TRANSLOCATION OF ^{14}C-LABELED COMPOUNDS IN WHEAT AND BEAN PLANTS AS AFFECTED BY WATER STRESS[1]

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Sucrose-^{14}C was applied to the primary leaf of fully turgid wheat (two cultivars) or bean plants. Distribution of the radioactivity was determined after an elapse of up to 7 or 9 days. Watered plants were compared with plants not receiving water after the isotope was applied. Distribution patterns proved to be similar in droughted plants and controls; the largest amount of radioactivity was in the newly developing leaf of the wheat plant or in new leaves and lower stem of the bean plant. Water stress caused a significant shift from ^{14}C-labeled ethanol-insoluble compounds to ethanol-soluble ones. The shift was especially prominent in leaves but was found also in other plant parts. Results confirm those of other studies where proteins and nucleic acids were found to decline markedly when plants were subjected to drought.

When radioactive 2,4-D (1), sucrose (2), or phosphate (3) is applied to leaves of water-stressed plants, translocation of the radioactivity is greatly reduced. Interpretation of experiments of this kind are complicated by the fact that uptake and transport within the leaf must occur before there is transport to some other plant part. It cannot necessarily be assumed that uptake and transport of such materials within water-stressed leaves is the same as within fully turgid ones.

Therefore experiments were designed whereby ^{14}C-labeled sucrose was applied to fully turgid plants which were subsequently subjected to water stress and redistribution was determined. An attempt was made to correlate translocation with drought hardiness. Two wheat cultivars (hardy and nonhardy) were compared with a drought-susceptible bean.

METHODS AND MATERIALS

Two cultivars of hard red winter wheat *Triticum aestivum* L. cv. Ponca (drought-susceptible) and cv. KanKing (drought-hardy) and bean (*Phaseolus vulgaris* L. cv. Stringless Green Pod) were grown in sterilized vermiculite in clay pots. The plants were watered weekly with half-strength Hoagland's nutrient solution and otherwise as needed with tap water. All plants were grown under Gro-lux fluorescent lights (11,000 lux) for 12 hr daily at a temperature of 20 C for wheat, and 25 C during the day and 20 C at night for bean.

Plants were used at about 3 weeks of age (before emergence of the third leaf of wheat or with fully expanded primary leaves and 2-3 cm trifoliolate leaf of the bean plants). Two μl containing 2.45 μg of sucrose labeled uniformly with ^{14}C (1 μci) containing 0.5 mg/l of wetting agent (Multifilm X-77) was applied 5 mm from the tip of the primary wheat leaf or in the middle of one side of a bean primary leaf.

After the designated time period plants were harvested, cut into parts, frozen, and lyophilized. Wheat plants (six per treatment) were subdivided into roots, crowns (1 cm above roots), sheaths (from crown to attachment of primary leaf), area of primary leaf where isotope was applied, tip and base of primary leaf, and other leaves. Bean plants (three per treatment) were subdivided into roots, area of primary leaf where isotope was applied, each primary leaf, stem below primary leaves, stem and apex above primary leaves. Relative leaf water content was also determined on two other plants of each series (4).

Each part was weighed after lyophilization and extracted with 5 ml boiling 80%

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ethanol for 15 min. One-half milliliter (0.5 ml) of this was placed in 15 ml scintillation counting liquid made up of xylene: *p*-dioxane: ethanol (5:5:3) containing 80 g/l naphthalene, 5 g/l 2,5-diphenyloxazole and 50 mg/l, 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene. Radioactivity was determined on a Nuclear-Chicago Scintillation Spectrometer and corrected for quenching. Insoluble radioactivity was determined either by a Geiger counter (wheat plants) or homogenization and suspension in scintillation counting liquid (bean plants).

Specimens of each treatment were also lyophilized intact and pressed against x-ray film for radioautograms.

RESULTS

Wheat plants

Radioautograms of intact plants following the application of ^{14}C-sucrose showed that the roots, leaf sheaths, crowns, and developing leaf received substantial amounts of the translocated ^{14}C. The second leaf which had completed growth at the time of application contained very little radioactivity. The new leaf that emerged on control plants during the course of the experiment contained substantial amounts of ^{14}C. This leaf did not emerge on water-stressed plants.

Plants comparable to the ones used for radioautography were cut into various parts and radioactivity quantitatively determined after each part was extracted with 80% ethanol (Table 1). Statistical analysis indicated no significant differences between wet and dry treatments for roots, crowns, or sheaths. In all these cases there were significant decreases (at the 1% level) in radioactivity between days 1 and 5. There were significant differences (at the 1% level) between wet and dry treatments in the leaves, although the changes in the turgid plants with time were not significant. The notable result of these experiments was the elevated radioactivity in the leaves which were subjected to water stress.

