The Soluble Sugars Determination in Cucurbitaceae Species under Water Stress and Recovery Periods

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Abstract

The soluble sugars detected in leaves of the two cucumber cultivars from Turkey, Beit Alpha 434 (B. 434) and Cengelkoy (C.M.C) and Cucumis melo var. flexuosus (L.) Naudin (Acur - snake cucumber), Cucurbita pepo L. (summer squash) and Ecbalium elaterium (L.) A. Rich. (squirting cucumber) grown in conditions of normal water supply and under moderate (MS) and severe (SS) water stress levels, in addition, the effect of recovery phases investigated 4days (4d) fully rewatering. GLC (gas liquid chromatography) analysis of sugars showed that Cucumis sativus and related species contained other sugar compounds such as mannitol (man.), inositol (inos.) and raffinose (raf.) in addition to glucose (glu.), fructose (fru.) and sucrose (suc.). The total sugar contents of the two cucumber cultivars were generally similar under control conditions. In the other species the total sugar contents were about double those in the cucumbers. Fructose and glucose accounted for more than 50% of the sugar content in control plants of all species, reaching 86% in C. pepo, together with varying proportions of mannitol, inositol, sucrose, and raffinose. The proportions of the hexoses, fructose and glucose tended to decrease with stress in two cucumber cultivars compared with control plants. In the other Cucurbitaceae species, the proportion of hexoses showed no changes with water stress, apart from an apparent rise in Acur under MS. The raffinose percentages were lower in extracts prepared after the recovery time, showed no differences between previously stressed and unstressed plants whereas inositol formed similar proportion of total sugars at the end of stress treatments.

Key words:

Introduction

Water stress, resulting from the withholding of water, changes the physical environment for plant growth and has major effects on crop physiology[31,2]. In research aimed at improvements of crop productivity, the development of high-yielding genotypes, which can survive unexpected environmental changes, particularly in regions dominated by water deficits, has become an important subject. The development of better varieties for dry conditions involves extensive selection and testing for yield performance, and has resulted in drought resistant cultivars of many crops such as wheat, barley, rice, maize, sorghum and soybean[8,21]. Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis, and interference with many other basic metabolic processes[32]. By maintaining turgor in tissues, osmotic adjustment may allow growth to continue at low water potential.

In leaves and stems at least, solute accumulation does not fully compensate for the effects of limited...
water supply on cell enlargement. Turner & Jones[59] stated that the rate of development of stress has a major effect on the degree of osmotic adjustment. Osmotic adjustment is an important drought adaptation mechanism in many crop plants[35]. The soluble sugars and other carbohydrates in the leaves of water stressed plants are altered in quantity and quality may act as a metabolic signal in the response to stress[30,23,12]. However, the role of sugars is not totally clear in this signalling[4] which effected variously depending on stress intensity either in accumulation of concentration of sugars or decreasing of soluble sugars. There are many examples of increase in sugar content in response to water stress, have been recorded, such as accumulation of total soluble sugars in wheat[27] in desiccation-tolerant South African grasses[48], in the nodule of alfalfa[22], sucrose accumulation in wheat[15], fructose, glucose, and sucrose accumulation in wheat[25], soluble and total sugars and oligosaccharides increasing in two sesame cultivars[17], sucrose accumulation in drought tolerant wheat varieties[28], increased sugar concentration in reproductive tissues in maize[50], a marked hexose accumulation (glucose and fructose) in all organs in maize[29] under water stressed plants.

In the contrary, there are some reports of stable or decreasing levels of total sugars or of individual soluble sugars[44], e.g. reducing sugars in the grain of water -deficient maize[61], “marked effect on carbohydrate metabolism” in Citrullus lanatus[9], sucrose in cotyledons of soybean[62], in the green leaf tissues the diurnal - light period levels of the raffinose family oligosaccharides stachyose and raffinose and non – structural carbohydrates (galactinol, sucrose, hexoses and starch) decreased in variegated coleus (Coleus blumei Benth.) whereas drought had little effect on soluble carbohydrate content in the other part of non – photosynthetic white leaf tissues[42], and was no difference in glucose and fructose levels between the wilted (incubated) and turgid bean leaves as well as depletion of starch concentrations was observed in plants of bean exposed to drought stress[41,55] whereas starch accumulated in stress-adapted leaves of cotton[1] however, only one report indicates the concentrations of sugars at different levels of water stress and in recovery phases[25,27].

