NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS* L.) COMPARATIVE STUDIES

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ABSTRACT

The aim of this study was to saturate the narrow-leafed lupin genetic map with sequence-defined markers. This approach facilitates comparative mapping of the *Lupinus angustifolius* genome with those of other legume species. The task has been carried out within the framework of the GRAIN LEGUMES Integrated Project of the 6 EU Framework Program (GLIP). Sequence-defined molecular markers were designed for conserved genomic regions of model legumes (*Medicago truncatula, Lotus japonicus)* as well as crop legume species. New methods applied in marker generation (CAPS/dCAPS, *Cel* I, SNaPshot) facilitated faster and more reliable analyses. Testing of the 466 primer pairs provided by GLIP and collaborative Scientific Institutions (University of Aarhus, Denmark; Montana State University, USA; Murdoch University, Australia) resulted in successful mapping of 63 markers. Moreover, preliminary results of the synteny between the narrow-leafed lupin and *M. truncatula* genomes were considered. Localisation of orthologs from *M. truncatula* chromosomes was found for markers with known genomic annotation.

KEYWORDS

Lupinus angustifolius, STS marker, genetic map, comparative mapping

INTRODUCTION

Narrow-leafed lupin (*L. angustifolius)* was selected as a representative species of the *Lupinus* genus for comparative mapping studies due to its relatively low chromosome number and a moderate genome size (Naganowska *et al*. 2003). Among all the lupin crops the range of its cultivation is the widest and covers not only the area of its Mediterranean origin, but also Northern Europe and Australia.

The aim of this study was to compare the narrow-leafed lupin and model legume *M. truncatula* genomes to assess their synteny level. This task was carried out within the framework of the GRAIN LEGUMES Integrated Project, 6 EU Framework Program (GLIP).

MATERIALS AND METHODS

The mapping population, consisting of 89 F_8 recombinant inbred lines developed by the single seed descent method from the cross combination 83A:476 x P27255, was provided within the framework of official cooperation with Dr Hua'an Yang (DAFWA). The genomic DNA from leaf tissue of mapping population plants was isolated using a DNeasy Plant Mini Kit (Qiagen), according to the protocol provided by the manufacturer. Four sets of primer pairs were tested in lupin. The first group, designed to amplify single or low copy genes on the basis of *M. truncatula* and *P. sativum* conserved sequences, was provided by GLIP. The second set consisted of PriFi primer pairs (degenerated primers) designed in collaboration with Prof. Jens Stougaard, (University of Aarhus, Denmark) to develop legume family anchor markers (Fredslund *et al*. 2006). The next group of primer pairs from *M. truncatula*, *L. albus* and soybean conserved regions (MLG primers) was designed and provided in collaboration with Prof. Richard Oliver (Murdoch University, Australia). The forth set of primer pairs tested in lupin concerned the gene-specific, cross-legume sequences and was tested in collaboration with Prof. Norman Weeden (Montana State University, USA).

Digestion with restriction enzymes (CAPS/dCAPS markers) as well as the *CEL*I enzyme (Oleykowski *et al*. 1998) and the SNaPshot genotyping method (Applied Biosystems) were used to facilitate scoring of marker allele segregations within the mapping population. Sequencing of PCR products (AbiPrism3130XL Genetic Analyser, Applied Biosystems) was performed to detect single nucleotide polymorphisms (SNPs). STS markers were localised on the existing narrow-leafed lupin genetic map based on the MFLP markers (Boersma *et al*. 2005). Linkage studies were accomplished using computer program MapManager, vs. QTXb20 and MapChart v. 2.2 (Voorrips 2002).

RESULTS AND DISCUSSION

From the total number of 315 primer pairs provided by GLIP (IGABC, Hungary) segregation of 47 markers was analysed in the mapping population (DxW) (polymorphism level 15%). Amplified products differed in size (24 markers) or represented the present/absent segregation type (9 markers).

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Fig. 1. GLIP and MGL markers and their homologous loci in *Medicago truncatula.*

Marker name in lupin	LG lupin	Genomic	EST or CDNA	Medicago chromosome	Genetic map marker name	Physical map bac
mtmt_GEN_00258	1	AC126019	TC101046	4	002H07	mth2_22P22
mtmt_GEN_00447	1	AC135233	TC94522	4	004A07	mth2_14C14
psat_EST_00171	gr19	At3g22200.1	CD858669	4		mth2-161l14
LG80	gr19		TC219033	4		mth2-16c13
mtmt_GEN_00024_04_1	gr34	AC124214	TC90673	4	SQEX	mth2-36a23
mtmt_GEN_00361_01_1	gr34	AC146651	TC98210	4		mth2-113d3
mtmt_GEN_00092_01_1	6	AC146572	TC79484	5	h2_104c10b	mth2-104c10
mt_DEG_3530	6	CR931733	TC104778	5	h2_10g3e	mth2-10g3
mtmt_EST_03188_06-08	gr25	CT033769	TC104830	5		mth2-133o10
mt_EST_03217	gr25	CR931731	TC110410	5	h2_83l19b	mth2-83l19

In the case of 14 markers, polymorphism was detected at the single nucleotide level. Finally 42 markers were mapped on the MFLP linkage map and 5 of them remained unlinked.

