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Protocols

A Novel Method for In Vitro Culture of Plants: Cultivation of Barley in a Floating Hydroponic System

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Abstract. We describe a system for hydroponically growing plants that is based on using a polyethylene (PE) granulate as the floating body. The procedure can be performed in all suitable vessels (e.g., bowls, basins, glasses) and is independent of the culture area. Barley plants (*Hordeum vulgare*) grown in this hydroponic system had no difference in the uptake of mercuric and cadmium ions when compared with plants grown on a defined agar medium. The simple and inexpensive method can be used for the isolation of leaf tissue and large amounts of root tissue. In addition, numerous experiments can be run in parallel.

Key words: *Hordeum vulgare*, heavy metal ions, hydroponics, polyethylene granulate

Abbreviations: PE, polyethylene.

Introduction

Growth conditions of plants may impair the reproducibility and significance of a designed experiment. In addition to phytotronic cabinets and exposure chambers supplying the quality, quantity, direction, and temporal variation of the artificial light (Thiel et al., 1996), plant nutrition is important to ensure a defined and reproducible experimental system. Hydroponics is commonly used to grow plants directly in granulated substrates like clay (Blähton) or in highly specialized vessels on a rockwool- or agar-based system (Gibeaut et al., 1996; Heidenreich, 1999; Huttner and Bar-Zvi, 2003; Tocquin et al., 2003). These methods are often associated with a high expenditure of work and difficulty in setup. Moreover, for important questions related to the uptake of additives (e.g., xenobiotics, heavy metal ions), these systems are not suitable because such additives may interact with rockwool or clay. For *Arabidopsis thaliana*, a floating sponge system for the growth of individual plants has been described (Arteca and Arteca, 2000).

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A patent application has been filed for the cultivation method (The Patent Office, London; 0325019.8).

Aluminium tolerance of barley was tested by using a 50-mL syringe system, which allowed the growth of 5 seedlings (Feng et al., 1997). However, for growing large numbers of plants, these systems are limited.

Here we describe a simple hydroponic floating system that is based on the use of polyethylene (PE) granulates. Advantages include the easy handling, fast maintenance, and low cost. Hydroponics is appropriate for heavy metal ion uptake studies running numerous parallel experiments. The method should also be suitable for other experiments with a large number of conditions.

Materials and Methods

Plant material

The *H. vulgare* cv. Barke (BSA-Nr. 1582) used in this work is an awned, double-lined summer barley that was bred by means of a Libelle × Alexis cross (Saatzucht Breun GdBr, Herzogenaurach, Germany).

Pretreatment of barley caryopses under axenic conditions

- Incubate the caryopses for 1 min in ethanol (70%) and then for 1 min in distilled water.
- Incubate the caryopses twice for 5 min in NaOCl (10%), containing 0.1% Triton X-100.
- Use distilled water to wash the caryopses 10 times for 1 min.
- Swell up the caryopses for 24 h with distilled water at room temperature.

Culture medium

To the autoclaved MS-basal medium (Sigma), $HgCl_2$ (0.1 M) or $Cd(NO_3)\cdot 4H_2O$ (0.1 M) was applied at about 55°C. The final heavy metal ion concentrations were 10, 20, 30, and 40 µM.

Hydroponic floating system

- Place the low-density PE-granulate (Basell) in a glass vessel (2-4 cm in height).
- Mix the swelled caryopses with PE-granulate (1:10 [v/v]) and apply the mixture onto the PE-granulate of the glass vessel. Alternatively, the caryopses may also be applied directly onto the PE-granulate.
- Pour the culture medium into the glass vessel at its brim.

Growth on agar

- Pour the culture medium containing 1.5% agar into Phytotray II culture vessels (Sigma).
- Apply the swelled caryopses onto the agar.

Culture conditions

Seedlings were grown for 6 d in a controlled environment cabinet (relative humidity, 70 ± 5%) under a 16/8 h day-night cycle with a photosynthetic active

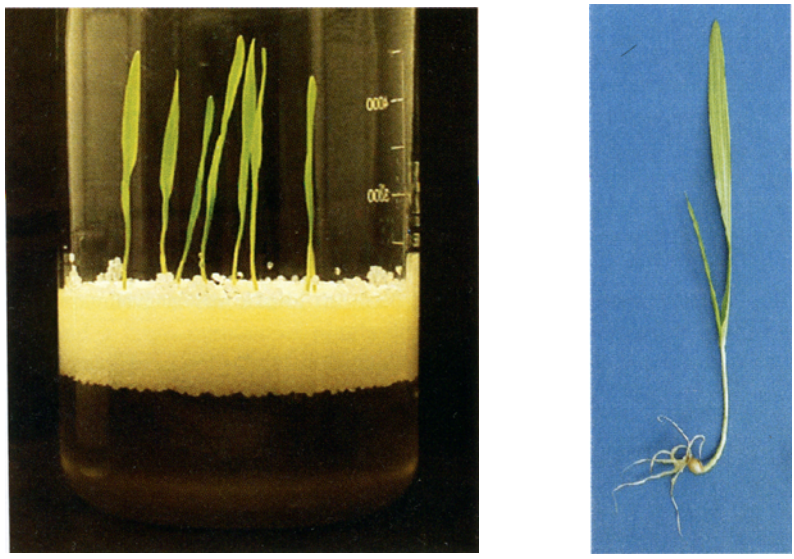

Figure 1. (A) Hydroponically grown barley plants were grown (as described in text) in the floating PE-granulate system for 6 d. (B) Close-up of a single barley plant.

radiation of 116 $\mu M \cdot m^{-2} \cdot s^{-1}$ from 06:00-22:00 h middle European summer time and a temperature of 24/20°C.

