Planta (Berl.) 103, 126–128 (1972)

A Specific Bioassay for the Inhibition of Flowering

JENNET BLAKE
Wye College (University of London), Ashford, Kent, U.K.

Received October 18/November 8, 1971

Summary. A bioassay for the inhibition of flowering involving the *in vitro* culture of excised, partially-induced, apices of *Viscaria candida* is described. This bioassay has been used to detect flowering inhibition in extracts from *Kalanchoe blossfeldiana*.

Introduction

The search for the natural substances promoting and inhibiting flowering in plants has been considerably handicapped by the lack of a specific assay for such substances. This paper describes a bioassay for the detection of natural flowering inhibition in *Kalanchoe blossfeldiana* (a short-day plant) using excised apices of *Viscaria candida* (a long-day plant).

Materials and Methods

Viscaria candida is a long-day species (Blake, 1966) which remains indefinitely in the vegetative rosette condition under short-days, and has a minimum induction period depending partly on light intensity. In midsummer flowers can be initiated with an induction period of 3 days of continuous light (8 h natural daylight and 16 h tungsten light in every 24 h), and an increase in the induction period gives an increase in the number of flowers formed (Taylor, unpublished data). In winter or in a growth room the same effect is obtained with a slightly longer induction period.

In the routine assay *Viscaria* seedlings were grown in a soil-less compost for about six weeks under short days of 8 h of natural or artificial light, by which time the plants had about 5 pairs of expanded leaves. They were then given 5 days of induction under continuous tungsten light, supplemented with 8 h of fluorescent light in every 24 h. The terminal apex was then excised and grown in culture under non-inductive (i.e. short-day conditions) for 3–4 weeks. At excision the apices were about 0.5–2.0 mm in height with 2 pairs of leaf primordia.

The culture method was as described previously (Blake, 1969) and used a liquid medium containing sucrose, major and minor nutrients, EDTA, 8-OH-quinoline, gibberellic acid and a range of vitamins[1]. The excised apex was placed

1 Nutrient medium (quantities per litre); sucrose, 20 g; ammonium nitrate, 1.65 g; potassium nitrate, 1.90 g; calcium chloride (dihydrate), 0.44 g; magnesium sulphate, 0.37 g; potassium dihydrogen phosphate, 0.17 g; boric acid, 1.55 mg; manganese sulphate, 5.57 mg; zinc sulphate, 2.15 mg; potassium iodide, 0.21 mg; sodium molybdate, 0.06 mg; copper sulphate, 0.006 mg; cobalt chloride, 0.006 mg; ferrous sulphate, 27.8 mg; sodium EDTA, 75.6 mg; 8-OH-quinoline, 0.3 mg; gibberellic acid, 1 mg; thiamin, 4 mg; nicotinic acid, pyridoxin, calcium pantothenate, riboflavin, biotin, choline, ascorbic acid, adenine, menadione, all at 1 mg.

on a filter paper bridge dipping into 6 ml of medium, and one apex was placed in each 7.5 × 2.5 cm tube, made of borosilicate glass, and covered with polypropylene. 16 replicates were normally used for each treatment.

The treatment was given by adding to the medium various concentrations of aqueous extracts of *Kalanchoe blossfeldiana* tissue (Pryce, 1972) and the entire solution was sterilised by dispensing through a 0.45 μ Millipore filter. After 3–4 weeks in culture the apices were dissected and the state of the apex was recorded as vegetative, transitional or flowering. For analysis the two latter categories were combined and an estimate of the significance of the number of cultures remaining vegetative against those not vegetative was made using chi-square.

Results and Discussion

The assay was developed in experiments in which three treatments containing the basic medium with the addition of (a) no extract (b) leaf extract from short-day treated (flowering) or (c) from long-day treated (vegetative) *Kalanchoe* were normally used. When the extracts were added at varying concentrations in different experiments these did not affect the assay response unless the concentration was so high that growth of the apices was inhibited.

The response to the short-day extract was generally very similar to that where extract was omitted. In all cases the long-day extract was inhibitory to the expression of the flowering response and this is shown in Table 1 which gives a typical set of results. All cultures had produced

Table 1. *The effect of Kalanchoe extract on partially induced apices of Viscaria*

	No extract	Short-day (flowering) extract[a]	long-day (vegetative) extract[a]
Number vegetative	3	4	12
Number transitional	1	0	3
Number flowering	11 } 12	10 } 10	1 } 4
Probability level of significant difference[b]	$P = 0.05$	$P = 0.05$	$P = 0.01$
Mean number of expanded leaf pairs present at dissection	4.2	4.1	3.8

[a] Each replicate tube contained the equivalent of 1.22 gm fresh weight of *Kalanchoe* leaves.

[b] Significant difference from that expected, using chi-square test.

approximately the same number of expanded leaves by the time of dissection indicating that there was no growth inhibition of the apices.

Although this assay takes four weeks to carry out, it has several distinct advantages; (1) the flowering inhibition is being directly assayed

and not assessed through some other inhibitory response as, for example, a germination or hypocotyl test; (2) compared with most other long-day species *Viscaria* has an induction period which is comparatively short, yet long enough to obtain reproducible partial induction; (3) *Viscaria* plants can be grown to provide uniform assay material which can be cultured under highly controlled conditions with a large number of replicates occupying a small area.

The use of this assay to isolate gallic acid as a flowering inhibitor is described elsewhere (Pryce, 1972).

This work was carried out under a grant given to Professor W. W. Schwabe by the Science Research Council. I am most grateful to the Council for their support, and also to Professor Schwabe who suggested the project and gave encouragement during its execution. I am also indebted to Miss Gillian Russell for her skilled technical help. The Agricultural Research Council provided experimental facilities.

Note. In the papers Blake (1966 and 1969) a reference was given to Murashige and Skoog (1962). This should have read: Linsmaier, E. M. and Skoog, F.: Organic growth factor requirements of tobacco tissue cultures. Physiol. Pl. **18**, 100—127 (1965).

References

Blake, J.: Flower apices cultured *in vitro*. Nature (Lond.) **211**, 990–991 (1966).

— The effect of environmental and nutritional factors on the development of flower apices cultured *in vitro*. J. exp. Bot. **21**, 113–123 (1969).

Pryce, R. J.: Gallic acid—its identification as a natural inhibitor of flowering from *Kalanchoe blossfeldiana*. Phytochem. In press (1972).

Dr. Jennet Blake
Wye College
University of London
Near Asford, Kent, U.K.