Use of the QTL approach to the study of soybean trait relationships in two populations of recombinant inbred lines at the F_7 and F_8 generations

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Received: 14/02/2006, Accepted: 26/06/2006

This work aimed to identify the quantitative trait loci (QTL) associated with photosynthesis and growth and productivity traits of soybean and to study possible associations between these traits by the analysis of coincidence of QTL in linkage groups (LGs). Thus, populations of recombinant inbred lines (RILs) of the F_7 and F_8 generations derived from the cross between the varieties BARC-8 and Garimpo were used. The traits evaluated were net assimilation rate of CO_2 under saturating light (A_{sat}), potential photosynthesis rate (P_{max}), leaf area (A), specific leaf area (SLA), specific leaf nitrogen (N); root (W_R), nodule (W_N), stem (W_{ST}), leaf (W_L), pod (W_P) and plant dry mass (W_T); nodule (n_N), seed (n_S), and pod number (n_P); seed fresh mass per plant (W_S), one-hundred seed fresh mass (W_{HS}) and seed protein percentage (P%). It was possible to identify the following QTL associated with the following soybean traits: SLA, A_{sat}, N, W_R, W_{ST}, W_L, W_T, W_P, W_{HS}, n_S and n_P, indicating that the RIL population has a great potential for mapping loci associated with quantitative traits of the soybean crop. The correlations between the soybean traits were partially confirmed by coincidence of QTL.

Key words: *Glycine max*, correlation, genes, photosynthesis, quantitative trait loci.

Identificação e uso de QTL no estudo de correlações entre carateres quantitativos em populações RILs de soja nas gerações F_7 e F_8: Este trabalho objetivou identificar locos de carateres quantitativos (QTLs) assosciados com fotossíntese, crescimento e produtividade de soja com a finalidade de identificar possíveis associações entre estas características pela análise de coicindência de QTLs nos respectivos grupos de ligação (GL). Para isto, foram usadas populações de linhagens recombinantes endogâmicas (RILs) das gerações F_7 e F_8 derivadas dos cruzamentos entre as variedades BARC-8 e Garimpo. As características avaliadas foram taxa de assimilação líquida de CO_2 sob luz saturante (A_{sat}), taxa de fotossíntese potencial (Pmax), área foliar (A), área foliar específica (SLA), nitrogênio foliar específico (N); número de nódulos (n_N), sementes (n_S) e vagens (n_P); massa fresca de sementes por planta (W_S), massa fresca de 100 sementes (W_{HS}) e percentagem de proteína da semente (P%). Foi possível identificar os seguintes QTLs associados com as características: SLA, A_{sat}, N, W_R, W_{ST}, W_L, W_T, W_P, W_{HS}, n_S e n_P, indicando que as populações de RIL apresentam grande potencial para mapeamento de locos associados com caracteres quantitativos em soja. As correlações entre os caracteres analisados foram parcialmente confirmadas pela coincidência de QTL.

Palavras-chaves: *Glycine max*, correlações, genes, fotossíntese, locos de caracteres quantitativos.

INTRODUCTION

Identification of QTL ("Quantitative Trait Loci") or controllers of quantitative traits associated with production and the conditions where such an association is lacking, may help develop gene-based simulation production models for a particular crop (Boote et al., 2001). Thus, the positioning of QTL directly associated with production can be compared with those associated with dry mass accumulation in other parts of the plant.

Few studies have been carried out aimed at identifying QTL associated with important physiological traits, such as photosynthetic rate, and soybean growth and productivity traits, simultaneously.

Traditional physiological studies approach the relationships between physiological traits without taking into account the genetic basis. Prioul et al. (1997) points out that if any association inconsistency is established in these studies it cannot be explained in terms of compartmentalization or interactions. Besides, it cannot be determined whether such correlations are induced by causal relationships between the traits or by other factors, such as linkage proximity between genes with opposing actions. Lebreton et al. (1995) and Price et al. (2002) used the analysis of coincidence of QTL to test causal relationships between plant traits associated with plant resistance to drought.

Physiological studies may benefit from the understanding of how quantitative traits are genetically controlled. Thus, it is necessary to identify various QTL along the genome for a simultaneous test of coincidence between various traits. One should also look for significant QTL for the traits under different environmental conditions. If two traits are correlated, there is a great chance of having QTL associated in the same chromosome region (Prioul et al., 1997).

This work aimed to: (i) identify QTL associated with photosynthesis, growth and productivity traits of the soybean; (ii) identify likely associations between these soybean traits by using the analysis of coincidence of QTL and (iii) evaluate the population of RILs to use it as a mapping population of loci associated with different soybean traits.

