Localization of New Monogerm and Late-bolting Genes in Sugarbeet using RFLP Markers[1]

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ABSTRACT

A single *M-m* gene controlling multi-monogerm phenotype has been described and mapped into linkage group 2. In this study a second monogerm locus, designated m^2, was analyzed in a segregating population from a cross between monogerm and multigerm lines of Russian origin (S22 and No.14, respectively). The m^2 gene was located using RFLP markers previously mapped in the sugarbeet (*Beta vulgaris* L.) genome. Seventeen RFLP probes were selected and 56 F_2 plants examined. Nine RFLP markers were located in all linkage groups except group 2. Segregation patterns of these nine RFLP markers and the monogerm and late-bolting traits indicated independence. The other eight RFLP probes indicated an association between monogerm and late-bolting in linkage group 2. The linear order of the markers considered in this analysis was identical to the order reported by others. However, the distance between some RFLP markers was not consistent with previous reports. The m^2 gene was mapped in the same linkage group (group 2) as the *m* gene. The distance between the m^2 and *m* genes was 32.2 cM. These results confirmed our supposition, based on genetic experiments, that these genes are different from each other. The *lb* gene from the Russian lines and the late-bolting gene reported in other germplasm appeared to be identical.

Additional Key Words: *Beta vulgaris* L., gene localization, mapping.

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In the generative stage, a monogerm sugarbeet plant has only a single flower per floral axis; whereas, a multigerm plant has fused flower clusters. The late-bolting trait delays bolting; the transition from vegetative to generative stage. The *M-m* locus controls the mono-multigerm trait (Savitsky, 1952). A series of dominant alleles (M^{l}, M^{br}, M and M^{Z}) control the multigerm phenotype and the single recessive allele *m* results in the monogerm phenotype (Savitsky, 1954). The gene controlling late-bolting (*Lb-lb*) is linked with the *M-m* gene with reported cross-over rates of 25.5% (Savitsky, 1952) and 11% (Shavrukov, 1990).

RFLP analysis provides an accurate method for detecting and locating genes. There are no reports linking the *Lb-lb* gene to sugarbeet molecular markers. The monogerm gene, *M-m*, was located in two RFLP linkage maps (Barzen et al., 1995; Schumacher et al., 1997). In spite of a different numbering of the linkage group, both papers reported the gene on the same chromosome. The *M-m* gene is located in linkage group 2, according to the most recent designation of sugarbeet chromosomes (Schumacher et al., 1997). All monogerm germplasm used in these RFLP experiments originated from Germany. RFLP analysis has also been used in studies of Japanese monogerm breeding lines (Shavrukov et al., 1997). German, Japanese, and American monogerm germplasm is believed to have a common origin and the monogerm (*m*) allele discovered by Savitsky (1952). Russian monogerm sugarbeet is thought to be of different origin. Genetic polymorphism of different monogerm types has been reported (Shavrukov and Khanov, 1992) and three additional recessive alleles of the *M-m* gene have been described (Shavrukov, 1997a). The appearance of multigerm plants in some hybrids involving Russian monogerm germplasm and also hybrids between Russian and American monogerm lines suggests that different monogerm genes are involved (Shavrukov, 1997a, 1997b). This hypothesis is supported by the results of Konovalov (1992). In his experiment a linkage between the *Adh* and *M-m* loci was observed in progeny from a cross with East-European monogerm germplasm; whereas, independent segregation of the same traits was observed in progeny from a cross with an American monogerm line.

The objectives of this study were to detect and locate the late-bolting (*lb*) and monogerm (m^2) genes from Russian sugarbeet and to compare their location to those indicated in published linkage maps including the *M-m* and *lb* loci.

MATERIALS AND METHODS

The segregating F_2 population used for this analysis was derived from a cross between monogerm inbred line S22 with late bolting tendency

and multigerm inbred line No. 14 with normal time of flowering, both originated from the Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia. Flowers of one plant from each parental line were manually emasculated and isolated with paper-bags. In spite of the self-incompatibility of both lines, the completeness of emasculation was observed in each flower before crossing. The 25 F_1 progeny examined had normal time of flowering and multigerm phenotype; however, the number of flowers per cluster was less than in line No. 14. F_1 plants were self-pollinated, and F_2 seeds were sown in the field in May. After harvesting in September and vernalization at 4°C for five months, F_2 stecklings were replanted in the field for leaf collection and phenotype characterization. Fresh leaves were collected from one-month old F_2 plants and used immediately for DNA extraction. Monogerm and late-bolting phenotypes were recorded visually after the beginning of flowering. Individuals had distinct mono or multigerm phenotypes. The difference in bolting time between normal and late bolting progenies was 7-10 days. A total of 103 plants was observed for monogerm and late-bolting phenotypes, but only 56 plants were used for RFLP analysis.

