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Root sugar level in luffa and bitter melon is not referential to their flooding tolerance

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Abstract. The effects of soil flooding for 8 days on root and leaf carbohydrate levels in relation to flooding tolerance in two cucurbitaceous plants: luffa (*Luffa cylindrica* Roem. cv. Cylinder #2), a flood-tolerant species, and bitter melon (*Momordica charantia* L. cv. New Known You #3), a flood-intolerant species, were investigated. With flooding, the levels of starch in luffa and bitter melon roots decreased rapidly, indicating that root starch was readily mobilized under flooding conditions. Significant accumulation of leaf starch was observed only in the intolerant bitter melon suggesting reduced phloem transport. Sucrose and hexose (glucose and fructose) levels in flooded roots of both species increased several fold with initial flooding, and were maintained at a higher level than those of non-flooded control during the flooding period. These results implied that starch accumulation in leaves does not result in the deficiency of sugar in flooded roots, and root sugar level is not critical for the flooding tolerance of luffa and bitter melon.

Keywords: Bitter melon; Carbohydrates; Flooding tolerance; *Luffa cylindrica*; *Momordica charantia*.

Introduction

The tolerance of a plant to anoxia or soil flooding has been postulated to be determined by the level of sustainable sugar in the root. Saglio et al. (1980) suggested that under anoxia, there is a positive relationship between adenylate energy charge and metabolic activity through the anoxic fermentation pathway, that is, the higher the metabolic activity, the higher the energy charge that will be maintained under anoxia. The anoxic fermentation in roots flows through glycolysis, and the fermentation rate in roots was thus thought to be controlled by the available supplement of soluble sugars. Vartapetian (1991) reported that exogenous glucose postponed the onset of ultrastructural disintegration of excised pea root cells. Zhang and Greenway (1994) found that exogenous sugar prevent depletion of sugar supplies in beetroots, and enhance ethanolic fermentation. Wample and Davis (1983) concluded that the accumulation of starch in leaves of flooded sunflower plants is a reflection of reduced phloem transport. Those studies generally implied that flooding results in the reduction of phloem transport, causing a further accumulation of photosynthate in leaves and a deficiency of carbohydrates in roots. Carbohydrate availability in roots during flooding might be a key factor determining the flood-tolerance of a plant. However, the observation of large accumulation of sucrose in roots of flooded alfalfa, a flood-intolerant species (Barta, 1988; Castonguay et al., 1993), suggested that the level of sugar in roots might not be a limiting factor to root anaerobic metabolism. Therefore, whether the sugar level in a root determines the tolerance of plant to flooding or not needs further elucidation.

In the present study, the carbohydrate levels in leaves and roots of luffa (*Luffa cylindrica* Roem. cv. Cylinder #2, a flood-tolerant species) and bitter melon (*Momordica charantia* L. cv. New Known You #3, a flood-intolerant species, Liao and Lin, 1994) were compared. We intended to analyze the relationship between sugar level in flooded roots of luffa and bitter melon and flood-tolerance.

Materials and Methods

Material Preparation

Seeds of luffa (*Luffa cylindrica* Roem. cv. Cylinder #2) and bitter melon (*Momordica charantia* L. cv. New Known You #3) were purchased from Known You Seed Company (Kaohsiung, Taiwan). The soaked seeds were germinated in vermiculite for 7 days, and the germinated seedlings were planted individually in a 1.6 dm^3 plastic pot filled with a 2:1:1 (on volume basis) mixture of vermiculite, perlite, and peat. The seedlings were cultivated in a nylon net house with glass roof without temperature control. During the experimental periods, the averaged day/night temperatures were 30/26°C. Approximately 0.3 g per pot of a complete fertilizer (N:P:K=20:20:20), Hyponex No. 2 (Hyponex Co., Inc., Copley, Ohio, USA), was supplied to potted plants every week until the application of the treatments. Plants reaching the twelve-leaf stage (approximately 4 weeks) were used for the experiment.

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Flooding treatment was conducted by immersing potted plants of each species in tap water and maintaining the water level about 2 cm above the soil surface throughout the experiment. At various flooding intervals (2,4,6 and 8 days) four plants of nonflooded control and four of the flooded treatment were taken for each independent measurement of following experiments. The value of each measurement represent results of 3 replicates (N=12) and was expressed as mean ± standard deviation.

