SUCROSE METABOLISM AND GROWTH IN TRANSPLANTED LOBLOLLY PINE SEEDLINGS [1]

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Abstract. Loblolly pine (*Pinus taedu* L.) seedling height, root collar diameter (RCD) and the specific activities of three sucrose metabolizing enzymes, namely, sucrose synthase (SS), acid invertase, and neutral invertase, were measured to assess seedling responses to transplant stress. Bare-root nursery-grown loblolly pine seedlings were lifted and transplanted immediately into nearby nursery beds in February 199 1. It was found that transplanted seedlings grew less in height and RCD and had lower SS activity than nontransplanted controls during the first 7 months after transplanting. However, the seasonal patterns for seedling growth and SS activity were not changed by transplanting. Stems, with SS as the dominant sucrose metabolizing activity, were a stronger growth sink than taproots between spring and early fall. Taproot SS activity decreased from spring to summer and increased again in late summer. Toward late fall when terminal buds were set and RCD growth stopped, taproot became a dominant growth sink. It was concluded that i) SS was the dominant enzyme for sucrose metabolism in actively growing pine stems and. taproots; ii) there were decreases in seedling growth and SS activity in transplanted seedlings as compared with nontransplanted controls; and iii) SS can be used as a biochemical indicator for growth sink strength and for stress caused by transplanting.

Introduction

In the Southern United States nearly 1 billion loblolly pine seedlings are lifted from nursery beds and transplanted onto natural forest sites and former agricultural lands each year (Mangold and others, 1992). Successful plantings depend upon seedlings surviving the stresses imposed by the transplanting process. One of these stresses is water stress caused by loss and damage of fine roots and mycorrhizae, lack of hydraulic continuity between soil and root or within the plant transpiration system, and poor absorption by suberized roots (Burdett and others, 1984; Sands 1984; Marx and Hatchell, 1986; Grossnickle 1988; Johnsen and others, 1988). Thus, it is essential for transplanted seedlings to utilize carbohydrates for new root growth to reestablish soil and water contacts in a short period of time after transplanting (Johnsen and others, 1988).

The carbohydrate source for new root growth varies with tree species. For example, Sitka spruce (*Picea sitchensis* (Borg.) Carr.) seedlings use root starch reserves for new root growth whereas Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings depend on current photosynthates (Philipson 1988).

How transplanted pine seedlings metabolize carbohydrates to survive and resume growth in forest soils is not clear. Sucrose is the major form of translocated carbon in plants including *Pinus* species (Shiroya and others, 1962; Zimmermann and Brown 197 1). The roles of three sucrose metabolizing enzymes have been reported with various annual crops (Claussen and others, 1986; Hubbard and others, 1989; Sung and others, 1989a; Xu and others, 1989a), deciduous tree seedlings (Sung and others, 1989b) and germinating *Pinus edulis seedling* (Murphy and others, 1992). The hypothesis we have proposed is that transplanted loblolly pine seedlings adjust their sucrose metabolism both spatially within the plant and temporally over the seasons in order to survive bare-root transplanting and to initiate growth. Here we will show that sucrose synthase (SS) was the major enzyme in pines for sucrose metabolism and that transplanting stress can be expressed biochemically with SS activity.

Materials and Methods

Plant Materials

Loblolly pine (*Pinus taedu* L.) seeds from mixed lots were stratified at 4 °C for 60 days and sown in April 1990 in beds (60 x 4 x 4 ft) at the Whitehall Nursery in Athens, Georgia. Nursery

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cultural practices were as described by Kormanik and others (1992). Nursery practices were designed to meet but not exceed the biological needs of the seedlings. Pine seedling growth was regulated by manipulating regimes of water and mineral nutrition instead of mechanically clipping seedling tops or through repeatedly wrenching roots as many nurseries do. In late February 1991, 2000 1-0 nursery-grown seedlings with well-set terminal buds, root collar diameter (RCD) equal to or greater than 1.2 in, and at least four first-order lateral roots were selected and their tap roots trimmed to 8 in. These seedlings were immediately transplanted into adjacent unoccupied nursery beds to minimize possible confounding factors, such as seedling handling, storage, and variations in growth environments. Control seedlings remained in the original beds and were thinned from 26 seedlings/ft^2 to the same density, 6.5 seedlings/ft^2, as the transplanted seedlings. Thereafter, all seedlings received regular watering.

