Mapping and stability of QTLs for seed weight in common beans under different environments [1]

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ABSTRACT

The objectives were: 1 – to identify, under different environments, RAPD markers linked to loci that control the 100 seed weight of common beans; 2 – to evaluate the existence of interactions involving QTL´s with planting dates and locations; 3 – to compare detection procedures of markers linked to QTLs utilizing the methods of mapping and multiple regression. One hundred and ninety-six recombinant inbred lines derived from the cross of cultivars Carioca x Flor de Mayo were evaluated under two traditional sowing dates for common beans in 1996, 1997 and 1998, in Lavras and Patos de Minas, Brazil. For the phenotypic evaluation of the families, seven field experiments were conducted. The experimental design used was a 14 x 14 partially balanced Square Simple Lattice. Results indicated that interaction of QTLs by location were significant, and some stable QTLs were identified. Multiple regression analysis identified a greater number of QTL-linked markers than the process of composite interval mapping. There was no coincidence between results obtained with the two methods studied. Molecular markers which were considered of greater potential use on marker-assisted selection for seed weight were OPN-02 (1445 pb) e OPM-06 (1096 pb).

KEY WORDS: *Phaseolus vulgaris*, seed weight, QTLs x environments interactions, RAPD.

INTRODUCTION

Seed Index or the weight of 100 seeds is a characteristic that is used as an indicator of seed size, and hence is considered a primary component of grain yield. Ramalho et al. (1993) stated that the former characteristic is controlled by a small number of genes, which implies more limited responses to environmental influences, consequently leading to higher trait heritability and greater easiness for selection when compared to selection for yield grain *per se*.

A main difficulty in QTL mappings is the fact that several genetic and environmental factors affect the final phenotype expression. Thus the experimental design utilized for this purpose must involve measurement and genotyping of a great number of individuals or families in order to allow the necessary precision for QTL identification (Ferreira and Grattapaglia, 1996; Paterson, 1998).

The field bean belongs to a species with a relative small genome (Arumuganatham and Earle, 1991) bearing predominantly simple copy sequences (Talbot et al., 1984). Such features make it easier to obtain molecular markers uniformly distributed throughout the genome, which in turn increases the possibility of QTLs detection, as it is possible to obtain highly saturated genetic maps. Besides that, the population sample size is also of paramount importance for the consistency of the information generated in the mapping process. In this way, the map resolution as well as the capacity of determining the sequence of the markers is directly related to the population sample size.

A great contribution to the mapping methodologies was the use of recombinant inbred lines (Burr et al., 1988). Young (1994) considered these sorts of populations a better option for building up of molecular maps. The author comments that the major problem of F2 plant populations, and backcrosses as well, is their ephemeral existence, for the seeds originated from such individuals would constitute new populations. The ideal situation would be to work with permanent populations for genetic mapping and the utilization of recombinant inbred lines would be the best strategy (Young, 1994), especially because the multiplication of the genotypes would permit also studies of the interactions of the QTLs with the environment. Research studies with field beans

describe the existence of strong genotype-by-environment interactions for the main characteristics important for the genetic improvement of the species (Melo et al., 1997).

The genetic mapping of the field bean genome started with the development of an empiric linkage map in which the majority of the markers were of the morphologic type plus a few isoenzimatic markers (Basset, 1991). After that, Vallejos et al. (1992), as well as Nodari et al. (1993) constructed maps based mainly on RFLP markers, covering respectively, 82% and 69% of the field bean genome.

The objectives of this research were: 1 – to map, in different planting dates and locations, QTL-linked RAPD markers responsible for the control of seed index in a segregant population of field beans; 2 – to evaluate the existence of Location–by–QTL interaction and 3 - to compare the processes for detection of QTL-linked markers, utilizing the method of composed-interval mapping and the method of multiple regression analysis.

MATERIAL AND METHODS

One hundred and ninety six families from the cross of parents Carioca (Genome 3) and Flor de Mayo (Genome 6) were utilized in this study. Starting with the F1 generation, the subsequent generations (F_2 trough F_5) were obtained and conducted by the bulk-population breeding method. On generation F_5, seeds from 196 individual plants were randomly harvested which in turn, originated 196 $F_{5:6}$ families. These families were then multiplied for one cycle, originating the $F_{5:7}$ families.

The families were evaluated in the two traditional sowing seasons, in 1996, 1997 and 1998, at two locations: Lavras (at the experimental area of the Biology Department of Universidade Federal de Lavras) and Patos de Minas (at the Experimental Station of Empresa de Pesquisa Agropecuária de Minas Gerais, EPAMIG), both located in the State of Minas Gerais, Brazil.

