Theor Appl Genet (1985) 70:207–212

Phytotron experiments in *Pisum*

1. Influence of temperature on the flowering behaviour of different genotypes

W. Gottschalk

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

Received October 24, 1984; Accepted December 4, 1984
Communicated by H. F. Linskens

Summary. The flowering behaviour of 17 *Pisum* mutants and 20 recombinants was studied under three different temperatures using long-day phytotron conditions. A constant low temperature of 12.5 °C led to a strong delay in flowering in all the genotypes tested but distinct relative differences could be found between them. Relative differences were also present with regard to speed of ontogenetic development under a permanent high temperature of 25.5 °C or under an alternating change between low and high temperature. Under the low temperature, recombinants R 20D and R 20E, carrying gene *efr* for earliness, entered the flowering period more than 4 weeks later than the donor of *efr*, demonstrating thereby a negative influence of one of the other mutant genes on *efr*. The high temperature of 25 °C influenced the flowering behaviour of 4 fasciated genotypes negatively – in contrast to the other strains studied. The plants of recombinant R 405 produced only tiny flower buds under these conditions. None of the plants of recombinant R 142F flowered under either the constant low or high temperature – they need the change of low and higher temperature for normal flower formation. The experiments show that most of the genotypes tested react specifically to the three temperature conditions offered to them.

Key words: *Pisum* mutants and recombinants – Phytotron experiments – Gene-ecology – Temperature

1 Introduction

Is is well known that mutants often differ from their mother varieties with regard to their adaptation optima to diverse ecological conditions.

This problem has been studied in wheat and garden peas by cultivating the same genotypes in regions with different climatic conditions: almost each genotype was found to have its specific reaction to a given environment. Similar but more precise findings were obtained in barley and peas under controlled phytotron conditions. A great deal of work in this field has been done by Gustafsson and co-workers with early flowering mutants of *Hordeum vulgare* using the Stockholm phytotron (Dormling et al. 1966, 1975; Dormling and Gustafsson 1969; Gustafsson et al. 1973a, b, 1974). We have carried out similar experiments with *Pisum sativum* (Gottschalk 1978, 1981a, b, 1982, 1983; Gottschalk and Kaul 1975, 1980).

In our previous phytotron studies, the influence of photoperiod on flowering behaviour of a great number of *Pisum* genotypes was studied and complicated interactions were observed. In the present paper, the influence of temperature is discussed. Both mutants and recombinants homozygous for several mutant genes were used. In this way it was possible to discern distinct interactions between specific genes in addition to the general reaction of the genotypes to the three temperature conditions offered to them. For obtaining these recombinants, some of our mutants were crossed with fasciated lines homozygous for more than 20 genes which mutated more or less simultaneously in the same embryos during irradiation. The respective F_1 hybrids were highly heterozygous and a great number of different recombinant types arose in the segregating F_2 and F_3 families. They were selected and developed into pure lines.

2 Material and methods

The ontogenetic development, particularly the flowering behaviour, of 17 X-ray induced *Pisum* mutants and 20 recombinants was studied in the phytotron under three different temperatures:

Trial 1: constant temperature of 12.5 °C
Trial 2: constant temperature of 25.5 °C

Trial 3: 9.00 p.m. to 6.00 a.m. 12.5 °C
6.00 a.m. to 10.00 a.m. 12.5 °C ⟶ 25.5 °C
10.00 a.m. to 4.00 p.m. 25.5 °C
4.00 p.m. to 9.00 p.m. 25.5 °C ⟶ 12.5 °C

The plants were grown under long-day conditions with 18 h light (30,000 lux) and 6 h darkness; humidity was about 60%.

Fifteen seeds per genotype were sown in Mitscherlich pots. Two weeks after germination any sickly plants were eliminated and only 10 plants per pot were allowed to continue ontogenesis. The following criteria were evaluated:

number of days to flowering
position of the lowest fully developed flower at the stem
position of the lowest pod
seed production per plant
plant height, internode length, degree of branching
fresh weight and dry matter of the shoot system.

