Theor Appl Genet (1988) 75:344–349

Phytotron experiments in *Pisum*

2. Influence of the photoperiod on the flowering behavior of different genotypes *

W. Gottschalk

Institut für Genetik der Universität Bonn, Kirschallee 1, D-5300 Bonn, Federal Republic of Germany

Received July 2, 1987; Accepted September 2, 1987
Communicated by G. Melchers

Summary. The flowering behavior of 59 *Pisum* mutants and 228 recombinants was studied in the phytotron in four different photoperiods (continuous light, long-day 18/6 h, short-day 12/12 h, extreme short-day 6/18 h). There was no or little difference in the response of the genotypes to long-day and permanent light, whereas great differences were observed between long- and short-day 12/12 h and between the two short-day trials. About half the genotypes tested were unable to survive or to flower in extreme short-day. Some recombinants, however, had an almost normal development under these unfavorable conditions. Gene *fis* controls the photoperiodic reaction of the plants: they are unable to flower in short-day. Gene *fds* negatively influences gene *efr* for earliness: it causes a strong delay of flowering of *efr* recombinants in long-day and suppresses the formation of functionable flowers in short-day. Most of the genotypes tested showed a specific reaction to the four photoperiods different from that of the mother variety and the other genotypes. The practical aim of our phytotron experiments is the preselection of *Pisum* genotypes which might be suited for cultivation in countries with short-day climate.

Key words: *Pisum* mutants and recombinants – Phytotron experiments – Gene-ecology – Photoperiod – Suppressor genes

Introduction

The reaction of different genotypes to specific ecological conditions can reliably be analysed in the phytotron. Only a single environmental factor is altered in different trials, whereas all other conditions remain unchanged. Any differences in the behavior of the genotypes tested must therefore be due to that altered factor.

Since 1980, mutants and recombinants of our *Pisum* collection have been studied under various phytotron conditions. Their reaction to different thermoperiods has already been discussed (Gottschalk 1985). In the present paper, the flowering behavior of a great number of *Pisum* genotypes under the influence of four different photoperiods is described.

The influence of the photoperiod on the flowering behavior of different plant species and the genetic basis of flowering have been intensively discussed by Murfet (1977). Investigations on the photoperiodic reaction of different *Pisum* varieties were carried out by Marx (1974, 1975, 1978). He used his findings for classifying the genotypes as follows:

K type: late in long-day, a modest quantitative delay of flowering in short-day
G type: late in long-day, a dramatic, almost qualitative delay in short-day
I type: insensitive to differences in day length

The mother variety of our mutant material is medium late in long-day and about 10 days later in short-day; thus, it belongs to the K type of Marx's system. The genotypes studied differ considerably with regard to their flowering behavior under the various photoperiodic conditions used. Moreover, distinct mutant genes of the *Pisum* genome were found to control the photoperiodic behavior and the formation of functionable flowers in dependence on the photoperiod.

* This paper is dedicated to Professor Karl-Ernst Wohlfarth-Bottermann on his 65th birthday

Material and methods

The flowering behavior of 59 X-ray and neutron-induced mutants, and of 228 recombinants of the variety "Dippes Gelbe Viktoria" (DGV) of *Pisum sativum* was studied in the phytotron under four different photoperiods. The recombinants are homozygous for several mutant genes; their genetic constitution is known to us. They arose either by crossing mutants with each other (R numbers), by crossing mutants with recombinants (RM numbers) or by crossing recombinants with each other (RR numbers). The following phytotron conditions were used:

Long-day 18/6 h

Photoperiod:	4.00	to	22.00	full light (30 klx)
	22.00	to	4.00	darkness
Thermoperiod:	21.00	to	6.00	15 °C
	6.00	to	10.00	15 °C → 25 °C
	10.00	to	16.00	25 °C
	16.00	to	21.00	25 °C → 15 °C
Humidity:	60%			

Short-day 12/12 h

Photoperiod:	6.00	to	18.00	full light
	18.00	to	6.00	darkness

Thermoperiod and humidity as in the long-day trials

Short-day 6/18 h

Photoperiod:	10.00	to	16.00	full light
	16.00	to	10.00	darkness

Thermoperiod and humidity as in the long-day trials

Continuous light

Thermoperiod and humidity as in the long-day trials

Ten normal developed plants per genotype were evaluated. Many genotypes were repeatedly studied under the same phytotron conditions and no or only small differences of the mean values for the various traits evaluated were found.