For the phenotypic evaluation of the families, seven experiments were conducted (two during the winter season at Lavras, one at Patos de Minas also in the winter, two in the dry season in Lavras and two also in the dry season of Patos de Minas). The experimental design utilized for all experiments was a partially balanced 14x14 square lattice, with two replications. Each experimental plot was comprised of two 2m-long rows spaced apart 50cm, sowed with 15 seed per meter. The cultural practices were the normal ones recommended for the crop, with the application of irrigation whenever needed. The Seed Index for each experimental unit was obtained by weighing a sample of one hundred apparently healthy seeds and was expressed in grams.

Initially, an analysis of variance was performed for each experiment individually, considering as random the genotypic effects. Later, a group analysis was run, utilizing the adjusted means of the individual analyses, in which the effects of growing seasons and locations were considered as fixed. The analysis of the group of experiments and the mean square expectations followed the partitioning proposed by Vencovsky and Barriga (1992). From the estimated mean square expectations, the genotypic and phenotypic variances were estimated, as well as a few population genetic parameters [broad-sense heritability, genetic coefficient of variation and the among-family "b" coefficient, according to Vencovsky and Barriga (1992)]. As for heritability, the upper and lower limits of the confidence intervals were estimated with confidence coefficient of $1-\alpha = 0,95$ (Knapp et al., 1985).

DNA extraction and the obtaining of the markers were carried out according to the procedures described by Duarte et al. (1999). Forty-two primers were used, which generated at least one polymorphic band amongst families. These 42 primers yielded 92 polymorphic amplification products (bands). The Chi-square test (χ^2) of the 92 markers indicated that twenty markers did not show Mendelian segregation and hence had to be discarded; out of the 72 remaining markers (bands), nine were discarded due to the fact that they incurred many lost observations. The 63 remaining markers were the ones finally considered and analyzed in this study.

A multiple regression analysis was run, involving all markers that presented Mendelian segregation for each experiment individually. The same was done with the means of each location and growing season separately, besides the analysis of all means over all experiments.

The multiple linear regression on the markers was performed according to the procedures outlined by Edwards et al. (1987), who considered the markers as independent variables and the phenotypic traits as dependent variables. Markers selected as of major importance were the ones obtained through significant partial F values ($P<0,05$) and the proportion (as a percentual) of the variation explained due to such markers was estimated by means of the partial

determination coefficient (Draper and Smith, 1981).

A genetic linkage map was constructed with the RAPD polymorphic markers exhibiting Mendelian segregation by means of the MAPMAKER software (Lander et al., 1987), version 3.0. For that purpose, a lod score (log of odds-ratio) of 4.8 was utilized, with maximum distance of 50 cM, which yielded an α value of approximately 1%. A Haldane map-function (1919) was employed in order to calculate the genetic distances in centimorgans (cM).

After the assembling of the RAPD-markers molecular map, the QTL mapping was achieved by means of the QTL Cartographer version 1.01 software for Windows. The mapping procedure was done individually for each experiment, using the averages for each location and growing season separately and also analyzing averages of all experiments.

The method of composed-interval (CIM) (Zeng, 1993), which is a combination of interval mapping and multiple linear regression, was utilized considering the markers as the regression variables. A stepwise procedure was employed for the selection of the regression variables for the multiple regression within the CIM mapping, by means of a lod score of 2.6 so that a QTL was assumed to be involved in the genetic control of the trait in question.

RESULTS AND DISCUSSION

Phenotypic evaluation

The parentals of the cross under analysis are of little divergence and are both classified as small-seeded genotypes. The analyses of the Seed Index of the families under evaluation (Table 1) as well as the analysis of the group of experiments (Table 2), demonstrate that in all experiments the family averages were attributable to the class of small seed. Such small variability in the population (Figure 1) was probably due to the parents in this study, which were similar in regard to seed weight.

In this investigation, since none of the parents had large seeds, the alleles of major effect do not occur in the population under evaluation, and the differences found were probably due to the presence of modifier genes which posses alleles of minor effect. Thus, despite the small genetic divergence there is the possibility of detecting QTLs responsible for seed weight without the interference of major-effect genes.

The identification of such minor-effect alleles via molecular markers may add up to the detection of major-effect alleles by means of the phenotypic evaluation, and thus increase the efficiency of the selection procedure and enhance the selection gains through a program of marker-assisted selection that utilizes the molecular information associated with the phenotypic information with the final objective of increasing crop yield.