MATERIAL AND METHODS

This study involved 118 Recombinant Inbred Lines (RILs) derived from a cross between the soybean varieties BARC-8 and Garimpo.

The F_6 generation of the RILs was obtained by Soares (2000), by single seed descent from the F_2 generation. The F_7 and F_8 generations were obtained from the F_6 generation by self-pollination. The F_7 and F_8 generations together with the parental lines constituted the genetic material used in this work. Each RIL and the parental lines were represented by five plants, cultivated in a greenhouse. The F_7 and F_8 generations of the RILs and the parental lines were cultivated in a nursery. Sowing was carried out in December 1999 and 2000 for the F_7 and F_8 generations, respectively. Plastic pots containing a 2.5 L of a soil:manure:sand (3:1:1, w/w/w) mixture were used. Approximately 330 kg $N.ha^{-1}$, 64 kg $P_2O_5.ha^{-1}$ and 141 kg $K_2O.ha^{-1}$ were incorporated into the soil mixture in 1999. In order to evaluate whether a significant interaction exists between the genotype and the environment with respect to the identification of QTL, nitrogen fertilization was suppressed for the F_8 generation in the year 2000.

Net photosynthesis under saturating light (A_{sat}) conditions was measured in fully-expanded leaves, located at the upper third of the plant, containing at least one pod at the seed filling stage (between R5 and R6), using an infrared gas analysis system (IRGA), model Li-6400 (Li-Cor, Lincoln, NE), at 1500 μmol m^{-2} s^{-1} of photosynthetic photon flux (*PPF*) and a temperature of 25°C, as described by Griffin and Luo (1999). Leaf disc samples of 10 cm^2 of area were removed for determination of the O_2 evolution rate under saturating CO_2 and light conditions, i.e., the potential photosynthetic rate (P_{max}), expressed in μmol $O_2.m^{-2}.s^{-1}$, using a LD2 oxygen electrode (Hansatech Ltd, King's Lynn, U.K.) regulated to maintain radiation intensity on the leaf disc between 1500 and 1800 $\mu mol.m^{-2}.s^{-1}$ of PPF, and a CO_2 saturating condition using a 2 M solution of potassium carbonate at 25°C. An additional leaf disc sample was removed from the F_8 generation, which, combined with the first sample, constituted the material for analysis of specific leaf nitrogen, expressed as g $N.m^{-2}$ (*N*), using the Kjeldahl method, as described by Lugg and Sinclair (1981).

During the seed filling period (between R5 and R6) for two plants of each RIL, following leaf disc sample collection, leaf area (*A*) was estimated using a leaf area integrator ΔT area meter, model MK2 (AT Delta-T Devices Ltd.). After 48 h of drying at 70°C until constant mass, dry mass was determined using a precision balance and expressed in grams of the following plant parts: stem (W_{ST}), leaves (W_L), roots (W_R), pods (W_P), nodules (W_N) and total (W_T) and number of nodules (n_N), seeds (n_S), and pods (n_P); seed fresh mass per plant (W_S), one-hundred seed fresh mass (W_{HS}), with W_N and n_N being evaluated only in the F_8 generation. Based on the primary data of leaf dry mass and leaf area, specific leaf

area (*SLA*) was calculated representing the leaf area (m^2) per Kg leaf weight. Productivity traits were measured (Fehr and Caviness, 1977) on the three remaining plants at the R8 development stage plus 10 days, and expressed as fresh mass of the seeds per plant in grams (W_S), one-hundred seed fresh mass in grams (W_{HS}) and production quality in protein content (%P), using the Kjeldahl method (Instituto Adolfo Lutz, 1985).

The experiment was arranged in a completely randomized design. Phenotypic variance ($\hat{\sigma}_f^2$) was estimated by the ratio between the mean square of the treatments or genotypes (MSG) and the number of replicates (k) considered for each trait while the genotypic variance ($\hat{\sigma}_g^2$) was estimated using the following equation:

$\hat{\sigma}_g^2 = (MSG - MSR) / k$,

where MSR is the mean square of the residue.

Heritability in its broadest sense (h^2) was estimated by the equation: $(\hat{\sigma}_g^2/\hat{\sigma}_f^2)$ x 100.

Variance analysis of the phenotypic data was performed for the traits evaluated in the generations and for different environments to determine the existence of significant genetic variability between the lines and interaction with the environment.

Phenotypic correlation matrices were built between the traits evaluated. The phenotypic correlations between the traits were compared with the coincidence, or not, of QTL identified for the respective phenotypes.

