Assignment of 3 Genetic Linkage Groups to 3 Chromosomes of Narrow-Leafed Lupin

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Abstract

The legume genus, Lupinus, has many notable properties that make it interesting from a scientific perspective, including its basal position in the evolution of Papilionoid legumes. As the most economically important legume species, L. angustifolius L. (narrow-leafed lupin) has been subjected to much genetic analysis including linkage mapping and genomic library development. Cytogenetic analysis has been hindered by the large number of small morphologically uniform chromosomes (2n = 40). Here, we present a significant advance: the development of chromosome-specific cytogenetic markers and assignment of the first genetic linkage groups (LGs) to chromosomal maps of L. angustifolius using the bacterial artificial chromosome (BAC)—fluorescence in situ hybridization approach. Twelve clones produced single-locus signals that “landed” on 7 different chromosomes. Based on BAC-end sequences of those clones, genetic markers were generated. Eight clones localized on 3 chromosomes, allowed these chromosomes to be assigned to 3 LGs. An additional single-locus clone may be useful to combine an unassigned group (Cluster-2) with main LGs. This work provides a strong foundation for future identification of all chromosomes with specific markers and for complete integration of narrow-leafed lupin LGs. This resource will greatly facilitate the chromosome assignment and ordering of sequence contigs in sequencing the L. angustifolius genome.

Key words: BAC-FISH, BEST marker, chromosome marker, linkage map, Lupinus angustifolius

Lupinus species belong to the legume family (Fabaceae), one of the 3 largest Angiosperm families, and have features typical of the family, most notably the ability to fix nitrogen symbiotically with bacteria known as rhizobia (e.g., Rhizobium or Bradyrhizobium) (Howieson et al. 1998). Four Lupinus species are grain crops, with the most important being L. angustifolius L. (narrow-leafed lupin). Various Old and New World lupins have been the subject of cytogenetic analyses including chromosome counting and measuring (Naganowska and Ładoń 2000; Maciel and Schifino-Wittmann 2002; Conterato and Schifino-Wittmann 2006) and genome size estimation (Naganowska et al. 2003; Naganowska et al. 2006). Fluorescence in situ hybridization (FISH) has been applied to many plant genomes as a useful tool for chromosome analysis (Kulikova et al. 2001; Pedrosa et al. 2002, 2003; Pagel et al. 2004; Jiang and Gill 2006). Ribosomal DNA (rDNA) FISH probes have been used in a number of lupin species (Naganowska and Zielinska 2002; Hajdera et al. 2003; Naganowska and Zielinska 2004; Kong et al. 2009). Nuclear genome of L. angustifolius is partitioned into many chromosomes (2n = 40), which are small and morphologically uniform, making cytogenetic analysis and identification challenging (Kaczmarek et al. 2009). FISH analysis using rDNA probes has proved unsuccessful in identifying all L. angustifolius chromosomes.
One solution may be to conduct FISH using bacterial artificial chromosome (BAC) clones as probes (a procedure known as BAC-FISH) that enables physical localization of relatively large fragments of nuclear DNA directly on chromosomes. BAC probes can serve various purposes, such as obtaining chromosome-specific landmarks and assignment of genetic linkage groups (LGs) to corresponding chromosomes (Islam-Faridi et al. 2002). However, BAC clones of *L. angustifolius* have not so far been used for LG and chromosome assignment.

Over the last decade, intensive efforts have been made to construct lupin genetic maps. For *L. angustifolius*, maps were established based on microsatellite fragment length polymorphism (MFLP) (Boersma et al. 2005) and gene-based markers (Nelson et al. 2006). Recently, Nelson et al. (2010) constructed a consensus genetic map consisting of >1100 loci based on combined data from the previous 2 maps. This new reference map for *L. angustifolius* comprises 20 main LGs (narrow leafed lupins [NLLs]) and 3 small unlinked clusters (Clusters), which together with the BAC library of *L. angustifolius* genome constructed by Kasprzak et al. (2006) provides the resources required for detailed integrative genome analysis of narrow-leaved lupin using the BAC-FISH approach. In this study, we established a set of BAC-based cytogenetic landmarks that allowed unambiguous identification of 7 *L. angustifolius* chromosomes. Additionally, these BACs were used for generation of genetic markers to find LGs corresponding to particular chromosome pairs.