The experimental precision may be considered relatively high with the exception of experiment 3. Nevertheless, due to the low genetic variability of the population for this trait (Figure 1), the estimates of the "b" parameter and broad-sense heritability were reduced in some experiments (1, 3 and 7). Even considering the little divergence between the parents and consequently the small variability within the population, in some situations (experiments 2, 4 and 5) it was clearly observed higher probabilities of success under selection. This was also evident from the large estimate of heritability found in the analysis of the group of experiments.

The group analysis for Seed Index (Table 2) over all locations and growing seasons, indicated significant interactions for both family x growing season and family x location. The correlation between family means for the two growing conditions was 69,72, and for the two locations was 68,90%, which indicates that part of the interactions for this characteristic are of the complex type. However, a major part of this interaction is of the simple type, i.e., it is due to the differences in the magnitudes of response and not to the response of the families to the change of the environment (Ramalho et al., 1993). Thus, the higher the correlation, the greater are the chances of the superior genotypes in one environment to be also superior in another environment, making the selection procedure easier. Nevertheless, it is paramount that the evaluation of the populations for this trait is performed in several locations as well as in different growing seasons, because some of the families may present specific behavior for each of the situations considered.

In order to confirm the effect of the family-by-environment interaction on Seed Index, it was calculated the heritability also considering all model factors as random (Vencovsky and Barriga, 1992). In this way, a heritability estimate totally free of the interaction effect on the genetic variance was obtained. The heritability estimate in such condition was 74.29%, which demonstrated the large effect of the interaction on bean seed weight phenotypic expression. Hence the necessity of evaluating this trait under several growing seasons and locations, because the family performance is dependent upon those environmental factors.

Table 1. Analysis of variance and estimative of genetic coefficient of variation (CVg) and the among-family "b" coefficient, broad-sense heritability (h_a^2), with the upper (LU) and lower limits (LL) of the confidence intervals for weight of 100 seed (g) in seven experiments.

Source of Variation	QM [1/]						
D.F.	E1	E2	E3	E4	E5	E6	E7
Trat. Aajusted	195	14.61[2/]	12.44[2/]	13.55[2/]	19.49[2/]	17.07[2/]	12.73[2/]	20.57[2/]
Effective error	169	5.79	1.41	6.24	2.56	2.54	2.60	6.79
Mean		24.88	22.61	20.53	27.85	25.50	25.56	26.70
CVe (%)		9.67	5.24	12.16	5.74	6.25	6.30	9.76
CVg (%)		8.44	10.04	9.30	10.44	10.57	8.80	9.83
b		0.88	1.91	0.76	1.82	1.69	1.40	1.01
h_a^2 (%)		60.37	88.66	53.95	86.86	85.12	79.58	66.99
LL (h_a^2)		47.00	84.84	38.41	82.43	80.10	72.69	55.86
LU (h_a^2)		70.46	91.55	65.67	90.21	88.91	84.77	75.39

[1/] E1: experiment in Lavras, generation F_7, winter, 1996; E2: Lavras, F_8, dryland, 1997; E3: Patos de Minas, F_8, dryland, 1997; E4: Lavras, F_9, winter, 1997; E5: Patos de Minas, F_9, winter, 1997; E6: Lavras, F_{10}, dryland, 1998; E7: Patos de Minas, F_{10}, dryland, 1998; [2/] Significant at the 1% probability level based on F test.

In this manner, in tasks involving QTLs mapping for this character, one should try to identify both QTLs that are only expressed in determined situations and those QTLs of greater consistency, i. e., those that express themselves in a majority of environments.

Multiple linear regression analysis

The multiple linear regression analysis for weight of 100 seed (Table 3) in the first experiment (Lavras - F_7 - winter - 1996) identified seven markers linked to QTLs that participate in the genetic control of this character, of which three were in the direction of reducing the expression of the trait whereas four were in the direction of augmenting it. The marker OPM-06[1] (the superscript refers to the length of the do DNA segment utilized as a marker itself and the smaller the number, the longest the segment) was the one which best explained the genetic variation (12.54%) of the character in this experiment; all markers together were able to explain 32.89% of the variation. Therefore, in this experiment the RAPD markers could explain practically a third of the total genetic variability, which demonstrated that they might be important instruments to increase the efficiency of the selection process.

Only two QTL-linked markers were detected in the second experiment, both acting in the direction of reducing the character expression. They were not identified in the previous experiment and none of them explained more than 3% of the genetic variation.

