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ORIGINAL PAPER

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Mapping of a spontaneous mutation for early flowering time in maize highlights contrasting allelic series at two-linked QTL on chromosome 8

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Abstract Only a few mutations affecting flowering time have been detected in maize. We analyzed a spontaneous early mutation, *vgt-f7p*, which appeared during production of the inbred line F7. This mutation shortens the time from planting to flowering by about 100 growing degree days (GDD), and reduces the number of nodes. It therefore seems to affect the timing of meristem differentiation from a vegetative to a reproductive state. It was mapped to a 6 cM confidence interval on chromosome 8, using a QTL mapping approach. QTL analysis of a mapping population generated by crossing the mutant F7 line (F7p) and the Gaspé flint population showed that *vgt-f7p* is probably allelic to *vgt1*, a QTL described in previous studies, and affects earliness more strongly than the Gaspé allele at *vgt1*. Global analysis of the QTL in the region suggested that there may be two consensus QTL, *vgt1* and *vgt2*. These two QTL have contrasting allelic effects: rare alleles conferring extremely early flowering at *vgt1* vs. greater diversity and milder effects at locus *vgt2*. Finally, detailed syntenic analysis showed that the *vgt1* region displays a highly conserved duplicated region on chromosome 6, which also plays an important role in maize flowering time variation. The cloning of *vgt1* should, therefore, also facilitate the analysis of the molecular basis of variation due to this second region.

Introduction

The timing of flowering is a key factor in the adaptation of plants to environmental conditions. It often varies markedly within species, highlighting evolutionary processes generating diversity and leading to contrasted local adaptation. Maize (*Zea mays* L.) illustrates these phenomena. It was domesticated approximately 7,000 years ago in the Yucatan Peninsula of Mexico, which is subject to typical tropical conditions—short days and warm temperatures (Beadle 1939). When cultivated in temperate rather than tropical regions, tropical varieties flower very late (Gouesnard et al. 2002). This late flowering results largely from high sensitivity to photoperiod, rendering these cultivars non-adapted to cultivation in long-day conditions. However, this tropical material was used to breed the varieties that made possible the southward and northward expansion of the crop. The development of these new varieties involved the use of processes triggering flowering in longer days and at lower temperatures. These efficient processes made it possible to adapt the crop for cultivation in the extremely cool regions of Canada and southern Chile. As a result, the Gaspé population, the earliest flowering maize variety known, requires only about 45 days to reach flowering when planted at the beginning of May, in the typical temperate conditions of the Paris Basin. A typical tropical population might require up to 135 days to reach flowering in the same conditions, with some extreme varieties not even flowering before the first frosts (Gouesnard et al. 2002). Overall, flowering time in maize varieties varies continuously between these two extremes and depends on the latitude and temperature conditions of the area cropped.

The development of molecular markers at the end of the 1980s made it possible to map the quantitative trait loci (QTL) involved in flowering time variation. This technique has been used for many other traits (Kearsey and Farquhar 1998), and has provided insight into the genetic basis of flowering time variation. Some QTL studies have focused on flowering time (e.g., the *vgt1* and *vgt2* QTL described by Vladutu et al. 1999), whereas others have dealt with traits such as grain yield, disease or insect resistance, but have recorded data for flowering time. A considerable body of data for QTL mapping is therefore available, from studies involving crosses

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between parental lines with different flowering times. Chardon et al. (2004) showed that the data currently available (313 QTL in total) could be interpreted statistically as corresponding to 62 consensus QTL, some of which appear to have major effects. However, little is known about the molecular bases of these QTL. Spontaneous mutants have frequently been detected for kernel characteristics, plant architecture and other traits. In contrast, only a few spontaneous mutants for flowering time have been identified in maize (Neuffer et al 1997), the principal exception being the *id1* mutation (Colasanti et al. 1998; McSteen et al. 2000). Induced mutations have also rarely been analyzed, *erl* mutations being among the few reported so far (Neuffer, 1994 pers com).

In this study, we used a mutant discovered by Maurice Polacsek in 1980 as a starting point for our investigation of the determinants of flowering time variation. The initial wild-type inbred line (F7) used to generate this mutant was still available, facilitating evaluation. However, no map position or near-isogenic material was available for this mutation. We therefore began by mapping the mutation, hereafter referred to as the *vgt-f7p* mutation, based on the similarity of its phenotypic effects to those of *vgt1* and *vgt2* (Vlatudu et al. 1999). The position of this mutation on chromosome 8 was compared with the QTL information available for the region of interest. This led to the development of a new mapping population, produced by crossing the F7p mutant line and Gaspé, to narrow down the location of the determinants of flowering time in this chromosomal region. Finally, we evaluated the synteny between this region and its duplicated counterpart on chromosome 6 in maize (Gaut and Doebley 1997), and the corresponding segment of rice chromosome 5 (Salse et al. 2004).

Material and methods

Discovery and evaluation of the *vgt-f7p* mutation

Inbred line F7 was developed at INRA Versailles from the Lacaune traditional population, released in 1956 and used as a parent in numerous early hybrids cultivated in northern Europe. The early mutant identified in 1980 (F7p) was subsequently multiplied by repeated self-pollinations. Anthesis occurs about seven days earlier in this line than in the F7 line. We investigated this effect and the dominance of the mutation in the F7 and F7p lines and the F7p×F7 F_1 hybrid.

Genetic material and phenotypic evaluation for mapping the *vgt-f7p* mutation

We crossed the wild-type line (F7) and the mutant line (F7p) with the inbred line F2 in 1997, to develop mapping populations. We used line F2 for these experiments because (i) it had been used in several other mapping experiments in our laboratory (e.g., Bouchez et al. 2002), (ii) it has a similar flowering time to F7, and (iii) it diverges considerably from F7 in terms of molecular markers (e.g., Dubreuil et al. 1996), despite being generated from the same Lacaune population. Line F2, like F7, was developed at INRA Versailles and was extensively used as a parental line in the early production of hybrids, following its release in 1956. F7p×F2 and F7×F2 F_1 hybrids were self-pollinated in 1998 to produce F_2 populations. In 1999, 150 F_2 plants from each population were self-pollinated to produce F_3 families.

