit per lateral branch) at different node positions numbered from the base to the apex on each branch. Female flowers at the selected positions were hand-pollinated to obtain fruits and other flowers were removed. Fruits were generated in this way either at node 3 (near the basal part of the branch), node 7 (in the middle part) or node 11 (near the apex).

The pollination date was recorded for each selected flower. Days after pollination (DAP) were subsequently used as time units for reporting the experiments. Leaves were also numbered from the base to the apex for each lateral branch. The growth stages of different leaves at different times after pollination are summarised in Table 1. At DAP 0, leaf rank 3 (near the basal part) was fully expanded while leaf ranks 8 (in the middle) and 12 (near the apex) were young and still growing. On DAP 12, leaf 3 was mature (but not senescent). Leaf 8 was fully expanded and leaf 12 was still in the process of expanding. On DAP 27, leaf 3 showed first signs of senescence (yellow patches on the leaf), leaf 8 was mature but not senescent and leaf 12 was fully expanded. Labelling (i.e. feeding of 3rd, 8th or 12th leaf by 13CO2) was carried out at DAP 27 for all fruit position treatments.

Therefore, at labelling time, there were different aged leaves but with approximately similar size (about 350 cm²), which completely covered the labelling chamber area. Fruits were not weighed but they all had similar length (about 25 cm) at labelling time. Therefore, the 13C-label found in a particular fruit should reflect the amount of 13C-labelled photoassimilates translocated from source leaves to sink (fruit), and so provide a measure of the effect of leaf or fruit position on the amount of photoassimilates reaching a fruit. Light intensity was not measured inside the greenhouse during the plant growth. However, it was continuously recorded at a meteorological station located 50 m from the experimental glasshouse.

2.2. Leaf gas exchange measurements

Gas exchange measurements were conducted at 3 DAP (0, 12 and 27) on 3 different leaf positions (3rd, 8th and 12th leaves). For each combination of DAP and leaf position, leaves of 30 plants were used (2 cultivars, 3 replicated blocks and 5 plants in each experimental unit). These plants were taken from the set of plants with the fruit retained at the 7th node position. A portable gas-exchange system (LI6400, LICOR INC. Lincoln, Nebraska, USA) was used for these measurements. Leaf net photosynthesis (Aₚ) and transpiration (E) were measured under ambient CO₂ concentration (400 μmol mol⁻¹) at a photosynthetic photon flux density (PPFD) of 1000 μmol photons m⁻² s⁻¹ and air temperature in the chamber of 25 °C. The air flow rate in the chamber was 500 μmol s⁻¹. The leaf-to-air vapour pressure deficit (VPD) was maintained at about 1 kPa during the gas exchange measurements. Stomatal conductance (gₛ) and intercellular to ambient CO₂ concentration ratio (Cᵢ/Cₐ) were calculated according to von Caemmerer and Farquhar (1981).

2.3. 13C-labelling

To study the source–sink relationship, we conducted labelling experiments (with the heavy stable isotope of carbon, 13C) on DAP 27 using a labelling chamber coupled to the Licor-6400 gas exchange system. Previous works have shown that at this stage fruits start to accumulate sugars and can be considered as highly active and fast developing sinks (Lingle and Dunlap, 1987), so, even small amounts of 13C-labelled photoassimilates can be detected. For each plant, the whole leaf from the 3rd, 7th or 12th node position was placed in a specially designed labelling chamber and fed with 13CO₂ for 4 h under natural light conditions in the greenhouse. In total 18 plants (2 cultivars, 3 fruit positions and 3 leaf ranks) were used for this purpose. For each plant, only one of the two trained lateral branches was used for 13CO₂ feeding. Leaf temperature in the labelling chamber was maintained at 25 °C by circulating temperature-controlled water inside the double-walls of the chamber. A 99% 13C-labelled CO₂ source (Eurisotop, Saint Aubin, France) was used for labelling experiments without any dilution. Since the gas analyser system cannot detect the heavy carbon isotope, the CO₂ concentration parameters of the Licor were fixed before labelling using non-labelled CO₂ to get 400 μmol mol⁻¹ in the labelling chamber during the labelling period. For the same reason, it was not possible to determine the leaf net photosynthesis rate during the 4 h labelling. However, the gas exchanges were determined using normal CO₂ on similar leaves of similar plants and at similar developmental stages as those used for labelling. PPFD in the labelling chamber was not controlled, but corresponded to the light level inside the glasshouse. Some simultaneous
measurements outside and inside the greenhouse showed that the light intensity is reduced by approximately 30% and up to 70% inside the greenhouse compared with the outdoor values, on sunny and cloudy days, respectively (data not presented). Therefore, the light at plant level was lower than the values recorded at the meteorological station reported here. The chamber wall would reduce even more the light reaching the leaf.

