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INTERVAL MAPPING OF QTLs CONTROLLING SOME MORPHOLOGICAL TRAITS IN PEA.

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Abstract: A linkage map of pea was constructed based on a 104 RIL population derived from the cross combination Wt10245 x Wt11238. The map, which consisted of 204 morphological, isozyme, AFLP, ISSR, STS, CAPS and RAPD markers, was used for interval mapping of the QTLs controlling the stem length and internode number of pea. In the characterization of a given QTL, we included an identification of its position with reference to the flanking markers, an estimation of the part of variance explained by it, and a determination of gene action. Six QTLs per trait were identified as demonstrating linkage to ten intervals on five linkage groups. As many as seven QTLs influencing the analysed traits were mapped on linkage group II, indicating the important role of this region of the pea genome in plant height control.

Key Words: Genetic Map, *Pisum sativum*, Plant Height, QTL Mapping

INTRODUCTION

There was a conflict between the Mendelian theory of monogenic inheritance and the observation that traits in nature in many cases exhibit continuous variation. It was eventually resolved by the concept that quantitative inheritance can result from the segregation of multiple genetic factors, modified by environmental effects [1]. As molecular marker technology has advanced, genetic maps covering the whole genome have become available.

Pea is one of the few legume species widely adapted to cultivation and also very useful as a model crop in genetics. The genetic map of pea has been developed gradually over time, from the initial version given by Lamprecht in 1948 up to the latest, published in 1998 by the *Pisum* Mapping Committee [3]. Using statistical analysis, the variation of a quantitative trait can be dissected into the effect of individual QTLs linked to markers on a genetic map. The interval mapping method postulates a single QTL at the position on the chromosome

corresponding to the maximum of the likelihood ratio of the QTL at that point to that of no QTL at that point [2].

In pea, as in other crops, the majority of economically important agronomic characteristics are controlled in a quantitative fashion. However, the range of research on quantitative traits in pea is still limited. A few reports on this area concerned seed weight [4], seed cotyledon colour [5] and *Aphanomyces* root rot resistance [6]. In this paper, we present the results of mapping the QTLs affecting plant height using an earlier constructed pea genetic map [7]. Plant height is a complex trait, influenced by the environment, and in pea cultivation severe losses in seed yield can be caused by a lodging stem that is too long.

MATERIALS AND METHODS

Plant material

A total of 104 (F_4) RILs derived from 114 individual F_2 plants were used in this analysis. The plants were assessed in field trials. The parental lines were selected on the basis of contrasting monogenic morphological traits, as well as significant differences in the expression of quantitative traits. The female parent – Wt10245 – is a cultivar with a long stem and the male parent – Wt11238 – (=WL1238) is a tester line, often exploited in our genetic studies, with a genotype including morphological and isozyme markers in all its chromosome linkage groups.

Map construction and QTL mapping

240 markers were used for linkage analysis. The fit to the codominant 1:2:1 or dominant 3:1 ratio was tested by χ^2 analysis using the computer program Linkage-1 [8]. The genetic map was constructed as described earlier [7]. The trait means and the analysis of variance (F-test) were done using the Genstat computer program [9]. QTLs were detected by interval mapping using MAPMAKER/QTL [10]. The significance level required to declare a QTL was set at $LOD \geq 2.1$

RESULTS AND DISCUSSION

A genetic linkage map consisting of 204 markers (140 AFLPs, 24 RAPDs, 10 ISSR, five CAPSs, one STSs, 11 isozymes and 13 morphological markers) was developed from a mapping population of 104 RILs (F_4). Nine linkage groups were obtained (Fig. 1). Eight of them correspond to previously known linkage groups [3]. In addition, there was one unidentified linkage group (VIII) containing AFLP and ISSR markers without anchor loci. The created map spans 2416 cM with an average distance of 12 cM (Kosambi units) between adjacent markers. However, the length of almost 50% of the map intervals is less than 10 cM, and only 1.5% of the intervals are longer than 30cM. The constructed map was used for QTL identification.

Table 1 contains the results obtained from the analysis of variance for stem length and internode number of 102 inbred lines (F_4) derived from the Wt10245 \times Wt11238 cross combination. Significant differences between all the inbred lines as regards all the examined traits were sufficient grounds for carrying out a further study, aimed at QTL mapping and effect estimation.

