

Prevention of leaf yellowing in Asian soybean rust infected plants is associated with green cotyledon color and the infection index

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Abstract Because the six major known resistance genes to Asian soybean rust (ASR) are pathotype-specific, they rarely offer durable resistance to all ASR pathogens. Thus, other types of resistance such as field resistance or tolerance that confer broad-spectrum resistance to ASR pathogens are required for soybean breeding. The Chinese soybean variety Lu Pi Dou has a green cotyledon color (CC) and a no leaf yellowing (LY) phenotype was previously identified during ASR infection of this strain. This phenotype may reduce yield

losses of soybean cultivars infected with ASR infections if it is independent of pathotype-specific major resistance genes and undesirable phenotypes. Thus, to identify associations of LY and CC and other color traits and loci that are linked to the six known ASR resistance genes, recombinant inbred lines (RILs) and backcross (BC) BC₂F₅ lines were derived from Lu Pi Dou and BRS184 plant strains. Comparisons of LY, CC, and genotypes of simple sequence repeat (SSR) markers revealed that the absence of LY is tightly associated with green CC in accordance with the expression of the genes *d1* and *d2*. However, although variations of LY in RILs with yellow CC predominantly reflected the infection index (IFI), no associations were observed between LY and pubescence color, pod color, seed coat color, or flanking SSR markers for six known ASR resistance loci. An association of LY with CC and IFI was also shown in data from BC populations. Therefore, prevention of LY during ASR infection of Lu Pi Dou was primarily dependent on the double-recessive genotype *d1d1d2d2* for CC and secondarily on low IFI.

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Introduction

The fungus *Phakopsora pachyrhizi* is the causal agent of Asian soybean rust (ASR) and is one of the most serious

economic threats for soybean growers (Goellner et al. 2010). It has caused severe losses to soybean production in South America (Yorinori 2008). Foliar disease is characterized by rapid leaf yellowing (LY) and premature defoliation, which result in yield reductions from both fewer and smaller seeds. Hence, the development of soybean varieties that are resistant to ASR is considered the most economical management strategy for this disease (Hartman et al. 2005; Ribeiro et al. 2007; Arias et al. 2008). However, the six known major *P. pachyrhizi* ASR resistance genes *Rpp 1–6* (Hyten et al. 2007, 2009; Garcia et al. 2008; Silva et al. 2008; Li et al. 2012) are pathotype-specific and are not always durable (Oliveira et al. 2005). Moreover, some of these genes are not effective in South America because of the presence of highly virulent and divergent ASR populations (Yamanaka et al. 2010, 2011; Akamatsu et al. 2013). Therefore, provision of multiple effective ASR resistance genes (*Rpp1–6*) in a single soybean cultivar and/or use of low specificity resistance modalities such as field resistance or tolerance to ASR are considered ideal strategies for providing widespread resistance against the diverse *P. pachyrhizi* populations in South America (Lemos et al. 2011; Yamanaka et al. 2013b).

In a previous study (Yamanaka et al. 2011), ASR infection promoted LY in both resistant and susceptible varieties during severe infection with the highly virulent ASR population, BRP-2. However, the two Chinese soybean varieties Lu Pi Dou and Hei Dou showed LY preventative phenotypes, despite being highly susceptible to ASR infection. These two varieties also showed a minor characteristic of a green cotyledon color (CC) of seeds. Hence, this LY resistant phenotype may reduce yield losses of soybean cultivars and maximize seed productivity under conditions of severe infection with ASR if it is independent of the major pathotype-specific resistance genes and does not produce undesirable phenotypes.

Green or yellow CC is reportedly associated with the unlinked loci *d1* and *d2* in soybean (Woodworth 1921), and the double-recessive genotype (*d1d1d2d2*) preserves most leaf thylakoid proteins during senescence and leads to maintenance of green color (Guiamét et al. 1991; Guiamét and Giannibelli 1996). These loci were mapped (Ott et al. 2013) on the linkage groups (LG) D1a (Chromosome 1) and B1 (Chromosome 11). In addition, the soybean orthologous STAY-GREEN (*SRG*) genes *GmSGR1* and *GmSGR2*, which encode chloroplast proteins, were recently identified to be responsible for

the green-cotyledon/stay-green phenotype in *d1d1d2d2* soybean mutants (Nakano et al. 2014). Moreover, several candidate pleiotropic effects of *d1d1d2d2* with the gene *G*, which preserves chlorophyll in the mature seed coat, were associated with agronomic and physiological characters such as seed yield and stomata conductance (Guiamét et al. 1990; Luquez and Guiamét 2001). However, the *d1d1d2d2* genotype was more susceptible to water stress than wild type (Luquez and Guiamét 2002). In addition, Young and Ross (1978) demonstrated co-inheritance of the green CC phenotype and non-chlorotic responses to brown spots (*Septoria glycines*) in disease resistant soybean plants.