In order to allow time for translocation of labelled carbon, plants were harvested 48 h after labelling. Thus the $^{13}$C-label in sampled organs corresponds to the residual label after 4 h of labelling and additional 48 h of translocation. Samples collected from plants for analysis were source leaf, fruit, and shoot apex (including very young emerging leaves near the apex). These were immediately frozen in liquid nitrogen and stored at $-20\degree$C until they were freeze-dried and then finely ground with a Retsch MM200 mortar (Bioblock Scientific, Illkirch, France). Similar leaves from non-labelled plants at the same stages as the labelled ones were also sampled as reference material, also in order to check any re-assimilation of labelled respired CO$_2$ by non-labelled leaves. An aliquot of 1 mg per sample powder weighed in stain capsules (Courtage Analyse Service, Mont Saint-Aignan, France) was used for $^{13}$C analysis with an isotope ratio mass spectrometer, IRMS (VG Optima; Micromass, France) coupled to an elemental analyser (Carlo Erba; NA 1500, Milan, Italy) at the Institut de Biologie des Plantes (IBP, Orsay). Carbon isotope composition ($\delta^{13}$C) was calculated as deviation of the carbon isotope ratio ($^{13}$C/$^{12}$C) from the international standard (Vienna Pee Dee Belemnite): $\delta^{13}$C = [($R_{\text{sample}} - R_{\text{standard}}$)/$R_{\text{standard}}$] × 1000. A laboratory standard (glutamic acid) was measured twice every 6 samples in order to correct for the drift of the IRMS.

$^{13}$C atom % ($\%$) of both labelled and unlabelled samples calculated as the ratio of $^{13}$C in a given sample to the total carbon isotopes, i.e. $\%$ = $^{13}$C/($^{13}$C + $^{12}$C), were used to determine the $^{13}$C-excess as a $^{13}$C atom % difference between labelled and unlabelled (control) plants as follows:

$$^{13}$C-Excess = $\%$labelled − $\%$control

2.4. Statistical analysis

For gas exchange data analysis, a factorial model based on a completely randomised block design was used (3 DAPs, 2 cultivars × 3 leaf positions × 3 replications × 5 observations per experimental unit = 270 data). Data were analysed using the SAS statistical programme (SAS Institute Inc., Cary, NC, USA), and means were compared by Duncan’s multiple range tests at the 5% probability level. Values were expressed as mean ± SE (standard error). There were no replications for $^{13}$C-labelling data to do statistical analysis. However, the trends were similar for both cultivars; they could thus be taken as replicates.

Pearson’s correlation coefficient was determined for the correlation between different variables. The difference in $\delta^{13}$C between the two cultivars was tested with non-paired $t$-tests on the full set of data obtained from all experiments (n = 9 leaves or fruits per cultivar) as well as with a paired $t$-test for identical combinations of labelled leaf/retained fruit (n = 9 combinations). Statistical models for the isotopic signatures of the organs were determined with generalised linear models (GLM) using photosynthesis at 1000 mmol photon m$^{-2}$ s$^{-1}$, outdoor 4-h integrated PPDF, an index of the difference in the nodal position of labelled leaf and retained fruit and cultivar as independent variables. All combinations of these 4 variables and less than 4 variables were tested. The most parsimonious model was chosen with the Akaike Information Criterion, AIC (Akaike, 1973).

3. Results

3.1. Leaf gas exchange

The values of leaf net photosynthesis ($A_{\text{net}}$), transpiration rate ($E$), stomatal conductance for water vapour diffusion ($g_{s}$), and intercellular to ambient CO$_2$ concentration ratio ($C_{i}/C_{a}$) measured on 3 leaf ranks of the two melon cultivars at three dates after pollination (DAP) are shown in Table 1 and Fig. 1. In this experiment fruits were maintained on the 7th node of each lateral branch in both cultivars, a procedure which has traditionally been used by Iranian melon growers for centuries.

