Genetic analysis of several flowering and branching mutants in *Pisum sativum* L.

a thesis submitted by
Estri Laras Arumingtyas

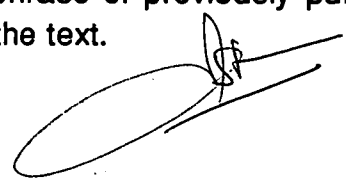
in partial fulfillment of the
requirement for the degree of
Master of Science Studies

Plant Science

University of Tasmania
Hobart
May 1992

Declaration

To the best of my knowledge and belief, this thesis contains no material which has been submitted for the award of any other degree or diploma; nor does it contain any paraphrase of previously published material, except where due reference is made in the text.



Acknowledgments

The present study forms part of an ongoing program of research on flowering and branching genes in pea organised by my supervisor, Dr. I.C. Murfet, whose help and guidance throughout my studies is greatly appreciated. F_1 and F_3 seeds, generated previously in the branching program by R. Floyd, M. Gregory and I. Murfet, were used in sections of the present study. In addition, some results from previous studies by these workers have also been incorporated into parts of the thesis. Such contributions by other workers are duly acknowledged, e.g. in Tables 4.2 and 5.2 and Fig. 4.1. In the flowering work, daily tagging of flowers was a shared effort between Dr. Murfet and myself. The data in Table 3.2 also come from a collaborative study in which one set of control lines was used for comparison with three flowering mutants being studied by myself and several other lines being investigated by Dr. Murfet. The authors and suppliers of the mutant lines used in this study are shown in Table 2.1. I am most grateful to Drs S. Blixt, N. Naidenova, K.K. Sidorova, Prof. Dr. W.K. Swiecicki and Dr. M. Vassileva for making available these lines. I have appreciated the technical assistance of Peter Bobbi, Leigh Johnson, Naomi Lawrence, and Katherine McPherson. I thank my husband Joni Kusnadi for his assistance throughout my experimental work and thesis preparation, for his support and encouragement, and my daughter Annisa A. Kusnadi for her understanding. I also wish to thank IDP for financial assistance. Finally, I thank the staff and students of the Botany Department who have assisted in various ways during this project and helped create a relaxed and friendly atmosphere in which to work.

Table of Contents

List of abbreviations

CrO%	crossing over value (=recombination value, recombination fraction)
cM	centimorgan; unit of recombination
DN	day neutral; DN flowering class (Murfet, 1985): FI and FT unaffected by photoperiod.
ED	early developing flowering class (Murfet, 1971a): FI and FT early and unaffected by photoperiod.
EI	early initiating; EI phenotypic class (Murfet, 1971a): FI early and unaffected by photoperiod but FT delayed in SD.
EMS	ethyl methane sulphonate
FD	node of first developed flower
FI	node of first flower initiation
FLR	flower-leaf relativity (Murfet, 1985).
FP	node of first pod
FT	flowering time, days from sowing to first open flower
G	Geneva-type flowering class (Marx, 1968, 1969) = class LHR (Murfet and Marx, 1976)
G2	G2 flowering class (Marx, 1968, 1969); classifies EI (Murfet and Marx, 1976)
h	hour
I	Insensitive type flowering class (Marx, 1968, 1969)
K	Kopetz type flowering class (Marx, 1968, 1969) = class L (Murfet and Marx, 1976).
L	late flowering class(Murfet, 1971a, 1985): quantitative delay in FI and FT in SD
LD	long day (s)
LDH	long day high response flowering class of sweet pea (Ross and Murfet, 1985a)
LDI	long day intermediate flowering class of sweet pea (Ross and Murfet, 1985a)

LDP	long day plant
LE	leaves expanded (number of)
LHR	late high response flowering class (Murfet, 1971a, 1985): very large delay in FI and FT in SD
M	mutant
N, n	number
P	probability
RCV	recombination value (=CrO% or recombination fraction)
RN	reproductive nodes (number of)
SD	short day (s)
SDP	short day plant
SE	standard error
t	Students t test
TL	total length of main stem
TLL	total lateral length = sum of length of all laterals
TLLE	total lateral leaves expanded
TN	total number of nodes with expanded leaves
VEI	very early initiating flowering class (Murfet, 1971a, 1985)
VL	very late flowering class (Murfet, 1971a)
WT	wild type

EMS	Ethyl methane sulphonate
NEU	Nitroso ethyl urea
NMU	Nitroso methyl urea

Abstract

Three flowering mutants and 17 branching mutants were studied.

The three induced early flowering mutants showed monogenic inheritance. Mutants L167 and M2/176 are the result of recessive mutations at the established loci *Lf* and *Sn*, and the mutant alleles appear equivalent to *lf* and *sn*, respectively. Mutant M2/137 is the result of a single gene partially recessive mutation at a new flowering locus, for which symbol *ppd* (photoperiod response) is proposed. *Ppd* is located on chromosome 1 about 32 cM from *A*.

Fifteen of the 17 branching mutants studied were shown to be single gene recessive mutants while the node of inheritance for two mutants, K319 and K586, was not clear. K319 is possibly a single gene dominant mutant, whereas K586 is possibly a single gene, recessive mutant with weak expression which needs the right conditions to produce laterals.

Based on the results of allelism tests, the 17 branching mutants were grouped into 7 series. Mutants WL5147, WL5237, WL5918, Wt15236 and Wt15240 are all allelic and the result of mutation of gene *Rms* since WL5237 is the type line for *rms*. Ten further mutants represent mutation at four new *ramosus* loci designated *rms-2* (mutants WL5951 and K524), *rms-3* (mutants WL6042, K487, and K564), *rms-4* (mutants K164 and Wt15242) and *rms-5* (mutants Wt10852, Wt15241 and Wt15244). The remaining two mutants, K319 and K586 do not appear to be allelic with each other or *rms-1, 2, 3, 4* or *5* (*rms* = *rms-1*).

CHAPTER 1
INTRODUCTION: GENETICS OF FLOWERING AND BRANCHING

1.1 Flowering genes in pea

Modern genetics was started when Mendel (1865) established the laws of inheritance using seven traits of pea (*Pisum sativum* L.). In contrast to these seven traits, he found that the flowering time of F_1 hybrids stood between that of both parents.

Barber (1959), using a joint genetic and physiological approach, found that the *Sn-sn* gene pair was responsible for the difference between early and late flowering in pea. He proposed that gene *Sn* controlled production of a flower delaying substance called colysanthin. Two polygenic systems were also proposed by Barber, one modifying the expression of *Sn*, and the other altering the node of first flower by a physiological mechanism other than by colysanthin. Rowlands (1964) proposed a simple polygenic system to control flowering. He also suggested *Sn* as an effective factor which was dominant for delaying flowering and whose effect increased under SD.

Generally F_2 populations show continuous variation for flowering in field conditions. However, Tedin and Tedin (1923) obtained a discontinuous bimodal distribution for flowering node in one F_2. They found that the number of early and late plants was consistent with a single factor difference with dominance of late, and named the gene responsible for late flowering *Sn*.

A relationship between flowering time and flower colour was reported by Hoshino (1915). White (1917) used symbol *Lf* for a dominant gene for late flowering that is linked to gene *A* which confers coloured flowers. Lamprecht (1961) shows this arrangement in his linkage map of *Pisum*. Pellew (1940) and Wellensiek (1969, 1972) found an indication of multiple alleles for flowering. Marx (1968,1969) developed a system of phenotypic classes. He recognised four photodependent response classes, I, G2, K and G. I plants have a low flowering node, are day neutral, and have only a small number of reproductive nodes. G2 plants are also day neutral for flowering node and flower as early as I plants under LD, but they have many reproductive nodes and show delayed senescence under SD. K plants flower somewhat later than I and G2 plants under LD. The flowering node of K plants is delayed under

SD, but the reproductive phase remains short. G type plants are like K-type plants under LD but their vegetative phase and life span is greatly prolonged by SD. Murfet (1971a, 1971b) also used two variables to separate several response classes. Using photoperiod and variables FI and FT he distinguished six phenotypic classes ED, EI, L, LHR, VEI and VL. However, class VL was subsequently merged with class LHR (Murfet,1975). Comparing Marx' classification (Geneva classes) and Murfet's classification (Hobart classes), Murfet and Marx (1976) found that G corresponds to LHR, and K to L, ED plants classified I and G2 plants classified EI. Hobart lines 60, 59, 24 and 63 represented classes EI, ED, L and LHR, respectively. Murfet (1971a,1971b) proposed three dominant genes S_1, S_2 and E which controlled the phenotypic differences between classes ED, EI and L. He also redefined *Lf* equal to S_1 and *Sn* equal to S_2, respectively (Murfet, 1971b). A fourth dominant gene *Hr* was found to control the difference between the LHR and L phenotypic classes (Murfet, 1973).

At least 8 major genes are now known to control flowering in pea. Five of them (*Lf*, *E*, *Sn*, *Dne* and *Hr*) are relevant to the present investigation. White's *Lf* locus (Hoshino's *A*, 1915) has at least four naturally occurring alleles Lf^d, *Lf*, *lf* and lf^a (Murfet, 1971b, 1975). It is proposed that these *Lf* alleles determine the threshold level of flowering signal necessary to trigger flower initiation at the shoot apex, with Lf^d specifying the highest threshold and lf^a the lowest threshold (Murfet, 1971c, 1975). The minimum node of flower initiation for Lf^d, *Lf*, *lf* and lf^a is 15, 11, 8, and 5, respectively (Murfet, 1978, 1985). The *Lf* locus is located on chromosome 1 about 10 units from *A*, the basic locus for anthocyanin production (Hoshino, 1915; Lamprecht, 1961; Murfet, 1971b,1975). The flowering locus *No* was symbolized by Wellensiek (1972) and three alleles, No^h,No^m and *no*, proposed. A further allele, no^l, was later added by Uzhintseva and Sidorova (1979) . Although the linkage test by Wellensiek (1972) showed no linkage of *no* with *a*, but weak linkage with *fa* on chromosome 4, Murfet (1978) found that the breeding data very clearly indicated a genotype of *lf E Sn Hr* for the *no* type line, and using marker *A* there appeared to be no functional difference between *no* and *lf*. Two other induced mutants, *efr* (early flowering x-radiation mutant) of Gottschalk (1978),

and *pra* (early flowering EMS mutant) of Monti and Scarascia-Mugnozza (1967,1970,1972), have also been traced to the *Lf* locus (Murfet, 1978). The symbol *lf* (White, 1917) has priority over *no*, *efr* and *pra* (Murfet, 1978, 1985).

Non-inductive photoperiod conditions were used by Barber (1959) to magnify the differences between response types. He used SD conditions to distinguish a dominant gene, *Sn*, with several effects. *Sn* increased flowering node, conferred the ability to respond to photoperiod and vernalisation, delayed the appearance of the first leaf with more than two leaflets, and decreased the internode length. Rowlands (1964) and Murfet (1971a, 1971b) found evidence of a major gene that had similarities to those described by Barber for *Sn*. This gene appears to control synthesis of flower inhibitor (Barber, 1959; Murfet, 1971a, 1971c; Murfet and Reid, 1973). Gene *Sn* was localised by Weeden, Kneen and Murfet (1988) on chromosome 2 close to the amylase locus, *Amy-1*. A second gene, *Dne*, conferring response to photoperiod was identified by King and Murfet (1985). *Dne* has the same action as *Sn* and it acts in a complementary manner (King and Murfet, 1985). *Dne* is located on chromosome 3, 5 units from *st* (reduced stipules; King and Murfet, 1985; Murfet, 1987). *Sn Dne* activity is reduced by LD and low temperature (Barber,1959; Murfet and Reid, 1974) and falls as the plant ages (Murfet, 1971b; Reid and Murfet,1977).

The activity of the *SnDne* system appears to be modified by *E* and *Hr*. *E* operates in the cotyledons to reduce *SnDne* activity in the early stages of seedling growth (Murfet, 1971c). *Hr* acts later in the life cycle to maintain *SnDne* activity (Reid and Murfet, 1977). The action of *E* was shown by studies with line 60 that has phenotype EI and genotype *lf E SnDne hr* (Murfet, 1971c). L60 flowers at about nodes 9-11. However, the development of the bud initiated was retarded in SD as a result of *Sn Dne* activity in the shoot. The LHR class (Murfet, 1971a) is a phenotype conferred by combination of *Hr* and *Sn* (Murfet, 1973). Gene *E* is located on chromosome 6 (Murfet, 1971b), while *Hr* is located on chromosome 3 approximately 7 units from *M* (marbled testa; Murfet, 1973, 1988).

Other genes, which will not be included in this study, are *veg* (vegetative) which prevents the plant flowering in any circumstances (Gottschalk, 1979;

Reid and Murfet, 1984), *dm* (diminutive) which is responsible for a 2-fold to indefinite flowering delay in homozygous recessive plants (Murfet, 1989), *gi* (gigas) which causes plants to flower much later than the initial line (Murfet, 1989, 1990), *fds* (flower development suppressor) which causes flower buds to fail to develop (Gottschalk, 1982, 1988; Murfet, 1990) and *det* (determinate) which causes the shoot to cease growth soon after the onset of flower formation (Marx, 1986; Swiecicki, 1987; Murfet, 1989; Singer et al., 1991).

1.2 Flowering genes in sweet pea

Little and Kantor (1941) showed that the difference between the winter and summer flowering habit in sweet pea (*Lathyrus odoratus* L.) was determined by a single pair of alleles, *Dn* - *dn*, with dominance of the summer flowering habit. Subsequently, Ross and Murfet (1985) reported that there are three flowering classes in sweet pea according to the response to photoperiod. Day neutral (DN) plants are essentially day neutral and correspond to the early (winter) flowering group. Classes LDI and LDH are long day types with an intermediate and high response to photoperiod, respectively. These classes correspond to the Curthbertson (spring) and late (summer) groups. The class differences are determined by three alleles *dn*, Dn^i, and Dn^h, which in homozygous condition give phenotypes DN, LDI,and LDH, respectively. Dn^h seems to be fully dominant over Dn^i and *dn*, but Dn^i seems to be partially dominant over *dn*. The alleles at this locus have pleiotropic effects on branching (see section 1.5). Dn^h was suggested as equivalent to either *Sn* or *Dne* in *Pisum*, or to act at another point in the synthesis pathway for a graft-transmissible flower inhibitor. A second flowering gene, *Sp*, also influences sensitivity to photoperiod in sweet pea (Ross and Murfet, 1988) . *Sp* acts in a complementary manner with Dn^h to confer the (LDH) summer flowering phenotype and a near obligate LD requirement for flowering in the unvernalised state. It was suggested that *Sp*, like Dn^h, controls a step in the biosynthetic pathway to produce a flower inhibitor in SD. Mutants *sp* and Dn^i each diminish the response to photoperiod, and genotypes *sp* Dn^h and *Sp* Dn^i confer a similar LDI (spring-flowering) phenotype. It was suggested that those mutations impose only partial blocks in the biosynthetic pathway. Therefore, like Dn^i, *sp* is a leaky mutant.

Response to photoperiod is further reduced in genotype *sp* Dn^i which flowers intermediate between the LDI and DN (winter flowering) phenotypes. Gene pair *Sp/sp* is hypostatic to *dn* and genotypes *Sp dn* and *sp dn* both have a DN phenotype; *dn* therefore appears to cause the most severe block to the biosynthetic pathway.

Locus *Sp* also has pleiotropic effects on branching. Like Dn^i, gene *sp* reduced basal branching. On the other hand, like Dn^h, *Sp* is associated with increased basal branching.

1.3 Flowering genes in other species

Arabidopsis thaliana has been studied quite intensively . Genes governing flowering time have been localised on chromosomes 1, 4, and 5. Late flowering genes *fb*,*fe*, and *ft* were separately located on chromosome 1, *fca* on chromosome 4, and *fg* and *fy* on chromosome 5 (Koorneef et al., 1983). Using forty-two independently induced mutants from early ecotype Landsberg erecta, Koornneef et al. (1991) identified late flowering mutations at 11 loci with distinct positions on 4 of the 5 *A. thaliana* chromosomes. Loci *gi*, *fe*, *fha* and *ft* are located on chromosome 1; *fpa* and *fve* on chromosome 2; *fca*, *fd* and *fwa* on chromosome 4; and *co* and *fy* on chromosome 5. Mutants at the *co* locus are allelic with *fg*, and *gi* is allelic with *fb*. Mutant *fy* is moderately recessive; *fca*, *fe*, *fd*, *fha*, *fpa*, and *ft* are almost completely recessive; *fve* is a slightly recessive mutant; *fwa* is partially dominant, and *co* is an intermediate mutant. Mutants at loci *fca*, *fpa*, *fve* and *fy* have a large response to vernalisation and photoperiod, whereas mutants *fd*, *fe*, *ft*, and *fwa* have a pronounced response to photoperiod but only a small or no response to vernalisation. On the other hand mutants *co-3* and *gi-3* are day-neutral and do not respond to vernalisation. Four other flowering mutants: *tfl1* (terminal flower), *elf* (early flowering) *1*, *2* and *3* were isolated by Zagotta et al (1992). Mutant allele *tfl1*, which shows response to photoperiod, is recessive for terminal flower but semi dominant for early flowering. Mutations at the *elf1* and *elf2* loci are inherited as single-gene recessives and SD conditions cause a delay to floral initiation in these genotypes. The fourth mutant *elf3* is day-neutral and shows single-gene recessive inheritance.

Work on geranium (*Pelargonium x hortorum*) showed genetic variation in flowering time and early flowering appeared to be inherited as a dominant single gene character (Hanniford and Craig, 1982). It was also reported that geranium is a day neutral plant.

Belliard and Pernes (1985) studied flowering of *Pennisetum typhoides*. They found that there were SDP, LDP, and DN plants which flowered in 9 hour, 16 hour, and all day lengths, respectively. Line 23 D2B1 only flowered if the day length was less than, or as long as, 13 hours, whereas cv. Ligui flowered under all daylengths. The F_1 hybrid flowered under all daylengths but slightly later than Ligui. It was proposed that the ability to flower in LD was controlled by a single locus designated *I* with two alleles. *I* was dominant over *i* and 23D2B1 was *ii*, and Ligui was *II*.

1.4 Branching genes in pea

Studies of the genetics of branching in *Pisum* date from Lamprecht (1950). He found evidence of digenic control of the trait. He proposed genes *Fr* and *Fru*, with the double recessive condition responsible for four or more basal branches (secondary stems), whereas all other genotypes expressed three or less branches. He also recognised that branching was extremely easily modified and strongly dependent on such environmental conditions as spacing, light conditions and temperature. In 1968, Blixt conducted a similar study to Lamprecht's, but found a different result. He proposed a phenotypic ratio of 9:7 in which *Fr fru*, *fr Fru* and *fr fru* had the same phenotype (four or more branches) and *Fr Fru* had a phenotype of three or less branches. He suggested a linkage relationship between *Fr* and *St* on chromosome III with a CrO value of 37.7±2.2%. *Fru* was located to chromosome IV with a distance of 28.1 units from *Pro*, and 32 units from *Vim*, with a gene order of *Le-Pro-Fru-Vim* (Blixt, 1968).

Monti and Scarascia-Mugnozza(1967) recognised another branching mutant after they treated seeds of "Parvus" with diethyl sulfate. The mutant was characterised by earliness (flowering at nodes 4-6 , while "Parvus" flowered at nodes 13-14), increased branching and low seed set. Two new phenotypes segregated when the mutant was backcrossed with "Parvus" and two lines were selected to represent these phenotypes. One line flowered very early

(nodes 4-6) and was designated P745d-p. The other line was highly branched (30 times higher than "Parvus") and designated P745d-r. Both mutants have been found to segregate as monogenic recessive characters independently of each other. The symbols *pra* (*praecox*) and *ram* (*ramosus*) were proposed for the flowering and branching mutants, respectively. The *ramosus* mutation was tested for linkage using marker genes from line WL851. Linkage with *oh* on chromosome II was demonstrated, with a CrO value of 17.81±2.87%.

Blixt (1976), obtained another branching mutant from Parvus, after treating the cultivar with X-irradiation. The mutant was shorter (76 cm), had more branches (4.2 basal), was 5 days later flowering, and had 4% higher protein content than the initial line Parvus (height 145 cm, and 1.7 basal branches). The mutant designated *rms* (*ramosus*) was identified as recessive and located on chromosome III between *fas* and *m* with a distance of 12.3 units and 22.4 units, respectively.

Uzhintseva and Sidorova (1979) obtained a digenic mutant, K319, from cv. Torsdag which showed both early flowering and increased branching. They concluded that the mutant allele responsible for the branching was dominant because the F_1 plants of cross K319 x Torsdag produced laterals.

Several other genes in *Pisum* are proposed to control the angles at which laterals arise from the main stem; *asc* (*ascendens*) results in branches that arise at ± 45 ^{o} to the main stem, *ho* (*horizontalis*) causes horizontal outgrowth of basal laterals, and *pro* (*procumbens*) initially causes horizontal outgrowth of the basal laterals but the laterals subsequently revert to a 45 ^{o} angle of growth.

A pleiotropic effect of flowering genes on branching habit has been reported for *Pisum*. Photoperiodic lines usually have a higher tendency to produce basal laterals than day neutral lines (Doroshenko and Rasumov, 1929). The flowering gene *Sn* causes a significant increase in the number basal branches which are generally not present on *sn* plants (Murfet and Reid, 1985). King and Murfet (1985) reported that the *Sn Dne* combination which conferred photoperiodicity, also affected branching. This effect, both on flowering and branching was further increased by gene *Hr* (Ross, 1983). Flowering genes *Lf* and *veg* have an effect on increased production of aerial

laterals (Reid and Murfet,1984; Murfet and Reid,1985). Genes such as *Lf*^{d} or *veg*, which delay or prevent flowering, increase the number of sites from which aerial laterals can arise (Floyd and Murfet, 1986).

Length genes also seem to influence the production of basal and aerial laterals in pea. Floyd (1985) found that nana plants, which have very short internodes, only produced basal laterals, whereas dwarf plants, which have slightly longer internodes than nana plants produced a lesser number of basal laterals but also produced aerial laterals. Tall plants, which have longer internodes than nana or dwarf plants, produced aerial laterals but not basal laterals in Floyd's study.

1.5 Branching genes in sweet pea

Ross and Murfet (1985a, 1985b) recognised that there was a difference in branching habit among three flowering classes of sweet pea. DN segregates (genotype *dndn)* seldom produced laterals. In SD, LDH ($Dn^{h}Dn^{h}$) segregates branched profusely from the basal nodes, while LDI ($Dn^{i}Dn^{i}$) segregates were intermediate in branching tendency. It was suggested that either the flowering genes had a pleiotropic effect on branching habit or there was a very close linkage between separate branching and flowering genes that involved two loci each with three alleles. The latter case was considered unlikely. Ross and Murfet (1988) examined the interactions between a branching gene *b* (bush) in sweet pea and the two flowering genes Dn^{h} and *Sp* which govern the response to photoperiod in this species (section 1.2). The mutant bush (*b*) segregates generally produced at least twice as many laterals as *B* segregates. In certain circumstances they also showed a delay in flower initiation. On the other hand, flowering mutants Dn^{i} and *sp* reduced basal branching, and *dn* largely prevented basal branching, in either *b* or *B* plants.

1.6 Branching genes in other species

The existence of genes governing ramification in other plants has not been widely explored. In common bean (*Phaseolus vulgaris*) a spindly branched mutant has been identified. This mutant does not alter the number of branches occurring on the plant, but causes a characteristic reduction in the

size and strength of branches. This gene, designated *sb*, was first reported by Awuma and Basset (1988). Additional information given by Basset (1990) said that there were three mimic mutants of *sb* which were non-allelic to each other.

CHAPTER 2
MATERIALS AND METHODS

2.1 Lines used

The mutant lines studied, their alternative names, their initial lines, the mutagenic agents used, the phenotype of the mutants, and their sources, are listed in Table 2.1. Details of the initial lines and reference lines used in the study are provided in Table 2.2.

2.2 Growing conditions and methods

Tests were conducted in the phytotron at Hobart. The plants were grown in 14 cm slimline pots at a rate of 1 per pot. The potting medium consisted of a 1:1 mixture (based on volume) of vermiculite and 10 mm dolerite chips, covered by a 4 cm layer of sterilized 1:1 peat and sand mixture. A small section of the testa was removed from seeds prior to planting to enable the seeds to imbibe freely and germinate more evenly. The seeds were coated with fungicide (Thiram) and sown at a depth of 2 cm. The potting mixture was watered daily until just before seedling emergence. After the seedlings were fully emerged, they were watered every 2 days for the first 2-3 weeks, and after that, daily. Nutrient was applied every week in the form of Hoaglands or Aquasol. The main shoot and major laterals were trained up vertical strings. For the flowering study, laterals were excised every 2 days.

The studies involved 4 photoperiods, 8 h (8 h daylight and 16 h of darkness), 12 h (8 h natural daylight, 4 h mixed incandescent-fluorescent light, and 12 h darkness), 18 h (natural daylight extended to 18 h by mixed incandescent-fluorescent light and 6 h darkness) and 24 h (8 h daylight and 16 h of incandescent light at 3 $\mu molm^{-2}s^{-1}$). Night temperature was 16^{o}C and day temperature was generally in the range of 20-25^{o}C.

To determine inheritance, the mutant lines were crossed with the initial line. For the flowering study, the mutant lines were also crossed with one or more of the following standard lines of known genotype : L59 (*lf E sn Dne hr*), L73 (*Lf E sn Dne hr*), K218 (*Lf E Sn dne hr*), L60 (*lf E Sn Dne hr*), L53 (*lf e Sn Dne hr*), and L24 (*Lf e Sn Dne hr*). These lines also served as

standard/reference lines for flowering phenotypes DN, EI and L, respectively (Murfet 1971a, 1985; Table 2.2). The linkage test for the flowering mutant 2/137 was done by crossing with line 111 which carries 14 marker genes (*A b cp fa gp i n r s st te tl wb wlo*). For the branching study, allelism tests were done by making crosses of the mutants in all possible combinations. Branching mutants not already characterised by Floyd (1985) were characterised using his procedures.

The segregation data were analyzed using Chi-square. The joint segregation Chi-square was obtained from a 2X2 contingency table. The recombination fraction for linked genes was calculated using the Product Ratio method and Stevens (1939) Tables. The significance of the difference between means was determined by Students t test.

2.3 Variables scored and definitions of traits measured.

Node of flower initiation (FI) and flowering time (FT) were used as the main indices of flowering. Counting from the first scale leaf as node 1, FI is the number of the first node on the main stem to bear a flower initial, regardless of whether or not that initial subsequently developed into a mature flower. Since the first flower buds sometimes aborted, the number of the lowest node to bear a fully developed flower also was recorded and designated FD. Because this flower does not always set, the number of the lowest node to bear a pod was also recorded and designated FP. Flowering time (FT) refers to the number of days from sowing to first open flower. The number of reproductive nodes (RN) refers to the number of nodes on the main stem to bear flower initials, fully developed flowers or pods. The total number of nodes on the main stem (TN) includes all nodes to bear a fully expanded leaf. The flower/leaf relativity (FLR) was used to measure the degree to which flower bud development lags behind or runs ahead of leaf expansion (Murfet, 1982, 1985) If a flower opened at the same time as the subtending leaf became fully expanded, the FLR value was taken as zero. If the leaf above was already expanded the FLR value was -1. The degree of leaf expansion was estimated on decimal scale after Maurer et al (1966) and if the subtending leaf was only half expanded at the time the flower opened FLR was +0.5. The variables FI, FD, FP, FT, RN, and FLR were recorded solely from main stems.

