## Study of the Translocation of the Methyl Group in *Triticum aestivum* (Gramineae)

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#### Abstract

The incorporation and the translocation of the methyl group in winter wheat was investigated during the early germination period. Absorption of S-methyl-L-methionine- $^{14}$ CH<sub>3</sub>, or its methyl group, by wheat roots was ascertained and uptake of the tagged methyl group was observed in the seed parts for 1, 2, and 3 days' germination and in the root and shoot by the third day of germination using liquid scintillation studies. Autoradiographic analysis of labeled tissue suggests translocation of the methyl group to meristematic regions and to the walls of the elongating cells in the region of cell elongation in root tissue.

# Introduction

Transmethylation, the transfer of a methyl group in toto, is by far the major pathway for the formation of biologically important methylated compounds. Methionine, the key compound in this transmission of the methyl group, may be formed by the methylation of homocysteine from either S-adenosyl-L-methionine or S-methyl-Lmethionine. This homocysteine methyltransferase (EC 2.1.1.10) has been demonstrated in Aerobacter aerogenes (9), Escherichia coli K-12 (10), Saccharomyces cerevisiae (11), jack bean meal (1), and in extracts of the seeds of a number of higher plants, including pea, lima bean, and corn (14). In these seed extracts it was demonstrated that S-methyl-L-methionine is a more efficient methyl donor than S-adenosyl-L-methionine. In addition, these studies are significant because they are the first demonstration of S-methyl-L-methionine:homocysteine methyltransferase activity in a system which possesses the substrate in detectable amounts.

A second direct transmethylation reaction, methionine S-methyltransferase (EC 2.1.1.12), has been studied in jack bean roots (4), pea, pumpkin, kohlrabi and sesbania seedlings (12). A recent comparative study of homocysteine methyl transferase and methionine Smethyl transferase systems in winter wheat has shown these two enzyme systems to be active in the dry seed, soaked seed, and the parts of the germinating wheat seedling and to display peaks at various days over the fourteen day germination study (2).

All of the studies cited above suggest general distribution of Smethyl-L-methionine in higher plants and thus far the key role described for S-methyl-L-methionine is that of a methyl donor in its conversion to methionine. It is the contention of several researchers that S-methyl-L-methionine serves as a methyl donor for compounds other than homocysteine. Sato, Bonner and workers analyzed the fate of sulfur and methyl-labeled S-methyl-L-methionine supplied *in vivo* to oat seedling sections (8). These workers found that the total recovery of the methyl group of methyl-labeled S-methionine in several products was less than the recovery of the sulfur-labeled compound and suggested that the methyl groups of S-methyl-L-methionine were used in some reaction in which the sulfur does not participate. The recent comparison of homocysteine methyltransferase and methionine S-methyltransferase systems in winter wheat has suggested that peak activities were related to the seedlings' requirements for methyl groups in the formation of various compounds (2). S-methyl-L-methionine could be transferring methyl groups to nucleic acid bases, histones, or pectic substances. There is much literature to support the methylation of these structures. The following study was undertaken to study the role of the methyl group donated by S-methyl-L-methionine in winter wheat.

## Materials and Methods

All common laboratory chemicals used were reagent grade commercial products. Labeled compound, L-methionine-(methyl-<sup>14</sup>C) sulfonium iodide, was purchased from International Chemical and Nuclear Corporation, Irvine, California.

The experimental plant used in this study, *Triticum aestivum*, ssp. *vulgare* (Vill., Host) MacKey variety Gaines/CI 13448 was stored in the seed state under vacuum at 4 C. After disinfection and soaking, seedlings were grown in a growth chamber for varying germination periods.

### Uptake of S-methyl-L-methionine- $^{14}CH_3$ by roots:

To establish absorption of S-methyl-L-methionine-<sup>14</sup>CH<sub>3</sub> by wheat roots an experiment such as that done by Sato (7) was performed. Entire plants and excised roots were incubated in a solution of S-methyl-L-methionine-<sup>14</sup>CH<sub>3</sub> (specific activity of this solution was  $4 \times 10^{-2} \,\mu \text{Ci}/\mu \text{M/ml}$ ). In order to monitor uptake by the plant tissue, aliquots of this solution were removed daily. This solution was placed in a Triton X-100 scintillation fluid and counted in a Beckman model LS-150 liquid scintillation spectrometer.

