Adenylate patterns of autumn-sown sugar beet differ from spring-sown sugar beet. Implications for root quality

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Received 20 September 2004; revised 22 November 2004


Sugar beet (Beta vulgaris L) is generally cultivated using two different planting and harvest patterns. In northern zones, spring sugar beet is sown in spring and harvested in autumn, whereas in subtropical latitudes, autumn sugar beet is sown in autumn and harvested in summer. The industrial quality of the root is frequently higher in spring-sown sugar beet crops. In order to explore physiological changes associated with this fact, this study has been focused on the seasonal changes of adenosine 5’-triphosphate and adenosine 5’-diphosphate levels in the storage roots of sugar beet plants, as an index of its metabolic status. The results obtained correspond to a different metabolic status of spring and autumn sugar beet at the moment of harvest. The adenylate patterns of autumn beets suggested a functional and active respiratory system. On the contrary, the patterns shown by spring beets corresponded to those we would expect to see in plants becoming dormant. The proline and glucose contents, which decrease the industrial quality of the root, and the respiratory rate measured in autumn-sown sugar beets, were nearly twice those of spring-sown sugar beets. The combination of an active respiratory system, which allows the carbohydrate catabolism and the synthesis of stress molecules, with the environmental factors at the time of the harvest, could be the underlying physiological mechanism causing some of the differences between spring- and autumn-sown sugar beet crops.

Introduction

Sugar beet is a short-term crop of about 6 months grown in the temperate regions of many European countries of the Northern Hemisphere for sugar production. In northern zones, the crop is usually sown in spring and harvested in autumn (spring sugar beet). On the contrary, in subtropical latitudes, such as Mediterranean countries and North African countries, sugar beet is sown in autumn, taking advantage of winter rain, and is harvested at the beginning of the summer (autumn sugar beet). In northern zones, a major limitation to autumn sowing is too low temperature in winter; conversely, extremely high summer temperature is the main obstacle to spring sowing in subtropical latitudes. For this reason, the climate conditions determine the pattern of sowing. In addition, climate has an impact on the yield and economics of the crop. In autumn-sown sugar beets, low winter temperature increases the risk of bolting, and high summer temperature makes water supply necessary and decreases recoverable sugar because of the accumulation of soluble non-sucrose constituents (reducing sugars, sodium, potassium and amino-nitrogen) (Campbell 2002).

Abbreviations – ADP, adenosine 5’-diphosphate; ATP, adenosine 5’-triphosphate; fw, fresh weight.
Sugar beet is grown using both the above-outlined planting and harvest patterns in Spain: spring beet in northern and central zones and autumn beet in the southern zone. The industrial quality of the root is frequently higher in spring-sown sugar beet crops (Gordo 2004). In order to explore physiological changes associated with these patterns, this study has been intended to investigate the seasonal variation of adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP) levels in the storage roots of sugar beet plants, as an index of its metabolic status.

The adenylate patterns of spring-sown sugar beets, grown in Russia, have been reported by Shugaev and Bukhov (1997). They showed an increase of ADP level in the storage root during the last period of the growth. Moreover, during the development of the storage root, the ADP content gradually increased, while respiration rate decreased (Shugaev and Bukhov 1997). In plants, ADP availability is one major factor controlling mitochondrial electron-transport activity (Beevers 1974). Plant respiratory rates are controlled by the cellular level of ADP; ADP initially regulates the rate of electron transfer and ATP synthesis, which in turn regulates citric acid cycle activity, which finally regulates the rate of the glycolytic reactions (Oliver and McIntosh 1995). Shugaev and Bukhov (1997) concluded that a simultaneous decline in respiration rate and increase in ADP level provide the evidence that respiratory control has to be reduced in the cells of mature storage organs and that reduced ADP availability and/or respiratory control do not determine low respiratory activities of mature storage roots. On the contrary, low mitochondrial activity could cause an increase in ADP level. Indeed, the mitochondrial activity in the storage roots has been reported to decrease at the end of the first year of plant development (Shugaev 2001).

