EFFECTS OF HIGH TEMPERATURE AND GENOTYPE ON THE GROWTH OF EXCISED ROOTS OF *ARABIDOPSIS THALIANA*

By T. F. Neales*

Summary

The effects of a high temperature (31·5°C), and the addition of vitamins (thiamine plus biotin) on the growth of excised roots of four clones of *A. thaliana* derived from the strains EST, Pi, BLA, and 1018/6 are described. Growth response of the roots to these treatments was different from that of the whole plant of the same strain. The vitamin requirement of the roots of strain 1018/6 was greater at 31·5°C than at 27°C.

I. Introduction

Langridge and Griffing (1959) and Langridge (1965) have shown that plants of several strains (EST, Pi, BLA, and 1018/6) of the small crucifer *Arabidopsis thaliana* change their requirements for vitamins with temperature when grown in aseptic culture. Langridge (1965) concluded that biotin enhances the growth of strains Pi and BLA at high temperatures (31·0 and 31·5°C) to a relatively greater extent than at lower (25°C) temperatures; also that strain 1018/6 has a thiamine requirement only below *c.* 27°C. Above this temperature thiamine inhibits the growth of 1018/6.

This type of evidence has been used (Langridge and Griffing 1959; Langridge 1963) in support of Bonner's (1957) hypothesis that the deleterious effects on growth of supra- and suboptimal temperatures may be ameliorated by supplying certain metabolites. Lang (1963), Ketellapper (1963), and Langridge (1963) have discussed some of the evidence for, and the implications of, this hypothesis regarding the interaction between intermediary metabolism and environmental temperature.

It was found (Neales 1968) that excised root cultures of strains Pi and 1018/6 of *A. thaliana* differed nutritionally and in growth rate from wild type EST. The aim of the present experiments was to determine whether changes in vitamin requirements for the growth, at high temperatures, of the excised roots of EST and of its nutritional mutants Pi, BLA, and 1018/6 were similar to those reported for the whole plants.

II. Materials and Methods

Clones were derived from aseptically grown seedlings of four strains of *A. thaliana*: the wild type, EST, and three nutritional mutants of it—BLA, Pi, and 1018/6 (Langridge 1965). Cultures of excised roots of these four clones were established and maintained in liquid culture by methods previously described (Neales 1968). Roots were grown in 250-ml conical flasks, each containing 150 ml of medium, in the dark in incubators regulated to various temperatures (±0·2°C). Root inocula weighed *c.* 20±5 mg, and the fresh weights of the roots were determined after growth for 42 days.

* Botany School, University of Melbourne, Parkville, Vic. 3052.

In one experiment the effect of temperature on the growth of EST roots was measured. At 27°C its roots have no vitamin requirement (Neales 1968). In a second experiment roots of clones of the four strains were grown in the presence or absence of thiamine and biotin at approximately the optimal temperature for growth (27°C) and also at the supra-optimal temperature of 31·5°C. These two temperatures were chosen after consideration of the results of the first experiment and the results of Langridge (1965) and Langridge and Griffing (1959).

III. Results

(a) Growth Response of Excised Roots of Strain EST to Temperature

The results are given in Table 1. The optimal temperature for an increase in fresh weight was 25°C, and 29°C for an increase in dry weight. Growth at 31·5°C was

TABLE 1

EFFECT OF TEMPERATURE ON GROWTH OF EXCISED ROOTS OF STRAIN EST

Each result is the mean of five replicates

Temperature (°C)	Mean Fresh Weight (mg)	Mean Dry Weight (mg)
10·0	70·6±10·7	11·0
20·0	177·2±16·0	26·0
25·0	217·5±13·2	30·1
29·0	191·0± 5·4	30·3
31·5	96·6±11·2	19·5
35·0	39·8± 3·1	8·3

44% of that at the optimal temperature. This degree of growth inhibition by the high temperature was similar to that induced in whole plants (Langridge and Griffing 1959).

