園学雑. (J. Japan. Soc. Hort. Sci.) 68 (6): 1155 1157. 1999.

SHORT COMMUNICATION

Effect of Water Stress on Oxalic Acid Concentrations in Spinach Leaves

Nobuo Sugiyama, Masashi Hayashi and Mitsunori Uehara
Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113

Summary

The changes in oxalic acid concentration in response to water stress were monitored to evaluate the contribution of oxalic acid to osmotic adjustment. Potassium and oxalic acid are the predominant solutes in spinach leaves. The addition of polyethylene glycol (PEG) 6000 to the nutrient solution significantly decreases osmotic potential at full turgor (π_{100}) in rapidly growing leaves. The decrease in π_{100} in response to PEG treatment can be accounted for by the accumulation of potassium, phosphate, nitrate, sugars, and amino acids, but not of oxalic acid. These results indicate that oxalic acid plays a minor role in osmotic adjustment, although potassium oxalate is a major osmoticum in spinach leaves.

Key Words: osmotic adjustment, oxalic acid, potassium, *Spinacia oleracea* L.

Introduction

Spinach leaves (*Spinacia oleracea* L.) accumulate large amounts of oxalic acid, which are considered a potential health risk (Libert and Franceschi, 1987). Therefore, much effort has gone into decreasing oxalic acid content in spinach leaves. Since oxalic acid exists mainly as soluble potassium (K^+) and sodium (Na^+) salts (Osmond, 1963), it has been widely accepted that oxalic acid may play a role in turgor generation and osmotic adjustment (Libert and Franceschi, 1987). Oxalic acid concentrations in spinach leaves increase in response to water stress (Tone and Uchiyama, 1989), but whether an increase in oxalic acid concentration on a fresh weight basis represents osmotic adjustment or is merely the result of tissue dehydration is unknown.

Watanabe et al. (1987) found that exposure to -0.46 MPa solutions containing polyethylene glycol (PEG) 1500 for 10 days causes the concentration of water-soluble oxalic acid to decrease in expanded leaves while the concentration in rapidly growing leaves increases. They did not measure the concentrations of solutes other than oxalic acid in either leaf type, but their results suggest that leaf position on the stem or leaf age determines the type of solutes that accumulate in response to decreasing external water potential, as found by Colmer et al. (1995). However, little attention has been paid to the kinds of solutes that accumulate in spinach leaves under moderate water stress conditions.

We evaluated the contribution of oxalic acid to osmotic adjustment in plants subjected to moderate water stress.

Received; March 10, 1999. Accepted; June 7, 1999.

Materials and Methods

Pre-germinated spinach seeds (*Spinacia oleracia* L. var. Okame) were sown in vermiculite and transferred to solution culture when the cotyledons were fully developed. Four plants per container were cultured with 8 liters of a nutrient solution. The composition of the standard nutrient solution has been described elsewhere (Okutani and Sugiyama, 1994). Solutions were renewed every 3 to 5 days. Two weeks after sowing, the containers were transferred to a growth room maintained at 25/20 ℃ (day/night) with a 12-h photoperiod and 65% relative humidity. Photon flux at plant level was 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

When leaf 5 reached 1 cm (day 0), the nutrient solutions in half the containers were changed to solutions containing 120 $g \cdot kg^{-1}$ PEG 6000: the remaining containers were refilled with standard nutrient solution. Leaves were numbered consecutively as they emerged; the younger leaves thus had larger numbers. Cotyledons were not numbered. On day 3, the PEG concentration in the nutrient solutions was increased to 200 $g \cdot kg^{-1}$ PEG 6000. Calculating from the osmotic potentials (π) of pure PEG solutions according to Michel and Kaufmann (1973), 120 and 200 $g \cdot kg^{-1}$ PEG 6000 solutions at 25℃ correspond to -0.42 and -0.93 MPa, respectively. On day 6, plants in four containers of each treatment were harvested 3 h into the light period and divided into individual leaves. For the determination of relative water content (RWC), leaf discs (0.785 cm^2) were excised from leaves 4 and 7 on day 6, weighed, and floated on water for 4 h at 25℃ under dim light, after which the leaf discs were reweighed and then dried at 70 ℃ for 48 h. RWC was calculated as follows:

$$RWC = (\text{fresh wt} - \text{dry wt})/(\text{turgid wt} - \text{dry wt}) \times 100$$

The remainder of the leaves was frozen in liquid nitrogen. After thawing, sap samples were collected using a hand press. The π values of sap samples from leaves 4 and 7 were measured using a dew point microvoltmeter (HR 33T; Wescor Inc., Logan, Utah, USA) calibrated with 0.5 mol • liter^{-1} NaCl solution (π = -2.34 MPa). Osmotic potentials at full turgor (π_{100}) were estimated as follows, according to Wilson et al. (1979):

$$\pi_{100} = \pi\ (RWC - B)/(100 - B)$$

Here, B, the bound water content, was assumed to be 10% according to Wilson et al. (1980). Osmotic adjustment was evaluated as the difference in π_{100} between control and PEG-treated plants.

