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Maximal Biomass of *Arabidopsis thaliana* Using a Simple, Low-Maintenance Hydroponic Method and Favorable Environmental Conditions[1]

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The advantages of *Arabidopsis thaliana* (L.) Heynh. for genetic studies are well known, but its diminutive stature and associated low biomass at maturity make it a challenging species for complementary physiological and biochemical studies. Hydroponic culture can significantly increase plant growth and produce uniform, stress-free root and shoot material that can be harvested throughout the life span of the plant. However, many shy away from the use of hydroponic culture because of the perceived difficulties in set-up and maintenance. Although other methods for the hydroponic culture of Arabidopsis have been reported (Rodecap et al., 1994; Delhaize and Randall, 1995; Hirai et al., 1995), they suffer from various shortcomings, including poor aeration, loss of root material, overcrowding, excess manipulation, and less-than-favorable environmental conditions. In this paper we describe an easy, low-maintenance method of hydroponic culture for Arabidopsis that combines the use of rockwool culture for uniform seedling establishment and a closed system of solution culture for the duration of plant growth. In addition, some consideration is given to temperature and light conditions that favor biomass production.

The most difficult part of hydroponic culture for Arabidopsis is to establish a good root system, because young seedlings are prone to hypoxic stress from water logging. Rockwool (GrodanHP, Agro Dynamics, East Brunswick, NJ) provides an excellent, well-aerated rooting environment that is a far superior medium for reliable and uniform seedling establishment compared with other media we have tried, including cheesecloth, blue blotter paper, brown germination paper, filter paper, fiberglass matting, agar, and soil- or vermiculite-filled straws. Rockwool is a mixture of igneous rock and limestone that is heated and spun into mats. Even when saturated, rockwool holds about 15% air space.

Containers and tops for hydroponic culture must be opaque to produce healthy roots and discourage growth of algae. Surfaces can be painted with either epoxy or vinyl paint or covered with aluminum foil. In places where foil could be exposed to the nutrient solution, an underliner of Parafilm (American National Can, Greenwich, CT) should be used to prevent Al contamination. Black paint or aluminum foil are suitable for controlled environment chambers, but white epoxy paint is best for greenhouse conditions to reduce radiant heat load. Tops for containers should also have suitable holes for supporting the rockwool rooting medium and for removal of plants. Plastic grids, such as those used as diffusers in lighting fixtures (1.5 × 1.5-cm grid), can be cut to fit inside the lip of small, 1-L plastic containers. We prefer to use larger containers, such as 32-L low-density polyethylene tubs (Rubbermaid, Wooster, OH) covered with acrylic tops (Fig. 1). The tops are cut from a large sheet of acrylic, 0.32 cm thick, purchased from a local plastics dealer, and holes are cut to hold 35 individual plants in removable, plastic plugs. The plugs, purchased from a hardware store, are made from 3.81-cm-diameter test plugs for polyvinylchloride pipe used in plumbing for drinking water. A 1.27-cm-diameter hole is drilled in each plug to accept the rockwool, and the plugs are painted with vinyl or epoxy paint. Cylinders of rockwool (3 × 1.5 cm) are cored from a slab and placed in the plugs or grids to a depth that allows contact with the solution with about 2 cm remaining above the solution. Dry seeds can be sprinkled on the rockwool or wet seeds can be pipetted after treatment in cold water. After the seeds are sown, the rockwool is wetted with a wash bottle to help disperse and settle the seeds, and then the entire surface is wetted. Plants are thinned to one plant per plug beginning on d 2 after germination and as needed thereafter.

Solutions must be well aerated and mixed. Pumps and air stones purchased from an aquarium store are suitable for most uses and can provide a continuous air supply. Continuous aeration is not necessary and may even inhibit root growth by the constant agitation. A more desirable situation is to bubble solutions intermittently from an oil-free compressed air source.