Forty seven primers generated by the PriFi program and previously analysed in common bean and groundnut were also tested in lupin. PCR products were sequenced to assess homology to the expected gene region in *A. thaliana* or *M. truncatula* as well as to detect polymorphism at the sequence level. The marker sequence analyses made it possible to detect 18 SNPs. As a result, 16 PriFi markers were localised on the MFLP map and 2 markers remained unlinked.

From a set of 110 MLG primer pairs tested in lupin 11 markers showed single nucleotide polymorphisms (SNPs) and 3 had already been localised on the lupin map. Furthermore, the analysis of 52 gene-specific, cross-legume primers was carried out, which resulted in mapping of two additional markers.

In the analyses of single nucleotide polymorphisms the *CEL* I method, SNaPShot genotyping assay as well as restriction enzyme digestions were applied. The *CEL* I method was rejected due to its low throughput. The application of degenerate primers gave the best results in term of the successful amplification and polymorphism rates (38% of the polymorphic markers). The total number of markers localised through our efforts on the narrow-leafed lupin genetic map is now 63 (Fig. 2). The map is currently composed of 679 markers connected into 21 main groups and 5 additional, minor groups. The total map length is 1994 cM and the average distance between adjacent markers is 2.94 cM. Genetic length of the 21 main linkage groups is now approximately 200 cM longer than the published map (Boersma *et al*. 2005). Localisation of the new markers on the existing lupin MFLP map increased its length, whereas it decreased the average distance between markers (642 markers versus 454 markers in the published map, and 2.94 cM versus 3.4 cM for the published map).

Newly mapped markers facilitate comparative mapping studies within legumes. It needs to be stressed that while synteny between closely related species is high, its degree declines with an increasing phylogenetic distance (Choi *et al*. 2004). In our study the narrow-leafed lupin genetic map was compared with *M. truncatula* chromosomes (www.medicago.org). Localisation of the homologous loci on the *M. truncatula* chromosomes was found for the markers with known genomic annotation, provided by GLIP and Murdoch University.

Using this approach an approximate position in *M. truncatula* was established for 43 out of 45 lupin markers. Comparative study results indicated that lupin linkage group 1 (LG1) as well as 2 additional groups 19 and 34, have syntenic regions to *M. truncatula* Chromosome 4. Furthermore, *Medicago* Chromosome 5 shared two homologous markers both with group LG6 and 25 (Fig. 1). On the other hand, it was observed in our study that loci originating from different LGs of the *L. angustifolius* map were syntenic with a single *M. truncatula* chromosome. This might support a hypothesis of polyploidisation followed by chromosomal rearrangements in the *L. angustifolius* genome (Phan *et al*. 2007).

Comparative mapping studies between the narrow-leafed lupin and *M. truncatula* were already undertaken by Nelson *et al*. (2006). Nelson's gene-based map contained a total of 382 loci. Those authors considered regions to have conserved synteny when at least 3 homologous markers were mapped in both species. In our research we found a maximum of two loci shared between the lupin and model plant genomes as a result of a lower marker number used in comparisons. Utilisation of the remaining available markers in comparative studies as well as further genetic mapping of new sequence-defined markers should support these results in the future. At this time it is also difficult to confront the comparative mapping results obtained by Nelson and his co-workers with our results, as both lupin maps are based on different markers. However, we

observed that marker UNK7 located in our study in LG14 on the MFLP map is also present on the gene-based map in LG12 (Nelson *et al*. 2006). Both teams also found its homologous locus on *Medicago* Chromosome 5. Furthermore, the other marker PRAT is shared between both lupin maps. It was mapped in LG2 on the MFLP map and in LG6 on the gene-based map, but we could not find its homologous locus in the *Medicago* map. Both markers might serve as reference markers in the integration of both versions of the lupin maps.

Although it is too early to draw a conclusion about syntenic blocks in the lupin and *Medicago* genomes, our data complement previous studies on *L. angustifolius* (Nelson *et al*. 2006). Integration of the maps as well as the development of new STS markers to be used in synteny studies are desirable. Further studies on conserved genome synteny in the future may facilitate transfer of knowledge among related species of the legume family.