Tissue sampling

Leaves and roots of 6-day-old plants were harvested and weighed to determine the fresh weight. Samples were frozen in liquid nitrogen and stored at –80°C.

Heavy metal ion quantification

Total mercury and cadmium content of leaves and roots was determined by means of sector field inductively coupled plasma mass spectrometry after acid digestion of about 100 mg of dried sample material (Schramel and Wendler, 1998).

Results and Discussion

We describe here a simple hydroponic system for the growth of barley. The use of hydroponic culture has several advantages for molecular, biochemical, and physiological experiments. Handling a PE-granulate as a floating body is simple, and no interactions with the applied heavy metal ions were observed. By using this system, we were able to grow barley in 50- to 3000-mL glass vessels (Figure 1A). The roots of the plant were anchored in the PE-granulate and grew into the liquid medium. Adaptation of the system for growing plants in increased or reduced vessels can be managed by using a proper amount of the PE-granulate. In addition to barley, caryopses seeds of other plants can be sown by adapting the PE-granulate lamination, as well as the PE-granulation. Individual plants can be removed from

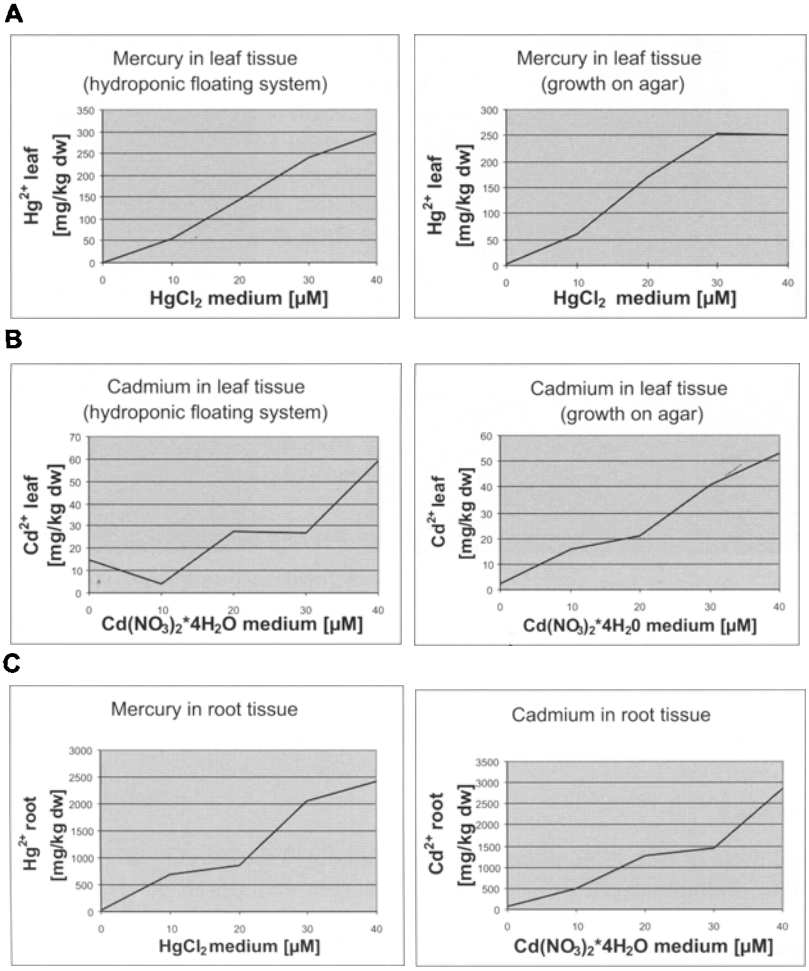

Figure 2. Accumulation of heavy metal ions in barley plants grown hydroponically or on agar. (A) Accumulation of Hg^{2+} in leaves. (B) Accumulation of Cd^{2+} in leaves. (C) Accumulation of Hg^{2+} and Cd^{2+} in roots after hydroponic growth.

PE-granulate and transferred to another vessel for growth under a changed medium. Sterilizing the PE-granulate is not necessary; however, it might be treated as the seed material. For our experiments, aeration of the medium was not necessary. If aeration is essential, we recommend using a cork ring for the growing area. The free space between the vessel edge and the cork ring can then be used to insert a commercial aeration system. For harvest, plants can easily be pulled out of the PE-granulate, and the root system and leaves can be separated for analysis in further studies (Figure 1B).

After using this system, we compared the uptake of Hg^{2+} and Cd^{2+} in barley with the uptake in plants grown on agar medium. In Figure 2A and B, the concentration of both metal ions in barley leaves is given. The correlation between the

metal ion concentration in the medium and the concentration in the leaf tissue was positive. The concentrations in plants grown in the floating hydroponic system were comparable with the concentrations in plants grown on agar (Figure 2A, B). The concentration of Hg^{2+} in the roots was increased by a factor of 10 compared with the leaf tissue, and of Cd^{2+} by a factor of 60 (Figure 2). This indicates a better transfer from roots to leaves for Hg^{2+} compared with that for Cd^{2+} because the concentrations of both metal ions were about the same in the roots (Figure 2C).

In conclusion, this floating hydroponic system can be used for the growth of plants independent of the size of the seeds. The system is easy to handle and inexpensive, and a large number of plants can be grown in parallel. In addition, the system is easy to manipulate with the application of xenobiotics or chemicals, plants can be harvested without damaging the root system, and the root system and leaves can be separated for further study.

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