Phenotypic analysis of F_3 families was carried out in 2000 for the F7×F2 population and in both 2000 and 2001 for the F7p×F2 population, the three parental lines (F7, F7p and F2) and the F7p×F7 F_1 hybrid—further referred to as parental checks. Each year, F_3 families and parental checks were planted in the first week of May at Gif-sur-Yvette (Paris Basin), on elementary plots, in a complete two-block design, with populations as sub-blocks and families randomized within sub-blocks. An elementary plot consisted of a single 4.2 m row of 20 plants. Rows were 0.8 m apart, giving a planting density of 6 pl/m^2. We used two replicates for each family (one per block) and six replicates for parental checks (three per block).

Temperature was recorded throughout the growing season at a meteorological station about 500 m away from the trial site. Growing degree days (GDD, °C) since planting were calculated from daily minimum and maximum temperatures (Bonhomme et al. 1994). Date of pollen shed (DPS) was evaluated as the number of GDD (°C) from planting to the date at which 50% of the plants in a plot had produced anthers. Silking date (SD) was evaluated as the number of GDD (°C) from planting to the date at which 50 % of the plants in a plot had silks emerging from the primary ear shoot. Node number (ND) was recorded as mean leaf number for five plants in the middle of a plot. For the determination of node number, it was necessary to mark the fifth leaf on each of the plants assessed while the first leaf was still visible (i.e., before total senescence).

Development of a F7p×Gaspé mapping population and phenotypic evaluation

Based on these results, we developed a new mapping population for investigating the relative positions and possible allelic relationships between the *vgt-f7p* mutation and a major QTL on chromosome 8 initially discovered by Koester *et al.* (1993) and then further analyzed by Vlatudu et al. (1999) and Salvi et al. (2002). These studies had shown that the Gaspé Flint population carries an allele shortening the interval between sowing and flowering by about six days, and that this allele is present at a major QTL (*vgt1*) (Salvi et al. 2002). Analysis of molecular markers showed that the Gaspé Flint population is typical of the "Northern Flint" genetic group (Rebourg et al. 2003) but flowers much earlier than other typical accessions from this group.

F7p was crossed with Gaspé in 1999, and two F_1 ($=S_0$) plants were self-pollinated in 2000 to produce F_2 plants. $F_{2:3}$ families were then produced by self-pollinating 111 and 54 plants within each F_2 population. These families were evaluated in 2002, using the experimental approach as described above.

Statistical analyses of field trials

Statistical analysis of phenotypic data was carried out by analysis of variance, using the GLM procedure of SAS. We estimated family values with the LSMEANS option. Parental checks were compared in pairs, using the dpiff option. Genetic variances within populations were then estimated with the VARCOMP option of SAS.

Genotyping of parental materials and mapping populations

We isolated DNA from the F7, F7p, and F2 lines, the Gaspé x F7p hybrid and each $F_{2:3}$ family. DNA was isolated from a bulk of leaf tissue from 15 seedlings (about 14 days old) in each case. Oligonucleotide primers for SSRs were obtained from MaizeGDB (http://www.maizegdb.org/ssr.php) and from a private consortium in two cases (cm32 and cm61). Reaction mixtures consisted of 50 ng of template DNA, 40 µmol of each of the forward and reverse primers, 0.5 mmol of $MgCl_2$, 10× PCR buffer and 5 units/µl *Taq* polymerase. The SSR protocol of the Maize Mapping Project (http://www.maizegdb.org/documentation/maizemap/ssr_protocols.php) was used for amplification. PCR products were separated by electrophoresis in 3% Metaphor agarose gels stained with ethidium bromide, run at 140 V for about 3 h. Gels were viewed and recorded using a Kodak Digital Camera with an ultraviolet light filter. We screened 1200 SSR markers evenly distributed throughout the genome. No polymorphism was observed between F7p and F7, confirming that these two lines are isogenic.

Mapping procedures

All genetic maps were constructed using MAPMAKER version 3.0b (Lander et al. 1987). Loci were assigned to linkage groups with a minimum LOD score of 3.0 and a maximum Haldane distance of 40 cM. Three-point linkage analysis was performed for each linkage group. The order of markers on each chromosome was checked, using the "ripple" option. QTL analyses were performed with PLAB-QTL software (Utz and Melchinger 1996), using a classical composite interval mapping strategy (Jansen 1993; Zeng 1994). Permutation tests were run for each population to identify thresholds that correspond to 10% experiment-wide risks of detecting at one least position with a "significant effect" under a global null hypothesis that no QTL is present in the regions considered. Note that this threshold is lower in experiments restricted to the analysis of a single chromosome (F7p×Gaspé and F7×F2 populations).

Meta-analysis of QTL results

The QTL positions estimated in this study were compared with those found in other studies, using a strategy similar to that described by Chardon et al. (2004). We used Biomercator software (Arcade et al. 2004) to obtain, by projection, a synthetic map including all markers used in QTL studies. QTL positions and their corresponding confidence intervals are projected onto this reference map and can be visualized. We then carried out a meta-analysis, as described by Goffinet and Gerber (2000), to determine the most likely number of consensus QTL for the region. Finally, we estimated the positions of consensus QTL and their confidence intervals.

Analysis of the synteny of the *vgt-f7p* mutation region with the rice and maize genomes

The bnlg1599 SSR, which was strongly associated with the *vgt-f7p* mutation, was sequenced. The sequence obtained was used as a query in BLAST searches against japonica rice BAC sequences (http://www.gramene.org/db/searches/blast). A homologous sequence was identified in the AC079022 and AC093921 BAC, mapping to the start of chromosome 5 (t 4.6 and 6 cM, respectively). BAC gene annotations were taken from the TIGR annotation database (http://www.tigr.org/tigr-scripts/e2k1/irgsp.spl). We then looked for homologous sequences in maize EST databases, using BLAST processes with default parameters (Altschul et al. 1997). Six maize clones were identified and mapped on a reference maize map (constructed using IBM and LHRE populations, Falque et al. 2003), using an RFLP protocol described elsewhere (Causse et al. 1996). Four of these clones mapped to chromosomes 8 and 6, confirming interspecific synteny between maize chromosome 8 and rice chromosome 5 and intraspecific synteny between maize chromosomes 8 and 6. We investigated this intraspecific synteny further by searching for a paralogous location on the other chromosome for each of the RFLP markers mapped to the regions of chromosomes 8 and 6 concerned in the Genoplante program (Falque et al. 2005). The re-examination of RFLP mapping autoradiographs led to the identification of four additional links between the two regions.