Leaves of Cucumis sativus L. and other species of the Cucurbitaceae contain, besides the main sugars glucose, fructose and sucrose, oligosaccharides such as raffinose, stachyose and verbascose[39,40,18,19]. According to Smart and Phart[54] large amounts of sugars of the raffinose family are utilised as readily available energy sources during germination cucumber. In some members of this family (e.g. C. pepo) lipid breakdown during germination showed temperature dependence and its utilisation was inhibited at lower temperature. Glycolysis occurred at the stages of germination examined and was particularly active during gluconeogenesis[56,58]. In C. pepo, immature importing leaves are extremely active in hydrolysing raffinose, stachyose and verbascose entering their tissues from the phloem, but are incapable of any detectable synthesis or accumulation of these sugars[57].

In terms of Turkish agricultural products, only Citrullus, Cucumis and Cucurbita have been planted nearly all over the country[14,37]. Ecballium (squirtling cucumber) is a wild plant frequently encountered in Mediterranean countries and rarely cultivated[24]. The aim of the present work was to investigate the effect of various growth and physiological responses of different levels of water stress and of subsequent recovery periods, following rewatering, on the soluble sugars of Cucurbitaceae species.

Materials and methods

Growth of plants and applying water stress

The cucumber (Cucumis sativus L.) cultivars B. 434 (Beit alpha 434) and C.M.C (Cengelköy Misir carsisi -untreated seed) from Turkey were selected growth and two different water stress levels after preliminary screening comparing 9 varieties of Cucumber. Seeds of cucumbers were germinated in trays containing compost for between 10-17 days, then transplanted singly into pots (9 cm diam, 7 cm height) containing equal amounts of compost (85 g) and grown under fluorescent tubes giving an irradiance of 63-72 µmol.m⁻².sec⁻¹ and a day/night temperature of 22 ± 2 C respectively and relative humidity 43-56%. The following investigation examined the effects of water loss on the biomass and morphology of the different parts of plants, based on their fresh and dry weights, according to methods of Roberts et al.[44] and Beadle.[5]. The parameters analysed included: plant height (PH), number of leaves (nl), leaves fresh weight (lfw), leaves dry weight (ldw), stem fresh weight (stfw), stem dry weight (stdw), shoot fresh weight (sfw), shoot dry weight (sdw), root fresh weight (rfw), root dry weight (rdw), total leaf area (LA), water potential of leaves (σ); relative water content (RWC) of the leaves of two cucumber cultivar and three related species. After their first leaves were fully expanded, the plants were exposed to different stress levels, as determined from from previous experiment which carried out with and without plant, following equation of Paquin & Mehuys[36]:

Water holding capacity= (W1-W2)x/100 where; W1 is the soil weight when water-saturated, W2 is the soil dry weight x/100 is the desired level of moisture.

\[
\text{Water holding capacity} = \left( \frac{W1 - W2}{100} \right) \times \text{water held} 
\]
Determination water potential and water deficits

The pot weights corresponding to soil moisture contents after 8d (52.4%) and 18d (37.3%), i.e. the periods of drying which induced MS and SS of cucumber plants in the first, were calculated according to the equation of Paquin & Mehuys[36]:

\[
\text{Water holding capacity} = \frac{(W1-W2)X}{100}
\]

where \(W1=\) soil weight when water-saturated, \(W2=\) soil dry weight and \(X=100\) is the desired level of water.

Water potentials of leaves were determining using pressure chamber method and recommendations of Scholander et al.[47]; Boyer[10,11]. The young expanded leaves from each plant were taken and examined by following procedure. Values were converted to MPa (1 MPa = 10 bars).

Water deficit from the two cucumber cultivars was determined according to the methods of Weatherley,[60,61]; Barrs and Weatherley,[3]; Barrs,[4]; Sanchez-Diaz and Kramer,[46]; Slavik,[53] Beadle et al.[6]. The fresh weight of 10-15 leaf segments (1cm\(^2\)) was determined and the tissue immediately put into petri dishes containing distilled water at the room temperature (20 °C) for 12 h. The segments were then drained on blotting paper, quickly weighed to determine of turgid weight and finally dried in oven at 75 °C for 12 h for dry weights. Relative water content determined as follows:

\[
\text{Relative water content} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100
\]

The work investigated the effect of two different levels of water stress and of subsequent recovery periods, following rewatering four days recovery stage to test on the soluble sugars of two cucumber varieties and related species. Water stresses (MS, SS) and the recovery (RW) stages of the seedlings were compared with their own control plants. Each of the experimental groups was evaluated statistically to avoid against growth fluctuations of the appearance between the treatments.

