Short Communication

A Simple Procedure to Overcome Polyethelene Glycol Toxicity on Whole Plants

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ABSTRACT

A procedure is described that can be used to minimize toxic effects of polyethylene glycol (PEG) to plants. The procedure is based on recycling nutrient solutions containing PEG-6000 through two plant cultures. Tomato plants grown in −0.3 megapascals PEG solutions used after two growth cycles exhibited minimal toxic effects. Long-term responses like dry matter production and chlorophyll content as well as short-term responses like CO_2 fixation rates and leaf conductance were severely inhibited by fresh PEG-6000 and only slightly reduced by recycled PEG-6000. Complete osmotic adjustment was obtained with tomatoes grown in recycled but not in fresh PEG solutions.

Polyethylene glycols are neutral polymers available in a wide range of mol wt and highly soluble in water. They have been used by numerous investigators as an osmotic agent either for whole plants or for plant tissues, cells, and organelles. Mc-Clendon and Blinks (15), showed as early as 1952 that PEG could be used as an osmoticum to replace sugar or salts for the preparation of red algal plastids. The use of PEG became a popular technique to reduce water potentials (ψ_w) of nutrient solutions to a predetermined constant water stress without its being taken up by plants. It was used satisfactorily by several investigators for various species (7, 9, 10, 18, 23), in which the response to PEG was attributed to a decrease in osmotic potential with no decisive toxic effects.

A very common problem using PEG was, however, its toxicity to plants. Such toxicity was sometimes ascribed to the presence of metallic ions like aluminum (11) or an ionic organic compound (3). Although such contaminants can be removed by ion exchange resins, gel filtration, or dialysis, toxicity was not always prevented (3, 12, 18, 21). Plant roots are probably not completely impermeable to PEG, and its toxicity might be due to uptake (2, 17, 21) and translocation throughout the plant (9, 11, 12). Some investigators (11, 12, 17) claim that it is transported without being broken down and that molecular size will determine the rate of its transport and location (8, 9, 12). It was suggested by Lawlor (12) that PEG blocks water pathway and thus induces desiccation. Toxic effects of PEG were attributed to inhibited phosphorus transport across the root to the xylem (2). Others (21, 24), however, claimed that PEG may be contaminated with phosphorus, so that high phosphorus concentrations in nutrient solution lead to high rates of uptake. Mexal *et al.* (17) suggested that the main damage to plants caused by PEG resulted from low O_2 solubility even in dilute PEG concentrations and slow O_2

transport to roots. Other investigators (6, 13, 14, 18) reported injury to plants much beyond the osmotic effect but suggested no cause.

In contrast to whole plants, excised pine xylem tissue (25), detached soybean ovules (20), and tomato cell lines (4) responded to the ψ_w induced by PEG with no symptoms of toxicity. A procedure was developed to minimize toxic effects of PEG on whole plants. This is based on recycling nutrient solutions containing PEG through two tomato plant cultures. Recycled PEG had minimal toxic effects on new tomato plants.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill cv Hosen Eilon) seeds were germinated in vermiculite that was presoaked with deionized H_2O and drained. Seedlings were transferred to 36 × 30 × 10 cm deep containers 10 to 12 d after emergence (2 leaf stage). Each container held 10 L of continuously aerated, half-strength Hoagland solution. Deionized H_2O was added every 2 to 3 d to replace water lost by transpiration. The nutrient solution was changed every 10 to 14 d.

The transferred seedlings, were placed in a growth chamber maintained at 25°C with a 550 μE m^{-2} s^{-1} (400–700 nm) for 13 h/d. After establishment, seedlings were thinned to 10 plants per container.

The first study was initiated at the 5 to 6 leaf stage, with three completely randomized treatments. The treatments consisted of nutrient solution only (control) and nutrient solutions containing either NaCl or freshly prepared PEG-6000 (purchased from Sigma). The NaCl and PEG cultures were adjusted to an osmotic potential (ψ_s) of 0.4 −MPa as determined by a freezing point osmometer. The osmometer was precalibrated with standard NaCl solutions. Our calibration was found to be in close agreement with those of Michel and Kaufman (19).

Plants were harvested before and 5 d after the differential treatments were started. Dry weight of plant tops was determined at each harvest on five groups of two plants each. At the second harvest leaf water potential, CO_2 fixation rate, leaf conductance, and Chl content were determined on the youngest, fully expanded leaves. Leaf ψ_w was determined immediately after leaf detachment as previously described (16). A microchamber, designed to measure $^{14}CO_2$ uptake (5), was used to measure CO_2 fixation of intact leaf discs. Leaf conductance of water vapor through the lower leaf surface was determined with a steady state porometer. Chl content was analyzed spectrophotometrically using 80% acetone leaf extracts (1).