**Material and Methods**

Seeds of *L. angustifolius* cv. Sonet were obtained from the Polish *Lupinus* Gene Bank in the Breeding Station Wiatrowo (Poznan Plant Breeders Ltd, Poland). Seeds of mapping population consisting of 89 F8 recombinant inbred lines (RILs) of a cross between a breeding line 83A:476 and cv. Sonet were obtained from the Gene Bank in the Breeding Station Wiatrowo (Poznan Plant Breeders Ltd, Poland). Seeds of *L. angustifolius* cv. Sonet were obtained from the Breeding Station Wiatrowo (Poznan Plant Breeders Ltd, Poland).

BAC clones were originated from the library of *L. angustifolius* cv. Sonet nuclear genome (Kasprzak et al. 2006). Clones used for LG/chromosome assignment analysis are listed in Supplementary Table 1.

**Selection of BAC Clones**

Hybridization probes for BAC library screening were prepared based on DNA sequences originated from genetic markers closely linked to disease resistance genes: AntjM1 and AntiM2 markers linked to anthracnose resistance gene, *Laur-1* (Yang et al. 2004; You et al. 2005), Ph258M2, a marker linked to *Phomopsis* stem blight resistance gene, *Phr-1* (Yang et al. 2002), and RustM1, a marker linked to lupin rust resistance trait (Sweetingham et al. 2005). As the markers were developed by MFLP technique (Yang et al. 2001), they contained microsatellite motifs: (TTG)$_6$ in AntjM1, (AAC)$_6$ in Ph258M2, and (GA)$_7$ in RustM1. Additionally, the AntiM2 marker encoded the nucleotide binding site sequence GLPLAL. Subsequently, the specific primer pairs were designed to test incorporation of these repetitive motifs into probe sequences: AntiM1F: TGG-TTTGTGCTAAGCTATTTG, AntiM1R: CAACACATAT-GTTAGAACATCTAAG, AntiM2F: CAAAATTTTCCTGGAACAAAA, AntiM2R: AAAACTCTATTTACTATGTC- AA, Ph258M2F: GTCATTCTCTGTGATCGAAC, Ph258M2R: GTAGTGACTGAAGAACTTACAC, RustM1F: TAAACATTCTACCTTCTT, and RustM1R: AACACATAGTGCTCAAAAA.

In addition, one hybridization probe was constructed on the basis of the symbiotic receptor kinase gene sequence fragment (*symRK*), which is one of the key genes of the common signaling cascade involved in plant–microbial symbiotic associations, such as arbuscular mycorrhiza and nodulation (Endre et al. 2002; Stracke et al. 2002). The primer pair used was as follows: SRK-F1: CTCGAACCT- GAAGGTTTTTGGAGA and SRK-R5: CTTAACC-TAACCTTGGTCAAGGC.

All probes were amplified by polymerase chain reaction (PCR) using *L. angustifolius* cv. Sonet genomic DNA as a template. The PCR products were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and radiolabeled by random priming HexaLabel DNA Labeling Kit (Fermentas, Ontario, Canada) with the presence of 50 μCi [α-32P]-deoxyctydine 5′-triphosphate.

**BAC Library Screening**

High-density DNA macroarrays on Hybond-N$^+$ 22.2 × 22.2 cm nylon filters (Kasprzak et al. 2006) were hybridized with probes for 16 h at 65 °C in a hybridization solution composed of 5× standard saline citrate (SSC) (0.75 M NaCl, 0.075 M sodium citrate), 5× Denhardt’s solution (0.1% w/v Ficoll-400, 0.1% w/v polyvinylpyrrolidone, 0.1% w/v bovine serum albumin [BSA]), and 0.5% w/v sodium dodecyl sulfate (SDS). Three high stringency washes in 0.1× SSC and 0.1% SDS at 65 °C for 30 min were conducted. For RustM1 and Ph258M2 probes, the temperature of washes was reduced to 60 °C. After hybridization, macroarrays were exposed for 24–48 h to BAS-MS 2340 imaging plates (Fujifilm) and scanned using a FLA-5100 phosphoimager (Fujifilm). Verification of clones that gave positive hybridization signals was performed by PCR using the same sets of primers that were applied for probe amplification.

