

**Table 1** Effects of Controlled Growth Conditions on the Photosynthetic Behaviour of Succulent Plants Capable of Crassulacean Acid Metabolism

Species and tissue	Growth period (weeks)	Temperature day/night (°C)	Day length (h)	Illumination	Photosynthetic mode	Diurnal malate change ( $\mu\text{mol g}^{-1}$ fresh wt)	$\delta^{13}\text{C}$ value (‰)
1. <i>K. daigremontiana</i> plantlets	0	—	—	—	—	—	−20.1
young plants	3	20/25	8	sunlight	C <sub>3</sub> -like	25	−24.0
young plants	3	30/15	8	sunlight	C <sub>4</sub> -like	53	−19.8
2. <i>K. daigremontiana</i> mature leaves	10	24/19	16	sunlight + artificial*	C <sub>3</sub> -like	20	−18.0, −18.4
mature leaves	10	27/15	12	artificial†	C <sub>4</sub> -like	110	−13.3, −13.3
3. <i>K. blossfeldiana</i> mature leaves	12	26/20	15	sunlight + artificial*	C <sub>3</sub> -like	‡	−26.4, −26.4
mature leaves	12	27/17	9	artificial†	C <sub>4</sub> -like	‡	−19.1, −18.4
4. <i>M. crystallinum</i> control, mature leaves	10	glasshouse conditions		sunlight	C <sub>3</sub> -like	0.1	−28.3
0.35 M NaCl, mature leaves	10	glasshouse conditions		sunlight	C <sub>4</sub> -like	38	−22.5

\* Approximately  $6 \times 10^5$  erg  $\text{cm}^{-2} \text{s}^{-1}$  (400–700 nm) with day length extended with low intensity tungsten lamps.

† Approximately  $3 \times 10^4$  erg  $\text{cm}^{-2} \text{s}^{-1}$  (400–700 nm) from tungsten lamps and fluorescent tubes.

‡ Not measured here, see ref. 10.

Our experiments show that a variety of environmental conditions may regulate the proportion of carbon gained by either pathway. It is likely that the availability of water may be of paramount importance. Kluge and Fischer<sup>18</sup> showed that droughting *Bryophyllum tubiflorum* rapidly reduced its ability to fix CO<sub>2</sub> in the light (the C<sub>3</sub>-like component). Consistent with these observations, we have found that droughting mature *K. daigremontiana*, grown on the 20/25° C treatment of experiment one, made the  $\delta^{13}\text{C}$  value less negative. After 9 weeks of drought, the control  $\delta^{13}\text{C}$  value of  $-22.8 \pm 0.8\text{‰}$  moved to  $-16.2 \pm 0.3\text{‰}$  as the plants relied increasingly on the dark CO<sub>2</sub> fixation; the C<sub>4</sub>-like component of photosynthesis. Similar data have been obtained for the  $\delta^{13}\text{C}$  values of *Optunia inermis* throughout its range in eastern Australia; in arid regions of central southern Queensland,  $\delta^{13}\text{C}$  values for this species range between  $-11$  and  $-13\text{‰}$  and in the more temperate, higher rainfall areas of coastal southern New South Wales we have found values of  $-16$  to  $-18\text{‰}$ . Environmental control of the photosynthetic options of these plants has brought about predictable changes in the  $\delta^{13}\text{C}$  value.

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<sup>1</sup> Bender, M. M., *Phytochemistry*, **10**, 1239 (1971).

<sup>2</sup> Lerman, J. C., in *Proc. Eighth Int. Conf. Radiocarbon Dating* (in the press).

<sup>3</sup> Smith, B. N., and Epstein, S., *Pl. Physiol. Lancaster*, **47**, 380 (1971).

<sup>4</sup> Tregunna, E. B., Smith, B. N., Berry, J. A., and Downton, W. J. S., *Can. J. Bot.*, **48**, 1209 (1970).

<sup>5</sup> Troughton, J. H., in *Proc. Eighth Int. Conf. Radiocarbon Dating* (in the press).

<sup>6</sup> *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.) (Wiley-Interscience, New York, 1971).

