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An Improved System of Subjecting Plants to Water Stress

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Abstract. An improved system of plant cultivation at stable and specific levels of polyethylene-glycol (PEG, mol. mass 1400–1600) – induced water stress has been described. To set up this system a perforated tubular glass vessel containing soil to support seedling growth was wrapped externally first with a layer of macroporous silica gel-G and then with three layers of a dialysis membrane of a lower exclusion limit (2000 mol. mass). Effects of 8 days of PEG – induced stress have been studied on uptake and translocation of N and P and growth of barley (*Hordeum vulgare* L. cv. KN 16) seedlings. Some of the noteworthy improvements of the system were exclusion of PEG from the plant consequent upon use of silica gel-membrane combination, shorter time (2 days) for the soil-plant-air continuum to attain steady state, and stability of the plant water potential over a period of a few days.

Maintaining plants at specific levels of water stress is one of the most difficult problems in plant water relations research. In the most up-to-date device the plants, after membrane-encasing of their root system, are grown in nutrient solution containing an osmotic agent such as polyethylene-glycol (PEG) (TINGEY and STOCKWELL 1977). Membrane-encasing of the root system produces reliable stress effects as a result of selective minimization of PEG penetration into plants but regulates passage of water and nutrients to the roots (COX and BOERSMA 1967, GRAHAM-BRYCE 1967, ZUR 1967). However, the minimum time necessary for the soil-plant-air continuum to attain a steady state, membrane effects on nutrient uptake and translocation, and if membrane excludes PEG from the plant, need further investigations. The objective of the present work was, therefore, to examine these aspects with a further improved device for solution cultivation of plants at specific levels of water stress stable over a period of a few days.

MATERIAL AND METHODS

The device of plant cultivation prior to stress consists of a perforated tubular glass vessel, henceforth called the seedling vessel (Fig. 1). Barley (*Hordeum vulgare* L. cv. KN 16) seeds which had imbibed water for 6 h were

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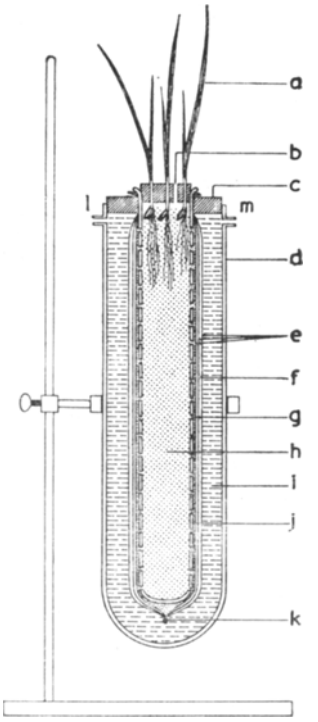

Fig. 1. Membrane-silica gel device for water stress. — a: barley seedlings, b: hole for root aeration, c: rubber lid, d: solution vessel, e: three membrane layers, f: seedling vessel, g: perforations, h: soil, i: PEG + nutrient solution, j: silica gel, k: knotted membrane, l and m: inlet and outlet.

planted in the moistened soil of the seedling vessel. The seedlings, directed through holes in the lid of the vessel, received 10 ml/vessel of half-strength ARNON and HOAGLAND (1943) nutrient solution on alternate days from 3 days after planting until water stress treatments. The seedlings were kept under 500 cd m^{-2} 1 m above leaf surface, 16 h light 8 h dark cycle at 80% relative humidity. The day/night temperatures were 28—20/18 °C.

A day prior to water stress treatments (6 days from planting) approximately 2 mm thick aqueous slurry (1 : 2, m/v) of macroporous silica gel — G (0.2 to 0.5 mm, 35—70 mesh) was coated on the outer surface of the seedling vessel with seedlings in place. After 24 h from gel coating the seedling vessel was safely inserted into 3 layers of a semipermeable membrane (Sigma, dialysis sacs, minimum exclusion limit 2000 mol. mass). The following 3 stress systems were employed. The seedling vessel was either wrapped, (1) with 3 layers of the membrane only (M), or (2) with both, the membrane and the silica gel (MS), or (3) with neither the membrane nor the silica gel (NM).

On the seventh day from planting the seedlings were subjected to 8 days of water stress (including 2 days for equilibration of the stress system) by immersing the seedling vessel in a larger vessel containing — 0.05 (control plants, receiving nutrients only), —0.1, —0.5, and —1.0 MPa solutions consisting of nutrients and PEG (mol. mass 14 000—16 000) prepared according to PARMAR and MOORE (1968). To make up for the water lost as a result of absorption by and transpiration from the plant and also to keep the solution water potential at a constant level, the respective solutions were

allowed to flow slowly from a reservoir through the replicate assembly of each treatment interconnected by polyethylene tubing (not shown in the Fig.).