Because most cultivated soybeans have yellow CC, disease resistance may be associated with genes that are independent of the green CC, and these could be used to breed soybeans with yellow CC. Thus, in the present study we clarified the associations of ASR-associated LY with CC and various other traits and genes using an independent population of recombinant inbred lines (RILs) and backcrossed lines that were derived from the cross between Lu Pi Dou and the ASR susceptible LY variety BRS184.

Materials and methods

Plant materials

Two experiments were conducted in this study. For the first experiment, 70 recombinant inbred lines (RILs, F_6) were developed using F_2 -derived single seed descent (SSD) from the cross of Lu Pi Dou \times BRS184. Lu Pi Dou is susceptible to ASR but has no LY phenotype during ASR infection, whereas BRS184 is a susceptible variety that shows LY during ASR infection (Yamanaka et al. 2011). A total of 70 F_6 lines (one plant each of 70 RILs) were evaluated for their CC (green/yellow), pubescence color (brown/white), and pod color (dark/light colored), and DNA was then extracted from samples for analyses of simple sequence repeat (SSR) markers. F_7 seeds were harvested from each F_6 plant and were evaluated for CC and seed coat color (green/yellow). Degrees of black pigmentation on seed coats were not evaluated, and three F_7 plants from each of 70 RILs were sown to evaluate degrees of LY and to calculate the infection index (IFI).

In the second experiment, a total of 58 BC_2F_5 plants were generated from a cross of the single F_3 plant with

the lowest degree of LY (1.0, see below), which was derived from the same cross used for the first experiment. BRS184 was used as the recurrent and pollen-producing parent. During backcrossing and selfing, plants showing no or low LY ($1.0 \leq LY < 1.5$) were selected and used to produce the following generation. All 58 BC₂F₅ plants were evaluated for their CC, degree of LY, and IFI and were then sampled for DNA extraction and SSR marker analysis.

Evaluation of leaf yellowing and infection index

The Japanese ASR isolate T1-2 (Akamatsu et al. 2013; Yamanaka et al. 2013b; Hossain et al. 2015) was used in LY and IFI experiments. A spore suspension of T1-2 was prepared at the concentration of 115,000 spores/mL and was used to inoculate plants grown in a greenhouse according to Yamanaka et al. (2013a). Three weeks after inoculation, degrees of LY and IFI scores on three trifoliate leaves from each plant were averaged using the scale and formula for IFI described by Yamanaka et al. (2013a). Mean values from three F₇ plants from each RIL and those from a single BC₂F₅ plant were included in subsequent statistical analyses.

Simple sequence repeat (SSR) markers

To analyze the CC-related loci *d1* and *d2*, the SSR markers Sat_160 and Sat_272, which were approximately 1 cM from the *d1* locus of the linkage group (LG) D1a (Chromosome 1) and approximately 14 cM from the *d2* locus in the LG B1 (Chromosome 11), respectively, were chosen based on the linkage map GmComposite2003 in SoyBase (Grant et al. 2010). The six SSR markers Sat_117, Satt366, Sat_263, AF162283, Sat_280, and Satt324 were used to detect *Rpps* 1–6, respectively (Hyten et al. 2007, 2009; Garcia et al. 2008; Silva et al. 2008; Li et al. 2012). The SSR markers Satt408 and BE806308 (Grant et al. 2010) were polymorphic between the parents and were expected to be linked with *d1* and *d2*, respectively, and were used in addition to Sat_160 and Sat_272. PCR and band detection were performed as described by Yamanaka et al. (2013a).

