

CYTOGENETIC MAPPING OF THE *LUPINUS ANGUSTIFOLIUS* GENOME

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ABSTRACT

The narrow-leaved lupin (*Lupinus angustifolius* L.), a species with valuable agricultural traits, has been lately a subject of cytogenetic studies. The present work is a continuation of genome mapping of the narrow-leaved lupin by molecular cytogenetics. The first bacterial artificial chromosome (BAC) library of the *L. angustifolius* genome, as well as information from its genetic maps have contributed to these studies. Two cytogenetic techniques were used by the authors: 1) fluorescence *in situ* hybridisation with BAC clones (BAC-FISH); and 2) primed *in situ* labelling (PRINS) with genetically mapped sequences. Several approaches have been applied, combining cytogenetic data with genetic mapping information. Preliminary results are presented, aimed at the assignment of selected linkage groups to individual *L. angustifolius* chromosomes.

KEY WORDS

Lupinus angustifolius, molecular cytogenetics, chromosomes, BAC-FISH, PRINS

INTRODUCTION

Studies of the *Lupinus* genome organisation contribute to the basic knowledge on lupins and may facilitate their exploitation as valuable crops. Lately, within the program of structural genomics, our research group has conducted cytogenetic analyses of the lupin genome. First flow cytometric measurements of genome size, made on a large group of lupins from the Old and New World, were published (Naganowska *et al.* 2003a, 2006), together with the interspecific relationship analysis within geographical groups and some evolutionary conclusions. Other studies concerned the optimisation of fluorescence *in situ* hybridisation (FISH) for physical mapping of the *Lupinus* genome (Naganowska *et al.* 2003b) and the localisation of rRNA genes in chromosomes of all the Old World species (Naganowska and Zielińska, 2002). Further studies were focused on the narrow-leaved lupin (*Lupinus angustifolius* L.) as a species of agricultural value, with a small genome and a relatively low chromosome number within the genus. Several repetitive sequences were verified as FISH markers (Naganowska and Kaczmarek, 2005). The first genomic bacterial artificial chromosome (BAC) library of *L. angustifolius* was

constructed by our group (Kasprzak *et al.* 2006), which has created a new dimension in structural genomics of that species. BAC clones from the library have been exploited as molecular probes for FISH (BAC-FISH) (Kaczmarek, 2007). Alternatively, for mapping of short DNA fragments (e.g. sequences from genetic maps) in the narrow-leaved lupin chromosomes, two techniques were optimised: PRINS (primed *in situ* DNA labelling) and C-PRINS (cycling PRINS) (Kaczmarek *et al.* 2007). Additionally, the use of a computer program for chromosome measurement allowed us to construct the first ideogram of *L. angustifolius* chromosomes, including a range of cytogenetic markers (Kaczmarek, 2007). As genetic maps for *L. angustifolius* have been developed recently (Boersma *et al.* 2005; Nelson *et al.* 2006), the current research of our group is aimed at the integration of lupin genetic and cytogenetic maps by combining linkage information with cytogenetic analysis. In this paper we present preliminary results concerning the assignment of *L. angustifolius* linkage groups to appropriate chromosomes using molecular cytogenetics techniques.

MATERIALS AND METHODS

The plant material consisted of seeds of narrow-leaved lupin (*L. angustifolius* L., cv. Sonet, 2n = 40) obtained from the Polish *Lupinus* Gene Bank at the Breeding Station Wiatrowo of the Poznań Plant Breeders Enterprise. The procedure of permanent squash preparations was based on protocols developed by Schwarzacher and Heslop-Harrison (2000), with some modifications for lupin chromosomes.

Three main approaches have been applied in order to assign the linkage groups to individual chromosomes: 1) localisation of BAC clones from the genomic BAC library in metaphase chromosomes by BAC-FISH; 2) sequencing of BAC clone ends to generate genetic markers, which may also be localised in chromosomes by PRINS; 3) the localisation of genetic markers in metaphase chromosomes by PRINS.

BAC clones were selected from the nuclear genome BAC library of *L. angustifolius* L. cv. Sonet on the basis of hybridisation with probes for molecular markers (Kasprzak, 2008). A set of clones contained markers for genes of resistance to pathogenic fungi, others – markers for nodulation genes (Table 1).

Table 1. Examples of sequences used for cytogenetic mapping.

| BAC-FISH | | PRINS | |
|----------------|------------------------------------|----------------|--------------------|
| BAC clone | Characteristics (marker sequences) | genetic marker | linkage group (LG) |
| 142D13, 109D22 | 1 Anthracnose + phomopsis + rust | psat_EST_00175 | LG2 |
| 112F11 | 2 Anthracnose + phomopsis + rust | mtmt_GEN_00092 | LG6 |
| 11G3, 136C16 | nodulation | | |

The isolated BAC DNA was labelled with digoxigenin-11-dUTP, biotin-11-dUTP or tetramethylrhodamine-5-dUTP (for direct FISH), using the standard nick translation protocol (Nick Translation System, Roche), and used as molecular probes for BAC-FISH reactions. The FISH procedure was based on the protocols of Schwarzacher and Heslop-Harrison (2000), with some modifications. The hybridisation mixture was denatured at 95°C for 8 min; chromosome and probe DNAs were denatured together at 75°C for 8 min. Hybridisation was carried out overnight at 37°C. Probes labelled with digoxigenin-11-dUTP or biotin-11-dUTP were detected with anti-digoxigenin-FITC (Roche) or streptavidin-Cy3 (Sigma-Aldrich), respectively. Chromosomes were counterstained with DAPI. The preparations were mounted in a Vectashield antifade solution (Vector Laboratories) and examined with an OLYMPUS BX 60 Research System Microscope. The images were acquired with a black and white CCD camera, interfaced to a PC running the analysis 3.0 software (Soft Imaging System).