In contrast, the adenylate patterns of autumn-sown sugar beet have not been reported so far. To explore whether or not some of the differences between spring and autumn sugar beets could be related to a differential metabolic status that could be reflected in ATP and ADP levels, the pattern of adenylate changes in storage roots was studied. The obtained results demonstrate a different metabolic status between spring and autumn sugar beet at the moment of harvest. The consequences of such a difference are discussed below.

Materials and methods

Plant material and growth conditions

Commercial varieties of sugar beet (Claudia, Ramona and Monatunno) were supplied by AIMCRA (Asociación de Investigación para la Mejora del Cultivo de la Remolacha Azucarera) and used throughout the trials. Plants were harvested in the field in several locations near Seville (southern Spain). Experiments were carried out along three consecutive seasons (1999–2000, 2000–2001 and 2001–2002). Plants grown in a greenhouse close to the laboratory were analysed in parallel during the 2000–2001 season. Details of the field trials are summarized in Table 1.

Sampling procedure

At each sampling date, plants were harvested and samples were prepared following their arrival at the laboratory. Each sample consisted of slices from three to four different storage roots. Samples were immediately frozen in liquid nitrogen.

Adenylate extraction

Frozen samples (0.3 g of storage roots) were ground in a mortar with 5 ml of 0.6 M HClO₄ (Bukhov et al. 1995) and adenylates extracted for 30 min at room temperature. The acid suspension was adjusted to pH 7.6 with 2 M K₂CO₃, and the residue was removed by centrifugation. Supernatants were stored at −35°C.

ADP determination

The ADP content was determined after ADP-to-ATP conversion (Schultz et al. 1993). A 30-μl aliquot of the frozen supernatant was mixed with 30 μl of a mixture containing 47 mM Tris-HCl buffer, pH 8, 4.7 mM MgCl₂, 38 mM KCl, 0.5 mM phosphoenolpyruvate and 50 U ml⁻¹ pyruvate kinase (EC 2.7.1.40). The reaction mixture was incubated for 1 h at 35°C, and the ATP produced was then measured bioluminometrically. The ADP content was calculated as the difference between the ATP measured after and before the incubation with pyruvate kinase. Triplicate assays of ADP were routinely run, and mean values were displayed. The full conversion of ADP to ATP was assessed with pure exogenous ADP (Sigma, St Louis, MO). The amount of light produced in the bioluminescent assay was the same when assaying ATP as that corresponding to an equimolar ADP concentration after ADP-to-ATP transformation. The recovery of 1 or 10 μM ADP added to mixtures containing different quantities of ATP and ADP was always greater than 95%. When the ADP content was negligible, a value of 0.1 ng g⁻¹ fresh weight (fw) was assigned to ADP.
ATP determination

The assay of ATP, or ADP converted to ATP, was performed with a luciferin-luciferase ATP bioluminescent assay kit (Sigma), using a Luminoskan TL (LabSystems Oy, Helsinki, Finland) luminometer. Preliminary measurements were performed in the presence or in the absence of an internal standard of ATP because of the sensitivity of luciferase to inhibition by salts. The addition of 0.87 μM exogenous ATP to four samples containing 5.58, 4.72, 0.37 and 0.12 μM endogenous ATP gave a measurement of 0.89 ± 0.03 μM (mean ± se). Triplicate assays of ATP were routinely run, and mean values are displayed. The interassay coefficient of variation (n = 7) was 7.6%, and the intra-assay coefficient of variation (n = 7) was 4.1%.

Proline and glucose quantification and respiration rate measurement

Proline was quantified by the method of Bates et al. (1973). The concentration of glucose was determined with the Glc test kit from Sigma. Respiration rate was measured in slices of storage roots using a Clark type oxygen electrode (Hansatech Instruments Ltd, Norfolk, UK).