Results

The reaction of a gene for earliness and of some genes for apical stem fasciation of the *Pisum* genome was found to be of particular interest with regard to both problems of basic research in the field of interactions between mutated genes and the agronomic utilization of the genotypes tested. The two groups of genotypes are separately discussed in the present paper.

Comparison of long- and short-day

Genotypes with gene efr for earliness. Recombinant R 46C of our collection is homozygous for genes *efr* for earliness and *bif-1* for dichotomous stem bifurcation. According to Murfet (1978), *efr* is identical with gene lf^a of the *Pisum* world collection. R 46C was used for crosses with other mutants in order to produce *efr* recombinants of different genotypic constitutions. The flowering behavior of 58 of them under long- and short-day phytotron conditions is presented in Fig. 1.

Under the long-day field conditions of West Germany, R 46C flowers 7–11 days earlier than its mother variety. A similar behavior was observed in the 18/6 h phytotron trials. Many of the recombinants tested showed in the phytotron the same flowering behavior as R 46C, demonstrating that the respective other mutant genes present in their genomes do not influence *efr* in its action. Quite a number of *efr* recombinants, however, entered flowering period later or considerably later. RM 865B, for instance, flowered 14 days later than R 46C due to the negative influence of one of its mutant genes on *efr*. Some of the *efr* genotypes show a very specific behavior and are separately discussed (Fig. 2).

In the short-day trials with only 12 h light per day, almost all the genotypes tested needed a longer time to reach the flowering stage due to the lower amount of light available for photosynthesis. The differences between the long- and short-day values are given in Fig. 1. Some of the *efr* recombinants showed no or only small differences in the two trials; RM 869 flowered in short-day even earlier than in long-day. Most of the genotypes studied, however, were in short-day considerably later than in long-day. The findings clearly

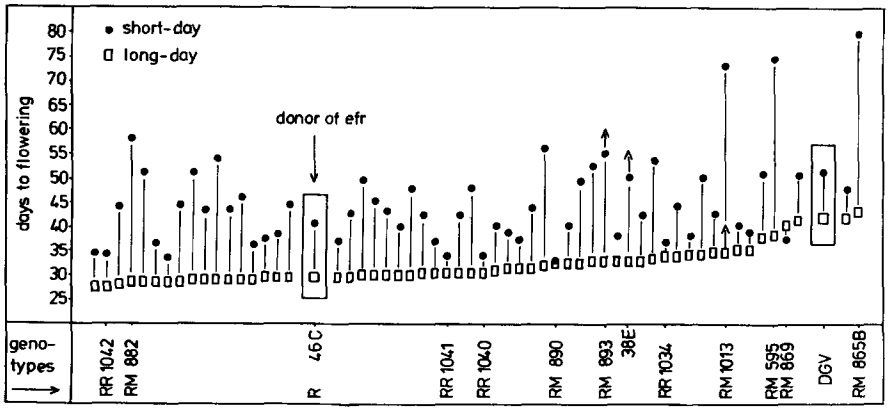

Fig. 1. The flowering behavior of 58 different *efr* recombinants under long- and short-day phytotron conditions (18/6 h and 12/12 h). Each dot represents the mean value of 10 plants. The means are ordered according to the reaction of the genotypes in long-day. They are compared with those of recombinant R 46C, the donor of gene *efr* for earliness, and with the control values of the mother variety "Dippes Gelbe Viktoria" (DGV)