Lateral outgrowth was quantified by measuring the length of all lateral branches and the number of leaves expanded on each lateral. The sum of these measurements for a plant is termed TLL (total lateral length) and TLLE (total lateral leaves expanded), respectively. Total number of nodes with expanded leaves (TN) and total length (TL) were also measured for the main stem in branching experiments.

Table 2.1. Mutant pea lines used in this study

Mutant line	Alternative names	Initial line	Mutagenic agent	Phenotype	Author	Supplied by
K164	WL 5847	Torsdag	EMS	Branching	K.K. Sidorova	S. Blixt
K 319	L 109	Torsdag	NEU	Branching Early Flowering	K.K. Sidorova	K.K. Sidorova
K 487	WL 5861	Torsdag	NMU	Branching	K.K. Sidorova	S. Blixt
K 524	WL 5864	Torsdag	EMS	Branching	K.K. Sidorova	S. Blixt
K 564	WL 5867	Torsdag	EMS	Branching	K.K. Sidorova	S. Blixt
K 586	WL 5868	Torsdag	EMS	Branching	K.K. Sidorova	S. Blixt
WL 5147	-	Weitor	R	Branching	S. Blixt	S. Blixt
WL 5237	-	Parvus	R	Branching	S. Blixt	S. Blixt
WL 5918	II/77	Raman	15 krad gamma	Branching	M. Vassileva	S. Blixt
WL 5951	L 162	Parvus	EMS 0.35 %	Branching	S. Blixt	S. Blixt
WL 6042	IV/107	Meteor	5 krad γ + EMS 0.2 %	Branching	M. Vassileva	S. Blixt
Wt 10852	-	Paloma	0.014 % NEU	Branching	W.K. Swiecicki	W.K. Swiecicki
Wt 15236	-	Paloma	-	Branching	W.K. Swiecicki	W.K. Swiecicki
Wt 15240	-	Kaliski	0.014 % NEU	Branching	W.K. Swiecicki	W.K. Swiecicki
Wt 15241	-	Paloma	-	Branching	W.K. Swiecicki	W.K. Swiecicki
Wt 15242	-	Paloma	0.014 % NEU	Branching	W.K. Swiecicki	W.K. Swiecicki
Wt 15244	-	Porta	170 r Nf	Branching	W.K. Swiecicki	W.K. Swiecicki
L 167	M1/178	Ramonsky 77	100 r Nf	Early Flowering	M. Vassileva	S. Blixt
M2/137	-	Borek	15 krad gamma	Early Flowering	N. Naidenova	M. Vassileva
M2/176	-	Borek	-	Early Flowering	N. Naidenova	M. Vassileva

Table 2.2. Details of initial lines for mutants and reference lines used in this study

Line	Other names	Phenotype for length and flowering°	Flowering genotype
Borek	-	Dwarf, L	*Lf E Sn Dne hr*
Kaliski	Wt 4042	Tall, L	*Lf E Sn Dne hr**
Meteor	L 136	Dwarf, DN	*lf sn Dne hr*
Paloma	Wt 3527	Dwarf, L	*Lf E Sn Dne hr**
Parvus	L77	Tall, L	*Lf E Sn Dne hr**
Porta	Wt 3519	Dwarf, L	*Lf E Sn Dne hr**
Raman	WL 2168	Dwarf, L	*Lf Sn Dne hr*
Ramonsky 77	WL 2164, L152	Tall, L	*Lf E Sn Dne hr*
Torsdag	L107	Tall, L	*Lf E Sn Dne hr**
Weitor	WL 1263	Tall, L	*Lf Sn Dne hr*
L24	WL 2681	Dwarf, L	*Lf e Sn Dne hr**
L53	WL 2683	Dwarf, L	*lf e Sn Dne hr**
L59	WL 1793	Dwarf, DN	*lf E sn Dne hr**
L60	WL 2684	Dwarf, EI	*lf E Sn Dne hr**
L73	WL 2689	Dwarf, DN	*Lf E sn Dne hr**
L111	Marx A 875-55-0	Dwarf, L	*Sn Dne hr*
K218	L 110	Tall, DN	*Lf E Sn dne hr**

°Flowering phenotype as defined by Murfet (1971a, 1985). DN= day neutral; EI= early initiating, early photoperiodic; L= late photoperiodic.

*Genotype from Murfet (1971b, 1978, 1985, 1991), Murfet and Groom (1984), and King and Murfet (1985).

CHAPTER 3
GENETICS OF FLOWERING MUTANTS

3.1 Early mutant L167

3.1.1 Nature and phenotypic characterization

Mutant L167 has a similar flowering phenotype to L60, the standard EI line, whilst the initial line (L152) has a similar flowering phenotype to L24 the standard late type (Fig. 3.1). In SD (8 h), the FI of L152 was delayed 8 - 11 nodes, and the FT was delayed 15-25 days, compared with the values in LD (24 h) (Table 3.1). SD delayed FT in the mutant by 9-15 days but FI was unaffected by photoperiod (Table 3.1)

3.1.2 Crosses made and results

The F_1 of cross L152 x L167 had a similar phenotype to the initial line but FI was slightly earlier (Fig. 3.2). The F_2 progeny segregated into two classes, late and early, corresponding to the two parents, L152 and L167, respectively (Fig. 3.2). The observed numbers of 22 late and 9 early, are in good accordance with a 3:1 ratio (X^2 = 0.27). These results indicate the mutant is recessive and monogenic.

Cross L167 x L73 (*A Lf E sn Dne hr*) gave rise to F_1 plants that were intermediate between L167 and its initial line, L152, in the terms of FI and FT and which had an EI phenotype (Fig.3.3). The F_2 progeny segregated into three phenotypic classes L, EI and DN (Fig.3.4). Most of the white flowered (*aa*) segregates were located in the EI class near the L167 parent and the standard EI line, L60. (Figs. 3.3 and 3.4).

3.1.3 Discussion and conclusion

A change in phenotype from L in the progenitor to EI in the mutant could be explained in two ways. The progenitor could have genotype *lf e Sn Dne hr* with mutation of *e* to *E*. This hypothesis is argued against by the fact that the mutant allele is recessive. Alternatively, the progenitor could have genotype *Lf E Sn Dne hr* with mutation of *Lf* to *lf*. This hypothesis is supported by the fact that the mutant allele is recessive and showed strong linkage with the allele for white flowers in cross L73 (*A Lf E sn Dne hr*) x L167 (putative genotype *a lf E*

Sn Dne hr). Locus *Lf* is about 10 cM from the basic gene for anthocyanin (*A*) on chromosome 1 (White 1917, Murfet 1971b, 1975). In cross L73 x L167 the EI segregates were distinguishable from the DN segregates on the basis of RN while the L segregates were distinguishable on the basis of FI (Fig.3.4). In the EI class 9 segregates had white (*aa*) flowers and 19 had red (*A*-) flowers. In contrast, in the L class 14 segregates had red flowers and only 1 had white flowers. These numbers indicate the two traits have not assorted independently although Chi-square of 3.55 is not quite significant at the 0.05 level. The tendency for *Lflf* heterozygotes to flower intermediate between the two pure forms, as seen here for the F_1 plants of crosses L152 x L167 and L73 x L167, is by now well documented (Murfet 1971b, 1975, 1991). Presumably most of the red flowered EI segregates in the 73 x 167 F_2 were also *Lflf* heterozygotes.

It is concluded on the basis of the above results that the progenitor L152 has genotype *Lf E Sn Dne hr* and that mutation of *Lf* to an allele approximately equivalent to *lf* has occurred in L167.

3.2 Early mutant M2/176

3.2.1 Nature and phenotypic characterization

Data for FI and FT in SD and LD, placed mutant 2/176 in the phenotypic range of reference DN and ED lines (L59,L73 and K218) (Table 3.2). Mutant M2/176 had a slightly higher FI than L59, but a lower FI than lines L73 and K218. The other characters recorded in Table 3.2, also indicate that the mutant has phenotype DN.

3.2.2 Crosses made and results

The cross between mutant 2/176 and the initial line (Borek) gave rise to an F_1 that was late and like Borek (Fig. 3.5). The F_2 segregated into 40 late and 7 early plants, corresponding to the two parents, Borek and M2/176 respectively (Fig.3.5). These numbers are in agreement with a 3:1 ratio (X^2=2.56, P>0.1).

The allelism test with L59 (*lf E sn Dne hr*) showed that the mutated gene in M2/176 was allelic with *sn.* The F_1 from that cross was early flowering (FI=10-11) like both parents (Fig.3.6). The F_1 plants of cross L 73 (*Lf E sn Dne*) x M2/176 had a similar early flowering, day neutral phenotype to the two

parents (Fig.3.7), again indicating that mutant M2/176 has genotype *sn*. The red (*A*-) and white flowered (*aa*) plants in the latter F_2 flowered about the same node but there was a small but significant ($P<0.05$) difference in flowering time (Table 3.3). Cross Borek x L60 (*lf E Sn Dne hr*) gave rise to a Borek-like F_1 but with slightly lower FI (Table 3.4;Borek = 25 nodes, F_1 = 22 nodes). This result showed that Borek carries gene *Lf*. The cross Borek (*Lf ? Sn Dne hr*) x L53 (*lf e Sn Dne hr*) was made in order to genotype Borek at the *E* locus. The F_1 was late (L-type). The F_2 segregated 35 late (L type) and 5 early (EI type) plants. One EI type F_2 plant bred true in F_3 (n=15). This result indicates Borek possesses gene *E*.

3.2.3 Discussion and conclusion

Borek has a late photoperiodic (L type) phenotype, mutant M2/176 has an early, day neutral phenotype, and the mutation is inherited as a monogenic recessive. These results indicate that Borek has genotype *Sn Dne hr* and that a recessive mutation has occurred at *Sn*, *Dne* or an unknown locus determining response to photoperiod. The result for crosses M2/176 x L59 (*sn Dne hr*) and M2/176 x L73 (*sn Dne hr*) show that the mutation is allelic with *sn*. Borek could have genotype *Lf E, Lf e* or *lf e* . Genotype *Lf* is indicated by the fact that the F_1 of cross Borek x L60 (*lf E Sn Dne hr*) is late flowering (*Lf* is epistatic to *E*; Murfet 1971b) and by the fact that there was no indication of linkage between the genes controlling flower colour (*A/a*) and flowering node in cross M2/176 x L73 (*A Lf*) [locus *A* is about 10 cM from locus *Lf* on chromosome 1 (White 1917, Murfet 1971b,1975)]. The fact that the F_2 of cross Borek x L53 (*lf e Sn Dne hr*) segregated some early phenotype plants and that at least one of these bred true in F_3, shows that Borek carries gene *E*, since a pure breeding early phenotype can only occur on this background (*Sn Dne*) if there is a combination of *lf* and *E*.

In conclusion, Borek has genotype *Lf E Sn Dne hr* and mutant M2/176 has resulted from mutation of *Sn* to *sn*.

3.3 Early mutant M2/137

3.3.1 Nature and phenotypic characterization

Mutant M2/137 was induced in cv Borek (Table 2.1). Borek has an L-type

phenotype (section 3.2), i.e it is late flowering with a quantitative response to photoperiod. The FI of Borek rose from 15-16 in a 24 h photoperiod to 27-30 in an 8 h photoperiod (Table 3.2). Mutant M2/137 showed an early flowering DN phenotype with similar characteristics to representative DN lines L59 (*lf E sn Dne hr*), L73 (*Lf E sn Dne hr*) and K218 (*Lf E Sn dne hr*) (Table 3.2). Mutant M2/137 had an FI of 11 in both 24 h and 8 h conditions, the first flower initials developed into open flowers in short days, and FT was only very slightly delayed in short days. The phenotype matched closely that of K218 (Table 3.2)

3.3.2 Crosses made and result

Crossing the mutant with the initial line (Borek) in SD conditions gave evidence that there was one gene segregating as the scattered plot of F_2 plants based on FT and FLR (Fig. 3.8) showed segregation into two groups with 10 early segregates like the mutant, and 36 late segregates like the F_1 and Borek. These results fit a 1: 3 ratio (X= 0.26). The recessive class bred true in F_3 (Table 3.5). The F_1 was intermediate in the terms of FI, FT and FLR between the initial line and the mutant (Table 3.5 and Fig.3.8).All F_2 plants were genotyped by growing F_3 progeny.

The mean FI of homozygous wild type, heterozygous, and homozygous mutant F_2 plants was 23.75±1.05, 20.67±0.47 and 12.10±0.18, respectively. Dividing the difference from the mean for heterozygotes to the mid-point between homozygous wild type and mutant by the difference from the mutant or wild type value to the mid-point, showed the degree of dominance of the wild type allele to be 0.48.

Cross L73 (*Lf E sn Dne hr*) x M2/137 gave an F_1 progeny with a late phenotype in SD (FI=19-20; Fig. 3.9). The F_1 phenotype was very different from both early parents, indicating that the mutant allele in M2/137 is not allelic to *sn*.

Line K218 (*Lf E Sn dne hr*) and mutant M2/137 are both early, essentially day neutral types (Table 3.2). However, in SD the F_1 of cross K218 x M2/137 was later flowering than both parents and the F_1 plants had more nodes than either parent although the FT and TN values were not as high as those of the initial line, Borek (Fig. 3.10). These results indicate that the mutant allele is not allelic to *dne*. The F_2 progeny consisted of 19 segregates like the two parents

and 29 segregates like the F_1 (Fig.3.10); these numbers fit a 7:9 ratio (X^2= 0.34, 0.7>P>0.5) and indicate segregation of two complementary genes. The fact that not one F_2 plant flowered later than FI 16 is rather inexplicable, since in the presence of *Lf* (from K218 and M2/137) at least some plants should be homozygous for the wild type alleles for photoperiod response and have a genuine L phenotype like the Borek controls.

Linkage tests with 15 marker genes (*A b cp fa gp i k n r s st te tl wb wlo*), showed that the mutated gene in M2/137 was located on chromosome 1, 31.82 ± 5.21 units from the *A* locus (Table 3. 6)

3.3.3 Discussion and conclusion

The genotype of Borek was shown to be *Lf E Sn Dne hr* in section 3.2. Like mutant M2/176, M2/137 is also a day neutral mutant induced in cv Borek. Since Borek has a late photoperiodic phenotype the mutation seems to be at a locus governing photoperiodicity, either *Sn* or *Dne or* a novel locus. Crosses of M2/137 with L73 (*Lf E sn Dne hr*) and K218 (*Lf E Sn dne hr*) showed that the mutant was not allelic with *sn* or *dne*, since the F_1 of each cross showed complementation. This conclusion was supported by the F_2 data from the cross with K218. These results indicate that M2/137 is the result of mutation at a previously unnamed locus. The symbol *Ppd* (Photoperiod response) is proposed. The results of the linkage test localized *Ppd* on chromosome 1, 31.82 ± 5.21 units from *A*, which also supports the conclusion that the locus is novel. The other genes governing the response to photoperiod, *Sn* and *Dne* are located on chromosome 2 close to the amylase locus *Amy-1* (Weeden, Kneen and Murfet 1988), and on chromosome 3, 5 units from *st* (reduced stipules) (King and Murfet 1985, Murfet 1987), respectively. This new gene has a different character from both *Sn* and *Dne*. *Sn* is known to be fully dominant over *sn* (Murfet 1971a) and *Dne* seems to show complete dominance over *dne* (King and Murfet 1985), whereas *Ppd* was only partially dominant over the mutant allele *ppd* (degree of dominance = 0.48).

Table 3.1 The node of flower initiation (FI) and time in days to first open flower (FT) of several plants of lines L152 and L167 in LD (24 h) and SD (8 h)

Line		24 h				8 h			
L152 [a]	FI	15	14	14	15	23	25	23	23
	FT	40	36	35	39	56	60	55	57
L167 [a]	FI	10	10	9	10	-	-	-	-
	FT	32	32	34	31	46	43	44	46
L152 [b]	FI					30	25	26	
	FT					93	75	80	
L167 [b]	FI					10	10	10	
	FT					67	64	64	

[a] Planted 14-1-1991
[b] Planted 25-4-1991

Table 3.2. Means of several reproductive traits for two early flowering mutants, M2/176 and M2/137, their initial line Borek, and several reference lines with known flowering genotypes. The plants were grown in the phytotron at Hobart under a 24 h (8 h daylight + 16 h incandescent light at 3 μmol m^{-2} s^{-1}) or an 8 h (8 h daylight + 16 h dark) photoperiod. Temperature day 23-25 °C, night 16 °C.

Line (Genotype) Phenotype	Photo-period	FI	FD	FP	FT	TN	RN	Pods	Seeds	N
L59 *(lf E sn Dne hr)*	24	9.0	9.0	9.0	32.8	11.0	3.0	3.0	20.0	4
Early, day neutral	8	9.3	9.3	10.0	35.8	13.8	5.5	4.0	22.5	4
L73 *(Lf E sn Dne hr)*	24	12.7	12.7	12.7	36.7	17.0	5.3	3.7	21.3	3
Day neutral	8	12.0	12.0	12.0	36.7	17.3	6.3	7.3	36.0	3
K218 *(Lf E Sn dne hr)*	24	12.0	12.0	12.0	34.0	15.7	4.7	5.3	16.3	3
Day neutral	8	12.0	12.0	12.0	37.5	21.0	10.0	16.0	33.0	2
L60 *(lf E Sn Dne hr)*	24	11.0	11.0	11.0	36.3	15.3	5.3	6.0	31.5	4
Early photoperiodic	8	10.7	12.3	12.7	49.7	37.7	28.0	33.5	106.0	3
Kaliski *(Lf E Sn Dne hr)*	24	15.0	15.0	15.0	40.8	19.5	5.5	6.3	28.3	4
Late photoperiodic	8	22.5	22.5	22.5	59.0	36.5	15.0	24.0	74.3	4
Borek	24	15.5	15.5	15.5	42.8	18.0	3.5	3.8	18.8	4
Late photoperiodic	8	28.3	28.3	28.3	75.8	38.5	11.3	22.0	86.0	4
M2/176	24	11.0	11.0	11.0	33.8	13.0	3.0	2.8	14.8	4
Day neutral	8	11.0	11.0	11.0	36.0	15.0	5.0	4.0	19.3	4
M2/137	24	11.0	11.0	11.0	34.5	13.8	3.8	3.0	10.5	4
Day neutral	8	11.0	11.0	11.0	37.8	17.0	7.0	7.8	24.5	4

FI node of flower initiation counting from the first scale leaf as node 1.
FD node of first developed flower.
FP node of first pod.
FT flowering time; days from sowing to first open flower.
TN total number of nodes (with expanded leaves) on main stem.
RN number of reproductive nodes on main stem.
Pods and Seeds (number per plant).
N number of plants.

Table 3.3 Node of flower initiation (FI) and time in days to first open flower (FT) for F_2 plants of cross M2/176 X L73. Photoperiod 12 h.

Genotype	FI mean ±SE	FT mean ±SE
A-	12.23±0.09	44.05±0.30
aa	11.88±0.23	42.25±0.56

Differences : FI *aa* and *A-* = 0.35 nodes, t = 1.44, P>0.05
FT *aa* and *A-*=1.82 days, t = 2.82, P<0.05

Table 3.4 The FI and FT of Borek, L60, and the F_1 of cross Borek X L60. Photoperiod 8 h

Line	FI			FT		
Borek	25	25	26	77	77	80
L60	11	11	11	53	47	48
F_1	23	21	22	70	63	64

Table. 3.5 Distribution of flowering node for cross M2/137 X Borek F_1, F_2, F_3 and parents; SD (8 h)

Generation		Node of first initiated flower																					
		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Borek																1		1	1	1				
M2/137		2	2																					
F_1												1	1											
F_2	R	2	7	1																				
F_2	H					3	1					1	9	6	4									
F_2	D								1	2					2	1	5	1						
F_3		20	18	1																				
F_3		57	20	9	26	35	7	3	2	1	11	26	46	21	6	4	2	2	2		2	2		
F_3				4	4	23	9	2			1	5	19	40	10	4	3	4	7		4	1	1	3

R : homozygous recessive, H : heterozygous, D : homozygous dominant

Table 3.6. Joint segregation data for the new flowering locus (shown as +/-) and 15 marker genes obtained from the F_2 of cross L111XM2/137.

Genes	Phenotype				Total	Seg1	Seg2	Joint Seg	RCV±SE	Phase
	A+	A-	a+	a-		X^2	X^2	X^2	(%)	
A/a vs *+/-*	75	15	20	16	126	0.86	0.01	10.70**	31.82±5.21	C
B/b vs *+/-*	62	13	13	2	90	3.33	3.33	0.14	45.64±8.28	R
Cp/cp vs *+/-*	74	28	21	3	126	2.38	0.01	2.34	36.47±7.61	R
Fa/fa vs *+/-*	78	25	17	6	126	3.06	0.01	0.03	51.35±6.58	R
Gp/gp vs *+/-*	68	21	27	10	126	1.28	0.01	0.17	52.55±6.49	R
I/i vs *+/-*	75	24	20	7	126	0.86	0.01	0.03	51.26±6.59	R
K/k vs *+/-*	72	21	23	10	126	0.10	0.01	0.78	55.56±6.26	R
N/n vs *+/-*	75	21	20	10	126	0.10	0.01	1.62	>57.00	R
R/r vs *+/-*	68	25	27	6	126	0.10	0.01	0.99	42.92±7.19	R
S/s vs *+/-*	87	29	8	2	126	19.57***	0.01	0.12	45.95±6.98	R
St/st vs *+/-*	70	23	25	8	126	0.10	0.01	0.00	49.63±6.71	R
Te/te vs *+/-*	68	25	27	6	126	0.10	0.01	0.99	42.92±7.19	R
Tl/tl vs *+/-*	63	23	32	8	126	3.06	0.01	0.67	44.67±7.07	R
Wb/wb vs *+/-*	72	27	23	4	126	0.86	0.01	1.77	39.25±7.44	R
Wlo/wlo vs *+/-*	74	24	21	7	126	0.32	0.01	0.00	50.38±6.65	R

** P<0.01, *** P<0.001

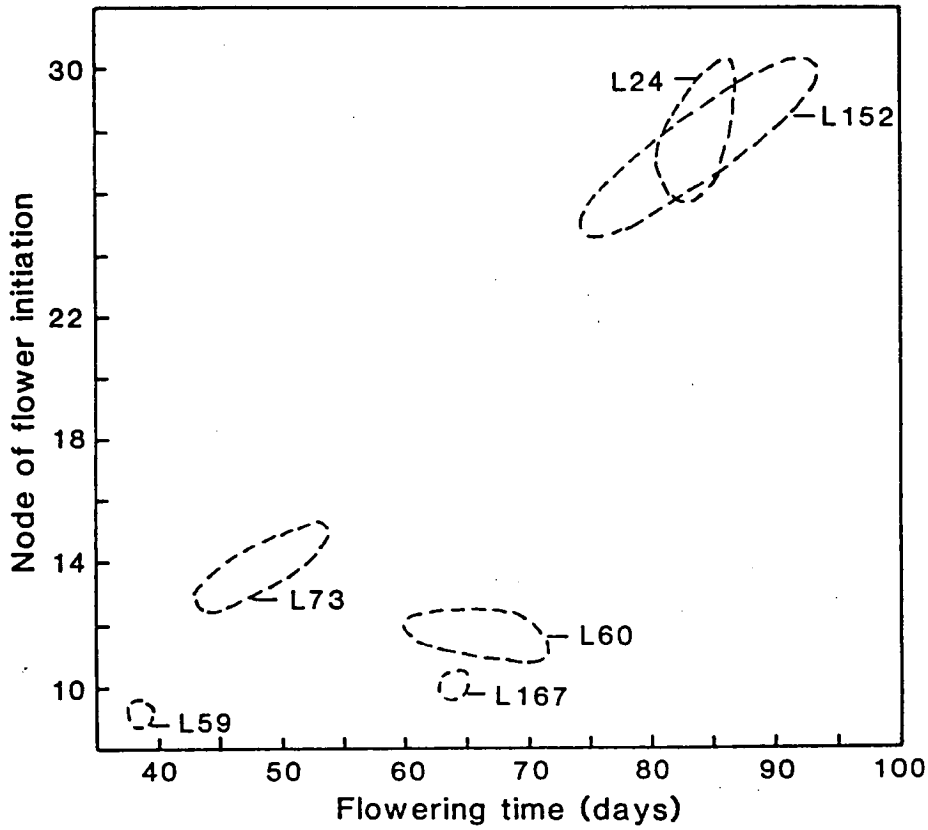


Fig. 3.1. Node of flower initiation (FI) and flowering time (FT) of L152 (initial line), L167 (mutant line) and standard lines L59 (ED), L73 (DN), L60 (EI) and L24 (L). Photoperiod 8 h.

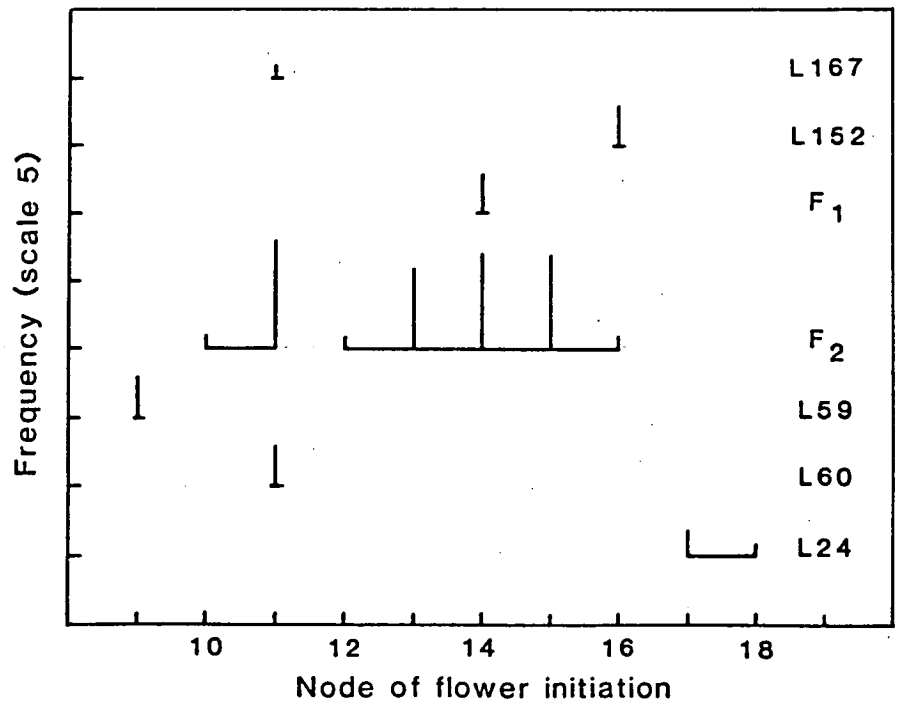


Fig. 3.2. Frequency distribution of node of flower initiation (FI) for L167 (mutant line), L152 (initial line), the F_1 and F_2 of cross L167 x L152, and standard lines L59 (ED), L60 (EI) and L24 (L). Photoperiod 18 h.