In the third experiment, four linked markers were identified, three in the direction of reducing and one of increasing seed weight. OPO-12 was the marker which explained the most genetic variation (7.83%) in this experiment. It is worth noting that in this trial, the marker OPM-06[1] presented an opposite effect to the one identified in the first experiment, changing from a positive to a negative effect. These two experiments were conducted in totally different locations and times of the year; a fact which may have caused a great enhancement of the family-by-environment interaction effect, hence leading to an inversion of the response of the QTL, identified by this marker.

Table 2. Joint Analysis of variance and estimative of genetic coefficient of variation (CVg) and the among-family "b" coefficient, broad-sense heritability (h_a^2), with the upper (LU) and lower limits (LL) of the confidence intervals for weight of 100 seed (g).

Source of variation	D.F.	QM
Locations (L)	1	646.20 [1/]
Season (S)	1	3339.3 [1/]
Families (F)	195	56.08 [1/]
F x L	195	10.80 [1/]
F x S	195	9.70 [1/]
F x L x S	195	6.08 [1/]
Mean error	1183	3.99
Mean		24.80
CVe (%)		8.05
CVg (%)		7.77
b		0.97
h_a^2 (%)		92.88 (74.29) [2/]
LL (h_a^2)		91.11
LU (h_a^2)		94.21

[1/] Significant at the 1% probability level based on F test; [2/] Heritability considering all model factors as random.

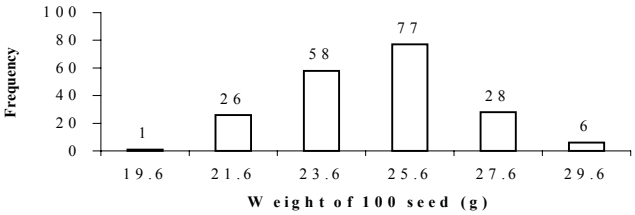

Figure 1. Frequency of Distribution for weight of 100 seeds in the families from the cross of parents Carioca and Flor de Mayo.

In the fourth experiment, *three* linked markers were found (two acting for reducing and one for increasing phenotypic expression), but none of these explained more than 4 % of the total genetic variation. No marker linked to QTLs was identified in the fifth experiment for the trait in question. In the sixth experiment, two markers were found acting in the direction of the reduction of phenotypic expression, but they both explained only a small fraction of the genetic variation. As for the seventh experiment, four QTLs linked-markers were found for the desired trait, all of them in the direction of reducing expression and again, explaining only a small fraction of the variation for the character.

The overall regression analysis, considering only the experiments conducted at the location of Lavras, has identified three QTLs-linked markers for the control of weight of 100 seeds, all of them acting in the direction of reducing the expression of the trait. The OPN-07[3] marker was the one that explained the greater portion of the genetic variation (7.26%), specifically at this location. This marker had already been identified in two other experiments and, in the first one, carried out in Lavras, it explained 6.0 % of the genetic variation of the trait in question. Therefore this marker may be useful as an aid for selection at Lavras, especially when the objective is the reduction of field-bean seed size and when one of the parents involved in a cross is the same as in the population utilized in this study.

The regression analysis for all experiments conducted at the location of Patos de Minas also identified three QTL-linked markers that control seed index, all of them also in the direction of reducing phenotypic expression. The OPS-13[2] marker was the one that explained the greater fraction of the genetic variation of this trait (4.33%) for this location. This marker had already been identified in another experiment (7) conducted at Patos de Minas, in which it has explained 3.57 % of the genetic variation for weight of 100 seeds. The analysis over all experiments at Lavras and Patos de Minas has not identified any common marker to both locations, which indicates how difficult it is to obtain progress for both sites simultaneously, reinforcing the results obtained in the previous interaction analysis, in which a significant family-by-location effect for the trait in study was detected (Table 2).

The regression analysis of the group of experiments conducted during the winter season, identified two QTL-linked markers for the control of seed index, in which one of them was in the direction of reducing trait expression and the other in the direction of enhancing it. The OPN-07[3] marker was the one that explained the major part of the genetic variability (8.23%) for that growing season. This marker had already been identified in two other experiments, the first of which, conducted during the winter season,where it explained 6.0% of the genetic variation for weight of 100 seeds. This marker was also identified in the group analysis of the experiments conducted at Lavras.

The group analysis considering only the experiments conducted in dryland conditions identified only one QTL-linked marker (OPN-02[1]) for the control of the trait in question in the direction to reduce the character and explained less than 4% of the genetic variability among families for this sowing time. This marker was also identified in experiment number six, as well as in the group analysis of both the locations of Lavras and Patos de Minas, but in all cases it was responsible for less than 4.5% of the total genetic variation of the trait.