TABLE 2

EFFECTS OF STRAIN, TEMPERATURE, AND VITAMIN SUPPLEMENT ON THE FINAL FRESH WEIGHT (MG) OF EXISED ROOTS OF A. THALIANA: ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F
All treatments	15	114595	14·10***
Strain (A)	3	183899	22·6***
Temperature (B)	1	463449	57·0***
Vitamins (C)	1	270630	33·3***
Interactions			
$A \times B$	3	110732	13·6***
$A \times C$	3	12235	1·5 (n.s.)
$B \times C$	1	63225	7·8**
$A \times B \times C$	3	339	0·04 (n.s.)
Error	64	8129	
Total	79		

** $P < 0.01$.   *** $P < 0.001$.   n.s., not significant.

(b) Growth Response of Excised Roots to Supra-optimal Temperature and Vitamin Supplements

The experimental design used was a randomized block $(4 \times 2 \times 2 \times 5)$, with the following variables:

(1) genotype (EST, Pi, BLA, and 1018/6);

(2) temperature (27 and 31·5°C);

(3) vitamins (none, and plus 0·1 μg/ml each of biotin and thiamine);

(4) replicates (five).

One of the 80 flasks was contaminated with microorganisms, and a missing-plot technique was used. The coefficient of variability of the whole experiment was 34·2%. The analysis of variance (Table 2) indicated that the effect of all treatments together was highly significant. The first-order effects of strain, temperature, and vitamins and the second-order interaction effects of strain × temperature and vitamins × temperature were also highly significant.

TABLE 3

EFFECTS OF STRAIN, TEMPERATURE, AND VITAMINS ON ROOT FRESH WEIGHT, *W*

Pooled data

Strain ($n = 20$)	W (mg)	Temperature ($n = 40$)	W (mg)
EST (control)	317	27°C (control)	340
Pi	334 (n.s.)	31·5°C	188***
1018/6	278 (n.s.)	Vitamins ($n = 40$)	
BLA	124***	None (control)	205
		Plus biotin and thiamine	322***

*** $P < 0·001$.   n.s., not significant.

Growth response of excised roots of the strains EST, Pi, and 1018/6 to the individual treatments were all similar and significantly greater than that of BLA (Table 3). An increase in the temperature from 27 to 31·5°C had an overall effect of significantly reducing growth by 45%, and the supply of biotin and thiamine to the

TABLE 4

RELATIVE EFFECT OF AN INCREASE IN TEMPERATURE FROM 27 TO 31·5°C AND OF VITAMIN SUPPLEMENT ON THE ROOT GROWTH OF FOUR STRAINS OF *A. THALIANA*

Growth at 27°C (control) has been taken as 100 in each instance, and the significance of the differences from the control has been calculated from the statistical analysis of the individual values of root weight

Strain	Fresh Weight		Dry Weight	
	No Vitamins	Plus Vitamins	No Vitamins	Plus Vitamins
EST	39***	32***	48	44
Pi	120 (n.s.)	90 (n.s.)	149	108
BLA	112 (n.s.)	49*	142	69
1018/6	32***	41*	38	72

* $P < 0·05$.   ** $P < 0·01$.   *** $P < 0·001$.   n.s., not significant.

liquid medium significantly increased growth by 57%. An analysis of the significant interactions, strain × temperature and vitamin × temperature (Table 4) shows that the expression of the overall inhibitory effect on root growth of an increase in temperature from 27 to 31·5°C depends upon genotype and the presence or absence of vitamins.

The higher temperature of $31 \cdot 5°C$ inhibited the growth of strains EST and 1018/6 by 59–68% in the presence or absence of vitamins (Table 4). Root growth of strains Pi and BLA in the absence of vitamins was not significantly affected by the temperature increase from 27 to $31 \cdot 5°C$, but in the presence of vitamins this temperature increase significantly reduced the root growth of BLA, but not Pi (Table 4). Likewise (Table 5),

TABLE 5

RELATIVE EFFECT OF VITAMIN SUPPLEMENTS ON ROOT GROWTH OF FOUR STRAINS OF *A. THALIANA* AT 27 AND $31 \cdot 5°C$

Growth of roots without vitamin supplement (control) has been taken as 100 in each instance, and the significance of the differences from the control has been calculated from the statistical analysis of the individual values of root weight

Strain	Fresh Weight		Dry Weight	
	27°C	$31 \cdot 5°C$	27°C	$31 \cdot 5°C$
EST	128 (n.s.)	104 (n.s.)	116	108
Pi	163**	123 (n.s.)	160	115
BLA	357**	157 (n.s.)	327	160
1018/6	185***	234*	143	268

* $P < 0 \cdot 05$.   ** $P < 0 \cdot 01$.   *** $P < 0 \cdot 001$.   n.s., not significant.

the growth of all strains, except EST, was enhanced by biotin and thiamine at 27°C, but at $31 \cdot 5°C$ this vitamin supplement significantly enhanced the growth only of strain 1018/6. There was no evidence in any strain of an inhibitory effect on growth of the vitamin supplement, as Langridge (1965) reported for whole plants of EST and 1018/6 strains. Thus single gene mutations (BLA, Pi, and 1018/6) of the wild type EST affect the form of the growth response of their excised roots to supra-optimal temperatures ($31 \cdot 5°C$), as well as their growth rate (Table 3) and vitamin requirements (Table 5).