The recombinants used are homozygous for different mutant genes or gene groups and have been studied under field conditions for many generations; their genetic constitution is known to us. The reaction of the mutants and recombinants was compared to that of the variety 'Dippes Gelbe Viktoria' (=DGV) which was used as the initial line for our radiation genetic experiments.

3 Results

The flowering behaviour of 32 *Pisum* mutants and recombinants under the three temperature conditions offered to them is shown in Figs. 1–3. The genotypes are ordered according to their behaviour under "normal" phytotron conditions (12.5 °C during night, 25.5 °C during daytime). The material is subdivided into the following groups, which are discussed separately:

genotypes with gene *efr* for earliness
genotypes with gene *dgl* for leaf degeneration
genotypes with genes *bif-1* and *bif-2* for stem bifurcation
genotypes with apical stem fasciation
other mutants showing a specific flowering behaviour under the phytotron conditions used.

Under the permanently given low temperature of 12.5 °C (trial 1), the control plants of the mother variety needed 77 days for reaching the flowering period – this was 39 days more than under the permanently given high temperature of 25.5 °C (trial 2). This behaviour was in general observed in all the genotypes tested but clear relative differences between some of them and the mother variety were found. Details can be seen from the graphs; they are discussed for the various groups. Most genotypes grown under low night and high daytime temperatures (trial 3) showed a flowering behaviour very similar to that of trial 2. Thus, the ontogenetic development is only influenced a little by the low night temperature provided that a high temperature during the daytime is available. The differences between control material in trials 2 and 3 was only 4–5 days but here also clear differences were found between distinct genotypes.

3.1 Early flowering genotypes

Mutant 46A of our *Pisum* collection, homozygous for gene *efr*, flowers about 10 days earlier than its mother variety under Middle European field conditions. By natural cross-pollination, recombinant R 46C arose homozygous for *efr* and gene *bif-1* causing dichotomous stem bifurcation. This recombinant was used for crosses with different mutants and more than a hundred early flowering recombinant types of different genotypic constitution were selected and developed into pure lines. Seven of them are considered in the present paper (Fig. 1).

The plants of recombinant R 46C, the donor of gene *efr*, reached the flowering period, in the three trials, 62.6, 29.2 and 32.7 days after sowing. The corresponding control values of the mother variety were 76.7, 37.6 and 42.0 days. Figure 1 shows that these relations are altered in some genotypes. The plants of recombinant R 831 (*efr, bif-1, dim*, causing a diminishing of all plant organs), for instance, showed approximately the same flowering behaviour as R 46C in trial 3, whereas they were 6 days earlier in trial 2. In trial 1, however, they were 5 days later than R 46C. Thus, one of the mutant genes, present in the genome of R 831, influences gene *efr* positively under high temperatures whereas this gene, or another one, has a negative effect under low temperature conditions.

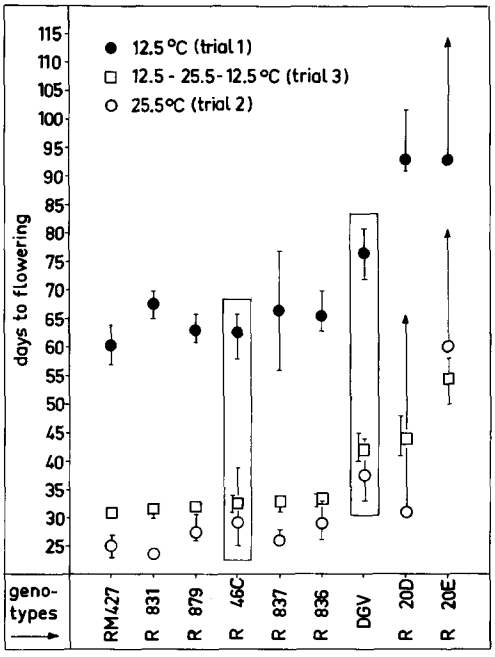

Fig. 1. The flowering behaviour of the mother variety 'Dippes Gelbe Viktoria' (DGV) and of eight recombinant lines containing gene *efr* for earliness and other mutant genes under three different phytotron conditions. Recombinant R 46C is the donor of *efr*. Each dot gives the mean value of 10 plants