Results

Evaluation of the phenotypic effects of the mutation

Comparison of the inbred lines F7 and F7p and of the F7p×F7 F_1 hybrid for the three traits of interest in 2000 and 2001 (Table 1) demonstrated that the mutation

Table 1 Evaluation of the effect of the *vgt-f7p* mutation in the 2000 and 2001 trials DPS: date of pollen shed, SD: silking date, ND: node number

	F7p (mutant)	F7 (wild type)	F7p×F7	F2 (check)
SD (GDD, °C)	702 ± 12	794 ± 12	752 ± 12	831 ± 12
DPS (GDD, °C)	699 ± 11	777 ± 11	740 ± 11	844 ± 11
ND	10.2 ± 0.2	11.7 ± 0.2	11.3 ± 0.2	13.8 ± 0.2

decreased time to flowering by 92 and 78 GDD (°C), for SD and DPS, respectively. It also significantly decreased the number of nodes (by 1.6 nodes), consistent with effects on the timing of apical meristem differentiation from the vegetative to the reproductive state. The phenotype of the F7p×F7 F_1 hybrid was intermediate between those of F7 and F7p for all traits, and significantly different from that of both parents, suggesting an additive effect of the mutation. The F2 line used as a parent in the mapping experiments flowered slightly later than F7 (+ 47 and + 68 GDD, for SD and DPS, respectively, Table 1) in the 2002 and 2001 trials, consistent with previous findings (Alain Charcosset, *pers. com.*).

Trait variation in the F7p×F2 and F7×F2 populations and mapping of the *vgt-f7p* mutation

Evaluation of the F7p×F2 and F7×F2 populations in the trials in 2000 showed that the F7p×F2 population flowered earlier (−53 and −70 GDD for SD, and DPS, respectively) and had fewer leaves (−0.7) than the F7xF2 population (Table 2). The difference between these two populations was similar to that expected for an additive effect of the mutation (half the mutation effect) on SD and ND and larger than expected for DPS. As expected, genetic variance was also higher for the F7p×F2 population (about double) than for the F7×F2 population (Table 3). For the F7p×F2 population, genetic variances were similar in 2000 and 2001, despite a much larger environmental variance in 2001. A combined analysis for both years showed significant genetic × environment variance, the magnitude of which was about half that of the genetic variance. We investigated trait distribution within the F7p×F2 population for individual years and for both years considered together. A moderate trend towards bimodality was observed for SD and ND (Fig. 1).

We selected markers on the basis of position and ability to reveal polymorphism between F7 and F2 on agarose gels. These were used to develop an initial map of 96 markers for the F7p×F2 population, covering all the maize chromosomes (results not shown). QTL analysis with this map identified four QTL, one of which had a major effect (accounting for 42% of phenotypic variation). This locus mapped to chromosome 8, between markers bnlg2046 and bnlg1599. We then used eight markers well spread out over chromosome 8 to characterize the F7×F2 population (Fig. 2). No QTL was identified in this region for the F7×F2 population, confirming that the major QTL found in the F7p×F2 population corresponded to the mutation. We then looked for additional markers on chromosome 8 to complement the F7p×F2 map; eleven markers were finally used for QTL detection (Fig. 2). The results of the QTL analysis conducted on this final map of chromosome 8 are presented in Table 4. They show that the mutation had a strong effect on the three traits analyzed and accounted for up to 55% of total phenotypic variation for DPS. For the three traits of interest, the additive effect of the F7p allele (Table 4) was about half the difference between F7 and F7p (Table 1), as expected under the hypothesis of no epistatic effect between the mutation and other QTL. No significant dominance was detected at this position.

Table 2 Average performance of F7×F2 and F7p×F2 populations (same traits as in Table 1) in trials conducted in 2000 and 2001, date indicated within brackets

	F7×F2 (2000)	F7p×F2 (2000)	F7p×F2 (2000–2001)
SD (GDD, °C)	807 ± 5	752 ± 7	769 ± 6
DPS (GDD, °C)	789 ± 4	746 ± 6	763 ± 5
ND	12.9 ± 0.1	12.2 ± 0.1	12.3 ± 0.1

Table 3 Structure of variance within F7×F2 and F7p×F2 populations (same traits as in Table 1) in trials conducted in 2000 and 2001, date indicated within brackets. σ^2_e is the environmental error variance, σ^2_g is the genetic variance and h^2 is heritability (at family mean level). For F7p×F2 evaluated in 2000–2001 trials, σ^2_{ge} is the genotype × environment (year) variance

	F7×F2 (2000)			F7p×F2 (2000)			F7p×F2 (2000–2001)			
	σ^2_e	σ^2_g	h^2	σ^2_e	σ^2_g	h^2	σ^2_e	σ^2_g	σ^2_{ge}	h^2
SD (GDD, °C)	174	995	0.85	238	1981	0.89	454	1336	655	0.75
DPS (GDD, °C)	193	526	0.73	157	1295	0.89	308	867	460	0.74
ND	0.15	0.20	0.57	0.12	0.44	0.78	0.10	0.48	0.20	0.77