Sampling for enzyme assays began in March 1991 and lasted until December 1991. There were two replicates for each of the 13 sampling dates with 10 to 40 seedlings per sample. Results reported here are averages of the two replicates. Variations in enzyme activities between the two replicates were less than 15 percent at all times.

Two hundred seedlings from each treatment were tagged in June 1991 and heights and RCD were measured monthly. Additional seedlings from each treatment were measured and then lifted for enzyme analyses in order to establish relationship between growth and enzyme activities.

Tissue Preparation for Enzyme Extraction

Root cambial zone tissues were obtained by peeling the bark from taproots and scraping off the inner (xylem-side) cambial tissues with a razor blade. The reason for choosing cambial zone tissues over entire organs for enzyme studies was that cambial tissues are the tissues most indicative of radial tree growth (Zimmerman and Brown 1971; Sung and others., 1989b). The same procedures were followed to obtain stem cambial zone tissues. Stems from the seedlings sampled for root enzyme activities were used. Tissues were taken only from the portion of stem formed during the Arst year to ensure that tissues from control and transplanted seedlings were comparable. At each sampling date, about 3 g fresh weight of each cambial tissue was obtained.

Enzyme Extraction Procedures

Cambium tissues were immediately placed in liquid N_2 and powdered with a pestle and mortar. Extraction buffer was added to powdered tissues at a 5:1 ratio (v/w) along with 1 percent (w/v) insoluble polyvinylpyrrolidone (PVP), 1 percent (w/v) Dowex-1 chloride form, 0.1 mM phenylmethylsulfonyl fluoride, and sand. The extraction buffer was similar to that by Sung and others (1989b) with 200 mM Hepes/NaOH (pH 7.8), 3 mM Mg acetate, 5 mM dithiothreitol (DTT), 10 percent (v/v) glycerol, and 1 percent (w/v) soluble PVP-40. The homogenate was passed through one layer of Miracloth and centrifuged at 34,000 g for 20 min at 4 °C. The supematant protein was concentrated with 70 percent ammonium sulfate. The pellet was resuspended in a solution of 25 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, and 15 percent (v/v) glycerol and then desalted on a Sephadex G-25 column equilibrated with the same suspension medium. Recovery from the ammonium sulfate concentration step was between 90 and 100 percent for all enzymes tested.

Enzyme Assays

Sucrose synthase (SS), acid invertase (AI), and neutral invertase (NI) were assayed from the same soluble extracts by previously described procedures (Xu et al., 1989a) with minor modifications. SS was assayed with 100 mM sucrose, 0.5 mM UDP, and 1 mM PPi in a two-step enzyme assay. Changes in OD at 340 nm at 25 °C were monitored continuously with a Beckman DU-7 spectrophotometer for SS. AI and NI were assayed with 25 and 100 mM sucrose, respectively. Reaction mixtures were incubated at 25 °C for 15 min and then boiled for 7 min to stop the reaction. All AI incubation mixtures were neutralized with 2 M NaOH before boiling. Activities for all enzymes were proportional to the amounts of each extract. Invertase activities were linear with up to 60 min incubation time. Enzyme specific activities were expressed as nmol per g fresh weight per min.