Quantification of oxalic acid was made by high-performance liquid chromatography (Okutani and Sugiyama, 1994). Sugars and malic acid were quantified by enzymatic methods according to the manufacturers (Boehringer Mannheim, Mannheim, Germany). Total α-amino nitrogen was determined by the ninhidrin method. K^+ and Na^+ were quantified by flame photometry, and calcium (Ca^{2+}) and magnesium (Mg^{2+}) by atomic absorption spectrometry. NO_3^-, Cl^-, SO_4^{2-} and $H_2PO_4^-$ were quantified by ion chromatography.

Results

Interveinal chlorosis and scorching appeared in three of 16 plants immediately after they were transferred to nutrient solutions containing PEG 6000. In this study, plants with no visible symptoms of chlorosis or scorching were harvested. The fresh weight of leaf 4 reached a maximum on day 3, that of leaf 7 continued to increase until day 6 (data not shown).

The π_{100} in leaf 7 was higher in control plants than in PEG-treated plants, but there was no significant difference in π_{100} in leaf 4 (Table 1). In both leaves 4 and 7, K^+ and oxalic acid are the predominant solutes in spinach leaves and accounts for 64 to 71% of total solutes (Table 2). The concentrations of oxalic acid did not differ significantly between PEG-treated and control plants (Table 2). On the other hand, PEG-treatment caused significant increases in the concentrations of K^+, NO_3^-, $H_2PO_4^-$, amino acids, and sucrose in leaves (Table 2). Changes in K^+, NO_3^-, and $H_2PO_4^-$ accounted for 80% of the increase in total solutes.

Discussion

Osmotic adjustment, estimated as the difference in π_{100} between control and water-stressed plants, can occur by the following three processes: an accumulation of osmotically active solutes, a change in bound water content, and a change in the ratio of leaf turgid weight to dry weight (Wilson, 1980). Wilson et al. (1980) demonstrated that 70 to 80% of the osmotic adjustment in grass leaves can be accounted for by the accumulation of

Table 1. Relative water content and osmotic potentials (π_{100}) measured at full turgor (i. e. at 100 % RWC) in the leaves of spinach plants grown for 6 days in standard nutrient solutions (control) or in solutions containing polyethylene glycol 6000 (PEG). Values are given as means ± SD. The differences in π_{100} between control and PEG-treated plants indicate osmotic adjustment.

	Leaf 4		Leaf 7	
	Control	PEG	Control	PEG
RWC (%)	78.0 ± 1.6	73.3 ± 2.5	73.8 ± 1.5	67.0 ± 1.9
π_{100} (MPa)	-0.832 ± 0.050	-0.918 ± 0.070	-0.657 ± 0.037	-0.851 ± 0.013

Table 2. The concentration of different solutes in mol • liter^{-1} in the leaves of spinach plants grown for 6 days in standard nutrient solutions (control) or in solutions containing polyethylene glycol 6000 (PEG). Values are given as means ± SD. Concentrations are expressed on the basis of full turgor (i. e. at 100 % RWC).

Solutes	Leaf 4		Leaf 7	
	Control	PEG	Control	PEG
K^+	0.176 ± 0.013	0.209 ± 0.018	0.165 ± 0.007	0.213 ± 0.003
Mg^{2+}	0.025 ± 0.001	0.029 ± 0.002	0.019 ± 0.001	0.018 ± 0.001
Oxalic acid	0.143 ± 0.006	0.137 ± 0.009	0.101 ± 0.004	0.102 ± 0.003
Malic acid	0.014 ± 0.002	0.015 ± 0.003	0.004 ± 0.000	0.005 ± 0.001
Cl^-	0.020 ± 0.002	0.021 ± 0.003	0.015 ± 0.001	0.017 ± 0.001
NO_3^-	0.035 ± 0.004	0.051 ± 0.010	0.029 ± 0.004	0.039 ± 0.010
SO_4^{2-}	0.007 ± 0.001	0.009 ± 0.001	0.004 ± 0.001	0.006 ± 0.000
$H_2PO_4^-$	0.031 ± 0.009	0.045 ± 0.007	0.021 ± 0.005	0.042 ± 0.006
Amino acids	0.008 ± 0.001	0.013 ± 0.002	0.006 ± 0.001	0.013 ± 0.001
Sucrose	0.009 ± 0.001	0.012 ± 0.002	0.006 ± 0.001	0.009 ± 0.001
Hexoses	0.002 ± 0.000	0.002 ± 0.000	0.004 ± 0.000	0.006 ± 0.002
Sum	0.465 ± 0.017	0.544 ± 0.017	0.373 ± 0.004	0.470 ± 0.014

solutes; the remainder of the adjustment results from an increase in bound water content. Our study shows that a significant decrease in π_{100}, resulting from the active accumulation of solutes, occurs in rapidly growing leaves, but not in fully expanded ones, when spinach plants are exposed to PEG 6000 solutions for 6 days. A sensitive response of π_{100} to water stress in rapidly growing leaves is in agreement with the observations of Kameli and Lösel (1995) on durum wheat seedlings.