RFLP probes used in this study were divided into three classes: A) probes 7, 49, 117, and 181; probes 7, 49, and 117 are closely linked (1-3 cM) with the *M-m* locus, and probe 181 is located 28 cM from the *M-m* locus in linkage group 2 (Barzen et al., 1995); B) probes pKP748, pKP810, pKP876, and pKP948 which are also located in linkage group 2 (Pillen et al., 1992, 1993; Schumacher et al., 1997); and C) random RFLP probes pKP490, pKP498, pKP727, pKP830, pKP847, pKP878, pKP994, pKP1004, and pKP1151 from the other eight linkage groups (Pillen et al., 1992, 1993; Schumacher et al., 1997).

Genomic DNA was isolated from 5 g of fresh leaf tissue, according to CTAB-protocol (Doyle and Doyle, 1990), and adjusted to a concentration of about 0.5 μg/μl by comparing the intensity of fluorescence in a transilluminator to that of known quantities of ϕX-174 marker. The DNA was digested overnight with *Eco*RI, *Eco*RV, *Hind*III, *Xba*I, *Bgl*II, or *Bam*HI. After electrophoresis separation in 0.8% agarose gel and Southern blotting, the DNA was fixed on a Hybond nylon membrane. Non-radioactive labeling of RFLP probes, hybridization and signal detection were conducted as prescribed by the manufacturer (Alk-Phos Kit, Amersham, England). The mapping function of Haldane (1919) was used to transform recombination rates into map units.

RESULTS

The 103 F_2 plants were phenotypically assigned to one of four classes: 20 - monogerm and late-bolting; 5 - monogerm and normal bolting; 0 - multigerm and late-bolting; and 78 - multigerm and normal bolting. The monogerm trait segregated as expected, if controlled by a single gene, but the segregation pattern for late-bolting was slightly different from the theoretically expected result. These two morphological traits appeared to be linked with 4.85% cross-overs, indicating a distance of 5.0 cM in the linkage map.

Segregation ratios indicated independence between monogerm or late-bolting and the nine RFLP probes randomly located in the eight linkage groups (Fig.1). Hybridization with eight RFLP probes located on chromosome 2 revealed that the m^2 gene was located in linkage group 2, as reported previously for the *M-m* locus (Barzen et al., 1995; Schumacher et al., 1997). The distances between the m^2 and *lb* loci, and the RFLP probes are presented in Fig.2A. Other genetic maps with the same markers and monogerm gene are inserted in Fig.2, for comparison (Barzen et al., 1995; Pillen et al., 1992, 1993; Schumacher et al., 1997; see also Fig. 2B, 2C and 2D).

The monogerm gene m^2 identified in our experiments was positioned in the fragment between RFLP probes pKP748 and pKP876. The *m* and m^2 genes are 32.2 cM apart in maps A and D (Fig.2). Two probes closely linked with the *M-m* locus (7 and 117) were clustered and mapped at a distance of 24.3 cM from the m^2 locus. Two other probes from the same class (181 and 49) were more closely linked with the m^2 gene (19.8 cM and 15.0 cM, respectively). An RFLP clone from the second class (pKP748) was mapped on the same side of m^2 as these four probes and was the closest to the m^2 locus (14.5 cM). Three probes from the second class (pKP876, pKP948, and pKP810) were mapped on the opposite side of the monogerm gene m^2 at distances of 30.3 cM, 39.8 cM and 43.1 cM, respectively (Fig.2).

DISCUSSION

The monogerm trait from the Russian sugarbeet line S22 is controlled by a gene different from the monogerm gene identified in other sugarbeet germplasm. The *m* and m^2 monogerm genes were derived from separate origins and represent different genetic pools. This was demonstrated by the presence of bi-germ progenies in crosses between American and Russian monogerm lines (Shavrukov, 1997a; 1997b). RFLP linkage maps indicated a distance of 32.2 cM between the *m* and m^2 loci.

