Water Stress, Rapid Polyribosome Reductions and Growth

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ABSTRACT

Measurements of the water status of various plant tissues exposed to differing levels of salts for 1 hour were made using the recently developed Campbell J-14 press (Logan, Utah). Values obtained with the press were found to correlate well with estimates of relative water content, and experiments with 3-day-old pumpkin seedlings showed that detectable changes in press values of cotyledon tissues could be obtained within 5 minutes following salt- or desiccation-induced stress.

Polyribosome levels were measured in tissues from various plant species following short duration water stress. A small reduction in polyribosome percentage was obtained in cotyledons of 3-day-old pumpkin seedlings which were exposed to an osmotic potential (NaCl) of -4 bar for 10 minutes, but more pronounced changes were found after 30 minutes of stress. Shoot tissues of peas, barley, wheat, and safflower following 20- or 30-minute salt- or desiccation-induced stress yielded extracts with reduced polyribosome levels; however, 30 minutes of exposure of cotton and pumpkin seedlings to -6 bars did not result in altered polyribosome percentage of extracts from roots. Studies using shoot tissues from pumpkins and peas showed that polyribosome percentages and growth rates of both plants were reduced in proportion to loss of tissue water. These plants differed in their sensitivity to stress in that polyribosome content and growth rate reductions were both nearly twice as severe per unit of water loss in peas as in pumpkins. These data along with those obtained by others suggest that growth rate reductions may be directly proportional to reductions in polyribosome levels during water stress.

Plant water deficits are known to reduce growth rates and to alter the metabolism of certain compounds (e.g. proline) of most plant species in similar ways (10). Rapid changes in polyribosome levels are also known to occur in mosses (3, 6). In other studies with higher plants, a number of workers have shown that polyribosome levels (4, 12, 13) and protein synthesis (7, 12, 13) are reduced following stress; but in most cases, specific efforts were not made to determine how quickly these changes could occur. The study reported here was initiated to determine if polyribosome levels of several higher plants could be rapidly and consistently reduced by stress and to gain a better understanding of the possible relationships existing among polyribosome changes, cellular water status, and growth rates.

MATERIALS AND METHODS

Water status measurements of tissues were determined using the Campbell J-14 press (Campbell Scientific, Logan, Utah, 84321). In these determinations, the end point of the press value was considered to be the point at which water was expelled from uncut surfaces. Since the apparatus has become available only recently, a number of experiments were conducted to insure that any value obtained by the procedure could be correlated to another measurable parameter of water status, relative water content. For this comparison, plants of the type used for polyribosome extractions were exposed (in light) to nutrient solutions containing added NaCl equivalent to 0, -2, -4, and -8 bars osmotic potential. Press values for six separate tissues and fresh weights (FW) of four replicate populations of plants were measured after 1 hr. For determination of RWC, the weighted tissues were allowed to imbibe water from wet Kimtowels (Kimberly-Clark) for 3 hr; they were next blotted, reweighed to determine saturated weights (SW), and then dried at 70 C overnight for dry weight (DW) evaluations. RWC was calculated as

\[
RWC = \frac{FW - DW}{SW - DW} \times 100
\]

When water status measurements were made in conjunction with polyribosome studies, press values were obtained within 3 min of the time of harvest for polyribosome extraction.

Polyribosomes were isolated from cotyledons of 3- and 4-day-old pumpkin, *Cucurbita pepo* L. var. Small sugar, and 1-week-old safflower, *Carthamus tinctorius* L.; leaves of 5-day-old wheat, *Triticum vulgare* L. var. INIA66R, and 8-day-old barley, *Hordeum vulgare* L. var. Hi Proly; shoots of 6- to 8-day-old peas, *Pisum sativum* L. var. Alaska; and roots of 3-day-old pumpkin and cotton, *Gossypium barbadense* L. var. P-19 seedlings. Pumpkin seedlings were grown in a 12-hr light 12-hr dark cycle at 36 C (800 ft-c, Sylvania Gro Lux) and cotton seeds were germinated in darkness at 30 C. All other seeds were germinated and grown in vermiculite at 26 C in a 13-hr light/11-hr dark cycle (2000 ft-c, fluorescent plus incandescent). Upon removal from vermiculite, roots were washed and seedlings transferred to aerated modified Hoagland solution (8) for temp-

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2 Abbreviation: RWC: relative water content.
perature equilibration 2 to 4 hr prior to treatment. Plants were stressed by: (a) addition of NaCl to the nutrient solution; (b) blotting the roots and maintaining seedlings in the light at 26 C (36 C for pumpkin); or (c) cutting seedlings at ground level and holding at 26 C in the light. Following the stress treatment, tissues were chilled immediately using liquid N₂ and stored at −45 C. Usually, a portion of these were extracted for ribosomes on the day of treatment; but since there were several replications, many of the samples were analyzed the next day. Comparative studies showed that ribosome percentages were not affected by this storage.