For the second study, seedlings were grown in freshly prepared PEG-6000 nutrient solution for 10 to 12 d and then discarded. The PEG solution was then filtered to remove extraneous root

Table I. *Effect of PEG-6000 and NaCl Added to Nutrient Solutions on Dry Weight Production, Chlorophyll Content, Leaf ψ_w and Conductance, Photosynthetic CO_2 Fixation and Cl^- Accumulation*

Plants were grown for 4 d in half-strength Hoagland solution containing fresh PEG-6000 or NaCl at a final concentration equivalent to 0.4 MPa. Values are means of five replicates ± SE of the means.

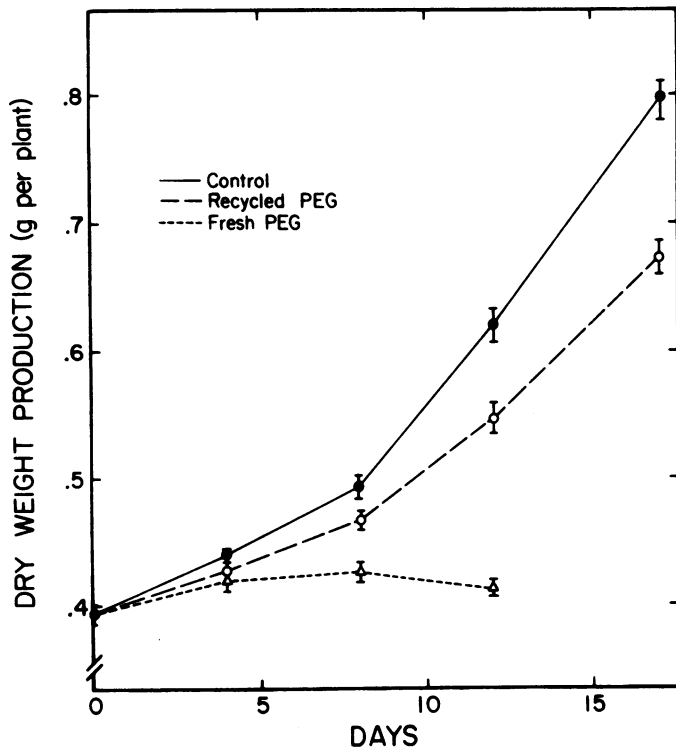|	Dry Wt Production	Chloride Content	Leaf ψ_w	Leaf Conductance	Rate of CO_2 Fixation
	$mg \cdot d^{-1} \cdot plant^{-1}$	$mg \cdot g\ fresh\ wt$	MPa	$mm \cdot s^{-1}$	$\mu mol \cdot m^{-2} \cdot s^{-1}$
Control	43.0 ± 3.7	1.20 ± 0.10	0.40 − 0.03	8.59 ± 0.70	0.337 ± 0.023
PEG	7.0 ± 1.0	0.95 ± 0.10	0.62 − 0.05	2.68 ± 0.38	0.015 ± 0.003
NaCl	36.2 ± 3.3	1.32 ± 0.11	0.63 − 0.05	6.00 ± 0.68	0.288 ± 0.042

FIG. 1. Effect of recycled and fresh PEG-6000 (−0.3 MPa) in nutrient solution on growth of tomato shoots. The points are means of five replicates (each of two plants) ± SE of the means.
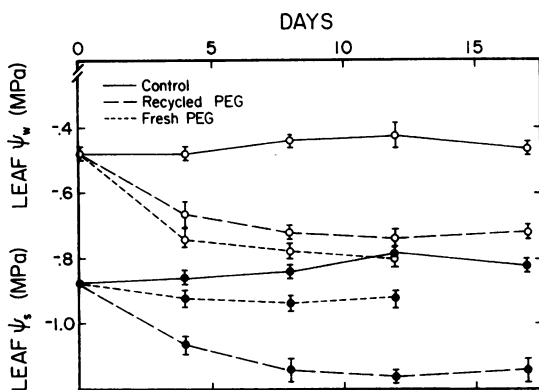

FIG. 2. Leaf ψ_w and ψ_s of tomato plants grown in Hoagland solution, recycled and fresh Hoagland solution containing PEG-6000 at a concentration equivalent to −0.3 MPa. The points are means of five replicates ± SE of the means.