Results and Discussion

Field performance of transplanted seedlings is usually evaluated at the end of the first growing season based on the percent of seedling survival and seedling growth. Seasonal growth patterns of these transplanted seedlings are generally not followed. Furthermore, from the sucrose metabolism aspect,

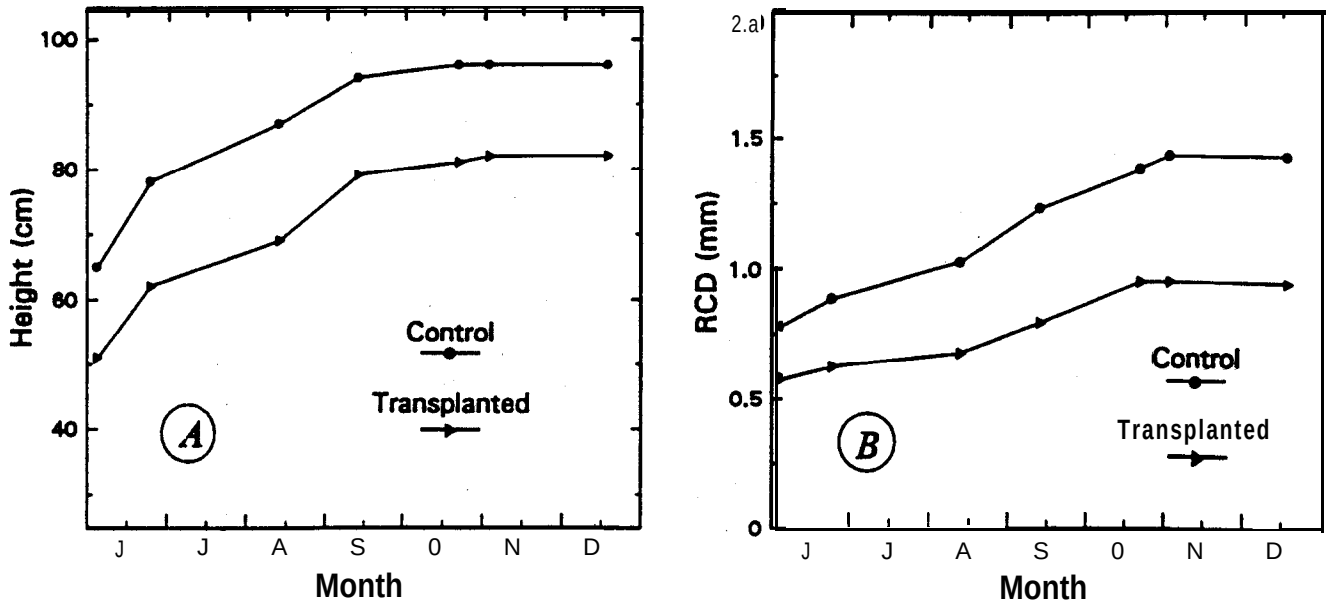

Figure 1. Cumulative growth of nontransplanted (control) and transplanted loblolly pine seedlings. (A) Seedling height growth. (B) Seedling root collar diameter growth. Average seedling height and RCD at transplanting was 31 an and 3.77 mm.