Statistical analysis

Comparisons of observed and expected segregation ratios of SSR marker genotypes, differences in SSR

marker determined haplotypes (*d* loci), and frequencies of CC in RILs were identified using Chi square (χ^2) analyses. One-way analysis of variance (ANOVA) was performed to identify significant relationships between LY and pubescence color, pod color, seed coat color, and CC-related genes *d1*, *d2*, and *Rpps* 1–6 in RILs and between LY and CC frequencies in BC₂F₅ plants. Numbers of green, segregating, and yellow CCs in RILs and BC₂F₅ plants were recorded as 2, 1, and 0, respectively, and regression analyses were performed to estimate associations between LY and CC.

Results

Leaf yellowing and cotyledon color in recombinant inbred lines

In the first experiment, no or low LY ($1.0 \leq LY < 1.5$) due to infection after the Japanese ASR isolate T1-2 was observed in the parental variety Lu Pi Dou and in approximately 24 % of RILs (Fig. 1). However, infection with T1-2 resulted in widespread LY in 76 % of the RIL population, which included the parental variety

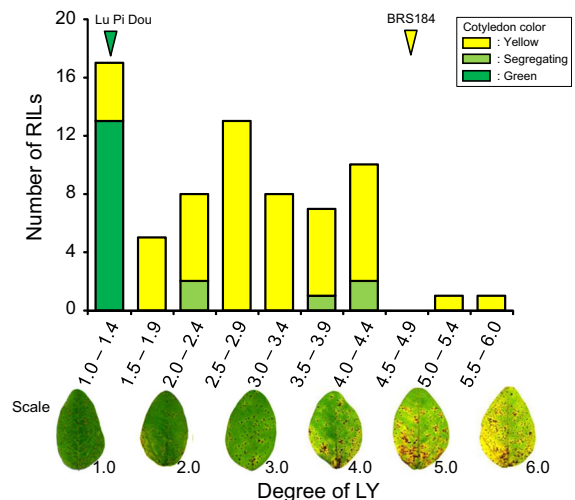


Fig. 1 Numbers of recombinant inbred lines (RILs) with differing degrees of leaf yellowing (LY). Color separation in the graph is based on phenotypes of cotyledon colors among F₇ plants. Values for parents and the LY scale (Yamanaka et al. 2011) are shown on the top and the bottom of the figure, respectively. (Color figure online)

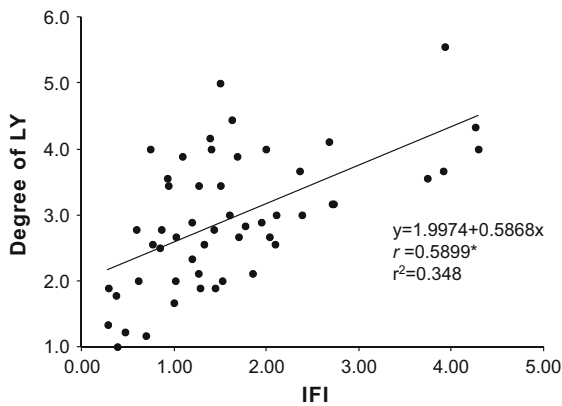


Fig. 2 Scatter diagram of leaf yellowing (LY) and infection index (IFI) in 52 recombinant inbred lines (RILs) with yellow cotyledon. *significant at the 0.1 % level; *t* test

BRS184. Degrees of LY among RILs were distributed from 1.0 to 5.6, and only two RILs showed higher levels of LY than BRS184 plants (Fig. 1). The CC of single F_6 plants was identical to their F_7 progeny (data not shown), except for five RILs. Specifically, single F_6 plants had yellow CC, whereas these five F_7 progeny RILs showed segregation of yellow and green CC in each line (shown as “segregating” CC in Fig. 1 and subsequent Tables). All the 13 RILs with green CC showed no (LY = 1.0 in 12 lines) or almost no yellowing (LY = 1.1 in one line; Fig. 2; Table 1). In contrast, LY was observed in BRS184 and RILs with yellow or segregating CC (Fig. 1; Table 1). Average LY values in segregating and yellow-fixed RILs were 3.2 (range 2.0–4.4) and 2.9 (range 1.0–5.6), respectively, and traces to 10 % of leaflet area showed yellowing. Significantly differing degrees of LY were observed between the three groups of RILs (green, yellow, and segregating; $P < 0.001$, Table 1). Among RILs, CC explained 34.9 % of variance in LY (Table 1), but four RILs had yellow CC but no LY.