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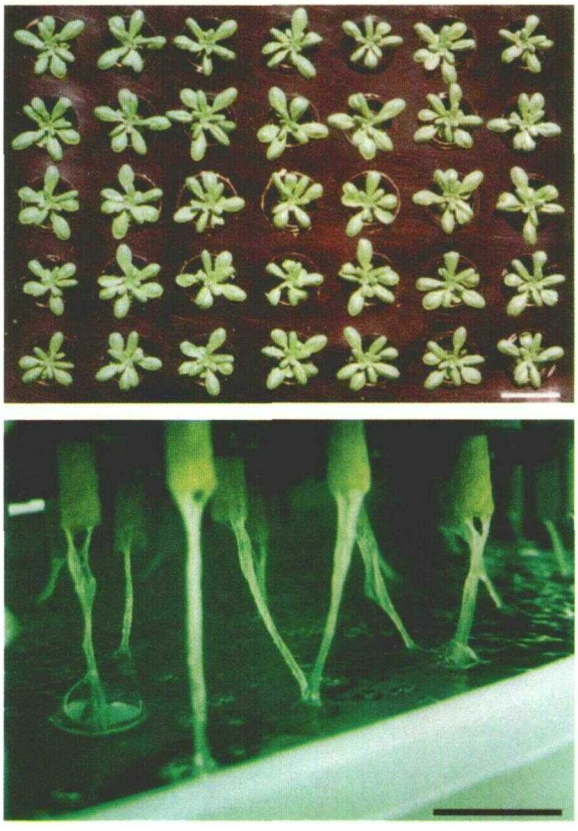

Figure 1. Three-week old *A. thaliana* (Landsberg *erecta*) grown in solution culture. Top, A black acrylic top with space for 35 plants and the plugs used to support the rockwool and plants. Bottom, The roots, the rockwool rooting medium, and bubbling of the solution. The tub holds 32 L of solution, and the dimensions are 53 × 38 × 22 cm. Rockwool plugs, 3 × 1.5 cm, are cored from a slab of rockwool with the "grain" of the rockwool in the long axis. Note how this method provides good uniformity of the shoots and roots. Bars = 5 cm.

For the 32-L tubs we bubbled air through a 30-cm-long air stone for 5 min every 30 min.

In the often-cited one-quarter-strength modified Hoagland solution (Hoagland and Arnon, 1950; Johnson et al., 1957; Epstein, 1972), the macronutrients are reduced to one-quarter strength to avoid possible osmotic effects, ion toxicity, and adverse interactions of certain nutrients, whereas the micronutrients are kept at full strength to prevent depletion. In comparison, the formulations used in most commercial hydroponic operations today are much closer in concentration to the full-strength Hoagland solution. With these considerations in mind, we have formulated a solution with about one-third the concentration of macronutrients and the full concentration of micronutrients reported for the solution culture of lettuce (Resh, 1995). These changes were made to prevent depletion of nutrients in long-running experiments while maintaining a low osmotic pressure. The hydroponic nutrient solution that we use comprises the following macronutrients: 1.25 mM KNO_3, 1.50 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 0.50 mM KH_2PO_4, and the following micronutrients: 50 μM KCl, 50 μM H_3BO_3, 10 μM $MnSO_4$, 2.0 μM $ZnSO_4$, 1.5 μM $CuSO_4$, 0.075 μM $(NH_4)_6Mo_7O_{24}$, 0.1 mM Na_2O_3Si, and 72 μM Fe-diethylenetriamine pentaacetate (Sequestrene 330, Ciba-Geigy, Greensboro, NC). The final solution pH is 6.0, and the electroconductivity is 0.66 dS m^{-1}. Even though Si is not recognized as an essential mineral for plants, we include Si because it is naturally present in the soil solution and in the plant cell wall and it may confer mechanical strengthening, improved nutritional balance, and pathogen resistance (Epstein, 1994).