Table II. *Rate of CO_2 Fixation, Leaf Conductance and Chlorophyll Content of Tomato Seedlings Subjected to Fresh and Recycled PEG Solutions*

Plants were transferred to a nutrient solution containing PEG-6000 which was either fresh or previously used for two crops of the same species. Values are means of five replicates ± SE of the means.

Time of Exposure	Osmoticum	CO_2 Fixation Rate	Leaf Conductance	Chl Content
d		$\mu mol \cdot m^{-2} \cdot s^{-1}$	$mm \cdot s^{-1}$	$mg \cdot g^{-1}$ fresh wt
5	None	0.504 ± 0.033	7.4 ± 0.5	1.35 ± 0.08
	Recycled PEG	0.537 ± 0.028	8.2 ± 0.7	1.34 ± 0.11
	Fresh PEG	0.058 ± 0.019	2.6 ± 0.2	0.78 ± 0.10
20	None	0.520 ± 0.027	9.3 ± 0.9	1.24 ± 0.07
	Recycled PEG	0.550 ± 0.033	10.5 ± 0.9	1.43 ± 0.10

material and additional nutrients, equivalent to 0.25-strength Hoagland solution, were added to each container to replace those used by the previous plants. Another set of plants was then placed in the same containers and also grown for 10 to 12 d and then discarded. The PEG solution was filtered again and 0.25-strength Hoagland solution added.

At the time the third set of plants were transferred to the recycled PEG solution, two additional treatments were set up: a control (nutrient solution only) are one containing freshly prepared PEG-6000. Both the freshly prepared and the recycled PEG solutions were adjusted to the same ψ_s of −0.3M. Plants were at the 5 to 6 leaf stage when treatments were initiated.

After treatment initiation, five groups of plants were sampled about every 4 d to determine leaf ψ_s, ψ_w, and dry matter production (on two plants per group). The ψ_s of leaf sap expressed after freezing and thawing was determined with an osmometer. CO_2 fixation rate, leaf conductance, and Chl content were measured on the 5th and 20th d of treatment.

RESULTS AND DISCUSSION

The exposure of tomato plants to 0.4 MPa PEG decreased their growth rate by about 6-fold during a 4-d period and caused a breakdown of Chl (Table I). Salinity had no effect on total Chl content while on a weight basis it was slightly increased as growth was somewht retarded. A partial osmotic adjustment was obtained in both osmotica, although the decrease in leaf ψ_w was less than the decreased external ψ_s. Leaf conductance, however, was much more drastically reduced by PEG than by salt. The rate of CO_2 fixation decreased over 20-fold and was much more severely affected than any other parameter measured.

A logarithmic growth pattern was obtained for tomato plants grown on recycled PEG solution (−0.3 MPa) similar to that obtained for control plants, although the rate was somewhat lower (Fig. 1). Plants which were transferred to fresh PEG of an identical ψ_s grew at a very low rate and died about 12 d after

transfer. The plants in the recycled PEG solution adjusted completely to the decrease in external ψ_s since leaf ψ_s decreased by 0.3 MPa within 8 d and $(\psi_s - \psi_s)$ was similar to that of the control (Fig. 2). In the fresh PEG solution the rate that ψ_s decreased was much less and osmotic adjustment was not complete. However, the toxic effect of PEG is apparently not related to plant water status because a positive $(\psi_w - \psi_s)$ was maintained throughout.

CO$_2$ fixation rate and leaf conductance were unchanged when plants were grown in the recycled PEG for either 5 or 20 d after transfer (Table II).

Recycling of PEG solutions through two preliminary growth cycles of a species may serve as an efficient procedure to detoxify PEG solutions. The solution could then be used as an osmotic medium for plant growth at least down to 0.3 MPa. Neither long-term responses like growth, nor short-term ones like CO$_2$ fixation rates were drastically reduced in the recycled PEG solution (Fig. 1; Table II). The fact that recycled PEG solution could be used indicates that the main toxicity is probably caused by contaminants or PEG molecules of relatively low mol wt that can be removed by plants. Artificial means for purification which would separate molecules, mainly on a size basis, were shown to be not successful in eliminating toxicity (3, 12, 21). This implies that removal of the toxic molecules is probably based not only on size but also on structural parameters. Growing different species other than tomatoes in PEG recycled with tomato plants was less successful than when tomatoes were used as experimental plants, suggesting that even a compatibility between species and PEG may be required. A possibility that the two initial cycles of plants excreted a substance which was taken up by the later plants inducing a protective response can, however, not be excluded.

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