Leaf extraction procedure

Leaf extracts were analysed by GLC, in order to identify and quantify most of the sugars present. The weighed tissue was plunged into 5 ml 80% ethanol at 70 C in McCartney bottles and kept in water bath for 15 minutes. The extract was transferred to another clean McCartney bottle by means of a Pasteur pipette. The process was repeated twice more. In green tissue, the removal of all remaining color is an indication of the removal of all ethanol-soluble constituents, including carbohydrates. The volume of extract was reduced under a stream of air to 2-3 ml.

Clearing and deionizing the extract

Dowex method

1 ml Dowex-50 which is a strongly acidic cation exchange resin and 1 ml Dowex-1, a strongly basic anion exchange resin (respectively, 100-200 mesh, 8% cross-linked in the Cl and H\(^{+}\) forms, Sigma Chemical Company Ltd., Poole, Dorset, UK) are greatly improved by acid and alkaline washes. The both resins were suspended in 2 volumes of 4 M HCl, 10% NaOH respectively and incubated in a water bath at 100 C for 1 h, then transferred onto a filter paper in a Buchner funnel and washed until the filtrate was at pH 7. After boiling the resin was again washed with distilled water in the Buchner funnel and resuspended with 2 M HCl. This procedure was repeated twice. The resin was resuspended in 1 M NaAc (Sodium acetate) with 3 changes of 2 volumes, and the finest particles were discarded each time. The resin was transferred to a Buchner funnel and washed using 0.1 M acetic acid until the pH of the washings equalled that of the acetic acid.

Preparation of the ion-exchange columns

Ion-exchange columns were prepared from 1 ml pipette tips with a small amount of glass wool in the outlet. The columns were washed with distilled water and loaded with 1 ml of either resin on top of the glass wool. Each Dowex-50 column was so placed that it could drip onto a Dowex-1 column, collecting the neutral fraction in a clean scintillation vial. After the sample had entered the Dowex-50 column it was the washed through both the columns using 4x300 µl distilled water.

GLC analysis of sugar

The deionised extract was prepared for GLC analysis using the method of Holligan & Drew[20]. The extracts were transferred to 5 ml pear shaped flasks then completely dried in a stream of air and stored in a desiccator until required for silylation. Samples of standard sugar solutions (100 µl of 1 mg/ml 50 % ethanol) were similarly prepared. Each sample was dissolved in 0.8 ml anhydrous pyridine, then 0.2 ml of N-trimethylsilylimidazole - TSIM (C6H12NSI) - added as the silylating reagent. The flasks were placed in a water bath in 60-70 °C for 30 min. . After cooling the samples were transferred to small injections vials for GLC. Extractions in the injection vials were injected to the system: Varian 3500 GC with FID detector, Varian 8035 Autosampler, on board integration package, J&W 30m X 0.25 mm ID DB column. All peak areas from traces peaks, corresponding to retention times
of standard sugars, were printed out by the chart recorder.

The sugars used as standards were as follows: arabinose, fructose, glucose, mannitol, sorbitol, inositol, sucrose, maltose, trehalose, raffinose, cellobiose, melibiose and gentiobiose. Not all listed sugars were used for routine analysis; others were injected when attempting to identify unknown peaks. Leaving aside sugars detected at very low levels (<1% of total sugars), changes in the following sugars during water stress and recovery were examined: glucose, fructose, mannitol, inositol, sucrose and raffinose.

**Statistical analysis**

The data obtained from experiments, were subjected to one way analysis of variance (ANOVA), using Minitab 11 for Windows, followed by Tukey’s range test 5% to determine significance of differences between means. Means are indicated with standard error (± s.e.).

**Results and discussions**

The effect of water stress on vegetative growth

The growth responses of two cultivars of cucumber which are suitable for greenhouse and field conditions were compared under control, MS and SS conditions (Table 1). Control values for PH, nl, lfw, ldw, stdw and rdw were similar in both cultivars but stdw and leaf area seemed to be slightly higher in B. 434, whereas rfw was slightly higher in C.M.C. Both MS and SS significantly decreased PH in C.M.C but the effect of two levels of stress was similar. B. 434 showed significant decrease in height under SS and an intermediate, non-significant reduction under SS. Ifw was significantly reduced at both levels of stress in C.M.C but only under SS in B. 434. LA and ldw decreased significantly under SS in both C.M.C and B. 434 while MS, resulted in intermediate values, between SS and Cplants.