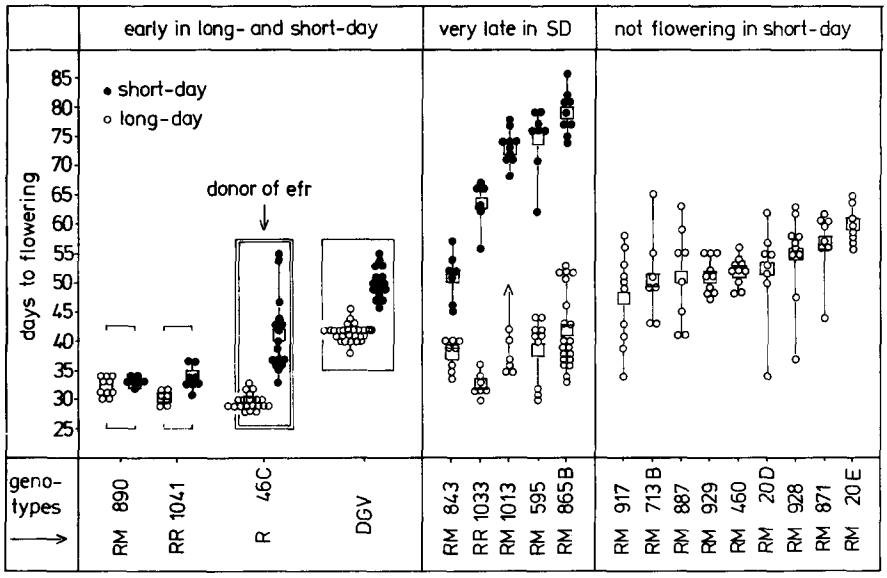

Fig. 2. The flowering behavior of 16 *efr* recombinants under long- and short-day phytotron conditions as compared with R 46C, the donor of gene *efr* for earliness, and with the mother variety DGV. Each dot gives the value for one plant; the squares are the mean values for the respective genotypes

demonstrate that the different *efr* genotypes investigated reacted very specifically to the two photoperiods: each genotype showed its specific response, although all of them are homozygous for gene *efr*. This holds true not only with regard to the beginning of flowering, but also in regard to other criteria evaluated (Gottschalk 1987).

The 73 different *efr* recombinants tested can be subdivided into three groups as follows:

Group I: early in long- and short-day;
Group II: later in long-day, very late in short-day;
Group III: late in long-day, not flowering in short-day.

Representatives of these groups are considered in Fig. 2.

Recombinant R 46C, the donor of gene *efr*, shows approximately the same difference between long- and short-day reactions as the mother variety. The plants of RM 890, however, show a certain tolerance against the lower amount of light: there was practically no difference in the flowering behavior in the two photoperiods. One of the genes present in its genome influences *efr* positively in short-day, resulting in the higher degree of earliness as related to R 46C.

The 5 *efr* recombinants of the second group are already in long-day later than R 46C, whereas they are so late in short-day that they would be without any agronomic value in countries with short-day climate. The delay, as compared to R 46C, was 4 to 5 weeks in recombinants RM 1013, RM 595 and RM 865B. Thus, the effect of *efr* gets completely lost through a mutant gene present in the genome of the genotypes of group II. Its negative effect on *efr* appears in short-day considerably stronger than in long-day. The suppressor gene has not yet been identified; it has provisionally been designated as *fds* (flower development suppressor; Gottschalk 1983). Under its influence, only minute flower buds are formed during the first half of the flowering period which do not undergo any further development. Fully developed flowers are produced in later ontogenetic stages.