Cotyledon color and *d* loci in recombinant inbred lines

Haplotypes of RILs were determined using the two SSR markers Sat_160 and Sat_272, which are linked to *d1* and *d2*, respectively, and the corresponding segregations of CC within each haplotype are shown in Table 2. The ratio of green to segregating to yellow CC in 70 RILs was 13:5:52 and corresponded with the

theoretical ratio of 16.4:2.2:51.4. Moreover, green CC reflected the two independent double recessive loci ($P = 0.1435$, Table 2). The number of RILs with nine classes of marker haplotypes fitted to the number of RILs with two unlinked loci ($P = 0.7461$). Twelve of 13 RILs with green CC had the AA haplotype (both alleles from Lu Pi Dou), whereas only one RIL with green CC had the BA haplotype (BRS184 allele at Sat_160 and Lu Pi Dou allele at Sat_272), reflecting potential recombination between Sat_160 and *d1*. In contrast, 52 RILs with yellow cotyledons had AA, AB, BA, HB, BH, or BB haplotypes (Table 2). The HH (H, heterozygous) haplotype was not identified among the present RILs.

Leaf yellowing and infection index in recombinant inbred lines

No significant associations were found between the degree of LY and any of the three color characters, the eight SSR markers that were linked to the two *d* loci, or the six *Rpp* loci (Table 3). However, regression analysis indicated a significant ($P < 0.001$) positive correlation ($r = 0.5899$) between IFI and LY among RILs with yellow CC (Fig. 2).

Leaf yellowing, cotyledon color, and infection index in the BC_2F_5 population

Minimum LY values (LY = 1.0) were observed in BC_2F_5 plants with green CC. In contrast, plants with yellow CC showed various degrees of LY and an average value of 3.7. Significant differences in degrees of LY were observed between BC_2F_5 plants with green and yellow CC ($P < 0.001$, Table 1). In this population, the observed segregation for CC was 28 green to 30 yellow (Table 1). In the analyses of segregation of the two *d2* locus-linked SSR markers BE806308 and Sat_272 linked, 27 of 28 plants with green CC were homozygous for the Lu Pi Dou allele at Sat_272, whereas seven of 28 plants were heterozygous or homozygous for the BRS184 allele at BE806308. The other one BC_2F_5 plant with green CC was homozygous for the Lu Pi Dou allele at the BE806308 locus and heterozygous at the Sat_272 locus. These segregations indicate that *d2* is located between BE806308 and Sat_272, although the linkage to Sat_272 appears

Table 1 Comparisons of leaf yellowing (LY) among recombinant inbred lines (RILs) and BC₂F₅ populations that differ in cotyledon color (CC)

Population	CC	n	Degree of LY		F value	R ²
			Average	SD		
RILs (F ₇)	Green	13	1.0	0.0	24.61*	0.349
	Segregating	5	3.2	1.1		
	Yellow	52	2.9	1.0		
	Total	70				
BC ₂ F ₅	Green	28	1.0	0.0	88.87*	0.613
	Yellow	30	3.7	1.5		
	Total	58				

* Significant at $P < 0.001$ level

Table 2 Haplotypes of recombinant inbred lines (RILs) determined using two simple sequence repeat (SSR) markers that are linked to *d* loci and frequencies of each cotyledon color (CC) type

SSR marker haplotype in F ₆ ^a		Cotyledon color in F ₇			Total		Haplotype number ^b	
Sat_160 (<i>d1</i>)	Sat_272 (<i>d2</i>)	Green	Segregating	Yellow	Observed	Theoretical	χ^2 value	Probability
A	A	12	1	2	15	16.423	5.1065	0.7461
A	H	0	1	0	1	1.060		
H	A	0	3	0	3	1.060		
A	B	0	0	18	18	16.423		
B	A	1	0	15	16	16.423		
B	H	0	0	1	1	1.060		
H	B	0	0	2	2	1.060		
B	B	0	0	14	14	16.423		
H	H	0	0	0	0	0.068		
	Observed	13	5	52	70	70		
	Theoretical	16.423	2.186	51.389	70			
	χ^2 value ^c	4.3369						
	Probability ^c	0.1435						

^a A Lu Pi Dou homozygote; B BRS184 homozygote; H heterozygote

^b χ^2 value and probability of the expected frequency of the SSR marker haplotypes

^c χ^2 value and probability of the expected frequency of CC phenotypes

stronger. In contrast, all BC₂F₅ plants with Sat_160 and Satt408 were homozygous for BRS184 and Lu Pi Dou, respectively, indicating that Satt408 is more tightly linked with *d1* than Sat_160 and that all BC₂F₅ plants had Lu Pi Dou alleles and were homozygous for *d1*.