In C.M.C, stdw and rdw tended to decrease under stress but not significantly, whereas both parameters showed a significant reduction under SS in B. 434. In C.M.C, rfw and rdw were significantly reduced by MS as well as SS but in B. 434, these values decreased significantly only under SS. The effects of MS and SS on relative water content (RWC), were investigated in the selected cucumber cultivars. Under moderate stress, the RWC of C.M.C was unchanged from the control value but it decreased significantly under MS. The RWC in control leaves of B. 434, which was lower than that of C.M.C controls and only slightly higher than in SS leaves of C.M.C, decreased significantly under SS. In MS conditions, the RWC of B. 434 was intermediate between C and SS values but was not significantly different from either of these. Leaf water potential (Y) significantly decreased in SS plants of C.M.C compared to MS and C (-0.76, -0.63, -0.56 MPa respectively), whereas MS and SS each caused significant reduction of (Y) in B. 434, compared to controls. (-0.82, -0.67, -0.53 respectively) (Table 1).

Fructose and glucose accounted for more than 50% of the sugar content in control plants of all species, reaching 86% in *C. pepo*, together with varying proportions of mannitol, inositol, sucrose, and raffinose (Table 2). However, some peaks remained unidentified in GLC analyses, including a relatively large peak, lying between trehalose and raffinose, with retention time slightly less than raffinose.

The proportions of the hexoses, fructose and glucose tended to decrease with stress in two cucumber cultivars compared with control plants. Sucrose and raffinose which each formed 13% and 8% respectively of leaf sugars in control C.M.C but only 6% and 2%, respectively, in B. 434 tended to increase with stress in both cultivars but with high error values, particularly for sucrose. In SS B. 434, the percentage of raffinose under SS was slightly lower than control treatments (Table 2).

In the other Cucurbitaceae species, the proportion of hexoses showed no changes with water stress, apart from an apparent rise in Acur under MS. In Acur, sucrose and raffinose formed less than 10% of total sugar in control leaves and sucrose showed a slight tendency to rise with SS, whereas raffinose slightly decreased under MS and SS treatments. *C. pepo* had smaller proportions of sucrose and raffinose and both sugars showed a slight but non-significant increase with severe water stress. *Ecballium* leaves had similar proportions of sucrose and raffinose to C.M.C, slightly higher than the other plants, but no significant changes in these sugars were detected under MS or SS conditions. Mannitol which was generally less than 5% of sugars estimated in all species (except for control C.M.C, where it gave a mean of 12 ± 5% and is probably unreliable), showed no change with water stress. Inositol generally formed between 13% and 21% of total sugars, except in leaves of *C. pepo*, where it was proportionately less, and showed no significant changes with water stress (Table 2).

Sugar changes after recovery period

After 4d of growth under normal water supply, following the stress treatments, the total sugar content of leaves of all plants recovering from severe stress treatments was found lower than in unstressed controls. The total amounts of sugars in previously MS *C. pepo* and *Ecballium*, tended to increase to higher levels than in control leaves (Table 3). The
The growth parameters of previously MS and SS leaves were generally lower in leaves of C. pepo recovering from stress appeared lower than in controls.

The raffinose percentages in both control and previously stressed plants were generally lower in extracts prepared after the recovery time and showed no clear differences between previously stressed and unstressed plants. Inositol formed generally similar proportions of total sugars to those recorded at the end of the stress treatments. C.M.C recovering from stress had slightly higher percentages of inositol than controls, similar to those in the stress period, while B. 434 had equal percentages of inositol in leaves previously exposed to MS or SS and in controls from both stress and recovery. In Acus, inositol remained lower in previously stressed leaves than in those of control plants.

**Disscussion**

Water shortage significantly affects extension growth and the root-shoot ratio at the whole plant level[38,7]. Sharp & Davies[51] pointed out that biotechnological improvement of crop performance cannot be achieved until the genes, and gene products, responsible for drought tolerance have been identified and that “this requires a thorough understanding of the physiological, biochemical and physiological perturbations induced by a restricted water supply”. According to Rambal & Debussche[43], changes in plant conductance under water stress are attributable to effects on the roots and xylem. Turner & Jones[59] stated that the rate of development of stress has a major effect on the degree of osmotic adjustment. Osmoregulation and turgor maintenance permit continued root growth and efficient uptake of soil moisture[51].

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**Table 1: Summary of responses of growth parameters to water stress in two greenhouse and field cucumber under control (C), moderate (MS) and severe stress (SS) conditions.

<table>
<thead>
<tr>
<th>Plant height (cm)</th>
<th>B. 434</th>
<th>ACUR</th>
<th>ECBAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>51.2± 23.1</td>
<td>30.5± 16.7</td>
<td>24.0± 12.3</td>
</tr>
</tbody>
</table>

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**Table 2: Sugar composition (% of total sugars ± s.e.) of leaves of cucumber cultivars and related species grown under control (C), moderate (MS) and severe stress (SS) conditions.