The buffers used for homogenization (buffer 1: 0.25 M sucrose, 0.2 M tris-HCl, 60 mM KCl, 30 mM MgCl₂, pH 8.5) and gradient preparation (buffer 2: 40 mM tris-HCl, 20 mM KCl, 10 mM MgCl₂, pH 8.5) were those of Davies and Larkins (5).

Approximately 1 g of tissue was homogenized in an ice-cold mortar and pestle with a total of 10 ml buffer 1 containing 3% (v/v) Triton X-100 (Rohm and Haas). The homogenate was centrifuged at 30,000g (max) in a Sorval SS-34 rotor for 10 min at 0 to 4 C and the ribosomes present in the supernatant were fractionated in various ways. Usually, they were partially purified by layering the 30,000g supernatant over a 3-mL cushion of 1 M sucrose in buffer 2 and centrifuging at 200,000g (100,000g in early experiments) for 2 hr in a Beckman Ti-50 rotor held at 2 to 4 C. The pellet was resuspended in 1 to 3 ml buffer 1; and 0.1-ml aliquots were layered onto freshly prepared discontinuous gradients (0.6, 1.2, 1.2, and 1.5 ml of 1.75, 1.31, 0.88, and 0.44 M sucrose in buffer 2, respectively) which were centrifuged at 175,000g in a Spincos SW 50 rotor at 2 to 4 C for 60 to 75 min. In some cases, 0.1 ml of the 30,000g supernatant was layered directly onto freshly prepared discontinuous gradients (0.6, 1.2, 1.8, and 1.0 ml, respectively, of the solutions used above) and centrifuged for 105 min at 175,000g in a Spincos SW 50 rotor. In early studies with pumpkin cotyledons, ribosomes in the 30,000g supernatant were precipitated on ice by rapidly adding 1 M acetic acid to pH 5.1, centrifuged quickly, and resuspended in 4 ml buffer 1. Subsequent centrifugation was the same as that used for partially purified ribosomes. Following centrifugation through a discontinuous gradient, solutions were floated from the tubes using 2 M sucrose and monitored at 254 nm with an ISCO model UA-2 UV analyzer.

RESULTS AND DISCUSSION

Effect of Salinity and Desiccation on the Water Status of Various Plants. As part of the test for determining the suitability of the Campbell J-14 press for water status determinations, a number of plants were exposed for 1 hr to nutrient solutions containing added NaCl equivalent to 0, −2, −4, and −8 bars osmotic potential and values obtained with the press were compared with plant RWC and solution water potential values. In all species, press results were found to be inversely related both to RWC determinations (Fig. 1A) and to solution water potentials (Fig. 1B). On occasion, the Campbell press could detect little response to the imposed stress conditions as can be seen in one of the two experiments with Alaska peas (curve b' in Fig. 1B); the low press values in this experiment occurred when RWC values were high (encircled points, Fig. 1A) indicating further that the Campbell press reflects the degree of water stress experienced by these green tissues. The sensitivity and ease of operation of the press were clear and superior to those of RWC determinations which often showed little change despite the fact that some tissues were clearly wilted. In barley leaves, e.g., a RWC decrease of only 2.5% resulted in an easily detectable 4-atm change in press results. Since the press values are known to correlate well with pressure bomb determinations of water potential (see as green, light-green tissues are used (G. Campbell, Washington State University, personal communication), the apparatus was used in all further determinations of water status.

In order to determine how quickly the water status of plant shoots changes following exposure of roots to saline media, 3-day-old pumpkin seedlings were placed in nutrient solutions at osmotic potentials of −2, −4, and −8 bars. In all treatments, rapid increases in press values of the cotyledons were noted between 5 to 20 min following exposure to salt, and by 30 min they appeared to equilibrate (Fig. 2A). When pumpkin seedlings were desiccated by blotting the roots and allowing them to dry, press values again increased rapidly (Fig. 2B). The high variance of measurements obtained after 20 min of stress suggests that the seedling populations were heterogeneous in response to water stress.