The 9 *efr* genotypes of group III were found to flower in long-day even later than the recombinants of the second group. Their specific peculiarity, however, consists in the fact that they are unable to produce functionable flowers in short-day. Only the minute flower buds just mentioned are formed. This reaction is again due to the suppressor gene. So far, 15 *efr* recombinants of our collection were found to show this short-day reaction. They do not have a specific discernible gene in common. It obviously has no visible effect and only becomes discernible under distinct photoperiodic conditions. The differences in its negative influence on *efr*, i.e. the different degrees of lateness of the respective recombinants of groups II and III, are obviously due to differences in their genotypic constitution.

Fasciated genotypes. The high-yielding fasciated mutants are useless for a cultivation in countries with short-day climate: they do not flower under these ecological conditions, or they are so late that their seeds do not ripen before the plants die. These genotypes are homozygous for more than 20 mutant genes, most of them being identical in the genotypes of this group. They have been used for producing many different fasciated and non-fasciated recombinants. The flowering behavior of 18 fasciated genotypes under long- and short-day phytotron conditions is given in Fig. 3.

Five of the fasciated mutants studied were considerably later in the long-day phytotron trials than under long-day field conditions. They began flowering 4 to 6 weeks later than the control material, whereas they are only a few days later in the field. In the short-day trials, they remained vegetative. When the phytotron conditions were changed from short- to long-day, the plants produced flower buds 8 to 10 days later. This is not only a confirmation of our findings obtained in some African countries, in Brazil, and at different locations in India, but it is evidence that these mutants have a gene controlling their photoperiodic reaction. They need long-day to produce flowers. This gene has been designated as *fis* (flower initiation suppressor; Gottschalk 1981).

It would be very useful if the stem fasciation, combined with good seed production, could be utilized in short-day countries. Therefore, 125 different fasciated recombinants were tested under short-day phytotron conditions with regard to their flowering behavior. As expected, they began flowering later than in long-day, but there were strong differences between the genotypes in this respect (middle part of Fig. 3). Recombinant R 657, for instance, showed the same flowering behavior in the two photoperiods, whereas differences of up to two weeks were observed in other genotypes.

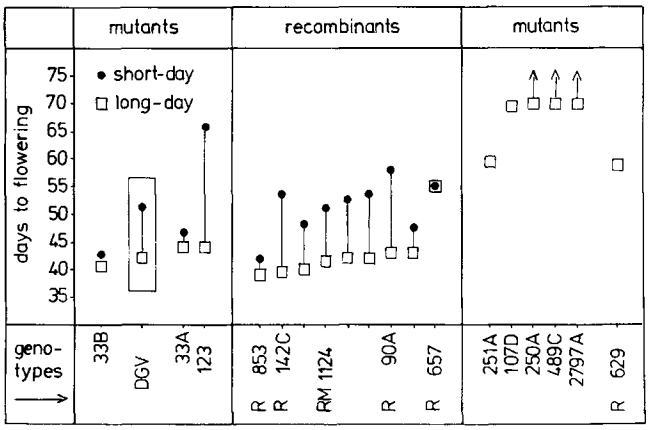

Fig. 3. The flowering behavior of eight fasciated *Pisum* mutants and 10 fasciated recombinants under long- and short-day phytotron conditions. The six genotypes of the third group did not produce any flowers in short-day

The flowering behavior of 33 fasciated recombinants under short-day phytotron conditions is given in Fig. 4. Almost half of them flowered simultaneously with the mother variety, or even earlier. All these genotypes derive from fasciated mutants, which do not flower in short-day due to the presence of gene *fis* (Fig. 3). This gene has been removed through crosses with other mutants. Some of them were found to be high-yielding in Germany and are currently being tested in India with regard to flowering and seed production.

Comparison of long-day and continuous light

Fifty-two mutants and recombinants of our collection were comparatively studied under long-day phytotron conditions 18/6 h and at continuous light. Thirty-five of these genotypes are homozygous for gene *efr* for earliness; they are compared with recombinant R 46C, the donor of *efr*, in Fig. 5. Twenty of them did not differ in their flowering behavior under the influence of the two photoperiods. Most of the others showed only very small differences in that they entered the flowering period at continuous light a few days earlier than in long-day. This also held true for R 46C and the mother variety. The same reaction was observed in 17 other genotypes, 13 of them containing genes for stem fasciation (Fig. 6).