The measured traits of two cultivars were not statistically different (Table 2) thus data of both cultivars are used together when needed. Net photosynthesis rates of the 3rd and 8th leaves (19.28 ± 0.69 and 19.70 ± 0.49 mmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively) were higher than net photosynthesis of the 12th leaf, i.e. the youngest leaf (16.08 ± 0.80 mmol CO$_2$ m$^{-2}$ s$^{-1}$). The highest values of stomatal conductance for water vapour diffusion were observed at DAP 0 (0.47 ± 0.005 mmol m$^{-2}$ s$^{-1}$) and on the 3rd and 8th leaves ($g_{s}$ = 0.45 ± 0.038 and 0.41 ± 0.044 mmol m$^{-2}$ s$^{-1}$, respectively), the differences with leaf 12 and with DAP 12 and 27 being statistically significant (Table 2 and Fig. 1B and E). $C_{i}/C_{a}$ differed among leaves and DAPs, the 3rd leaf having significantly higher $C_{i}/C_{a}$ (0.75 ± 0.01) than the other leaves. In agreement with stomatal conductance values, the lowest $C_{i}/C_{a}$ value was noted for the 12th leaf. The highest $C_{i}/C_{a}$ (0.77 ± 0.009) was found at DAP 12 and the lowest at DAP 0 (0.67 ± 0.03) (Table 2 and Fig. 1C and F).

It is clear (Fig. 1A and D) that at the pollination time of the female flower of 7th node, earlier produced leaves (e.g. 3rd leaf) had the highest photosynthesis activity and can probably be considered as the main assimilate source for coming new fruits. At this time, later produced leaves (e.g. 8th and 12th leaves) had not yet reached their maximum photosynthetic activity. While photosynthesis rate of the 3rd leaf decreased during the fruit growing period (i.e. DAP 27), 8th and 12th leaves may play an important role in fruit feeding.

3.2. $^{13}$C-labelling

As comparison, $^{13}$C data are summarised in Table 3 as mean $\delta^{13}$C values and corresponding $^{13}$C atom % in control and labelled plants, as well as $^{13}$C-excess in labelled relative to control plants. Mean values are calculated using all combinations of leaf ranks and fruit positions.

$^{13}$C-excess in leaves (Fig. 2A), fruits (B) and shoot apex (C) 48 h after a 4-h labelling period vary with the fruit position maintained in both melon cultivars. The $^{13}$C-label translocated from the source leaf to the sink (i.e. fruit or vegetative tissues) varied with the position of the fruit retained after thinning. Regardless of the cultivar, all plants show a similar pattern. However, the variation is higher in Suski-Sabz compared with Jalali-Zard (Fig. 2A and B), mainly when leaf 8 was labelled. The shorter the distance between labelled leaf and sink fruit, the higher the $^{13}$C-excess detected in the leaf (Fig. 2A). $^{13}$C-excess in leaves is much higher (more than 100% for some cases) than in fruits and in shoot apexes (less than 10%), except for leaf 8 when fruit was set at node 7. In addition, it is obvious that the $^{13}$C-excess in leaf 3 is the lowest in all situations (Fig. 2A). For all experimental plants, the $^{13}$C-label in the fruit was the highest when fruits were set near to the labelled leaf (Fig. 2B); $^{13}$C-excess in fruits being the highest when leaf 8 is feeding a fruit at node 7 (9% and 7.5% for Suski-Sabz and Jalali-Zard, respectively) and next highest when leaf 12 is feeding a fruit at node 11 (6% and 5% for Suski-Sabz and Jalali-Zard, respectively) (Fig. 2B). It seems that increasing the distance between fruit position and shoot apex...
Fig. 1. Changes in (A, D) the leaf net photosynthesis rate ($A_n$) (B, E) stomatal conductance for water vapour diffusion ($g_s$), and (C, F) the intercellular to ambient CO$_2$ concentration ratio ($C_i/C_a$), of 3 leaf ranks during the developmental stages of two Iranian melon cultivars, Suski-Sabz (left panel) and Jalali-Zard (right panel). Different leaf ranks are indicated by different symbols: rank 3 (white), rank 8 (black) and rank 12 (grey). DAP corresponds to the days after pollination. Only 2 fruits per plant were allowed to set (i.e. one fruit per lateral branch) on similar position (7th node position). The bars correspond to the standard errors (5 plants × 3 replications, n = 15).