**BAC DNA Isolation**

BAC DNA was isolated from single *Escherichia coli* colonies by a miniprep method using QIAPrep Spin Miniprep Kit (Qiagen) according to the protocol of Farrar and Donnison (2007) and digested using restriction enzyme *Not*I (Roche Diagnostics, Basel, Switzerland). The quality and size of inserts were estimated by pulsed field gel electrophoresis (PFGE). To estimate insert size, Lambda ladder PFG marker (New England Biolabs, Ipswich, MA) and O’GeneRuler 1 kb Plus DNA Ladder, ready-to-use (Fermentas) were used as fragment size markers.
Mitotic Chromosome Preparation

Chromosome preparations were made from the root meristem tissue according to the protocol for mitotic chromosome squashes provided by Jenkins and Hasterok (2007) with minor modifications resulting from specificity of the narrow-leaved lupin material. To synchronize and accelerate germination, the seeds were air-dried in tap water at 25 °C overnight prior to transfer on moistened filter paper in petri dishes at 25 °C. Seedlings with 1.5–2.0 cm root length were treated in chilled tap water (2–3 °C) for 24 h to accumulate cells at metaphase. Excised roots were drained and immediately fixed in freshly made 3:1 ethanol/glacial acetic acid mixture and stored at −20 °C until use. Roots were digested in enzyme solution comprising 40% (v/v) pectinase (Sigma, St. Louis, MO), 3% (w/v) cellulase (Sigma), and 1.5% (w/v) cellulase “Onozuka R-10” (Serva, Heidelberg, Germany) for 3–4 h at 37 °C. Chromosome preparations were made from dissected meristematic tissue on alcohol-cleaned slides in one drop of 60% acetic acid and frozen. Preparations were postfixed in 3:1 ethanol/glacial acetic acid, dehydrated in 99.8% ethanol for 20 min, and then air-dried. The slides were quality-controlled under a phase-contrast microscope (BX41, Olympus) and used for FISH.

Fluorescence In Situ Hybridization

FISH was carried out according to the protocol of Jenkins and Hasterok (2007) with minor modifications. BAC DNA was labeled with digoxigenin-11-dUTP and/or tetramethylrhodamine-5-dUTP (Roche Diagnostics) by nick translation reaction as described by Jenkins and Hasterok (2007). In brief, the slides were pretreated with RNase (100 µg/ml) in 2× SSC in a humid chamber at 37 °C for 1 h, washed 3 times in 2× SSC at room temperature (RT), and treated with pepsin (50 mg/ml) at 37 °C for 12 min. Slides were dehydrated in ethanol series (50, 70, 100%) and dried at RT. Hybridization mixture (50% deionized formamide in 0.1× SSC, 0.5% SDS, sonicated salmon sperm DNA in 50–100× excess of the probe, 75–200 ng probe per slide) was denatured at 90 °C for 9 min, then applied to the chromosome preparation, and denatured together at 78 °C for 10 min using thermal cycler for in situ hybridization (Twin Tower, PTC-200, MJ Research). Hybridization was carried out in a humid chamber at 37 °C for 20–24 h. Posthybridization washes were conducted in 15% deionized formamide in 0.1× SSC at 42 °C, which is the equivalent of 82% stringency (Schwarzacher and Heslop-Harrison 2000). Immunodetection of digoxigenated DNA probes was carried out with fluorescein isothiocyanate-conjugated antidigoxigenin primary antibodies (Roche Diagnostics). Chromosomes were counterstained with 2 µg/ml 4′,6-diamidino-2-phenylindole (Sigma) in Vectashield antifade mounting medium (Vector Laboratories, Burlingame, CA). Slides were examined with an OLYMPUS BX 60 microscope using Cell_F software, the images were captured using a CCD monochromatic camera, tinted in Wasabi (Hamamatsu Photonics), and superimposed using Micrografx (Corel) Picture Publisher 8 software.