The overall regression analysis (all experiments considered) identified three QTL-linked markers for the control of weight of 100 seed, all of them acting in the direction to reduce the character expression and all explaining less than 4.5% of the among-family genetic variation. The three considered together explained 9.79% of this variation. The marker OPN-02[1] was again identified as related to a QTL, which indicates that, it is indeed a quite stable QTL linkage in spite of its small effect. This result is in direct contradiction to Tanksley's suggestion (Tanksley, 1993), i.e., that the QTLs of major effects are the most stable ones. However, the stability criterion adopted by that author seems to be different from the one that was utilized in the present research. The author considered stable the QTLs that were detected in different environments, however in our study those QTLs were considered stable when identified in several environments but also kept a consistent effect overall the majority of them.

The interactions observed between family and location as well as between family and growing

Table 3. Multiple linear regression analysis for weight of 100 seed utilizing stepwise procedure.

Markers	Parameters estimatives	R^2 partial	Standard error	t teste [1/]
1 - LAVRAS - F_7 – WINTER – 1996				
Intercepto	24.88		0.63	39.80
OPE-20 [2/](1585 pb)	0.85	0.0362	0.38	2.22
OPM-06[1] (1096 pb)	1.39	0.1254	0.39	3.55
OPN-07[3] (1698 pb)	-1.39	0.06	0.42	-3.33
POR-02[1] (1230 pb)	0.92	0.0204	0.39	2.35
OPG-04 (1259 pb)	-1.25	0.0296	0.41	-3.06
OPN-02[2] (436 pb)	-1.01	0.0337	0.40	-2.56
OPS-16 (640 pb)	0.88	0.0236	0.39	2.24
2 - LAVRAS. F_8. DRYLAND. 1997				
Intercepto	23.56		0.35	66.90
OPE-15[1] (851 pb)	-1.00	0.0279	0.42	-2.41
OPO-19[1] (1636 pb)	-0.85	0.0271	0.42	-2.05
3 - PATOS DE MI NAS. F_8. DRYLAND. 1997				
Intercepto	21.90		0.43	50.48
OPE-20[2] (891 pb)	-1.20	0.0312	0.46	-2.63
OPM-06[1] (1096 pb)	-0.89	0.0393	0.43	-2.09
OPO-12 (1412 pb)	-1.56	0.0783	0.51	-3.05
OPH-03[3] (1112 pb)	1.10	0.0272	0.50	2.19
4 - LAVRAS. F_9. WINTER. 1997				
Intercepto	58.11		0.50	116.57
OPE-20[2] (891 pb)	-0.89	0.0271	0.43	-2.09
OPN-07[2] (1445 pb)	1.07	0.0294	0.42	2.56
OPO-13[2] (1318 pb)	-1.30	0.0361	0.44	-2.97
5 - PATOS DE MINAS . F_9. WINTER. 1997 – No marker linked to QTLs was identified				
6 - LAVRAS. F_{10}. DRYLAND. 1998				
Intercepto	27.12		0.47	57.10
OPI-07 (631 pb)	-0.87	0.0254	0.44	-2.00
OPN-02[1] (1445 pb)	-1.12	0.042	0.43	-2.62
..7 - PATOS DE MINAS . F_{10}. DRYLAND. 1998				
Intercepto	29.58		0.69	42.79
OPN-07[3] (1072 pb)	-1.08	0.024	0.54	-1.99
OPD-08 (759 pb)	-1.31	0.0408	0.53	-2.47
OPN-02[1] (1445 pb)	-1.24	0.0259	0.56	-2.23
OPS-13[2] (1122 pb)	-1.60	0.0357	0.54	-2.99
MEAN – LAVRAS				
Intercepto	33.79		0.25	133.02
OPE-20[2] (891 pb)	-0.47	0.037	0.22	-2.17
OPN-07[3] (1072 pb)	-0.76	0.0726	0.22	-3.46
OPN-02[1] (1445 pb)	-0.49	0.0265	0.23	-2.12
MEAN – PATOS DE MINAS				
Intercepto	25.90		0.45	57.39
OPN-02[1] (1445 pb)	-0.69	0.028	0.41	-1.67
OPN-02[2] (436 pb)	-1.23	0.0262	0.41	-2.97
OPS-13[2] (1122 pb)	-1.12	0.0433	0.42	-2.65
MEAN – WINTER				
Intercepto	36.14		0.27	136.29
OPM-06[1] (1096 pb)	0.58	0.0317	0.25	2.29
OPN-07[3] (1072 pb)	-0.75	0.0823	0.25	-2.96
MEAN – DRYLAND				
Intercepto	24.60		0.32	77.23
OPN-02[1] (1445 pb)	-0.95	0.0395	0.38	-2.47
MEAN – ALL EXPERIMENTS				
Intercepto	30.18		0.30	101.27
OPE-20[2] (891 pb)	-0.51	0.0429	0.25	-2.01
OPN-07[3] (1072 pb)	-0.56	0.0298	0.26	-2.20
OPN-02[1] (1445 pb)	-0.64	0.0252	0.27	-2.34

