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Induction of Frost Hardiness in Stem Cortical Tissues of *Cornus stolonifera* Michx. by Water Stress

II. BIOCHEMICAL CHANGES[1]

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PAUL M. CHEN[2] AND PAUL H. LI
Laboratory of Plant Hardiness, Department of Horticultural Science and L.A., University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

A decrease of protein, RNAs, and starch, and an increase of sugar were observed in 3-day water-stressed red osier dogwood plants (*Cornus stolonifera* Michx.) when the frost hardiness increased from −3 to −6 C. As the frost hardiness increased to −11 C after 7 days of treatment, the starch continuously decreased, however, the proteins and RNAs increased with a continuous increase of sugar. Further water stress treatment had little effect on the changes of these chemicals. Control plants in short days showed similar gradual biochemical changes in patterns. From the results of frost hardiness increases, the pattern of biochemical changes, and the mechanism of the increased freezing resistance, it appears that the water stress and short days accomplished essentially the same physiological end(s) in inducing frost hardiness in red-osier dogwood.

Plants respond with many protective adaptations toward water stress. Different species have different types of responses. There is no universal mechanism of drought tolerance; however, it has been suggested that an increasing tolerance to one unfavorable environmental factor helps the plant to endure other unfavorable effects (7). There is ample evidence that similar biochemical changes occur when plants are undergoing cold hardening or are subjected to water stress (9, 11). For instance, the increase in protoplasmic viscosity by water stress (19) corresponds to the seasonal augmentation in total protoplasm during cold acclimation (18). One of the major adaptations of plants in response to water stress is the ability to synthesize nucleic acids (10) and therefore, to synthesize proteins during hardening (8). The increased resistance of the protoplast to both desiccation and freezing appears to be associated with the accumulation of soluble sugars which may play a protective role to stressed tissues (16, 22).

The studies on physiological and biophysical aspects in association with the induction of frost hardiness in red osier dogwoods (*Cornus stolonifera*, Michx.) by water stress have been reported previously (4, 5). In this study, biochemical changes in stem cortical tissues produced by water stress are presented.

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[2] Present address: Department of Crop Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

MATERIALS AND METHODS

Preparation of Plant Materials. Red osier dogwood plants (*C. stolonifera* Michx.) were propagated and treated as described previously (4). Stems of each treated plant were collected separately. Stem cortical tissues were peeled, weighed, and then frozen by liquid N_2. Tissues were then freeze-dried, ground into 70 mesh size, and stored at −20 C until analysis.

Evaluation of Frost Hardiness. The survival of stem cortical tissues to controlled freezing stress was evaluated as previously described (4).

Extraction and Determination of Nucleic Acids. Nucleic acids were extracted by tris-phenol (13) and chromatographed on methylated-albumin Kieselguhr (MAK) column (14) using a linear gradient of 0.4 to 1.2 M NaCl in 0.5 M phosphate buffer (pH 6.5). Tenaciously bound RNA (TB-RNA) was removed from the column with 100 ml of 1.5 M NH_4OH. Relative quantities of each type of nucleic acid were determined by calculating the A_{260} units of each peak ($\Sigma A \times$ ml/tube) and using yeast-RNA as the reference.

Total Protein Determination. One g dry weight powder was first extracted with 80% ethanol by shaking for 0.5 hr at room temperature. The residues were then quantitatively filtered and washed four times with 80% ethanol. The residue was then oven-dried at 75 C. Nitrogen in the residue was measured by the Kjeldahl method (1) and total protein was calculated by 6.25 × residue-N.

Total Soluble Sugars and Starch Analysis. Total soluble sugars were referred to the sugars presented in the 80% ethanol extract. They were estimated by the method of Dimler *et al.* (6). Starch remaining in the residues was extracted by boiling water and measured according to the method of Arnoff (2).

RESULTS

During a 21-day water stress treatment, stressed plants and controls, either in LD or SD regime, showed an increase of different types of RNAs and a constant level of DNA (Table I). After 3 days of treatment, stressed plants had a lower level of RNAs than controls; but, after 7 days and thereafter, a higher level of RNAs was observed. The total RNA after 3 days of water stress in LD and SD was 8.5 and 17.5% lower, respectively. After 7 days, water stress resulted in an increase of 18.1% of total RNA in LD plants and 2.8% in SD plants as compared with their controls. Additional 14 days of treatment had little effect on the nucleic acid metabolism. SD controls always had a higher level of RNAs than the LD controls with a similar increasing rate of RNAs during a 21-day treatment of water stress.

Table I. *Changes of Different Fractions of Nucleic Acids and Survival Temperatures of Stem Cortical Tissues of Red Osier Dogwood Plants Grown under LD or SD Regimes after 3, 7, 14, and 21 Days of Controlled Watering Treatments*

Within each column, means without letters in common are significantly different at the 5% level by Duncan's Multiple Range Test.