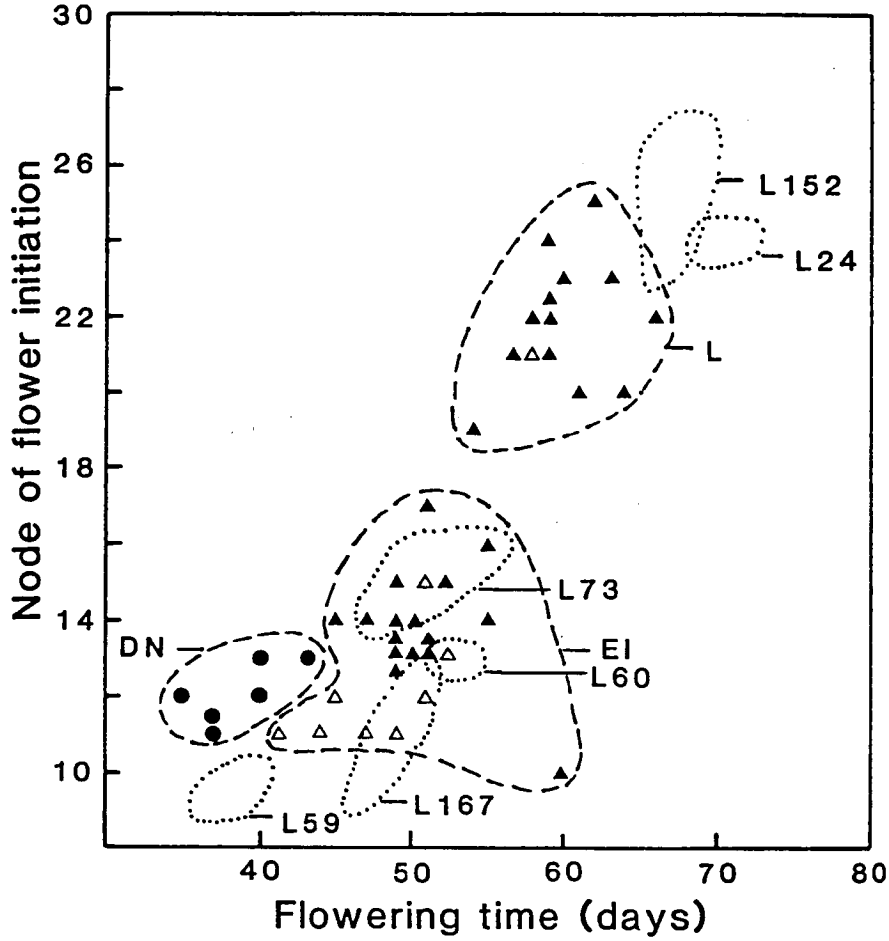


Fig. 3.3. Node of flower initiation (FI) and flowering time (FT) for L152 (initial line), L167 (mutant line), standard lines L59 (ED), L60 (EI), L73 (DN) and L24 (L), the F_2 of cross L167 x L73 (*A Sn* = ▲ ; *a Sn* = △ ; *A sn* = ●). Photoperiod 8 h.

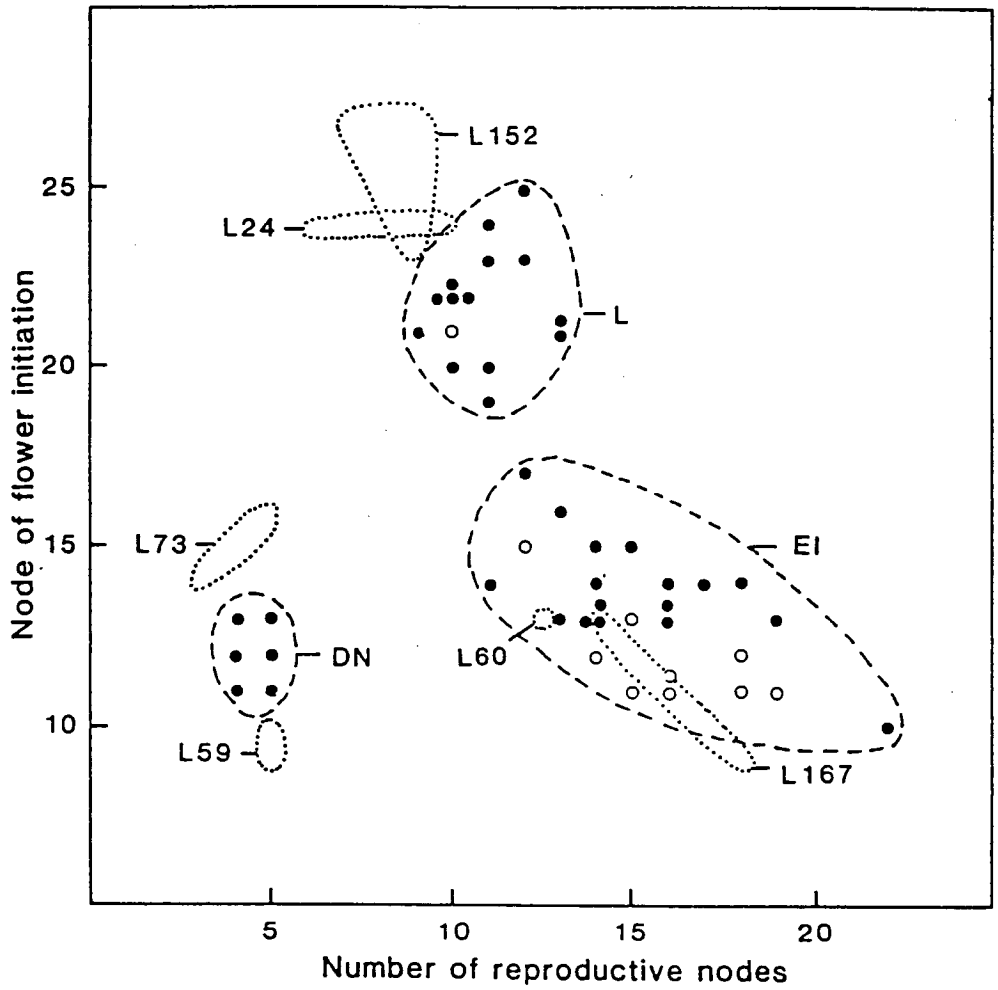


Fig. 3.4. Node of flower initiation (FI) and number of reproductive nodes (RN) for L152 (initial line), L167 (mutant line), standard lines L59 (ED), L60 (EI), L73 (DN) and L24 (L), and the F_2 of cross L167 x L73 (● = *A-* ; ○ = *aa*). Photoperiod 8 h.

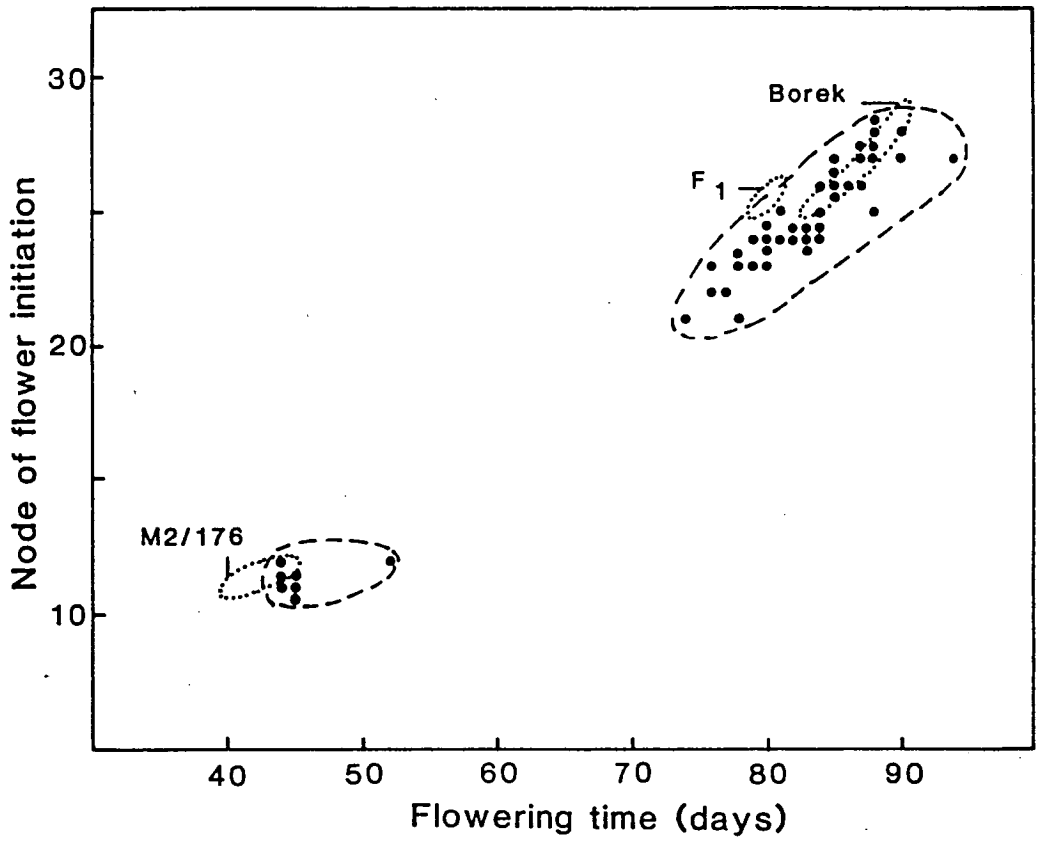


Fig. 3.5. Node of flower initiation (FI) and flowering time (FT) of M2/176, Borek and the F_1 and F_2 of cross M2/176 x Borek. Photoperiod 8 h.

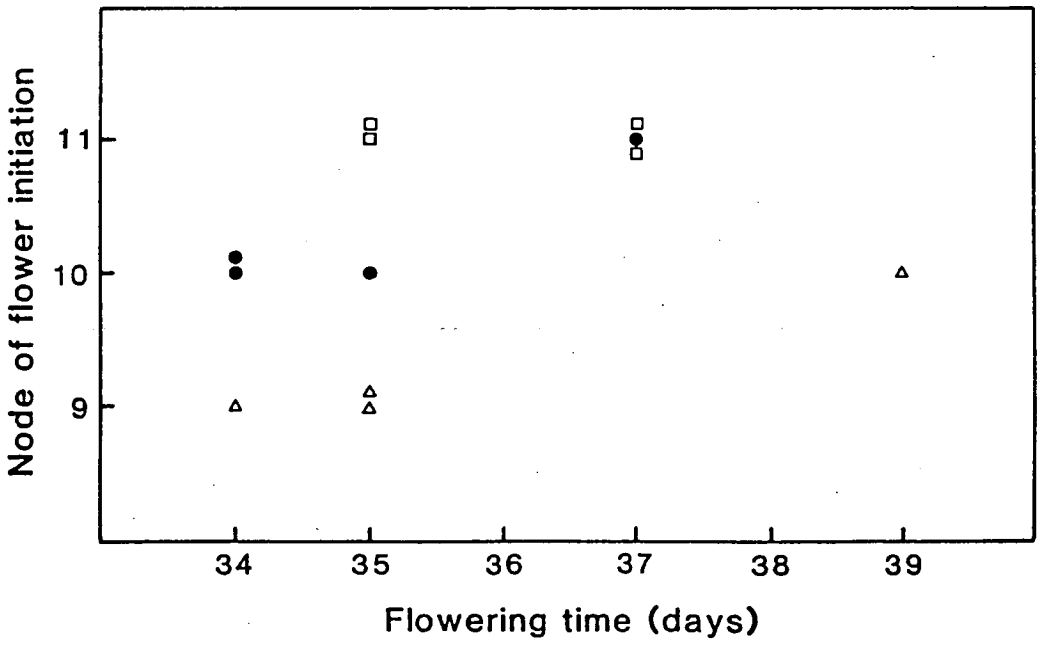


Fig. 3.6. Node of flower initiation (FI) and flowering time (FT) of M2/176 (□), L59 (△) and the F_1 (●) of cross M2/176 x L59. Photoperiod 8 h.

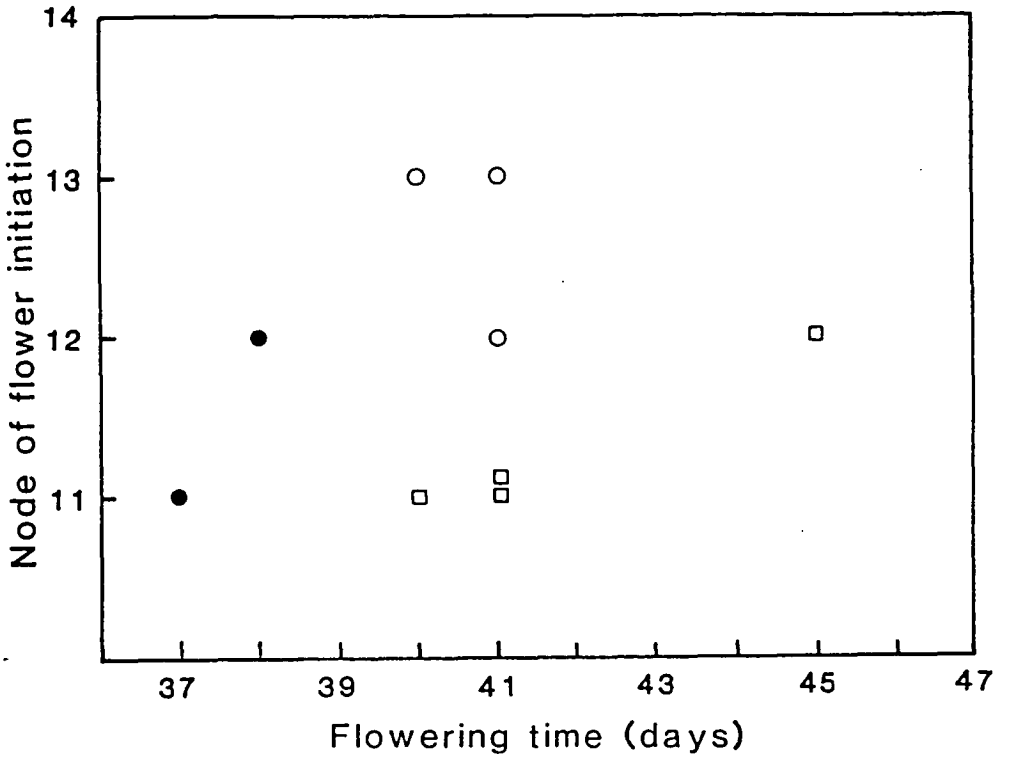


Fig. 3.7. Node of flower initiation (FI) and flowering time (FT) of M2/176 (□), L73 (○), and the F_1 (●) of cross M2/176 x L73. Photoperiod 8 h. L73 and the F_1 were planted on 31-3-1991 and M2/176 control planted on 25-4-1991.

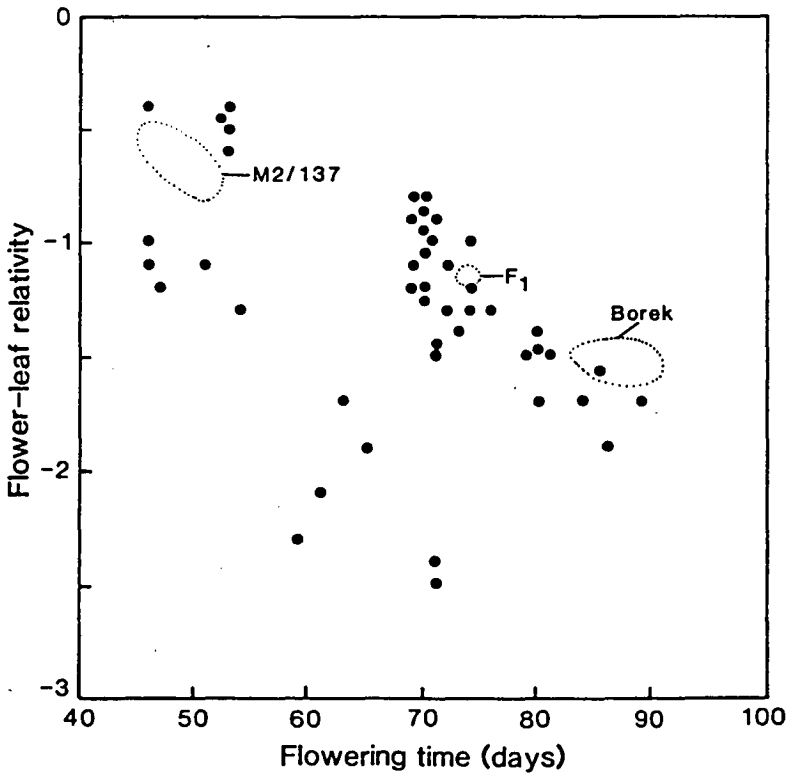


Fig. 3.8. Flowering time (FT) and flower-leaf relativity (FLR) of M2/137, Borek, and the F_1 and F_2 of cross Borek x M2/137. Photoperiod 8 h.

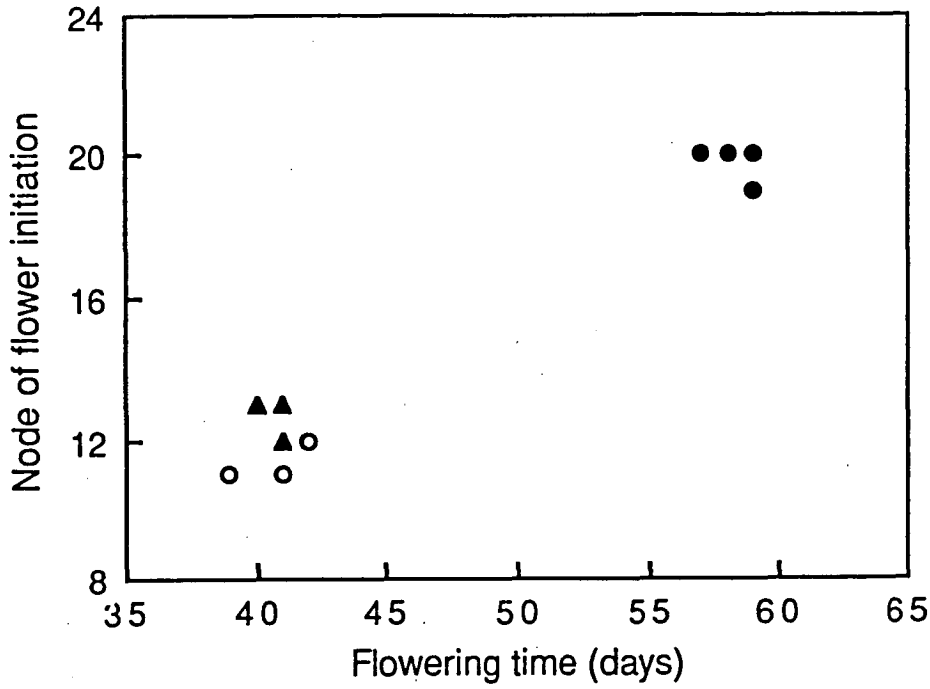


Fig. 3.9. Node of flower initiation (FI) and flowering time (FT) of M2/137 (○), L73 (▲) and the F_1 (●) of cross M2/137 x L73. Photoperiod 8 h.

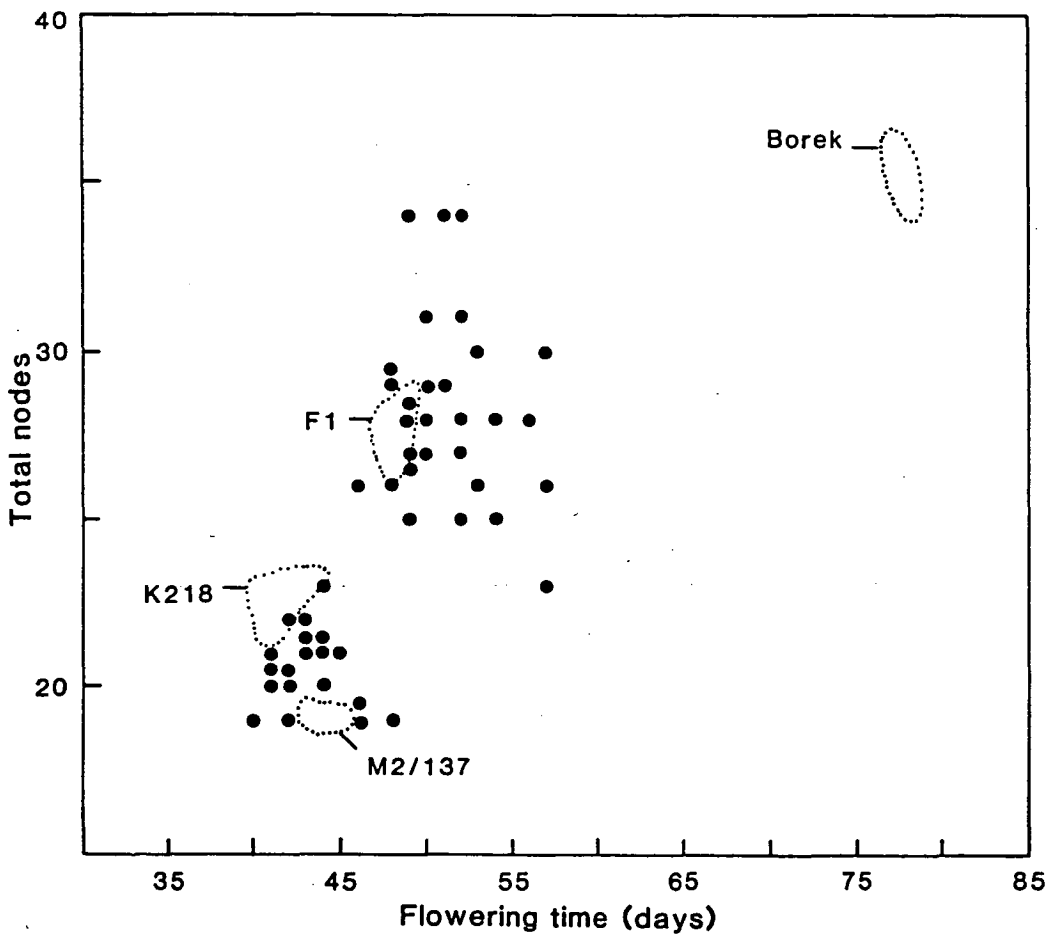


Fig. 3.10. Flowering time (FT) and total nodes (TN) for M2/137, K218, Borek, and the F_1 and F_2 of cross M2/137 x K218. Photoperiod 8 h.

CHAPTER 4
GENETICS OF BRANCHING MUTANTS

4.1 Introduction

Seventeen branching mutants were studied in the present program. Nine mutants (K164, K319, K487, K524, K564, K586, WL5147, WL5918 and WL5951) had previously been characterised phenotypically by Floyd (1985; Fig. 4.1). In addition, mutants Wt10852, Wt15236 and Wt15242 had been characterised by M. Gregory (unpublished). The remaining five mutants (WL5237, WL6042, Wt15240, Wt15241 and Wt15244) were characterised in present study by growing plants to maturity in both LD and SD conditions. With the exception of K319 and K586, all mutants were clearly expressed in LD conditions, and an 18 h photoperiod was used for inheritance studies on these mutants. In the case of K319 and K586, SD conditions were chosen as giving the best chance of exposing segregation of the mutant type.

Blixt (1976) found the branching habit of WL5237 is conferred by a recessive allele, *rms* (*ramosus*). Mutant K487 is also inherited as a monogenic, recessive (Floyd,1985; I.C. Murfet unpub.). Floyd (1985) was unable to obtain a clear answer as to the mode of inheritance of K586. Based on F_1 and F_2 data , M. Gregory (unpub.) concluded mutants Wt10852, Wt15236 and Wt15242 were the result of recessive mutations at three separate loci.

The studies reported in Chapt. 4 therefore had the following aims:

1) To characterise the branching habit of WL5237, WL6042, Wt15240, Wt15241 and Wt15244.
2) To identify the mode of inheritance of mutants K164, K319, K524, K564, K586, WL5147, WL5918, Wt5951, Wt15240, Wt15241 and Wt15244 by crossing with the initial line and growing F_1, F_2 and F_3.
3) To prove monogenic recessive inheritance of mutants Wt10852, Wt15236 and Wt15242 by growing the F_3 generation from F_2s raised by M. Gregory.

Clear segregation of wild type and mutant type plants was often apparent well before the plants reached maturity. Data on laterals were usually recorded when clear segregation become obvious and final measurements at plant maturity were not made unless necessary.

4.2 Phenotypic characterization and genetic control

Mutant K164

This mutant was characterised by Floyd (1985) as a complete branching type which produced lateral branches from all nodes below FI (Fig. 4.1). However, in the present study this mutant produced both basal and aerial laterals, but with gaps at nodes 9 and 11 in LD, and at node 7 and from nodes 15 to 21 in SD (Table 4.1). Both the basal and aerial laterals were strong. The laterals bear a large number of small pods (Floyd, 1985). The plants were generally shorter and less robust than the initial line (Torsdag).

The F_1 of cross K164 x Torsdag (F_1 seeds were made by R. Floyd) was non branching like Torsdag. The F_2 segregated into two classes (Fig. 4.2). Cutting the TLL value at 15 cm gave a clear separation into 34 plants similar to Torsdag and 13 plants similar to the mutant K164 (Fig. 4.2). These numbers are in good accordance to 3:1 ratio (X^2= 0.18, 0.5 <P<0.77). The mutant-type F_2 segregates bred true in F_3 (Table 4.2). Mutant K164 is therefore inherited as a monogenic recessive.

Mutant K319

This mutant was characterised by Floyd (1985) as a basal branching type (Fig. 4.1). In the present study the mutant did not produce laterals in an 18 h photoperiod but K319 did tend to produce both basal and aerial laterals when grown in SD. The aerial laterals arose just below FI.

F_1 and F_2 generations were grown for cross K319 x Torsdag in SD but did not give a clear answer concerning the mode of inheritance. The majority of F_2 plants branched extensively (Fig.4.3) which is in accordance with the conclusion of Uzhintseva and Sidorova (1979) that the branching habit of K319 is the result of a dominant mutation.

Mutant K487

This mutant was characterised as an incomplete/gap branching type (Fig. 4.1, from Floyd, 1985). In SD laterals that arose from basal nodes were usually stronger than the aerial laterals (Table 4.1). In LD (18 h), the mutant generally did not produce basal laterals (Table 4.1) but if basal laterals did occur in LD

they were usually weaker than in SD.

Floyd (1985) concluded from F_1 and F_2 data from cross K487 x Torsdag that mutant K487 is inherited as a monogenic recessive (Table 4.2). Murfet (unpub.) confirmed this result by showing that F_2 segregates with the K487 type branching habit bred true in F_3 (Table 4.2). Floyd found the branching habit of K487 was dissimilar to that of WL 851 which has branching genes *pro*, *fr* and *fru* according to Blixt (1968).

Mutant K524

This mutant was characterised by Floyd (1985) as a basal branching type. However, in LD both basal and aerial laterals were observed in the present study (Table 4.1). This mutant also had curved pods, and a weaker appearance and shorter main stem than the initial line.

Cross K524 x Torsdag gave rise to an F_1 that was similar to Torsdag , and the F_2 segregated into 35 plants like Torsdag and 13 mutant-type plants (Fig. 4.2). These numbers fit a 3:1 segregation ratio (X^2= 0.11, P=0.81). The mutant-type F_2 segregates bred true in F_3 (Table 4.2). Mutant K524 is therefore inherited as a monogenic recessive.

Mutant K564

K564 was characterized by Floyd (1985) as a complete branching type and this was confirmed in the present study (Table 4.1). The mutant is not as strong as the initial line , and it has a shorter and thinner main stem (Floyd, 1985).

F_1, F_2 and F_3 data from cross K564 x Torsdag (Table 4.2 and Fig. 4.2) show the mutant is inherited as a monogenic recessive.

Mutant K586

This mutant was characterized as a basal branching type by Floyd (1985). In present study the mutant mostly produced a strong basal lateral (Table 4.1), which had a length of 60-80 % of main stem height in LD and SD. This mutant was not easy to study because the basal lateral was not always present if the conditions were not suitable.