Table 2
Leaf net photosynthesis rate ($A_n$), transpiration rate (E), stomatal conductance to water vapour diffusion ($g_s$) and internal to ambient CO$_2$ concentration ratio ($C_i/C_a$), determined using gas exchange system (Licor 6400) on intact leaves, three times during the growth period in the greenhouse on 2 Iranian melon cultivars. The time of the measurements is indicated as days after pollination (DAP). The mean values for each cultivar are the means of independent measurements on 15 plants: 3 DAPs, 3 leaf ranks, 3 replications and 5 plants in experimental unit (n = 135). For each leaf rank, the mean values include measurements of all replications and observations of both cultivars at all DAPs (n = 90). For each DAP, the mean values include all replications and observations of both cultivars and all leaf ranks (n = 90). Within each trial, and for each parameter, mean values followed by different letters are significantly different (P < 0.05) according to Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$A_n$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>E (mmol m$^{-2}$ s$^{-1}$)</th>
<th>$g_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>$C_i/C_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suski-Sabz</td>
<td>18.47 ± 0.64 a</td>
<td>3.75 ± 0.18 a</td>
<td>0.4 ± 0.035 a</td>
<td>0.74 ± 0.01 a</td>
</tr>
<tr>
<td>Jalali-Zard</td>
<td>18.25 ± 0.61 a</td>
<td>3.49 ± 0.17 a</td>
<td>0.37 ± 0.031 a</td>
<td>0.70 ± 0.01 ab</td>
</tr>
<tr>
<td>Leaf rank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19.28 ± 0.69 a</td>
<td>4.00 ± 0.22 a</td>
<td>0.45 ± 0.038 a</td>
<td>0.75 ± 0.01 a</td>
</tr>
<tr>
<td>8</td>
<td>19.70 ± 0.49 a</td>
<td>3.73 ± 0.19 a</td>
<td>0.41 ± 0.044 a</td>
<td>0.72 ± 0.01 ab</td>
</tr>
<tr>
<td>12</td>
<td>16.08 ± 0.80 b</td>
<td>3.13 ± 0.21 a</td>
<td>0.3 ± 0.034 b</td>
<td>0.69 ± 0.02 b</td>
</tr>
<tr>
<td>Time (DAP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16.71 ± 0.92 c</td>
<td>3.31 ± 0.31 b</td>
<td>0.47 ± 0.05 a</td>
<td>0.67 ± 0.03 b</td>
</tr>
<tr>
<td>12</td>
<td>19.52 ± 0.58 a</td>
<td>4.06 ± 0.13 a</td>
<td>0.38 ± 0.032 b</td>
<td>0.77 ± 0.009 a</td>
</tr>
<tr>
<td>27</td>
<td>18.84 ± 0.61 b</td>
<td>3.50 ± 0.15 b</td>
<td>0.31 ± 0.031 b</td>
<td>0.72 ± 0.01 a</td>
</tr>
</tbody>
</table>
results in less competition between them in assimilate import and higher potential of apex for absorption of photoassimilates.

3.3. Relation between light intensity, leaf photosynthesis and \(^{13}\)C-label in leaves and fruits

The \(^{13}\)C-excess in leaves follows the light conditions during the corresponding labelling periods (i.e. PPFD values are those recorded in a meteorological station outside the greenhouse); that is the \(^{13}\)C-label of the leaves increased when PPFD was high and followed a similar pattern in both cultivars (Fig. 3). Although linear relationships were obtained between these two parameters (not shown), the \(R^2\) was low (\(R^2 < 0.25\)) and regression results were not significant (\(P > 0.17\)).

Paired tests on the differences between the two cultivars (paired by leaf/fruit combination) did not indicate any significant difference for all organs (Table 4). None of the GLM models for leaf \(^{13}\)C or fruit \(^{13}\)C showed a decreased AIC when cultivar was added as additional factor. Thus, there is no indication of significant differences between the two cultivars and all subsequent analyses were done on data pooled for the two cultivars, resulting in \(n = 2\) for the different experiments. In addition, the labelling level depends on the labelled leaf photosynthesis, and since the photosynthesis values were not significantly different between the cultivars (see SE values on Table 2 and Fig. 1), we expect that the labelling should result at similar levels too.