It can be concluded from these results that the additional amount of light has no, or only a very small, effect on the flowering behavior of most of the genotypes tested. Strong differences were found in only a few cases. The respective genotypes flowered at con-

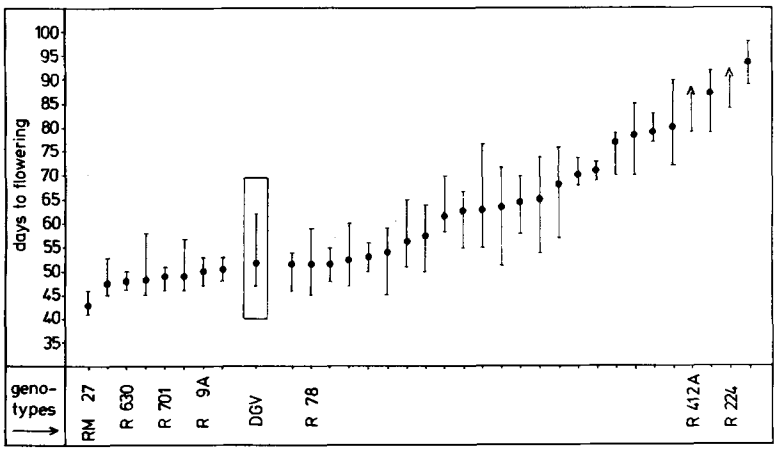

Fig. 4. The flowering behavior of 33 fasciated *Pisum* recombinants under short-day phytotron conditions. Some of them flowered as early as the mother variety DGV or even earlier. The genotypes considered in the graph were not yet tested in long-day

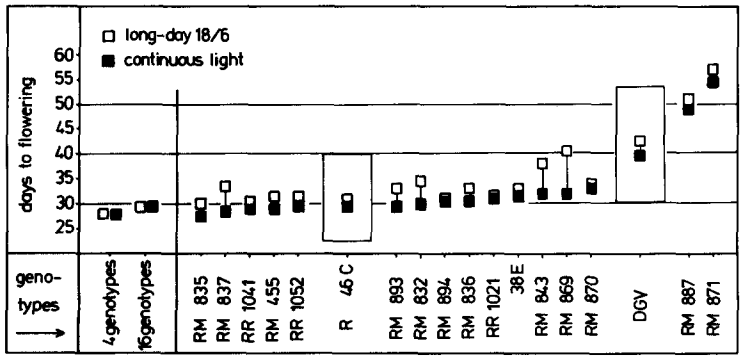

Fig. 5. The flowering behavior of 35 different *Pisum* recombinants, homozygous for gene *efr* for earliness in combination with other mutant genes, under long-day phytotron conditions and at continuous light. The genotypes are compared with the mother variety DGV and with recombinant R 46C, the donor of gene *efr*. Mutant 38E is a double mutant homozygous for *efr* and a gene for long internodes

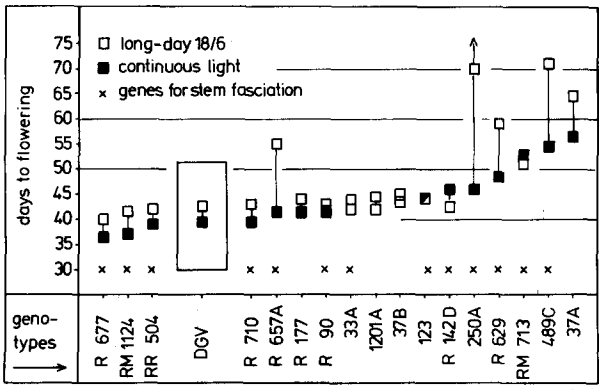

Fig. 6. The flowering behavior of seven *Pisum* mutants and 10 recombinants under long-day phytotron conditions and at continuous light as compared with that of the mother variety DGV. Many of the genotypes tested have genes for stem fasciation

tinuous light 3 to 4 weeks earlier than in long-day. If we compare these findings with the short-day results, it becomes clear that we have to interpret them not in the sense of a positive reaction to continuous light; on the contrary, it is a negative reaction to lower amounts of light:

24 h light: late (1 or 2 weeks later than DGV);
18 h light: very late (3 or 4 weeks later than DGV);
12 h light: not flowering.

It should be mentioned that a negative effect of the permanently given light on the flowering behavior could not be observed in any of the 53 genotypes studied.

Comparison of normal and extreme short-day

Three mutants and 29 recombinants were studied under extreme short-day conditions with only 6 h light per day. Only three of the 20 control plants of DGV reached the flowering stage; all the others died as a consequence of insufficient amount of light. Thus, the initial line shows a very low degree of tolerance against these unfavorable ecological conditions. Two mutants and five recombinants showed a similar behavior. In another group of six recombinants, some flower buds appeared, but they did not develop into functionable flowers. The fasciated mutant 489C and recombinant R 142F were still in a vegetative state when the trial was terminated 83 days after sowing. At that time, some *efr* genotypes already had fully ripened seeds.

Out of the 33 genotypes tested, only 17 flowered to some extent, but not all plants reached the flowering stage. In Fig. 7 their mean values are compared with the means obtained under the influence of the other three photoperiods used. The material is subdivided into two groups according to the presence or absence of gene *efr* for earliness. Recombinant R 46C, the donor of this gene, reacted as negatively as its mother variety. A completely different behavior was observed for the recombinants RM 840, RM 427 and RM 430. Their ontogenetic development was relatively normal and they produced small amounts of seed, thus demonstrating a high degree of tolerance to these highly unfavorable photoperiodic conditions. From eight other *efr* recombinants, some plants produced a small number of flowers but died before seed ripening. An almost normal flower formation showed some fasciated recombinants considered in the right hand part of the graph.

The differences between the results of the 6/18 h and 12/12 h short-day trials were, in most genotypes, studied more than those found between the 12/12 h and 18/6 h trials. The low amount of light per day is a strong handicap even for those genotypes which are able to undergo full ontogenetic development until seed ripening. The most interesting result of these experiments, however, is the confirmation that distinct genotypes of our collection reacted very specifically to unfavorable ecological conditions.

The influence of the photoperiod on the flowering behavior becomes particularly clear in Fig. 7, showing the reaction of 17 *Pisum* genotypes to four different photoperiods in the phytotron. From mutant 123 and recombinant R 46C, only three mean values are given