When the F_2 of cross K586 x Torsdag was grown in SD, only a low number of mutant types were detected (4 out of 64 plants). The deficiency of

mutant types may have resulted from unsuitable conditions. The mutant may be inherited as a monogenic recessive but Floyd (1985) also was unsuccessful in obtaining a clear indication as to the mode of inheritance of this mutant.

Mutant WL5147

This mutant was classified by Floyd (1985) as a complete branching type where the laterals arose from all nodes below FI both in SD and LD (Fig. 4.1). In the present study, in LD, no basal laterals occurred at nodes 1-4, and in SD, only one aerial lateral was produced (Table 4.1). This mutant showed a large decrease in height compared with the progenitor, and it had a weaker appearance (Floyd, 1985).

The cross WL5147 x Weitor gave rise to an F_1 that was similar to Weitor. Two F_2 populations were grown. The first F_2 segregated into 41 wild types and 6 mutant types, and the second F_2 segregated into 25 wild types and 7 mutant types (Table 4.2, Fig. 4.2). There is a deficiency of mutant types in the F_2 but the deviation from a 3:1 ratio is not significant ($X^2 = 2.87$, $P>0.05$). Mutant F_2 plants bred true in F_3 (Table 4.2). These results indicate that mutant WL5147 is inherited as a monogenic recessive.

Mutant WL5237

This mutant showed a similar appearance to WL5147. The laterals arose from node 3 to 25 in SD and from 3 to 16 in LD with gap at node 8 in LD and 17 in SD (Table 4.1, Fig. 4.4). This mutant matches Floyd's criteria for a complete branching type. The initial line, Parvus, also produced weak laterals with one aerial lateral that was quite strong in SD (Table 4.1, Fig. 4.5). Blixt (1976) found the mutant to have a height about half that of the progenitor (Parvus), the stem was thinner, and the mutant had, on average, 4.2 basal branches compared with 1.7 in the progenitor. The phenotypic characters in the present study were consistent with what Blixt found except that the observed height of the mutant was 70-75% of the initial line in SD and LD (Table 4.3).

Blixt (1976) found that the mutant was monogenic recessive. He symbolised the mutant allele *rms* (*ramosus*), and showed that *rms* was located on chromosome 3 about 12 units from *fa* (fasciated).

Mutant WL5918

This mutant was reported to be a basal branching type by Floyd (1985). The mutant was quite strong, with strong basal branches (Table 4.1) that usually grew to the same height or even taller than the main stem in SD and 80-90% as high as the main stem in LD.

The cross WL5918 x Raman gave rise to an F_1 that was similar to the initial line, Raman. Two F_2 populations were grown. The first F_2 segregated 41 wild types and 5 mutant types, and the second F_2 segregated 27 wild types and 5 mutant types (Fig. 4.2). These results show a significant deficiency of mutant types (X^2 testing 3:1 = 5.93, $P<0.05$, Table 4.2). F_2 mutant types bred true in F_3 (Table 4.2). It is concluded that the mutant is inherited as a monogenic recessive. More extensive data are necessary to establish whether the deficiency of recessive segregates is a consistent feature of segregation for this pair of alleles.

Mutant WL 5951

This mutant was reported to be a basal branching type by Floyd (1985; Fig. 4.1) . In the present study, the laterals that arose from basal nodes were strong, and the mutant differed from the initial line mainly in terms of increased basal branching (Table 4.1).

Cross WL5951 x Parvus gave rise to a wild-type F_1. The F_2 segregated into two classes, corresponding to the two parents. In this F_2, a frequency distribution for TLL did not permit clear separation but a two way plot of total length of main stem (TL) against total lateral length (TLL) allowed the wild type and mutant type segregates to be distinguished clearly (Fig. 4.6). The F_1, F_2 and F_3 results (Table 4.2) indicate monogenic recessive inheritance of the mutant.

Mutant WL6042

Meteor (L136) is listed in the Weibullsholm accession list as the progenitor of mutant WL6042. However, Meteor is an early flowering, day neutral cultivar while mutant WL6042 has a late flowering, photoperiodic habit (Table 4.3). Either WL6042 is the result of at least two separate mutations or it is, in fact,

derived from a late, photoperiodic, dwarf cultivar. The latter view is favoured, and Raman was chosen as the most likely true progenitor. Meteor is presumed to have genotype *sn*. The recessive mutant allele *sn* confers a day neutral habit and a marked reduction in the ability to produce basal laterals (Murfet and Reid, 1985). This is reflected in the fact that Meteor did not produce laterals in LD or SD (Fig. 4.7). In contrast, mutant WL6042 produced strong basal laterals in both LD and SD (Table 4.1, Fig. 4.8) and it classifies as a basal branching type as proposed by Floyd (1985). In SD, the strongest basal laterals were longer than the main stem (Fig. 4.8), and the mutant produced more pods ($t = 5.86$, $P<0.01$) and seeds ($t = 5.44$, $P<0.01$) on the laterals than on the main stem (Table 4.4).

Cross WL 6042 x Raman gave rise to an F_1 like Raman. The F_2 segregated into 30 wild types and 18 mutant types (Fig. 4.2) and there was therefore a significant excess of mutant types above those expected on the basis of a 3:1 ratio ($X^2 = 4.0$, $P< 0.05$; Table 4.2). The mutant-type F_2 plants bred true in F_3 (Table 4.2) and monogenic recessive inheritance of the mutant is proposed.

Mutant Wt10852

This mutant has a basal branching phenotype (Table 4.1) with basal laterals arising from nodes 1 and 2 in SD and LD. The laterals were usually as strong as, or stronger than, the main stem.

Based on F_2 data from cross Wt10852 x Paloma, M. Gregory (unpub.) obtained clear evidence of monogenic recessive inheritance of mutant Wt10852 (Table 4.2). F_3 data confirmed that conclusion (Table 4.2).

Mutant Wt15236

This mutant has a basal branching phenotype both in LD and SD (Table 4.1) but with a tendency to produce weak aerial laterals in LD. The main stem and laterals were usually thinner and less robust than the shoot of the initial line, Paloma. Basal lateral shoots were sometimes taller than the main stem.

F_2 data from cross Wt15236 x Paloma (M. Gregory unpub.) indicated mutant Wt15236 to be the result of a monogenic recessive mutation (Table 4.2). F_3 data confirmed that conclusion (Table 4.2).

Mutant Wt15240

This mutant produced strong aerial branches in both SD and LD (Fig. 4.9). In contrast, the initial line Kaliski produced only weak aerial branches (Fig. 4.10). In SD, Wt15240 produced one basal branch, and aerial branches commencing at most nodes above node 8 and below FI (Fig. 4.9). In LD, aerial branches arose from nodes 11 to 15 (Fig. 4.9). The main stem was thinner and shorter compared with the initial line, Kaliski, and the mutant had shorter internodes (L_{1-6}, L_{1-9} and L_{1-12}, $P<0.05$) in both SD and LD conditions (Table 4.3). In LD, there was no significant difference in the number of pods ($t = 2.31$, $P>0.05$) and seeds ($t=0.19$, $P>0.05$) on the laterals and on the main stem of mutant plants. However, in SD, the pods and seeds were mostly produced on the laterals (Table 4.4). In contrast, the initial line Kaliski produced most of its pods and seeds on the main stem.

Cross Wt15240 x Kaliski gave an F_1 similar to Kaliski. The F_2 segregated into 33 wild type and 15 mutant type plants (Fig. 4.2). These observed numbers fit a 3:1 ratio ($X^2 = 1.00$, $0.5>P>0.2$), indicating Wt15240 is the result of a monogenic recessive mutation. F_3 data confirmed that conclusion (Table 4.2).

Mutant Wt15241

This mutant is a basal branching type and it produced laterals from nodes 1 and 2 in LD and nodes 1-4 in SD (Table 4.1, Fig. 4.11). These laterals were usually almost as strong as the main stem. In contrast, the initial line, Paloma, did not produce laterals in LD, but sometimes produced one strong basal lateral in SD (Table 4.1, Fig. 4.12). The mutant was earlier flowering ($t = 6.20$, $P<0.001$ in LD; $t = 6.90$, $P<0.001$ in SD) than the initial line Paloma and in LD it had 1.2-1.3-fold longer internodes (L1-4, $t = 5.03$; L1-6, $t = 7.06$ and L1-9, $t = 5.57$, $P<0.001$ in each case) (Table 4.3). In SD, the mutant had 1.1-1.2-fold longer internodes than the initial line but the differences were not significant ($t = 1.49$-2.68, $P>0.05$). In SD, this mutant produced more pods and seeds from the laterals than from the main stem, while the initial line produced pods and seeds mostly from the main stem (Table 4.3).

Cross Wt15241 x Paloma gave an F_1 similar to Paloma. The F_2 segregated into 33 wild type and 15 mutant type plants (Fig. 4.11). These

observed numbers fit a 3:1 ratio (X^2 = 1.00, 0.5>P>0.2), indicating Wt15241 is the result of a monogenic recessive mutation (Table 4.2). F_3 data are not presently available.

Mutant Wt15242

This mutant has a basal branching phenotype (Table 4.1). The mutant is less robust than the initial line, Paloma. Mutant Wt15242 showed very severe growth abnormalities and leaf damage in SD conditions.

F_2 data from cross Wt15242 x Paloma (M. Gregory unpub., Table 4.2) indicated mutant Wt15242 to be the result of a monogenic recessive mutation. F_3 data confirmed that conclusion (Table 4.2).

Mutant Wt15244

Mutant Wt15244 has a basal branching phenotype (Fig. 4.14) with strong branches in both LD and SD. The initial line, Porta, usually produced 1 to 2 basal branches in SD and sometimes also in LD (Fig. 4.15). However, these laterals were neither as numerous nor as strong as those produced by the mutant. The mutant produced 4 to 6 basal laterals from nodes 1, 2 and 3. Some of these laterals were usually similar to the main stem in diameter and length. The mutant had shorter internodes than the initial line in SD and LD conditions although the difference in SD was not statistically significant (Table 4.3). In LD, the total length of the mutant was also shorter than for the initial line (t = 5.04, P<0.01; Table 4.3). In SD conditions, the mutant produced more pods and seeds on the laterals than on the main stem, but in LD, no significant differences were found in the number of pods and seeds on the laterals and main shoot. The initial line produced more pods and seeds on the main stem than on the laterals in LD, but followed the same pattern as the mutant in SD (Table 4.4).

Cross Wt15244 x Porta F_1 was similar to Porta. The F_2 segregated into two classes with 35 wild type and 7 mutant type plants (Fig. 4.16). These numbers fit a 3:1 ratio (X^2 = 1.56, 0.3>P>0.2). Mutant type F_2 plants bred true in F_3 (Table 4.2). These results indicate monogenic recessive inheritance.

Summary

Based on Floyd's (1985) method for grouping branching mutants, the 17

mutants studied are grouped as follows:

- Basal branching type : K586, WL5918, WL6042, Wt10852, Wt15236, Wt15241, Wt15242, Wt15244.
- Aerial branching type : Wt15240.
- Gap branching/incomplete branching type : K164, K319, K487, K524, WL5951.
- Complete branching type : K564, WL5147, WL5237.

Mutant K164 that was classified as a complete branching type by Floyd (1985), is here classified as a gap branching type, since in the present study a gap phenotype was observed in the two photoperiods used.

The initial lines for these mutants are classified as follows: non branching type (Meteor); basal branching type (Weitor, Raman, Paloma, and Porta) and aerial branching type (Kaliski, Parvus, and Torsdag).

The mutants studied all showed a clear monogenic recessive inheritance, except K319 and K586. Mutant K319 showed an indication of being a dominant mutant. K586 is a weak branching mutant and the mode of inheritance is presently not clear.

Table 4.1: The node of origin of laterals and lateral length (cm) for branching mutants and their initial lines. Measurements were taken from mature plants.

Mutants	Photo-period	Node of origin																										N
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Torsdag	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0	3.4	69.4	-	-	-	-	-	-	-	-	-	1
K164	18	-	-	-	-	-	-	63.7	1.5	0.5	-	24.2	-	44.1	23.4	22.2	27.1	13.8	-	-	-	-	-	-	-	-	-	2
	8	-	-	-	72.7	64.5	66.1	50.6	-	47.8	47.5	47.0	100.6	41.2	35.9	40.4	-	-	-	-	-	-	-	27.0	27.4	-	-	2
K319	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	8	-	54.1	69.4	-	-	-	-	-	-	-	-	119.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
K487	18	-	-	-	-	-	-	1.5	1.7	38.1	-	-	37.9	31.9	61.1	63.6	43.7	-	-	-	-	-	-	-	-	-	-	2
	8	-	-	128.2	69.5	112.2	148.3	-	-	-	40.7	77.6	1.9	72.1	63.5	72.5	52.4	33.2	21.7	-	-	-	-	-	-	-	-	2
K524	18	-	-	49.8	-	-	-	-	-	-	31.7	12.3	31.1	27.6	27.8	9.9	10.5	-	-	-	-	-	-	-	-	-	-	2
	8	-	152.2	140.8	28.3	18.8	-	-	-	-	-	-	-	-	-	-	-	-	-	11.4	-	7.8	-	-	-	-	-	2
K564	18	-	-	-	-	0.8	29.8	44.3	27.4	42.8	-	2.1	8.6	23.5	31.4	30.7	28.0	-	-	-	-	-	-	-	-	-	-	2
	8	-	-	-	57.3	42.3	46.8	19.8	44.0	45.5	36.9	39.8	32.5	34.4	67.7	69.9	1.6	35.7	1.6	4.8	-	-	-	-	-	-	-	2
K586	18	-	-	72.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	8	-	30.7	126.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Weltor	18	-	-	-	-	-	-	-	-	-	3.3	0.8	0.8	1.8	19.7	2.1	-	-	-	-	-	-	-	-	-	-	-	2
	8	-	-	44.6	-	-	-	-	-	-	-	-	6.5	34.2	3.2	2.4	0.8	0.7	-	-	-	-	-	-	-	-	-	2
WL5147	18	-	-	-	-	-	71.6	54.8	17.8	2.3	0.6	16.8	35.6	32.6	30.8	27.6	8.0		-	-	-	-	-	-	-	-	-	2
	8	-	-	89.9	57.7	99.5	53.6	62.0	-	-	9.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Parvus	18	-	-	-	-	-	-	-	-	-	8.9	-	-	8.4	5.6	12.6	3.9	1.3	-	-	-	-	-	-	-	-	-	2
	8	-	-	28.1	-	-	-	-	-	-	1.2	2.7	-	47.1	116.3	1.8	2.8	15.5	4.6	6.8	1.6	13.2	5.8	1.8	10.7	12.2	2.3	2
WL5237	18	-	-	-	20.9	44.6	64.5	63.3	11.6		64.7	57.0	50.4	47.8	35.0	32.1	99.2	5.3	-	-	-	-	-	-	-	-	-	4
	8	-	-	-	132.1	58.9	112.4	50.7	89.9	107.2	99.9	97.3	88.0	81.0	64.1	55.6	59.5	35.9	-	32.4	33.5	28.4	24.3	16.3	13.5	8.3	3.9	2
WL5951	18	-	49.6	135.6	41.7	8.1	-	-	-	0.8	27.1	17.9	21.9	18.1	16.0	9.8	7.9	-	-	-	-	-	-	-	-	-	-	2
	8	-	-	311.4	-	-	-	-	-	-	-	27.5	12.2	15.2	1.5	11.2	11.8	-	4.3	8.9	7.4	6.0	2.3	-	-	-	-	2
Raman	18	-	-	17.6	-	-	-	-	-	-	-	-	-	-	2.2	-	1.0	-	-	-	-	-	-	-	-	-	-	2
	8	-	81.5	147.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
WL5918	18	-	14.3	31.7	64.0	61.9	5.3	-	-	12.9	-	-	0.6	0.3	0.7	1.3	0.8	-	-	-	-	-	-	-	-	-	-	2
	8	-	89.9	151.2	31.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
WL6042	18	-	12.0	51.4	20.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	8	-	38.2	119.5	78.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Paloma	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	8	-	-	48.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Wt10852	18	-	24.5	29.1	-	-	1.8	-	0.4	-	-	1.2	1.8	0.4	0.4	0.3	0.2	-	-	-	-	-	-	-	-	-	-	2
	8	-	68.9	105.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Wt15236	18	-	20.9	41.9	27.0	7.9	5.3	7.3	3.6	-	3.6	3.9	4.7	2.8	2.5	0.4	0.4	-	-	-	-	-	-	-	-	-	-	2
	8	-	47.8	116.2	42.1	29.5	10.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Wt15241	18	-	22.1	32.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
	8	-	14.2	33.9	56.2	17.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Wt15242	18	10.8	10.6	19.2	19.3	14.8	13.3	6.8	-	-	-	2.2	3.1	1.9	2.3	0.3	0.3	-	-	-	-	-	-	-	-	-	-	2
	8	-	37.5	114.6	63.5	29.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Kallski	18	-	-	-	-	-	-	-	-	-	-	-	-	2.5	2.5	13.5	-	-	-	-	-	-	-	-	-	-	-	4
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.7	-	-	-	-	-	-	3
Wt15240	18	-	-	-	-	-	-	-	-	-	-	-	18.5	38.4	60.6	59.1	47.1	-	-	-	-	-	-	-	-	-	-	4
	8	-	-	62.9	-	-	-	-	-	-	108.0	57.3	93.6	112.0	105.0	93.2	74.6	36.0	36.3	29.4	-	-	18.5	16.0	-	-	-	2
Porta	18	-	-	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	8	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Wt15244	18	-	7.7	33.3	13.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	8	-	51.4	107.1	50.3	-	-	-	-	-	-	0.7	0.4	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	4

Table 4.2. Results of crosses between branching mutants (phenotype M) and their initial lines (phenotype WT).

Cross	F_1	F_2 segregation WT	M	Chi-square testing 3:1	F_3 from M-type F_2
K164 X Torsdag	WT	34	13	0.18	M
K487 X Torsdag[a]	WT	46	18	0.33	M
K524 X Torsdag	WT	35	13	0.11	M
K564 X Torsdag	WT	38	10	0.44	M
WL5147 X Weitor	WT	66	13	2.86	M
WL5918 X Raman	WT	67	10	5.93*	M
WL5951 X Parvus	WT	34	14	0.44	M
WL6042 X Raman[b]	WT	30	18	4.00*	M
Wt10852 X Paloma[c]	WT	42	11	0.51	M
Wt15236 X Paloma[c]	WT	36	8	1.09	M
Wt15240 X Kaliski	WT	33	15	1.00	M
Wt15241 X Paloma	WT	33	15	1.00	
Wt15242 X Paloma[c]	WT	43	15	0.02	M
Wt15244 X Porta	WT	35	7	1.56	M

* $P<0.05$

a F_1 and F_2 data Floyd (1985); F_3 data I.C.Murfet (unpub.)

b Meteor is listed as the initial line but Raman was used because both WL6042 and Raman have an L-type flowering phenotype. Meteor is a DN type

c F_1 and F_2 data M. Gregory (unpub.)

Table 4.3. Mean ± SE for node of flower initiation (FI), stem length (cm) between node 1 and nodes 4, 6, 9, 12, and total length of stem (TL) for five branching mutants and their initial lines.

Lines	FI	L1-4	L1-6	L1-9	L1-12	TL	N
			Photoperiod 18 h				
Parvus	17.00±0.00	8.50±1.00	23.90±1.50	52.85±1.85	81.60±1.90	174.30±4.53	2
WL5237	15.75±0.25	8.58±0.43	22.23±0.79	43.05±1.57	67.35±1.74	133.08±2.86	4
Meteor	9.25±0.25	2.95±0.24	8.53±0.58	23.33±1.01	34.50±1.37	33.60± 1.60	4
WL6042	17.00±0.00	2.43±0.21	6.20±0.24	12.30±0.46	20.38±0.67	46.40± 1.44	4
Kaliski	16.00±0.00	7.50±0.50	22.28±0.97	56.28±1.28	89.05±0.80	178.23± 2.27	4
Wt15240	16.25±0.25	5.10±0.31	17.03±0.21	45.05±0.39	72.58±1.07	167.85± 4.85	4
Paloma	17.33±0.33	1.87±0.09	4.63±0.12	11.93±0.29	23.30±0.40	52.90± 2.15	3
Wt15241	15.13±0.13	2.51±0.09	5.78±0.11	13.86±0.19	24.08±0.28	40.44± 1.33	8
Porta	15.75±0.48	3.03±0.19	6.95±0.33	17.43±0.32	27.28±0.63	42.33± 1.75	3
Wt15244	16.00±0.00	2.48±0.14	5.93±0.18	13.23±0.34	20.33±0.31	33.15± 0.51	4
			Photoperiod 8 h				
Parvus*	27.00±1.00	8.50±1.00	20.50±1.30	41.00±0.40	64.90±0.10	235.65±3.05	2
WL5237*	26.00±0.00	8.30±0.40	19.70±0.30	33.55±0.55	51.10±0.06	169.15±2.45	2
Meteor*	9.25±0.25	2.83±0.23	7.35±0.48	18.38±1.22	33.25±0.76	40.33± 1.52	4
WL6042*	24.00±0.00	2.70±0.00	5.43±0.19	9.07±0.30	12.73±0.52	43.67±11.63	3
Kaliski*	21.67±0.33	6.43±0.38	18.17±0.56	43.30±1.04	69.87±1.81	248.93± 2.72	3
Wt15240*	26.00±0.00	4.95±0.75	14.70±0.50	32.40±0.70	51.00±0.10	199.55± 3.05	2
Paloma	22.00±0.58	2.13±0.18	4.63±0.35	10.57±0.67	16.97±1.11	69.17± 4.21	3
Wt15241	18.00±0.00	2.43±0.09	5.63±0.13	11.73±0.15	19.10±0.46	57.60± 4.07	3
Porta*	21.50±0.50	2.73±0.26	5.97±0.50	12.50±1.15	18.00±1.17	39.10± 8.83	3
Wt15244*	19.75±0.25	2.80±0.21	5.63±0.25	10.55±0.38	15.68±0.70	43.40± 1.08	4

*Transferred to LD (18 h) at age 48 days.

Table 4.4. The mean ± SE for the number of pods and seeds on the laterals and main shoot of five branching mutants and their initial lines.

Line	Laterals		Main shoot		N
	No. of pods	No. of seeds	No. of pods	No. of seeds	
		Photoperiod 18 h			
Parvus	3.00±1.00	6.50±1.50	5.50±1.50	33.00±6.00	2
WL5237	25.00±0.71	47.25±4.17	4.75±1.25	16.25±4.21	4
Meteor	0.00±0.00	0.00±0.00	2.00±0.00	10.25±0.85	4
WL6042	4.50±0.50	11.50±0.87	3.50±0.29	10.50±1.44	4
Kaliski	1.25±0.75	2.75±1.70	6.00±0.41	21.50±0.50	4
Wt15240	9.25±1.03	19.00±1.35	5.75±1.11	19.75±3.71	4
Porta	0.25±0.25	0.25±0.25	3.50±0.65	11.25±1.80	3
Wt15244	3.75±0.63	9.25±1.89	2.00±0.41	4.75±1.03	4
		Photoperiod 8 h			
Parvus*	2.50±0.50	9.00±3.00	4.00±4.00	24.00±24.00	2
WL5237*	40.00±2.00	86.50±6.50	2.50±1.50	9.00±0.00	2
Meteor*	0.00±0.00	0.00±0.00	3.00±0.00	15.00±0.41	4
WL6042*	13.70±1.20	34.70±3.18	2.67±1.45	6.67±4.06	3
Kaliski*	0.67±0.67	2.00±2.00	8.33±0.67	36.00±6.03	3
Wt15240*	23.00±4.00	47.00±10.00	5.00±0.00	14.50±0.50	2
Paloma**	4.00±0.58	7.00±1.00	7.33±1.20	15.67±2.19	3
Wt15241**	11.00±0.58	20.33±1.33	2.67±1.33	6.33±3.28	3
Porta*	6.67±0.88	20.00±1.00	1.00±0.58	3.67±1.86	3
Wt15244*	11.30±0.48	24.00±2.58	2.50±0.87	7.25±2.14	4

*Transferred to LD (18 h) at age 48 days.
**LD plants were not scored.

Group 1

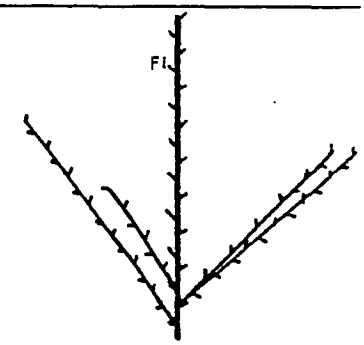
Non-branching types

Occasionally produce one basal and/or aerial lateral under 8 h PP.
Generally robust.
(K218, L136)

Group 2

Basal branching types

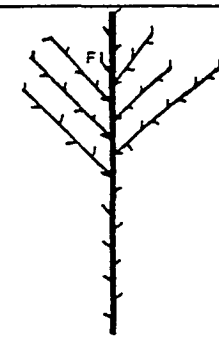
Produce basal laterals from nodes 1,2 and 3.
May produce up to 4 laterals at either of nodes 1 or 2.
Laterals usually reach 60-80% of main stem height.
(K586, WL5918, L78, WL6042, WL6013, WL1263, WL2168, WL851, K319, WL5951, K524)



Group 3

Aerial branching types

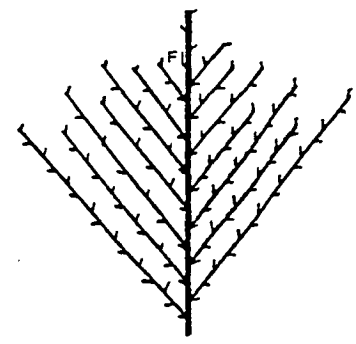
Produce only aerial laterals at nodes just below FI.
Laterals usually 30-40% of main stem height.
(L77, WL5969)



Group 4

Complete branching types

Produce laterals from all nodes below FI.
Laterals bear large numbers of small pods.
Plants generally shorter and less robust than Non-branching types.
(K564, K164, WL5147)



Group 5

Incomplete / Gap branching types

Produce laterals from most nodes but have a break in branching between nodes 3-8.
Size of break varies with photoperiod, being greatest under 24 h PP.
Laterals bear many small pods.
(L107, K487)

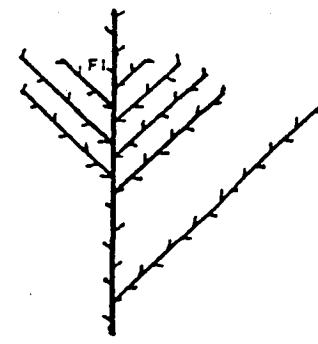


Fig. 4.1. Major branching phenotypes and classification for several branching mutants, initial lines and cultivars (from Floyd, 1985).