IV. DISCUSSION

(a) Comparison of the Vitamin Requirements and Sensitivity to Supra-optimal Temperatures of Excised Roots and Whole Plants of A. thaliana

The inhibitory effect of the temperature increase from 27 to $31 \cdot 5°C$ on the growth of excised roots of EST and 1018/6 strains was similar, and was not affected by the presence or absence of the vitamin supplement (Table 6); a temperature of $31 \cdot 5°C$ inhibited growth, relative to that at 27°C, by 68–59%. Inhibitory effects of approximately the same magnitude were recorded for whole plants of these two strains by Langridge (1965, Fig. 2). However, excised roots of strains Pi and BLA, which require biotin in whole-plant culture (Langridge 1965), responded to the supra-optimal temperature of $31 \cdot 5°C$ in a manner quite distinct from the responses of the whole plant. This was especially true for Pi. In the absence of vitamins, Langridge and Griffing (1959, Figs. 3 and 4) found that the growth of whole plants of BLA and Pi was inhibited by 78 and 90% respectively by a temperature rise from 27 to $31 \cdot 5°C$. However, in excised-root culture in the absence of vitamins a similar temperature rise *increased* growth, but not significantly so (Table 6): the "high-temperature lesion" (Bonner 1957) of whole plants was not present in the excised roots of these strains.

A similar conclusion can be reached by comparing data for excised roots in Table 6 with the results of Langridge (1965, Fig. 5), in which a temperature increase from 27 to 31·5°C inhibited the growth of whole plants of Pi by 64% in the absence of biotin and by 16% in the presence of biotin. The corresponding relative values for excised roots of this strain (Pi) were a 20% *increase* in growth in the absence of vitamins (not significant) and, in the presence of vitamins, a decrease in growth of 10% (Table 6). It is possible to conclude, therefore, that the high-temperature lesion in whole plants of Pi, which is reversed by the addition of biotin (Langridge and Griffing 1959; Langridge 1965) is due to the supra-optimal temperature of 31·5°C either increasing the requirement *of the shoots* for biotin, or due to this supra-optimal temperature restricting the movement of biotin from the roots to the shoots. Langridge and Griffing (1959) describe the morphological effects of the high-temperature lesion

TABLE 6

EFFECT OF VITAMIN SUPPLEMENT (BIOTIN AND THIAMINE) AND A TEMPERATURE INCREASE FROM 27 TO 31·5°C ON MEAN ROOT FRESH WEIGHT (MG) OF FOUR STRAINS OF *A. THALIANA*

$n = 5$

Strain	No Vitamins		Plus Vitamins	
	27°C	31·5°C	27°C	31·5°C
EST	411	161***	501	168***
Pi	252	302 (n.s.)	412	371 (n.s.)
BLA	67	75 (n.s.)	239	118*
1018/6	284	92***	524	215***

* $P < 0.05$.   *** $P < 0.001$.   n.s., not significant. Least significant differences for 5, 1, and 0·1% levels are 114, 152, and 197 mg, respectively.

in Pi as affecting shoot growth; there occurs "a breakdown of leaf growth . . . . .; in place of the second leaf pair are rudimentary undifferentiated structures (0·3 mm long); subsequent leaves are absent." Similar conclusions from a comparison of the growth of whole plants and excised roots of the biotin-requiring mutant BLA are less definite because the growth of excised roots of this mutant without vitamins (Table 6) was small in comparison with the variability of the experiment. However, the high sensitivity to supra-optimal temperatures of whole plants of this strain in the absence of biotin (Langridge and Griffing 1959; Langridge 1965) also seems to be absent in excised roots (Table 6).

(b) Evidence for an Increase in the Vitamin Requirements of Excised Roots of A. thaliana
at 31·5°C

The criteria used by Langridge and Griffing (1959) to detect the "chemical repair", by vitamins, of high-temperature lesions in whole plants of *A. thaliana* may be restated as follows:

(1) the increase in temperature (from an optimal to a supra-optimal level for growth) must, in the absence of a vitamin supplement, induce a significant reduction in growth, i.e. a high-temperature lesion must exist;

(2) at the supra-optimal temperature the addition of vitamins (or other supplement) must induce a significant increase in growth;

(3) the *relative* stimulatory effect on growth of this supplement at the supra-optimal temperature must be greater at the higher than at the lower optimal temperature.