For the mapping of the QTL to the soybean traits, the phenotypic values were associated with a molecular linkage map initially defined by Soares (2000) using microsatellite markers, which was later partially saturated with microsatellite markers (Oliveira, 2002) and RAPD markers (Miranda, 2002). This map used as reference the nomenclature for the linkage groups (LGs) described by Cregan et al. (1999) working with F_2 populations of an interspecific cross of *G. soja* and *G. max*. This map has 24 LGs totalizing 523.17cM containing 75 molecular markers. Of these, 54 markers are microsatellite types while 21 are RAPD types.

The mapping of microsatellite and RAPD markers and the establishment of the linkage groups were carried out using the program GQMol (Cruz and Schuster, 2000). The markers were grouped by using a *LOD minimum score* of 3.0 and a maximum recombination frequency of 0.40. Kosambi mapping distance was used for the conversion of the recombination frequency into centiMorgans (cM).

The linkage map data were used to map the QTL along the linkage groups by means of compound interval mapping (Zeng, 1994), using the Windows QTL Cartographer version 2.0 (Basten et al., 2002).

The cut-off point of maximum likelihood ratio to consider the presence of QTL by using the compound interval mapping was obtained by a permutation test, as reported by Deorge and Churchill (1996).

RESULTS

The individual variance analyses of each trait and the estimates of different genetic parameters in the F_8 generation (table 1) of the seventeen traits evaluated, shows that only one failed to present significant genetic variability. This is important, since the occurrence of genetic variability in a mapping population is one of the requirements for detecting QTL. Also, it can be seen in table 1 that the heritability values ranged from 18.73%, for nodule number, to 99.86%, for seed protein content.

QTL associated with 9 of the 14 traits studied were identified in the F_7 generation. These QTL were located at intervals within 10 of the 24 linkage groups named according to Cregan et al. (1999). A single interval was associated with *SLA* in the LG C1 whereas only 7.8% of the phenotypic variation of A_{sat} was explained by a locus located in the LG O (table 2). Overall, the QTL found to be associated with *A* and with traits related to biomass in the seed filling stage (*SLA*, W_{ST}, W_R, W_T and W_P) are located at five intervals in linkage groups A1, C1, C2, D1b + W and O (table 2). Besides, QTL located in other linkage groups (G, O, L, F and N) also explain a fraction of phenotypic variation of *A*, W_{ST}, W_R, W_T and W_P (table 2). Also in table 2, it can be observed that in the LG L, a QTL was found associated with W_{ST}. Moreover, a coincidence of QTL is noted in two linkage groups for the traits W_{HS} and n_S, a fact only observed for this association (table 2). On the other hand, it was not possible to identify coincident QTL between A_{sat} and the traits P_{max}, *SLA*, n_S and W_S despite the significant correlations shown in table 4. In the F_8 generation, QTL were identified associated with 10 of the 17 traits studied, at intervals located in 8 of the 24 linkage groups considered (table 3). A single molecular interval in LG A1 was identified as being associated with specific leaf nitrogen (*N*), while 14.72% of the phenotypic variation of A_{sat} was explained by the loci positioned in the LG J (table 3).

The traits *A*, W_L, W_{ST}, W_T and W_{HS} have compatible QTL, located in LG M, explaining 10.47, 8.86, 8.43, 13.78 and 20.73% of the phenotypic variations of these traits, respectively (table 3).

Table 1. Genotype (MSG) and residue(MSR) mean square, estimate of phenotypic variance ($\hat{\sigma}_f^2$), genotypic variance ($\hat{\sigma}_g^2$), heritability values (h^2) estimated in the F_8 generation of a population of recombinant inbred lines (RILs), not submitted to nitrogen fertilization and mean phenotypic values of the parental lines BARC-8 and Garimpo.

	N	*SLA*	A_{sat}	P_{max}	A	W_L	W_{ST}	W_R	W_P	W_N	W_T	W_S	W_{HS}	n_S	n_N	n_P	%P
MSG	0.43	387.00	95.58	289.45	0.01	50.50	70.65	14.34	62.50	0.37	479.55	125.78	13.68	5816.0	3481.9	1254.07	20.44
MSR	0.20	127.00	47.59	193.64	$1.0x10^{-3}$	8.25	8.85	9.32	28.86	0.25	99.54	33.22	4.49	1594.8	2829.7	322.43	0.03
F	2.22*	3.04*	2.00*	1.49*	5.43*	6.12*	7.98*	1.54*	2.17*	1.47*	4.81*	3.79*	3.05*	3.65*	1.23^{ns}	3.89*	739.69*
$\hat{\sigma}_f^2$	0.06	12.90	13.65	41.35	$2.7x10^{-3}$	25.25	35.32	7.17	31.25	0.19	239.78	41.93	4.56	1938.6	1740.9	418.02	10.22
$\hat{\sigma}_g^2$	0.034	8.67	6.86	13.69	$2.0x10^{-3}$	21.12	30.90	2.51	16.82	0.06	190.00	30.85	3.06	1407.0	326.1	310.55	10.21
h^2(%)	55.01	67.14	50.22	33.10	81.60	83.66	87.47	35.02	53.83	32.02	79.24	73.59	67.19	72.5	18.7	74.29	99.86
BARC-8	1.33	65.30	12.52	29.54	0.016	5.22	4.52	4.30	0.50	0.38	42.99	11.99	10.52	83.0	24.0	37.50	49.09
Garimpo	1.83	70.15	22.25	44.64	0.025	11.13	8.97	9.13	3.21	1.51	55.47	22.79	14.97	116.0	349.5	49.00	40.49