#### Detection of S-methyl-L-methionine- $^{14}CH_3$ in solubilized tissue:

Actual uptake of S-methyl-L-methionine- ${}^{14}CH_3$  was measured in solubilized tissue. Plants incubated in this labeled substance were rinsed well with distilled water, separated into seedling parts, layered with NCS tissue solubilizer and allowed to stand until solubilization was complete. Radioactivity was determined after adding Triton-containing scintillation fluid. To establish a difference between radioactive material absorbed and radioactive material remaining on the external parts of the seedling, both incubated unsolubilized and unincubated unsolubilized tissues were examined.

### Detection of S-methyl-L-methionine- $^{14}CH_3$ by autoradiograms:

Plants were incubated in S-methyl-L-methionine-<sup>14</sup>CH<sub>3</sub> in concentrations ranging from 0.1  $\mu$ Ci/ $\mu$ M to 18.0  $\mu$ Ci/ $\mu$ M for 5-120 minutes. After rinsing in distilled water, seedlings were fixed, dehydrated, infiltrated with paraffin, cut on the microtome at a thickness of 10 microns and placed on slides coated with gelatin-chrome alum (6). After deparaffination, the slides were ready for autoradiographic treatment and analysis. All photographic procedures were carried out in a darkroom under a safelight containing a Wratten series 2 filter. Prepared slides were dipped in Kodak NTB<sub>3</sub> liquid emulsion and stored in a light proof box until development. Following development and fixation, slides were stained in gallocyanin-chromalum (3, 13), rinsed, dehydrated and

In all previous described studies, tests were performed in duplicate and repeated tests were conducted in order to establish reproducibility.

### **Results and Discussion**

Table 1 shows a study of absorption of S-methyl-L-methionine- $^{14}$ CH<sub>3</sub> or its methyl group by wheat roots. At 24 hours the activity of the incubation solution for intact seedlings, previously germinated 1 or 2 days decreased about 33%. Decreases for periods longer than 24 hours were less, but were recorded at 62-67% at 96 hours. No loss of labeled material was noted in solutions lacking plant tissues. These data were interpreted to mean that S-methyl-L-methionine- $^{14}$ CH<sub>3</sub> or its methyl group, was being absorbed by the wheat roots.

 TABLE 1. Radioactivity of solution after incubation for intact seedlings and root fragments.

Incubation	Radioactivity (cpm) remaining after:						
Solutiona	24 hr	48 hr	72 hr	96 hr			
Control Day 1 <sup>b</sup>	2353	2410	2402	2500			
Intact seedling Day 1 <sup>b</sup>	1611	1227	1052	945			
Root fragments Day 2c	2221	2199	2223	2204			
Intact seedling Day 2c	1684	1332	996	839			
Root fragments	2122	2070	1868	1747			

a S-methyl-L-methionine-<sup>14</sup>CH<sub>3</sub> (specific activity of  $4 \times 10^{-2} \, \mu \text{Ci}/\mu \text{M/ml}$ ).

b 24-hour germination before incubation.

c 48-hour germination before incubation.

mounted.

The rapid loss in label of the incubation solution after 24 hours and declining change throughout the remaining incubation period suggest a metabolic function related to absorption of S-methyl-L-methionine. It was considered that a contrast of the incorporation of the methyl group with proteolytic activity during the period shown in Table 1 would provide some information about mobility of the methyl group in winter wheat. In 1971, Allamong (2) studied protease activity in germinating wheat seedlings over a 14-day germination period. This study showed a peak activity through day 4 in the root tissue. The comparison of Allamong's study of protease activity in germinating wheat seedlings with those of comparable growth in this study of the uptake of the methyl group indicates that mobility and uptake may be related over the 4-day span investigated. In addition, Allamong's work indicated a high level of S-methyl-L-methionine: homocysteine methyltransferase activity in the root for day 1, 2, and 3, followed by a drop to zero on the fourth day. These observations again suggest correlation of mobility and utilization of the S-methyl-L-methionine.

Results of the tissue solubilization of root, shoot, and seed parts of seedlings, 1 to 3 days' germination, after incubation in S-methyl-Lmethionine- $^{14}$ CH<sub>3</sub> are shown in Table 2. These data include a subtraction for background and a subtraction for residual radioactivity on the exterior of the seedling. These data indicate incorporation in the seed parts for day 1, 2, and 3; however, after subtraction, incorporation was indicated in the root and shoot only for day 3. Based on the previous study of protease activity in these tissues during early germination, S-methyl-L-methionine might not be expected until day 3 (2). This study supports this conclusion. Measured activity was quite high in the seed during day 1 and declines by day 2—at a time when protease activity indicates mobilization. By day 3, incorporation of the labeled S-methylmethionine was indicated in both root and shoot tissue. This pattern was repeated in duplicate studies and found to be a reproducible result.

