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Aseptic Culture of *Arabidopsis thaliana*: A Flowering Plant That Can Be Handled Like a Microorganism

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Arabidopsis thaliana (L.) Heynh. belongs to the tribe Arabideae of the Cruciferae. The plant occurs throughout Europe, East Africa, and Northern and Central Asia.

The relatively small size of the plant makes it easy to handle. When grown in soil, *A. thaliana* may reach a height of 15-20 cm. When grown in aseptic culture, it reaches a height of about 10 cm. *A. thaliana* has a very short life cycle, twenty-eight days, from seed sowing to flowering. The flowers are naturally self fertile and produce about 1,500 seeds per plant when grown in soil. Plants in sterile culture produce about 150 seeds per plant.

The small seeds of *A. thaliana* show 95 per cent germination. The seeds retain their ability to germinate for three years. Because of their small seed size, they have very little nutrient reserves. This makes the plants very sensitive to mineral deficiencies and potentially valuable for mineral bioassay. These advantages are enhanced by the relative ease with which the plants may be grown under sterile conditions. This precision of environmental control facilitates physiological experiments on growth and development, the addition of organic substances to the growth medium,

and the study of heredity-environment interactions.

Major mineral requirements were provided as Knop solution (Table I). The trace elements were iron, manganese, zinc, copper, boron, and molybdenum (Table II). The water

Table I
Macronutrients

Compound	Grams per Liter
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.8
KNO_3	0.2
KH_2PO_4	0.2
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2

Table II
Micronutrients

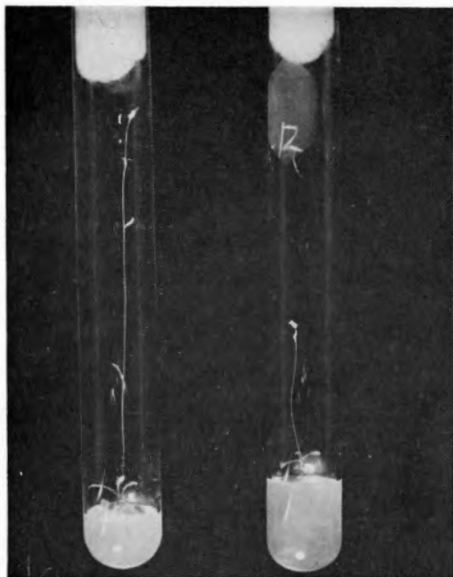
Compound	Grams per Liter
EDTA	0.05
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.06
$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$	0.11
$\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$	0.01
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.0003
H_3BO_3	0.0002

used was pyrex glass distilled. The mineral salt solution was adjusted to pH 6.2. The nutrient solution was solidified with 0.75 per cent agar. Besides providing support for the growing plant, agar makes oxygen more accessible. The melted medium was dispensed in 5 ml aliquots to 16 X 150 mm pyrex test tubes, which were then plugged with non-absorbent cotton wool and autoclaved at 15 pounds pressure for ten minutes.

The seeds were sterilized by immersing in a solution of absolute ethyl alcohol and hydrogen peroxide (1:1) for ten minutes and planted directly

with a platinum loop. When planting the seeds, care must be taken to lay the seeds on the surface of the agar; for if they were placed slightly below the surface, the plants failed to orient properly.

Chilling the seeds at 0-5 C for forty-eight hours insures uniform germination. They were then transferred to a growth chamber which was maintained at 26 C and supplied with continuous illumination of 800 foot candles. *A. thaliana* is a long day plant and as such requires more than sixteen hours of light a day.



A. thaliana growing in culture medium.

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