* = Significant F value; ^{ns} = non significant F value at 5% probability. Specific leaf nitrogen content, g $N.m^{-2}$ (*N*); specific leaf area $m^2.kg^{-1}$ (*SLA*); net assimilation rate of CO_2, $\mu mol.m^{-2}.s^{-1}$ (A_{sat}); potential photosynthesis rate, µmol de $O_2.m^{-2}.s^{-1}$ (P_{max}); leaf area, m^2 (*A*); dry mass of leaves, g (W_L); stem, g (W_{ST}); roots, g (W_R); pods, g (W_P); nodules, g (W_N) and total, g (W_T); fresh mass of seeds per plant, g (W_S); one-hundred seed fresh mass, g (W_{HS}); number of seeds per plant (n_S); number of nodules (n_N); number of pods (n_P) and seed protein percentage (P%)

The coincidence of the QTL in LG M, associated with the leaf area and biomass-related traits (W_L, W_{ST} e W_T), as well as to one-hundred seed dry mass (W_{HS}), could explain the significant correlations between these traits, as shown in table 4, ranging from 0.96 (W_{ST} x W_T) to 0.39 (W_{ST} x W_{HS}).

Productivity-related traits such as W_S, n_S and n_P, in general, do not have QTL in common with those identified for the other variables, except for one present in LG M associated with W_{HS}, as previously discussed. However, the likelihood of one interval located in the LG K, explaining 13.23, 24.51 and 7.6% of the phenotypic variation of W_S, n_S and n_P, respectively, was observed (table 3). Significant correlations above 0.88 observed among these traits (table 4) may be attributed to the coincidence of the QTL in the LG K. It should be taken into account that the nature of this correlation is phenotypic, thus, one portion may have an environmental basis.

When comparing tables 2, 3 and 4, it is not possible to identify coincident QTL among some traits with physiological associations described in the literature, namely: significant correlations between A_{sat} x P_{max}, *N* x A_{sat}, *N* x *SLA*, *SLA* x A_{sat} and A_{sat} with productivity components such as n_S and W_S.

DISCUSSION

The heritability for P% in the F8 generation was above the value of 73.40% estimated for this population in the F_6 generation under field conditions (Soares, 2000) while the heritability for A_{sat} of 50% shown in table 1 is above the value of 41% estimated by Harrison et al. (1981) for canopy-apparent photosynthesis during seed filling in G. max. On the other hand, Wiebold et al. (1981) observed a low to moderate (36% to 56%) heritability in the broad sense, for net photosynthesis of individual leaves, in the initial F_3 and F_4 generations using an infrared gas analysis system at temperatures varying from 29 to 31ºC.

Table 1 shows heritability values for W_{ST}, n_N e W_N of 87.47%, 18.70% e 32.02%, respectively. These values are in agreement with the observations of Santos et al. (2006) who estimated heritability values of 49% for stem dry mass, 30% for nodule number, and 33% for nodule dry mass, in a segregating population composed of 157 recombinant inbred lines derived from the cross between two soybean cultivars identified as contrasting for biological nitrogen fixation capacity. Therefore, it can be concluded that the evaluated traits are under environment effects, as well as that the present population can be used in studies aimed at evaluating heritability of such traits.

Nicolás et al. (2006) studying QTL associated with nodulation and stem dry mass of soybean plants in F_2 and F_3 generations observed a positive and significant correlation (0.91**) with W_N and nitrogen (N) content of the whole plants and a coincidence of QTL for W_N, W_{ST} e n_N in LG D1b+W, B2 and H/J. In this work we observed a positive and significant correlation of 0.29* between W_{ST} and N (table 4). However, as shown in table 3, we identified only one QTL associated with leaf area in LG D1b+W. A QTL associated with W_{ST} was identified in LG M and no QTL was identified for traits such as n_N and W_N.