Determinations of the radioactivity left after alcohol extraction were much less accurate (data not shown), but it appeared that, after the first day, the total radioactivity in a certain location did not change substantially; the observed changes in radioactivity were probably due to interconversion of soluble and insoluble ^{14}C compounds within that organ.

Bean plants

Similar experiments were performed on bean plants except that, in these, the spot on the primary leaf where the sucrose-^{14}C had been applied was removed, with a cork borer, one day after application. Translocation patterns also were followed by making radioautograms of representative intact plants (Figures 1 and 2).

Very little radioactivity was found in the opposite primary leaf (data not shown). Of the total radioactivity transported out of the leaf, over 50% was found in the stem section below the pri-

TABLE 1. *Radioactivity found in the 80% ethanol-soluble extract in various parts of the wheat plant after treatment of the primary leaf with ^{14}C-labeled sucrose on day zero.*[a]

Cultivar	Treatment	Relative water content (%)	Disintegrations per minute			
			Roots	Crowns	Sheaths	Leaves[b]
KanKing	1 day wet	91	5,610	1,910	4,922	8,180
	5 days wet	88	524	345	1,089	10,054
	5 days dry	79	870	430	1,865	7,691
	9 days wet	91	878	486	1,042	6,722
	9 days dry	12	1,017	868	2,575	25,220
		LSD (5%)	703	238	1,080	4,020
Ponca	1 day wet	94	6,896	2,410	6,640	8,624
	5 days wet	92	206	396	1,154	4,250
	5 days dry	55	1,644	535	2,670	11,701
	9 days wet	90	293	322	938	5,722
	9 days dry	10	1,421	705	2,030	13,447
		LSD (5%)	288	235	915	1,622

[a]Wet plants, watered daily as required; dry plants, not watered after day zero. Data = averages of 6 replicate plants. A one-way analysis of variance showed significant treatment differences; the 5 L.S.D. was used to compare treatments within each plant part.
[b]Excluding primary leaf.

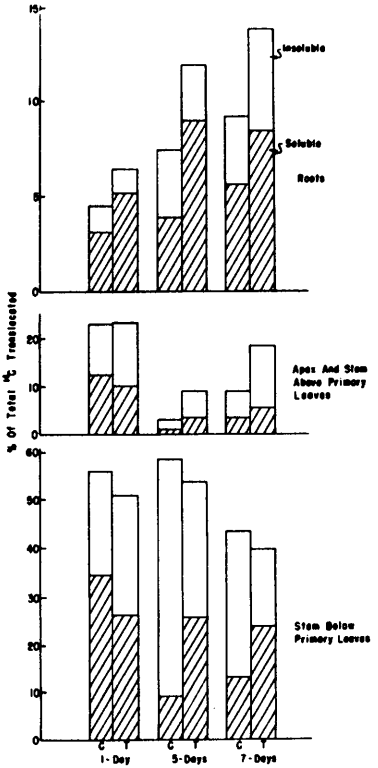

FIGURE 1. Percentage of the total amount of radioactivity translocated from the primary leaf, the site of application of ^{14}C-sucrose, to various parts of the bean plant at 1, 5 or 7 days following application. (C = watered controls; T = droughted.)

mary leaves (Table 2). The roots of the bean plants contained much less of the transported ^{14}C after one day than did the wheat plants. Between 1 and 5 days there were significant decreases in ^{14}C in the apex-upper stem fraction. In nearly all organs there were also significantly greater amounts (at the 1% level) of soluble ^{14}C in the 7-day droughted plants than in watered plants. The increase in total ^{14}C (both ethanol-soluble and -insoluble) was especially evident in both apex-upper stem,

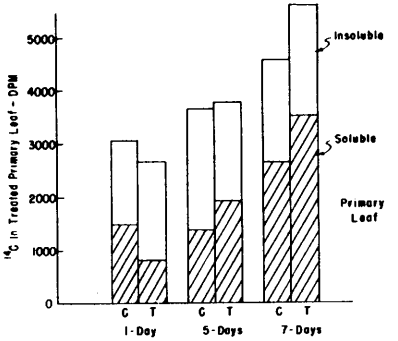

FIGURE 2. Radioactivity, in disintegrations per minute, of the primary leaf to which the isotope was applied, excluding the application spot. (C = watered controls; T = droughted.)

lower stem and root portions. Some of this difference may be due to a reduced metabolism in the droughted plants.