When analysis of regression of leaf \(^{13}\)C on PPFD was repeated for combined data obtained with the two cultivars, results for leaves 3 and 12 were non-significant (\(P > 0.12\)), while the analysis for leaf 8 resulted in a strong trend for increasing \(^{13}\)C with increasing PPFD just about non-significant (\(P = 0.054, R^2 = 0.56\)). The correlation analysis (Table 4) showed a highly significant (\(P = 0.0024\)) correlation between leaf and fruit \(^{13}\)C with \(r = 0.67\). Thus, roughly half of the variability in fruit \(^{13}\)C was explained by the residual label within the leaves at the time of sampling. Fruit \(^{13}\)C was correlated with light levels during labelling (\(r = 0.501, P = 0.034\), while leaf \(^{13}\)C showed weak (\(P = 0.076\) and 0.086) trends for association with photosynthetic activity and light levels during labelling (\(r = 0.429\) and 0.415, respectively).

Among the statistical models (GLM) evaluated with all combinations of the independent variables, the model relating leaf \(^{13}\)C to the rank of the labelled leaf and the light level during labelling resulted as the most parsimonious and explained 49.9% of the total deviance between the observed and predicted leaf \(^{13}\)C.

Among the GLMs evaluated with all combinations of the independent variables, the model relating fruit \(^{13}\)C to leaf \(^{13}\)C and the nodal distance between labelled leaf and retained fruit resulted as the most parsimonious and explained 59.4% of the total deviance between the observed vs predicted fruit \(^{13}\)C.

4. Discussion

The main aim of the present work was a better understanding of the traditional pruning-thinning practices (i.e. cutting the main stem and training two lateral branches, removing female flowers before and after 6th–8th node and retaining just one fruit on each lateral branch) of Iranian melon producers. Labelling experiments using \(^{13}\)CO\(_2\) were conducted to study the photoassimilate import from leaves to fruits in relation with leaf and fruit positions. Results clearly showed that in both cultivars the labelling level in retained fruits is higher when the adjacent leaf is labelled and that the fruits retained in the middle part of the branch receive the highest labelled photoassimilates, result which is in agreement with the traditional practices of melon producers in Iran.

4.1. Changes in net photosynthesis and stomatal conductance with leaf rank and age

Leaves are the main source of \(\text{CO}_2\) assimilation in melon plants. Leaf photosynthesis depends not only on environmental conditions such as temperature, air humidity, light intensity and carbon dioxide concentration, but also on internal regulation, and it can be altered by treatments affecting the source organ activity or sink demand as shown by Valantin et al. (1998) on cantaloupe. It is well known that photosynthetic rate changes with leaf age; generally low in young expanding leaves, it increases up to or slightly after full leaf expansion reaching a plateau and decreases then with leaf senescence, e.g. in tomato (Shishido et al., 1990) and cucurbit (Turgeon and Webb, 1975). Stomatal conductance generally shows a more or less similar pattern with leaf ageing (Shenxi and Luo, 2003). The relative changes in stomatal conductance (i.e. in \(\text{CO}_2\) supply from air into the leaf sub-stomatal cavities) and in leaf intrinsic photosynthetic capacity (i.e. in \(\text{CO}_2\) consumption in the chloroplasts) determines \(C_I/C_V\) values (Wong et al., 1979; Farquhar and Sharkey, 1982).

In the present work, for both cultivars, the photosynthetic rate of leaf 3 (basal leaf) was indeed the highest from DAP 0 to 12 and subsequently decreased presumably due to the beginning of the senescence of the basal leaves. In contrast, net photosynthesis of leaves 8 and 12 increased in parallel to expansion (maturity). Net photosynthesis of leaf 8 reached the plateau only at DAP 12 and stayed constant until DAP 27, while the leaf 12 (the youngest leaf analysed) reached its highest photosynthesis rate at DAP 27 only. Therefore, at DAP 12, both leaves 3 and 8 (and presumably the leaves with intermediate ranks) were mature and operated at maximum photosynthetic rates. As expected, the increase in net photosynthesis was associated with an increase in stomatal conductance. Stomatal conductance showed almost similar pattern as net photosynthesis mainly for Suski-Sabz, with a maximum value