A single interval was associated with *SLA* in the LG C1 whereas only 7.8% of the phenotypic variation of Asat was explained by a locus located in LG O (table 2). Taken as a whole, the QTL found to be associated with A and with traits related to biomass in the seed filling stage (*SLA*, W_{ST}, W_R, W_T and W_P) are located at five intervals, located in linkage groups A1, C1, C2, D1b + W and O (table 2). In addition, QTL located in linkage groups G, O, L, F and N also explain a fraction of the phenotypic variation of A, W_{ST}, W_R, W_T and W_P (table 2). A QTL was found in LG L associated with WST and a coincidence of QTL is noted in two linkage

Table 2. Intervals containing quantitative trait loci (QTL) associated with specific leaf area (*SLA*); net assimilation rate of CO_2 (A_{sat}); leaf area (A); dry mass of stem (W_{ST}), root (W_R), plant (W_T) and pods (W_P); one-hundred seed fresh mass (W_{HS}) and number of seeds per plant (n_S) identified in the F_7 generation of a population of recombinant inbred lines (RILs), derived from the cross between the varieties BARC-8 and Garimpo.

Traits	Linkage Groups	Interval	Size of Interval (cM)	QTLPosition (cM)	R^2 (%)	LR
SLA	C1	Satt139 ~ Satt476	11.2	8.00	9.90	13.71
A_{sat}	O	Satt241 ~ Satt345	2.1	0.23	7.80	10.66
	A1	Satt449 ~ Satt526	7.7	0.18	16.52	12.99
A	G	Satt594 ~ Satt303	6.4	0.18	7.66	10.31
	O	Satt241 ~ Satt345	2.1	0.23	11.00	14.35
	O	Satt241 ~ Satt345	2.1	0.23	7.30	9.78
W_{ST}	C2	Satt281 ~ Satt422	19.3	1.9	7.30	10.15
	L	Satt462 ~ Satt523	5.6	0.02	10.00	8.40
	F	Satt193 ~ Satt325	13.2	1×10^{-3}	7.17	10.40
W_R	O	Satt241 ~ Satt345	2.1	0.23	7.60	10.42
W_T	O	Satt241 ~ Satt345	2.1	0.23	8.10	10.86
	C2	Satt281 ~ Satt422	19.3	1.8	7.50	9.12
	A1	Satt449 ~ Satt526	7.7	0.14	11.80	8.48
W_P	D1b + M	Satt350 ~ Satt506	4.9	0.15	12.26	14.70
	N	Satt-091 ~ Satt549	5.4	0.05	13.27	18.79
	C1	OPACO2 ~ OPAN09	10.4	0.10	11.53	10.89
	G	Satt594 ~ Satt303	6.4	0.18	6.80	9.35
W_{HS}	O	Satt241 ~ Satt345	2.1	0.23	7.00	9.95
	K	Satt475 ~ OPAW09a	24.5	1×10^{-4}	9.40	11.67
n_S	O	Satt241 ~ Satt345	2.1	0.23	7.20	8.77
	K	Satt475 ~ OPAW09a	24.5	1×10^{-4}	7.70	9.24

R^2 represents the percentage of phenotypic variation, explained by the interval and LR (*Likelihood ratio*) or maximum likelihood ratio, significant at 5% probability. The brackets group two or more QTL associated with the same trait. QTL position in cM is given by the marker on the left.

groups for the traits W_{HS} and n_S, a fact only observed for this association (table 2). On the other hand, it was not possible to identify coincident QTL between A_{sat} and the traits P_{max}, *SLA*, n_S and W_S despite the significant correlations shown in table 4. A single molecular interval in LG A1 was identified as being associated with specific leaf nitrogen (*N*), while 14.72% of the phenotypic variation of A_{sat} was explained by the loci located in the LG J (table 3).

The traits *A*, W_L, W_{ST}, W_T and W_{HS} have compatible QTL, located in the LG M, explaining the values 10.47, 8.86, 8.43, 13.78 and 20.73% for the phenotypic variations of these traits, respectively (table 3).

The identification of QTL associated with *A* and biomass-related traits during seed filling (W_{ST}, W_R, W_T) in the linkage groups A1, C1, C2, D1b + W and O confirms, in part, the observation made by Mansur et al. (1993) that the LGs C1, C2 and D1 have loci associated with the traits leaf area and maturity of the soybean. The identification of a QTL associated with W_{ST} in the LG L confirms the observation that in this LG there is a QTL associated with WST (Santos et al., 2006) and plant height of *G. max* (Lee et al., 1996).