Polyribosomes from Control and Stressed Tissues of Various Plants. Representative profiles of polyribosomes obtained from the two plants most extensively used in this study, peas and pumpkins, are shown in Figure 3, A and C, respectively. In both
cases, a monoribosome peak, m, was found near the top of the gradient and the material in the polyribosome region, p, shifted to the monoribosome area when treated with ribonuclease (Fig. 3, B and D). In this study, distinctions were not made between mono- or polyribosomes from cytoplasmic, chloroplastic, or mitochondrial sources, and polyribosome percentage is calculated as \[ \frac{p}{(m+p)} \times 100. \]

Direct comparisons showed that polyribosome percentages obtained following resuspension of material pelleted through a sucrose cushion at 200,000g for 2 hr (the procedure used most extensively) were virtually identical to those obtained from clarified homogenates or from acetic-acid-precipitated ribosome fractions. Although the use of clarified homogenates was rapid, the presence of UV-light-absorbing material near the meniscus sometimes obscured the monoribosome peak. The acetic acid method yielded excellent preparations but only from pumpkin cotyledons. When material was sedimented through a cushion at 100,000g, the trends obtained following stress were the same, but the polyribosome percentages were exceptionally high. This is now known to result from partial sedimentation of the monoribosomes (11).

To test the hypothesis that water deficits would quickly and consistently reduce polyribosome percentages, a variety of plants were stressed rapidly, and attempts were made to monitor changes in their polyribosome content. Initially, roots of 3-day-old pumpkin seedlings were immersed in nutrient solutions at an osmotic potential of \(-4\) bars and cotyledons were harvested after 10, 20, or 30 min. In these studies, ribosomes were precipitated using acetic acid. The data of Figure 4 (top) as well as those of six additional experiments showed that exposure of roots to NaCl for 10 min effects a slight, but noticeable, reduction in polyribosome percentages of cotyledon extracts. The data of Figure 4 (bottom) show that reductions are more pronounced after 30 min; these reductions in polyribosome percentages are readily reversed when seedlings are transferred back into nutrient solutions that contained no NaCl. These results as well as those from two other studies showed that polyribosome percentages following a 30-min recovery period were slightly higher than those found in the control tissues.

Since well defined differences were found in pumpkin cotyledons after a 20- to 30-min stress, this period was routinely used to determine effects of water deficits on polyribosome levels of a number of other plants. Despite many attempts, we have been unable to obtain polyribosomes from leaves of mung beans, black-eyed peas, and red kidney beans, and from cotyledons of radishes. Representative results from studies using tissues which did yield polyribosomes are presented in Figure 5 and Table 1. These and all other data obtained from young, green tissues showed that salt or water stress for 20 or 30 min consistently effected a reduction in polyribosome content.

Although all studies showed that polyribosome percentages of shoot tissues were reduced by stress, polyribosome levels in roots were not affected by a 30-min exposure to an osmotic potential of \(-6\) bars (Table 1). These response differences be-

**Figure 3.** Sedimentation profiles of ribosomes from un-stressed pea and pumpkin tissues. Homogenates from 6-day-old pea shoots and 3-day-old pumpkin cotyledons were layered over 3.5 ml of 1 M sucrose and centrifuged at 200,000g for 2 hr. The pelleted ribosomes were resuspended and a portion of the ribosome suspension was mixed with an equal volume of pancreatic ribonuclease to yield a final ribonuclease concentration of 1 \(\mu\)g/ml. Sedimentation profiles of pea and pumpkin ribosomes were obtained after incubation of the undiluted, control ribosome suspensions (A and C, respectively) and the diluted ribonuclease-treated ribosome suspensions (B and D, respectively) at 20 C for 2 min.

**Figure 4.** Rapid and reversible reductions in polyribosome contents of pumpkin cotyledons from seedlings exposed to salinized nutrient solutions. Polyribosomes were precipitated with 1 M acetic acid as outlined in "Materials and Methods." Areas used for determinations of polyribosome contents and the resulting percentages are indicated. Top row: seedlings were transferred from control solution to a nutrient solution with an osmotic potential of \(-4\) bars for 10 and 20 min before cotyledons were harvested. Bottom row: in a separate experiment, seedlings were stressed for 30 min at an osmotic potential of \(-4\) bars (middle figure) or stressed for 30 min and later returned to control solution so that all treatments were harvested at the same time.