Extraction and Determination of Carbohydrates

At each sampling date, the taproots and the sixth fully expanded leaf of each individual plant were harvested around 11:00 AM. For root starch and sugar extraction, 1 g fresh weight of taproot tissue was ground in liquid nitrogen and extracted in 5 cm^3 of 80% ethanol in a water bath at 55°C for 3 h. The extract was then centrifuged at 5,000 *g* for 20 min. The supernatant was used directly for the determination of sucrose, glucose, and fructose. The pellet was resuspended in 2 cm^3 of 0.2 N KOH and boiled at 100°C for 30 min, and subsequently centrifuged at 5,000 *g* for 30 min. After centrifugation, the supernatant was adjusted to pH 5.5 with 1N acetic acid for starch assay (Rufty and Huber, 1983).

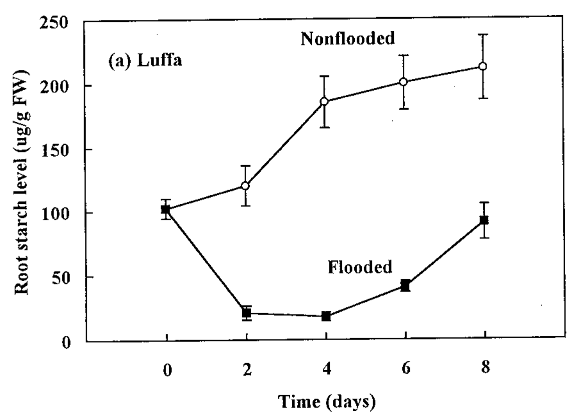
For leaf starch extraction, 0.5 g of leaf tissue from the sixth fully expanded leaf was ground into small pieces in liquid nitrogen and repeatedly extracted with 80% hot ethanol until the tissue was pigment-free. The pigment-free mixture was then homogenized with Polytron PT300 (Kinematica, Switzerland) at 12,000 rpm and centrifuged at 5,000 *g* for 20 min. The brownish pellet was subsequently treated using the procedure described previously for the roots.

All carbohydrates were determined enzymatically by the following means: Starch was done as in Rufty and Huber (1983). In brief, the starch sample was digested with amyloglucosidase solution at 55°C for 30 min. At the end of digestion, tubes were placed in boiling water for 1 min. and centrifuged, and the hexose, including glucose and fructose in the supernatant was analyzed enzymatically using hexokinase and glucose-6-p dehydrogenase. (Bergmeyer et al., 1974; Bernt and Bergmeyer, 1974). For sucrose analysis, the method of Jones et al. (1977) was adopted. Free glucose or fructose is first destroyed by boiling in alkali. Then, sucrose was acted upon by invertase, hexokinase, p-glucoisomerase, and glucose-6-p dehydrogenase in a single analytical step. The end product, NADPH, is followed spectrophotometrically at 340 nm or by its native fluorescence.

Results and Discussion

Among the biochemical changes observed in plants under flooding, high fermentative metabolism in roots has been known to be of importance in plant tolerance to flooding, through its supply of an energy charge high enough to sustain the metabolism in roots (Jackson and Drew, 1984; Mohanty et al., 1993). Thus, maintaining adequate levels of fermentable sugars in flooded roots is undoubtedly important for the long-term survival of plants under flooding.

Several studies have shown that the starch levels in intact roots of alfalfa and rice did not significantly vary with increased durations of flooding, indicating that root starch is not readily mobilized to fermentable substrates (Barta, 1988; Bertani et al., 1981). However, Perata et al. (1992) have recently reported that rice seeds can degrade starchy reserves under anoxia during germination, while wheat seeds do not germinate and are unable to degrade the starch in endosperm. These clearly distinctive behaviors were the consequence of a successful induction of α-amylase in rice seeds under anoxia but not in wheat seeds. Starchy reserves in roots are considered to be easily mobilized during flooding and could provide readily available sugars for anaerobic metabolism in flooded roots. In the present study, the starch levels in roots of both flood-tolerant luffa and flood-intolerant bitter melon markedly decreased at the early flooding stage (Figure 1a–b). The level in flooded luffa started to increase after 6 days of flooding (Figure 1a), while the level in flooded bitter melon remained low throughout the entire flooding period (Figure 1b). The variation of soluble sugars in roots of luffa and bitter melon during flooding period agreed well with studies on alfalfa by Barta (1988) and Castonguay et al. (1993) that the root starch can be mobilized to soluble sugars at the early stage



