Early flowering mutants Wt11790 and Wt11791 result from mutation at the *Lf* locus

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The early flowering mutant lines Wt11790 and Wt11791 were selected by Dr W.K. Swiecicki (Plant Breeding Station Wiatrowo) following irradiation of cultivar Porta (Wt3519) with 200 r Nf. I report here the results of tests which show that early flowering in both these lines results from mutation of *Lf* to *lf*.

Materials and methods

Tests were conducted in the phytotron at Hobart. Seed of Porta and the mutant lines was kindly provided by Dr W.K. Swiecicki. The genotypes of the Wiatrowo lines were determined by crossing with Hobart lines of known flowering genotype, namely line 2 (late photoperiodic, *Lf E Sn Dne hr*) and line 60 (early photoperiodic, *lf E Sn Dne hr*). Plants were grown in 14 cm slim line pots in a 1:1 mixture of vermiculite:dolerite chips topped with 3 cm of sterilised peat/sand potting mixture. Nutrient (Hoaglands #1) was supplied once a week. Lateral shoots were regularly excised. Node counts commenced from the first scale leaf as node 1. Node of flower initiation was taken as the first node on the main stem to bear a flower initial regardless of whether or not the bud developed into a mature flower. The F_1 progenies in Table 1 with parental control lines were sown 12 Mar 1990 and the F_2 progenies with parental controls were sown 10 Aug 1990. All plants received 8 h of daylight at 20-25°C and 16 h of dark in night compartments at 16°C. Data for the controls were very similar from the two plantings and the results are combined in Table 1.

Results and discussion

Preliminary tests showed that Porta and the mutants have phenotypes L and EI, respectively, as defined by Murfet (1), i.e. Porta is a late photoperiodic type showing a quantitative increase (6-8 nodes) in the node of flower initiation in short day conditions while the two mutants are typical early photoperiodic types in which node of flower initiation is unaffected by photoperiod but days to first open flower is substantially delayed in short days. The characteristics of lines belonging to these phenotypic classes are illustrated in more detail elsewhere in this issue (4).

The phenotypic classification of Porta and its derivatives, and the fact that Wt11790 and Wt11791 both flower at nodes 11 and 12, indicates two possibilities. Porta may have genotype *Lf E Sn Dne hr* with the mutants arising from forward mutation of *Lf* to *lf*, or alternatively, Porta may be *lf e Sn Dne hr* with the mutants arising from back mutation of *e* to *E.* The latter possibility is excluded by the fact that F_1 of the cross Porta (Wt3519) x L60 (*lf E Sn Dne hr)* was late flowering (flowering node 18-22, Table 1) which indicates Porta carries gene *Lf*. (*Lf* is epistatic to *E*; 2). The first hypothesis was substantiated by all remaining data. The crosses of Wt11790 and Wt11791 with L60 bred true in the F_1 and the F_2; all offspring flowered within the range of the parents (Table 1). The F_1 of cross Wt11791 (proposed genotype *lf E Sn Dne hr*) x L2 (*Lf E Sn Dne hr*) was late, flowering at nodes 24 and 25, while

Table 1. Distribution of node of flower initiation for Porta (Wt3519), early flowering mutants Wt11790 and Wt11791, Hobart lines 2 (*Lf E Sn Dne hr*) and 60 (*lf E Sn Dne hr*), and F_1 and F_2 progenies for crosses between the Hobart and Wiatrowo lines. In crosses 11790 x 2 and 11791 x 2 F_2 plants flowering at nodes 13-16 were genotyped by growing F_3. Photoperiod 8 h.