<sup>7</sup> Smith, B. N., *Bioscience*, **22**, 226 (1972).

<sup>8</sup> Berry, J., Troughton, J. H., and Björkman, O., *Carnegie Inst. Wash. Yb.*, **71**, 158 (1972).

<sup>9</sup> Whelan, T., Sackett, W. M., and Benedict, C. R., *Biochem. biophys. Res. Commun.*, **41**, 1205 (1970).

<sup>10</sup> Queiroz, O., *Physiol. Vég.*, **3**, 203 (1965).

<sup>11</sup> Neales, T. F., *Aust. J. biol. Sci.* (in the press).

<sup>12</sup> Sutton, B. G., and Osmond, C. B., *Pl. Physiol. Lancaster*, **50**, 360 (1972).

<sup>13</sup> Kluge, M., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 283 (Wiley-Interscience, New York, 1971).

<sup>14</sup> Ting, I. P., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 169 (Wiley-Interscience, New York, 1971).

<sup>15</sup> Avadhani, P. N., Osmond, C. B., and Tan, K. K., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 288 (Wiley-Interscience, New York, 1971).

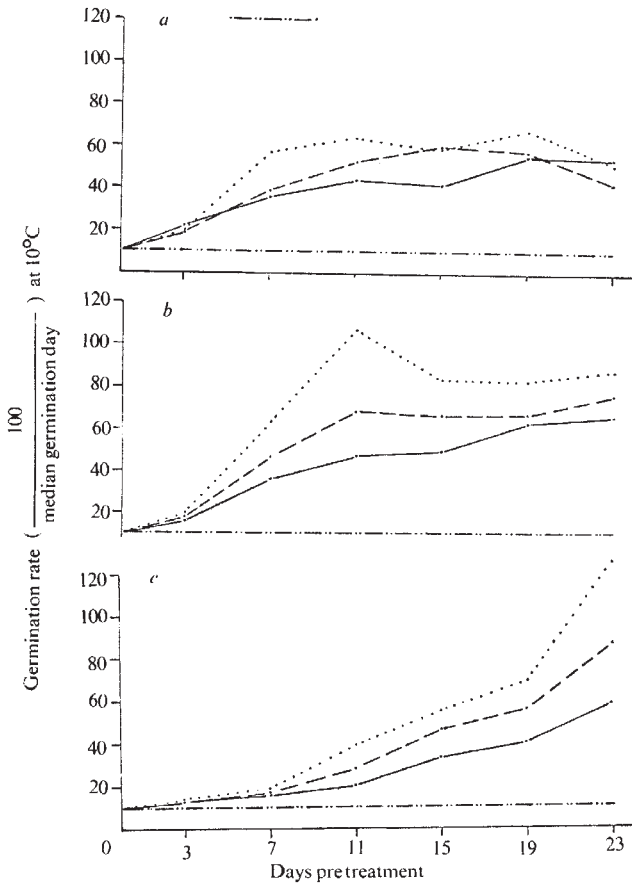
<sup>16</sup> Winter, K., and von Willert, D. J., *Z. Pflanzenphysiol.*, **67**, 166 (1972).

<sup>17</sup> Craig, H., *Geochim. Cosmochim. Acta*, **3**, 53 (1953).

<sup>18</sup> Kluge, M., and Fischer, K., *Planta (Berl.)*, **77**, 212 (1967).

## Accelerated Germination by Osmotic Seed Treatment

CROP seeds sown in cold soil are notoriously slow to emerge and it would obviously be desirable to shorten the time from sowing to seedling emergence and the time between the emergence of the first and the last seedling. This has occasionally been achieved by the pre-sowing seed treatment sometimes referred to as 'hardening'<sup>1-3</sup> or 'advancing'<sup>4</sup> involving repeated cycles of imbibition of a carefully controlled quantity of water, followed by drying back the seeds before the radicles emerge but results have not been consistent. There have been some successful attempts to replace this method by imbibition of seeds in salt solutions<sup>5-7</sup>; tomato seeds thus treated for 6 d exhibit a much higher level of RNA production during germination than untreated seeds<sup>8</sup>.