Table 3

Comparison of \(^{13}\)C data in different organs of two Iranian melon cultivars (Suski-Sabz and Jalali-Zard). All organs including leaves, fruits and shoot apex were sampled 48 h after a 4-h labelling period. For both melon cultivars, carbon isotope composition (\(^{13}\)C) calculated relative to international standard VPDB and corresponding \(^{13}\)C-atom values calculated as \% of both isotopes: \(^{13}\)C(\(^{12}\)C + \(^{13}\)C), as well as \(^{13}\)C-excess calculated as a \(^{13}\)C-atom difference between labelled and unlabelled (control) plants (see Section 2 for the equations used) are presented as means ± SE. Means are calculated using all leaf ranks, shoot-apex and 3 fruit positions maintained (\(n = 9\)).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Parameter</th>
<th>Leaf</th>
<th>Fruit</th>
<th>Shoot-apex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suski-Sabz</td>
<td>Control</td>
<td>(^{13})C (%)</td>
<td>(-29.10 \pm 0.27)</td>
<td>(-27.33 \pm 0.21)</td>
<td>(-28.59 \pm 0.20)</td>
</tr>
<tr>
<td></td>
<td>Atm (%)</td>
<td>(1.079 \pm 0.0001)</td>
<td>(1.0812 \pm 0.0002)</td>
<td>(1.0798 \pm 0.0002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>(^{13})C (%)</td>
<td>(792 \pm 147)</td>
<td>(1453 \pm 6.74)</td>
<td>(5.71 \pm 2.37)</td>
</tr>
<tr>
<td></td>
<td>Atm (%)</td>
<td>(1.972 \pm 0.159)</td>
<td>(1.127 \pm 0.007)</td>
<td>(1.118 \pm 0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(^{13})C-Excess</td>
<td>(0.89 \pm 0.16)</td>
<td>(0.046 \pm 0.007)</td>
<td>(0.038 \pm 0.003)</td>
<td></td>
</tr>
<tr>
<td>Zard-Jalali</td>
<td>Control</td>
<td>(^{13})C (%)</td>
<td>(-29.06 \pm 0.12)</td>
<td>(-27.94 \pm 0.45)</td>
<td>(-28.96 \pm 0.07)</td>
</tr>
<tr>
<td></td>
<td>Atm (%)</td>
<td>(1.079 \pm 0.0001)</td>
<td>(1.0805 \pm 0.0005)</td>
<td>(1.0794 \pm 0.00008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>(^{13})C (%)</td>
<td>(868 \pm 69)</td>
<td>(20.13 \pm 3.95)</td>
<td>(7.04 \pm 2.22)</td>
</tr>
<tr>
<td></td>
<td>Atm (%)</td>
<td>(2.055 \pm 0.074)</td>
<td>(1.133 \pm 0.004)</td>
<td>(1.119 \pm 0.002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(^{13})C-Excess</td>
<td>(0.976 \pm 0.074)</td>
<td>(0.053 \pm 0.004)</td>
<td>(0.040 \pm 0.002)</td>
<td></td>
</tr>
</tbody>
</table>
being higher for Jalali-Zard compared with Suski-Sabz (around 0.7 and 0.5 mol m\(^{-2}\) s\(^{-1}\), respectively). As a consequence, \(C_{i}/C_{a}\) remained almost constant (around 0.75) between DAP 12 and 27 for all leaves. In agreement with a higher stomatal conductance, \(C_{i}/C_{a}\) was slightly higher for leaf 3 of Jalali-Zard at DAP 0. At DAP 27, the 3rd leaf was older having lower photosynthesis thus maintaining \(C_{i}/C_{a}\) constant, despite a lower stomatal conductance. Our results are in agreement with those of literature clearly indicating the effect of leaf ageing on gas exchange parameters (Turgeon, 1989).