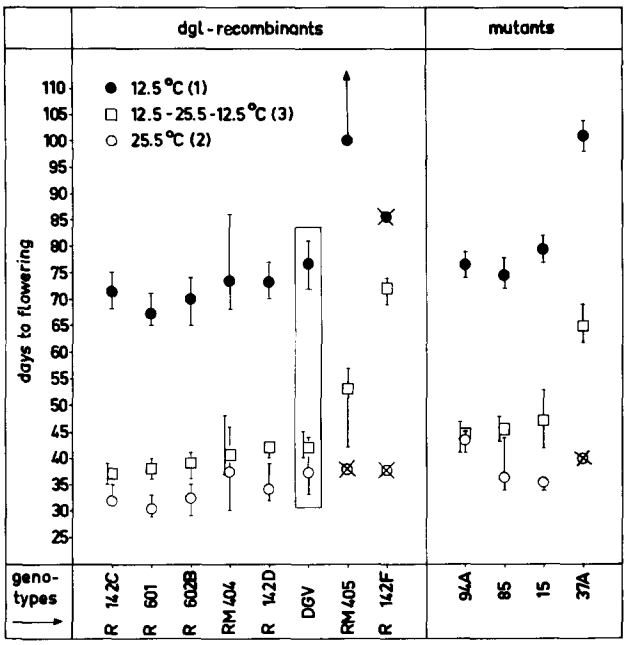

Fig. 2. *Left:* The flowering behaviour of seven recombinants homozygous for gene *dgl* for leaf degeneration and for other mutant genes in the phytotron. *Right:* The behaviour of four different mutants under the same conditions

Recombinants R 20D and R 20E are of particular interest. They were selected after having crossed R 46C with the fasciated mutant 489C homozygous for more than 20 mutated genes. Their genotypic constitution is as follows:

R 20D: *efr* for earliness (from R 46C)
bif-1 for dichotomous stem bifurcation (from R 46C)
ccr for reduced chlorophyll content (from 489C)
long III for very long internodes (from 489C)

R 20E: *efr* (from R 46C)
bif-1 (from R 46C)
short II for short internodes (from 489C).

Even under the relatively normal phytotron conditions of trial 3, they were not only considerably later than R 46C but even later than the mother variety not containing gene *efr* for earliness. This holds especially true for R 20E which formed fully developed flowers about 3 weeks later than R 46C and almost 2 weeks later than DGV. Induction of flower formation occurred at a normal stage of ontogenetic development but only tiny flower buds were produced at the lower nodes of the stem and these did not undergo further development. When grown constantly under 12.5 °C, the plants of R 20D entered the flowering period more than 4 weeks later than R 46C; R 20E was even later. Under the constant temperature of 25.5 °C, the plants of each of these two genotypes showed a very heterogeneous behaviour. The first plant of R 20D began flowering 2 weeks later than R 46C, the last ones had not yet flowered 53 days after sowing, when the trial was stopped. This tendency was even more pronounced in R 20E. The first two plants began flowering 4 and 5 weeks later than R 46C; the remaining 8 had not entered the flowering period when the trial was stopped. The specific reaction of recombinant R 20E becomes clear from Fig. 1: in all the other genotypes tested, the mean value for the trait "number of days from sowing to flowering" was in trial 2 (25.5 °C) lower than in trial 3 (12.5–25.5–12.5 °C). Recombinant R 20E, however, showed the opposite reaction.

3.2 Genotypes with leaf degeneration

The plants of mutant 142B, homozygous for gene *dgl*, are dainty and weak. The gene causes degenerative alterations of stipules and leaflets. These organs become brown and dry, dying during ontogenetic development. Only the leaves in the top region of the plants are a normal green and are able to perform photosynthesis. The seed production is extremely low due to the negative gene action. After having crossed mutant 142B with the fasciated mutant 489C, seven recombinant types, homozygous for *dgl* and for other gene groups, were selected. Their reactions on the three temperature conditions are presented in Fig. 2. Mutant 142 B, the donor of gene *dgl*, could not be included into the phytotron experiments because of a lack of seed material.