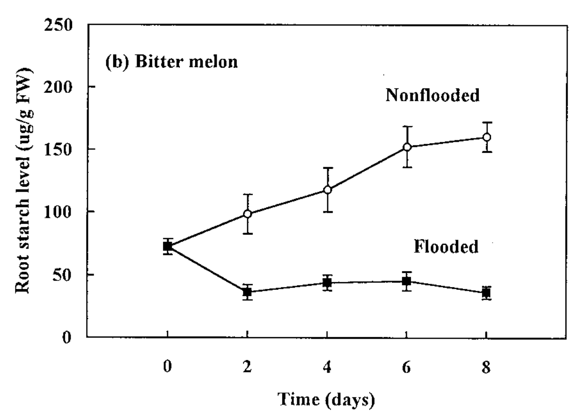


Figure 1. The effect of flooding on root starch levels in luffa (a) and bitter melon (b) during an 8-day period. Data shown are average ± SD of twelve individual measurements (N=12).

of flooding. Analysis of the soluble sugars in roots show that levels of sucrose and hexose (glucose and fructose) in flooded roots of both species increased 3.5–4.0 fold at the early stage of flooding, gradually decreasing after that and reaching levels similar to those of the controls (Figures 3a–b and 4a–b); afterward, the roots of flood-intolerant bitter melon were generally rotten, and plants had died (Liao and Lin, 1995; Su and Lin, 1996).

In comparison with the non-flooded controls, lower starch levels were found in leaves of flooded luffa during the entire flooding period (Figure 2a). In contrast, higher starch levels accumulated in flooded bitter melon after two days of flooding but began to decline after six days (Figure 2b), probably resulting from the respiration rate in leaves exceeding the carbon assimilation rate after long-term flooding as Liao and Lin (1994) suggested.

A continuous supply of fermentable substrates (sugars) to roots has been considered to be critical for long-term survival of pea, pumpkin, and several herbaceous plants under anoxia or flooding (Jackson and Drew, 1984; Saglio et al., 1980; Webb and Armstrong, 1983). Theoretically, if the translocation path is blocked during flooding, the assimilates in leaves will be unable to reach the roots, resulting in a sugar deficiency there. However, the sugar

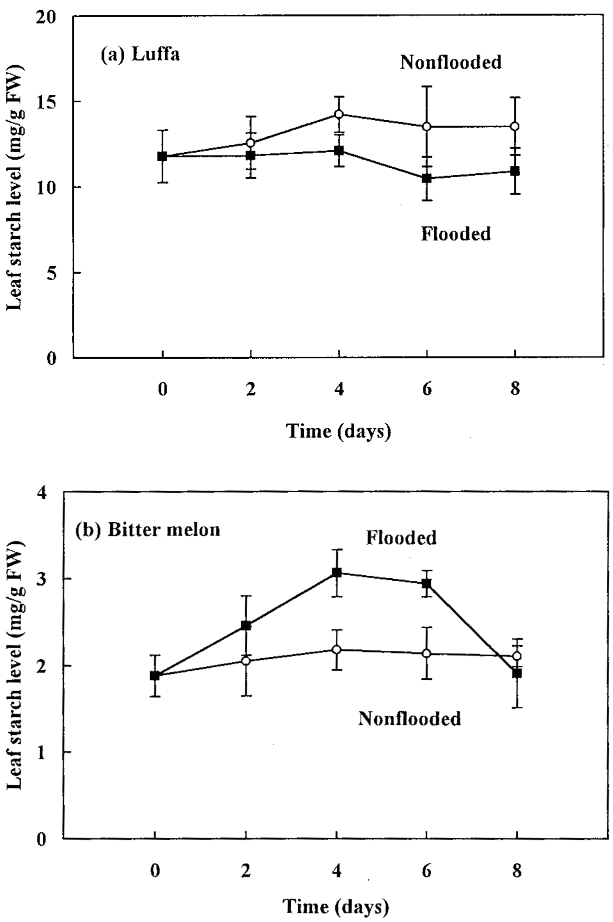


Figure 2. The effect of flooding on the leaf starch levels in luffa (a) and bitter melon (b) during an 8-day period. Data shown are average ± SD of twelve individual measurements (N=12).