CC explained 61.3 % of the variation in LY among BC₂F₅ plants (Table 1), and IFI and LY were highly correlated ($r = 0.8023$, $P < 0.001$) in BC₂F₅ plants with yellow CC (Fig. 3), indicating that the degree of LY in plants with the yellow cotyledon genotype was largely determined by IFI.

Discussion

In this study of unique Lu Pi Dou phenotypes, no LY was observed during infection of the Japanese ASR isolate T1-2. A similar phenotype was observed in our previous study after the same variety was infected with the Brazilian ASR population: BRP-2 (Yamanaka et al. 2011), although these two ASR pathogens have differing virulence in resistant varieties (Yamanaka et al. 2013b). The distribution of LY phenotypes in 70 RILs was continuous (Fig. 1). In addition, LY values in most of the RILs ranged between those of Lu Pi Dou

Table 3 Comparison of leaf yellowing (LY) between phenotypes of three traits and genotypes of eight simple sequence repeat (SSR) markers in 52 recombinant inbred lines (RILs) with yellow cotyledons

Trait/marker	Classes ^a	One-way ANOVA	
		F value	Probability
Pubescence color	Brown/white	0.27	0.60
Pod color	Dark/light	0.15	0.70
Seed coat color	Green/yellow	0.01	0.93
Sat_160 (<i>d1</i>)	A/H/B	0.90	0.41
Sat_272 (<i>d2</i>)	A/H/B	0.88	0.42
Sat_117 (<i>Rpp1</i>)	A/H/B	0.10	0.75
Satt366 (<i>Rpp2</i>)	A/H/B	1.17	0.32
Sat_263 (<i>Rpp3</i>)	A/H/B	0.72	0.49
AF162283 (<i>Rpp4</i>)	A/H/B	0.05	0.96
Sat_280 (<i>Rpp5</i>)	A/H/B	1.06	0.35
Satt324 (<i>Rpp6</i>)	A/H/B	1.76	0.18

^a A Lu Pi Dou homozygote, B BRS184 homozygote, H heterozygote for SSR markers

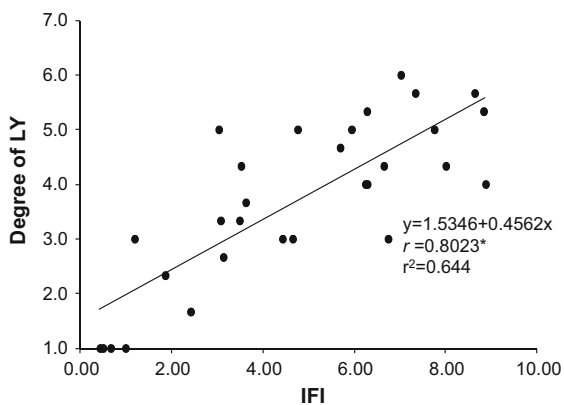


Fig. 3 Scatter diagram of leaf yellowing (LY) and infection index (IFI) in 30 BC₂F₅ plants with yellow cotyledon. * significant at the 0.1 % level; *t* test

and BRS184 (Fig. 2), indicating that Lu Pi Dou and BRS184 carry factors for preventing and promoting LY, respectively and that LY may be controlled by multiple factors or environmental variables. Most RILs with no or almost no LY had green CC, and all 13 RILs with green CC belonged to this class (Fig. 1). In addition, the degree of LY differed significantly between RILs with green, segregating, and yellow CC (Table 1), indicating that the loci for CC