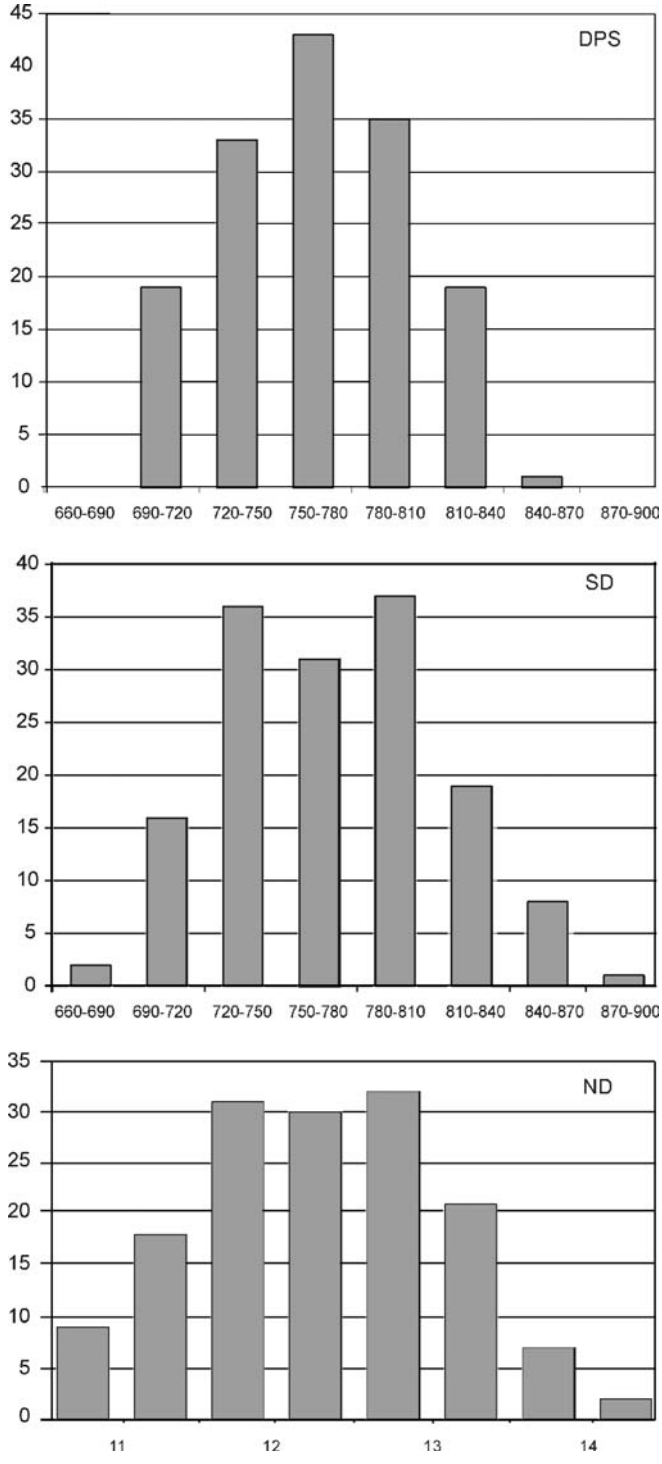

Fig. 1 Phenotypic distribution within the F7p×F2 population (mean for 2000 and 2001). DPS: date of pollen shed (GDD, °C), SD: silking date (GDD, °C), LN (ND): leaf number

Analysis of the F7p×Gaspé mapping population

The Gaspé population flowered much earlier than the other early parental material used in this study (more than 100 GDD earlier than F7p), as expected (Table 5). F7p×Gaspé F_3 families generally flowered very early and had small numbers of nodes, intermediate between the values for the F7p line and Gaspé population (Table 5). Highly significant genetic variation was observed for all traits. None of the families flowered earlier or had fewer nodes than Gaspé. Genetic variance was similar to that observed in 2000–2001 for the F7p×F2 population for DPS and smaller for SD and ND.

We mapped 14 markers on chromosome 8 for a population of 111 families derived from a single hybrid plant (Fig. 2). Initial QTL analysis gave very different results for the three traits. No QTL was detected for DPS, whatever the model used. One QTL was detected for ND at position 18. Two QTL were detected for SD, both in the region of interest, at positions 6 and 18. These two QTL had opposite effects, with position 6 in Gaspé and position 18 in F7p conferring early flowering. Position 18 corresponds to the position of the *vgt-f7p* mutation, as estimated above (Fig. 2). Given these results, and the limited power of QTL mapping in cases of linked QTL, we carried out complementary analyses for each trait, assuming two QTL, at positions 6 and 18. For each trait, we investigated whether the addition of the second QTL to the model accounted for a significantly larger proportion of the variation than the single-QTL model. We found that position 18 contributed significantly to the variation of all three traits, whereas position 6 contributed to the variation of SD and DPS only, with no significant effect on node number (Table 4). Linkage between these two QTL for SD and DPS makes it difficult to estimate a confidence interval for positions, so no confidence interval is included in Table 4. The effects of these two QTL are also not independent and must therefore be analyzed with care. However, they seem to act in opposite directions, with Gaspé and F7p contributing early alleles at positions 6 and 18, respectively.

Meta-analysis of available information for flowering time QTL on chromosome 8

We compared our results for flowering time QTL positions with those reported for this region in other studies. We took into account the QTL positions reported by Chardon et al. (2004), together with a QTL mapped in an F2×F252 population of 300 F_3 lines obtained by means of four intercrossing cycles (Moreau and Charcosset, personal communication). As we were specifically interested in this region, we also made use of the data of Bouchez et al. (2002) and analyzed eight additional markers. This lead to a total of 15 markers, two of which are also present on the F7p×F2 map (Fig. 2). Reexamination of QTL effects using this denser map confirmed that 29 % of phenotypic variation was explained by the flowering time QTL. This analysis also made it possible to decrease the size of the confidence interval for the position of this QTL from 22 to 12 cM. Numerous markers were mapped in the F7p×F2 population specifically to increase the precision of map comparison