Genomic DNA Isolation

Genomic DNA of individual plants of mapping population and parental lines was extracted from the young leaf tissue (3–4 leaves) using a DNeasy Plant Mini Kit (Qiagen) and the protocol provided by the manufacturer.

BEST Markers

BAC-ends were sequenced in both 3′ and 5′ directions. These sequences approximately 500 bp long have been submitted to GenBank and are available under accession numbers GS887821–GS887832. Primer pairs for BEST marker generation were designed basing on end sequences using free website software http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlasterHomeAd.

The PCR profiles were optimized for parental lines of mapping population 83A:476 and P27255 using temperature gradient or touchdown PCR methods to detect polymorphism (length or present/absent type). In the case of monomorphic product, the genomic DNA of both parental lines was purified by the QAquick PCR Purification Kit (Qiagen), PCR performed, and the amplicons directly sequenced (Faculty of Biology, Adam Mickiewicz University, Poznan, Poland), using the forward primer as the sequencing primer. Single nucleotide polymorphism (SNP) was detected using Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, MI). Digestion with specific restriction enzyme (Fermentas) was used to generate cleaved amplified polymorphic sequence (CAPS) markers, in order to distinguish parental alleles. Restriction enzymes were chosen using Sequencher 4.7 and NEBcutter program (Vincze et al. 2003).

Segregation of polymorphic markers among 89 F8 RILs of the mapping population was tested using electrophoretic separation of DNA fragments in 2% agarose gel with O′Range Ruler 100 kbp DNA Ladder as a size marker (Fermentas) and visualized by ethidium bromide staining. Sequences of BEST markers are accessible under accession numbers GF102179–GF102183 in GenBank.

Linkage Mapping

The new BEST markers were localized in the LGs of recently constructed genetic map of L. angustifolius genome (Nelson et al. 2010). Segregation data of new markers were introduced to the framework marker data set of the existing map, and LGs were reconstructed using Map Manager QTx20 software (Manly et al. 2001). The LGs were drawn in MapChart software (Voorrips 2002).

Results

Identification of Individual Chromosomes

The L. angustifolius BAC library was subjected to several independent hybridization procedures with probes designed
on the basis of the *SymRK* gene sequence and sequences of genetic markers linked to disease resistance genes for anthracnose (AntjM1 and AntjM2), lupin rust (RustM1), and Phomopsis stem blight (Ph258M2). Positive hybridization signals were verified by PCR, using primers specific for each probe and insert DNA from the selected BAC clones as a template. For each probe, analyzed PCR products with appropriate length and sequence complementary to the probe sequence were amplified. From among selected clones, 18 BACs identified using the markers linked to disease resistance genes and 4 BACs identified using the *SymRK* probe were chosen for FISH mapping. In addition, 21 BACs were randomly chosen from the library (Supplementary Table 1). All 43 BACs were separated by PFGE to estimate the size of inserts and the number of *Not*I restriction sites (examples of clones shown on Figure 1). Insert size ranged from 20 to 160 kbp with average size 77 kbp. Most clones possess no restriction sites for *Not*I within the insert with the exceptions of the 3B18 and 3N20 clones, each contain one site (Supplementary Table 1).