Sampling, for growth (oven-dry m., 80 °C, 24 h), measurement of plant water potential using whole leaves (SHARDAKOV 1948), determination of nitrogen (JENSEN 1955), phosphorus (KING and WOOTON 1959), extraction of PEG from fresh plant tissue (LAWLOR 1970) and its quantitative measurement (TINGEY and STOCKWELL 1977), was completed within 3 h from start of the light period. To assess if the plants were subjected to normal diurnal fluctuations, the plant water potential was also measured once daily during the latter half of the 8 h dark period.

RESULTS AND DISCUSSION

The membrane in the solution collapsed within 24 h against the wall of the seedling vessel. The interlinking silica gel layer provided good contact between the soil inside and the solution external to the seedling vessel. The perforated vessel provided passage of water and nutrients to the roots from the solution. Plant water potential attained a level less than the solution water potential within 2 days. No abrupt change in plant water potential during the stress period indicated absence of any membrane disintegration or bacterial degradation of PEG (ZUR 1967, HAINES and ALEXANDER 1975). Corresponding to solution water potential the steady state order of lowering of plant water potential was MS $<$ M $<$ NM. The elevated plant water potential during the dark period came to normal within 1 h after the light was switched on. Thus plants in our experiment were subjected to normal diurnal fluctuations.

PEG uptake by the NM plants was most significant. On the other hand, both M and MS systems hindered PEG uptake, but the latter did it significantly more effectively. PEG values (mg g^{-1} fresh m.) in plants of the NM, M and MS systems at -1 MPa stress level, respectively, were 6.5, 0.5, 0.1 for leaf tissue, and 8.5, 0.3, 0.08 for the root tissue. Thus the problem of PEG absorption (JANES 1974, LAWLOR 1970, RESNIK 1970) and its unmetabolized transport (LAWLOR 1970) did not exist effectively in the MS plants because of absence of PEG penetration. However, the insignificant amount of PEG still detectable in M plants may have resulted from the cumulative effect of penetration of some low mol. mass PEGs present in our batch of PEG polymer. Still lower concentration of PEG in the MS plants, the most desirable stress system, results also from some PEG adsorption on the silica gel surface. Therefore, the stress that develops in the MS plants is solely due to the effective lowering of the plant water potential external to the roots. In contrast, greater lowering of plant water potential in the NM system is due to lowering of the component solute potential as a result of PEG absorption. The solution water potential in our experiment did not change as the PEG solution was slowly removed from the solution vessel and continuously replenished from the reservoir.

Greatest stress-induced reduction in plant growth occurred in the leaves and least in the roots, the stems being intermediate in this respect (Table 1). Plants of the NM and the MS systems respectively suffered the greatest and least amounts of growth reduction. As the PEG solution need not be delib-

TABLE 1

*Changes in water potential, growth and concentration of nitrogen and phosphorus of barley seedlings at the end of 8 day stress period**

Water stress (solution Ψ [MPa])	Stress system†	Parameters††									
		Leaf Ψ [MPa]	Plant growth [% decrease over control]			Total nitrogen [mg g^{-1} dry matter]			Phosphorus [mg g^{-1} dry matter]		
			Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
	NM	−0.10				42.6	12.6	5.6	3.5	2.1	1.8
−0.05**	M	−0.10				42.7	12.7	5.3	3.5	2.0	2.0
	MS	−0.10				42.8	12.2	5.1	3.4	1.9	2.1
SE						2.3	2.4	1.3	0.5	0.3	0.2
LSD ($P = 0.05$)		NS				NS	NS	NS	NS	NS	NS
	NM	−0.55	15.5	15.0	6.5	—	—	—	—	—	—
−0.10	M	−0.20	11.2	9.5	3.5	—	—	—	—	—	—
	MS	−0.13	8.5	6.5	2.5	—	—	—	—	—	—
SE		0.07	1.6	1.0	1.6						
LSD ($P = 0.05$)		0.17	4.0	3.0	NS						
	NM	−1.00	30.5	23.0	8.5	43.0	12.6	6.0	2.0	1.2	0.8
−0.50	M	−0.71	15.5	20.0	6.2	43.5	23.1	10.5	7.5	3.5	4.5
	MS	−0.55	12.6	15.5	4.0	44.0	25.5	11.5	10.5	5.0	6.6
SE		0.07	0.8	1.5	2.5	1.5	1.2	0.3	0.4	0.2	0.2
LSD ($P = 0.05$)		0.15	2.4	4.6	NS	NS	3.6	0.9	1.0	0.6	0.6
	NM	−3.80	50.0	36.5	12.5	41.2	8.5	3.5	1.5	0.8	0.4
−1.0	M	−2.00	30.0	25.0	10.5	42.5	25.5	15.5	8.5	4.8	4.5
	MS	−1.60	25.5	20.5	4.5	43.8	30.5	16.7	12.5	7.6	7.5
SE		0.06	1.0	1.4	1.5	2.7	1.6	0.3	1.5	0.6	0.3
LSD ($P = 0.05$)		0.15	3.0	4.3	4.6	NS	5.0	0.7	4.6	1.5	0.7