Tissue	Radioactivity incorporated after germination day:						
	1	2	3				
Root	b	b	4608				
Shoot	b	b	143				
Seed	11699	465	4877				

TABLE 2. Radioactivity of incubated tissue.a

a Data represents individual seedling solubilized with NCS tissue solubilizer.

b \_\_\_\_\_ indicates net count (experimental-control) was equal to or less than zero. Control includes a factor for background and unsolubilized incubated tissue.

Autoradiograms were analyzed for the incorporation of the radioactive label and results are shown on Table 3. For each preparation, control slides of unincubated tissue, processed in exactly the same manner as the experimental tissue were included. The labeling of root meristematic tissue from days 1 through 4 was diffuse; label was observed throughout the tissue over both the cytoplasm and the nucleus and was not associated with any cell structure (Fig. 1). At times labeling was observed in the stele of the root as seen in cross sections. With a longer incubation time and an increase in unlabeled S-methyl-Lmethionine, day 1 and 2 root tissue showed an interesting pattern, which is susceptible of a greater range of interpretation. Silver grains were observed within the walls of cells in the region of cell elongation (Fig. 2). These grains were continuous from slide to slide. No such pattern was observed in control slides. Labeling of the aleurone layer was observed in day 1 and 2, an observation corresponding to the levels of S-methyl-L-methionine: homocysteine methyltransferase activity as described by Allamong (2). Labeling was not observed in shoot tissue during this study. No labeling of the nuclear components among the

cells of shoot, root and seed were observed. The contention that methyl groups are necessary for differential methylation of nucleic acids or histones in winter wheat during germination and during daily development was not evidenced over this short germination period.

Seedling part	Germination day	Incubation, minutes	Specific activity <sup>a</sup>	Tissue of incorporation <sup>b</sup>		
root	1	30	$18 \mu \text{Ci}/\mu \text{M/ml}$	meristematic		
root	1	60	$0.1 \mu \mathrm{Ci}/\mu \mathrm{M/ml}$	region of elongation, cell wall		
seed	1	60	$0.1 \mu Ci/\mu M/ml$	aleurone layer		
root	2	30	$18\mu Ci/\mu M/ml$	meristematic		
root	2	30	$18 \mu \text{Ci}/\mu \text{M/ml}$	stele		
root	2	60	$0.1 \mu \mathrm{Ci}/\mu \mathrm{M/ml}$	region of elongation, cell wall		
seed	2	60	$0.1 \mu \mathrm{Ci}/\mu \mathrm{M/ml}$	aleurone layer		
root	3	30	$18\mu Ci/\mu M/ml$	meristematic		
root	4	30	$18\mu Ci/\mu M/ml$	stele		
root	4	30	$18 \mu \mathrm{Ci}/\mu \mathrm{M/ml}$	meristematic		

TABLE 3.	Incorporation	01	label	into	specific	tissues	of	plant	parts.
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<sup>a</sup> Specific activity refers to the amounts of S-methyl-L-methionine-<sup>14</sup>CH<sub>3</sub> used in the incubation media.

b Tissue of incorporation determined by presence of silver grains in autoradiograms.

Observations of silver grains within the root tissue suggest that S-methyl-L-methionine is donating a methyl group to the formation of pectic substances within the cell wall. In 1957 Sato and others (7) presented evidence which for the first time showed that methyl esters of pectinic acid may be formed by a transmethylation reaction in higher plants. In this study, the methyl group of methionine was shown to be transferred intact to form pectinic acid methyl esters in radish plants. Further studies by this same group (8) indicated that methionine is not essential for methyl esterification since methionine sulfoxide and S-methyl-L-methionine as well as methionine act as methylating agents for pectin and protopectin in intact oat sections. Jakob and Tal (5) have recently described the incorporation of methyl groups from methionine into pectinic acid in germinating *Vicia faba*.

#### Conclusion

The results of this study indicate an absorption of S-methyl-Lmethionine- ${}^{14}CH_3$ , or its methyl group, by the roots of winter wheat seedlings. Absorption rate appears to be greatest within the first 24 hours of incubation, and appears to decrease on longer incubation. Liquid scintillation studies with solubilized tissues indicate incorporation in the seed parts for day 1, 2, and 3 and incorporation in the root and shoot for day 3. Autoradiographic analysis points to uptake in the seed's aleurone layer after 1 and 2 days' germination and in the root after 1, 2, 3, and 4 days' germination, with localization in the meristematic region and the walls of cells in the region of cell elongation.



FIGURE 1. Autoradiogram of root meristematic tissue (top). FIGURE 2. Autoradiogram of root tissue in the region of cell elongation (bottom).

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