The coincidence between QTL associated with leaf area and biomass related traits could explain the significant phenotypic correlations ranging from 0.22 (between W_{ST} and W_P) to 0.96 for W_{ST} and W_T (table 2). Except for an interval located in the LG O associated with seven of the nine traits, a separation of the QTL associated with W_{HS} and n_S from those related to the other traits was observed (table 2). The QTL grouping associated with n_S and W_{HS} may partially explain the negative correlation of -0.19 shown in table 4.

Another fact that must be emphasized concerns the P% correlations which were significant for various traits in the F_7 generation (table 4). However, these correlations were not confirmed for the F_8 generation. This fact may be explained,

Table 3. Intervals containing quantitative trait loci (QTL) associated with specific leaf nitrogen (*N*); net assimilation rate of CO_2 (A_{sat}); leaf area (*A*); dry mass of leaves (W_L), stem (W_{ST}) and total (W_T); fresh mass of seeds per plant (W_S); one-hundred seed fresh mass (W_{HS}); number of seeds per plant (n_S) and number of pods (n_P) identified in the F_8 generation of a population of recombinant inbred lines (RILs), derived from a cross between the varieties BARC-8 and Garimpo. This population was not submitted to nitrogen fertilization.

Traits	Linkage Groups	Interval	Size of interval (cM)	QTL Position(cM)	R^2 (%)	LR
N	A1	Satt449 ~ Satt526	7.7	1.70	16.85	10.73
A_{sat}	J	Satt215 ~ Satt183	4.4	2.00	14.72	12.82
A	D1b + W	Satt350 ~ Satt506	4.9	0.12	11.33	8.39
	M	OPAPO4a ~ OPAPO4b	16.1	0.02	10.47	10.48
W_L	M	OPAPO4a ~ OPAPO4b	16.1	1 x 10^{-4}	8.86	11.28
W_{ST}	M	OPAPO4a ~ OPAPO4b	16.1	0.01	8.43	9.97
W_T	N	Sat-091 ~ Satt594	5.4	0.05	7.30	8.46
	M	OPAPO4a ~ OPAPO4b	16.1	0.03	13.78	11.93
W_S	K	Satt475 ~ OPAW09a	24.5	0.20	13.23	8.68
W_{HS}	M	OPAPO4a ~ OPAPO4b	16.1	0.09	20.73	11.11
n_S	K	Satt475 ~ OPAW09a	24.5	0.24	24.51	10.87
n_P	C1	Satt139 ~ Satt476	11.2	5.00	7.30	9.71
	K	Satt475 ~ OPAW09a	24.5	0.24	7.60	9.48

R^2 represents the percentage of phenotypic variation explained by the interval and the LR (*Likelihood ratio*) or maximum likelihood ratio significant at 5% probability. The brackets group two QTL associated with the same trait. The position of QTL in cM is given by the marker on the left.

Table 4. Correlation matrix for the traits measured in the F_8 generation (superior diagonal) and F_7 generation (inferior diagonal) of the RILs (recombinant inbred lines) derived from the cross between the varieties BARC-8 and Garimpo. Specific leaf nitrogen, $g.m^{-2}$ (*N*); specific leaf area, $m^2.kg^{-1}$ (*SLA*); net assimilation rate of CO_2, $\mu mol.m^{-2}.s^{-1}$ (A_{max}); potential photosynthesis rate, $\mu mol.m^{-2}.s^{-1}$ (P_{max}); leaf area, m^2 (*A*); dry mass of leaves, g (W_L); stem, g (W_{ST}); roots, g (W_R); pods, g (W_P); nodules, g (W_N) and total, g (W_T); fresh mass of seeds per plant, g (W_S); one-hundred seed fresh mass, g (W_{HS}); number of seeds per plant (n_S); number of nodules (n_N); number of pods (n_P) and seed protein percentage(P%).