*Each mean is based on 6 observations; **control plants receiving nutrients only; †NM, M and MS respectively are non-membrane, membrane, and membrane-silica gel systems; ††nitrogen and phosphorus contents were not measured in plants on the −0.10 MPa stress level; SE = standard error; LSD = least significant difference; NS = not significant.

erately aerated, foaming and consequent lowering of the O_2 content of the viscous PEG solution (MEXAL *et al.* 1975) did not exist in our system. New root growth during the initial few days at low stress level (−0.1 MPa) indicated that O_2 requirement of roots was apparently met *via* O_2 diffusion through the soil around the root system rather than from the PEG solution. Considerably reduced root growth at the end of the stress period of 8 days was evidently elicited by elevated stress.

PEG in the solution differently affected N and P concentrations of plant tissue in the three stress systems. In the M and MS plants there was no marked effect of the PEG—induced stress on the N concentration of leaves, but the N concentrations of stems and roots were significantly elevated above controls; although to a much greater extent in the MS than in the M plants. In agreement with TINGEY and STOCKWELL (1977) changes in concentration of these elements in leaves, stems and roots of the MS plants indicated their uptake and translocation to be differently sensitive to stress. While uptake of N and translocation of both N and P were not impeded the translocation of N was retarded by stress.

Despite being conceptually similar to previously published reports (COX and BOERSMA 1967, GRAHAM-BRYCE 1967, TINGEY and STOCKWELL 1977) some of the noteworthy improvements achieved with our MS device may be mentioned. As the device does not involve manipulations of the plant system during the entire growth and stress period, chances of root injury and consequent increase in PEG uptake (LAWLOR 1970) do not exist. The semipermeable membrane with a much lower exclusion limit of 2000 mol. mass effectively excludes PEG from plants and, in contrast to 4 days (TINGEY and STOCKWELL 1977), the soil-plant-air continuum attains a steady state within 2 days. No change in solution water potential as a result of continuous removal and replenishment of the PEG solution, and observed stability of the plant water potential have been obtained with previous devices. The use of the membrane-silica gel system reduces many of the problems associated with solution culture of plants during water stress.

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BOOK REVIEW

EGAN, H., STOLOFF, L., CASTEGNARO, M., SCOTT, P., O'NEILL, I. K., BARTSCH, H. (ed.): ENVIRONMENTAL CARCINOGENS SELECTED METHODS OF ANALYSIS. VOL. 5. SOME MYCOTOXINS. — International Agency for Research on Cancer, Lyon 1982, 455 pp.

A large number of turkey poults and ducklings died on British farms in 1960 as a result of consuming groundnut meal. The death of the birds was caused by the liver damage resulting from toxic metabolic products of the mold Aspergillus flavus which contaminated the Brazilian groundnut. This publication, published jointly by the IARC, WHO and the United Nations Environmental Programme deals with the genotoxic effects of products of fungi (named mycotoxins) and with analytical methods for their monitoring. The introductory overviews present data on the genotoxic effects of various mycotoxins, a list of mold metabolites that have been reported to produce various types of cellular damage, further data on the International Mycotoxin Check Programme and a list of maximum tolerated aflatoxin levels in foods and feeds in various countries. Several papers are concentrated on the quantitative determination of aflatoxins, the first mycotoxins that were shown to be potent genotoxic compounds. Methods of thin-layer and high-performance liquid chromatography of the mycotoxins in peanuts and cottonseeds, in milk and cheese, in feeding stuff and in animal tissue are described in detail. Other mycotoxins than aflatoxins have received less attention, but can also evoke genotoxic effects. The publication also covers the following mycotoxins: Ochratoxin A, Sterigmatocystin, Patulin, Penicillic acid, Citrinin, Trichothecenes, Mycophenolic acid, Luteoskyrin and Rubratoxin B. Data on the genotoxic effects, occurrence and methods of analysis are presented for each compound.

Providing an invaluable reference source and guide, this IARC publication is extraordinarily useful for toxicologists and those studying the genotoxic effects of naturally occurring mutagens and/or carcinogens.

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