Photoperiod	Treatment	Period in Days	Survival Temp (°C)	Different Fractions of Nucleic Acids (mg per g dry wt.) rRNA	t-RNA	TB-RNA	Total RNA	DNA
Long Day (14 hrs light)	30ml/day	3	-6.0c	.516a	.137ab	1.054a	1.705a	.01a
		7	-10.5d	.838def	.161defg	1.217bc	2.216de	.01a
		14	-11.0d	.862def	.174gh	1.317cde	2.353ef	.01a
		21	-12.0d	.948fg	.189i	1.407ef	2.543hi	.01a
	Control	3	-3.0a	.563ab	.145abc	1.156ab	1.864b	.01a
		7	-3.0a	.588ab	.149bcd	1.143ab	1.876b	.01a
		14	-3.0a	.660bc	.152cde	1.233bc	2.041c	.01a
		21	-3.0a	.749cd	.164efg	1.225bc	2.139cd	.01a
Short Day (10 hrs light)	30ml/day	3	-6.5c	.558ab	.134a	1.080a	1.773ab	.01a
		7	-10.8d	.829de	.168fg	1.356ef	2.352ef	.01a
		14	-11.5d	.916ef	.188i	1.544g	2.650i	.01a
		21	-12.2d	1.038g	.208j	1.628g	2.871j	.01a
	Control	3	-3.2a	.663bc	.157cdef	1.333def	2.151cd	.01a
		7	-4.0ab	.766cd	.166fg	1.357ef	2.290e	.01a
		14	-5.0bc	.784d	.170fg	1.431f	2.386fg	.01a
		21	-6.5c	.841def	.184hi	1.437f	2.461gh	.01a

A general significant trend of total protein became evident in response to the water stress in LD and SD regimes (Fig. 1). The stressed plant had a lower level of total protein than the controls in both LD and SD regimes after 3 days of water stress. Total proteins were 11.3 and 2.5% lower in stressed plants in LD and SD regimes, respectively, as compared with controls. However, there was a net increase of 6.9% in LD regime and 10.8% in SD regime after 7 days. Further water stress treatments only resulted in the total protein being at high levels as compared with their corresponding controls. LD and SD control plants had a gradual increase in total protein during the 21 days, and the SD controls always maintained higher protein levels than LD controls.

In water-stressed plants, total soluble sugars increased to about 15% after 3 days both in LD and SD regimes as compared with their controls, and then increased to about 30% in LD and 17% in SD after 14 days. Thereafter, the increase of total soluble sugars was insignificant (Table II). Total soluble sugars in LD control plants remained unchanged after 21 days, however, they increased up to 20% in SD control plants. A typical decrease in starch corresponding to an increase of total soluble sugars was apparent in water-stressed plants both in LD and SD regimes (Table II). Starch decreased to 40% in water-stressed plants after 3 days, and then to 88% after 7 days. An additional 14 days of water stress treatment showed little further decrease in starch. Control plants in LD regime maintained a constant level of starch during 21 days, but SD controls showed a substantial decrease—a decrease of 70% starch.

DISCUSSION

The decrease in RNAs and proteins in water-stressed dogwoods after 3 days probably corresponds to the "reaction phase" as suggested by Stocker (20) and to "phase B" by Chen *et al.* (3). Many studies on the metabolic activities responding to water stress were conducted during the period at which the soil moisture content dropped rather rapidly. The decrease in soluble protein N_2 and RNA in sugar beet during drought was measured within the period when the soil moisture content continuously decreased to permanent wilting point after 10 days (17). The decrease in total protein and nucleic acids in drought-stressed wheat was observed through the period at which the soil was not watered until the plant died after 15 days (21). The increase in total RNA and proteins in water-stressed dogwoods after 7 days may parallel Stocker's "restitution (*i.e.* hardening) phase" (20) and "phase C" of Chen *et al.* (3). When the soil moisture had been stabilized at 22% field capacity after 26 hr water stress, Stocker (20) found that the protoplasm of *Lamium maculatum* showed a tendency to increase its structural viscosity and to revert to a firmer bonding. Chen *et al.* (3) also demonstrated that

there was an increase in protein nitrogen in citrus plants after the soil water content had been dropped to about 3% and maintained at the level thereafter. In our experiments, the soil moisture content was maintained at a low and relatively constant level after 7 days and thereafter (4).