K^+ and oxalic acid are the predominant solutes in spinach leaves, but exposure to PEG 6000 increases the concentrations of K^+, NO_3^-, and $H_2PO_4^-$, but not that of oxalic acid, when the concentrations are expressed at full turgor. These results provide evidence for the primary role of K^+ and a minor role for oxalic acid in the decrease in π_{100} under water stress conditions. It is possible that anions other than oxalic acid accumulate along with K^+ to maintain electroneutrality in the cytoplasm when plants are subjected to water stress conditions. It has been reported that K^+ is the major contributor to osmotic adjustment in sorghum (Jones et al., 1980) and wheat (Morgan, 1992). Kameli and Lösel (1995) reported that glucose accounts for 86% of the increase in π_{100} under water stress conditions. Tone and Uchiyama (1989) found that oxalic acid concentrations (fresh weight basis) in spinach leaves increase in response to water stress. It seems that such an increase in oxalic acid concentration is merely the result of tissue dehydration because oxalic acid concentrations at full turgor do not change in response to water stress conditions.

Literature Cited

Colmer, T. D., E. Epstein and J. Dvorak. 1995. Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-torelant wheat x *Lophopyrum elongatum* (Host) A. Löve amphiploid. Plant Physiol. 108: 1715–1724.

Jones, M. M., C. B. Osmond and N. C. Turner. 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. Aust. J. Plant Physiol. 7: 193–205.

Kameli, A. and D. M. Lösel. 1995. Contribution of carbohydrates and other solutes to osmotic adjustment in wheat leaves under water stress. J. Plant Physiol. 145: 363–366.

Libert, B. and V. R. Franceschi. 1987. Oxalate in crop plants. J. Agric. Food Chem. 35: 926–938.

Michel, B. E. and M. R. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51: 914–916.

Morgan, J. M. 1992. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol. 19: 67–76.

Okutani, I. and N. Sugiyama. 1994. Relationship between oxalate concentration and leaf position in various spinach cultivars. HortScience 29: 1019–1021.

Osmond, B. 1963. Oxalates and ionic equilibria in Australian saltbushes (*Atriplex*). Nature 198: 503–504.

Tone, S. and Y. Uchiyama. 1989. Effects of cultivation on the oxalic acid content of spinach (*Spinacia oleracea* L.). 1. Effects of cultivar, gibberellic acid treatment, plant density, growing period and soil moisture. Bull. Yamaguchi Agric. Exp. Sta. 41: 32–39 (In Japanese with English summary).

Watanabe, Y., M. Yoneyama and N. Shimada. 1987. Effect of water stress treatment on sugars, vitamin C and oxalate contents of spinach. Japan. J. Soil Sci. Plant Nutr. 58: 427–432 (In Japanese).

Wilson, J. R., M. J. Fisher, E. D. Schulze, G. R. Dolby and M. M. Ludlow. 1979. Comparison between pressure-volume and dewpoint-hygrometry techniques for determining the water relations characteristics of grass and legume leaves. Oecologia 41: 77–88.

Wilson, J. R., M. M. Ludlow, M. J. Fisher and E. D. Schulze. 1980. Adaptation to water stress of the leaf water relations of four tropical forage species. Aust. J. Plant Physiol. 7: 207–220.

ホウレンソウの葉のシュウ酸濃度に及ぼす水分ストレスの影響

杉山信男・林　真史・植原光稔

東京大学大学院農学生命科学研究科　113–8657　東京都文京区弥生

摘　要

水分ストレスを与えた場合にホウレンソウの葉のシュウ酸濃度がどのように変化するかを調べ，浸透調節に対するシュウ酸の寄与を明らかにしようとした．ホウレンソウの葉の主要な浸透物質はシュウ酸カリウムであった．ポリエチレングリコール 6000 (PEG)を培養液に加えると，生長中の葉の飽和水分条件下での浸透ポテンシャル(π_{100})は有意に低下した．PEGの添加によって起こるπ_{100}の低下はカリウム，リン酸，硝酸，糖，アミノ酸濃度の上昇によるものであり，PEGを添加してもシュウ酸濃度は上昇しなかった．これらの結果は，ホウレンソウの葉における主要な浸透物質はシュウ酸カリウムであるが，シュウ酸は浸透調節にほとんど寄与していないことを示している．