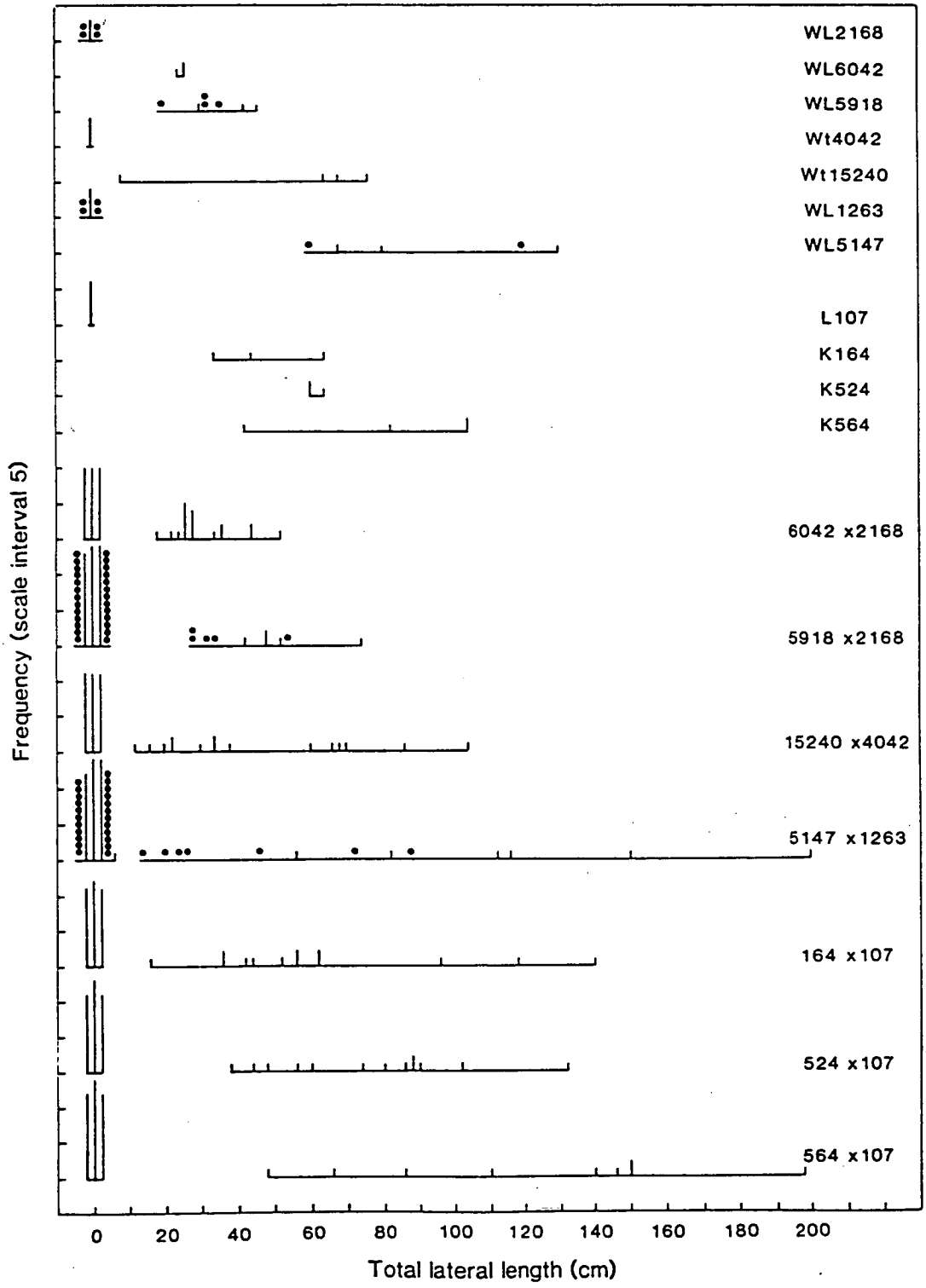


Fig. 4.2. Total lateral length (TLL) of mutants WL6042, WL5918, Wt15240, WL5147, K164, K524, and K564, their initial lines WL2168 (Raman), Wt4042 (Kaliski) and L107 (Torsdag), and F_2 populations from crosses between the mutants and their initial line. Photoperiod 18 h. (bar = first population; ● = second population).

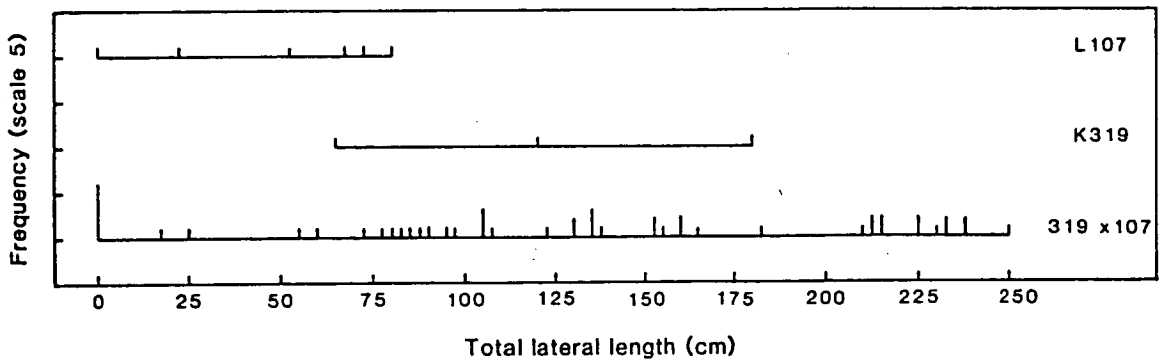


Fig. 4.3. Frequency distribution of total lateral length (TLL) for mutant K319, its initial line (Torsdag) and the F_2 of cross K319 x Torsdag. Photoperiod 8 h.

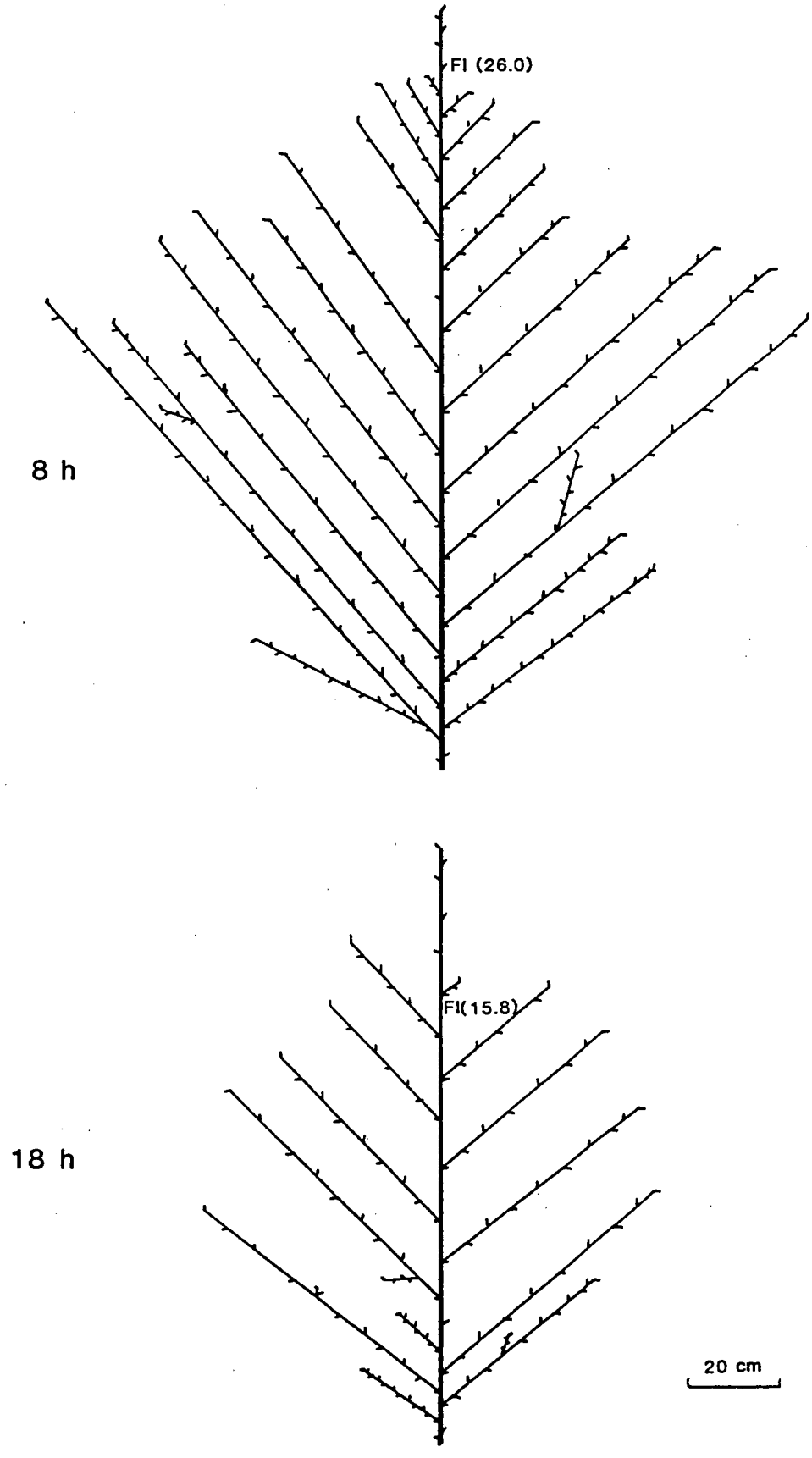


Fig. 4.4. Mutant WL5237 in 8 h and 18 h conditions. The length of the main shoot and laterals are drawn to scale, but individual internodes are not drawn to scale. Measurements were taken from mature plants.

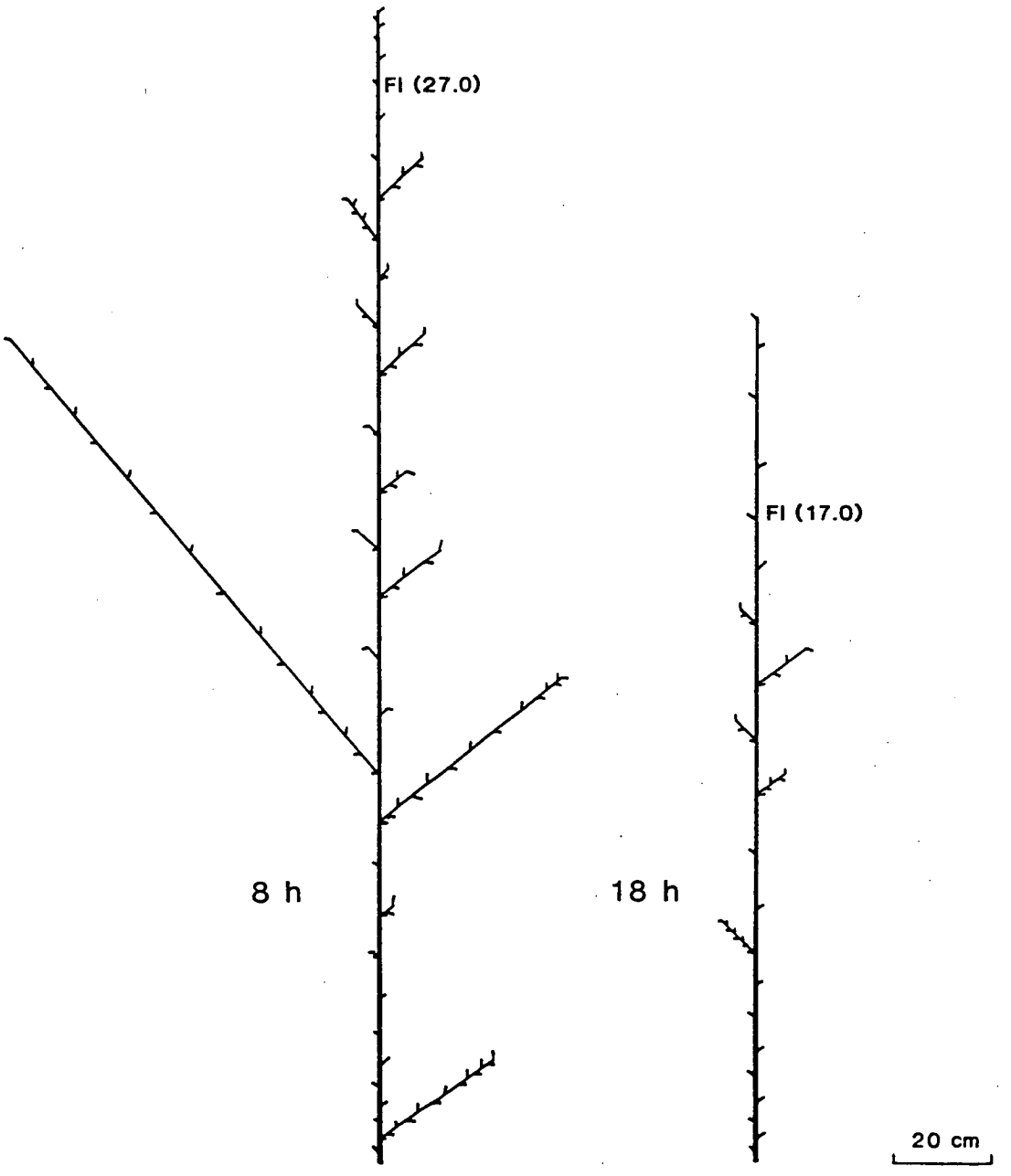


Fig. 4.5. Initial line Parvus (L77) in 8 h and 18 h conditions. The length of the main shoot and laterals are drawn to scale, but individual internodes are not drawn to scale. Measurements were taken from mature plants.

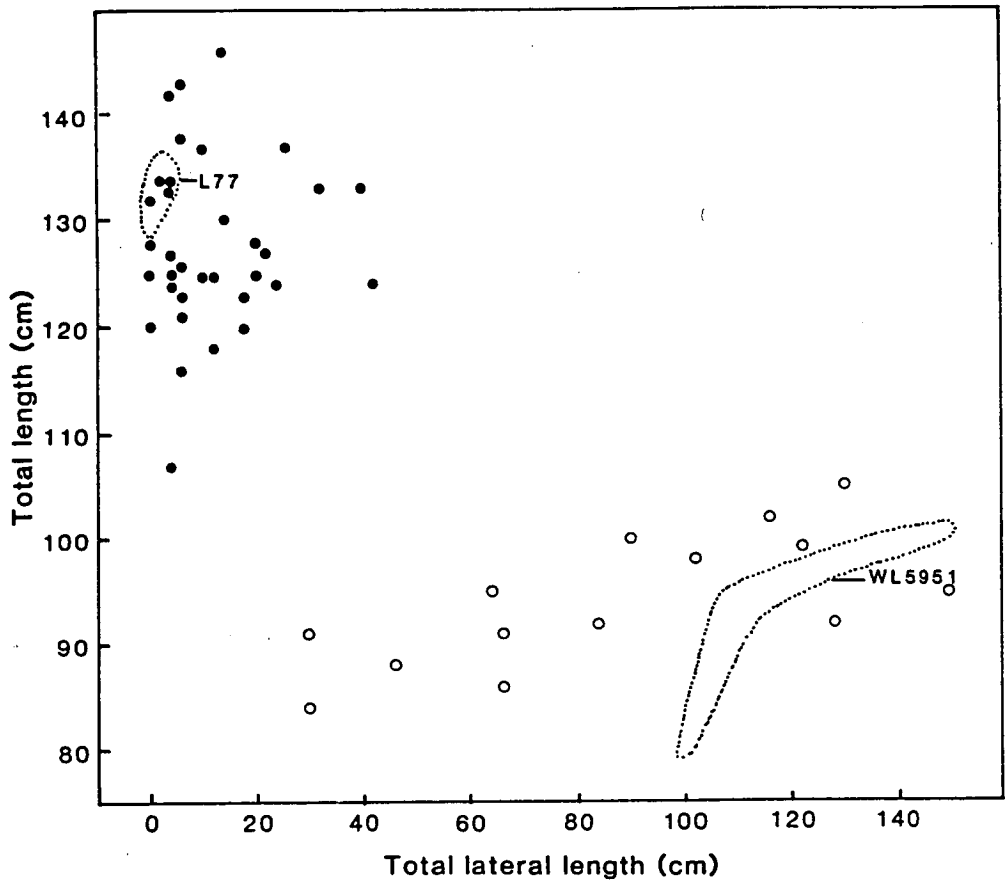


Fig. 4.6. Total length of stem (TL) and total lateral length (TLL) of mutant WL5951 and its initial line L77 (Parvus), and the F_2 of cross WL5951 x Parvus. Photoperiod 18 h.

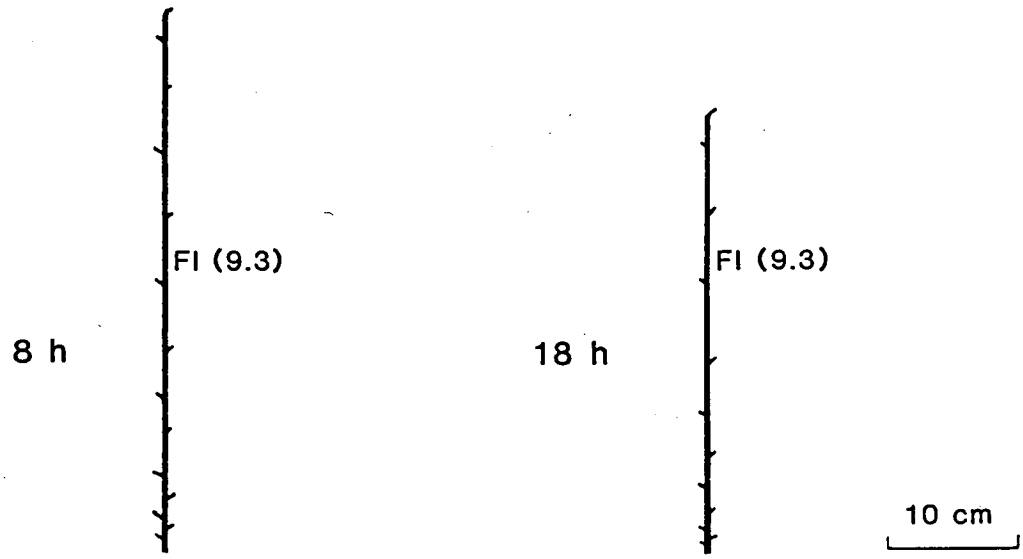


Fig. 4.7. Stem of L136 (Meteor) plants in photoperiods of 8 h and 18 h. Internodes are drawn to scale. Measurements were taken from mature plants.

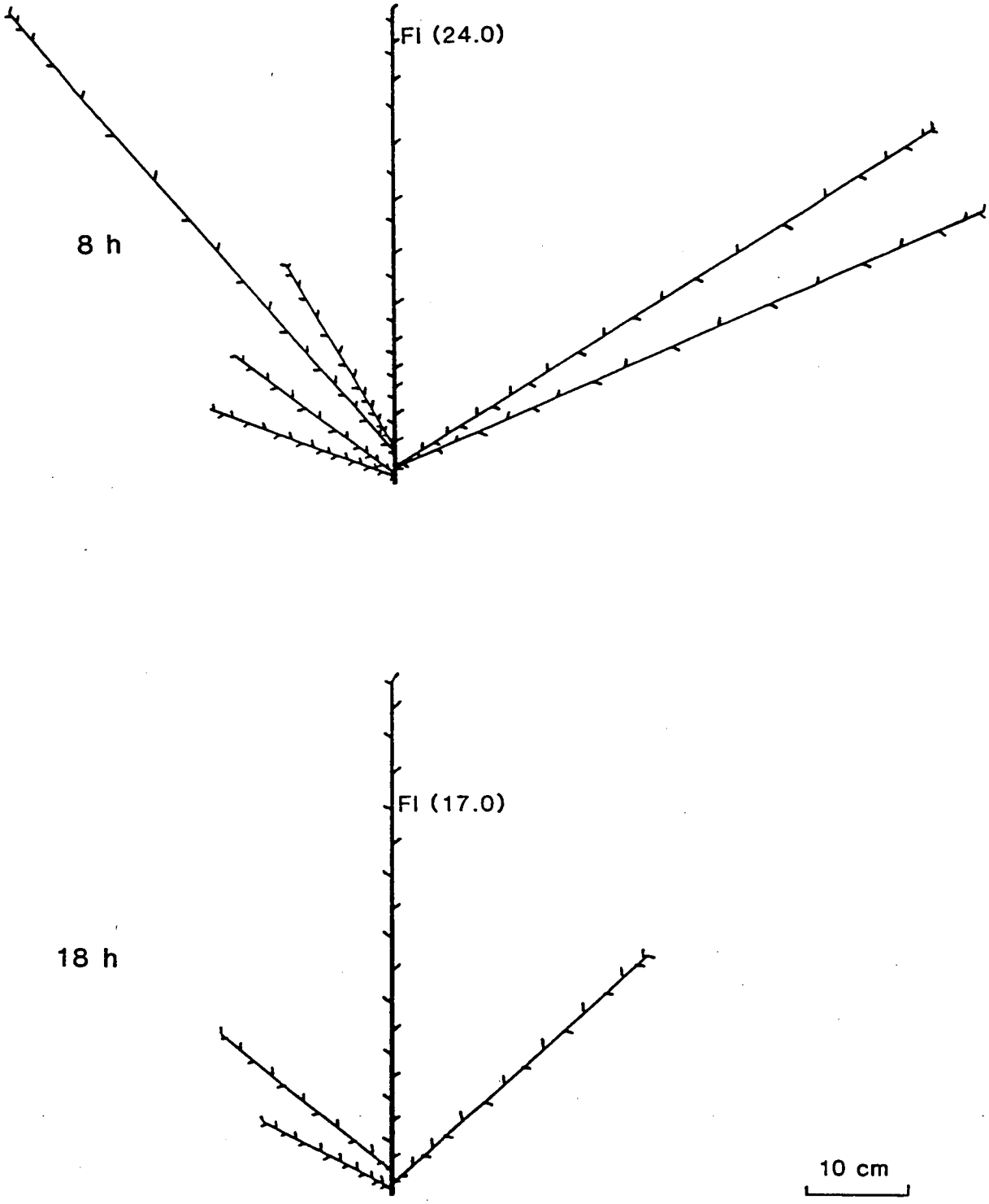


Fig. 4.8. Mutant WL6042 in 8 h and 18 h conditions. Internodes on the main shoot and laterals are drawn to scale. Measurements were taken from mature plants.

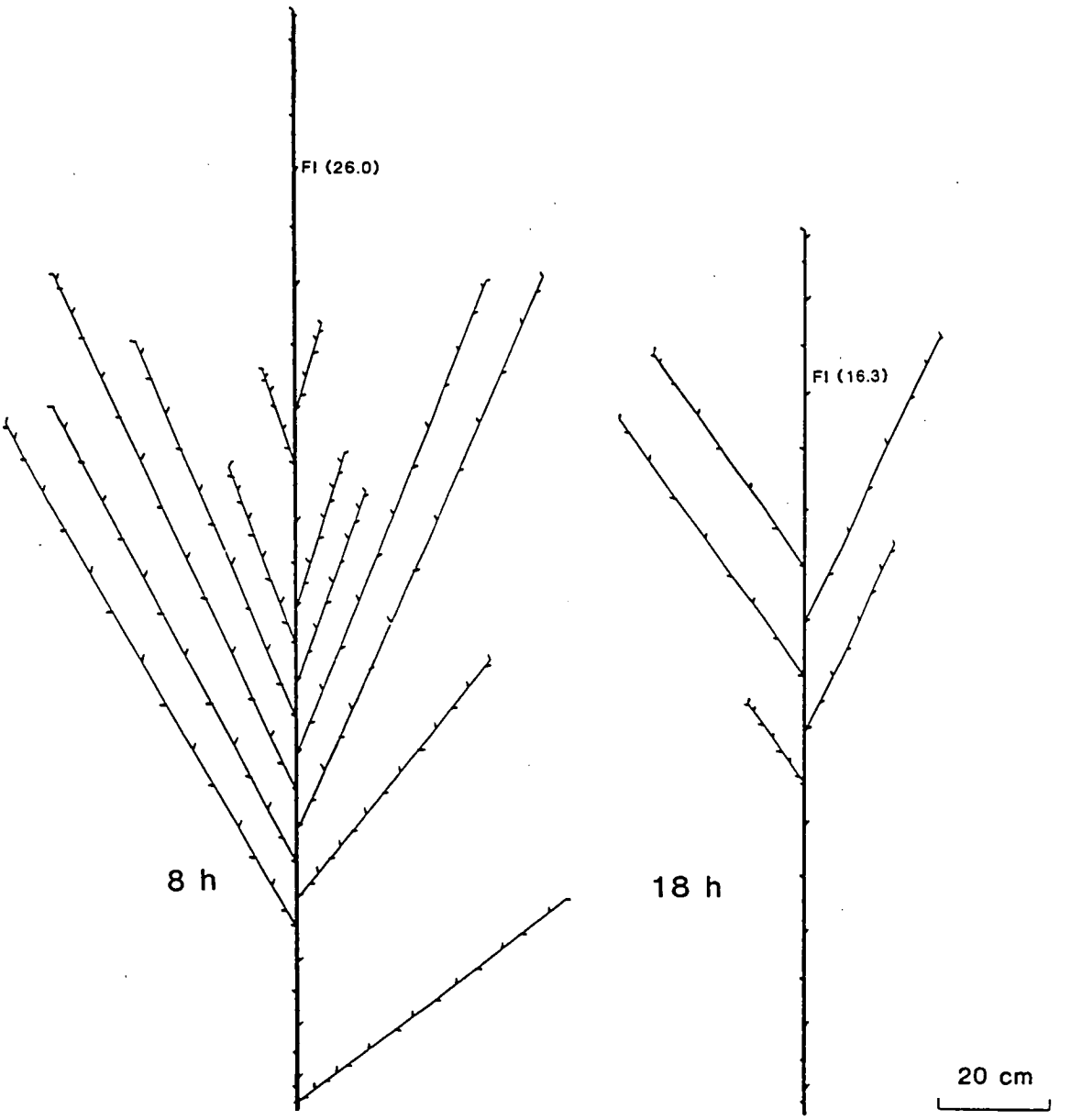


Fig. 4.9 Mutant Wt15240 in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

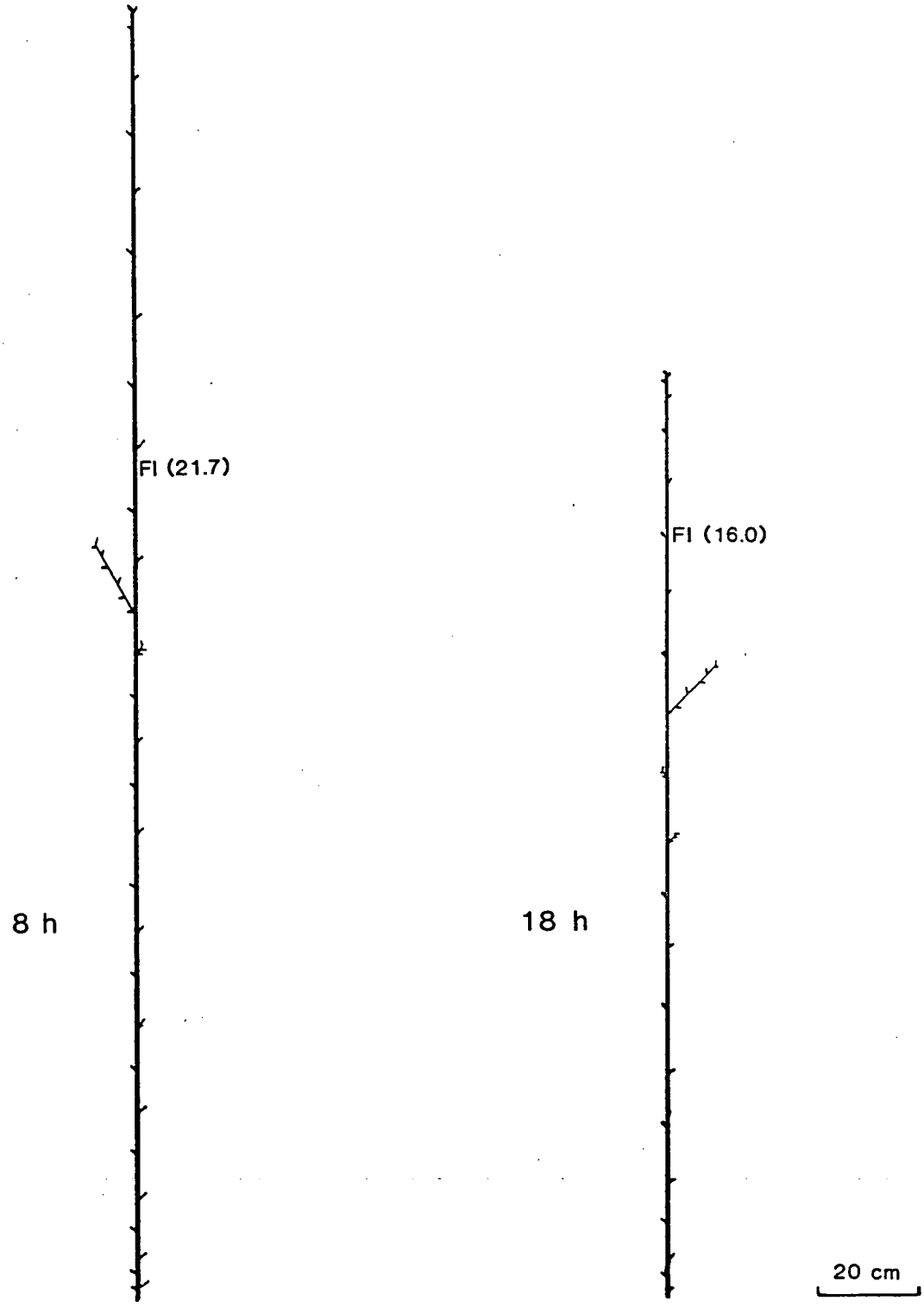


Fig. 4.10. Initial line Kaliski (Wt4042) in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