	N	*SLA*	A_{sat}	P_{max}	*A*	W_L	W_{ST}	W_R	W_P	W_N	W_T	W_S	W_{HS}	n_S	n_N	n_P	%P
P%	0.16^{ns}	0.18^{ns}	0.41*	0.27*	0.28*	0.30*	0.32*	0.31*	0.22*	0.21*	0.31*	0.50*	0.67*	0.52*	0.18^{ns}	0.52*	
n_P	0.41*	0.37*	0.34*	0.44*	0.59*	0.62*	0.62*	0.42*	0.36*	0.39*	0.57*	0.88*	0.43*	0.99*	0.31*		0.01^{ns}
n_N	0.27*	0.30*	0.20*	0.13^{ns}	0.34*	0.32*	0.31*	0.42*	0.22*	0.86*	0.35*	0.36*	0.21*	0.31*			
n_S	0.44*	0.39*	0.37*	0.44*	0.60*	0.63*	0.64*	0.43*	0.36*	0.38*	0.59*	0.89*	0.43*			0.96*	0.00^{ns}
W_{HS}	0.28*	0.37*	0.56*	0.45*	0.36*	0.38*	0.39*	0.44*	0.30*	0.20*	0.43*	0.53*		-0.19*		-0.18^{ns}	-0.05^{ns}
W_S	0.41*	0.41*	0.34*	0.41*	0.61*	0.60*	0.61*	0.42*	0.42*	0.43*	0.59*		0.18*	0.89*		0.88*	-0.01^{ns}
W_T	0.68*	0.74*	0.32*	0.42*	0.89*	0.94*	0.96*	0.82*	0.67*	0.41*		0.60*	-0.02^{ns}	0.62*		0.61*	0.02^{ns}
W_N	0.29*	0.26*	0.08^{ns}	0.07^{ns}	0.39*	0.38*	0.38*	0.46*	0.31*								
W_P	0.28*	0.33*	0.15^{ns}	0.15^{ns}	0.47*	0.45*	0.53*	0.34*			0.33*	0.14*	-0.27*	0.23*		0.22*	0.08^{ns}
W_R	0.69*	0.71*	0.37*	0.47*	0.71*	0.78*	0.76*		0.24*		0.93*	0.62*	-0.02^{ns}	0.62*		0.62*	0.03^{ns}
W_{ST}	0.71*	0.72*	0.29*	0.44*	0.92*	0.96*		0.92*	0.22*		0.96*	0.61*	-0.04^{ns}	0.62*		0.63*	0.00^{ns}
W_L	0.70*	0.72*	0.29*	0.44*	0.92*		0.92*	0.85*	0.23*		0.95*	0.55*	0.04^{ns}	0.54*		0.54*	-0.01^{ns}
A	0.65*	0.82*	0.22*	0.38*		0.90*	0.85*	0.82*	0.28*		0.88*	0.54*	-0.02^{ns}	0.57*		0.55*	0.04^{ns}
P_{max}	0.58*	0.46*	0.52*		0.31*	0.36*	0.41*	0.45*	0.13^{ns}		0.45*	0.50*	0.07^{ns}	0.53*		0.47*	0.01^{ns}
A_{sat}	0.43*	0.40*		0.59*	0.40*	0.39*	0.42*	0.51*	0.04^{ns}		0.46*	0.55*	0.13^{ns}	0.57*		0.54*	0.02^{ns}
SLA	0.74*		0.42*	0.13^{ns}	0.51*	0.32*	0.23*	0.27*	-0.08^{ns}		0.31*	0.05^{ns}	0.20*	0.07^{ns}		0.03^{ns}	-0.01^{ns}

* = Significant correlation; ^{ns} = non-significant correlation at 5% probability.

in part, by the significant genotype x environment interaction observed for this trait (table 5).

The comparison of tables 2 and 3 shows that the identification of QTL may vary as a function of the environment/generation considered. Only one QTL was detected in both environments, associated with the number of seeds per plant (n_s) located in LG K (tables 2 and 3), despite the genotype-environment interaction being significant (table 5). Thus, of the 34 QTL detected, 33 were considered environmental specific. This was also observed by Lee et al. (1996) when identifying QTL related with seed protein and oil contents at three different locations.

Although the associations between some physiological traits were recurrent in the literature, such as *N* versus A_{sat} (Lugg and Sinclair, 1981; Evans, 1983; Suan-Chin et al., 1985; Sinclair and Horie, 1989), in our study the associations *N* versus *SLA*(Lugg and Sinclair, 1981), *SLA* versus A_{sat} (Lugg and Sinclair, 1981; Beadle, 1993) and A_{sat} versus W_S (Harrison et al., 1981) could not be confirmed at the level of coincidence of QTL. Though Santos et al. (2006) related the presence of coincident QTL in LG C2 for WN and nN, this was not observed in the F_8 generation in the present work (table 3).

The analysis of photosynthetic CO2 rate assimilation in leaves and canopy can express the plant response to environment. However, none of these analyses gives information about the partition of dry mass or about competition between sinks that determine if the assimilated carbon will be converted, for instance, to oil, protein or carbohydrates (Beadle, 1993). In potato, although fruit development increased the rate of net photosynthesis, without affecting total dry matter yield, it accelerated plant maturity and decreased the partitioning of assimilates to leaves, stems and tubers (Tekalign, 2005). Kumudini (2002) concluded that the relationship between source and sink is a quantitative trait regulated by several factors. In the present study we observed independence of genomic regions associated with Asat from those associated with *A*, W_L, W_{ST}, W_T, W_{HS} W_S, n_N and n_P.

