A Molecular Marker for Early Maturity (Ku) and Marker-Assisted Breeding of Lupinus angustifolius

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ABSTRACT

Australia is the largest producer in the world of sweet narrow-leaved lupin (L. angustifolius) with approximately 1.5 million hectares planted annually in Western Australia. Increases in both area grown and in yields come mainly from the introduction of new cultivars. Using traditional breeding methods it can take up to ten years to release a new cultivar of crop but recently the use of genotypic analysis has become widespread to facilitate crop improvement. However, little is known of the genomic organization of this species beyond the presence and mode of inheritance of five major domestication traits. AFLPs are being used to construct a molecular linkage map and find molecular markers linked to traits of interest. To date, over 700 polymorphic loci have been identified in an F₂ mapping population. Linkage analysis, using MAPMAKER/EXP V3.0, has generated 21 linkage groups, including 2 AFLP markers linked to Ku (early maturity). A specific PCR test has been developed for this marker that can distinguish homozygous early and late maturing plants, as well as heterozygotes. Studies are continuing to find other markers, and to expand the linkage map.

KEYWORDS

Lupin, marker-assisted breeding, AFLPs

INTRODUCTION

Australia is the largest producer in the world of sweet narrow-leaved lupin (L. angustifolius) with approximately 1.5 million ha planted annually in Western Australia. Increases in both the area grown and in yields are mainly brought about by the introduction of new cultivars. Using traditional breeding methods it can take up to ten years to release a new cultivar. In order to facilitate crop improvement, by increasing the speed and efficiency of breeding strategies, the use of genotypic analysis is now becoming widespread. At present, little is known of the genomic organization of this species, which is diploid (2n=40), beyond the presence and mode of inheritance of five major domestication traits. These characteristics are early maturity (Ku), low alkaloids (lucundus), white flowers (leucospermus), reduced pod shattering (lentus - associated with red pigment in seed pod walls, and tardus) and permeable seed coats (mollus). Such traits are desirable in current domesticated cultivars, but not generally found in wild type plants. A cross between parents differing for all these traits would provide the most information for the first molecular mapping studies of this species.

MATERIAL AND METHODS

The F₂ progeny of a cross between an advanced breeding line (83A.476 - maternal parent) and a wild type (P27255 - paternal parent, from Morocco) were selected for this study as they segregated for all the aforementioned traits. DNA was extracted from 0.2g of fresh leaf tissue using a CTAB method (Rogers and Bendich, 1994). Fluorescently-labelled amplified fragment length polymorphisms (AFLPs), using EcoRI1 and Mse1 primers with 3 selective nucleotides after a pre-selective amplification with 1 selective nucleotide, were used to generate polymorphic loci (Vos et al., 1995). These loci, together with the phenotypic data, were then assembled into molecular linkage groups using MAPMAKER/EXP 3.0 (Lander et al., 1987), with an initial LOD score of 3.0. From the linkage groups constructed, molecular markers closely linked to agronomic traits of interest were selected, and the AFLP step repeated, using the appropriate primers and isotopic labeling (³²P). The desired fragment of DNA was isolated from a manual sequencing gel, cloned and sequenced. Primers were then developed from the DNA sequence, to create a specific PCR test for an individual characteristic. For the marker for Ku a further step was required to distinguish early and late maturing plants and heterozygotes - a restriction digest using the enzyme Mse1.

RESULTS AND DISCUSSION

To date, 705 polymorphic loci have been identified in this F₂ mapping population. Linkage analysis, at a LOD score of 3.0, generated 52 linkage groups covering approximately 1000cM, including two AFLP markers linked to Ku (early maturity). The locus M4E2A290 was selected and developed from a DNA fragment on an automated sequencing gel to a radiolabeled fragment (Fig 1). This fragment was isolated from the gel and amplified again. After cloning and sequencing this maternally derived fragment, specific primers were designed but these primers were found to amplify same size fragments (237bp) in both maternal (early maturing) and paternal (late maturing) genomic DNA. Sequencing of the paternal fragment

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and comparison showed that there were only 3 bases differing between the two, one of them being a T/C mutation, which left an Mse 1 restriction site intact in late plants, but was not present in early plants. An Mse1 restriction digest was added after the PCR, which allows homozygous, early maturing plants (having a single, unrestricted, 237bp DNA fragment) to be distinguished from the phenotypically identical heterozygotes (3 bands), and from the late maturing plants (2 bands) (Fig 2).

As this species has only 20 pairs of chromosomes, some of the linkage groups obtained must be on the same chromosome. By relaxing the LOD score to 2.0, more loci were included in the map, resulting in several of the groups being combined to give a total of 21 linkage groups, with only 64 of the 705 loci remaining unlinked. Work is now continuing with the mapping of additional 60-100 F2 individuals, which should allow tighter and more significant linkages to be found at the higher level of significance. As more markers linked to agronomic traits are identified, they too will be developed into specific PCR tests. Other traits under consideration are resistance to Anthracnose and to Phomopsis leptostromiformis (a fungus causing lupinosis in sheep). Further studies will involve cytological work, endeavoring to locate molecular linkage groups on chromosomes.

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LITERATURE CITED