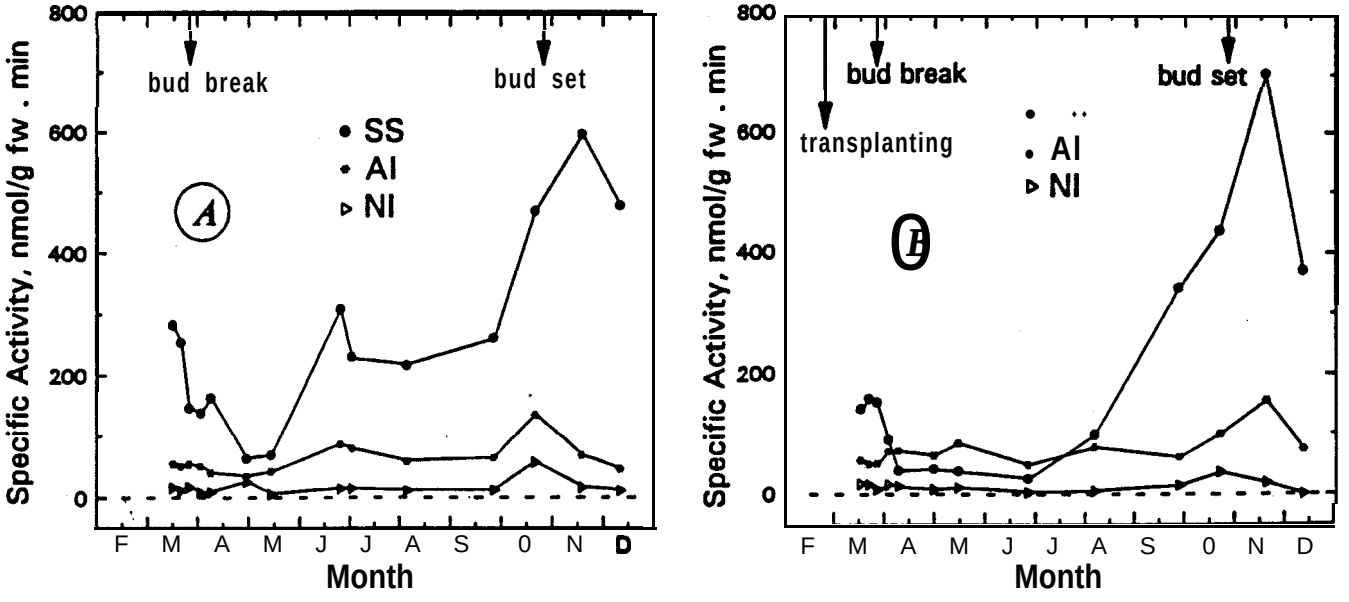

Figure 2. *Seasonal patterns of activity of sucrose metabolism enzymes* **in** *(A) nontransplanted* **control and** *(B) transplanted loblolly pine seedling taproot cambial tissues.*

Compared to taproots, nontransplanted loblolly pine seedling stems were active in sucrose metabolism from March to mid-November when RCD growth was also active (Figs. **1A &** B, **2A,** 3A). Thus, there was a spatial pattern with seedling SS activity in addition to temporal patterns observed with stem and root cambial tissues. These SS activity patterns coincided well with periodic growth patterns in loblolly pine stem and roots (DeWald and Feret 1988; Kuhn and Gjerstad 1991; Fig. **1A &** B). Therefore, it is possible to biochemically assess the growth status of an organ or plant by measuring SS activity.

Compared with roots of transplanted seedlings, stems of transplanted seedlings were much leas influenced by transplanting as measured by sucrose metabolizing enzyme activities (Fig. 3B). SS was still the dominant sucrose breakdown activity in the stems of transplanted seedlings. There was a 40 percent decrease in SS activity in transplanted stems as compared to a maximal 90 percent decrease in transplanted roots throughout the year (Figs. **2B,** 3B). No differences in SS activity between stems of transplanted and nontransplanted seedlings were found 7 months after transplanting (Figs. **3A,** 3B).

growth loss of transplanted seedlings when compared with nonlifted controls is not addressed. Figures 1A and 1B show the cumulative growth data of seedlings. Bud break of nontransplanted control seedlings occurred toward late March. Within the next 2 months seedling height doubled with another 50 percent increase by September when dormant terminal buds were set (Fig. 1A). Heights of transplanted seedlings increased at similar rates to those of controls from June to September (Fig. 1A). This indicates that height growth was only slowed down during the first three months after transplanting. RCD growth rate of nontransplanted seedlings increased until September, slowed during the following two months and stopped later in the year than height growth (Fig. 1B). Transplanting did not significantly change the temporal pattern of RCD growth except the amount of growth was less in transplanted seedlings (Fig. 1B).