Most of the *dgl*-genotypes flowered earlier than the mother variety DGV in all the three phytotron trials. The recombinants R 405 and R 142F, however, showed a very specific reaction. Their genotypic constitution is as follows:

R 405: *dgl* for leaf degeneration (from 142B)
long II for long internodes (from 489C)
a gene for apical stem fasciation (from 489C)
a gene for lateness (from 489C)

R 142F: *dgl* (from 142B)
long III for very long internodes (from 489C)
a gene for a stronger type of lateness than R 405 (from 489C)
a gene for deviating leaf colour (from 489C)

Full flowering of R 405 and R 142F occurred only in trial 3 but considerably later than in all the other genotypes of the group. The plants of R 405 entered the flowering period 11 days, those of R 142F even 30 days, later than the mother variety. Under 12.5 °C, the first two plants of R 405 began flowering more than 3 weeks later than DGV; all the other plants of this genotype had only small flower buds at that time. A mean value for this recombinant cannot be given because the trial was stopped. Under 25.5 °C, tiny flower buds were exclusively formed, none of which showed any further development although the plants were tall and fully developed. Recombinant R 142F showed an even more deviating reaction. There was no induction of flower formation in any of the tall and vigorous plants of this genotype in both these trials, i.e. under the constant low and high temperatures. All their growing points

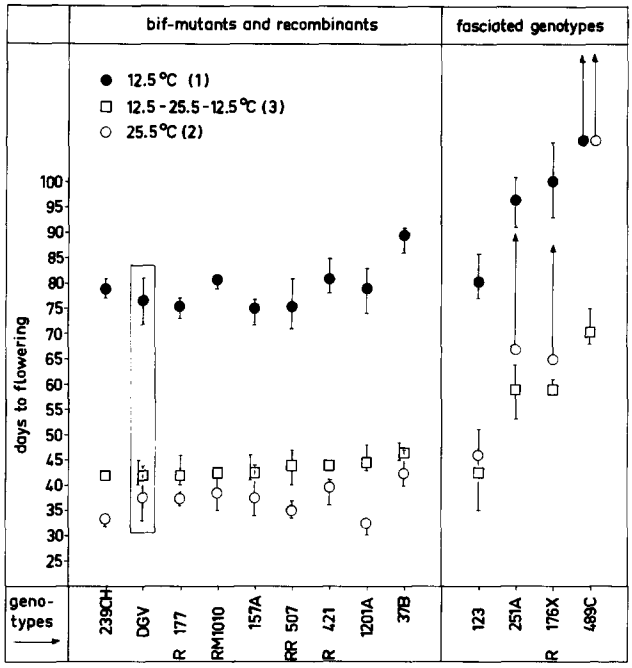

Fig. 3. The flowering behaviour of eight genotypes with dichotomous stem bifurcation and of four genotypes with stem fasciation under three different phytotron conditions

remained in the vegetative state, exclusively producing leaves. Thus, the plants of this genotype need the daily change of low and high temperatures in order to produce flowers. These results show that one of the mutant genes of R 142F specifically controls the reaction of the plants to the temperature conditions. This is certainly not gene *dgl* because all the other genotypes containing this gene do not show the reaction of R 142F.

3.3 Genotypes with stem bifurcation and stem fasciation

Gene *bif-1* causes dichotomous stem bifurcation in the upper part of the shoot. In mutants 239CH and 1201A, the gene shows reduced penetrance. An allele of *bif-1* with full penetrance is present in mutant 37B. Mutant 157A is homozygous for *bif-2* polymeric to *bif-1,* likewise showing reduced penetrance. Mutant 1201A was crossed with some other mutants of our collection giving rise to the bifurcated recombinants considered in Fig. 3.

With regard to the flowering behaviour under the three phytotron conditions used, most of the 8 bifurcated genotypes tested were similar to the mother variety or showed only small differences. The differences between the mean values for the number of days to flowering between trials 2 and 3 were very low in the control material DGV whereas they were much greater in mutants 239CH and 1201A. In these two mutants, the permanent high temperature caused a certain degree of earliness which was not observed in most of the other genotypes studied. The small degree of lateness, on the other hand, observable for mutant 37B in trials 2 and 3, appeared to be much more pronounced in trial 1. The mean values for the three trials were as follows:

DGV: 76.7, 37.6 and 42.0 days
37B: 89.6, 42.6 and 46.6 days.