### 4.2. Relationships between light intensity, leaf photosynthesis rate, \(^{13}\)C fixation by source leaves and distribution to sink fruits

Since our labelling experiments were conducted under natural light in the greenhouse, the changes in the light intensity could have influenced the photosynthetic activity during the labelling of different leaves affecting thus the label level of leaves and fruits for each experiment. Therefore, the labelling level in fruits of different node positions could be influenced not only by labelling level of leaves (leaf rank effect) but also by PPFD level and thus photosynthesis rate during labelling. In addition, as mentioned in Section 2, the light intensity in the greenhouse received by plants is less than that measured outside and is even more reduced inside the labelling chamber. Regarding the values of PPFD and its reduction inside the labelling chamber, the light at plant level was probably limiting for photosynthesis. However, the photosynthesis values we measured were reasonably high indicating that the plants were receiving enough light to have active photosynthesis. Because the \(^{13}\)C label in leaves was measured only 48 h after the 4-h labelling period during which the labelled photosynthases were partly exported to the sink tissues, it corresponds to the residual label in the leaves. Furthermore, labelled CO\(_2\) could be lost by plant organs via respiration. Although assimilation of respiratory released CO\(_2\) from labelled plants cannot be ignored, the isotopic signature of control plants sampled in parallel to the labelled ones did not indicate detectable assimilation of label originating from CO\(_2\) respired by the labelled plants. Unlabelled leaves, fruits and apex had \(\delta^{13}\)C of –28.78‰ to –29.64‰, –26.46‰ to –27.48‰ and –28.19‰ to –29.03‰, respectively (see mean values in Table 3), which are typical values for plants grown in ambient air. In addition apex and fruits of the control plants were less depleted in \(^{13}\)C than the leaves, a pattern usually found as a consequence of post-photosynthetic isotope fractionation (Badeck et al., 2005), while a significant absorption of label by leaves should lead to leaves that are less \(^{13}\)C-depleted than the other organs as is the case in the treated plants.

It seems that fruits formed near a leaf can stimulate its photosynthetic activity (C fixation) probably through reducing feedback effect of assimilates. For a given labelled leaf the highest level of \(^{13}\)C is clearly observed in leaves closest to the fruit position (Fig. 2A). Since in two treatments fruits were kept in basipetal position of labelled leaves (fruit on node 7 and labelling of leaf 8; fruit on node 11 and labelling of leaf 12), results indicate basipetal transportation of assimilates (Fig. 2B). Shishido et al. (1999) indicated that the source–sink relationship in tomato plants is independently formed between each source leaf and all sinks, or between each sink and all source leaves and sink strength varies due to its distance from and relative position to the source leaves (Shishido and Hori, 1991; Shishido et al., 1999). We had no treatment with fruit position exactly at the next acropetal node relative to the labelled leaf to investigate the relative importance of distance and relative position on the stem and this subject can be investigated in future studies. In all cases leaf labelling leads to a labelling of the shoot apex. The highest apex label was found when the closest leaf was labelled (Fig. 2C).

### 4.3. \(^{13}\)C transport from source (leaves) to sink (fruit) tissues

Sink position and distance from a leaf determines how a leaf participates in the fruit feeding. The direction of translocation of assimilates changes with the developmental stage of leaves. As reported by Dickson and Isebrand (1991), fully developed apical leaves export carbohydrates mainly towards younger growing leaves, basal leaves provide carbohydrates for the shoot and roots, and intermediate leaves export in both directions. In our experiment, when leaf 8 was labelled the \(^{13}\)C-excess measured in fruits 3 and 11 was approximately the same. Since fruit 3 has 5 nodes and fruit 11 has 3 nodes distance from leaf 8 labelled, similar \(^{13}\)C-excess in both fruits can be due to a greater tendency of melon plant for basipetal than acropetal translocation of assimilates. For large sinks such as fruits, the subtending leaf usually acts as the main supplier of assimilate.

Sink strength can be defined as ability of each sink to accumulate assimilates from individual source leaves (Shishido et al., 1999). According to our results, the share of each source leaf in feeding a given sink can also depend on its distance to the sink (Shishido and Hori, 1991; Shishido et al., 1999). In addition, roots and apical vegetative tissues could compete as sink organs with fruits for