The BAC-FISH experiments for all tested clones were performed at the same stringency conditions, without using fast-denaturing DNA fractions such as C$_0$ DNA (often applied to suppress weak or nonspecific hybridization signals). BAC probes that produced one strong signal without background were selected for FISH and used at least thrice in duplicate experiments to ensure that clones were localized specifically in a given chromosome. From a total number of 43 BACs, 12 clones (i.e., 7 carrying molecular markers and 5 randomly chosen) hybridized to single locus per genome, that is, giving unique FISH pattern on mitotic chromosomes and were defined as cytogenetic markers. The single locus BACs were colocalized by multiple dual or triple-color FISH experiments in various clone combinations (Table 1). All the chromosome-specific clones map to terminal or subterminal regions of chromosomes (Figure 2a–d and Figure 3a–c,e–f). Four clones (84D22, 111B08, 142C04, and 142D13) landed on the same chromosome arm (Figure 3b,c). The clones 111G03 and 136C16 hybridized to one arm of another chromosome, whereas clone 3B18 hybridized to the opposite arm of the same chromosome (Figure 3e). The 5 remaining cytogenetic markers were mapped as is shown in Table 1. It is worth noting that in 2 chromosomes more than one chromosome-specific BAC clones were mapped. Thirty-one clones, representing 72% of the total, hybridized to multiple sites across most or all chromosomes (examples shown on Figure 2e,f). These clones were excluded from analysis of assignment of LGs to particular chromosomes.

### Genetic Markers and Assignment of LGs to Particular Chromosomes

The 12 BAC-based cygenetic markers were assessed as genetic markers. Dominant polymorphisms (i.e., amplified product present or absent) were identified for 3 BEST markers (44J16_3, 142D13_3, and 1M23_5). Of the remaining 9 BEST markers, PCR amplified fragments derived from parental DNAs were found to contain SNPs in 2 cases (111G03_3 and 3B18_3). Based on these SNPs, CAPS markers were developed. No more sequence polymorphisms were found in the remaining 7 parental products, even though BAC-end sequences were extended using the same forward primer as for amplification of PCR product in the parental lines (Table 2). The 5 BEST markers developed were localized to 3 main LGs (NLL-06, NLL-08, and NLL-17) and one cluster (Cluster-2) of the reference *L. angustifolius* genetic map of Nelson et al. (2010) (Table 2, Figure 3d1–4).

### Discussion

FISH with clones containing large inserts of nuclear DNA, such as BACs, has proved to be an effective method for chromosome identification in many plants, including genomes with small morphologically indistinguishable chromosomes, for example, *Solanum tuberosum* (Dong et al. 2000) and *Brassica oleracea* (Howell et al. 2002) or with high chromosome number, for example, *Gossypium hirsutum* (Wang et al. 2007). Moreover, BAC clones can be successfully used as markers to integrate chromosomal and genetic maps (Jiang and Gill 2006).

Among the 43 clones screened, we obtained 12 BACs that were found to be useful as cygenetic markers. The largest number of BACs mapped to the same chromosome arm was 4 (84D22, 111B08, 142C04, and 142D13).

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**Figure 1.** PFGE of insert DNA from selected clones from *Lupinus angustifolius* BAC library. Clones were digested using the restriction enzyme, *Not*I. M1—Lambda ladder PFG marker and M2—O’GeneRuler 1 kb Plus DNA Ladder fragment size markers.
Moreover, these clones were used in physical mapping for contig assembly (Książkiewicz et al. 2008). Cytogenetic mapping of the 4 clones mentioned above supported the preliminary results of physical mapping. Mapping of BAC clones containing the same sets of MFLP-derived markers for disease resistance genes on different chromosomes can be explained that these sequences in L. angustifolius genome are present in relatively high copy number due to repetitive elements and only a part of them can be linked to analyzed traits (Książkiewicz et al. 2008). However, the analysis of structure and organization of disease resistance regions in L. angustifolius genome was not investigated in our study. Three other BACs landed on another chromosome. Two of them (111G03 and 136C16) hybridized to the same arm; the third clone (3B18) was localized in the opposite arm. This chromosome represents one of a few examples in L. angustifolius where 2 single locus BAC clones enabled unambiguous discrimination of 2 chromosomal termini. However, such precise cytogenetic mapping is not uncommon in some other plant species, for example, for all chromosomes in Cucumis sativus (Ren et al. 2009), 4 of 10 Sorghum bicolor chromosomes (Kim et al. 2005) although only 2 of 26 in the allopolyploid G. hirsutum (Wang et al. 2007). The chromosome colocalization of the 5 remaining single locus BAC clones was not tested for all combinations. Further simultaneous FISH mapping of various BAC combinations should establish more precisely their chromosomal localization. However, it has to be accepted that due to the relatively high chromosome number and lack of clear morphometrical markers, establishing a BAC-based L. angustifolius cytogenetic map will not be as straightforward as it was in species with considerably lower chromosome number and more asymmetric karyotype, such as for example Brachypodium distachyon (Hasterok et al. 2006).