While it is not possible to judge whether the 7-day dry plants used would have recovered if watered after the 7 days, comparable plants showed about 50% mortality.

DISCUSSION

A primary sink in the wheat plant is the newly developing shoot region, especially the leaf that is rapidly expanding, although substantial amounts were found in all plant parts except the second leaf which had finished expansion. A fairly substantial amount of the ^{14}C label which translocated out of the primary leaf of the bean plant remained in the lower portion of the stem. Plaut and Reinhold (5) also found substantial amounts of ^{14}C label in the first internode and hypocotyl regions when ^{14}C-sucrose was applied although their experiments were restricted to shorter time periods (15 min to 15 hr).

Internal water stress began to be evident in the bean plants after 3 days without water whereas in the wheat plants the relative water content did not start declining until after 4 days without water.

Water stress caused a striking increase in ethanol-soluble labeled materials in both wheat and bean plants. Previous work (1, 5) showed that translocation rates in water-

TABLE 2. *Radioactivity found in 80% ethanol-soluble and -insoluble fractions of various parts of bean plants after treatment of the primary leaf with ^{14}C-labeled sucrose on day zero.*[a]

Treatment	Relative water content (%)	Disintegrations per minute									
		Treated leaf		Petioles		Apex and stem above primary leaves		Stem below primary leaves		Roots	
		Sol[b]	Insol[c]	Sol	Insol	Sol	Insol	Sol	Insol	Sol	Insol
1 day wet	89	500	517	2024	1262	3346	2839	8830	6312	858	355
1 day dry	89	523	1189	1550	2287	3082	3695	7823	7449	1563	362
5 day wet	92	1469	2433	1297	4470	343	590	2841	5973	1233	1168
5 day dry	69	1315	1280	2533	1156	969	1419	6789	7315	2381	754
7 day wet	91	1400	1020	1122	1790	529	866	1977	4627	860	556
7 day dry	46	2549	1515	3488	2571	1937	4690	8294	5738	3095	1930
Standard error of mean		754	637	890	1218	634	1655	2943	4090	900	608

[a]Wet plants, watered as required; dry plants, not watered after day zero. Data = average of 3 plants per treatment.
[b]80% ethanol-soluble fraction.
[c]80% ethanol-insoluble fraction.

stressed bean plants were clearly reduced, although the total amount of ethanol-soluble materials transported may not be reduced proportionately because of the elevated levels of transportable compounds present in the leaves (6).

Total amounts of ^{14}C in the treated leaf of both wet and dry plants were higher after 5 and 7 days than in the one day sample. This would imply a transport from some other part of the plant, although it is not readily apparent just what the source was. The increased levels of ethanol-soluble ^{14}C observed in the water-stressed tissues fits with the known behavior of macromolecules such as proteins and nucleic acids which have been found to be rapidly degraded during water stress (7).

It is difficult to correlate these findings with drought hardiness. The behavior of the two wheat cultivars was very similar even though cv. KanKing is known to be much more drought hardy. Both wheat cultivars and the bean plants had elevated levels of ethanol-soluble ^{14}C as a consequence of severe water stress. Probably a considerable amount of this radioactivity was in the form of sugars and amino acids. Both groups of compounds increase in plants subjected to water stress (6, 8) and sugars have often been implicated in drought hardiness.

These results indicate that overall shifts between ethanol-soluble and -insoluble ^{14}C compounds or transport during stress are probably not the factors responsible for drought hardiness. The fate of nucleic acids and proteins (7) and the ability to resynthesize critical components is probably of primary importance.

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REFERENCES

1. E. BASLER, G. W. TODD, and R. E. MEYER, Plant Physiol. 36: 573-576 (1961).
2. Z. PLAUT and L. REINHOLD, Aust. J. Biol. Sci. 20: 297-307 (1967).
3. A. M. WILSON and C. M. MCKELL, Plant Physiol. 36: 762-765 (1961).
4. G. W. TODD, F. W. INGRAM, and C. A. STUTTE, Proc. Okla. Acad. Sci. 42: 55-60 (1962).
5. Z. PLAUT and L. REINHOLD, Aust. J. Biol. Sci. 18: 1143-1155 (1965).
6. D. G. ROUTLEY, Crop Sci. 6: 358-361 (1966).
7. G. W. TODD and E. BASLER, Phyton (Argentina) 22: 79-85 (1965).
8. I. M. VASSILIEV and M. G. VASSILIEV, Plant Physiol. 11: 115-125 (1936).