Seasonal Patterns of Carbohydrates and Proline in Jojoba Clones that Differ in Frost Susceptibility

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Abstract. Total nonstructural carbohydrates (TNC), starch, total soluble sugars, sucrose, and proline concentrations were monitored for 18 months in leaf tissue of two jojoba [*Simmondsia chinensis* (Link) Schneider] clones that differ in frost susceptibility. Seasonal changes in TNC and starch concentrations, with maxima in the winter and minima in summer, were significant. Sugar levels decreased from fall to spring and increased during early summer. The more frost-resistant clone (C-1) had significantly higher sugar concentrations during most of the study than the less frost-resistant clone (C-2). Proline concentrations largely followed the trends found for TNC. The C-1 clone had the higher levels of proline, except when C-2 was frost-injured. Growth trends were similar between C-1 and C-2, with a major growth flush from March to May. Relatively high levels of starch preceded growth flushes.

Fig. 5. (Top) ACC content of secondary roots of nonflooded or flooded tomato seedlings during a 120-hr treatment. Data were combined for both test species. Each data point represents the mean of measurements from eight plants. ○ = $Y_{\text{control}} = 8.97 + 0.030X$, $R^2 = 0.01$; ● = $Y_{\text{flood}} = -2.30 + 3.75X - 0.025X^2$, $R^2 = 0.57$. (Bottom) Ethylene evolution from flooded or nonflooded tomato seedlings PI 128644 or PI 406966 over 120 hr of treatment. Each data point represents the mean of measurements from four plants. ○ = Control of PI 128644: $Y = 1.02 + 0.0038X$, $R^2 = 0.04$; ● = flood of PI 128644: $Y = 0.034 + 0.45X - 0.0070X^2 + 0.000029X^3$, $R^2 = 0.48$; □ = control of PI 406966: $Y = 0.54 + 0.020X - 0.00045X^2 + 0.0000023X^3$, $R^2 = 0.42$; ■ = flood of PI 406966: $Y = 0.51 + 0.0015X - 0.0032X^2 + 0.000015X^3$, $R^2 = 0.54$.

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Freezing temperatures are a major limitation to jojoba cultivation in the United States. Freezes can cause complete loss of the crop (National Research Council, 1985). Freeze damage varies among plants (Coit, 1962; Yermanos, 1982; M. Younes, personal communication), and susceptibility to damage is altered by cultural practices, such as irrigation (Yermanos, 1982).

In plants other than jojoba, total nonstructural carbohydrates (TNC) and proline concentrations increase during cold acclimation (Lalk and Dorffling, 1984; Mezo-Basso et al., 1986; Yelenosky, 1979). Seasonal changes in TNC develop in jojoba near Tucson, Ariz. (Almeida, 1979; de Oliveira, 1983). TNC increase to a maximum in late winter and decrease to a minimum in summer. This pattern is similar in Australia (Reddy, 1980).

The objective of this study was to determine seasonal patterns in nonstructural carbohydrates and proline in two clones that differ in frost susceptibility.

Seven-year-old jojoba plants of two clones were selected for this study. The clone C-1 is rated more frost resistant than C-2 (Khalafalla, 1987). These clones were originally selected from within natural populations and propagated using stem cuttings before establishment in the field. Plants were grown under irrigated conditions at the Univ. of Arizona Casa Grande Overpass Farm. Leaves from node number 4 below the growing point were collected monthly between Oct. 1984 and Mar. 1986 from each of three plants of each

clone. Leaves from each plant were wrapped in aluminum foil and immediately immersed in liquid N. Frozen samples were lyophilized and ground to pass a 60-mesh sieve.

Total nonstructural carbohydrates (TNC) were analyzed using the procedure of Silveira et al. (1978); however, a 0.5% solution of the enzyme clarase (EC 3.2.1.1) (Miles Laboratories, Elkhart, Ind.) was used instead of a 0.3% solution of mylase 100 (Silveira et al., 1978). Total soluble sugars (TSS) were determined by the method of Yemm and Willis (1954) as modified by Riazi et al. (1985). Sucrose concentration was determined using the procedure of Handel (1968) as modified by Riazi et al. (1985). Starch concentration was calculated by subtracting TSS from TNC. The acid ninhydrin procedure as modified by Bates et al. (1973) was used for proline determination.