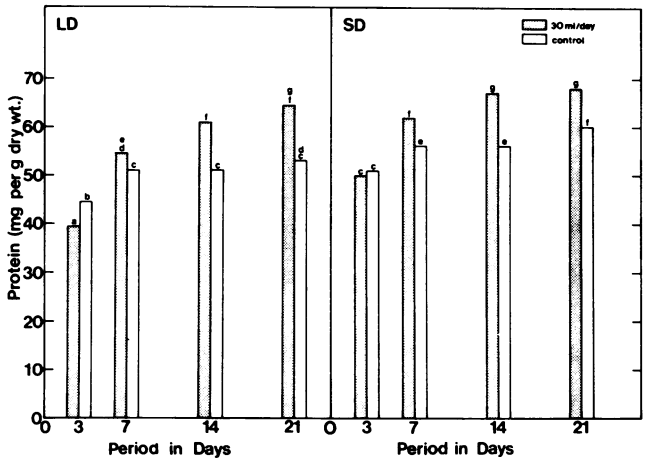

FIG. 1. Changes of the protein content in stem cortical tissues of red osier dogwood plants grown under LD or SD regimes after 3, 7, 14, and 21 days of controlled watering treatments. Means followed by the same letter do not differ significantly at the 5% level according to Duncan's Multiple Range Test.

The increase in RNAs and proteins in SD control plants as compared with LD control plants is a general phenomenon induced by short days. An increase in RNA accompanied by an increase in proteins had been reported during natural fall hardening in dogwoods when the daylength became shortened (12). A loss of tissue hydration in dogwoods normally occured under short days and was believed to be the result from decreased stomatal resistance and increased root resistance to water movement (15). It is conceivable that SD control plants first undergo a moderate rate of water stress caused by short day effect so as to decrease their water potentials (4), and then increase their macromolecules thereafter. Stocker (20) hypothesized that there was no reaction phase if the soil was allowed to dry very slowly. Instead, the restitution phase had sufficient time to counteract the reaction phase at the very beginning of water stress. When the olive and ligustrum leaves were dehydrated very slowly at 30 C, an immediate increase in RNA was reported (10). Thus, the increase in RNA and proteins in SD control plants may imply, to a certain extent, Stocker's restitution theory.

The increase in total soluble sugars concurrent with a decrease in starch in water-stressed plants indicates one of the adaptative reactions in plants responding to the unfavorable environmental factors. The accumulation of sugars during water stress may

Table II. *Changes of Starch, Total Soluble Sugars, and Survival Temperatures of Stem Cortical Tissues of Red Osier Dogwood Plants Grown under LD or SD Regimes after 3, 7, 14, and 21 Days of Controlled Watering Treatments*

Within each column, means without letters in common are significantly different at the 5% level by Duncan's Multiple Range Test.

Photoperiod	Treatment	Period in Days	Survival Temp (°C)	Starch (mg/g dry matter)	Total Sugar (mg/g dry matter)
Long Day (14 hrs light)	30ml/day	3	-6.0c	15.53d	130.10bc
		7	-10.5d	3.14ab	139.66cd
		14	-11.0d	1.98ab	150.84def
		21	-12.0d	1.39ab	154.66ef
	Control	3	-3.0a	25.61f	114.16a
		7	-3.0a	24.32f	117.34a
		14	-3.0a	26.14f	116.18a
		21	-3.0a	26.31f	117.34a
Short Day (10 hrs light)	30ml/day	3	-6.5c	15.13d	131.68c
		7	-10.8d	2.98ab	152.16ef
		14	-11.5d	1.77ab	155.34ef
		21	-12.2d	1.32a	162.34f
	Control	3	-3.2a	24.48f	110.24a
		7	-4.0ab	19.21e	119.17ab
		14	-5.0bc	13.04d	131.50c
		21	-6.5c	6.89c	144.84de

indicate that the sugar plays a protective role in the stressed tissues (22). In some plants, any treatments that increase the sugar content enhance their frost hardiness, and that decrease the sugar content lower their frost hardiness (23). The SD control plants also showed an increase in total soluble sugars and a decrease in starch as compared with LD control plants, however, the changing rates were much slower as compared with water-stressed plants. The accumulation of sugars in relation to an increase in frost tolerance may be explained as: (1) the osmotic effect which increases the avoidance of freeze-induced dehydration; and (*b*) the metabolic effect which produces unknown protective changes and leads to increase the tolerance of freeze-induced dehydration (11). The nuclear magnetic resonance study indicates that the increase in frost hardiness in water-stressed and SD control plants is due to a combination of tolerance and avoidance of freeze-induced dehydration (4). The accumulation of sugars in both water-stressed and SD control plants may contribute to the osmotic and also metabolic effects which, in turn, increase the freezing tolerance.

It may be concluded that water stress and SD accomplished essentially the same (nonadditive) physiological end(s) in inducing frost hardiness in dogwood plants. The rapid increase in frost hardiness by water stress appears due to a rapid metabolic adjustment, *i.e.* from disorganization to reorganization of macromolecules, which affects the rapid changes of osmotic potential and protoplasmic structure in order to adapt to a new unfavorable environment. SD-induced frost hardiness is due to an increase in synthetic activities although the rate of metabolic changes is much slower as compared with water stress treatments. Nevertheless, the biochemical changes induced by SD also affect the changes of osmotic potential and protoplasmic structure of the plant tissues during the hardening process.

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