Preliminary analyses aimed at mapping marker sequences by PRINS have been performed (Table 1). Two markers from among polymorphic STS markers, obtained within the framework of the 6 FP EU GRAIN LEGUMES, were added to the genetic map constructed earlier (Boersma *et al.* 2005) and were simultaneously used for PRINS in the mitotic chromosomes of *L. angustifolius*. The genomic DNA was isolated from young leaf tissue using a DNeasy Plant Mini Kit (Qiagen). Details of the PRINS procedure were based on the protocol of Kaczmarek *et al.* (2007), with some modifications. The preparations were examined and images were acquired similarly as it was described for BAC-FISH.

RESULTS AND DISCUSSION

We localised a set of BAC clones from the nuclear genome BAC library of *L. angustifolius*, by BAC-FISH in *L. angustifolius* chromosomes (Fig. 1). The results differed depending on the clone. Some BACs gave unambiguous clear signals on one chromosome pair (Fig. 1a, b, c); these signals showed the genome regions where the markers from the BAC clones were located. Furthermore, we may conclude that a specific chromosome contains not only the marker of interest, but also other markers from the same linkage group. A group of BAC clones showed hybridisation on several

pairs (Fig. 1b) or over all chromosomes (Fig. 1d). These results do not contribute to the assignment of linkage groups to chromosomes.

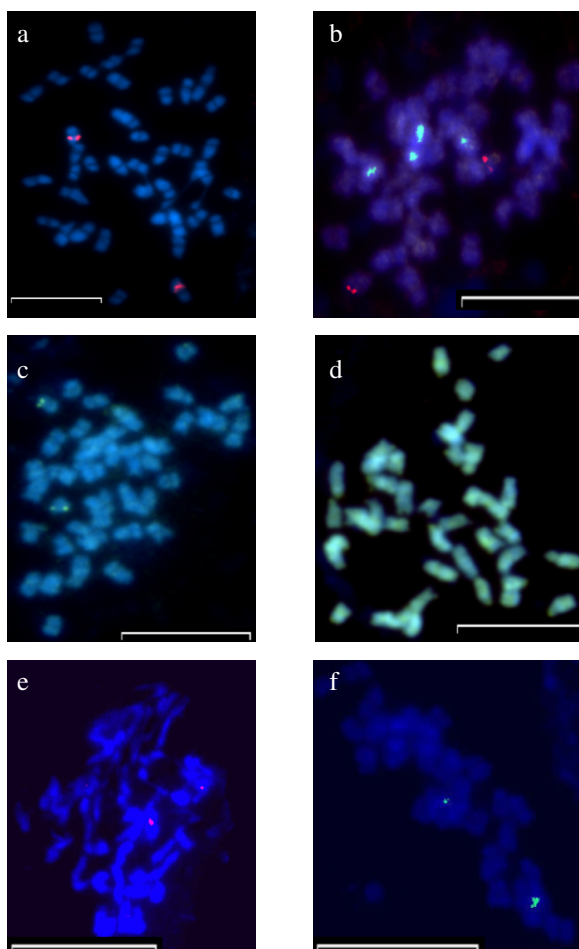


Fig. 1. Mitotic chromosomes of *Lupinus angustifolius* with fluorescence signals: a) BAC clone 142D13 (BAC-FISH); b) BAC clone 112F11 (green) and 136C16 (red) (BAC-FISH); c) BAC clone 111G3 (BAC-FISH); d) BAC clone 109D22 (BAC-FISH); e) genetic marker mtmt_GEN_00092 (PRINS); f) genetic marker psat_EST_00175 (PRINS). Scale bar: 10 µm.

In order to generate new genetic markers, sequencing of some BAC clone ends was performed. Until now, 10 markers based on these sequences were analysed; however, all of them were monomorphic.

Two genetic markers of the STS type were localised in chromosomes by PRINS. Each of them gave clear signals on a single chromosome pair. Thus, linkage groups LG2 and LG6 could be assigned to appropriate chromosomes (Fig. 1e, f).

Another PRINS approach has concerned the analysis of several markers for domestication genes concerning such lupin traits as soft seeds (*Mollis*), white flower and seed colour (*leucospermus*), reduced pod-shattering (*lentus* and *tardus*) and early flowering (*Ku*). Information on cDNA sequences from the genetic map (Nelson *et al.* 2006) has been available for PRINS analyses. That part of the work is still in progress.

The continuation of our research will be focused on some regions of the narrow-leafed lupin genome containing genes of valuable agricultural traits. Combining FISH and PRINS techniques (e.g. two different techniques applied on the same preparation) should enhance efficiency of further studies. Approaches combining linkage information with cytogenetic analysis constitute the first step towards the integration of genetic and physical maps of *Lupinus angustifolius*.

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