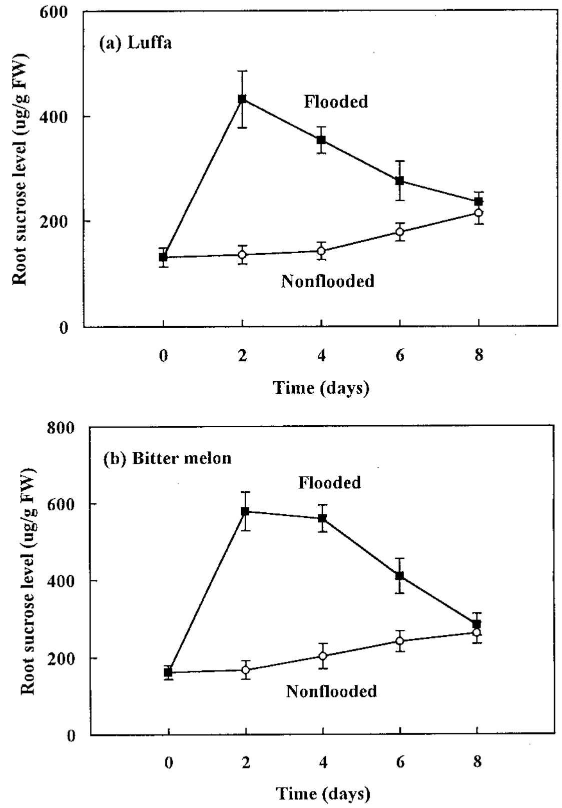


Figure 3. The effect of flooding on root sucrose levels in luffa (a) and bitter melon (b) during an 8-day period. Data shown are average ± SD of twelve individual measurements (N=12).

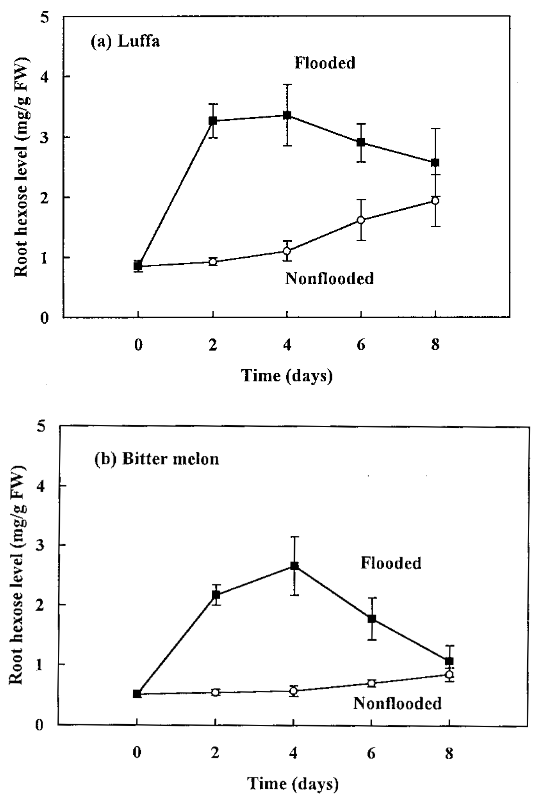


Figure 4. The effect of flooding on root hexose levels in luffa (a) and bitter melon (b) during an 8-day period. Data shown are average ± SD of twelve individual measurements (N=12).

level in flooded roots of bitter melon was higher than that in the nonflooded control (Figures 3b and 4b). Root starch tends to play a role in flooding tolerance at the early flooding stage. We, therefore, conclude that sugar levels in flooded roots are not related to the flood-tolerance of luffa and bitter melon.

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