[1/] Significant at the 5% probability level based on t test; [2/] The superscript refers to the length of the do DNA segment utilized as a marker itself and the smaller the number, the longest the segment.

season (Table 2), and the small variability for the characteristic (Figure 1), explain the small number of stable markers with large effect identified in this study.

Considering that small seed size is an undesirable trait, the majority of the QTLs identified by the markers, were not favorable for the genetic improvement of seed weight although selection for the absence of those markers could help for obtaining of genotypes with larger seeds, despite a lesser efficiency.

RAPD-markers Linkage Map

Seven linkage groups on the field bean genome were established with the markers utilized in this study (Table 4). The bean species presents a basic chromosome number of eleven, therefore this should result in an expected minimum number of linkage groups. However, as the number of markers used in this study was relatively small, the low marker saturation of the map is the most probable explanation for the small number of linkage groups.

It was obtained, in average, groups with 112.24 cM, the smallest one with 16 cM and the longest with 252 cM. Therefore, the markers were mapped, on average, with 20.15 cM intervals within each linkage group, the smallest interval with 4.2 cM (linkage group 2, between markers OPO-20^3 and OPO-20^2) and the greatest with 63.1 cM (linkage group 4, between markers OPN-02^2 e OPO-1O^3). Sutber et al. (1992) reported that the maximum advantage of the interval analysis in relation to the point analysis occurs when linked markers are reasonably far apart from each other (a distance between 15 and 35 cM). In such conditions, some exchanges will probably occur between markers and the QTL, which might be compensated by the interval analysis. When the marker loci are too distant (> 35 cM), the interval analysis also should constantly be inefficient towards the detection of QTL in the interval between two marker loci.

In its totality, it was possible to map a region corresponding approximately to 785.7 cM. Considering that the size of the field bean genome is estimated at about 1200 cM (Vallejos et al., 1992), it is estimated that this research was able to map around 66 % of the species genome, although with a low marker saturation. Such coverage may be considered satisfactory compared to other studies (Nodari et al., 1993; Vallejos, 1994).

In this research, the linkage map obtained involved a relatively small number of RAPD markers, which led to a low saturation molecular map. Thus the map presents some uncovered regions that make the identification of QTLs difficult. On the other hand, one could find the amount of information quite acceptable for the maps assembled from RAPD markers if they are built up with high saturation. According to Lanza et al. (2000), a map should be

Table 4. Mapping of the molecular linkage for the common bean utilizing RAPD markers (Random Amplified Polimorphic DNA) and recombining inbred from a bi-parental crossing, Lavras-MG, 2000.

Linkage groups	Markers RAPD (size)	Distance (cM)
1	OPF-10 (1000 pb)	0.0
	OPM-06$^{1\ 1/}$ (1096 pb)	58.1
	OPH-03^3 (1112 pb)	89.8
	OPI-03 (955 pb)	104.1
	OPE-20^2 (891 pb)	133.2
2	OPI-06 (480 pb)	0.0
	OPR-12^4 (794 pb)	24.9
	OPO-10^1 (2951 pb)	38.2
	OPO-20^5 (741 pb)	76.1
	OPO-20^3 (1585 pb)	88.3
	OPO-20^2 (1698 pb)	92.5
	OPO-20^4 (933 pb)	102.9
3	OPN-10 (851 pb)	0.0
	OPM-06^3 (575 pb)	16.0
4	OPG-16^2 (676 pb)	0.0
	OPO-04 (1905 pb)	49.5
	OPE-15^1 (851 pb)	70.3
	OPO-13^1 (1698 pb)	75.0
	OPG-16^3 (602 pb)	81.4
	OPS-13^2 (1122 pb)	88.6
	OPN-02^2 (436 pb)	116.0
	OPO-10^3 (525 pb)	179.1
	OPG-19 (851 pb)	213.1
	OPN-07^1 (1698 pb)	236.0
	OP0-12 (1412 pb)	252.0
5	OPN-07^5 (661 pb)	0.0
	OPN-07^3 (1072 pb)	11.8
	OPG-16^1 (851 pb)	56.1
	OPS-13^1 (1950 pb)	81.0
	OPS-10^2 (562 pb)	92.5
	OPR-02^2 (832 pb)	115.5
	OPR-12^3 (1000 pb)	131.8
	OPO-19^2 (1412 pb)	159.3
	OPAA-09 (1059 pb)	204.3
6	OPR-02^1 (1230 pb)	0.0
	OPB-18 (1412 pb)	29.7
	OPS-16 (640 pb)	48.3
7	OPH-03^4 (759 pb)	0.0
	OPR-12^1 (3200 pb)	29.0