A striking feature of all single locus BACs mapped in this study is their localization to distal chromosomal regions. A similar pattern of single-locus BAC localization was observed in Medicago truncatula (Kulikova et al. 2001) and Lotus japonicus (Pedrosa et al. 2002). It may be attributed to tendency of subterminal and terminal regions of chromosomes in many species to be the most gene dense, with lowest concentrations of repetitive DNA sequences (Schmidt and Heslop-Harrison 1998). The findings of Ito et al. (2000), who revealed that cDNA-based hybridization probes preferentially mapped in the distal regions of L. japonicus chromosomes, seem to support this hypothesis. In the present study, 2 BAC clones, that is, 111G03 and 136C16, known to carry SymRK gene sequence, were also found to hybridize with a distal, predominantly euchromatic, chromosome region. The single-locus BACs contrast with those which produce multiple hybridization signals along all chromosomes in the complement of L. angustifolius (Figure 2e,f). Although these clones cannot be utilized as chromosome-specific markers, they contribute to general characterization of the L. angustifolius genome. Such dispersed signals of

<table>
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<th>Table 1</th>
<th>Pairs of BACs used for FISH to obtain chromosome-specific markers in Lupinus angustifolius</th>
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<tbody>
<tr>
<td>111G03</td>
<td>136C16</td>
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<td>y</td>
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<td>n</td>
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<td>n</td>
<td>y</td>
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</table>

y, pairs of BAC clones hybridized to the same chromosome pair; n, pairs of BAC clones hybridized to different chromosome pairs; n/a, not analyzed.

Figure 2. Localization of selected BAC clones on Lupinus angustifolius chromosomes using FISH; (a) 111B08 (green), (b) 84D22 (red), (c) 142D13 (green) and 2B3 (red), (d) 6E05 (green), (e) 119M19 (green), and (f) 120E23 (red), both carrying repetitive DNA sequences widespread in the genome. Chromosomes counterstained with 4’,6-diamidino-2-phenylindole (blue). Bars: 5 μm.
hybridization are most likely produced by repetitive DNA sequences, widely distributed in the genome. They were also commonly observed in other legume plants, such as *Phaseolus vulgaris* (Pedrosa-Harand et al. 2009) and *Glycine max* (Lin et al. 2005). Use of specific fast-denaturing DNA fractions, such as C<sub>0</sub><sup>t</sup> DNA, to suppress hybridization of the probe components containing repetitive DNA was found effective in some species, for example, *B. oleracea* (Howell et al. 2002) and *G. hirsutum* (Wang et al. 2007). This procedure increased the number of BAC clones producing effectively single-locus signals and thus facilitated the process of cytogenetic mapping. On the other hand, the blocking efficiency of C<sub>0</sub><sup>t</sup> DNA fractions depends on the amounts of repetitive DNA within certain BACs (Dong et al. 2000) and therefore can be insufficient in some experiments (Kim et al. 2002). However, it may be worthwhile to consider that about 25% of BACs used in this study produced single-locus signals without any sophisticated suppression of potential cross-hybridization.

