SHORT COMMUNICATIONS

FLOWERING IN *PISUM*. THE EFFECT OF COTYLEDON REMOVAL ON GENOTYPES *lf E Sn hr* AND *lf e Sn hr**

By I. C. MURFET†

Abstract

Photoperiod does not affect the flowering node of intact plants of the early pea genotypes *lf e sn hr* and *lf E Sn hr* but cotyledon removal converts *lf E Sn hr* into a photoperiodically sensitive form flowering with the late genotypes under short days. Cotyledon removal lowered the flowering node in late plants of genotype *Lf e Sn hr* and *lf e Sn hr* but this effect was thought to be indirect. The particular *lf e Sn hr* variety used normally gives a large proportion of early plants. Removal of one cotyledon significantly lowered the percentage of early plants (from 46 to 9%) and with both cotyledons removed all plants were late. These results demonstrate that gene *E* and the penetrance modifiers of *Sn* are operative in the cotyledons and that *Sn* is active in the shoot under short days. They show that the cotyledons of both early and late lines of peas act as a source of flower promoter and are consistent with the proposal that *Sn* forms a flower inhibitor and that flowering in *Pisum* is determined by the balance between inhibitor and promoter.

Introduction

Evidence for a flower promoter in the cotyledons of early peas has been brought forward by Haupt (1952, 1957, 1958, 1969) and he suggests, along with Köhler (1965) that control of flowering in *Pisum* may be explained without postulating a flower inhibitor. In contrast several other workers (Paton and Barber 1955; Sprent and Barber 1957; Barber 1959; Johnston and Crowden 1967; Amos and Crowden 1969) claim evidence for a flower inhibitor in the cotyledons of late varieties. Murfet (1971*c*) found evidence from grafting studies of both a promoter and an inhibitor and suggested that flower initiation is determined by the balance between these two substances. Genetic control of flowering in peas is largely determined by the interaction of four major genes *Lf*, *E*, *Sn* (Murfet 1971*a*, 1971*b*), and *Hr* (Murfet 1973). It is suggested that *Sn* is responsible for the formation of an inhibitor in the cotyledons and shoot, that *E* lowers the level of inhibitor in the cotyledons, that *Lf* increases the sensitivity of the apex to inhibitor, and that *Hr* blocks the diminution of *Sn* activity which seems to occur with age. The *Sn* gene confers the ability to respond to photoperiod and genotype *lf e Sn hr* is a quantitative long-day plant.

Two genetically different early varieties are used in the present experiments. They react in different ways to cotyledon removal and their behaviour adds support

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† Botany Department, University of Tasmania, Box 252C, G.P.O. Hobart, Tas. 7001.

to the model outlined above. In addition the possible site of action for the penetrance modifiers of *Sn* (Murfet 1971*b*) has been tested by removing the cotyledons of a low penetrance late line. The delicately poised threshold situation in this line also allows us to obtain some experimental evidence of a flower promoter in the cotyledons of a late pea, something which was assumed by Murfet (1971*c*).

Materials and Methods

Growing techniques were as described by Murfet (1971*c*). For long days the natural photoperiod of 14–15 hr was extended to 18 hr by light from a mixed incandescent–fluorescent source giving an intensity of about 430 lux at plant height. Short-day treatments received 8 hr of natural light. Night temperatures were in the range 15–20°C and day temperatures mostly between 20–28°C. Flowering node refers to the node of first initiated flower counting from the cotyledons as zero. For the character nodes expanded (NE) a leaf was considered expanded when it reached stage 0·8 on the scale of Maurer *et al.* (1966). Only mainshoots were used in the analysis. In experiment 1 the cotyledons were excised on the fifth day from the start of germination for line *58* and the sixth day for lines *60*, *61a*, and *24*. In experiment 2 the cotyledons were excised on the fourth day. These times correspond to approximately the same developmental stage (plumule 10–15 mm), the variation arising from differences between varieties in experiment 1 and a higher germination temperature in experiment 2.