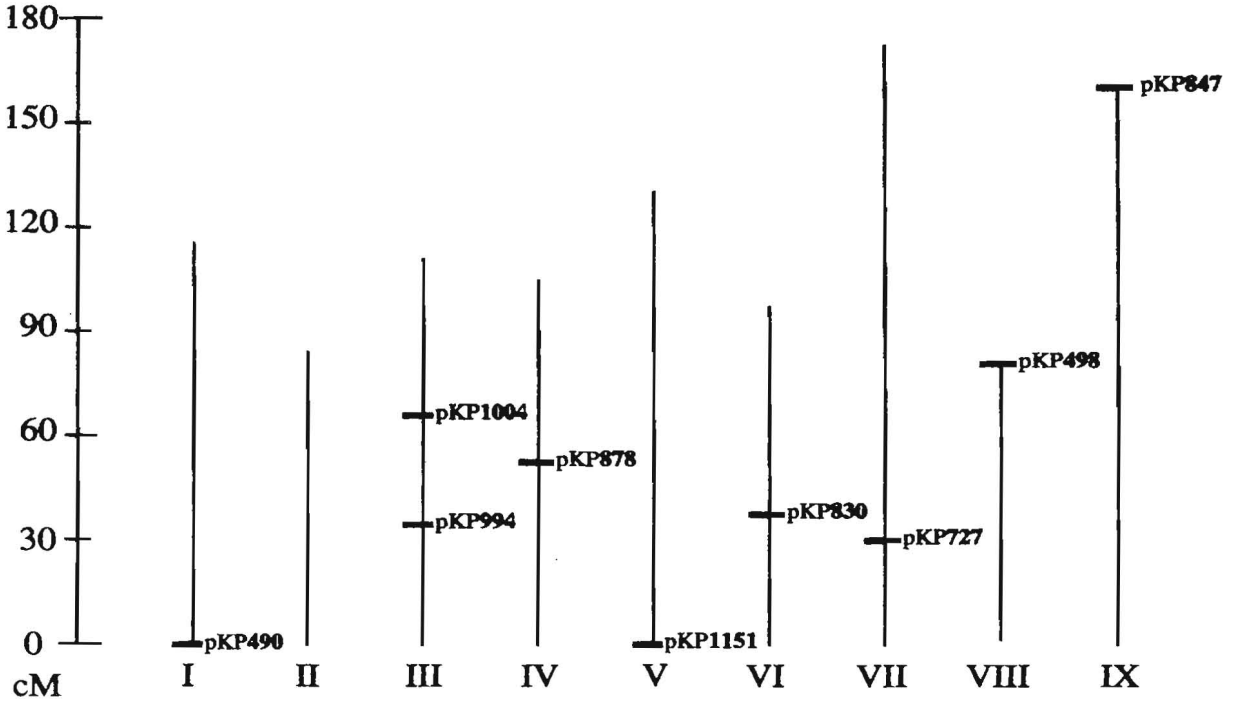

Fig. 1. Nine RFLP probes located in eight linkage groups (Pillen et al. 1992, 1993) with independent segregation of monogerm and late-bolting.