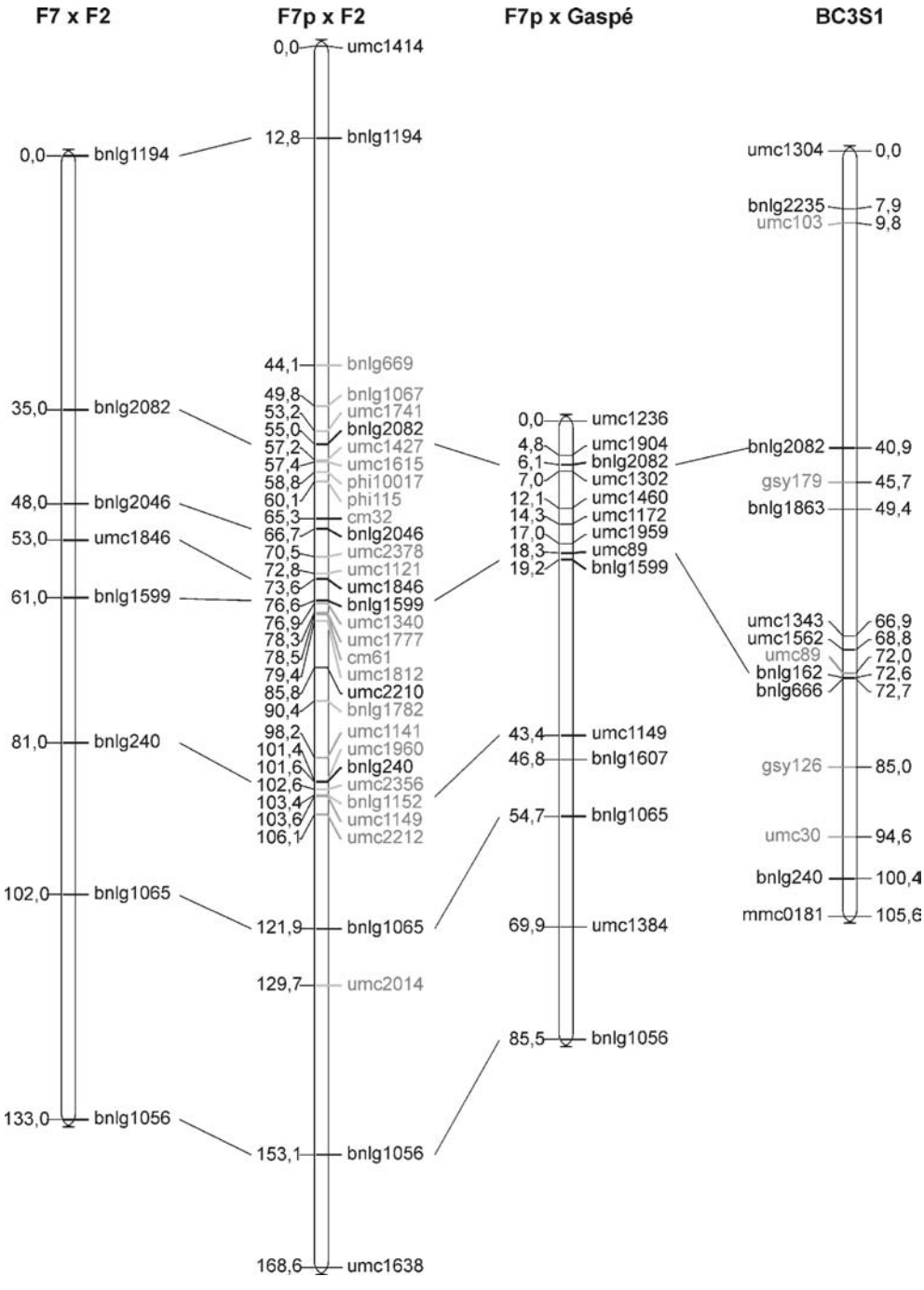

Fig. 2 Genetic maps of chromosome 8 developed for the study. For the F7p×F2 map, dark letters indicate the markers used for QTL analysis (see Table 4), whereas gray letters indicate additional markers analyzed to facilitate comparison with other maps. These additional markers were projected onto the QTL map. For the F2×MBS847 map (BC3S1), markers in gray indicate RFLP markers considered in the initial QTL study of Bouchez *et al.* (2002), and markers in black indicate additional SSR markers analyzed in our study

(Fig. 2). We made particular efforts to compare our results with those obtained by Vladutu et al. (1999) and Salvi et al. (2002).

The projection of QTL positions onto the same reference map (Fig. 3) demonstrated major differences in precision. The most precise positions were those reported in this study for F7p×F2, Gaspé×F7p (position 18) and the position of *vgt1* reported by Vladutu et al. (1999) and Salvi et al. (2002). Meta-analysis concluded to two "consensus" QTL in this region and suggested that *vgt-f7p* and *vgt1* probably correspond to a single QTL. If we consider only the most precise position estimated for a given population, all the other QTL can be attributed to a second consensus QTL, corresponding to the *vgt2* position described by Vladutu et al. (1999).

Discussion

Effect of the *vgt-f7p* mutation and relationship to *vgt1*

Comparison of the F7p mutant line with its wild-type progenitor F7 showed that the *vgt-f7p* mutation greatly reduced time to flowering. It also strongly decreased the

Table 4 QTL detected in populations F7p×F2, Gaspé×F2 and MBS847×F2 BC_3-S_1. Positions refer to maps presented in Fig. 2