Sucrose Metabolism in Taproot Cambial Tissues of Nontmnsplanted and Transplanted Seedlings

Throughout the study, SS was the dominant sucrose cleavage enzyme, followed by AI and NI, in nontransplanted loblolly pine seedling taproot cambial tissue (Fig. 2A). Neither invertases showed much oscillation with time whereas SS activity had a definite seasonal pattern (Fig. 2A). SS activity decreased 3- to 4-fold during the period from bud breakage to stem elongation in April and May. From July to September SS activity resumed to before bud breakage levels. There was 100 percent increase in root SS activity after bud set in late September and this activity remained constant through December. This seasonal pattern of SS activity in pine roots was similar to that of sweetgum (*Liquidambar styraciflua* L.) taproot except the latter decreased to minimal levels after leaf abscission in late November (Sung and others, 1989b). Since loblolly pine needles still photosynthesize in winter in the South (McGregor and Kramer 1963; Drew and Ledig 1981), there should be a continuous supply of sucrose to sink tissue, such as pine taproots, for growth in winter (Drew and Ledig 1981; DeWald and Feret 1988; Khuns and Gjerstad 1991). SS has been associated with growth and storage of starch in sucrose importing agriculture crop sinks (Claussen and others, 1986; Sung and others, 1989a). Our results indicate that active root growth in winter is associated with high levels of root SS.

There were no significant changes in either AI or NI activities throughout the season in roots of transplanted seedlings compared with their controls (Fig. 2B). However, there were 50 percent decreases in SS activity in taproots of transplanted seedlings two and half weeks from transplanting (Fig. 2B). These decreases were more pronounced during the period of active shoot elongation. In late June, nontransplanted seedling roots were 10 times more active in SS activity than the transplanted roots (Figs. 2A, 2B). It took taproots of transplanted seedlings 7 months to resume their SS activity comparable to the nontransplanted seedlings. The inability of a taproot of transplanted seedling to metabolize sucrose via SS is obviously a response to transplanting stress. Transplanting stress was also observed toward the end of the year when there was less SS activity than that of the nontransplanted controls. Therefore, our results suggest that growth loss and stresses experienced by transplanted seedlings can be expressed with SS activity.

From April to June when SS 'activity was at minimal level, AI activity was the dominant sucrose cleavage activity in transplanted seedling roots (Fig. 2B). It has been speculated that due to its higher affinity toward sucrose, compared to that of SS, AI could act as sucrose scavengers in tissues undergoing strong competition for sucrose (Loboda and others, 1990). For example, when there is a great demand for sucrose in actively elongating shoots during spring and summer, AI will be more efficient than SS in metabolizmg limited amounts of sucrose available to roots of transplanted seedlings. Thus, transplanted seedlings could survive this critical period and become active when sucrose supply is more available during fall.

Sucrose Metabolism in Stem Cambial Tissues of Nontransplanted and Transplanted Seedlings

Besides roots and new shoots, stems also are a sucrose sink in loblolly pine seedlings. Similar to root cambial tissues, stem cambial tissues contained three sucrose metabolizing enzymes with SS the dominant activity throughout the year (Fig. 3A). Except in the month of December, SS activity was 9- and 25-fold as much as AI and NI activities, respectively (Fig. 3A). The latter two enzymes did not fluctuate with season. Stem SS activity, however, had a temporal pattern similar to root SS except the former decreased drastically to minimal level in mid-December. During April through August, the period of active new shoot growth, SS activity in previous year stems was maintained at constant levels and was only half that in spring and early fall (Fig. 3A).