<table>
<thead>
<tr>
<th>Proximity</th>
<th>Leaf (\delta^{13})C</th>
<th>Fruit (\delta^{13})C</th>
<th>(A_n)</th>
<th>PPFD (Σ 4h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximity</td>
<td>0.331</td>
<td>0.041</td>
<td>0.596</td>
<td>-0.192</td>
</tr>
<tr>
<td>Leaf (\delta^{13})C</td>
<td>0.179</td>
<td>0.67</td>
<td>0.429</td>
<td>0.415</td>
</tr>
<tr>
<td>Fruit (\delta^{13})C</td>
<td>0.873</td>
<td>0.002</td>
<td>0.441</td>
<td>0.501</td>
</tr>
<tr>
<td>(A_n)</td>
<td>0.009</td>
<td>0.076</td>
<td>0.067</td>
<td>0.018</td>
</tr>
<tr>
<td>PPFD (Σ 4h)</td>
<td>0.444</td>
<td>0.086</td>
<td>0.034</td>
<td>0.945</td>
</tr>
</tbody>
</table>
import of assimilates from the basal and apical leaves, respectively. Therefore, the accumulation of photoassimilates in fruits 3 and 11 seems to be limited by both root and shoot apex as competing sinks. Furthermore, in leaf 3, labelled carbon accumulation was limited by reduced net photosynthesis presumably because of the start of senescence of the basal leaves. When data were used to make an average of $^{13}$C-excess for each fruit (regardless to rank of labelled leaf) a higher $^{13}$C-label in fruit 7 than in fruits 3 and 11 for both cultivars was observed (Fig. 4). According to Hughes et al. (1983), each melon fruit imports photosynthates mostly from the closest leaves. These authors reported that there is a source limited situation around fruit position (i.e. considerable demand for assimilates) while there is a sink limited situation near the apex, where there are more leaves and no close by fruit–sink. The results for light response of different leaf ranks (Fig. 3) indicate that this could have been the case also in the current experiment. Higher sensitivity of leaf 8 to PPFD than the other positions (leaves 3 and 12) can be taken as an indication that this leaf is located in part of stem where it is least limited by sink strength.

Even in presence of other source leaves, fruits acropetal as well as basipetal to labelled leaves up to a distance of 9 internodes probably received assimilates produced by the labelled leaves as evidenced by the increase in $^{13}$C-excess in fruits for all combinations of labelled leaves and retained fruits. It is improbable that fruit labels could derive from re-assimilation by leaves close to the fruit of label respired by a distant labelled leaf given that no evident labelling could be seen in control plants. A quantitative assessment
of the role of re-fixation of respired CO₂ needs to be done with future experiments. However, the relative level of label that was found in the fruits decreased with increasing distance between source leaf and fruit. The GLM model fruit δ¹³C (leaf δ¹³C, nodal distance between labelled leaf and retained fruit) explained 59.4% of the deviance between modelled and observed fruit δ¹³C. It was the better model (lower AIC) as compared to the model relating fruit δ¹³C to leaf δ¹³C only and added 14.5% points to the deviance explained by the latter. Thus, transport towards the base as well as the tip of the stems across all studied combinations of leaf and fruit positions and variation of the relative sink strength of fruits for leaves at varying distance was indicated with the current experiment.

5. Conclusion

Our experimental data and correlation analysis indicated that distance, photosynthesis, and probably relative position of a given leaf can affect its share in feeding of a given fruit. According to our results that in both cultivars the net photosynthesis of basal leaves (i.e. leaf 3) decreased (presumably because of the beginning of senescence), the ¹³C label was distributed from apical leaves (i.e. leaf 12) mainly to the growing apical tissues, and that ¹³C-excess was the highest in intermediate leaves (i.e. leaf 8) and fruits (i.e. fruit 7), we could answer to the initial objectives by concluding that: (i) the proximity between source leaf and sink fruit not only favours the amount of label transferred to the sink fruit but probably also increases the source strength and (ii) among all treatments (combinations of labelled leaf rank and fruit position maintained) fruits retained in an intermediate position (e.g. 7th node) seems to be the most efficient sink (i.e. obtaining a high supply of labelled assimilates). The second conclusion is in agreement with traditional pruning–thinning practices by melon producers in Iran and could explain the largest fruits they harvest using such practices. 

¹³C-labelling combined with mass balance studies will allow deeper analysis of source–sink relationship in these plants.

Acknowledgements

The authors thank Laurent Vanbostal for setting up a labelling chamber specially designed for melon leaves, Marlène Lamothé for isoare analysis at the technical platform ‘Métabolisme–Métabolome’ of IFR 87 (IBP, Orsay) and the staff of the greenhouse (ESE, Orsay) for preparation of the experimental plot and for plant culture. The Iranian Ministry of Science, Research and Technology is acknowledged for the fellowship to TB to carry out this work in Orsay as a part of his PhD.

References