Population	Trait	Chr.	Upper marker of interval (position)	Lower marker of interval (position)	Position and confidence limits	LOD	R^2	Effect[a]
F2 × F7p	DPS	1	Bnlg1615 (133)	umc1335 (155)	148(132–188)	2.7	8.0	−9.3
F2 × F7p	DPS	3	Phi3741 (32,6)	mmc031 (53)	40(16–50)	2.4	7.1	−12.6
F2 × F7p	DPS	8	umc1846 (74)	bnlg1599 (77)	76(74–80)	25.7	54.6	−37.1
F2 × F7p	DPS	10	Phi117 (0)	bnlg1451 (20)	0(0–10)	3.5	10.3	9.5
F2 × F7p	ND	1	bnlg1615 (133)	umc1335 (155)	136(130–150)	2.9	8.5	−0.2
F2 × F7p	ND	1	umc1335 (155)	umc129 (188)	182(164–196)	3.2	9.5	−0.2
F2 × F7p	ND	1	bnlg1016 (231)	bnlg176 (268)	258(234–270	2.5	7.5	0.2
F2 × F7p	ND	4	Phi021 (76)	bnlg121 (118)	92(66–118)	2.5	7.5	−0.2
F2 × F7p	ND	8	bnlg1599 (77)	umc2210 (86)	78(74–82)	23.5	51.4	−0.8
F2 × F7p	SD	1	umc1335 (155)	umc129 (188)	166(144–180)	5.0	14.2	−17.2
F2 × F7p	SD	3	bnlg110 (168)	phi047 (195,6)	194(182–194)	4.7	13.7	15.3
F2 × F7p	SD	8	umc1846 (74)	bnlg1599 (77)	76(70–82)	19.1	45.2	−41.5
F2 × F7p	SD	10	umc1336 (107)	bnlg1028 (141)	108(94–124)	3.6	10.5	13.1
F7p × Gaspé	DPS	8	bnlg2082 (6)	umc1460 (12)	6(–)	1.6	6.5	−17.42
F7p × Gaspé	DPS	8	umc89 (18)	bnlg1599 (19)	18(–)	1.5	6.1	18.44
F7p × Gaspé	ND	8	bnlg2082 (6)	umc1460 (12)	6(–)	0.4	1.7	−0.151
F7p × Gaspé	ND	8	umc89 (18)	bnlg1599 (19)	18(–)	2.4	9.2	0.404
F7p × Gaspé	SD	8	bnlg2082 (6)	umc1460 (12)	6(–)	1.7	6.6	−19.9
F7p × Gaspé	SD	8	umc89 (18)	bnlg1599 (19)	18(–)	2.5	9.7	26.99
F2 × MBS847	SD	8	bnlg1863 (49)	umc1343 (67)	58(52–64)	16.2	29.0	0.83

[a]Additive effect contributed by parental line L_1 for a cross described as $L_1 \times L_2$

Table 5 Characteristics of F7p × Gaspé population evaluated in 2002 (same traits as in Table 1); values of parents F7p and Gaspé, F7p×Gaspé hybrid, μ is the mean for the population, σ^2_e is the environmental error variance, σ^2_g is the genetic variance and h^2 is heritability

	F7n (check)	F7p	Gaspé	F7p×Gaspé	μ	σ^2_e	σ^2_g	h^2
SD (GDD, °C)	839	767	661	675	721	166	1022	0.92
DPS (GDD, °C)	837	771	666	677	723	166	786	0.90
ND	11.9	10.0	9.3	10.6	10.5	0.29	0.20	0.59

number of nodes (1.6 fewer nodes), indicating that the mutation affected flowering time by accelerating differentiation of the shoot apical meristem from the vegetative to the reproductive state. Mapping of this mutation, using a segregating population generated from a cross with line F2, confirmed the magnitude of these effects and made it possible to map the mutated gene to a 6 cM interval in the bin 8.05 region of chromosome 8. This position excludes several potential candidate flowering time genes, including the *HASTY-like* gene, associated with the *early phase change* (*epc1*) mutation mapped to the bin 8.02 region (Vega et al. 2002). Meta-analysis of the QTL results available for this region showed that the *vgt-f7p* mutation is either allelic to the *vgt1* mutation or affects a gene very close to *vgt1*. The results obtained for a specific mapping population generated by crossing F7p and Gaspé and carrying *vgt1* are consistent with this hypothesis. They also suggested that the *vgt-f7p* mutation has a stronger effect than *vgt1* (−0.4 nodes). Current progress towards the positional cloning of *vgt1* (Fengler et al. 2003; Salvi et al. 2002) and the strict isogenicity of F7 and F7p should make it possible to determine whether the two mutations are allelic and to determine their molecular bases.

Far fewer spontaneous mutants with strong effects have been detected for flowering time than for other traits in maize, with several tens of kernel trait mutants known, for example (Neuffer et al. 1997). The only spontaneous mutants known are the late *id1* mutation (Colasanti et al. 1998; Singleton 1946), the early *epc1* mutation and the early *vgt1* mutation specific to the Gaspé population. The vgt-*f7p* early mutation, which was the starting point of this study, occurred more than 20 years after fixation of the genotype of its wild-type progenitor, F7. It is therefore a distinct mutation. Our finding that the *vgt-f7p* mutation is probably allelic to *vgt1*, one of the two known early mutations in maize is therefore of particular importance. The *id1* mutation has also been identified independently at least twice in studies based on non-targeted approaches (Colasanti et al. 1998; Singleton 1946). These results suggest that *Id1* and *vgt1* play a specific role in the determinism of flowering time and that these genes display little redundancy.