Twelve branches, three from each of the four cardinal directions, were tagged on each plant at the beginning of the study and monthly average shoot tip elongation was determined.

There were 24 nights of 0C or lower during the first winter of the study. The lowest temperature was -6C on 1 Feb., ≈ 3 weeks before sampling in February. Leaves as far down on the stem as node 4 were freeze-damaged on C-2. Damaged tissue was included in the C-2 sample and likely accounted for elevated levels of proline found in leaves of this clone during these months. Sampled leaves of C-1 for these months showed no frost injury.

A completely randomized design with three replications was used. For each sample date, means were compared using the method of least significant differences.

Seasonal pattern of TNC and starch concentrations were similar for C-1 and C-2 (Fig. 1 A and B). Maximum TNC levels approximated 240 and 340 mg·g⁻¹ dry weight in the first and second winters of the study,

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Fig. 1. Composition of leaf tissue of two jojoba clones differing in frost susceptibility (Oct. 1984 to Mar. 1986, Tucson, Ariz.). (A) Total nonstructural carbohydrates. (B) Starch. (C) Total soluble sugars. (D) Sucrose. Each point is the mean $\pm$ SD of three replicates. Clone 1 (O) is more resistant than clone 2 (*).

respectively, and a minimum of 95 to 140 $\text{mg}\cdot\text{g}^{-1}$ dry weight during June to August. Maximum starch concentrations approximated 170 and 240 $\text{mg}\cdot\text{g}^{-1}$ dry weight in the first and second winters, respectively, and the minimum concentration was 80 $\text{mg}\cdot\text{g}^{-1}$ dry weight during June to August. In two samples (Apr. and June 1985) the TNC level of C-1 was significantly less than that of C-2 ($P = 0.01$). Levels of TNC found in this study are similar to those found in jojoba growing in natural populations near Tucson (Almeida, 1979; de Oliveira, 1983). In Australia, Reddy (1980) also found similar seasonal patterns; however, his concentrations of 53 to 125 $\text{mg}\cdot\text{g}^{-1}$ were significantly lower than those found in this study or by Almeida (1979) and de Oliveira (1983). In India, maximum levels of soluble carbohydrates and starch occur in the summer, with a minimum in winter (Prasad and Iyengar, 1985). Jojoba growth flushes occurred in both spring and summer and were preceded by a build-up of starch similar to that found for citrus (Jones and Steinaker, 1951). Reduction in shoot growth rates were associated with a decline in TNC during the spring. A less abrupt drop in TNC occurred during the summer growth.

Significant differences in concentration of TNC and starch were observed between the two winter seasons. In the 2nd year, the average concentration during November to March was 46% higher than in the previous winter. Similar data were obtained in two consecutive years by de Oliveira (1983). We have no explanation for this pattern. Growth patterns for the 2 years were similar, as were air temperatures and plant water potential as measured by a Scholander pressure bomb (Scholander et al., 1965). Jojoba plants can be alternate-bearing, which could cause different yearly patterns for carbohydrates; however, in this study, seed were not produced.

Seasonal concentration patterns of TSS and sucrose (Fig. 1 C and D) were similar in C-1 and C-2. However, the actual concentrations were higher ($P = 0.01$) for the more frost-resistant C-1 (10 months out of 17 for total sugars, 5 months out of 17 for sucrose). Unlike the case for TNC and starch, TSS and sucrose levels increased during the summer.

Proline concentrations were the highest in the winter and lowest in the summer. Concentrations were generally higher in C-1 than C-2 (Fig. 2). Clone-2 had a higher proline concentration than C-1 only in Feb. and Mar. 1985, when C-2 was severely damaged by frost. In this instance, proline is regarded as a symptom of damage rather than an adaptive response. Proline levels found in this study are higher than those found in salt-stressed jojoba cuttings (Rasoolzadegan, 1980) and in 65 desert plant species (Mohammed and Sen, 1987).

The results of our study support large seasonal changes in total nonstructural carbohydrates in jojoba and proline as a stress indicator. The increased levels of sugars and proline may play a protective role in freeze situations (Levitt, 1980).

Fig. 2. Proline concentration in leaf tissue of two jojoba clones which differ in frost susceptibility (Oct. 1984 to Mar. 1986, Tucson, Ariz.). Each point is the mean $\pm$ SD of three replicates. Clone 1 (○) is more resistant than clone 2 (●).

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