	Node of flower initiation																					
Line or cross	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Wt3519												2	2	2								
Wt11790		6	2																			
Wt11791		5	2																			
L2																	3	2		1	1	
L60	1	7																				
3519 x 2 F_1																3						
3519 x 60 F_1									1	-	-	1	2									
11790 x 2 F_1						2	1	-	-	-	-	-	-	-	1	2						
11791 x 2 F_1															1	5						
11790 x 60 F_1		4	1																			
11791 x 60 F_1		5	1																			
11790 x 2 F_2 *lf*		15	1	1																		
Lf				2	2	2	4	-	-	1	3	3	5	1	1	-	2	3		1	-	1
11791 x 2 F_2 *lf*		15	2																			
Lf					2	-	-	-	-	1	2	2	3	5	4	2	1	6	3			
11790 x 60 F_2	2	21	1																			
11791 x 60 F_2	3	17	4																			

Table 2. Dihybrid segregation data from the F_2 of crosses between Hobart line 2 (*A Lf*) and Wiatrowo lines 11790 (*a lf*) and 11791 (*a lf*).

	Observed numbers					Chi-square (df=l)				
Cross	*A Lf*	*A lf*	*a Lf*	*a lf*	Total	*A-a*	*Lf-lf*	Joint	Recomb. fract.	SE
11790 x 2	30	1	1	16	48	2.8	2.8	39.7***	3.8	2.9
11791 x 2	28	4	3	13	48	1.8	2.8	22.0***	13.9	5.5

***P< 0.001

the F_2 segregated into 29 late plants flowering at nodes 19-28, 17 clearly early plants flowering at nodes 11-12 and 2 intermediate plants flowering at node 14. F_3 data (n=12 for each progeny) showed conclusively that thesetwo intermediate plants were *Lflf* heterozygotes because the majority of their offspring were phenotypically late. Thus the observed F_2 segregation of 31 *Lf*- and 17 *lflf* segregates is in agreement with the expected 3:1 ratio (Table 2, $\chi^2 = 2.78$, $P > 0.05$). The *Lf* locus is on chromosome 1 and shows fairly strong linkage with the basic gene for anthocyanin production, *A* (recombination fraction about 10%; 2, 3, 5). Cross Wt11791 (*a lf*) x L2 (*A Lf*) is in the coupling phase and the close linkage between the two loci (Table 2) is consistent with previous results and supports the conclusion that the late/early flowering difference in this cross is attributable to segregation at the *Lf* locus.

As expected, cross WT11790 x L2 gave similar basic results to cross Wt11791 x L2 (Tables 1 and 2) except that there was an increased frequency of heterozygous *Lflf* plants flowering in the intermediate zone of nodes 13-16. This was already apparent in the Wt11790 x L2 F_1 where 3 of the 6 plants flowered at nodes 15-16 and 3 flowered at nodes 24-25 (Table 1). Eleven F_2 plants flowered in the intermediate zone and F_3 data again confirmed that 10 of these were *Lflf* heterozygotes. The joint segregation data again support the conclusion that the late/early flowering difference is attributable to segregation at the *Lf* locus because strong linkage is evident between the flowering gene concerned and marker *A* (Table 2).

The F_2 data from crosses L60 (*A lf*) x Wt11790 *(a If)* and L60 x Wt11791 (*a lf*) indicate that the mutant *lf* alleles in the two Wiatrowo lines are of very similar strength to the naturally occurring *lf* allele in the reference early photoperiodic line, L60. In cross 60 x 11790 the mean flowering node for *A*- segregates was 10.94 ± 0.09 (n=19) and 11.00 ± 0 (n=5) for the *aa* segregates. In cross 60 x 11791 the comparable data were 11.19 ± 0.14 (n=16) and 10.75 ± 0.16 (n=8). The slightly earlier flowering of the *aa* segregates in the latter cross may suggest lf^{11791} is a slightly earlier allele but that suggestion is not supported by the data for crosses with line 2 where it is cross 2x11790, not 2x11791 which contains the highest frequency of *Lflf* heterozygotes flowering in the intermediate zone. The tendency for heterozygotes for *Lf* alleles to flower intermediate between the pure forms is on record (2, 3).

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