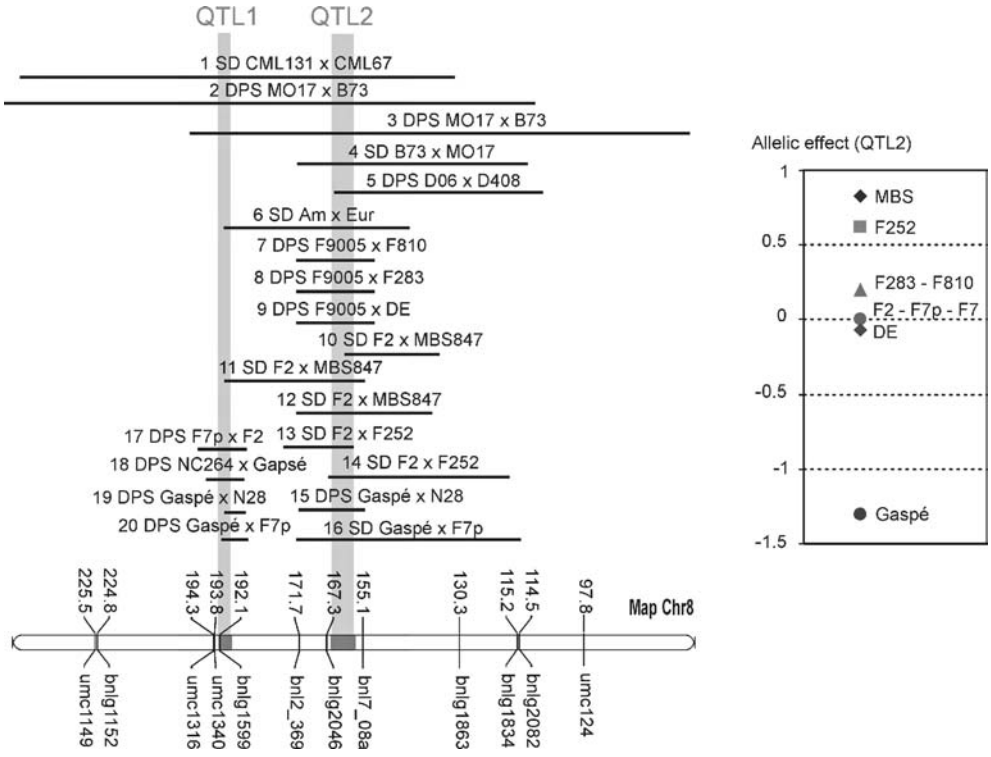

Fig. 3 Synthetic representation of maize chromosome 8 QTL results for flowering time. QTL confidence limits were projected onto the same reference map (horizontal black lines). Each QTL is identified by a number, followed by trait acronym (either SD or DPS) and the parental lines of the cross. Gray vertical rectangles correspond to the confidence limits of consensus QTL (QTL1 and QTL2), as identified by meta-analysis. Approximate synthetic estimates of the allelic effects at QTL 2 are presented in the box on the right. QTL numbers correspond to the following studies: (1) Groh et al. (1998), (2–3) Stuber et al. (1992), (4) Beavis et al. (1994), (5) Bohn et al. (2000), (6) Rebaï et al. (1997), (7-8-9) Blanc et al. (2004), (10) Moreau, Poupard and Charcosset (*unpublished result*), (11) Méchin et al. (2001), (12) Bouchez et al. (2002), (13) Bouty, Moreau et al. (2004), (14) Moreau et al. (2004), (15–19) Vladutu et al. (1999), (16-17-20) this study, (19) Koester et al. (1993)

Contribution of the *vgt1-vgt2* region to flowering time variation in maize

Flowering time is simple to score and is usually recorded in maize mapping populations, even if this trait is not the primary trait studied. Large amounts of data are therefore available for maize flowering time QTL. Chardon et al. (2004) recently analyzed publicly available results and identified 313 individual QTL, 16 of which are located in the bin 8.04–8.05 region of chromosome 8. We carried out a meta-analysis, including these data and the results obtained in this study. This analysis led to the identification of two consensus QTL. Individual QTL can be attributed to one of these two QTL, corresponding to the *vgt1* and *vgt2* QTL described by Vlatudu et al. (1999).

These two QTL have very different effects. QTL corresponding to *vgt1* have very strong effects on both flowering time and node number, but appear to be restricted to material derived from the Gaspé population or the F7p line investigated here. The only known exception is a QTL with very strong effect discovered in private material, developed by Limagrain (Martinant et al. pers. com.). However, there may be unknown pedigrees relating this material to Gaspé, which has been widely used in breeding programs to increase earliness in maize. All the other QTL presented in Fig. 3 correspond to *vgt2*. Consistent with results of Vlatudu et al. (1999), our results for the F7p×Gaspé population showed that *vgt2* differs from *vgt1* in having only a limited effect on node number (Fig. 4 in Vlatudu et al. (1999)). We further investigated the contribution of *vgt2* to flowering time variation by identifying the parents of the populations displaying significant effects at this QTL (Fig. 3). Some of these parents are related. We evaluated the inheritance of chromosomal segments in the region of interest in these cases, using information obtained as part of a genetic diversity survey of maize inbred lines for 9 markers of chromosome 8: phi115, phi121, phi014, bnlg1031, bnlg1065, phi015, phi233376, bnlg1194, phi119 (Madur et al. *pers. com.*). We found that F9005, which was derived from a single cross between F2 and F252, probably inherited the F252 segment rather than the F2 segment. As no difference was found in the allelic effects of F2, F7p and F7 at *vgt2*, we can estimate the allelic effects of MBS847, F252, F283, F810, DE and Gaspé, with respect to those of F2, F7p and F7 (Fig. 3). Such

comparisons will require the development of specific statistical tests to determine which differences may be considered significant, and the extent to which results may be affected by epistatic effects between the QTL of interest and other QTL, the alleles of which vary between mapping populations. In the absence of such methods, we can speculate that the alleles at *vgt2* fall into at least three groups: (MBS847 and F252), (F283, F810, F2, F7, DE) and Gaspé, in flowering time order, from late to early. The parental lines of other populations cannot be attributed to these groups on the basis of information currently available. However, future analyses based on dense haplotyping of the *vgt2* region, following an approach similar to that proposed by Jansen et al. (2003) for QTL mapping in multiparental designs, may resolve this issue.

Vgt1 and *Vgt2* therefore differ considerably in the magnitude of effects, with maximum differences between alleles of approximately 8 days (2 × 41 GDD, see Table 4) and 2 days (Fig. 3), respectively. They also differ in the frequency of classes of allelic effects. Mutations at *vgt1* shorten the duration of the plant cycle considerably by reducing the number of leaves. Such mutations decrease light interception and are probably selected against in most environmental conditions, being favorable only in very cold conditions, such as those in Gaspésie, Quebec. Diversity at *vgt2* is associated with milder effects and has no major effect on leaf number, which may account for the more diverse effects observed.

Synteny with the rice genome and relationships between chromosome 8 and chromosome 6

Several QTL have been associated with heading date and photoperiodic response in rice. These QTL have been mapped to eight of the 12 chromosomes (Li et al. 1995; Lin et al. 1998; Yano et al. 2000; Yano and Sasaki 1997). Four have been identified and the genes involved were characterized (Yano et al. 2001). Determination of the complete genome sequence for rice will certainly make it easier to identify candidate genes for other QTL in rice. An analysis of synteny, comparing the order of sequences in the rice genome sequence with that in other cereals, can be used to identify candidate genes for QTL in other species. Studies of this type have been carried out in ryegrass (Armstead et al. 2004) and wheat (Yan et al. 2003).

