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Genotyping *Lupinus angustifolius* cultivars with SRAP molecular markers and degenerate primers

Eugeny Nikolaevich Sysoliatin, Natalia Vladimirovna Anisimova and Olga Gennadievna Babak

Abstract

We examined 18 combinations of SRAP primers with resistance gene analog (RGA) and chitinase degenerate primers in order to determine their utility for genotyping *L. angustifolius*. Primer pairs ResAn51-f/Me8, p-loop/Em5, TM/Me8, Chit3-r/Em5 were the most effective for detection of genetic polymorphism of different narrow-leaved lupine varieties.

Laboratory of Ecological Genetics and Biotechnology, Institute of Genetics and Cytology of NAS of Belarus, Minsk, Belarus

Corresponding author: Eugeny Nikolaevich Sysoliatin
E-mail: meeugeny@yandex.by

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Introduction

Narrow-leaved lupine (*L. angustifolius* L.) is a high-protein crop capable of symbiotic nitrogen fixation. There is an actual need for varieties of high-yielding, resistant to environmental conditions and diseases fodder lupine with a stable productivity over the years. Progress in improving the efficiency of lupine breeding programs is largely dependent on the improvement and application of modern molecular techniques and their appropriate combination with traditional methods.

Two types of molecular techniques have become particularly useful to identify markers linked to disease resistance genes to plant pathogens. Sequence-related amplified polymorphism (SRAP) primers target coding sequences in the plant genome (1,2). Resistance gene analog (RGA) (3,4) primers are useful markers for tagging resistance genes and were designed from conserved motifs of the "Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR)" resistance gene family.

Materials and Methods

SRAP-RGA technique is reported to be of great utility in detection and development of molecular markers, linked to plant resistance genes (2). The approach implies utilization of various combinations of RGA and SRAP primers in polymerase chain reaction (PCR). The aim of the investigation was to determine the possibility of using SRAP-RGA analysis for the detection of genetic polymorphism of different narrow-leaved lupine varieties. We also extended the method by implementation of different combinations of SRAP primers and degenerate primers, designed from conserved motifs of chitinases, enzymes involved in plant defense against pathogens (5).

Results

We have analyzed the collection of 12 samples of cultivated *L. angustifolius* by means of a total of 18 combinations of 2 SRAP primers with 5 RGA and 4 chitinase degenerate primers. Our results revealed polymorphism in 9 combinations of primers. The number of bands in the obtained electrophoretic profiles varied from 4 to 16. The number of polymorphic bands varied from 1 to 4.

Table 1. Characteristics of the primer combinations used for genotyping *L. angustifolius* L.

Primer combination	Number of bands		
	Total	Polymorphic	% of polymorphic bands
TM/Me8	**11**	**3**	**27.3**
TM/Em5	14	0	0
p-loop/Me8	7	1	14.3
p-loop/Em5	**14**	**4**	**28.6**
Chit1-f/Me8	12	1	8.3
Chit1-f/Em5	8	0	0
Chit1-r/Me8	14	0	0
Chit1-r/Em5	10	1	10
Chit3-f/Me8	16	2	12.5
Chit3-f/Em5	11	0	0
Chit3-r/Me8	13	0	0
Chit3-r/Em5	**15**	**4**	**26.7**
ResAn51-f/Me8	**10**	**3**	**30**
ResAn51-f/Em5	16	2	12.5
ResAn34-r/Me8	7	0	0
ResAn34-r/Em5	8	0	0
ResAn35r/Me8	3	0	0
ResAn35r/Em5	4	0	0

Discussion

Degenerate RGA primers TM, p-loop, ResAn are known to be used for the study of resistance gene analogs of narrow-leaved and yellow lupin (4). Their combinations with SRAP primers allow to obtain polymorphic fragments. Degenerate primers Chit1 and Chit3 were used previously for the study of chitinase genes of muskmelon (5). Nevertheless, they showed high levels of amplification with narrow-leaved lupin. The highest percentage of polymorphism was detected by ResAn51-f/Me8, p-loop/Em5, TM/Me8, Chit3-r/Em5 primer pairs (**Table 1**).

Conclusions

Based on the results we can conclude that the use of SRAP-RGA is a simple strategy for genotyping narrow-leaved lupine. By this method we can generate multiple polymorphic fragments associated with conserved domains of potential genes of resistance to phytopathogens. Further expansion of SRAP and degenerate primers spectrum can be useful for genotyping narrow-leaved lupine.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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