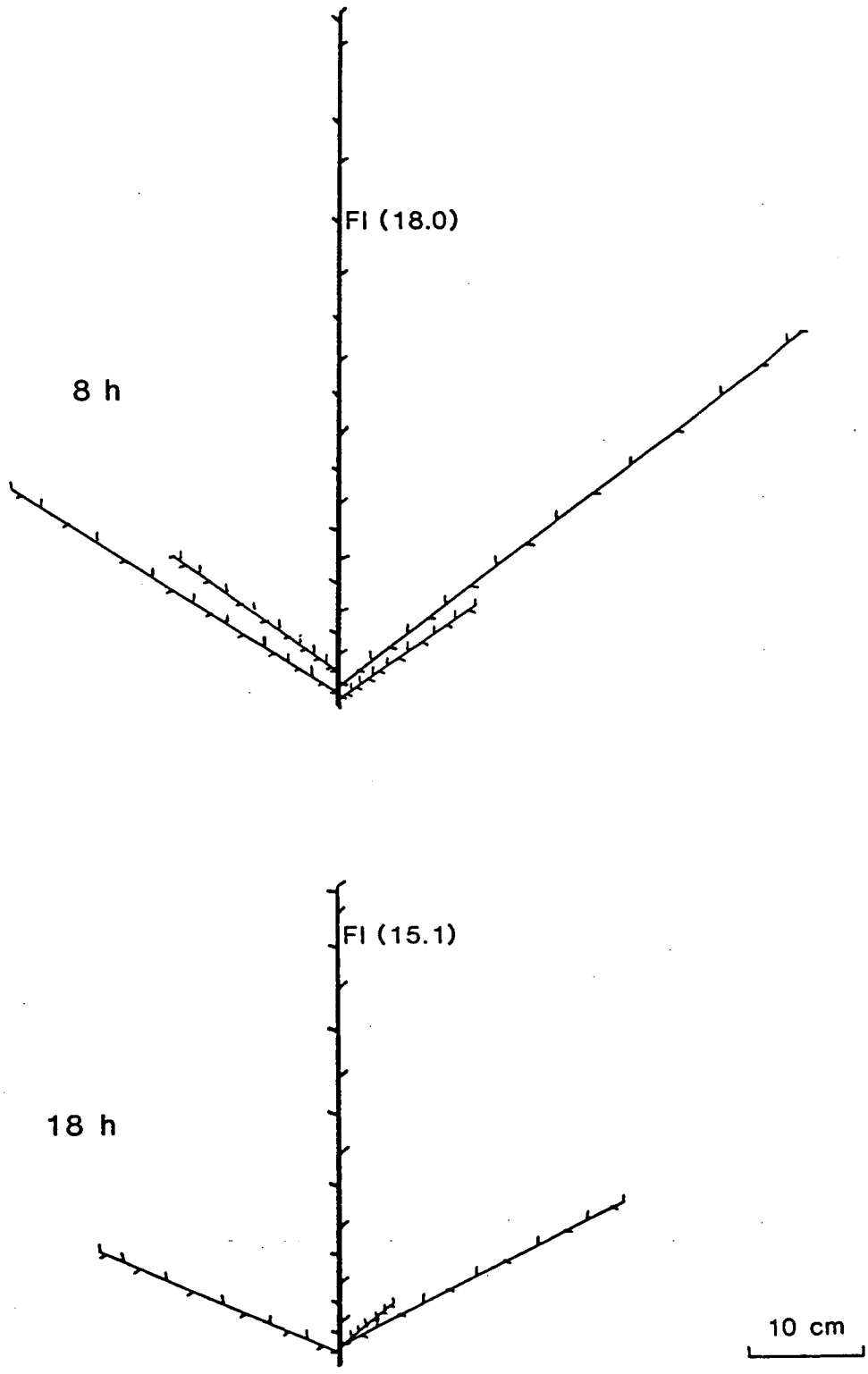


Fig. 4.11. Mutant Wt15241 in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

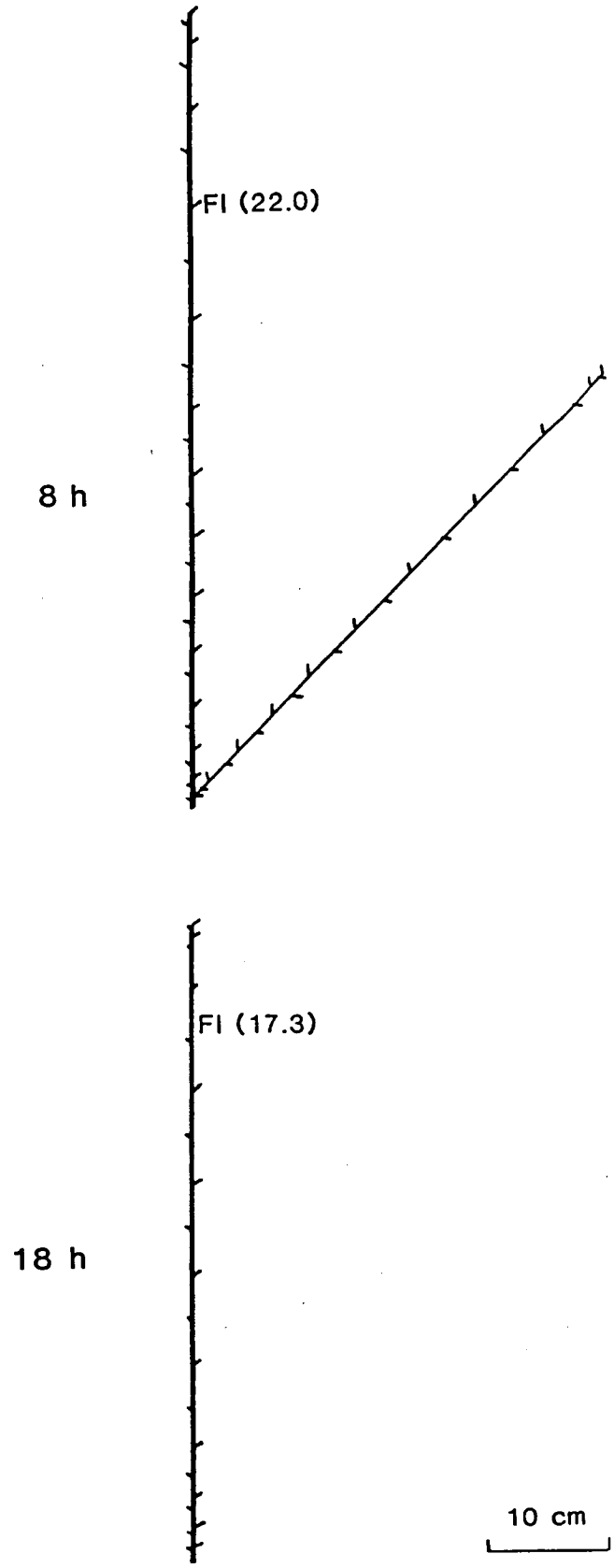


Fig. 4.12. Initial line Paloma (Wt3527) in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

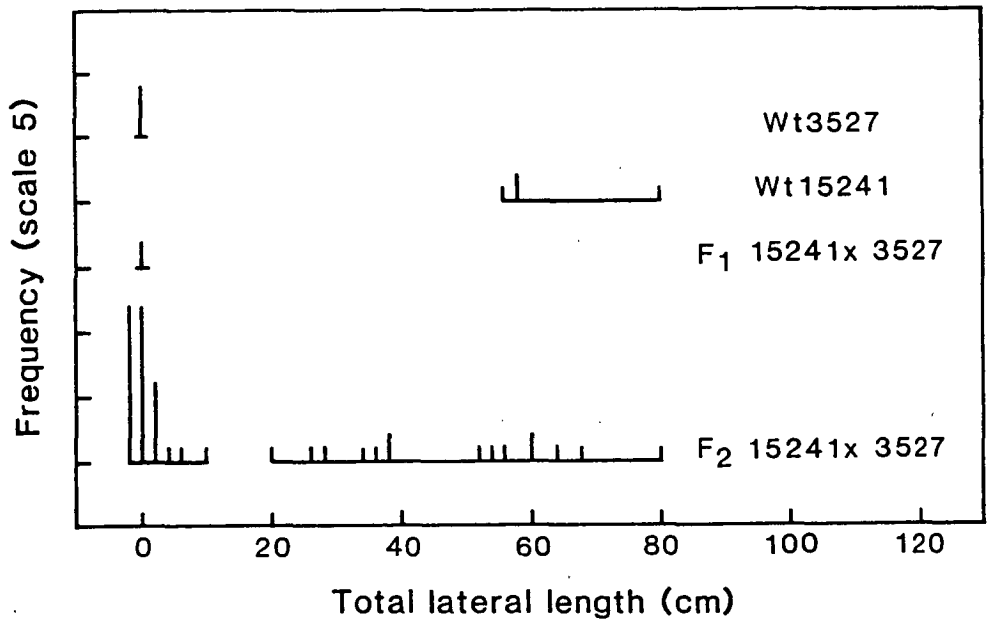


Fig. 4.13. Frequency distribution of total lateral length (TLL) for mutant Wt15241, its initial line Wt3527 (Paloma), and the F_1 and F_2 of cross Wt15241 x Wt3527. Photoperiod 18 h.

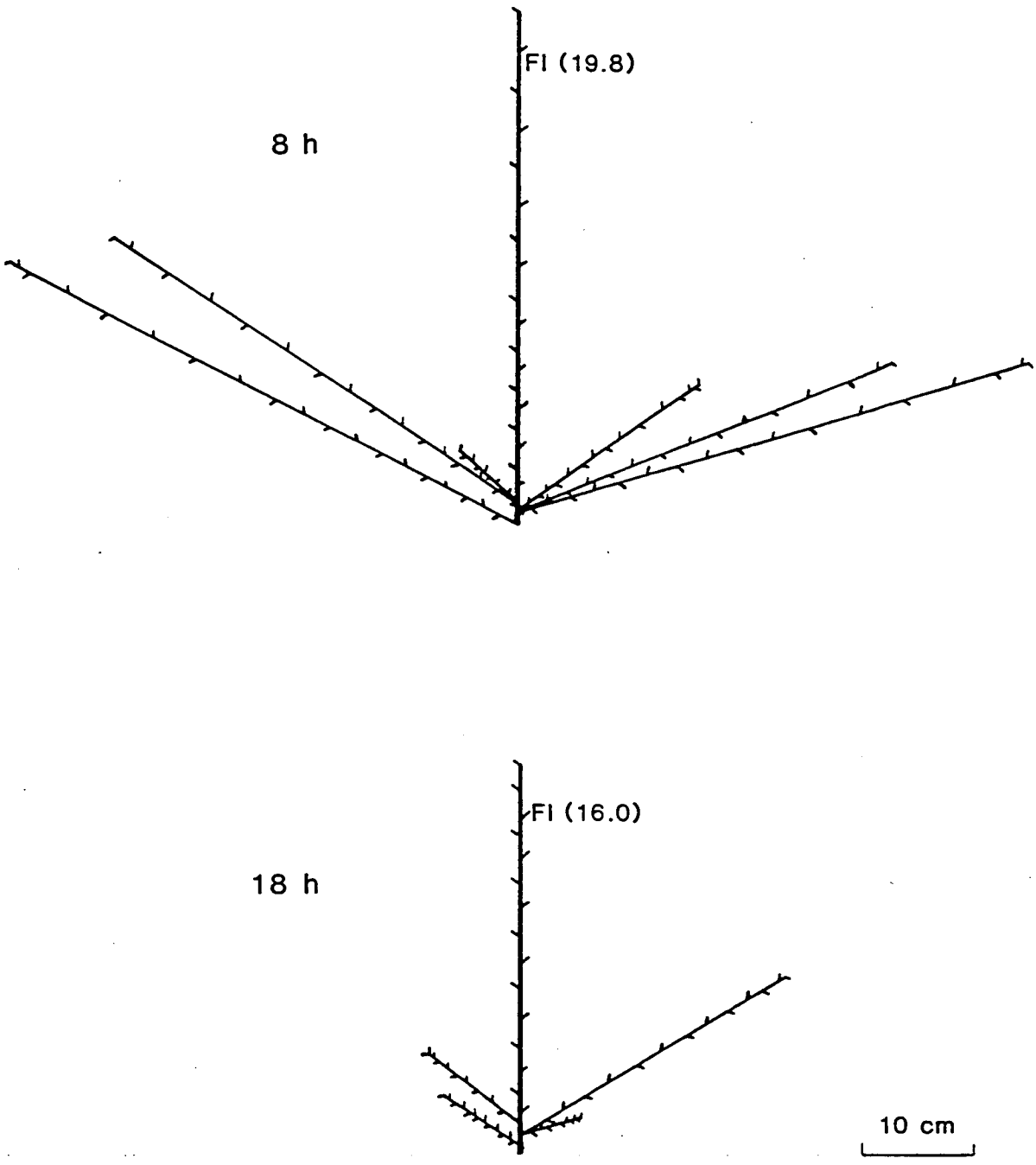


Fig. 4.14. Mutant Wt15244 in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

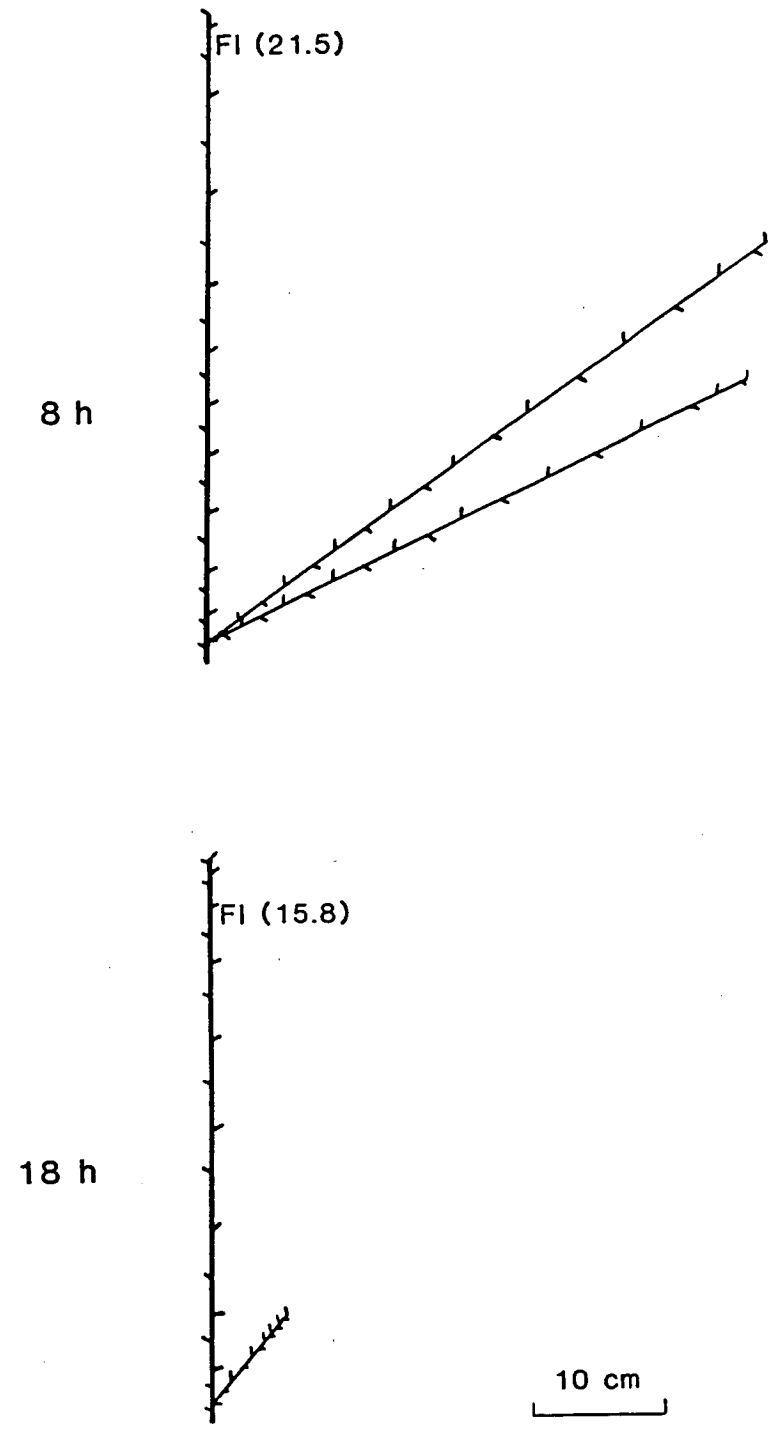


Fig. 4.15. Initial line Porta (Wt3519) in 8 h and 18 h conditions. Internodes are drawn to scale. Measurements were taken from mature plants.

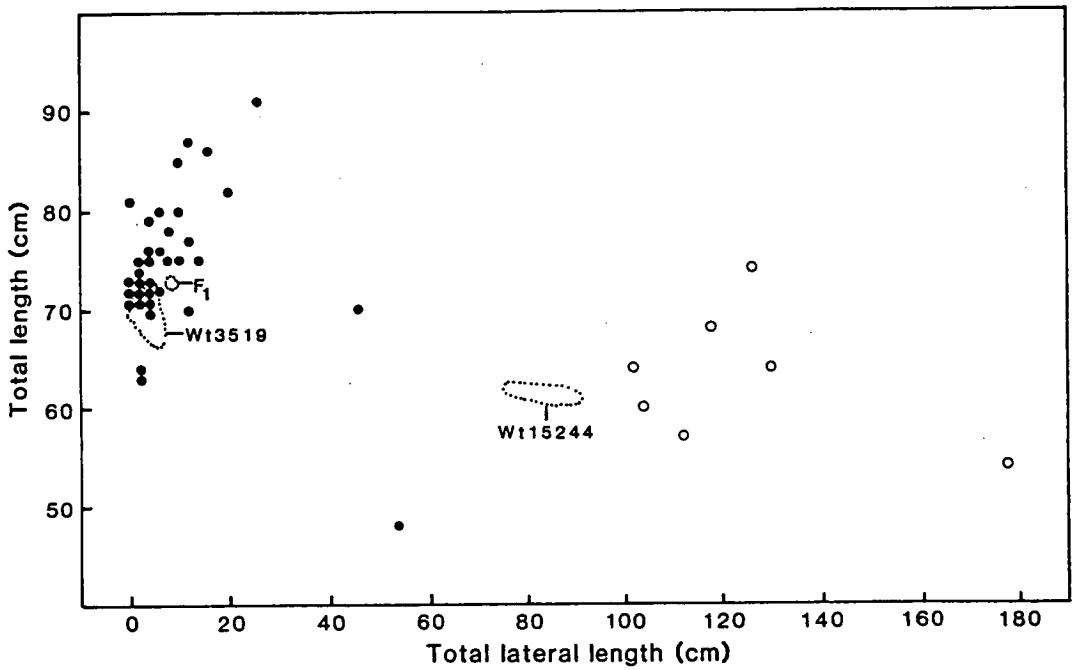


Fig. 4.16. Total length of stem (TL) and total lateral length (TLL) of mutant Wt15244, its initial line Wt3519 (Porta), and the F_1 and F_2 of cross Wt15244 x Porta. Open circles indicate strongly branching F_2 segregates which bred true in F_3.

CHAPTER 5
ALLELISM TESTS BETWEEN BRANCHING MUTANTS

5.1 Introduction and Methods

To test for allelism, the 17 branching mutants were crossed in all possible combinations. Reciprocal F_1s were made and grown for most crosses. In some cases F_1 seed was already available from crosses made by other workers at Hobart as indicated in Table 5.2. The tests were conducted over several sowing dates (Table 5.1). Line Wt15241 only became available after the program was well underway. Fifteen mutants were expressed clearly in a LD (18 h) photoperiod and these conditions were therefore used for tests involving only those mutants. Mutants K319 and K586 showed weak and variable expression and a SD (8 h) photoperiod was used for crosses involving these two parents in an endeavour to improve expression and recognition of a mutant phenotype. With the exception of K319, all mutant lines had an L-type flowering phenotype and a proven or supposed flowering genotype of *Lf E Sn Dne hr* (Table 2.2). These plants generally commenced flowering at node 15-18 in an 18 h photoperiod. Line K319 is a double mutant and shows both increased branching and earlier flowering (Uzhintseva and Sidorova, 1979). Early flowering in K319 is the result of a mutation of the type *Lf* to *lf* (Murfet, 1982b). K319 usually commenced flower initiation at node 11 or 12 regardless of the photoperiod. In pea, axils occupied by an inflorescence do not also produce a lateral branch. Thus K319 has fewer sites for aerial laterals than the other mutants and the branching ability conferred by the mutation may be underestimated in this early background. Some of the mutant lines were tall (*Le*) and others dwarf (*le*). The variable TLL (total lateral length) may therefore give a misleading impression when data are compared for a mix of tall and dwarf plants. To enable a fairer comparison, the ratio of TLL to TL (total length of main shoot) was used as the principal variable in the allelism tests.

A line representing the *ramosus* mutant *ram* (Monti and Scarascia-Mugnozza, 1967) was obtained toward the end of the present study. Because the *ram* mutant is poorly fertile and appears to act in a completely different manner to any of the 17 mutants in the study it was deemed neither practicable nor necessary to include it in the allelism tests.

5.2 Results and Discussion

The ratio of TLL : TL of all initial lines, mutants and F_1 hybrids from crosses between mutants is presented in Figs 5.1 to 5.9. If the F_1 hybrid was normal, similar to the respective initial line(s) of the mutants , the mutants are not allelic. On the other hand, if the F_1 hybrid was similar to one or both of the mutant parents, those mutants are allelic. However, not all the hybrids showed a clear cut phenotype (wild- type or mutant-type). F_1 plants from some crosses with mutants WL5951 and WL5237 showed a tendency to produce very weak laterals (Figs 5.1, 5.2, and 5.6). However, since the initial line, L77, also produced some laterals, the weak ramification tendency shown by the hybrids was not an evidence of allelism. A similar argument applies to the result of cross 15244 x 15236 (Fig. 5.5).

The F_1s from crosses 164 x 15242 (Fig. 5.3), 6042 x 487 (Fig.5.4), 6042 x 564 (Fig. 5.4) and 15240 x 5918 (Fig. 5.4) all showed quite a low TLL:TL ratio. This low ratio was probably caused by the winter conditions which reduced the vigour of the plants and the ability to produce laterals. However, the fact that some F_1 hybrids were similar to the respective tall mutant parents (K164, K487, K564 and Wt15240, respectively), indicates that in each of the above crosses the two mutants are allelic.

Crosses 15244 x 15241, 10852 x 15241 and 10852 x 15244 each gave rise to F_1s with a *ramosus* phenotype indicating all three mutants are allelic (Fig. 5.5). F_2 plants derived from the single F_1 plant from cross 10852 x 15244 all had a definite *ramosus* phenotype and all produced strong basal laterals, confirming that the two mutants are allelic. The F_2 showed two patterns of ramification (Fig. 5.10), and there is some evidence that there are two alleles with different strengths which control the ramification of Wt10852 and Wt15244. The alleles which cause ramification of Wt15244 and Wt15241 are stronger than the allele present in Wt10852.

Cross 564 x 586 (Fig. 5.6) gave ambiguous results. One F_1 plant had no laterals (ratio TLL : TL = 0) and the other F_1 plant had quite a high TLL : TL ratio (0.48). In the latter case the female parent was K564 and this F_1 plant may be the result of self-pollination (because K564 and K586 have the same initial line no differences in marker genes are available as a check). Moreover, in this

experiment the control plants of the mutant parent K586 also did not have laterals. It is therefore difficult to decide whether the two mutants are allelic or not. However, there is evidence that K564 is allelic with both WL6042 and K487 (Fig. 5.4). Since K586 did not show allelism with WL6042 (Fig. 5.4) or K487 (Floyd, 1985), it is more likely that K586 is not allelic with K564. However, to confirm this result, cross 564 x 586 should be repeated.

F_1 hybrids of crosses with K319 (Figs 5.7, 5.8 and 5.9) have laterals similar to K319. However, since the tendency to have laterals was shown by most of the F_1 hybrids, it is highly doubtful that the ramification is true evidence of allelism. More likely it is caused by the dominance of the K319 mutant allele as reported by Uzhintseva and Sidorova (1979).

The results for all allelism tests are summarized in Table 5.2. Based on these tests, the 17 mutants were grouped into 7 series (Table 5.3). Mutants WL5147, WL5237, WL5918, Wt15236 and Wt15240 are all allelic and the result of mutation of gene *Rms* since WL5237 is the type line for *rms* (Blixt, 1976). Ten further mutants represent mutation at four new *ramosus* loci designated *rms-2* (WL5951, K524), *rms-3* (WL6042, K487, K564), *rms-4* (K164, Wt15242) and *rms-5* (Wt10852, Wt15241, Wt15244). These 15 mutants all showed clear monogenic recessive inheritance (Chapter 4). The remaining two mutants, K319 and K586, do not appear to be allelic with each other or *rms-1, 2, 3, 4* or *5* (*rms* = *rms-1*) They are probably the result of mutation at two further loci but since neither Floyd (1985) nor myself have been able to obtain clear proof of monogenic inheritance, no symbols have been assigned. The K319 mutant will be transferred into a late flowering Torsdag background in order to examine segregation of branching without the confounding effect of early flowering.