Table 1. Details of the field trials. The level of application of nitrogen within each treatment was: RN, recommended level and EN, recommended level plus 300 kg ha⁻¹. Autumn sugar beets were sown in October and November. Spring sugar beets were sown in March. Sugar beets were irrigated from April to July or from April to September.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sowing</th>
<th>Location</th>
<th>Variety</th>
<th>Irrigation</th>
<th>Nitrogen (kg ha⁻¹)</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>Claudia</td>
<td>Yes¹</td>
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</tr>
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</tr>
<tr>
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<tr>
<td></td>
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<td>Yes¹</td>
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</tr>
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<td>2000–2001</td>
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<tr>
<td>Variety: Claudia vs Monatunno</td>
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<td>Claudia</td>
<td>Yes¹</td>
<td>RN (150)</td>
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<td>Irrigation: irrigated Claudia and Ramona non-irrigated Claudia and Ramona</td>
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<td>EN (450)</td>
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<td>EN (450)</td>
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<td>2001–2002</td>
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<tr>
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<td>Ramona</td>
<td>Yes¹</td>
<td>RN (188)</td>
</tr>
</tbody>
</table>

Statistical analysis

For comparison between treatments, data were subjected to one-way ANOVA, and Dunn’s multiple range test or t-test was used to compare mean values from two groups (SigmaStat 2.03, SPSS Inc., Chicago, IL). Means are considered to be significantly different at \( P < 0.05 \).

Results

The ATP and the ADP contents of autumn sugar-beet storage roots, from four field trials on commercial sugar beet crops at different locations in Southern Spain, were measured during the 1999–2000 (Fig. 1, closed triangles) and 2000–2001 (Fig. 1, open circles) seasons. In parallel, the adenylate patterns of Beta vulgaris cv. Claudia cultured in a greenhouse and carried in their pots to the laboratory were recorded in 2000–2001 (Fig. 2). Despite the variability caused by the specific growth conditions of each trial, the results were strikingly homogeneous, and a general pattern in adenylate changes can be outlined. The development of the storage root can be divided into several stages characterized by different ATP and ADP levels. At the beginning of root
development, an initial increase in both ATP and ADP was seen. The next stage (February–March) was characterized by an exponential growth of the roots at the end of February, when the temperature began to rise and the metabolic activity of the root was probably very high. As ADP content dropped, ATP content remained high; a maximum of ATP/ADP ratio could be seen at this stage. ATP and ADP contents declined gradually on fw basis as roots accumulated sugar, and the ATP/ADP ratio remained steady and low. Finally, at the end of the season (June–July), peaks in the ATP/ADP ratio were caused by a minimum of ADP content, probably reflecting momentary increases of metabolic activity in response to environmental changes. Collectively, these results show major differences in ATP and ADP levels of autumn sugar beet in comparison with that reported for spring sugar beet (Shugaev and Bukhov 1997).

A field trial was conducted in 2001–2002 with the two different planting and harvest patterns, and the adenylate contents of autumn beets (closed circles) and spring beets (open circles) from field crops in the same location were recorded (Fig. 3) to explore a possible link between major environmental signals and the stage of development of the root (dormant or not dormant). Measurements of spring beets from a different location (open triangles) in the last four dates of sampling were also included. Spring sowing caused a marked increase in both the ATP and ADP content of roots at the harvest date (October–November), which correlates with the results reported for Russian spring beets (Shugaev and Bukhov 1997). In contrast, both ATP and ADP values were markedly lower in autumn sugar beets at their corresponding harvest date (July–August). In addition, the ATP/ADP ratio was low and stable in spring plants; this made a difference from the highly variable ATP/ADP ratio of autumn plants at harvest date. It is worth noting that adenylate patterns of spring sugar beets cultured in two different locations (open circles and open triangles) were closely alike. This suggests that the differences between the metabolic status of autumn- and spring-sown sugar beets, reflected by the adenylate patterns, were determined by major environmental signals, such as photoperiod and wide temperature changes, and not with local environmental conditions particular to each specific field trial.

