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Field Crops Research 95 (2006) 355–366

Field Crops Research

www.elsevier.com/locate/fcr

Identification of quantitative trait loci controlling nodulation and shoot mass in progenies from two Brazilian soybean cultivars

Marisa F. Nicolás [a,b,1], Mariangela Hungria [b,*], Carlos A.A. Arias [a]

[a] *Universidade Federal do Paraná, Dept. de Genética, Centro Politécnico, Jardim das Américas,*
81531-990 Curitiba, PR, Brazil
[b] *Embrapa Soja, Cx. Postal 231, 86001-970 Londrina, PR, Brazil*

Received 16 March 2005; accepted 16 April 2005

Abstract

Nitrogen (N) demand of soybean [*Glycine max* (L.) Merrill] can be supplied via biological nitrogen fixation (BNF), however, higher yielding cultivars increase plant demand for N. Phenotypes differing for traits associated with biological nitrogen fixation result from the expression of the multiple genes of both the host plant and the microsymbiont, but limited studies have been done on the genetics of the soybean BNF. Integrated maps of soybean with simple sequence repeat (SSR) markers [Cregan, P.B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., Van Toai, T.T., Lohnes, D.G., Chung, J., Specht, J.E., 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39, 1464–1491.] offer an excellent opportunity for the identification of traits related to BNF. This study aimed at the identification of quantitative trait loci (QTLs) controlling BNF and nodulation in an F_2 population of 160 plants derived from an intraspecific cross between two Brazilian cultivars, Embrapa 20 × BRS 133, previously identified as having good potential for mapping of QTLs [Nicolás, M.F., Arias, C.A.A., Hungria, M., 2002. Genetics of nodulation and nitrogen fixation in Brazilian soybean cultivars. Biol. Fertil. Soils 36, 109–117.]. From 252 SSR markers tested in the parental genotypes 45 were polymorphic with high heterozygotes resolution. Mapping was performed with those 45 SSR markers for nodulation [nodule number (NN) and nodule dry weight (NDW)] and plant growth [shoot dry weight (SDW)] phenotypes in $F_{2:3}$ lines. A total of 21 SSR loci were mapped with a likehood of odds (LOD) value of 3.0 and a maximum Haldane distance of 50 cM, and were distributed in nine linkage groups with coverage of 251.2 cM. The interval mapping analysis with Mapmaker/QTL revealed two genomic regions associated with NN and NDW, with a contribution of putative QTLs of 7.1 and 10%, respectively. The regression analysis identified 13 significant associations between the marker loci and QTLs due to additive effects, with some of them being significantly associated with more than one phenotypic trait. Effects were observed in all variables studied, ranging from 2 to 9%. A one-way analysis of variance (ANOVA) also detected 13 significant associations, related to dominance effects. A two-way-ANOVA showed six epistatic interactions among non-linked QTLs for SDW, NN and NDW, explaining up to 15% of the trait variation and increasing the phenotypic

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Abbreviations: ANOVA, analysis of variance; cM, centimorgan; QTL, quantitative trait loci; SSR, simple sequence repeat

[*] Corresponding author. Tel.: +55 43 33716206; fax: +55 43 33716100.
E-mail addresses: marisa@lncc.br (M.F. Nicolás), hungria@cnpso.embrapa.br (M. Hungria).

[1] Present address: LNCC, Bioinformatics Laboratory, Av. Getulio Vargas, 333 Quitandinha, 25651-075 Petrópolis, RJ, Brazil.

doi:10.1016/j.fcr.2005.04.012

expression from 8 to 28%. The data obtained in this work establish the initial stage for additional studies of the QTLs controlling BNF and indicate that effective marker-assisted selection using SSR markers may be feasible for enhancing BFN traits in soybean breeding programs.

Keywords: Biological nitrogen fixation; *Bradyrhizobium*; Quantitative trait loci (QTL); *Glycine max*; Simple sequence repeats (SSR); Nodulation

1. Introduction

Nitrogen (N) demand of soybean [*Glycine max* (L.) Merrill] can be supplied via biological nitrogen fixation (BNF) through the inoculation with selected *Bradyrhizobium japonicum*/*B. elkanii* strains, eliminating the need for N fertilizers and resulting in an economy estimated in US$ 3 billion per crop season. However, as the mean yield in Brazil has increased from 1166 kg ha^{-1} in 1968/1969 to 2737 kg ha^{-1} in 2003/2004, plant demand for N has also increased (CONAB, 2004; Hungria et al., in press).

The importance of the plant genotype to the success of symbiosis is well known. Although pioneer studies have demonstrated differences among Brazilian genotypes in relation to BNF, in the last two decades breeders have often not evaluated the symbiotic performance, while paying more attention to disease resistance and yield (Hungria et al., in press). Consequently, differences among cultivars were observed when 152 genotypes recommended for different regions of Brazil were evaluated for BNF capacity, with some of them accumulating up to 4 times more NDW and 2.5 times more N in tissues (Bohrer and Hungria, 1998; Hungria and Bohrer, 2000).

The symbiosis between soybean and bradyrhizobia result from a complex process involving many genes of both partners that leads to the formation of N_2-fixing nodules in roots (Verma et al., 1992; Provorov and Vorob'ev, 2000). In relation to the microsymbiont, several laboratories have reported research progresses and relevant information has been obtained with the sequencing of the genome of *B. japonicum* strain USDA 110 (Kaneko et al., 2002). Nevertheless, the plant genes determining nodulation and BNF are still not well known, mainly, due to the large size of the plant genome. There are a few studies on nodulation characteristics described for "nitrate-tolerant-symbiotic" (nts) soybean mutants (Carroll et al., 1985; Gremaud and Harper, 1989; Akao and Kouchi, 1992). Advances were also achieved by the detection and isolation of several nodulin genes and plant proteins that are nodule enhanced or nodule specific, with functions in oxygen transport, cell wall architecture, sugar and N metabolism, among others (Verma et al., 1992). More recently, an integrated genetic linkage map of the soybean genome was published including some loci related to nodulation (*Rj1*, *Rj2* and *Nts* loci) (Cregan et al., 1999). Further progresses in the genetics have been obtained from studies with single nucleotide polymorphisms (SNPs) and bacterial artificial chromosome (BAC) libraries (Shoemaker et al., 2003) and will certainly come from the recent consortium established to sequence the genome of the soybean (Anonymous, 2004).

The information already available in genetic maps or the construction of new ones may lead to marker-assisted selection programs targeting desirable agronomic traits (Dudley, 1993). Important advances have been achieved by the characterization and mapping of QTLs in soybean and another crops.

In relation to the mapping of QTL controlling BNF, Nodari et al. (1993) detected four QTLs affecting nodule number in common bean (*Phaseolus vulgaris* L.), with some of them showing relation with effects of the pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Boscariol et al., 1998; Tsai et al., 2000), while others show relation with mineral N (Souza et al., 2000). In our laboratory, we first reported a study of quantitative trait inheritance of BNF in Brazilian soybean cultivars with different capacities of BNF: J-200 and Bossier (high), Embrapa 20 (medium) and BRS 133 (low). Two crosses, Bossier × Embrapa 20 and Embrapa 20 × BRS 133 have shown adequacy to use in studies of QTLs controlling BNF and nodulation (Nicolás et al., 2002). The objective of this work was to use