These criteria can be applied to the growth responses of excised roots of the four strains of *A. thaliana* to a temperature increase and to the addition of a vitamin supplement.

Criterion (1) applies to the response of root growth of strains EST and 1018/6 only (Table 6); no high-temperature lesion for root growth was detected in BLA and Pi. Criteria (2) and (3) apply only to strain 1018/6: at 31·5°C the addition of biotin and thiamine significantly ($P < 0·05$) increased the mean root growth from 92 to 215 mg. The relative effect of this vitamin supplement on growth (taking growth without vitamins as 100%) was 185% at 27°C and 234% at 31·5°C (Table 5). Hence it appears that the vitamin requirement of the roots of this thiamine-deficient mutant (Langridge 1955; Rédei 1965) is greater at the supra-optimal than at the optimal temperature. Thus the excised roots of this mutant (1018/6) possess a high-temperature lesion (Bonner 1957) at 31·5°C that can be repaired by the addition of biotin and thiamine, supplied together. However, the repair is partial, as Langridge and Griffing (1959) and Langridge (1965) reported for BLA and Pi, in that the addition of vitamins at 31·5°C does not completely restore the growth obtained at the optimum temperature of 27°C (Table 6). These growth data for the roots of the mutant 1018/6 are also at variance with those from the whole plants of this genotype (Langridge 1965, Fig. 2), in which the thiamine requirement of whole plants of 1018/6 was only manifest below *c.* 27°C. Above this temperature thiamine (3 mg/plant) was inhibitory. Thus it is apparent, as with strain Pi, that the growth response of excised roots and intact plants of the same genotype to vitamin supplements and supra-optimal temperatures are widely different. A possible explanation of this is that the growth requirements of shoot growth are different from those of the roots.

The demonstration of an increased vitamin requirement of roots of the mutant 1018/6 of *A. thaliana* at 31·5°C (Table 5) is similar to the results of Hutner *et al.* (1957) for *Ochromonas malhamensis*, which showed that the thiamine requirement of this unicellular flagellate alga, grown heterotrophically, "rose steeply" above 35·5°C. Also, Ketellapper (1963) demonstrated that a vitamin B mixture (which included thiamine) counteracted the effects of high temperatures on growth in *Lupinus nanus* and *Pisum sativum*.

V. Acknowledgments

VI. References

Bonner, J. (1957).—The chemical cure of climatic lesions. *Engng Sci. Mag.* **20**, 28.

Hutner, S. H., et al. (1957).—Growing *Ochromonas malhamensis* above 35°C. *J. Protozool.* **4**, 259.

Ketellapper, H. J. (1963).—Temperature-induced chemical defects in higher plants. *Pl. Physiol., Lancaster* **38**, 175.

LANG, A. (1963).—Achievements, challenges, and limitations of phytotrons. In "Environmental Control of Plant Growth". (Ed. L. T. Evans.) (Academic Press, Inc.: New York.)

LANGRIDGE, J. (1955).—Biochemical mutations in the crucifer *Arabidopsis thaliana* (L.) Heynh. *Nature, Lond.* **176**, 260.

LANGRIDGE, J. (1957).—The aseptic culture of *Arabidopsis thaliana* (L.) Heynh. *Aust. J. biol. Sci.* **10**, 243.

LANGRIDGE, J. (1963).—Biochemical aspects of temperature response. *A. Rev. Pl. Physiol.* **14**, 441.

LANGRIDGE, J. (1965).—Temperature-sensitive, vitamin-requiring mutants of *Arabidopsis thaliana*. *Aust. J. biol. Sci.* **18**, 311.

LANGRIDGE, J., and GRIFFING, B. (1959).—A study of high temperature lesions in *Arabidopsis thaliana*. *Aust. J. biol. Sci.* **12**, 117.

NEALES, T. F. (1968).—The nutritional requirements of excised roots of three genotypes of *Arabidopsis thaliana* (L.) Heynh. *New Phytol.* **67** (1), 159.

RÉDEI, G. P. (1965).—Genetic blocks in the thiamine synthesis of the angiosperm *Arabidopsis*. *Am. J. Bot.* **52**, 834.