**Fig. 1** Germination rate of onion seeds in response to osmotic pretreatment. Effect of water potential, temperature and duration of treatment. Seeds were placed on filter papers moistened with solutions of a polyethylene glycol of high molecular weight, Carbowax 6000 (Union Carbide) of -10, -12.5 or -15 bar osmotic potential<sup>9</sup> and at temperatures of 10, 15 and 20° C in the dark (batches of fifty seeds on four Whatman No. 1 filter papers in 8 ml of solution per 9 cm diameter Petri dish). After 3, 7, 11, 15, 19 and 23 d, four replicates of each of the treatments were thoroughly washed and surface dried. The seeds were then placed in other Petri dishes similarly prepared but with distilled water and were allowed to germinate at 10° C in the dark. . . . , -10.0 bar osmotic potential; · · · · · , -12.5 bar osmotic potential; · — · — · , -15.0 bar osmotic potential; — — — — — , untreated. a, 20° C; b, 15° C; c, 10° C.

By using a polyethene glycol of high molecular weight, we have avoided the complications of both 'hardening' and the use of salts. Promising results have been obtained in preliminary experiments on carrot (*Daucus carota* L.) and red beet (*Beta vulgaris* L.), but we report here only on onion (*Allium cepa* L.) seeds.

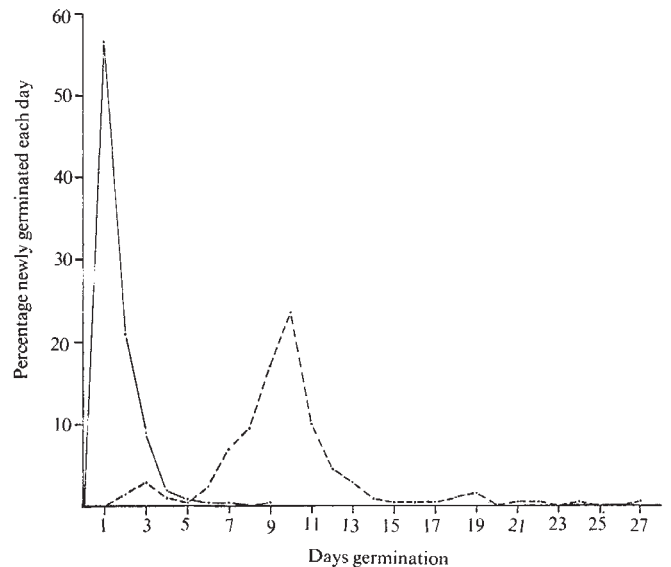
All osmotic pretreatments resulted in an increase in the rate of radicle emergence (Fig. 1) depending in magnitude on

all three components of the preparatory treatment, that is, osmotic potential, temperature and duration. There was no effect on the percentage of seeds germinated but certain treatment combinations permitted germination of some seeds in the osmotic solution (Table 1).

For every temperature and osmotic potential combination, increasing treatment duration initially increased the rate of subsequent germination. At 15° C and 20° C, however, an optimal duration was found beyond which there was no further benefit and in some cases an eventual decline. Almost all comparable rates of germination were lower after treatment at 20° C than 15° C. At 10° C the optimal duration may not have been reached in this experiment.

When seeds were treated at 10° C and 15° C, a lower osmotic potential resulted in a lower rate of germination and a longer treatment was needed for the seeds to reach any desired rate of germination. Treatment at the lowest temperature caused a slower increase in the rate of germination than treatment at the higher temperatures, but ultimately resulted in the highest rate achieved. The combination of -10 bar and 15° C for 11 d, however, was nearly as successful in increasing the rate of germination of the seed population as the best treatment; -10 bar at 10° C was 0.8 d, when the resulting median time from sowing to radicle emergence at a germination temperature of 10° C was 0.8 d, which compared with 9.3 d for untreated seeds (Fig. 2). It would be hard to better this result.

This technique has fascinating physiological implications,



**Fig. 2** Germination of onion seeds in response to 23 d osmotic pretreatment in PEG (Carbowax 6000) solution of -10 bar potential at 10° C. Germination at 10° C on filter paper moistened with water. The pretreatment increases both the rate and the uniformity of germination. —, Pretreated, washed and dried; - - -, untreated.

