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SHORT REPORTS

CARNITINE CONTENT OF GREENING BARLEY LEAVES

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; carnitine; greening leaves.

Abstract—The carnitine content of etiolated barley leaves doubled on exposure to light. After 24 hr exposure the carnitine content was the same as that of barley leaves grown in 18 hr days.

INTRODUCTION

The first report of the quaternary amine carnitine ($Me_3N^+CH_2CHOHCH_2COO^-$) in plants, wheat grain and alfalfa seedlings was by Fraenkel[1] using the unreliable *Tenebrio* bioassay. Panter and Mudd[2] used a spectrophotometric assay to detect carnitine in wheat grain, oat seedlings, cauliflower and avocado. McNeil and Thomas[3] employed the radioactive assay developed by Cederblad and Linstedt[4] to detect carnitine in germinating pea cotyledons. This communication reports the presence of carnitine in barley leaves.

RESULTS AND DISCUSSION

The amount of carnitine present in each extract is shown in Table 1. The carnitine content was lowest in the ungerminated seeds and the amount increased only slightly after germination for 2 days. The etiolated leaves grown in the dark for 7 days showed a further increase in carnitine content, and the amount of carnitine was more than doubled when these leaves were exposed to light for 24 hr. The carnitine content of these leaves was similar to that of 7-day-old green leaves of barley grown in alternating 18 hr light and 6 hr dark days. The amount of carnitine in the leaves did not increase after this period, although the chlorophyll content did increase. Thomas *et al.*[5] demonstrated that when carnitine was supplied to barley leaves greening in the light, the rate of chlorophyll production was enhanced. These workers also showed that the carnitine neither acted as a carbon source nor was it likely to act as a nitrogen source. Carnitine may play a role in the transport of acyl groups in the greening barley leaves, this role being similar to that proposed for mammalian systems[6]. Furthermore, carnitine, by accepting acyl groups from acyl CoA derivatives, may buffer the leaf cell acyl CoA/CoA pools in the manner proposed for animal cells[7].

Table 1. The carnitine content of barley grain, etiolated and green leaves of barley. Each result is the mean of four assays ± standard error

Specimen analysed	Carnitine (nmol/g fr.wt)	Chlorophyll (mg/g fr.wt)
Air-dry barley grain	0.83 ± 0.02	—
2-Day-old germinated barley	0.91 ± 0.05	—
7-Day-old etiolated leaves	1.6 ± 0.04	—
7-Day-old etiolated leaves exposed to light for 16 hr	2.2 ± 0.06	0.37 ± 0.03
7-Day-old etiolated leaves exposed to light for 24 hr	3.4 ± 0.11	0.49 ± 0.04
7-Day-old green leaves	3.5 ± 0.05	1.30 ± 0.04
14-Day-old green leaves	3.6 ± 0.09	1.52 ± 0.06

EXPERIMENTAL

Barley grain (*Hordeum vulgare* L. cv Zephyr 2M) was purchased from West Cumberland Farmers Ltd., Hexham, Northumberland. Before sowing, the seeds were immersed in running tap water for 8 hr. The imbibed seeds were planted in trays containing moist vermiculite and left in total darkness at 25° for 7 days. The trays of etiolated plants were then placed in continuous white light (6.4 W/m²) at 25° for 24 hr. The light source was 4 × 40 W Universal White fluorescent tubes. Some seed trays were sown with imbibed barley grain and maintained in 18 hr days at 25° (6.4 W/m²) for 7 or 14 days. Chlorophyll was determined by the method of ref.[8].

Carnitine assay. Barley leaves or germinated barley seedlings (10 g) were ground in a mortar with a pestle in 50 ml H_2O and a little acid-washed sand. After the initial grinding, 50 ml 1 M $HClO_4$ was added and the mixture ground again. The final mixture was filtered and the filtrate made 50% with respect to EtOH and neutralized with KOH. The filtrate was stood in ice for 1 hr and the ppt. removed by filtration. The filtrate was evaporated to dryness *in vacuo at* 35°. The residue was dissolved in H_2O to a final vol. of 4 ml. Dry grain was first ground in a mill before grinding the powder with H_2O and

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$HClO_4$ to extract the carnitine as before. To express the results on a fr. wt basis, 10 batches of air-dry grain (10 g each) were imbibed in 1% $CuSO_4$ for 24 hr. The $CuSO_4$ allowed imbibition but prevented germination. The grain was dried to constant wt at 110°. Fr. wt/air-dry wt ratios were found and the amount of carnitine/g fr. wt calculated. The assay of L-carnitine was according to the method of ref. [3], based on the principle of the technique used in ref. [4] employing acetyl CoA and carnitine-*O*-acetyltransferase (EC 2.3.1.7) (Sigma). The incubation medium was as described in ref. [3]. In this assay the percentage recovery of carnitine was 95–105% when known quantities of carnitine (2 nmol per g fr. wt) were added to extracts of plant material of known carnitine content. It was possible to determine as little as 10 pmol of L-carnitine with the assay procedure (accuracy ± 5%).

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PHOSPHATIDYLETHANOLAMINE IN WHEAT AND BARLEY LEAVES UNDER WATER STRESS

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Key Word Index—*Hordeum vulgare*; *Triticum aestivum*; Gramineae; barley; wheat; leaves; phosphatidylethanolamine; water stress.

Abstract—Phosphatidylethanolamine could not be detected in the leaves of less drought-tolerant varieties of wheat (S-308) and barley (BG-25) when the plants were subjected to water stress at tillering, ear emergence and grain filling stages. However, it remained unchanged in the more drought-tolerant varieties C-306 (wheat) and C-138 (barley). Upon release of stress by subsequent irrigation phosphatidylethanolamine reappeared in less drought-tolerant varieties.

INTRODUCTION

Water stress has inhibitory and sometimes devastating effects on almost all aspects of plant growth. Its adverse effects on carbohydrate [1], protein [2] and nucleic acid [3] metabolism are known, and recently [4] its effects on phospholipids have been studied. In a further study of the effect of water stress on leaf phospholipids some interesting observations were recorded concerning phosphatidylethanolamine.

RESULTS AND DISCUSSION

The phosphatidylethanolamine (PE) content of wheat varieties was in general higher than in the barley varieties. During growth, it decreased in both crops (Table 1). When irrigation was withheld for the creation of stress, PE disappeared in S-308 (wheat) and BG-25 (barley) while it remained more or less unchanged in the two other varieties. PE reappeared in S-308 and BG-25 upon release of stress by subsequent rewatering of plants stressed at tillering and ear emergence stages (Table 2).

PE is an important constituent of non-photosynthetic membranes such as mitochondria, endoplasmic reticulum, glyoxysomes and peroxisomes [5]. Changes in PE content have been reported during growth in pea and bean leaves [6] and wheat seedlings [7]. An inverse relationship between growth temperature and PE content has also been reported [8].

Under conditions of water stress, both in wheat