Line *58* has genotype *lf e sn hr* and phenotype ED (early developing) and line *60* has genotype *lf E Sn hr* and phenotype EI (early initiating). These two varieties both initiate flower buds at an early node but in the case of line *60* development of the lower flower buds is suppressed by short days. Line *24* (*Lf e Sn hr*) is a typical late variety (phenotype L) developed directly from cultivar Greenfeast by several generations of single plant selection. Line *58* is descended indirectly from cultivar Massey. Although lines *24* and *58* are phenotypically similar to cultivars Greenfeast and Massey respectively similarity of genotypes cannot be assumed. Further information on these lines and the system of phenotypic classification is given by Murfet (1971*a*, 1971*b*). Line *61a*, described for the first time here, is a white-flowered dwarf of genotype *lf e Sn hr* developed from Cross 57 (Murfet 1971*b*). This genotype is normally late but penetrance of *Sn* in terms of flowering node is subject to polygenic modification and line *61a* has been selected for a polygenic background giving 40–50% of EI plants. The phenotypic segregation into EI and L types does not represent a genetic difference as line *61a* should be fairly pure after seven generations of self-fertilization and single plant selection.

Results and Discussion

Cotyledon removal has led in several cases to a qualitative difference in behaviour suggesting a direct effect on the flowering process. Decotyledonized line *60* plants respond to photoperiod like late plants, flowering in the late region under short days (6–8 node delay in short days) whilst the flowering node of intact line *60* plants is unaffected by photoperiod (Tables 1, 2). These results show that gene *E* operates in the cotyledons, that *Sn* is active in the shoot under short days, and that the cotyledons are primarily responsible for the early flowering of line *60*. The decotyledonized line *60* plants were not significantly later than the intact controls under long days (Table 2) which suggests that *Sn* is inactive under long days and this point is now being examined in a specific study. The proportion of line *61a* plants flowering in the early region falls from 46% in intact plants to zero in plants from which both cotyledons were removed (Table 3) showing that the penetrance modifiers of *Sn* are operating in the cotyledons and that the stimulus for early flowering is coming from the cotyledons. The results for lines *60* and *61a* agree with the proposal (Murfet 1971*c*) that both early and late cotyledons supply flower promoter, the difference between

cotyledons of genotype *lf e sn hr*, *lf E Sn hr*, and *lf e Sn hr* depending on increasing levels of inhibitor in the order given. They also support Haupt's (1957, 1969) view

TABLE 1

EFFECT OF COTYLEDON EXCISION ON NODE OF FIRST INITIATED FLOWER (FI) IN FOUR PEA VARIETIES

The data are from experiment 1 in which the photoperiod was 8 hr and the cotyledons were removed at 5 days for line *58* and 6 days for lines *60*, *61a*, and *24*. $\bar{x}$ is the mean node of first initiated flower, S.E. the standard error, and *n* the number of plants

Variety	Character	Intact control			One cotyledon removed			Both cotyledons removed		
		$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*
Line *58*	FI	9·78	0·10	18	9·86	0·10	14	10·18	0·15	17
Line *60*	FI	10·83	0·06	36	11·00	0·17	34	16·75	0·23	36
Line *61a*	FI	20·23	1·10	35	23·74	0·61	35	21·24	0·25	34
Line *24*	FI	24·06	0·31	16	24·06	0·37	16	22·94	0·52	17
Line *61a**	FI	26·00	0·25	19	24·72	0·29	32	21·24	0·25	34
Line *61a**	NE†	24·79	0·22	19	23·47	0·19	32	18·44	0·19	34

* Data for late plants only—see Table 3. † Number of nodes expanded at 8 weeks.