Tab. 1. Means and the results of analysis of variance estimated for 104 RILs of pea.

Quantitative trait	Minimum	Mean	Maximum	F statistics
Stem length (cm)	22.40	80.22	125.40	8.92*
Number of stem internodes	13.80	21.53	26.40	3.41*

* – Significant at $\alpha = 0.001$

The interval mapping revealed 12 QTLs (six per trait) localised on linkage groups II, III, IV, V and VII. Seven of them were mapped on linkage group II, indicating an important role of this region of the pea genome in plant height control (Tab. 2). It is interesting that the *lst3* locus was localised on linkage group III close to region of the *le* locus, which has previously been suggested to decrease internode length.

Tab. 2. Locations of putative QTLs affecting stem length and internode number.

Trait	QTL symbol	Linkage group	Nearest marker	Distance* (cM)
Stem length	<i>lst1</i>	II	<i>afp15h</i>	0.0
	<i>lst2</i>	II	<i>wb</i>	4.0
	<i>lst3</i>	III	<i>C1506a</i>	12.0
	<i>lst4</i>	IV	<i>afp3b</i>	2.0
	<i>lst5</i>	V	<i>afp10i</i>	13.0
	<i>lst6</i>	VII	<i>Est-1</i>	0.7
Internode number	<i>int1</i>	II	<i>afp15h</i>	2.0
	<i>int2</i>	II	<i>C1508a</i>	6.5
	<i>int3</i>	II	<i>afp6c</i>	4.0
	<i>int4</i>	II	<i>wb</i>	4.0
	<i>int5</i>	II	<i>afp3k</i>	0.4
	<i>int6</i>	V	<i>U</i>	6.0

*The distance was measured from the nearest marker to the maximum LOD peak of a given QTL.

Likelihood intervals for two QTLs affecting stem length coincide closely with those of QTLs influencing internode number. This suggests that either some individual QTLs have a pleiotropic effect on both traits, or that different QTLs

are clustered together. The genetic resolution afforded by this experiment does not permit us to distinguish between linkage and pleiotropy using QTL mapping. Individually, these particular QTLs explained from 9.8 to 42% of the total variation for stem length, and from 29.9 to 52.4% for internode number. The alleles from parental line Wt11238 were responsible for a decrease in stem length (with one exception) and for an increase in internode number (with two exceptions). Detailed characteristics of each identified QTL are given in Tab. 3.

Tab. 3. Summary of the statistics for stem length and internode number detected by interval mapping using Wt10245 × Wt11238.

Trait	QTL symbol	LOD score	V _E (%)	a	d	d/a	GA
Stem length	lst1	2.5	11.2	11.6	-1.8	-0.2	A
	lst2	4.9	24.2	-12.8	18.7	-1.5	R
	lst3	4.4	42.0	-23.4	81	-0.3	A, R
	lst4	2.1	33.8	-13.5	24.0	-1.8	R
	lst5	2.1	31.2	-2.7	29.0	10.7	OD
	lst6	2.1	9.8	-8.4	12.3	-1.5	R
Internode number	int1	6.2	29.9	2.0	0.2	0.1	A
	int2	6.3	34.2	2.2	0.9	0.4	A, D
	int3	4.7	23.8	1.9	0.4	0.2	A
	int4	3.9	23.0	-1.0	2.3	-2.3	R
	int5	4.5	52.4	-2.3	2.9	-1.3	R
	int6	3.0	20.4	1.2	1.7	1.4	D, OD

LOD – logarithm of odds; V_E – variance explained; a – additive effect; d – dominance effect; GA – gene action: A – additive, R – recessive, D – dominant, OD – overdominant

QTLs exhibited gene action ranging from recessiveness to overdominance. In some cases, it was impossible to determine gene action unambiguously. The ability to estimate gene action is reduced by half with each generation of selfing as heterozygosity is lost. It is additionally limited by the number of individuals examined [11]. In fact, the estimation of gene action on the basis of results obtained for the F₄ generation can be misleading.

Nevertheless, our research provides insight into the genetic complexity of traits and identifies regions of the genome for further investigation.

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