Thus, the low temperature delays flowering period in mutant 37B more than in the other bifurcated genotypes.

The fasciated mutants 123, 251A and 489C are closely related to each other. They are homozygous for more than 20 mutant genes, most of them being identical in the three genotypes. Recombinant R 176X arose by natural cross-pollination between 489C and the narrow-leafed mutant 176A homozygous for gene *dim.* In the phytotron, the fasciated genotypes tested showed a very abnormal reaction (right side of Fig. 3).

Under the "normal" phytotron conditions of trial 3, mutant 123 was similar in its flowering behaviour to that of the control material DGV. Mutant 251A and recombinant R 176X began flowering more than two weeks, mutant 489C even 4 weeks, later. Under the low temperature of 12.5 °C (trial 1), approximately the same relations between the four genotypes were observed. The trial was stopped a hundred days after seed sowing. At that time, the plants of mutant 489C had only minute flower buds; they would have needed some weeks more for reaching full flowering. Of particular interest, however, is the response of the four fasciated genotypes to the permanent high temperature of 25.5 °C (trial 2). Under these conditions, the plants of mutant 123 needed about 4 days more than in trial 3 for reaching the flowering period. This delay was much more pronounced in 251A and R176X. The trial was stopped 70 days after seed sowing. The first two plants of recombinant R 176X began flowering 65 and 68 days after sowing. Of the 10 plants of mutant 251A, only one entered the flowering period 67 days after sowing. The other plants of these two genotypes and all the plants of mutant 489C had only formed very small flower buds at that stage of ontogenetic development.

The differences in the reaction of the bifurcated and the fasciated genotypes become clear in Fig. 3 if we compare the relative location of the mean values for trials 2 and 3. All the *bifurcated* genotypes reached the flowering period under the permanent high temperature earlier than under the combination of high daytime and low night temperature. In the four *fasciated* genotypes, however, the opposite behaviour is realized. Thus, the permanent temperature of 25.5 °C negatively influences the flowering behaviour of the four fasciated genotypes whereas it has a positive influence not only on the bifurcated but also on most of the other genotypes tested. This negative influence, however, is not combined with stem fasciation in general. It was not

observed in recombinants R 142C, R 142D, R 601 and R 602B homozygous for *dgl* and for genes causing stem fasciation (left side of Fig. 2).

3.4 Other mutants

In addition to the genotypes already discussed, nine other mutants were studied, most of them showing in principle the same reactions as their mother variety DGV. Four mutants with somewhat diverging behaviour are considered in the righthand part of Fig. 2. In the weakly fertile flower mutant 94A, the mean values of trials 2 and 3 are equal. Thus, the permanent high temperature does not lead to the more rapid ontogenetic development expected which would result in an earlier beginning of flowering. Of particular interest is mutant 37A, a micro-mutant morphologically similar to its mother variety. Under the "normal" phytotron conditions of trial 3, the plants were very late (beginning of flowering more than 3 weeks later than in DGV). A similar behaviour was observed in trial 1. In trial 2, however, the induction of flower formation occurred at such a late stage of ontogenesis, that only minute flower buds had been formed about 10 weeks after sowing when the trial was stopped. Thus, the permanent temperature of 25.5 °C influences the flowering behaviour of this mutant very negatively.

4 Discussion

For the present phytotron studies, a small number of radiation – induced *Pisum* mutants and recombinants was used. They show a relatively narrow genetic diversity as compared to the genetic differences existing between the agronomically utilized pea varieties or strains and the natural genetic variability of the species *Pisum sativum* with its genetically very divergent subspecies. It is therefore surprising that so many different reactions to the three different temperatures used were observed within this small group of genotypes. Almost each genotype studied shows its specific reaction to the phytotron conditions offered to it. This is in agreement with the results of many other gene-ecological investigations which have been carried out with our material for about 15 years in countries with different climates as well as under varying conditions in the phytotron. This holds not only true with regard to the flowering behaviour discussed in the present paper but also with regard to such other agronomically interesting traits as ripening time, seed production, and to some extent seed protein content and even amino acid composition of the proteins.