Linkage maps display a linear order of markers and the frequency of recombination between them. However, because of unequal distribution of crossover frequency along chromosome arms, genetic distance does not simply reflect physical distance (Harper and Cande 2000). BAC clones containing genetic markers were used for integration or assigning LGs and chromosomal maps for many important crop species, for example, *S. tuberosum* (Tang et al. 2009) and *Zea mays* (Danilova and Birchler 2008). The relationship between the genetic and chromosome map of *L. angustifolius* has not been analyzed so far. Recently, Nelson et al. (2010) published a reference map of *L. angustifolius*, which provides the foundation to assign LGs to their chromosomes. In this study, we present the first use of BEST markers to link genetic and cytogenetic maps of narrow-leaved lupin. We assigned 3 LGs (NLL-06, NLL-08, and NLL-17) and one small cluster (Cluster-2) to 4 chromosomes. This approach was focused on generating BAC-based chromosome-specific physical markers and

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**Figure 3.** *Lupinus angustifolius* mitotic chromosomes with BAC clones determined by FISH as chromosome-specific markers: (a) clone 44J16 (green), clone 142C04 (red); (b) 84D22 (green), 142C04 (red), and 142D13 (yellow); (c) 84D22 (green) and 111B08 (red). d1–d4: reciprocal assignment of 3 main LGs (NLL-06, NLL-08, and NLL-17) plus one small cluster (Cluster-2) to chromosomes of *Lupinus angustifolius* (LANG 06, LANG 08, LANG 17, LANG—unassigned chromosome, respectively) using BAC clones as FISH probes and templates for BEST markers. (e) 111G03 (green), 136C16 (red), and 3B18 (green, mapped in opposite arm of the chromosome), (f) 1M23 (green), 3B18 (red), and 8C03 (yellow, arrows). Chromosomes counterstained with 4’,6-diamidino-2-phenylindole (blue). Bars: 5 μm.
BEST-based genetic markers to relate the chromosomes with respective LGs. To date, chromosome nomenclature was not associated with any genetic maps of *L. angustifolius*.

We propose to number individual narrow-leafed lupin chromosomes (defined as LANG) according to numbers of their corresponding LGs. We use the most comprehensive *L. angustifolius* genetic map (Nelson et al. 2010) that not only combines the data from 2 previous maps (Boersma et al. 2005; Nelson et al. 2006) but is also enriched by 200 new markers. In the genetic part of the current research, 5 BEST markers were developed. Mapping dominant marker 44J16_3 in LANG 08 shows homology to *Lo. japonicus* chromosome Lj2 and Lj3, respectively.

In this study, a set of chromosome-specific BACs was developed to assign 3 main LGs to particular chromosomes of *L. angustifolius* genome. This can be considered as a starting point to assign the remaining LGs to the relevant chromosomes and eventually to construct a complex, integrated map of *L. angustifolius* genome, which can be useful for the lupin community in a variety of applications. It has to be stressed, however, that for species with high chromosome numbers, such an approach is laborious and time consuming, as in the case of *G. hirsutum* (Wang et al. 2007). Clones from the BAC library of *L. angustifolius* nuclear genome used in this study can be potentially an effective tool for comparative analysis of other *Lupinus* species and can be helpful in a genome sequencing project, particularly in the characterization of clones containing genes controlling agricultural traits. The importance of narrow-leafed lupin as a crop plant and the relative wealth of data concerning its genome lead to a potential role as a useful model species within the genus *Lupinus*. Preliminary experiments using heterologous landing of *L. angustifolius* BACs on chromosomes of related *Lupinus* species support this possibility (Lesniewska, unpublished data).

### Table 2  BAC clones used for developing BEST markers and characteristics of the markers used to assign LGs to chromosomes of *Lupinus angustifolius* (LANG)

<table>
<thead>
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<th>BAC clone</th>
<th>BEST marker</th>
<th>Marker type</th>
<th>Marker length (bp)</th>
<th>Extended sequence</th>
<th>LG</th>
<th>LANG</th>
<th>GenBank accession</th>
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<tbody>
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<td>111G03</td>
<td>111G03_5</td>
<td>Codominant</td>
<td>P1: 256 + 197</td>
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<tr>
<td>P2: 453</td>
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<td>17</td>
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<td>136C16</td>
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<tr>
<td>3B18</td>
<td>3B18_3</td>
<td>Codominant</td>
<td>P1: 231 + 33</td>
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<tr>
<td>P2: 264</td>
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P1: parental line 83A/476; P2: parental line P27255.

* BEST designation according to Nelson et al. (2010).
Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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References


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