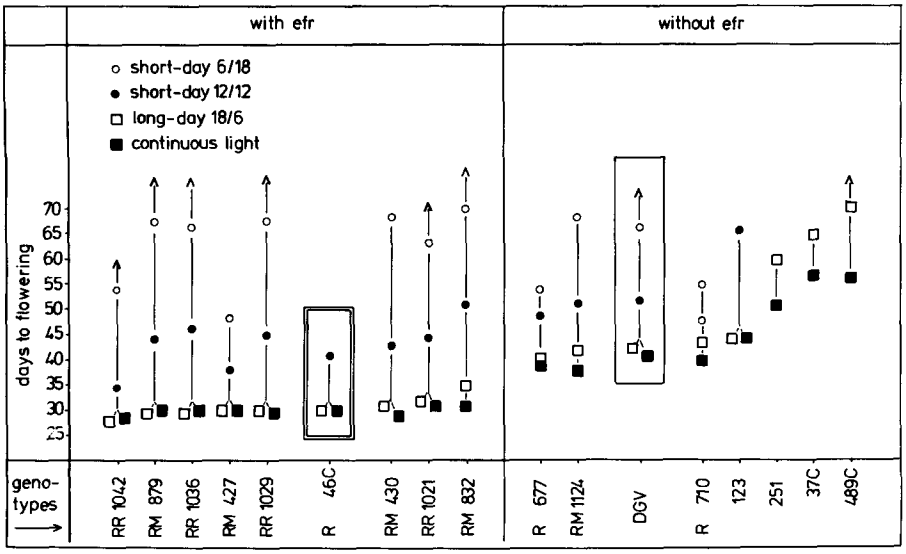

Fig. 7. The flowering behavior of four *Pisum* mutants, 12 recombinants and the mother variety DGV under four different photoperiods in the phytotron. R 46C is the donor of gene *efr* for earliness

because they did not flower in 6/18 h short-day. Mutants 37C, 251A and 489C did not flower in both 12/12 h and 6/18 h short-day. The graph demonstrates the small differences in the reaction to long-day 18/6 h and continuous light, and the strong differences between the long-day and the two short-day values.

Discussion

The main result of our gene-ecological investigations, carried out for more than 15 years with about 350 different genotypes of our *Pisum* collection, is the fact that most of them show a specific reaction to distinct ecological conditions different from that of other genotypes tested. The photoperiod is an especially important environmental factor for judging the selection value of genotypes in all cases in which the material is of agronomic interest for countries with short-day climate. This holds true for the garden pea, which is used for closing the protein gap in many vegetarian Asiatic countries.

Pisum sativum is principally a day-neutral species flowering in long- as well as in short-day, but there are strong differences with regard to the reaction of distinct genotypes to the two photoperiods. Under the influence of specific genes, the transition from the vegetative to the reproductive stage of the plants is suppressed or extremely delayed in short-day; other genes suppress the formation of functionable flowers in short-day. The action of these suppressor genes cannot be discerned in long-day. For discerning the whole breadth of the adaptibility of genotypes to diverse ecological conditions, the material should be tested under as many different environmental conditions as possible. Experience has shown that international cooperation in this field is very difficult, and does not always provide reliable findings. Fortunately, distinct problems of this kind can be studied in the phytotron. This holds particularly true for the dependence of the flowering behavior on different photoperiods. In this way, genotypes which may be of agronomic interest for countries with other climates can be preselected, and tested in the respective countries with regard to their usefulness. An impressive example is the behavior of some of our fasciated genotypes in countries with different climatic conditions. In the frame of these investigations, recombinant R 46C was found to be well suited for cultivation in Central India. Gene *efr* for earliness of this genotype has been used for developing a commercial variety.

References

Gottschalk W (1981) Induced mutations in gene-ecological studies. In: Induced mutations – a tool in plant breeding. IAEA, Vienna, pp 411–436

Gottschalk W (1983) A *Pisum* gene preventing flower development under short-day conditions. Egypt J Genet Cytol 12: 385–396

Gottschalk W (1985) Phytotron experiments in *Pisum* 1. Influence of temperature on the flowering behaviour of different genotypes. Theor Appl Genet 70:207–212

Gottschalk W (1987) The development of *Pisum* genotypes under long- and short-day phytotron conditions. Biol Zentralbl 106:207–218

Marx GA (1974) A P.I. accession showing sensitivity to photoperiod. Pisum Newslett 6:35

Marx GA (1975) Photo-dependent responses in *Pisum:* Impenetrant K type segregants and evidence of a polygene system. Pisum Newslett 7:26–27

Marx GA (1978) Internode length differences associated with differences in flowering response. Pisum Newslett 10: 43–45

Murfet IC (1977) Environmental interaction and the genetics of flowering. Annu Rev Plant Physiol 28:253–278

Murfet IC (1978) The flowering genes *Lf, E, Sn* and *Hr* in *Pisum:* Their relationship with other genes, and their descriptions and type lines. Pisum Newslett 10:48–52