**Table 1** Percentage Germination of Onion Seeds (cv. Excellent) During and After Osmotic Pretreatment\* in PEG (Carbowax 6000)

Pretreatment		Days					
Temp. (°C)	Potential (bars)	3	7	11	15	19	23
10	-10	0+96.0	4.0+88.0	9.0+83.5	10.5+82.0	15.0+79.0	9.0+80.5
	-12.5	0+93.5	1.0+91.0	0+91.5	6.0+86.0	2.0+85.5	3.0+88.5
	-15	0+90.5	0+94.5	0.5+93.0	1.5+92.0	1.0+85.5	1.5+89.5
15	-10	0+87.5	1.5+93.5	4.5+90.5	15.0+82.0	21.5+72.5	18.0+74.5
	-12.5	0+92.0	0+92.0	1.5+90.0	5.0+88.5	2.5+94.0	4.5+93.0
	-15	0+93.0	0+90.0	0+94.0	2.5+92.5	1.0+94.5	1.0+90.0
10	-10	0+93.5	0+92.5	0.5+94.0	0+88.5	5.5+84.5	3.5+91.0
	-12.5	0+92.0	0+90.0	0+89.5	0+89.5	0+90.5	0.5+94.0
	-15	0+96.0	0+93.0	0+92.0	0.5+90.5	0+89.5	0+88.0
Untreated		90.0.					

\* Different water potentials, temperatures and durations of treatment. Germination temperature 10° C.

and promises to be of considerable value in establishing crops from seeds sown in early spring.

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- <sup>1</sup> Henckel, P. A., *A. Rev. Plant Physiol.*, **15**, 363 (1964).
- <sup>2</sup> Austin, R. B., Longden, P. C., and Hutchinson, Jane, *Ann. Bot.*, **33**, 883 (1969).
- <sup>3</sup> Hegarty, T. W., *Hort. Res.*, **10**, 59 (1970).
- <sup>4</sup> Longden, P. C., *J. agric. Sci.*, **77**, 43 (1971).
- <sup>5</sup> Levitt, J., and Hamm, P. C., *Plant Physiol.*, **18**, 288 (1943).
- <sup>6</sup> Ells, J., *Proc. Am. Soc. hort. Sci.*, **83**, 684 (1963).
- <sup>7</sup> Oyer, E. B., and Koehler, D. E., *Proc. 17th Int. Hort. Cong., Maryland*, **1**, 626 (1966).
- <sup>8</sup> Koehler, D. E., thesis, Purdue University, USA (1967).
- <sup>9</sup> Manohar, M. S., *Planta (Berl.)*, **71**, 81 (1966).

## *In vitro* Control of *de novo* Flower, Bud, Root, and Callus Differentiation from Excised Epidermal Tissues

AMONG the plant species capable of neof ormation, a much larger number are capable of root and vegetative bud formation than of direct flower neof ormation. For most materials, the formation of buds or roots or flowers concerns different tissues, such as epidermis, perivascular or callus tissue of cambial origin, and each tissue maintains complex correlation with the surrounding tissues.

The study of the control of organogenetic differentiation is faced with certain difficulties because of the different tissues involved in the origin of different types of organogenesis and the complexity of the material: lack of criteria for a valid study of changes during organogenesis and for rigorous comparisons among the different types of organogenesis.

This problem was simplified when it was found that a few superficial cell layers excised and cultured *in vitro* have organogenetic potentialities which can be controlled at will. From very small explants of *Nautilocalyx lynchei* (5 mm × 2 mm), composed of one to five layers of foliar epidermal and subepidermal cells, mitoses without organogenesis (non-organogenetic differentiation), mitoses leading to the controlled formation of roots (Fig. 1) or vegetative buds (Fig. 2) (organogenetic differentiation) were induced from the same cellular layer, the epidermis, each cell of which divides<sup>1</sup>. Thus, by the *in vitro* culture of a few superficial cell layers, it is possible to show experimentally the capacity for callus formation and *de novo* formation of roots up to then apparently inhibited. In leaf or stem fragments, root formation is confined to perivascular tissue.