The climatic conditions at the time of harvest were quite different for autumn and spring sugar beet. The maximum temperature was 34–37°C and minimum temperature 17–19°C, and no rainfall was recorded from July to August 2002; in contrast, maximum temperature was decreasing from 25 to 16°C and minimum temperature from 17 to 7°C, and rainfall was 142 l m⁻² from October to November 2002 (Data from RIA, Red de Información Agroclimática de Andalucía, Consejería de Agricultura y Pesca de la Junta de Andalucía, Seville, Spain). The high temperature and inexistente precipitation when autumn sugar beet is harvested are conditions usually linked to heat and water stress. Proline and glucose content were significantly higher in autumn sugar beet roots when compared with spring sugar beet roots (Table 2). Proline is a compatible solute that has been
associated with osmotic adjustment in higher plant cells in response to drought stress (Yeo 1998). Glucose could accumulate as a remainder of sucrose mobilization in order to cope with stress, for example by providing a source for synthesizing proline and other compatible osmolytes. This could be one of the causes of enhanced metabolic activity in autumn sugar beet at the time of harvest. As one of the components of this increased activity, Table 2 summarizes that the respiration rate measured in slices from autumn sugar beet was nearly twice that of the spring sugar beet respiration rate.

**Discussion**

The ratio ATP/ADP is a fundamental regulatory and regulated value. High ATP/ADP values have been found in germinating seeds (Ching and Kronstadt 1972) and lower values in roots (Adams 1970) and green tissues (Bukhov et al. 1995). It appears that adenylate ratios of non-green plant cells are stabilized at high values as is the case in other living cells, but these ratios are stabilized at lower values in green cells (Pradet and Raymond 1983).

The adenylate patterns of autumn-sown sugar beets throughout their pre-harvest development are reported for the first time in this paper. Similar adenylate patterns were recorded (1) in different seasons (1999–2000, 2001–2002 and 2001–2002), (2) in plants from field trials (Fig. 1) and from a greenhouse (Fig. 2) and (3) irrespective of the specific sugar beet variety, nitrogen fertilization and irrigation of each trial (Table 1). Metabolic indexes of an underground organ, such as respiratory rate or catabolic enzyme activities, are difficult to measure in vivo. The quantification of adenylate levels, as shown in this work, seems to be an accurate method to estimate the metabolic status of the root. Despite the multiplicity of reactions that utilize the adenylates, similar patterns were recorded in autumn-sown sugar beet grown in the different agricultural seasons and subjected to the variety of conditions.
summarized in Table 1. Although respiratory activity is probably not the same in all the conditions studied, the ATP and ADP levels are very similar. ATP and ADP levels reflect the equilibrium between production and consumption and seem to be characteristic of a determinate stage of development, despite short-term variations of metabolic activity.

The pattern of sowing (autumn/spring) causes a substantial change in the adenylate patterns of sugar beet roots (Fig. 3). When the adenylate patterns of autumn-sown sugar beets are compared with those reported for spring sugar beets grown in Russia (Shugaev and Bukhov 1997) and with adenylate patterns of spring sugar beets grown in Southern Spain (reported in this paper), strong differences are evident. Spring beets are harvested at the end of the first year of development, when plants become dormant, and the adenylate patterns correspond to a low metabolic activity with high ATP and ADP levels, and a low and very steady ATP/ADP ratio. In contrast, autumn-sown sugar beets reach the harvest date with a high metabolic activity, presenting increased levels of proline, enhanced glucose accumulation and higher respiratory rate in storage roots. This situation corresponds to the low ADP level that is measured in summer.