It can be concluded from these experiences that many mutants of different crops, available in our collections and being without any practical value, could be utilized in countries with distinct climatic conditions. As their usefulness has only been tested under that climate in which they were selected, no information on the breadth of their ecological adaptability is available. Interesting examples are some mutants of our *Pisum* collection. The early flowering mutant 46A, homozygous for gene *efr*, is without any interest for German pea breeding because of its reduced seed production. In India, gene *efr* was used for developing a commercial variety not only because of the pronounced degree of earliness but also because of a certain degree of drought resistance of the mutant. This specific peculiarity, however, cannot be discerned under the climatic conditions of West Germany. The plants of mutant 142B, containing gene *dgl* for leaf degeneration, are semilethal in Germany, most of them not producing any seeds. In Egypt, the gene was found to be unable to express its negative action; the plants grow normally having full seed production. Mutants 1201A and 157A, homozygous for genes *bif-1* and *bif-2* for stem bifurcation, are likewise not able to express their mutant character in tropical and subtropical climates. Thus, it would not have been possible to select these agronomically useful mutants in mutation experiments carried out in these countries. Most of our fasciated mutants, finally, high-yielding under long-day conditions, are completely useless in countries with short-day climate because they are unable to flower there. All the mutants just mentioned have also been tested under different phytotron conditions demonstrating a broad field of reactions completey different from those of their mother variety and of other mutants of our collection.

In most of the phytotron experiments carried out in barley and pea, the photoperiod was found to influence the behaviour of distinct genotypes strongly. Our findings show that not only the temperature as such but also the thermoperiod can be of great influence on the flowering behaviour of specific genotypes. Most of the genotypes tested show a positive reaction to 25 °C as compared to 12 °C. In some mutants, however, a negative effect of the higher temperature with regard to the transition from the vegetative to the reproductive stage of the plants is observed. Of particular interest is the reaction of recombinant R 142F which is unable to flower under permanent high or low temperatures. The plants of this genotype need the thermoperiod, i.e. the alternative change of higher and lower temperatures for being able to enter flowering period.

The genotypes discussed in the present paper were also tested under other phytotron conditions, under different photoperiods, but also under higher temperatures in order to study their heat tolerance. A general discussion on their reaction to different ecological conditions will be given when these additional data are evaluated.

References

Dormling I, Gustafsson Å (1969) Phytotron cultivation of early barley mutants. Theor Appl Genet 39:51–61

Dormling I, Gustafsson Å, Ekman G (1975) Growth disorders and phenotype variability in phytotron-cultivated barley. Hereditas 79:255–272

Dormling I, Gustafsson Å, Jung HR, Wettstein D von (1966) Phytotron cultivation of Svalöf's Bonus barley and its mutant Svalöf's Mari. Hereditas 56:221–237

Gustafsson Å, Dormling I, Ekman G (1973a) Phytotron ecology of mutant genes. 1. Hereditas 74:119–126

Gustafsson Å, Dormling I, Ekman G (1973b) Phytotron ecology of mutant genes. 2. Hereditas 75:75–82

Gustafsson Å, Ekman G, Dormling I (1974) Variability, photoperiod and phenotypic trait. Hereditas 76:137–144

Gottschalk W (1978) Gene-ecological studies in *Pisum* mutants and recombinants. Genetika (Beograd) 10:43–61

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

Gottschalk W (1981b) *Pisum* genes controlling response to photoperiod. *Pisum* Newslett 13:14

Gottschalk W (1982) The flowering behaviour of *Pisum* genotypes under phytotron and field conditions. Biol Zentralbl 101:249–260

Gottschalk W (1983) Flowering behaviour of 18 *Pisum* genotypes under three different photoperiods. *Pisum* Newslett 15:21–22

Gottschalk W, Kaul MLH (1975) Gene-ecological investigations in *Pisum* mutants. 1. The influence of climatic factors upon quantitative and qualitative characters. Z Pflanzenzücht 75:182–191

Gottschalk W, Kaul MLH (1980) Gene-ecological investigations in *Pisum* mutants. 2. Comparative performance in Germany and North India. Theor Appl Genet 56:71–79