Table 5.1 Planting dates, conditions and scoring dates for plants in the allelism tests. Parents, initial lines and F_1s sown on each date are indicated in Figs 5.1 - 5.7

Planting date	Photoperiod	Scored (day)
16 - 1-91	SD 8 h	41
16 - 1-91	LD 18 h	41
23 - 5-91*	LD 18 h	61
21-10-91	SD 8 h	49
3-12-91	SD 8 h	63
3-12-91	LD 18 h	34

*Ramification was less well expressed in these plants probably due to decreased plant vigour resulting from poor light quality during the winter months of June and July

Table 5.2. Diallel table showing the results (A=allelic, NA=not allelic) of crosses among the 17 mutant lines

	WL5237	WL5918	WL5951	WL6042	K164	K319	K487	K524	K564	K586	Wt10852	Wt15236	Wt15240	Wt15241	Wt15242	Wt15244
WL5918	A	—														
WL5951	NA	*NA	—													
WL6042		NA	NA	—												
K164	NA	*NA	*NA	NA	—											
K319	NA	*NA	*NA	NA	*NA	—										
K487	*NA	*NA	*NA	A	*NA	*NA	—									
K524	NA	NA	*A	NA	*NA	*NA	*NA	—								
K564	NA	*NA	*NA	A	*NA	*NA	*A	NA	—							
K586	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	—						
Wt10852	°NA	NA	NA	NA	NA	NA	°NA	NA	NA	NA	—					
Wt15236	°A			NA			°NA				†NA	—				
Wt15240	A	A	NA	NA	NA	NA	NA	NA	NA	NA	†NA	A	—			
Wt15241	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	A	NA	NA	—		
Wt15242	°NA	NA	NA	NA	A	NA	°NA	NA	NA	NA	†NA	†NA	NA	NA	—	
Wt15244	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	†A	†NA	†NA	A	†NA	—
WL5147	A	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	A	A	NA	NA	NA

°crosses made by M. Gregory; F_1 grown by the author
*crosses made by R. Floyd ; F_1 grown by the author
†crosses made by I. Murfet ; F_1 grown by the author

Table 5.3. Allelic series of branching mutants

Series	Line	Locus
1	WL5147, WL5237*, WL5918, Wt15236, Wt15240	*rms-1*
2	WL5951, K524	*rms-2*
3	WL6042, K487, K564	*rms-3*
4	K164, Wt15242	*rms-4*
5	Wt10852,Wt15241,Wt15244	*rms-5*
6	K319	
7	K586	

*Type line for *rms* (= *rms-1*) (Blixt, 1976)

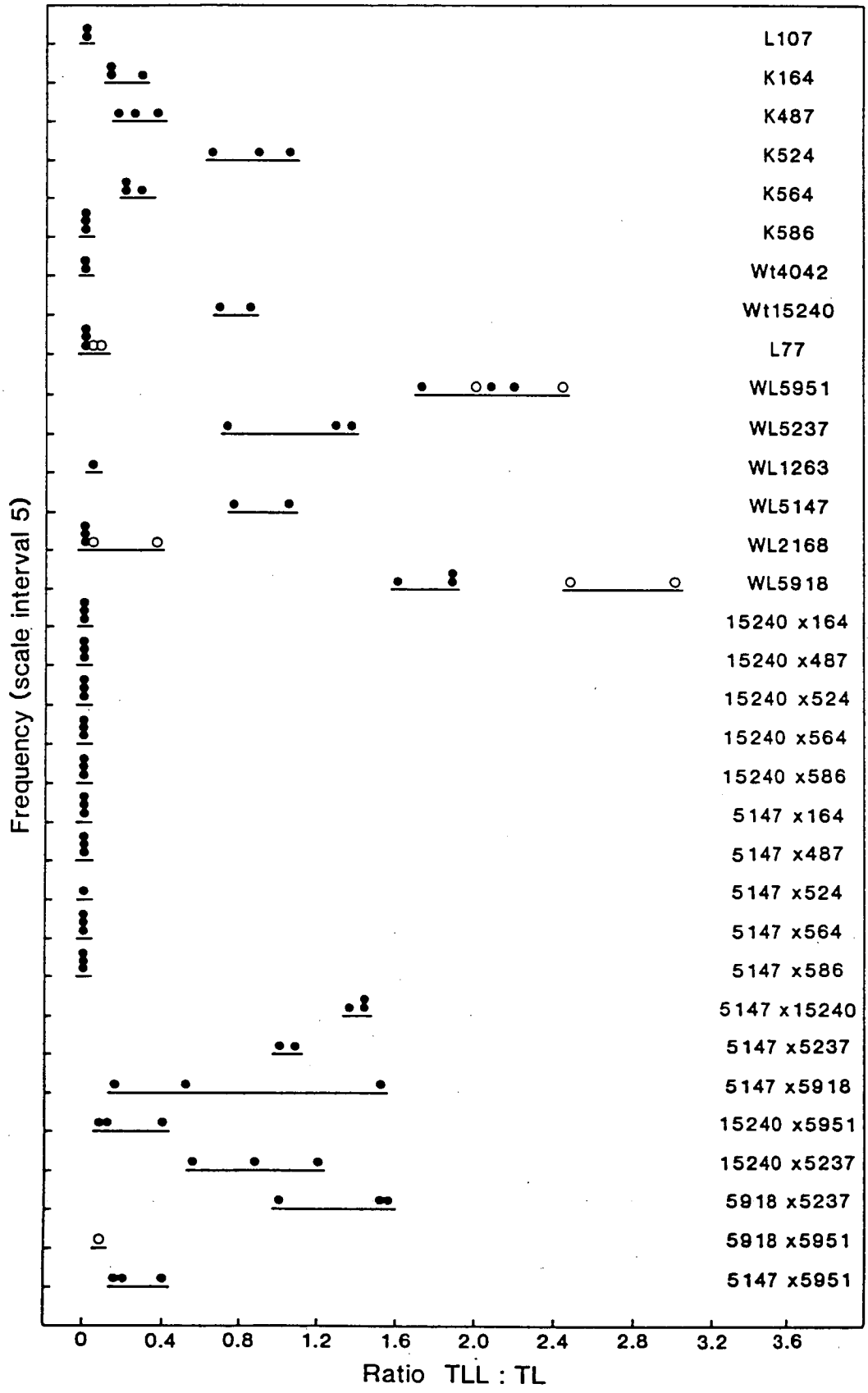


Fig. 5.1. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), L77 (Parvus), Wt4042 (Kaliski), WL1263 (Weitor), and WL2168 (Raman), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; o = planted 16-1-91)

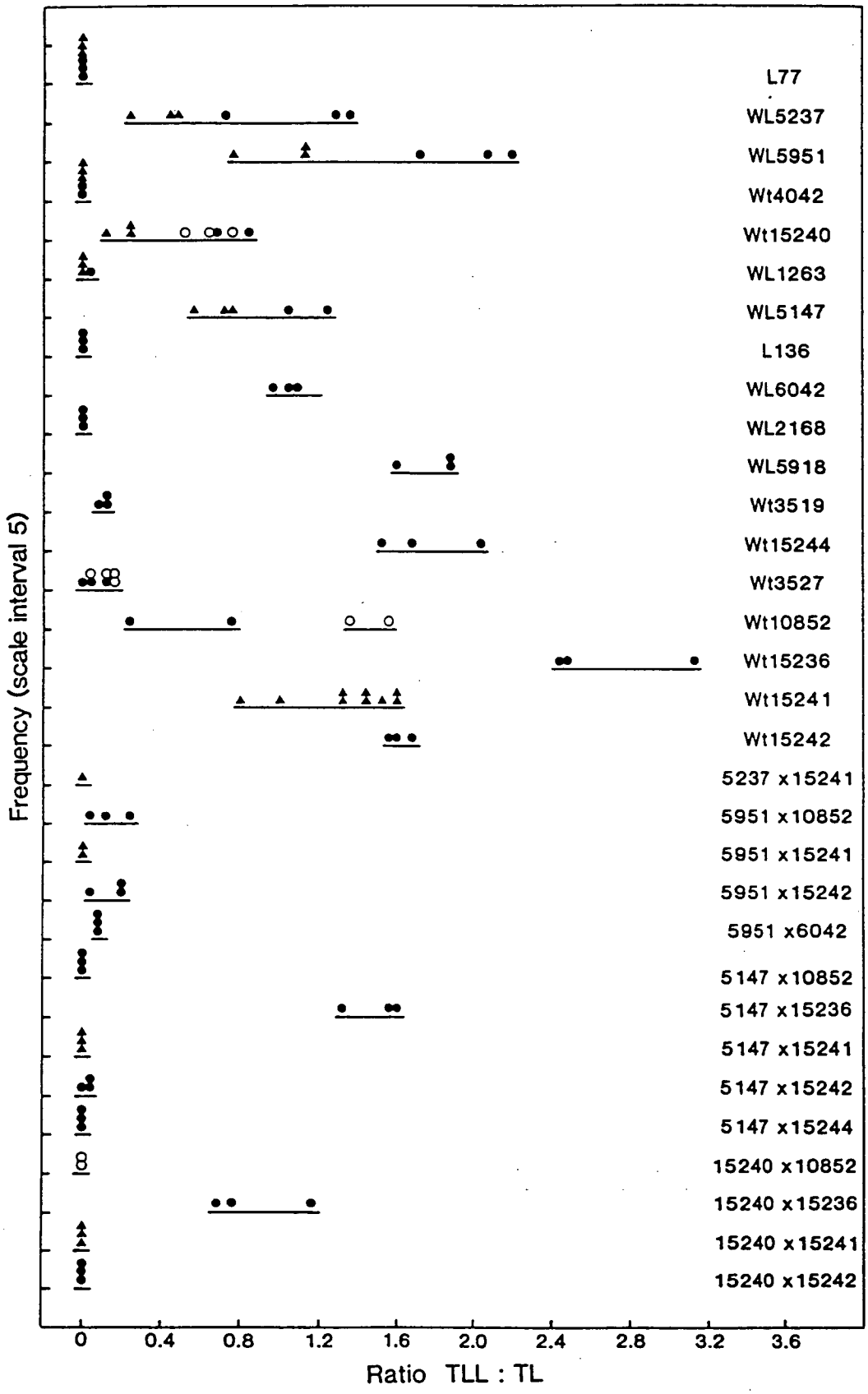


Fig. 5.2. Frequency distribution of the ratio TLL : TL for initial lines L77 (Parvus), Wt4042 (Kaliski), WL1263 (Weitor), L136 (Meteor), WL2168 (Raman), Wt3519 (Porta), and Wt3527 (Paloma), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; o = planted 16-1-91; ▲ = planted 3-12-91).

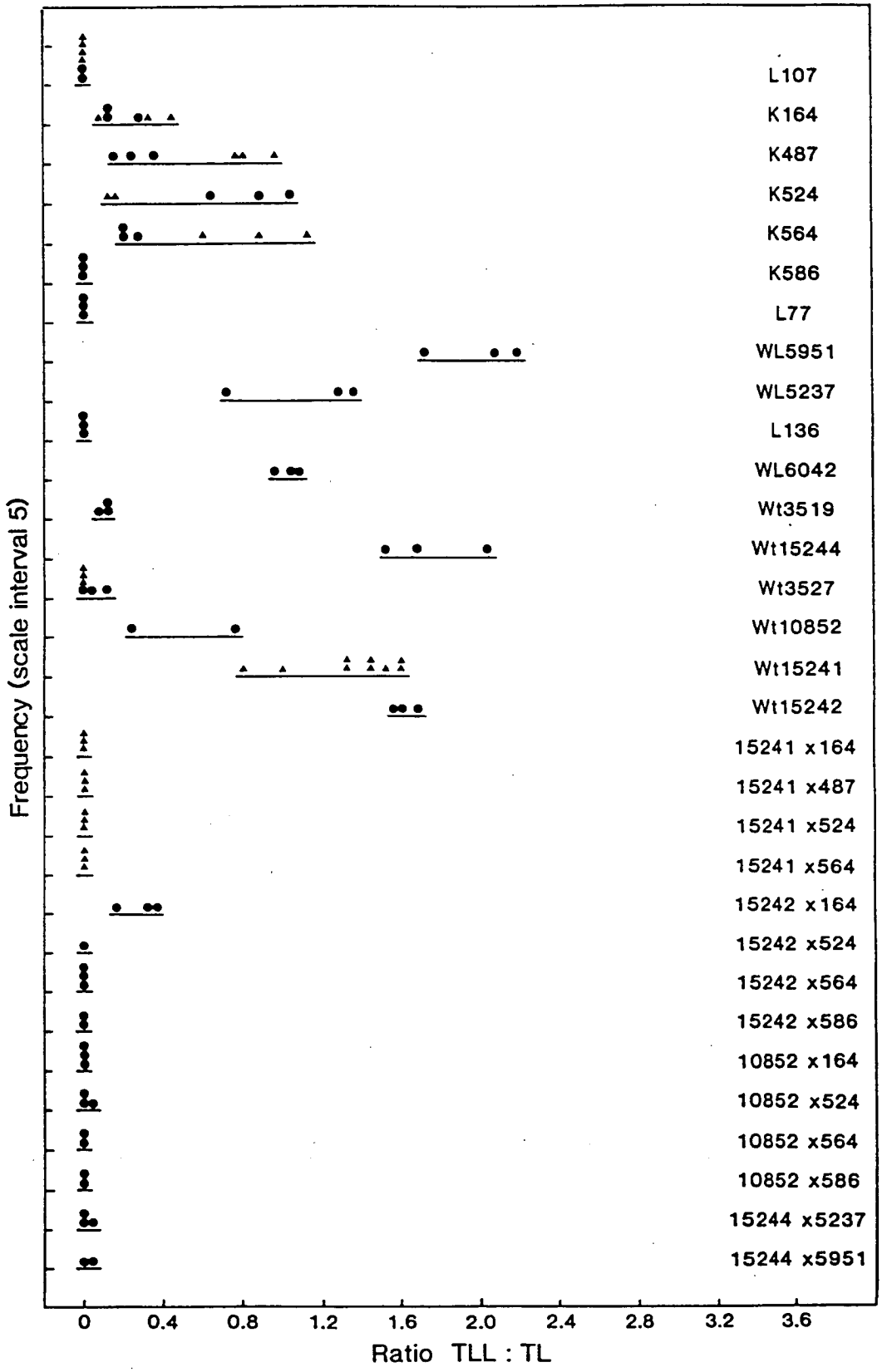


Fig. 5.3. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), L77 (Parvus), L136 (Meteor), Wt3519 (Porta), and Wt3527 (Paloma), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; ▲ = planted 3-12-91).

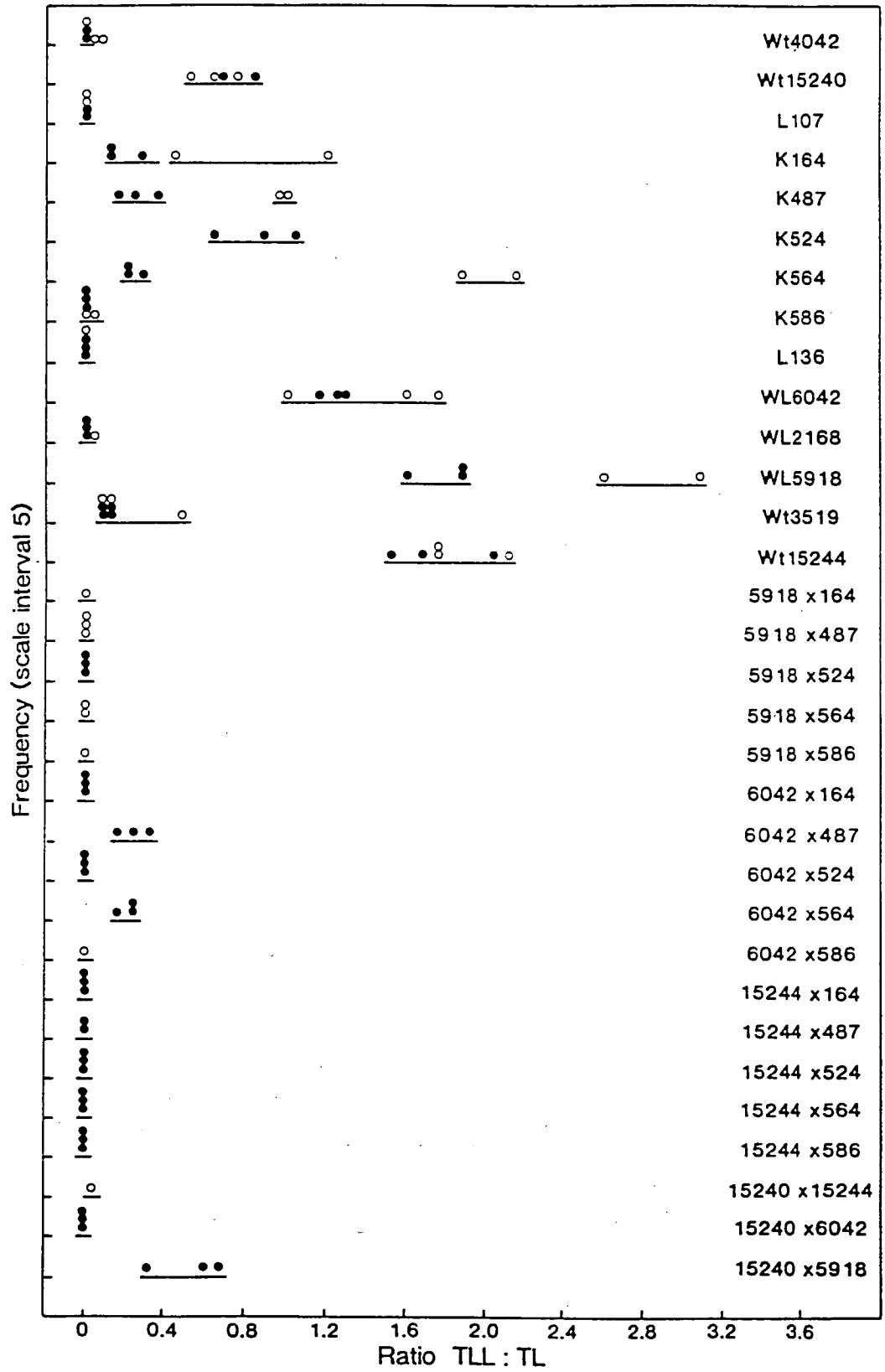


Fig. 5.4. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), Wt4042 (Kaliski), L136 (Meteor), WL2168 (Raman), and Wt3519 (Porta), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; o = planted 16-1-91).

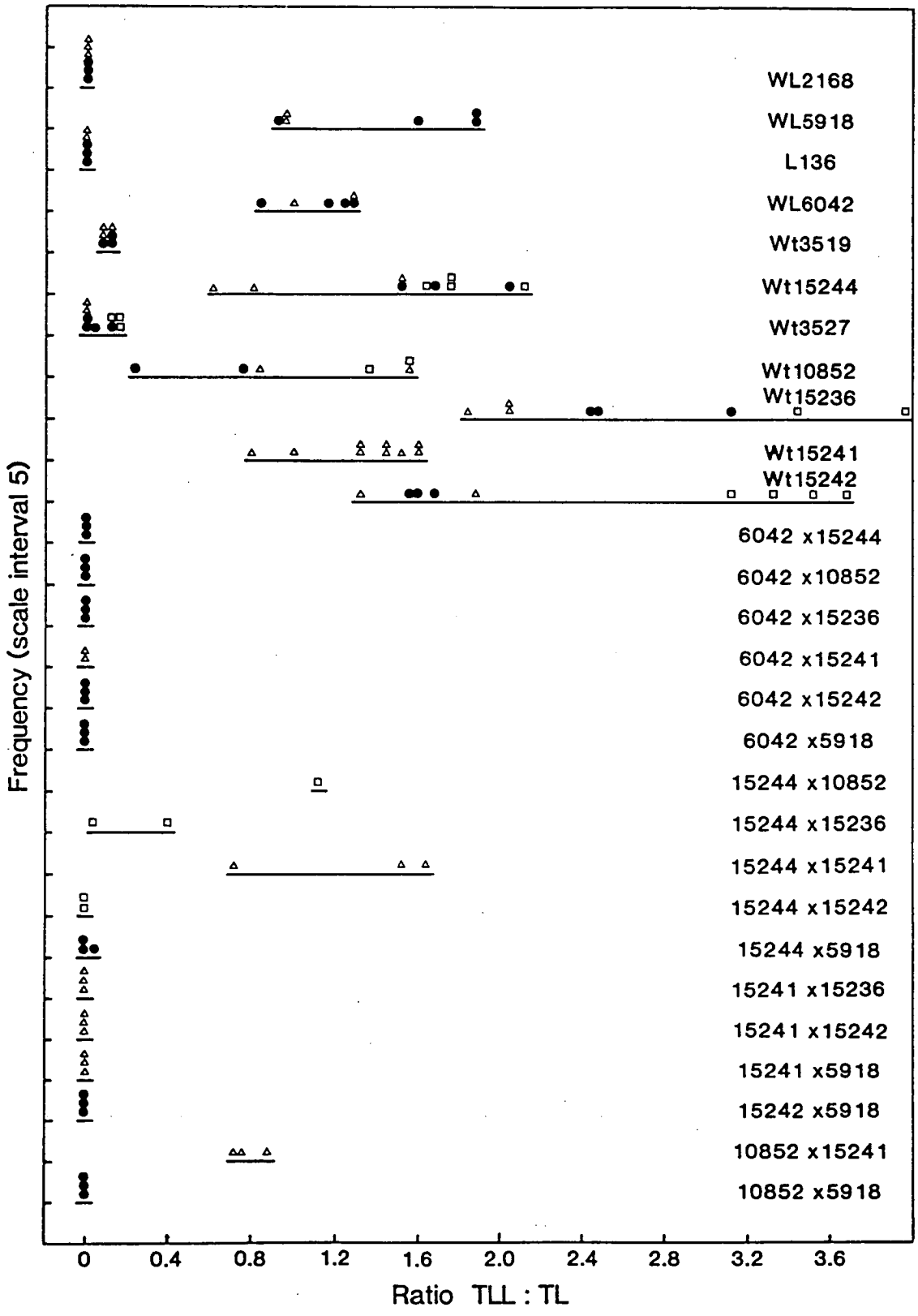


Fig. 5.5. Frequency distribution of the ratio TLL : TL for initial lines WL2168 (Raman), L136 (Meteor), Wt3519 (Porta), and Wt3527 (Paloma), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; △ = planted 3-12-91; □ = planted 16-1-91).

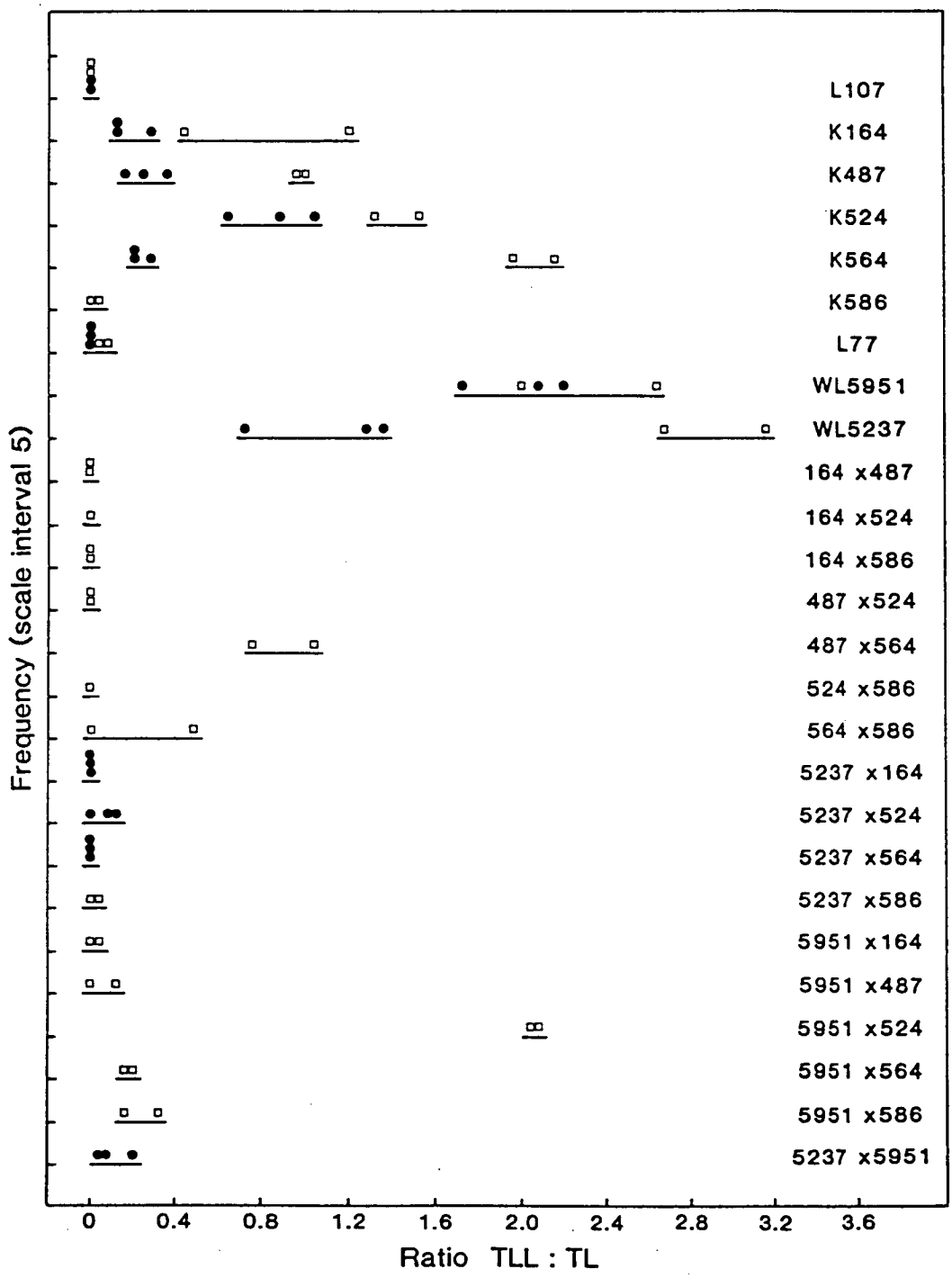


Fig. 5.6. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), and L77 (Parvus), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 18 h. (● = planted 23-5-91; □ = planted 16-1-91).