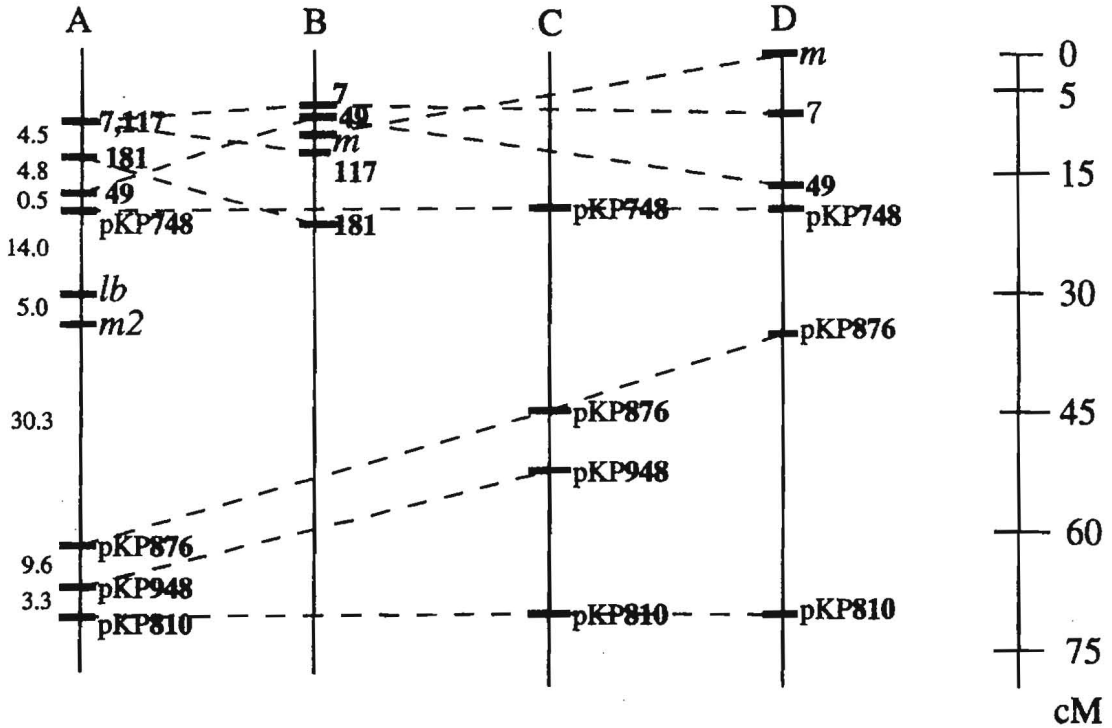

Fig. 2. RFLP map of m^2 and *lb* genes in linkage group 2(A) and its comparison to previous genetic maps: (B) Barzen et al., 1995; (C) Pillen et al., 1992, 1993; and D. Schumacher et al., 1997.

In this study, RFLP markers linked with the *m* locus in earlier studies had the same location as previously published. The locations of two probes (7 and 117) closely linked with the *M-m* locus coincided with the locations indicated by Barzen et al. (1995). On the other hand, probes 49 and 181 were located closer to the m^2 gene. Differences in RFLP loci mapping are probably due to differences in the genetic material used. For example, probe 49 was closely linked (2 cM) with the *m* gene from Koeln (Barzen et al., 1995) compared with a distance of 17.7 cM in lines from Kiel (Schumacher et al., 1997).

The linear order observed in our analysis of four RFLP loci located on chromosome 2 but not closely linked to the *M-m* locus was identical to that reported by Schumacher at al. (1997). The distance between RFLP markers pKP948 and pKP810 was shorter (3.3 cM) than the estimate of Pillen et al. (1992, 1993) (20 cM). The distance between probes pKP876 and pKP948 (9.6 cM) was identical to one map of Pillen et al. (1992) (9 cM) but different from another map of Pillen et al. (1993) (20 cM). The distance between probes pKP748 and pKP876 was the greatest discrepancy observed. In our experiments this distance was 44.8 cM, whereas the distance was estimated to be 36 cM, 28 cM, and 19.7 cM in three linkage maps presented in Fig. 2C and 2D (Pillen et al., 1992, 1993; Schumacher et al., 1997). Discrepancies in distance between RFLP probes are associated not only with the germplasm origin but with population size and conditions of individual experiments (Schumacher et al., 1997). A similar problem also was observed in linkage group 2 in *Beta vulgaris* ssp. *maritima* (Laporte et al., 1998). Taking into account the above mentioned differences in RFLP probe locations, the position of the second monogerm gene m^2 was quite different (32.2 cM) from the *m* gene described previously. This indicates that the monogerm gene m^2 is separate and district from the previously reported monogerm gene *m*.

The late-bolting gene *lb* was closely linked with the m^2 gene (5.0 cM). The distance between the *m* and *lb* genes in our experiment can be calculated either as 32.2 + 5.0 = 37.2 or 32.2 - 5.0 = 27.2 (cM). In the original publication on the association between *m* and *lb* loci, Savitsky (1952) estimated 25.5% cross-overs, this translates to linkage map distance of 28.5 cM. Therefore, 27.2 appears to be the more probable distance. There is no basis for comparing the *lb* gene from our experiment with the *lb* reported by Savitsky. Nevertheless, the similar linkage distance observed in our research (27.2 cM) and by Savitsky (28.5) suggests they are identical. Conflicting published information about the same linkage (11% of cross-overs) cannot be explained, but a linkage between m^2 and *S* (self-incompatibility) genes provides a possible explanation (Shavrukov, 1990). More

precise mapping of the m^2 gene as well as *lb*, *S*, and other genes that control morphological traits is the subject of further investigation. Molecular mapping will enable us to clarify some digenic inheritance patterns involving linked loci which otherwise might be difficult to understand.

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