The non-detection of coincident QTL for these traits may be explained by the non-saturated linkage map used in this study. The loci mapped in the present study cover a region of about 17.25% of the soybean genome (523.17 cM). Therefore, other markers are being used to better saturate the mapped regions. On the other hand, it is known that QTL for grain production, plant height and number of leaves of Z. mays interact significantly with the environment, presenting low genetic correlations and a small number of QTL mapped in a single region. The low number of stable QTL across environments imposes additional challenges to design marker-assisted selection in tropical areas, unless the breeding program is directed towards specific target areas (Milena et al., 2006).

A spatial QTL separation pattern is also observed, characterized by the independence of the QTL related to the traits Asat and N, in relation to the QTL associated with *A*, W_L, W_{ST}, W_T, and W_{HS} and in relation to the third QTL group related to the traits WS, n_s and n_P.

This result suggests that the models aiming to predict soybean productivity, using *SLA*, for instance, as independent variable, may present low efficiency. The identification of production-related QTLs may help to develop production simulation models based on genes for a particular crop (Boote et al., 2001).

Table 5. Analysis of variance of the data of the F_7 and F_8 generations for the mean squares of genotypes, environments and interaction (genotype x environment) for specific leaf area, $m^2.kg^{-1}$ (*SLA*); net assimilation rate of CO_2, $\mu mol.m^{-2}.s^{-1}$ (A_{sat}); potential photosynthesis rate, µmol de $O_2.m^{-2}.s^{-1}$ (P_{max}); leaf area, m^2 (*A*); dry mass of leaves, g (W_L); stem g (W_{ST}); roots, g (W_R); pods, g (W_P) and plant, g (W_T); fresh mass of the seeds per plant, g (W_S); one-hundred seed fresh mass, g (W_{HS}); number of seeds per plant (n_S); number of pods (n_P) and seed protein percentage (P%).

Trait	Genotype	Environment	Interaction (genotype x environment)
SLA	183.4^{ns}	115347.2*	225.1*
A_{sat}	64.9*	1848.9*	59.9^{ns}
P_{max}	316.6*	4680.4*	187.8^{ns}
A	2.6 x 10^{-3} ^{ns}	1.183*	2.7 x 10^{-3}*
W_L	31.6^{ns}	16855.6*	25.4*
W_{ST}	83.9*	5593.3*	44.1*
W_R	48.4^{ns}	44.5	275.1*
W_P	59.2^{ns}	5372.0*	49.2*
W_T	371.7*	1775.0*	241.1*
W_S	93.3*	884.7*	67.0*
W_{HS}	83.9*	5593.3*	44.1*
n_S	4692.6*	1773.7^{ns}	2701.1*
n_P	1101.8*	108.2^{ns}	580.1*
%P	24.0*	101.7*	13.0*

* = Significant at 5% probability; ^{ns} = non-significant correlation at 5% probability.

Therefore, only in the case of having coincident QTL for different populations/environments, saturated maps and confirmation between association of QTL with different traits could one foresee the use of these tools in breeding programs based, for instance, on marker-assisted selection. The use of RILs is of particular interest, as they could be replicated in many different environmental conditions. This work constitutes the first genetic study of photosynthesis-related traits upon soybean productivity, and has led to the description of important markers for breeding programs.

Therefore, there is a great potential for improving the integration of physiological studies with breeding programs, using mathematical models to predict soybean production, mainly based on the coincidence of QTL associated with production and physiological traits, such as CO2 net assimilation rate.

Thus, under the conditions this experiment was carried out, it can be concluded that:

a) It was possible to identify QTL associated with various soybean traits, namely: specific leaf area, net CO_2 assimilation rate under saturating light, specific leaf nitrogen, dry mass of roots, stem, leaves, total plant, one-hundred seed fresh mass, and number of seeds and pods per plant;

b) The correlations between the soybean traits were confirmed partially by the coincidence of the QTL;

c) The RIL population evaluated shows a great potential for mapping loci associated with quantitative traits of the soybean.

Acknowledgements: To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). To the Universidade Federal de Viçosa (UFV), particularly, to the Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), Unidade de Crescimento de Plantas (UCP) and the Graduate Program in Plant Physiology for the opportunity to develop this work. To Gerardo Cervigni, for his valuable help with the statistical analyses. To Mercês Bustamante, for her dedication and precious help with the determination of potential photosynthesis rate.

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