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Short Communication

THE CHROMOSOMAL LOCATION OF RYE AFLP BANDS

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Abstract: 23 AFLP bands were assigned to different rye chromosomes by means of two different sets of wheat-rye addition lines. Only one AFLP band could be assigned to 4R, and no specific AFLPs were found on the 5R chromosome. Only one AFLP band was explicitly assigned to 4R, and no specific AFLPs were found on the 5R chromosome. At least seven co-migrating AFLPs showed the same chromosomal location in both sets of addition lines. A total of 22 AFLPs were assigned to chromosome 1R using wheat-rye substitution lines. Six of them have counterparts in one of the addition lines analyzed, but only four have the same chromosomal location. Six and four of the total AFLPs located using addition (23) and substitution (22) lines segregated in the mapping population DS2 x RXL10, but only six were simultaneously assigned to the same chromosome by both approaches. Although co-migrating AFLPs could be located on different rye chromosomes using addition and substitution lines, we believe that AFLPs can be useful as rye chromosome markers.

Key Words: AFLPs, Chromosomal Location, Rye, Wheat-Rye Addition and Substitution Lines

INTRODUCTION

Secale cereale L. is a source of many genes conferring resistance to pathogens [1] or salt tolerance [2]. For breeding purposes, it is important to evaluate diagnostic markers working on divergent material. To ensure that marker-assisted selection is directed towards one or more genes conferring the trait, the markers should be associated with the relevant chromosomes. The co-migrating band approach [3, 4] using addition, substitution, translocation or unrelated

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mapping populations could be an alternative method when relative mapping populations are not available.

Disomic wheat-rye addition and substitution lines are valuable tools used, e.g., for the assignment of molecular markers to the chromosomes of rye. Recently, disomic and ditelosomic rye addition lines of wheat, centric wheat-rye translocation lines of wheat, and single D(R) substitution lines of hexaploid triticale were used to identify the chromosomes responsible for the production of dietary fibre and arabinoxylans [5], to locate the genes controlling aluminium tolerance [6] and identify the rye sequences expressed as the result of aluminium treatment, and to assign 22 RAPDs linked to aluminium-tolerance genes to rye chromosome arms [7]. Recently, 46 RAPD markers were located on rye chromosomes using wheat-rye addition lines [8]. The wheats carrying the substitution 1R(1D) and the rye-wheat aneuploid lines carrying the long arm of chromosome 1R were used in AS-PCR experiments to amplify the Glu-R1 gene subunits of rye [9].

Despite the evident benefits, the application of wheat-rye addition or substitution lines is limited to a low level of variability that could also be detected by most other molecular systems. Moreover, some bands of the same electrophoretic mobility could be detected in more than one line [10], making their usage inexplicit. A possible solution could be the exploration of the SSR [11] or AFLP [12] techniques, known to amplify many reproducible polymorphisms.

Although some AFLPs to the pollen fertility restoration genes in *cms-P* have been preliminarily assigned to chromosomes of rye [13] using wheat-rye addition and substitution lines and AFLPs, there is currently no published data on the chromosomal location of rye AFLPs using these materials. The purpose of this study is to evaluate the practicality of the AFLP technique in obtaining AFLP markers explicitly assigned to rye chromosomes.

MATERIALS AND METHODS

Plant material

A complete set of seven disomic additions of King II and Blanco ryes to Chinese Spring wheat were provided by Dr. A. J. Lukaszewski, University of California, Riverside, CA, USA. These two sets also included wheat-rye amphiploids; in the CS/King II set, chromosome 2R was represented by ditelosomic 2RL. Dr. Lukaszewski also provided a set of two substitutions of the same rye chromosome 1R for chromosomes 1A and 1C of hexaploid wheat Pavon 76. These lines are respectively referred to as 1R(1A) and 1R(1C). The mapping population DS2 x RXL10, in the form of total genomic DNA isolated from 127 F₂ plants, was provided by Dr. K. M. Devos from the John Innes Centre, Norwich, UK.

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique followed the procedure modified for rye [14].

Assigning bands to the chromosomes of rye

We only considered bands identified in amphiploids, absent in the appropriate wheat component and present within the analyzed wheat-rye addition lines. In the wheat-rye substitution lines, a band could be assigned to 1R if it was missing in Pavon76 and present in both substitution lines.

Mapping co-migrating bands

The AFLP fragments assigned to rye chromosomes in wheat-rye addition and/or substitution lines were tested for segregation in the DS2 x RXL10 mapping population, and mapped at LOD3.0 using MAPMAKER3.0 software.