[1/] The superscript refers to the length of the do DNA segment utilized as a marker itself and the smaller the number, the longest the segment.

considered complete when the number of linkage groups obtained through the marker analysis is equal to the basic chromosome number, or when all genetic markers mapped are linked, suggesting that all genome regions are thus represented.

Composed-Interval Mapping (CIM)

The mapping assembled with the first experiment (Figure 2) (Lavras during the winter) identified two QTLs. The first is present in the linkage group 2 and located at 1 cM from the beginning of the group (lod of 4.1). The second is also placed in linkage group 2 but located at 68 cM prior to the beginning of the group (lod of 5.6). When such results are compared with the RAPD-marker linkage map (Table 4), it may be concluded that the first QTL is located between the markers OPI-06 and OPR-12[4], at a distance of 1 cM from the first and of 23.9 cM from the second. The second QTL is placed between markers OPO-10[1] and OPO-20[5], at a distance of 29.8 cM from the first and of 8.1 cM from the second. In the regression analysis, none of those markers were identified as QTL-linked for the trait in question. In the second experiment, no QTLs were identified for the characteristic.

As for the third experiment (Patos de Minas, dryland) (Figure 3), one QTL was identified in linkage group 1, at 56 cM from the beginning (lod of 3.70). This QTL is linked between markers OPF-10 and OPM-06[1], at 56 cM from the first and at 2.1 cM from the second. In the regression analysis, only the marker OPM-06[1] was identified as linked to QTLs involved with the genetic control of the trait. In one of the experiments, this QTL was responsible for more than 12% of the genetic variation, which indicates its potential use for selection purposes. The result for this particular marker may be explained by its smaller distance in relation to the identified QTL (2.1 cM). In the other experiments, taken individually, no further QTL was identified which could be involved in the genetic control of the characteristic. Nevertheless, it is worth mentioning that in one experiment (Patos de Minas, during the winter) (Figure 4), one QTL was identified, with lod of 2.5, in the same position (linkage group 1, located 56 cM from the beginning) of the QTL detected in the third experiment.

Mappings assembled using the means of each location experiment individually (Lavras and Patos de Minas) have not identified any environment-specific QTL for Seed Index. However, when the means of winter cropping (Figure 5) were used, it was possible to identify one QTL in the linkage group 2, located 78 cM from the beginning (lod of 3.70). Such QTL is linked between markers OPO-20[5] and OPO-20[3], at 1.9 cM from the first and at 10.3 from the second. None of those markers were identified as QTL-linked in the regression analysis.

When the mapping was assembled with averages of the dryland experiments (Figure 6), one QTL was also identified in linkage group 1, located 54 cM from the beginning (lod of 3.90). This QTL is linked to the markers OPF-10 and OPM-06[1], 54 cM from the first and at 4.1 cM from the second, practically as it has already been detected in the third experiment, indicating a certain stability of this QTL.

When mapping was done utilizing the means of all experiments, no QTL was identified with lod greater than 2.6, but once again the QTL in linkage group 1 (Figure 7) as it was related previously, has presented

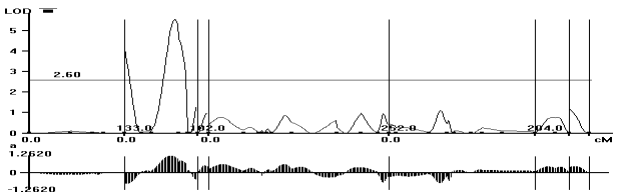

Figure 2. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, winter, Lavras, MG, 1996.

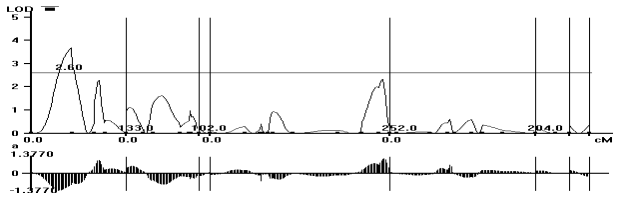

Figure 3. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, dryland, Patos de Minas, MG, 1996.