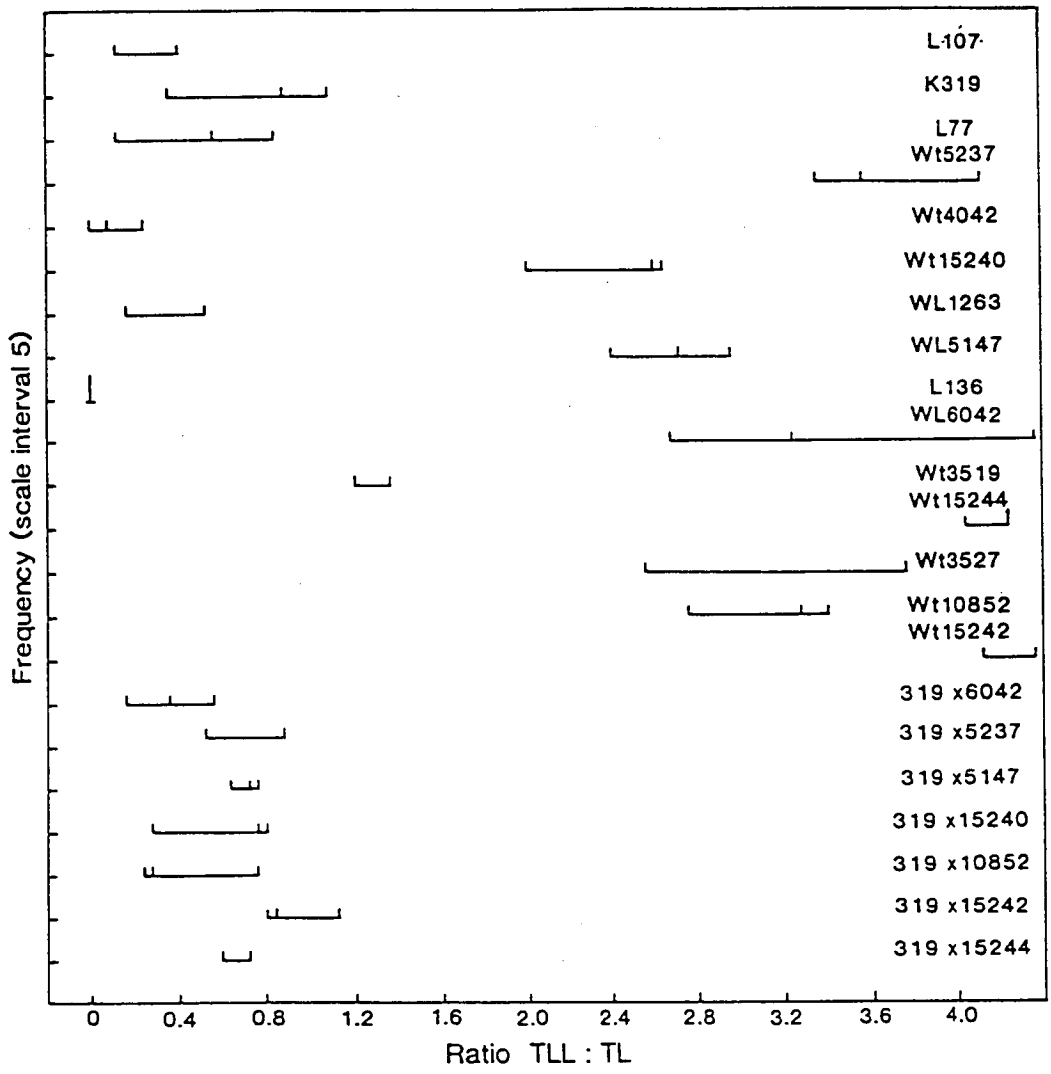


Fig. 5.7. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), L77 (Parvus), Wt4042 (Kaliski), WL1263 (Weitor), L136 (Meteor), Wt3519 (Porta), and Wt3527 (Paloma), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 8 h. Planted 21-10-91.

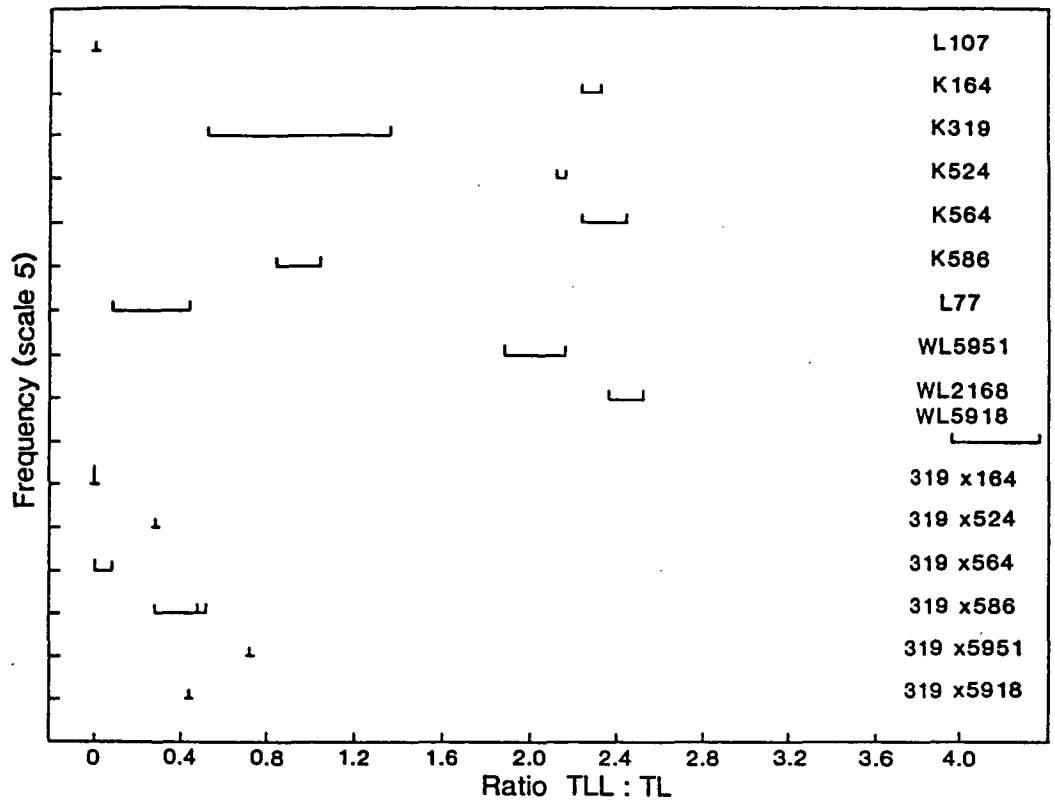


Fig. 5.8. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), L77 (Parvus), and WL2168 (Raman), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 8 h. Planted 16-1-91.

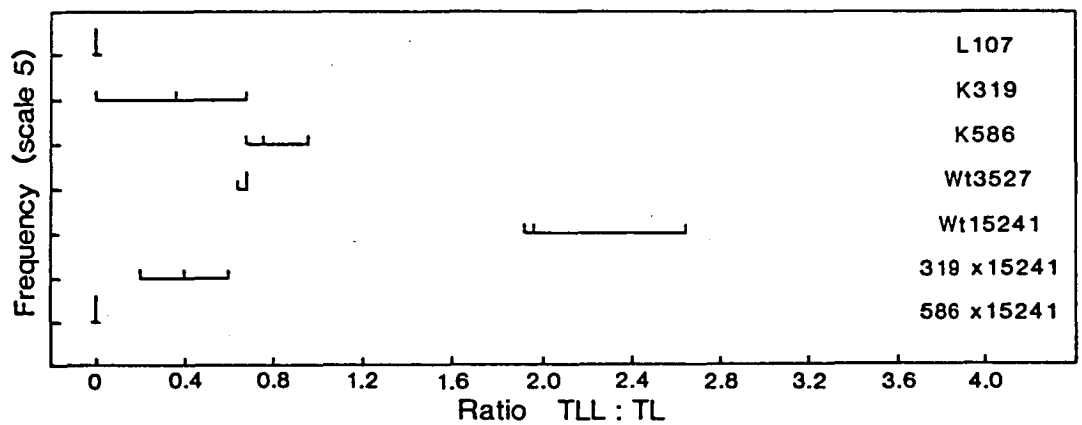


Fig. 5.9. Frequency distribution of the ratio TLL : TL for initial lines L107 (Torsdag), and L3527 (Paloma), several mutants derived from these progenitors, and F_1 hybrids from crosses among the mutant lines. Photoperiod 8 h. Planted 3-12-91.

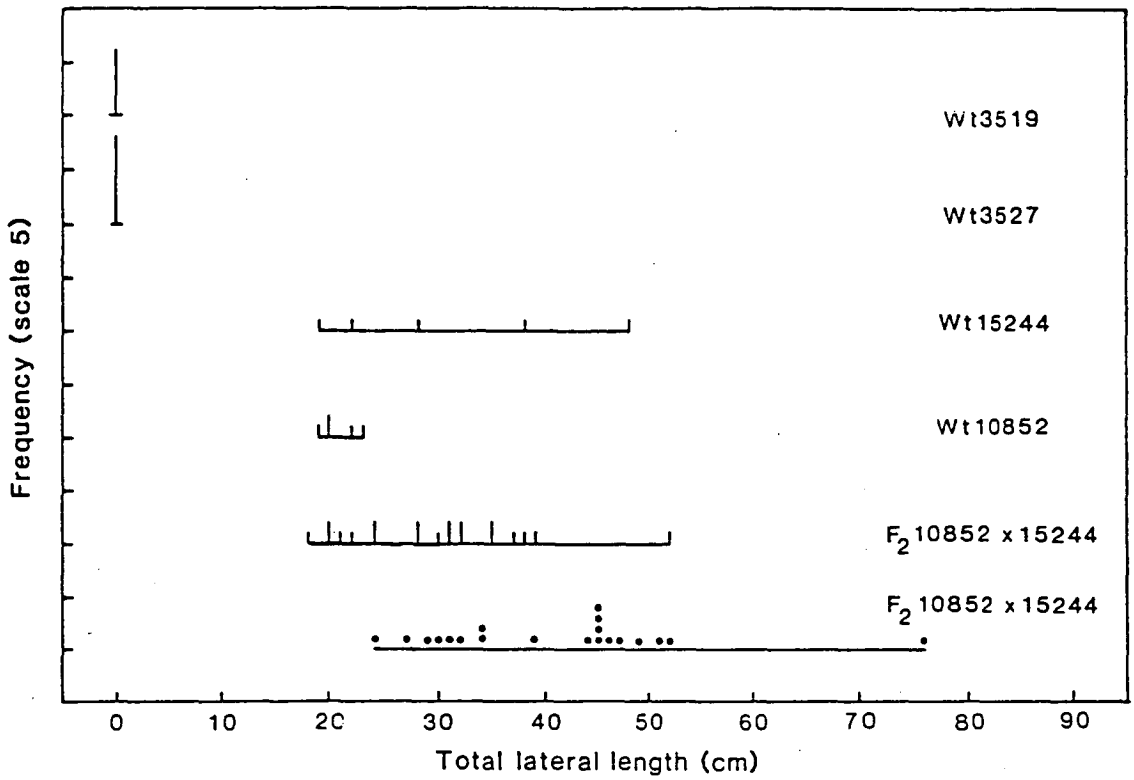


Fig. 5.10. Frequency distribution of total lateral length (TLL) for initial lines Wt3519 (Porta) and Wt3527 (Paloma), mutants derived from them, and the F_2 of cross 15244 x 10852 (bar = scored at age 34 days; ● = scored when the plant had senesced). Photoperiod 18 h. Planted 2-1-92.

CHAPTER 6
CONCLUDING DISCUSSION

In contrast to the initial line L152 (Ramonski 77), which has an L phenotype similar to the standard line L24, the early flowering mutant L167 has an El phenotype similar to the standard El line L60. In the cross L73 x L167, the white flowered F_2 plants tended to flower earlier than the red flowered plants, indicating that the mutant possesses gene *lf* consistent with White (1917). The tendency of the heterozygote (*LfIf*) to flower intermediate between the homozygous genotypes (*LfLf* and *IfIf*) in this mutant is consistent with results found previously (Murfet, 1971b, 1975, 1991). Most mutations at the *Lf* locus are from *Lf* to *lf* or lf^a. In the case of L167, the mutation is the same as that of L60 (*lf*) but could be of different strength since the Fl and FT are almost similar to L60. However, cross L167 x L60 needs to be done to gain more information about the strength of the mutant allele.

Mutant M2/176 was found to be allelic to *sn* (Chapter 3.2). The completely recessive behaviour of the mutant fits the nature of *sn* as stated by Murfet (1971a, 1971b). In day neutral plants, segregation at the *Lf* locus can not easily be distinguished without the presence of a marker such as gene *A*, and this marker was not available in the case of cross L59 (*a lf E sn Dne hr*) x M2/176 (*a Lf E sn Dne hr*). Therefore cross M2/176 x L73 (*A Lf E sn Dne hr*) was made and it was successful in detecting the possession of *Lf* by the mutant. The results of cross Borek x L60 also prove that Borek possesses *Lf* and they support the conclusion of a monogenic recessive mutation from *Sn* to *sn*. Genotyping Borek at the *E* locus, resulted in the finding that Borek possesses *E*, and this automatically also genotyped the mutant as having *E*.

Sn has been reported to control the production of a graft-transmissible flower inhibitor (Barber, 1959; Murfet, 1971c). Physiological experiments using the grafting technique need to be done to gain more evidence of an *sn* mutation at M2/176.

The results of the study on M2/137 gave evidence that a monogenic, incompletely recessive, mutation controls the day neutral nature of the mutant. This day neutral habit was the result of mutation at a new flowering locus (proposed name *ppd*) that controls the response to photoperiod. The allelism

tests with *sn* (L73) and *dne* (K218) failed to prove allelism with the mutant. Ratio 9 : 7 has been found in the F_2 of the latter cross and indicated complementary gene interaction. A linkage test, using markers from line L111, localized the *ppd* locus at about 32 units from *A* on chromosome 1. Linkage analysis using other markers on chromosome 1 needs to be done.

Mutant allele *ppd* seems to have a complex effect. Besides the incomplete dominance of the wild type allele over the mutant allele, this gene also seems to affect the expression of *Lf*. For example, the F_2 of cross M2/137 x K218 did not contain any plants as late as Borek. Again the F_3 of cross M2/137 x Borek contained only two homozygous dominant F_3 families with all members having a high flowering node like Borek. In these crosses there seems to be a suppression of *Lf* expression or possibly the phenomenon of impenetrance has occurred.

All branching mutants studied showed clear evidence of monogenic recessive mutation, except for the mutants K319 and K586. For mutant K319 indication of a dominant mutation was found consistent with the result found by Uzhintseva and Sidorova (1979). However, a clear segregation that matched the ratio 3 branching-type : 1 wild-type was not obtained. The lack of clarity could be caused by misclassification due to the fact that early segregates have less space to produce aerial laterals. To eliminate the effect of early flowering, a cross to the late line Torsdag has been started. Mutant K586 also did not give clear evidence of monogenic inheritance. However, in contrast to K319, the lack of clarity for mutant K586 seems to be the effect of unsuitable conditions. An 8 h photoperiod during the summer of 1991/1992 did not seem able to trigger the production of laterals. Therefore, the F_2 of cross K586 x Torsdag contained only 4 branching-type plants out of 48 plants. In contrast, Floyd (1985) used a 14 h photoperiod for the same cross and most of the F_2 plants produced laterals. He proposed that in mutant K586 a partially dominant gene controlled the production of laterals . However, the result in the present study does not seem to match to his proposal.

Allelism tests between K319 and K586 and the other mutants failed to obtain any evidence of allelism. The other 15 branching mutants were classified into 5 groups of alleles. The first group consists of mutants which are allelic with WL5237, Blixt's *rms* type line. Because the 5 groups are not allelic,

5 different symbols, *rms-1*, *rms-2*, *rms-3*, *rms-4* and *rms-5*, are proposed. Mutants at loci *rms-1*, *rms-3* and *rms-4*, include both tall and dwarf plants and some different branching types. For *rms-1*, WL5147 and WL5237 are classified as complete branching types, WL5918 and Wt15236 are classified as basal branching types, and Wt15240 is classified as an aerial branching type. For *rms-3*, WL6042 is a basal branching type, K487 is a gap branching type, and K564 is a complete branching type. For *rms-4*, K164 is a gap branching type, while Wt15242 is a basal branching type. Both *rms-2* lines (WL5951, K524) were tall and both were gap branching types. All three *rms-5* lines (Wt10852, Wt15241, Wt15244) were dwarf and all were basal branching types. The mixture of branching types resulting from a change at one locus shows that basal and aerial branching is controlled by one gene as stated by Floyd (1985).

In those series none of the dwarf plants (WL5918, WL6042, Wt10852, Wt15236, Wt15241, Wt15242 and Wt15244) produced aerial laterals, although a tendency to produce aerial laterals was shown in some cases. In contrast, most of the tall plants (K319, K487, K524, K564, WL5147, WL5951 and WL5237) produced aerial laterals, although in some mutants the aerial laterals were not strong. It seems that the length genotype influences the expression of laterals, as has been reported by Floyd (1985) and that as internode length increases plants tend to produce fewer basal and more aerial laterals.

In conclusion, a further mutation at each of the *Lf* and *Sn* loci has been identified in this study. The new mutants showed a similar character to the other mutants resulting from mutation at those loci. Another gene controlling the response to photoperiod has also been identified.

Fifteen of the 17 branching mutants studied were shown to be the result of a monogenic recessive mutation. Those mutants were assigned to five loci named *rms-1*, *rms-2*, *rms-3*, *rms-4* and *rms-5*. Two other mutants, K319 and K586 did not give a clear evidence of monogenic recessive inheritance. The expression of basal and aerial laterals of the branching mutants is controlled by one gene and seems to be influenced by the internode length genotype.

REFERENCES

Awuma, K and Bassett, M. J. (1988) Addition of genes for dwarf seed (*ds*) and spindly branch (*sb*) to the linkage map of common bean. *J. Amer. Soc. Hort. Sci.* **113** (3), 464-467.

Barber, H. N. (1959) Physiological genetics of *Pisum*. II. The genetics of photoperiodism and vernalisation. *Heredity* **13**, 33-60.

Bassett, M. (1990) Three mimic mutants for spindly branch in common bean and tests for linkage with other mutants. *Hort. Sci.* **25** (10), 1280-1281.

Belliard, J. and Pernes, J. (1985) *Pennisetum typhoides.* In: *Handbook of Flowering.* Vol. IV. Ed. Halevy, A. H. CRC Press, Boca Raton, Florida, pp.22-37.

Blixt, S. (1968) Linkage studies in *Pisum*. XII. Linkage relations of the genes *Fr* and *Fru* determining ramification. *Agri Hort. Genet.* **26** (3-4), 136-141.

Blixt, S. (1976) Linkage studies in *Pisum*. XV. Establishing the *Rms*-gene and the linkage of *Rms* and *Fas* in chromosome 3. *Agri Hort. Genet.* **34**, 83-87.

Doroshenko, A. V. and Rasumov, V. I. (1929) Photoperiodism of some cultivated forms in connection with their geographical origin. *Tr. Prikl. Bot. Genet. Sel.* **22**, 219-276.

Floyd, R. S. (1985) Branching in *Pisum sativum* L. Honours thesis. University of Tasmania.

Floyd, R. S. and Murfet, I. C. (1986) Branching in *Pisum*: Effect of the flowering and length genes. *Pisum Newsl.* **18**, 12-15.

Gottschalk, W. (1978) The genetic constitution of bifurcated mutants and recombinants. *Pisum Newsl.* **10**, 12.

Gottschalk, W. (1979) A *Pisum* gene preventing transition from the vegetative to the reproductive stage. *Pisum Newsl.* **11**, 10.

Gottschalk, W. (1982) The flowering behavior of *Pisum* genotypes under phytotron and field conditions. *Biol. Zbl.* **101**, 249-260.

Gottschalk, W. (1988) Phytotron experiments in *Pisum*. 2. Influence of the photoperiod on the flowering behavior of different genotypes. *Theor. Appl.*

Genet. **75**, 344-349.

Hanniford, G. G. and Craig, R. (1982) Genetics of flowering-geraniums for the future. *Sci. Agric.* **29**, 9.

Hoshino, Y. (1915) On the inheritance of the flowering time in peas and rice. *J. Coll. Agric. Hokkaido Imp. Univ., Sapporo*, **6**, 229-288.

King,W.M. and Murfet, I. C. (1985) Flowering in *Pisum*: a sixth locus, *Dne. Ann. Bot.* **56**, 835-846.

Koornneef, M., van Eden, J., Hanhart, C. J., Stam, P., Braaksma, F. J. and Feenstra, W. J. (1983) Linkage map of *Arabidopsis thaliana. J. Hered.* **74**, 265-272.

Koornneef, M., Hanhart, C.J. and Van der Veen, J.H. (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana. Mol. Gen. Genet.* **229**, 57-66.

Lamprecht, H. (1950) The degree of ramification in *Pisum* caused by polymeric genes. *Agri Hort. Genet.* **8** (1-2), 1-6.

Lamprecht, H. (1958) Über grundlegende Gene für die Gestaltung höherer Pflanzen sowie über neue und bekannte Röntgen-Mutanten *Agri Hort. Genet.* **16** (3-4), 145-195.

Lamprecht, H. (1961) Die Genenkarte von *Pisum* bei normaler Structur der Chromosomen. *Agri Hort. Genet.* **19**, 360-401.

Lamprecht, H. (1963) Zur Vererbung des ascendens-merkmals von *Pisum.* Genmanifestation und Chromosomenzugehörigkeit *Agri Hort. Genet.* **21** (3-4), 87-110.

Little, T. M. and Kantor, J. H. (1941) Inheritance of earliness of flowering in the sweet pea. *J. Hered.* **32**, 379-383.

Marx, G. A. (1968) Influence of genotype and environment on senescence in peas (*Pisum sativum* L). *BioScience* **18**, 525-506.

Marx, G. A. (1969) Some photo-dependent responses in *Pisum*. I. Physiological behavior. *Crop. Sci.* **9**, 273-276.

Marx, G. A. (1975) Photo-dependent responses in *Pisum*: impenetrant K-type segregants and evidence of a polygene system. *Pisum Newsl.* **7**, 26-27.

Marx, G. A. (1986) Linkage relations of *Curl, Orc* and "*Det*" with markers on chromosome 7. *Pisum Newsl.* **18**, 45-48.

Mendel, G. (1865) Versuche über Pflanzenhybriden. Verhand. Naturf. Ver. Brünn. 4,3-47. (English trans.)

Maurer, A. R., Jaffray, D. E. and Fletcher, H. F. (1966) Response of peas to environment. III. Assessment of the morphological development of peas. *Can. J. Plant Sci.* **46**, 285-290.

Monti, L. M. and Scarascia-Mugnozza, G. T. (1967) Mutazioni per precocita e ramosita indotte in pisello. *Genetica Agraria* **21**, 301-312.

Monti, L. M. and Scarascia-Mugnozza, G. T. (1970) Impiego di una mutazione per precocita in un programma di incrocio per il miglioramento genetico del pisello da industria. *Genetica Agraria* **24**, 195-206.

Monti, L. M. and Scarascia-Mugnozza, G. T. (1972) Richerce per miglioramento genetico per precocita in pissello. *Genetica Agraria.* **26**, 119-127

Murfet, I. C. (1971a) Flowering in *Pisum*: three distinct phenotypic classes determined by the interaction of a dominant early and a dominant late gene. *Heredity* **26**, 243-257.

Murfet, I. C. (1971b) Flowering in *Pisum*: a three gene system. *Heredity* **27**, 93-110.

Murfet, I. C. (1971c) Flowering in *Pisum*: reciprocal grafts between known genotypes. *Aust. J. Biol. Sci.* **24**, 1089-1101.

Murfet, I. C. (1973) Flowering in Pisum: *Hr*, a gene for high response to photoperiod. *Heredity* **31**, 157-164.

Murfet, I. C. (1975) Flowering in *Pisum*: multiple alleles at the *lf* locus. *Heredity* **35**, 85-98.

Murfet, I. C. (1978) The flowering genes *Lf, E, Sn* and *Hr* in *Pisum*: their relationship with other genes, and their descriptions and type lines. *Pisum Newsl.* **10**, 48-52.

Murfet, I. C. (1982a) Flowering in garden pea: expression of gene *Sn* in the field and use of multiple characters to detect segregation. *Crop Sci.* **22**, 923-926.

Murfet, I. C. (1982b) Present state of physiological and biochemical gene mapping. In: *Documentation of Genetic Resources: a Model.* Eds Blixt, S.

and Williams, J.T. IBPGR, Rome. pp. 45-51.

Murfet, I. C. (1985) *Pisum sativum* L. In: *Handbook of Flowering.* Vol. IV. Ed. Halevy A.H. CRC Press, Boca Raton, Florida, pp. 97-126.

Murfet, I. C. (1987) Linkage data for *dne* and chromosome 3 markers *b* and *st. Pisum Newsl.* **19**, 45.

Murfet, I. C. (1988) Linkage relationship of *Pisum* genes *st*, *m* and *hr. Pisum Newsl.* **20**, 29.

Murfet, I. C. (1989) Flowering genes in *Pisum.* In: *Plant Reproduction: From Floral Induction to Pollination.* Eds Lord, E.M. and Bernier, G., American Society of Plant Physiologists, Rockville, Maryland. pp. 10-18.

Murfet, I. C. (1990) Flowering genes in pea and their use in breeding. *Pisum Newsl.* **22**, 78-86.

Murfet, I. C. and Marx, G. A. (1976) Flowering in *Pisum*: Comparison of the Geneva and Hobart systems of phenotypic classification. *Pisum Newsl.* **8**, 46-48.

Murfet, I. C. and Reid, J. B. (1973) Flowering in *Pisum*: evidence that gene *Sn* controls a graft-transmissible inhibitor. *Aust. J. Biol. Sci.* **26**, 675-677.

Murfet, I. C. and Reid, J. B. (1974) Flowering in *Pisum*: the influence of photoperiod and vernalizing temperatures on the expression of genes *Lf* and *Sn. Z. Pflanzenphysiol.* **71**, 323-331.

Murfet, I. C. and Reid, J. B. (1985) The control of flowering and internode length in *Pisum*. In: *The Pea Crop: A Basis for Improvement.* Eds Hebblethwaite, P.D., Heath, M.C. and Dawkins, T.C.K. Butterworths, London. pp. 67-80.

Pellew, C. (1940) Genetical studies on the first reciprocal translocation found in *Pisum sativum. J. Genet.* **39**, 363-390.

Reid, J. B. and Murfet, I. C. (1977) Flowering in *Pisum*: the effect of genotype, plant age, photoperiod and number of inductive cycles. *J. Exp. Bot.* **28**, 811-819.

Reid, J. B. and Murfet, I. C. (1984) Flowering in *Pisum* : a fifth locus, *veg. Ann. Bot.* **53**, 369-382.

Ross, J. J. (1983) Flowering and branching in sweet pea. PhD. thesis.

University of Tasmania.

Ross, J. J. and Murfet, I. C. (1985a) Flowering and branching in *Lathyrus odoratus* L.: environmental and genetic effects. *Ann. Bot.* **55**, 715-726.

Ross, J. J. and Murfet, I. C. (1985b) A comparison of the flowering and branching control systems in *Lathyrus odoratus* L. and *Pisum sativum* L. *Ann. Bot.* **56**, 847-856.

Ross, J. J. and Murfet, I. C. (1988) Flowering and branching in *Lathyrus odoratus* L.: loci *sp* and *b*. *Theor. Appl. Genet.* **76**, 941-946.

Rowlands, D. G. (1964) Genetic control of flowering in *Pisum sativum* L. *Genetica*. **35**, 75-94.

Singer, S. R., Hsiung, L. P. and Huber, S. C. (1990) Determinate (*det*) mutant of *Pisum sativum* (Leguminosae: Papilionoideae) exhibits an indeterminate growth pattern. *Amer. J. Bot.* **77**, 1330-1335.

Swiecicki, W. K. (1987) Determinate growth (*det*) in *Pisum* : a new mutant gene on chromosome 7. *Pisum Newsl.* **19**, 72-73.

Tedin, H. and Tedin, O. (1923) Contributions to the genetics of *Pisum*. III. Internode length, stem thickness and place of the first flower. *Hereditas* **4**, 351-362.

Uzhintseva, L.P. and Sidorova, K.K. (1979) Genetic nature of early flowering pea mutants. *Genetika* **15** (6), 1076-1082.

Weeden, N. F. , Kneen, B. E. and Murfet, I. C. (1988) Mapping of the *Sn* locus to chromosome 2. *Pisum Newsl.* **20**, 49-51.

White, O. E. (1917) Studies of inheritance in *Pisum*. II. The present state of knowledge of heredity and variation in peas. *Proc. Am. Phil. Soc.* **56**, 487-589.

Wellensiek, S. J. (1969) The physiological effects of flower forming genes in peas. *Z. Pflanzenphysiol.* **5**, 388-402.

Wellensiek, S. J. (1972) Multiple alleles for late, medium, and early flowering. *Pisum Newsl.* **4**. 59.

Zagotta, M. T., Shannon, S., Jacobs, C. and Meeks-Wagner, D. R. (1992) Early-flowering mutants of *Arabidopsis thaliana*. *Aust. J. Plant Physiol.* (in press).