RESULTS

A total of 32 rye AFLPs with different sizes, found using the wheat-rye addition lines and 12 primer combinations, and 23 (CSB) and 22 (CSKII) AFLPs were present in the corresponding amphiploid and absent in CS. Of these, 22 (CSB) and 19 (CSKII) were polymorphic in the corresponding addition lines. 17 (CSB) and 7 (CSKII) rye AFLPs were located explicitly on rye chromosomes. These fragments were not evenly distributed among the rye chromosomes: 5, 3, 10, 1, 0, 3 and 2 AFLPs were located on the 1R, 2R, 3R, 4R, 5R, 6R, and 7R chromosomes, respectively. If we consider all the polymorphic bands, then the AFLPs are distributed in the following way: 13, 9, 16, 9, 6 and 5, respectively. The EAGG/MCAG primer pair was the most effective at generating specific locations (Fig. 1). Several AFLPs with the same size are amplified in different wheat rye addition lines. The EAGT/MCGT, EAGT/MCGC, EAGT/MCCC and EAGG/MCAG primer combinations generated at least two co-migrating AFLPs in CSB addition lines. Similarly, EAGG/MCG, EAGG/MCCG, EAGC/MCAA, EACA/MCTG, EAAT/MCTG, EAAT/MCGT and EAGT/MCTC simultaneously amplified several co-migrating bands in CSKII. Finally, EAGC/MCAA194 was present in the seven addition lines of the CSB set.

Only a few of the chromosome specific bands were amplified in the wheat-rye addition lines by the same primer combination, and even less were assigned to the same chromosome; i.e. EAGT/MCGT155 and EACT/MCCG145 fragments were amplified in both wheat-rye addition sets and EACT/MCCG145 was located to the same 3R chromosome in both cases. The EAGG/MCCG combination generated three bands, 272, 159 and 158 bases in length. They were identified in both sets of addition lines. Similarly, the EAGT/MCTC primers amplified a fragment (EAGT/MCTC175) that was common to the lines representing the 3R addition in CSB and CSKII. In total, only seven bands, shared between 3R, 4R and 5R and common for the corresponding wheat-rye addition lines, were identified.

A total of 35 polymorphic AFLPs were obtained using wheat-rye substitution lines with 11 primer combinations. However, only 22 bands could be assigned to chromosome 1R. Only seven AFLPs showed the same electrophoretic mobility as the fragments amplified in wheat-rye addition lines. In four cases

(EAGT/MCGT155, EAGG/MCAG283, EAGG/MCAG143 and EAAT/MCGT172), the co-migrating bands were identified in the line carrying the disomic addition of the first chromosome. The former three were assigned to 1R in CSB and the last to 1R in the CSKII lines. It should be noted that, at least in case of EAGT/MCGT155, there were differences in the assignment based on CSB and CSKII. Co-migrating bands were associated with the 1R and 3R chromosomes, respectively. A comparison of the remaining three bands was not possible since the appropriate signals were not amplified in the amphiploids; the remaining signals gave incomprehensive results.

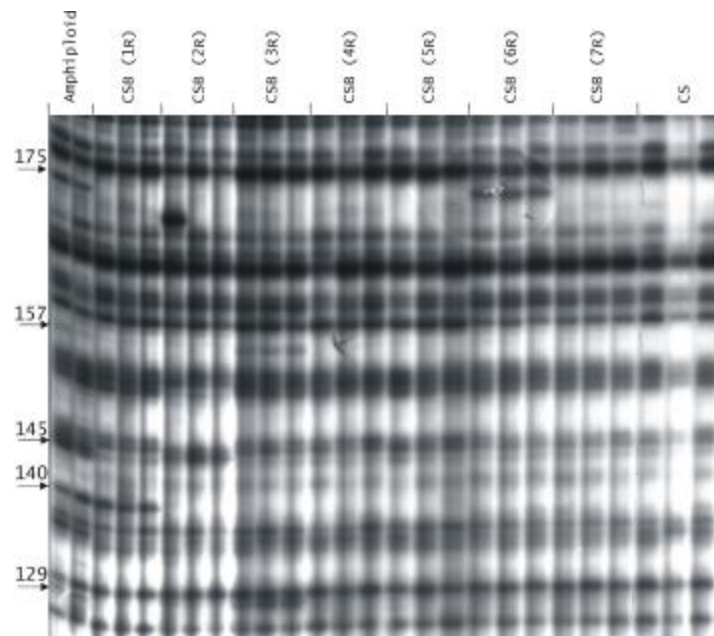


Fig. 1. AFLP patterns obtained with the EAGG/MCAG primer combination. Chinese Spring (CS); Blanco (B); amphiploid (CSB) and CSB disomic addition lines (1R to 7R). The AFLPs located on specific chromosomes are indicated by arrows with the size (base pair) of each band.