With such materials, certain fundamental problems in organogenesis, which are difficult to study using relatively large organ fragments, can be examined. For example, for *Nautilocalyx*, where bud and root neof ormations arise from epidermal cells, it would be possible to study the evolution and distribution of organogenetic centres by observing the thousands of cells on the epidermal surface to determine whether there is a special mode of distribution of these centres in relation to the presence of basal hair cells or stomata.

To the potentialities for bud, root and callus formation, we were able to add the direct *de novo* formation of floral buds on the explant without intermediate callus or leaf

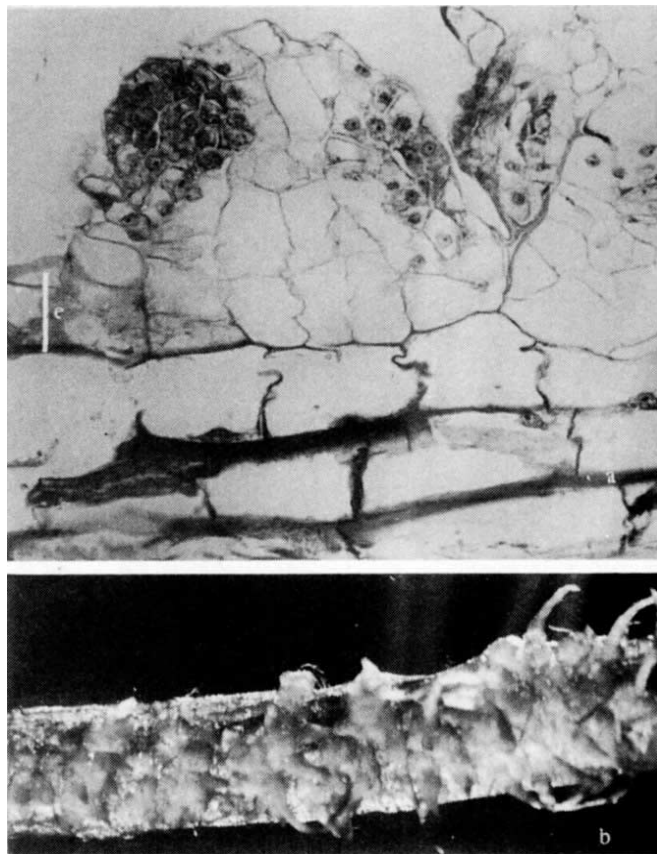


Fig. 1 a, Longitudinal section of an explant of *Nautilocalyx* showing the epidermal origin of the root primordia organized *de novo* from divided epidermal cells. ( $\times 160$ .) b, *De novo* formation of roots from epidermal tissue explants after 37 d. e, Epidermis.

formation by applying the techniques described for *Nautilocalyx* to explants composed of three to five layers of epidermal and subepidermal cells excised from floral ramifications of *Nicotiana tabacum* "W.38". These explants are excised with a scalpel from floral ramifications previously disinfected in a 7% hypochlorite solution for 10 min, and planted on an aseptic culture medium composed of mineral elements, vitamins, auxin, cytokinin and sucrose.

Depending on the relative concentrations of the auxin, cytokinin and sucrose and on certain environmental factors, such as light and temperature, different types of differentiation can be induced in 10 to 14 d: callus formation which has been maintained without organogenesis for 2 yr, root formation, vegetative shoot formation and floral bud formation (Fig. 3). This last type of organogenetic differentiation is the most interesting. It implies that stimulation of mitoses takes place in cells which had lost

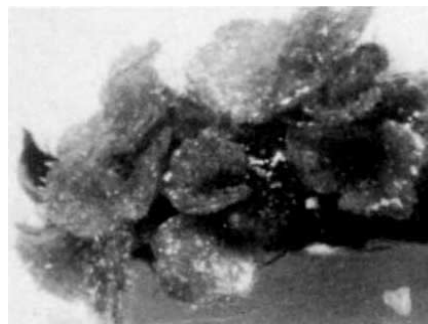


Fig. 2 Explant showing the *de novo* shoot formation, the surface covered with buds after 37 d. ( $\times 8$ .)