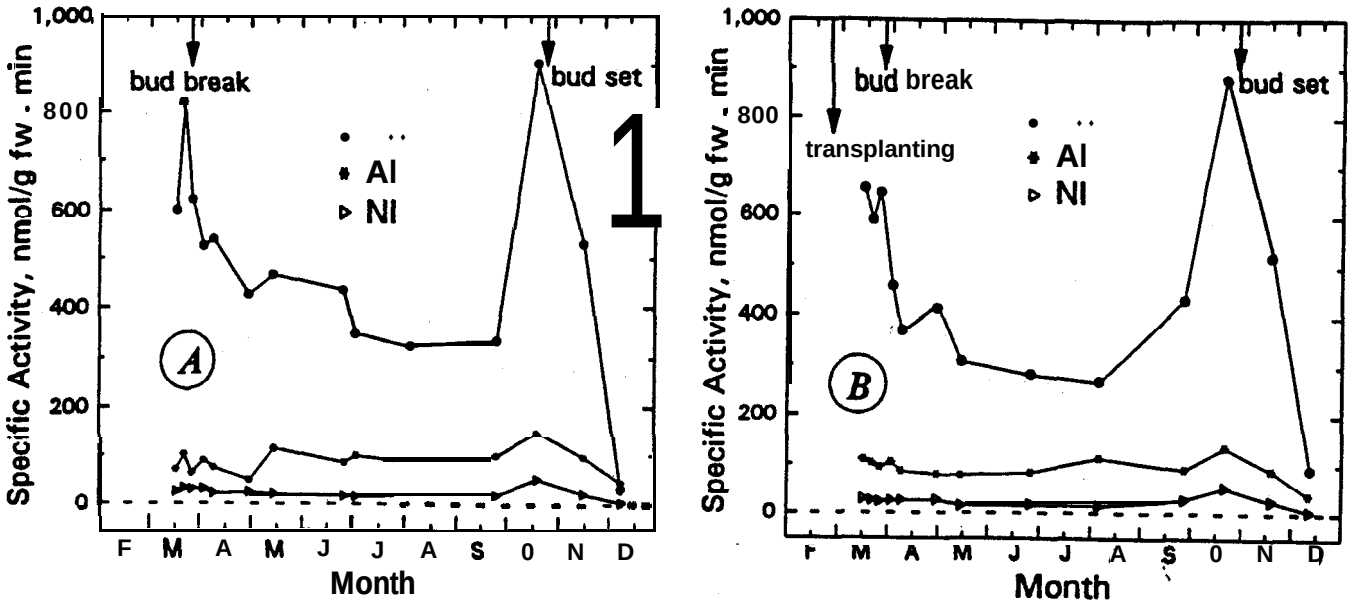

Figure 3. Seasonal patterns of activity of sucrose metabolism enzymes in (A) nontransplanted control and (B) transplanted loblolly seedling stem cambial *tissues.*

The transplanting process neither altered the spatial patterns of SS activity within a seedling nor did it affect the temporal patterns of SS activity in stems and roots. Only the level of SS activity was decreased by transplanting.

Use of Sucrose Synthase as Indicator of Physiological Status of a Seedling

To further demonstrate the feasibility of using SS activity as a biochemical indicator for the physiological status of seedlings under transplanting stress, individual seedlings were measured on June 4 and 24,199 1. Composite tissues from those seedlings with similar growth in RCD during these three weeks were collected and assayed for enzyme activities on June 24. Table 1 presents the relationship between RCD growth and sucrose metabolizing enzyme activities of nontransplanted and transplanted seedlings. Similar to results in Figs. 1B, 2A, 2B, 3A, and 3B, nontransplanted seedlings grew more in RCD and had higher levels of SS and AI activities than transplanted seedlings (Table 1). These data suggested further that SS can be used as an indicator for the physiological status of tissues. High SS activity is associated with rapid growth and low activity is associated with slow growth due to seasonal changes or transplanting stress.

Table 1. Relationship between second year pine seedling growth and sucrose metabolism enzyme activities.

Increase in RCD[a]		Enzyme Specific Activity		
		SS	AI	NI
		nmol/g fw. min		
Nontransplanted				
mm				
1.67	Stem	518	99	16
	Root	326	99	15
Transplanted				
0.35	Stem	268	52	16
	Root	28	a2	4

[a] RCD measurements were taken on June 4 and 24, 1992.

Conclusions

In this study, other stresses generally associated with transplanting process were minimized in order to examine transplanting stress more closely. It is obvious that even under these good growing conditions, transplanted seedlings still lagged 3 to 7 months behind nontransplanted controls in height growth, RCD growth, and sucrose synthase activity. From March to June a nontransplanted pine seedling allocates sucrose for growth between elongating new shoots, previous year stems and roots. Roots have the lowest activities within a seedling. Similarly, roots of transplanted seedlings were the least active in spring and summer. This trend lasted for almost 60 days longer than in the nontransplanted seedlings. This suggests that sucrose synthase may be used as a biochemical indicator for the physiological status of stressed loblolly pine seedlings.

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