Comparison of the relative positions of RFLP probes in maize and rice indicated strong synteny between the region investigated in our study on chromosome 8 and rice chromosome 5 (Fig. 4). This region of the rice genome also displays strong synteny with a region of maize chromosome 6 (Fig. 4), which has been reported to carry several QTL affecting flowering time and interpreted as a single consensus QTL on the basis of meta-analysis (Chardon et al. 2004). RFLP mapping confirmed the strong homology between these regions of chromosomes 6 and 8, suggesting that genes orthologous to *vgt-f7p* may correspond to the flowering time QTL on chromosome 8. However, no QTL affecting flowering time has been mapped to chromosome 5 in rice. Analysis of BAC sequences corresponding to the syntenic region identified no candidate gene clearly involved in controlling flowering in rice or in *Arabidopsis*. These results suggest that genes involved in maize are not conserved or do not affect flowering time in rice. However, the effect of the *vgt1*/*vgt-f7p* region is limited to very specific material in maize, so it is possible that such material exists in rice but has yet to be analyzed. Finally, rice BAC clones AC079022 and AC093921 contain 56 genes according to TIGR annotation. At least four of these genes may be indirectly involved in the genetic control of flowering time:

- bnlg1599 SSR. This sequence is similar to that of a gene encoding a chloroplast selective amino-acid channel protein correlated with cold acclimation in cereals (Baldi et al. 1999).
- *Pumilio*. This gene encodes an RNA-binding protein involved in the specification of posterior body plan in the *Drosophilia* embryo (Macdonald 1992; Wharton et al. 1998). The homologous maize sequence used for RFLP mapping was identified from EST extracted from apical meristem tissues, the apical meristem being the organ in which floral transition occurs.
- A gene with a *Ring H2* domain, probably encoding a transcription factor (Kosarev et al. 2002). Homologous EST sequences were extracted from meristem tissues.
- *DOF3*. This gene encodes a protein of the DOF protein family. The members of this family play critical roles as transcriptional regulators in plant growth and development (Yanagisawa 2002).

These four genes may be involved in *vgt1* and *vgt-f7p* mutations. Expression experiments should therefore be conducted to investigate the possible differential expression of these genes in maize meristem before and after floral transition. Such studies would constitute a first step towards testing the effects of these genes and analyzing the relevance of sequencing alleles from the F7p and F7 lines.

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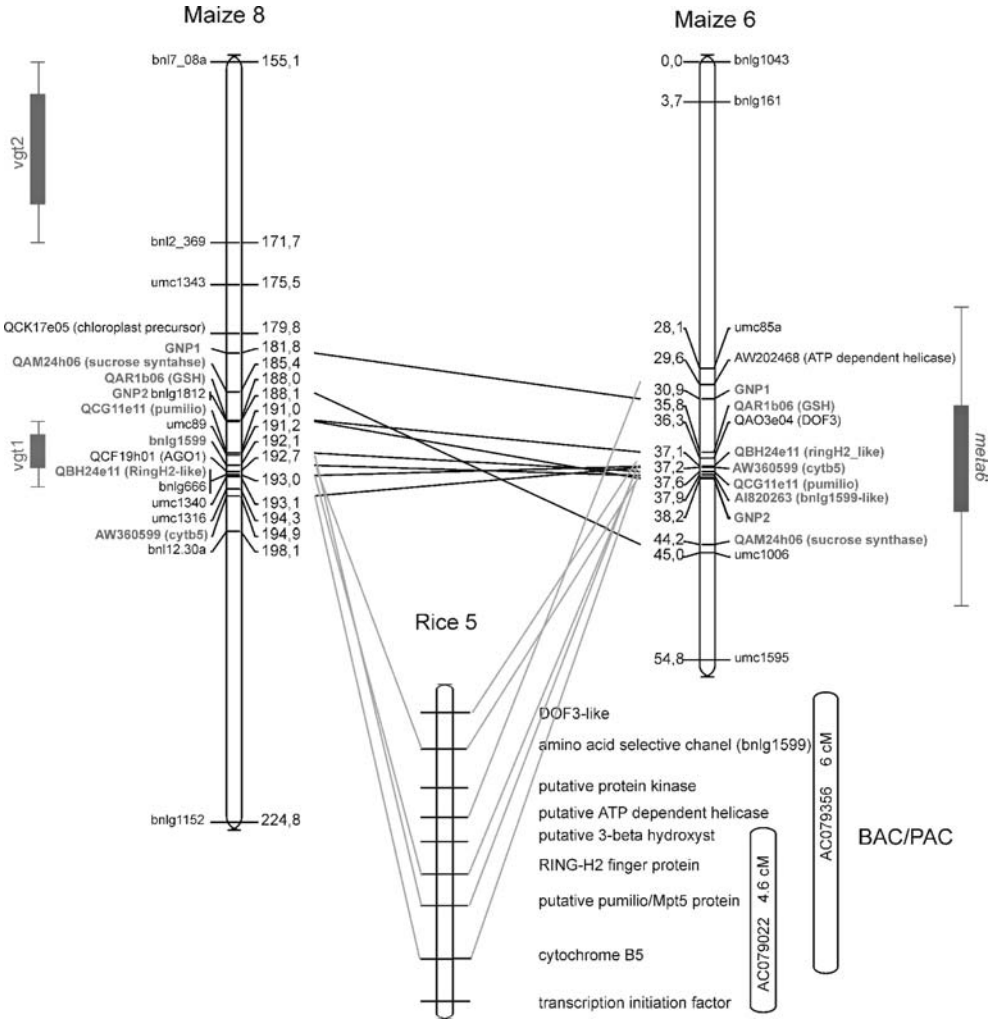

Fig. 4 Comparison between the genetic map of the *vgt-f7p* region of maize chromosome 8, the genetic map of consensus QTL (meta6) on maize chromosome 6 and the physical map of the homologous region on rice chromosome 5. Loci mapped on maize genome with the same RFLP marker are connected by dark lines. RFLP markers mapped on maize chromosomes and corresponding homologous sequence on the rice chromosome are connected by gray lines. All SSR loci (black characters) are identified according to standard international nomenclature. RFLP loci (gray characters) are identified by probe clone according to NCBI nomenclature, followed by summarized annotation within brackets. GNP1 and GNP2 indicate RFLP loci mapped in the framework of Génoplante, coded because of their value for other research programs

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