Many of the environmental effects on the growth and transition to flowering of Arabidopsis have been reviewed (Martinez-Zapater et al., 1994). Arabidopsis is a facultative long-day plant and there are many interactive effects of daylength, light intensity, light quality, and temperature on the transition to flowering. Generally, flowering is induced by 16-h photoperiods if the plants are sufficiently mature; however, under constant light plants can be forced to flower with only two to five leaves. At less than inductive photoperiods, the total photon flux determines the rate of development and time of flowering. We have observed considerable shortening of the flowering time in plants grown at 400 compared with 200 μmol quanta PAR m^{-2} s^{-1} with a 10-h photoperiod. Arabidopsis is commonly grown at 100 to 200 μmol quanta PAR m^{-2} s^{-1}; however, light saturation of CO_2 fixation occurs at about 600 μmol quanta PAR m^{-2} s^{-1} (Eckardt et al., 1997). Higher temperatures tend to reduce the time to flowering and leaf number. The typical temperature used in laboratories ranges between 16 and 25°C. We have observed that cv Landsberg *erecta* does well at 23°C, whereas cv Columbia shows some signs of stress at this temperature.

For plants to obtain maximal growth, a balance of environmental conditions must be found that delays flowering and yet produces rapid growth. We have observed that under an 8-h photoperiod plants grow large but slowly, whereas under a 14-h photoperiod plants grow more rapidly but flowering is induced early, thus reducing leaf biomass. Estimates of growth under various environmental conditions are difficult to find in the literature (but see Martinez-Zapater et al., 1994, and refs. therein). Most studies are conducted under inductive photoperiods, 16-h to continuous light, which produce plants with about 0.1 to 0.01 of the leaf biomass we report (Table I). Although we have not thoroughly examined all possible combinations of growth conditions (i.e. photoperiod, light intensity, and temperature), we have adopted conditions that provide vigorous vegetative growth as determined by fresh weight, leaf area, and

Table I. *Growth of A. thaliana (cv Landsberg erecta) in hydroponic culture under a 10-h photoperiod (400 μmol quanta PAR m^{-2} s^{-1}), 75% RH, and day/night temperatures of 21/18°C*

Data were obtained from five separate experiments comprising 163 plants. Only selected harvest dates are shown, and values are the means ± SE for individual plants.

D after Planting	Root		Shoot[a]		Raceme (Height)	Leaf (Area)
	Fresh wt	Dry wt	Fresh wt	Dry wt		
	g				*cm*	*cm^2*
25 (n = 10[b])	0.057 ± 0.011	0.005 ± 0.001	0.204 ± 0.022	0.022 ± 0.002	0.0	8.31 ± 0.73
32 (n = 9)	0.498 ± 0.057	0.032 ± 0.003	1.160 ± 0.069	0.121 ± 0.006	0.0	38.9 ± 1.35
39 (n = 5)	1.480 ± 0.112	0.097 ± 0.005	5.026 ± 0.213	0.508 ± 0.020	6.36 ± 0.62	130 ± 5.98
48 (n = 3)	2.917 ± 0.164	0.211 ± 0.010	10.94 ± 0.499	1.195 ± 0.037	16.6 ± 1.53	258 ± 6.69

[a] Leaves, stem, and raceme combined. [b] No. of individuals.

days to flowering (Table I). Under these conditions, germination from dry seed began on d 3, the transition to flowering began about d 30 to 32, and bolting commenced about d 33 to 34. It should be noted that the atmospheric concentration of CO_2 was closely controlled in these experiments and that elevated CO_2 concentrations (360 versus 1000 μmol/mol) decreased the days to flowering by about 5 d.

The advantages of hydroponic culture have long been recognized in the production of larger and more uniform plants than those grown in soil. Some of the reasons for this are inherent in the ample supply of nutrients and water and in the elimination of root restriction, which can significantly inhibit shoot growth (Peterson and Krizek, 1992). In addition, the excellent aeration of the root environment in hydroponic culture is considered to be a major factor that allows superior plant growth compared with soil-grown plants. For Arabidopsis a well-aerated and yet uniformly wet medium is critical for good germination and seedling establishment because the tiny seedlings are easily stressed by over- or underwatering; rockwool provides a medium for good seedling establishment. In addition to the obvious benefits of increased growth and elimination of stress factors, hydroponic culture also allows the easy harvest of root tissues for studies of growth, physiology, and biochemistry.

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