CONCLUSIONS

1. Most of the primer pairs amplified a monomorphic product. The application of restriction enzymes as well as the SNaPshot method may be a valuable tool for faster and more reliable analyses.
2. Legume family anchor markers showed the highest amplification efficiency and polymorphism levels (38% of polymorphic markers). This might be due to the degenerated character of primers, which facilitated their efficient DNA binding. These markers should be included in further comparative mapping studies.
3. The number of reference markers located so far on the lupin genetic map makes possible preliminary comparative mapping between the *L. angustifolius* and *M. truncatula* genomes; however, a higher number of shared loci would be desirable.

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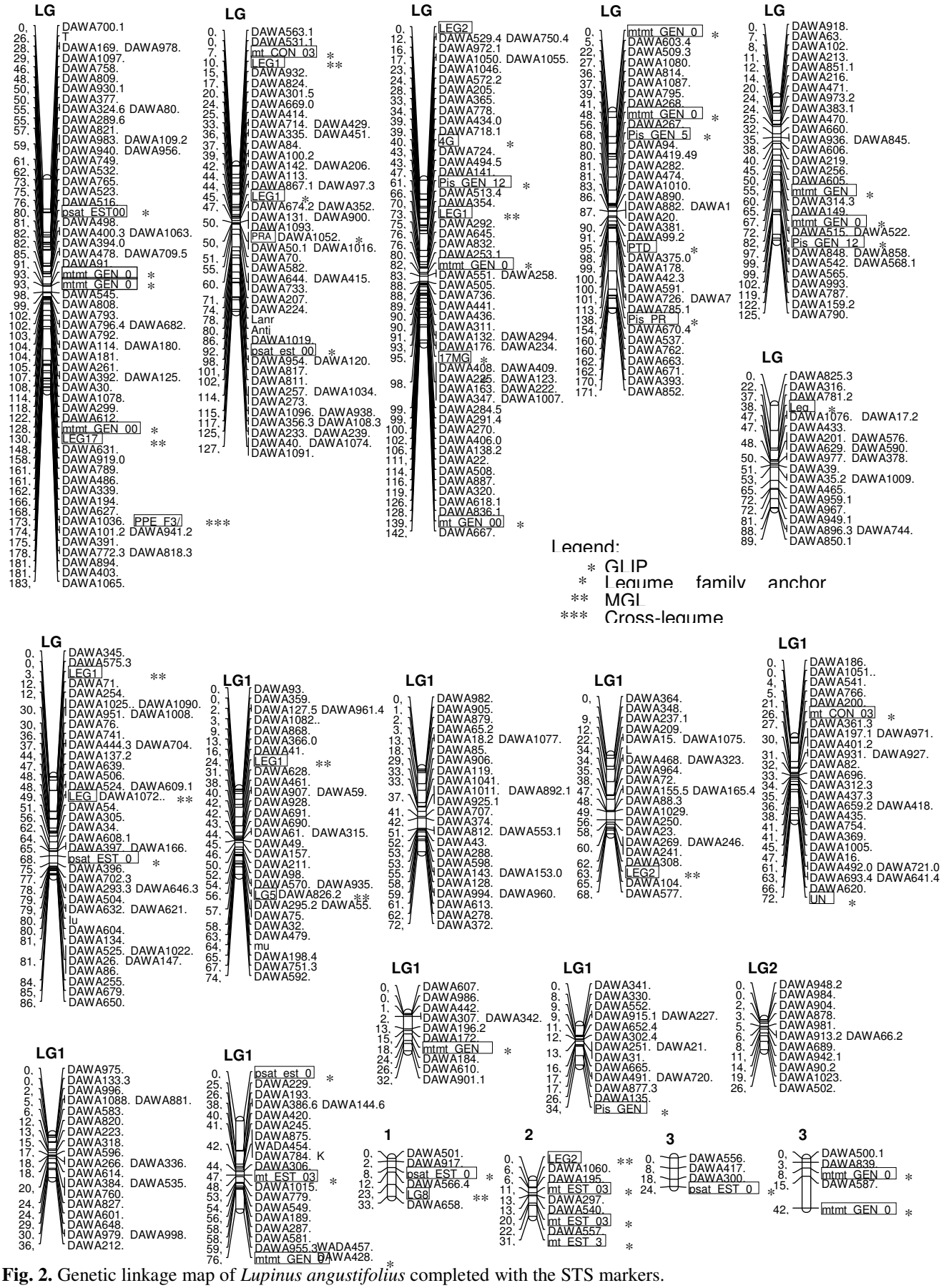

Fig. 2. Genetic linkage map of *Lupinus angustifolius* completed with the STS markers.