**Figure 5.** Polyribosome profiles from tissues of wheat (A), safflower (B), pea (C), and barley (D). Wheat and safflower seedlings were stressed by transferring from a control nutrient solution to one with a \(-4\) bar osmotic potential and incubating for 30 min; pea shoots were cut above the soil line and dried for 30 min. Polyribosomes were extracted as described in "Materials and Methods" and purified by centrifugation at 100,000g (safflower) or 200,000g (wheat, pea, and barley) for 2 hr. Polyribosome percentages, the areas used for their determination, and the stress values (when available) are indicated for each profile.
Table 1. Polyribosome Content of Control and Stressed Tissues from Roots and Shoots of Various Plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Treatment and Press Values</th>
<th>Mean % Polyribosomes</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Pea shoots</td>
<td>Cut, dried 20 min</td>
<td>63.6</td>
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<tr>
<td></td>
<td>Control = 7.1</td>
<td></td>
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<tr>
<td></td>
<td>Stress = 10.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut, dried 20 min</td>
<td>66.9</td>
</tr>
<tr>
<td></td>
<td>Control = 7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress = 11.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut, dried 30 min</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
<td>Control = 7.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress = 14.7</td>
<td></td>
</tr>
<tr>
<td>Barley leaves</td>
<td>Blotted roots dried 30 min</td>
<td>72.7</td>
</tr>
<tr>
<td></td>
<td>Control = 6.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress = 9.1</td>
<td></td>
</tr>
<tr>
<td>Pumpkin cotyledons</td>
<td>Blotted roots, dried 30 min</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td>Control = 7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stress = 10.7</td>
<td></td>
</tr>
<tr>
<td>Pumpkin roots</td>
<td>-6 bars (NaCl) 30 min</td>
<td>42.4</td>
</tr>
<tr>
<td>Cotton roots</td>
<td>-6 bars (NaCl) 30 min</td>
<td>31.4</td>
</tr>
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* Significant at 0.05 level, t-test.
** Significant at 0.01 level, t-test.

between shoot and root tissues are probably not due specifically to changes occurring in the chloroplasts since stress is known to reduce polyribosome levels in barley aleurone (2) and root tip cells (13), both of which have few, if any, chloroplastic ribosomes. Electron micrographs obtained by Armstrong and Jones (2) and in our own laboratory of pumpkin cotyledons and pea leaves (Matsuda and Rhodes, unpublished results) suggest that the concentration of 80S cytoplasmic ribosomes found along the rough endoplasmic reticulum is reduced by water loss. Differences between our data obtained with whole roots (Table I) and those obtained with root tips (9, 13) may be due to differences in the amount of membrane-associated ribosomes.

Polysome Content, Tissue Water Status, and Growth Rate. The occurrence of reproducible stress-induced reductions in polyribosome levels in various plants suggested that a proportionality may exist between polyribosome and water status reductions in shoot tissues. Such a relationship has been found for 3-day-old pumpkin and 8-day-old Alaska pea seedlings. Seedlings were transferred to nutrient solutions containing various concentrations of NaCl. After 30 min, press measurements were taken and samples harvested for polyribosome extraction. Values from a number of experiments together with the respective regression analyses are presented in Figure 6A. These data show a linear rate of reduction in polyribosome content with increasing levels of stress such that an increase of 1 atm in press value is accompanied by a 1.8% and 3.3% reduction in the polyribosome content of pumpkin cotyledons and Alaska pea shoots, respectively.

Differences in response of polyribosome percentage to reduced water levels in the two plant species prompted us to attempt a comparison of the growth rates of 3-day-old pumpkin and 7-day-old Alaska pea seedlings under water stress conditions. Because of the nature of the plants, comparisons of growth rates using the same type of shoot tissues could not be made. In addition, it was not feasible to measure detectable growth changes in the 30-min interval used to induce stress for polysome studies. Consequently, seedlings were transferred to nutrient solutions containing various amounts of NaCl, and pumpkin hypocotyl and Alaska pea shoot lengths were measured after 24 hr. Growth rates for these tissues and the press values of pumpkin cotyledons and Alaska pea leaves from a number of experiments are shown in Figure 6B. As these data indicate, the
growth rate of pumpkin and pea seedlings is reduced by 0.32 and 0.65 cm/day, respectively, for each 1-atm increase in the press value.

Interestingly, calculations based on data from Figure 6, A and B show that while polyribosome levels and growth of pumpkin are less affected by stress than Alaska peas, a striking similarity exists between polyribosome and growth changes: a 1% reduction in polyribosome percentage is associated with a 0.18 and 0.2 cm/day reduction in pumpkin and pea seedling growth rates, respectively. Although caution must be used in interpreting these results, it is of particular interest to compare them with data presented by Brandle et al. (4) and by Hsiao (9). Brandle and co-workers found that large reductions in water potential were required to produce detectable changes in polyribosome levels of black locust, a drought-hardy species. In contrast, Hsiao obtained large polyribosome percentage changes for relatively low water potential changes in corn, which is presumably a more sensitive species. With the added data from Figure 6, A and B, one might now postulate that polyribosome levels of drought-hardy species will decrease less for a unit loss of water potential than drought-sensitive species, and changes in polyribosome levels may provide a basis for predicting stress effects on growth. This hypothesis can be tested by further studies with a variety of plants at different stages of growth.

LITERATURE CITED