In the northern areas, where spring beets are grown, crops reach maturity for harvest when temperatures are rapidly decreasing towards winter. On the contrary, in Southern Spain, crops are harvested when temperatures and evaporative demands are high. High temperature could cause an imbalance between photosynthesis and respiration (Huang and Gao 2000), and also could cause a mobilization of carbohydrates stored in the root to supply for leaf net carbon losses. In the field, heat shock may arise in leaves when transpiration is insufficient. Drought stress causes stomata closure, limits transpiration and increases leaf temperature as a consequence (Shaw et al. 2002, Singla et al. 1997). Combinations of high light intensities, high temperatures and water deficits are known to severely inhibit photosynthesis and reduce crop productivity (Ludlow et al. 1990, Salvucci and Crafts-Bradner 2004). Indeed, drought stress has been reported to be the major factor causing yield loss of the sugar beet crop in the United Kingdom (Richter et al. 2001), Italy (Tognetti et al.

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**Table 2.** Proline, glucose and respiration rate in roots of autumn- and spring-sown sugar beet at harvest time. Autumn sugar beet was sampled from 15 July to 12 August 2002. Spring sugar beet was sampled from 7 October to 6 November 2002. Results are mean values ± SE (n = 6). fw, fresh weight.

<table>
<thead>
<tr>
<th></th>
<th>Autumn sugar beet</th>
<th>Spring sugar beet</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>4.79 ± 0.17</td>
<td>1.79 ± 0.14</td>
<td>P &lt; 0.001 (μmol g⁻¹ fw)</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.69 ± 0.16</td>
<td>0.30 ± 0.15</td>
<td>P = 0.017 (mg g⁻¹ fw)</td>
</tr>
<tr>
<td>Respiration rate</td>
<td>2.22 ± 0.26</td>
<td>1.23 ± 0.14</td>
<td>P &lt; 0.001 (μmol O₂ g⁻¹ fw h⁻¹)</td>
</tr>
</tbody>
</table>
Carbohydrates stored in the root could be mobilized and used to synthesize compatible osmolytes in response to drought stress (Yeo 1998).

Soluble non-sucrose constituents of sugar beet (sodium, potassium, amino-nitrogen and reducing sugars such as glucose and fructose) reduce sucrose crystallization in normal factory processes. Sucrose concentration and the ratio of sucrose to total soluble solids (sucrose plus impurities) determine the processing quality of sugar beet (Campbell 2002). The mobilization of stored carbohydrates in the root could increase the glucose content of the sugar beet as a consequence of sucrose catabolism, and nitrogen compounds, such as proline (Ghoulam et al. 2002) and glycine betaine (Shaw et al. 2002), would accumulate in response to drought stress. Indeed, proline and glucose content were significantly higher in autumn sugar beet roots when compared with spring sugar beet roots at the time of harvest (Table 2).

Autumn-sown sugar beets seem to have a full active respiratory system at the date of the harvest. A low mitochondrial activity, such as reported in spring-sown sugar beets at the end of the first year of plant development (Shugaev 2001), would have limited the capacity of the plant to metabolize stored sugars to cope with stressful environmental conditions. In autumn-sown sugar beets, these responses are possible. The quality of the root will then be dependent on the degree of stress suffered by the plant at the date of harvest. Drought stress seems to be the main factor leading to proline and glucose accumulation in autumn-sown sugar beet roots. Excess nitrogen supply also increases proline levels, partially by increasing leaf area index and exacerbating drought stress (E. T. Jiménez 2004, Thesis, University of Seville, Spain). The combination of an active respiratory system, which allows the catalysis of carbohydrates and the synthesis of stress molecules, with the climatic conditions at the time of the harvest, could be the underlying physiological mechanism causing some of the differences between spring- and autumn-sown sugar beet crops.

Acknowledgements – The authors thank AIMCRA for the supply of sugar beet plants from field trials performed by this society. This research was supported by grants FEDER IFD97-0893-CO3-01 from Subdirección General de Proyectos de Investigación Científica y Técnica of Spain, USE-MONTE P-96 from Universidad de Sevilla and Monte and by the Junta de Andalucía (PAI group CVI-134). JA Monreal was in receipt of a FPI fellowship from Universidad de Sevilla (Spain). The authors also thank Christopher Roland for the correction of the manuscript.

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