In total, the unique bands identified in the wheat-rye addition lines comprise 55.5% of the polymorphic bands; those that were amplified in two lines of the set, 27.8%; and the remainder were present in at least three lines. On average, a set of lines enables the generation of approximately 2% (calculated from the whole number of the identified AFLP fragments) of the chromosome specific bands. If all 65 polymorphic bands that could be assigned to different rye chromosomes are included, this figure rises up to 6%. The substitution lines generated 2% of the AFLP fragments that could be associated to the 1R.

The comparison of the DNA fragments assigned to rye chromosomes in CSB, CSKII and in substitution lines to the bands co-migrating in the DS2 x RXL10

population revealed that EAGG/MCAG140 was present in CSB on 1R, in substitution lines and mapped to 1RS in DS2 x RXL10. Similarly, EAAT/MCGT172 was associated with 1R in CSKII, substitution lines and mapped to 1RL in the mapping population. Markers EAGG/MCAG283 and EACA/MCTG198 were assigned to 1R and mapped to 1RS in the DS2 x RXL10 population. The results obtained with EAGG/MCCG159 (1R-4R-5R in CSB; 4R-5R in CSKII; and 1RS in DS2 x RXL10), EAGG/MCCG158 (1R-4R-5R in CSB; 4R-5R in CSKII; and 7RL in DS2 x RXL10), EAGG/MCAG175 (6R in CSB and 2RS in DS2 x RXL10), and EACA/MCTG112 (1R in substitution lines and 6RS in DS2 x RXL10) were inconsistent or not useful for mapping purposes.

DISCUSSION

Previously, it was demonstrated that the level of polymorphisms generated by the AFLP approach in unrelated inbred lines of rye may even reach 70% [15]. However, this figure was significantly lower in closely related inbred lines, and tended to 0% [16]. The level of polymorphic AFLPs changed from 5.7 to 33.3% in mapping populations of rye, depending on the primer combination [17]. Studies on wheat-rye addition lines carried out using the RAPD technique indicate that this level may reach 50% [8]. Our results show that the AFLP technique generates approximately 3% of the chromosome-specific bands.

The significant differences exhibited by the two techniques (AFLP and RAPD) with comparable materials and lower variability of the AFLP fragments could be attributed to the competition in the amplification of the short rye templates in the predominant wheat background. In our AFLP approach, the most suitable template of short sequences probably comes from the wheat background, and only rye-specific signals that are very abundant could be detected. This suggestion seems to be confirmed by our experiments with wheat-rye substitution lines where the wheat background is lower than in addition lines and 22 bands (in comparison to 5) were located on chromosome 1R. If so, many useful but less abundant polymorphisms could be lost when the wheat-rye addition lines are used.

Our current and previous studies [13] and those with the RAPD technique [8] demonstrate that the amplification of the DNA fragments on wheat-rye addition lines may result in the generation of co-migrating bands present simultaneously in several lines within a given set of the wheat-rye addition lines. In case of the AFLP and RAPD approaches, they comprise 44.7% and 15% of the polymorphic bands, respectively, and are mostly represented by bands amplified from two distinct chromosomes. Since many RAPD markers were successfully converted into SCARs and cloned sequences were usually homogenous or no data concerning band complexity was presented [18, 19], the lower number of the co-migrating bands generated with the RAPD technique could be attributed to the amplification of fragments that were relatively long (usually 600 to 1200bp) compared to AFLP-obtained fragments (100 to 300bp). The comparison of the

results presented here and those published by González [8] indicates that the AFLP bands may be more heterogeneous than the RAPDs. Numerous studies on cloning AFLPs seem to confirm their complexity [20] which is comparable with our results.

Our results demonstrate that the chromosome assignment of the bands identified in wheat-rye addition and substitution lines do not necessarily fit their mapped position. Moreover, many fragments mapped in the DS2 x RXL10 population and assigned to the rye chromosome in the addition and substitution lines differ in their location, indicating variation between plant materials. Nevertheless, following Mersem's [21] results, we tend to agree with the suggestion presented by González [8], that the unique bands co-migrating in different wheat-rye addition (substitution) lines, if converted into specific PCR, could be useful as markers for the chromosomes of rye. However, the number of useful AFLPs is relatively low compared to the number of RAPDs.

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