<table>
<thead>
<tr>
<th>C.M.C</th>
<th>B. 434</th>
<th>ACUR</th>
<th>ECBAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raf. 3± 1</td>
<td>7± 3</td>
<td>5± 1</td>
<td>3± 1</td>
</tr>
<tr>
<td>Suc. 17± 8</td>
<td>21± 8</td>
<td>43± 8</td>
<td>20± 10</td>
</tr>
<tr>
<td>Inos. 13± 6</td>
<td>22± 5</td>
<td>18± 2</td>
<td>21± 4</td>
</tr>
<tr>
<td>Glu. 30± 7</td>
<td>22± 5</td>
<td>15± 4</td>
<td>25± 6</td>
</tr>
</tbody>
</table>

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**Table 3: Summary of responses of growth parameters to water stress in two greenhouse and field cucumber under control (C), moderate (MS) and severe stress (SS) conditions.

<table>
<thead>
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<th>C.M.C</th>
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</tbody>
</table>
In the first part of the study morphological changes, such as the height of plants, no of leaves, total leaf area, leaf, stem, root fresh and dry weights, water potential and relative water content, were examined. The main target of that in order to gain a better understanding of underlying physiological responses, of cucumis cultivars, which showed different growth responses to different levels of water stress, were chosen for further biochemical studies such as solutes accumulation.

The Cucumis seedlings exposed to moderate and severe water stress, showed various different responses. Moderate water stress (52% moisture content in 85 g compost in pot) was not as effective as severe stress (37% moisture content in 85 g compost in pot) in causing significant morphological changes. However, even moderate stress affected some growth parameters significantly and to differing degrees in the cultivars studied. The both cultivars showed clear correlations between water stress and shoot growth, having shorter stem heights as well as smaller leaf area and total number of leaves. Other growth parameters, leaf, stem and root fresh and dry weight and shoot/root ratio gave different responses individually from plant to plant. Decrease in the dry weight of stressed leaves might be partly related to the strong depletion of starch. The explanations provided by the present data are not enough to interpret all morphological changes, since they depend on limited observations; however plant growth and inhibition of development are clearly affected by limited water availability in the soil. It is quite well documented that accumulation of solutes contributes to osmotic adjustment and physiological adaptation, allowing plants to maintain growth and survive environmental stresses such as water stress. However, the actual role of sugars in the metabolic changes remains to be established[49,16].

Leaves of Cucumis sativus L. and other species of the Cucurbitaceae contain, besides the main sugars glucose, fructose and sucrose, oligosaccharides such as raffinose, stachyose and verbascose[18,19,39,40]. GLC analysis of sugars showed that Cucumis sativus and related species contained other sugar compounds in addition to above three main sugars, as in some previous reports indicating the presence of raffinose, stachyose, and verbascose in Cucumis sativus, C. melo and Cucurbita pepo species. Total sugar content including mannitol, inositol and raffinose was found higher in the related species than in the cucumber cultivars. In addition, sucrose content was lower than fructose and glucose in all plants but higher than had appeared from the enzymatic analysis. Raffinose content was generally similar to sucrose and did not appear in high amount either under stress or after recovery periods. Mannitol had the smallest proportion among the sugars analysed and showed no correlation with water stress. Unfortunately, some peaks which appeared at retention times between those of trehalose and raffinose, remained unidentified, including a relatively large peak which occurred in all extracts. It is possible that this could be stachyose but preliminary attempts to check this were unsuccessful.

Conclusions

Analysis of sugar extracts by gas liquid chromatography showed that, besides glucose, fructose and sucrose, mannitol and raffinose were present in each of the species investigated. In addition some unidentified peaks were detected, including one with a retention time slightly less than raffinose. This peak was present in substantial amounts in all leaf extracts. It would be of interest to identify and quantify this compound if further time was available. Preliminary attempts to compare its retention time with stachyose which, with other sugars of the raffinose family, such as verbascose, might be expected in the Cucurbitaceae (Lewis & Smith, 1967; Lewis, 1984) were unsuccessful.

In conclusion, the above study has provided some understanding of the ways in which physiological and biochemical changes, as well as important morphological responses may allow different plants to tolerate and survive water stress. The cucumber cultivar B. 434 showed more resistance to mild water stress than the other cucumber C.M.C. However, both cultivars were significantly affected under severe drought conditions.

References