TABLE 2

INTERACTION OF COTYLEDON REMOVAL AND PHOTOPERIOD ON NODE OF FIRST INITIATED FLOWER IN LINE *60*

The data are from experiment 2 using 18 plants per treatment. The cotyledons were removed at 4 days

Treatment	Long day (18 hr)		Short day (8 hr)	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Intact control	11·67	0·18	11·39	0·14
Both cotyledons removed	12·17	0·19	20·56	0·39

TABLE 3

DISTRIBUTION OF NODE OF FIRST INITIATED FLOWER FOR LINE *61a*

The data are from experiment 1 in which the photoperiod was 8 hr and the cotyledons were removed at 6 days

Treatment	Node of first flower																	
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Intact control*	4	7	2	1	2								1	5	8	4	0	1
One cotyledon removed*		2	1								1	6	12	4	3	3	3	
Both cotyledons removed								1	13	9	3	4	4					

* Penetrance: control 0·54 and one cotyledon removed 0·91; difference significant at the 0·001 level.

that early cotyledons supply promoter and indeed the behaviour of line *60* bears a marked resemblance to that reported by Haupt for the early cultivar Kleine Rheinländerin. Since the results for line *58* are in agreement with those reported for cultivar

Massey by Johnston and Crowden (1967), the difference between the German and Australian results for early varieties is probably due to the use of different genotypes.

The very large response to removal of a single cotyledon in line *61a* (Table 3) compared with the complete lack of response to this treatment in line *60* (Table 1) emphasizes the underlying genetic and physiological differences between the phenotypically similar EI plants of the two lines. On the other hand the phenotypically dissimilar EI and L plants of line *61a* are thought to be fairly similar physiologically since they have the same genotype. The wide difference in flowering node is probably caused by the threshold nature of the flowering process and the balance of flowering hormones is seen as varying in a continuous manner from plant to plant but poised closely around the threshold at the time nodes 12–16 are being laid down in intact line *61a* plants. The balance of flowering hormones at the apex depends on the flow from two sources; under short days the supply from the shoot is definitely against flowering as a result of inhibitor produced by activity of gene *Sn*, whereas the cotyledons are supplying promoter and inhibitor in a proportion which just achieves a net apical balance in favour of flowering in many intact plants. Removing one cotyledon increases the relative contribution from the shoot and the balance is tipped against flowering in almost every case. The balance emerging from the line *60* cotyledons is more strongly in favour of flowering and the inhibitor from the shoot tissue can only maintain a balance against flowering when both cotyledons are removed.

There are two examples in which cotyledon removal has led to statistically significant quantitative changes. Firstly, removal of both cotyledons has caused a 0·4 node increase in the flowering node of line *58* (increase significant at the 0·05 level). Such a small difference could well arise indirectly and it is more important to note that cotyledon removal has had a relatively minute effect on line *58* compared with lines *60* and *61a*. The early flowering of decotyledonized line *58* plants under short days is to be expected since the shoots lack dominant *Sn*. Secondly, with the late plants of line *61a* removal of one cotyledon has promoted flowering by one node and removal of both cotyledons has promoted flowering by five nodes. These differences are significant at the 0·01 and 0·001 levels respectively. (Decotyledonized plants of line *24* are also earlier but the effect is not statistically significant.)

A reduced flowering node in late varieties as a result of cotyledon removal has been reported by other workers, e.g. Paton and Barber (1955), Moore (1964), Sprent (1966), Johnston and Crowden (1967), Amos and Crowden (1969), who have mostly interpreted the effect as evidence for a flower inhibitor in late cotyledons. This interpretation now seems untenable. Haupt (1954, 1956, 1969) and Köhler (1965) suggest the effect may be indirect, arising from a change in growth pattern and their view is supported here by the parallel reduction in growth rate and flowering node of decotyledonized plants (compare the last two rows of Table 1). In addition the major opposition to early flowering in line *61a* comes from the shoot not the cotyledons. Finally the cotyledons exert little influence in *lf* shoots after formation of the 15th or 16th node (Murfet 1971*c*). Under short days flowering seems to depend on the phasing-out of *Sn* activity with age and treatments which slow the growth rate may accelerate the aging process relative to the number of nodes or leaves formed. This may wholly or partly account for the reduced flowering node in the decotyledonized plants.

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