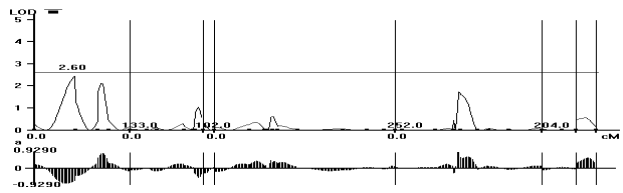

Figure 4. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, winter, Patos de Minas, MG, 1996.

a quite large effect (lod of 2.4), which indicates that there is a great possibility of finding a more stable QTL in this region. Such hypothesis is reinforced when the graphs with all maps are analyzed simultaneously (Figure 8). It can be observed the great number of lod score peaks in the region of linkage group 1, near the region where a very stable QTL was identified in this study.

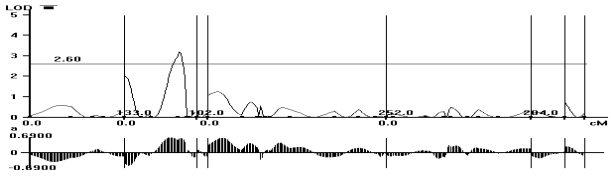

Figure 5. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, all experiments in winter, Lavras e Patos de Minas, MG, 1996/1997.

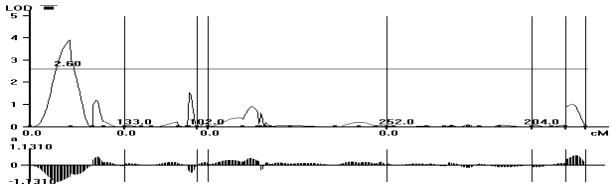

Figure 6. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, all experiments in dryland, Lavras e Patos de Minas, MG, 1997/1998.

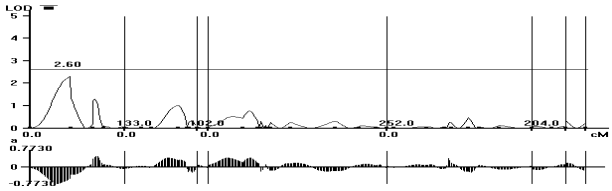

Figure 7. Mapping and aditive effect of QTLs for weight of 100 seed in common bean, all experiments, Lavras e Patos de Minas, MG, 1996/1998.

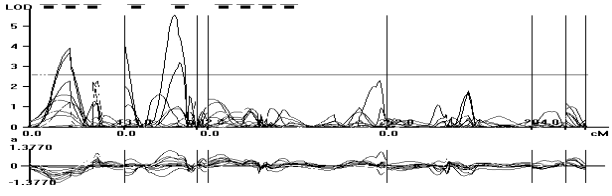

Figure 8. Mapping and aditive effect of QTLs for weight of 100 seed in the common bean, all experiments in different experiments (seasons, locals and years).

CONCLUSIONS

Interaction of QTLs by location were significant, but some stable QTLs were identified.

Multiple regression analysis identified a greater number of QTL-linked markers than the process of composite interval mapping. There was no coincidence between results obtained with the two methods studied.

Molecular markers which were considered of greater potential use on marker-assisted selection for seed weight were OPN-02 (1445 pb) e OPM-06 (1096 pb).

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RESUMO

Estabilidade e mapeamento de QTLs para peso de sementes do feijoeiro em diferentes ambientes

Os objetivos foram: 1- mapear, em diferentes épocas e locais, marcadores RAPD ligados a QTLs de peso de 100 sementes em feijoeiro; 2- avaliar a interação QTLs com locais e épocas de cultivos e; 3- comparar os métodos de mapeamento e regressão múltipla no processo de detecção de QTLs. Foram avaliadas 196 linhagens recombinantes, oriundas do cruzamento entre Carioca e Flor de Mayo, em duas épocas de cultivo do feijoeiro nos anos de 1996, 1997 e 1998, em Patos de Minas e Lavras, MG. Para avaliação fenotípica foram conduzidos sete experimentos utilizando o delineamento experimental em látice quadrado simples. Os resultados mostraram que a interação QTLs por locais foi expressiva, mas foram identificados alguns QTLs com maior estabilidade. O método da regressão múltipla identificou mais marcadores ligados a QTLs do que o processo de mapeamento por intervalo composto, não havendo grande concordância entre os resultados apresentados pelos dois métodos. Os marcadores que se mostraram mais estáveis e promissores para serem utilizados na seleção assistida por marcadores para peso de sementes foram OPN-02 (1445 pb) e OPM-06 (1096 pb).

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