predominantly determines the degree of LY. This conclusion was also supported in subsequent experiments with BC₂F₅ plants, which showed greater differences in LY between CC groups and greater explanatory power of CC (Table 1). Our preliminary test using 45 F₅ lines also revealed significant differences in LY between CC classes (data not shown). Moreover, analyses of the SSR markers that are linked to the known double recessive loci *d1* and *d2* (Woodworth 1921) indicated that green CC in Lu Pi Dou is controlled by these loci, although some recombination was observed between markers and CC phenotypes in RILs and BC₂F₅ plants (Table 2). In conclusion, LY prevention during ASR infection in Lu Pi Dou primarily reflects green CC controlled by double recessive loci for CC (*d1* and *d2*). Young and Ross (1978) also observed co-inheritance of green CC controlled by double recessive genes and non-chlorotic responses to brown spot caused by *Septoria glycines*. In addition, they observed non-chlorotic responses to *S. glycines* in plantlets of only 8 of 626 plant introduction (PI) lines, including the two ASR resistant lines PI 230970 (*Rpp2*) and PI 339866 (Hartwig and Bromfield 1983; Laperuta et al. 2008). The PI 79609 had green CC and black lesion brown spot disease symptoms without showing any LY. Moreover, the green CC variety Lu Pi Dou produced dark-colored (but highly sporulating) ASR lesions and showed no LY phenotype. Brogin (2005) also demonstrated the presence of a gene on LG C2 of the soybean variety FT-2 that conferred resistance against ASR and brown spot, potentially reflecting similar mechanisms of resistance.

Although LY prevention in Lu Pi Dou was shown to primarily reflect the green CC by the known double recessive loci *d1* and *d2*, large variations in LY were observed among plants with yellow CC in the two populations. In the present study, IFI, pubescence color, pod color, and seed coat color, as well as eight SSR markers that are linked to six known resistance loci (*Rpp1*–6) and two *d* loci were evaluated as additional indicators of the association between LY and yellow CC. However, pubescence color, pod color, seed coat color, and the six known ASR resistance genes were not associated with LY, and no association was found between LY and the two *d* loci markers, suggesting that each of *d* loci is not exclusive determinates of LY solely (Table 3). In contrast, positive associations of IFI and degrees of

LY were significant in both of the present experiments (Figs. 1, 2), indicating that IFI could be considered a determinate of LY in the progeny of Lu Pi Dou \times BRS184 plants. In particular, initial experiments identified four RILs with yellow CC and similarly low levels of LY (mean 1.2; range 1.0–1.3) to those of 13 RILs with green CC (Fig. 1). Moreover, the corresponding IFI values were the lowest (mean 0.45; range 0.28–0.69) among the 52 RILs with yellow CC (Fig. 2). These observations further indicate that IFI is a significant determinate of LY. Accordingly, among genotypes with yellow CC, IFI explained 64 % of the variance in LY in BC₂F₅ plants but only 34.8 % in RILs (34.8 %), potentially reflecting relative uniformity of the genetic background among BC₂F₅ plants and fewer minor genetic contributors to LY. Taken together, these data indicate that in addition to *d* loci, IFI and other genetic factor(s) influence LY.

Both of the experiments in this study indicated that the absence of the LY character in Lu Pi Dou is associated with green CC and is regulated by known double recessive loci (*d1d1d2d2*). Thus, introduction of LY prevention phenotypes of the green CC variety Lu Pi Dou into soybean breeding programs of the widely grown yellow CC varieties may not be possible. However, low IFI reduced LY in genotypes with yellow CC. Thus, low IFI is genetically determined and may be exploited to reduce LY due to ASR infection in soybean breeding programs.

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References

- Akamatsu H, Yamanaka N, Yamaoka Y, Soares RM, Morel W, Ivancovich AJG, Bogado AN, Kato M, Yorinori JT, Suenaga K (2013) Pathogenic diversity of soybean rust in Argentina, Brazil, and Paraguay. *J Gen Plant Pathol* 79:28–40. doi:10.1007/s10327-012-0421-7
- Arias CAA, Toledo JFF, Almeida LA, Pipolo AE, Carneiro GES, Abdelnoor RV, Rachid BF, Ribeiro AS (2008) Asian rust in Brazil: Varietal resistance. In: Kudo H, Suenaga K, Soares RM, Toledo A (eds) JIRCAS Working Report No. 58: Facing the challenge of soybean rust in South America. JIRCAS, Tsukuba, pp 29–30
- Brogini RL (2005) Mapeamento de genes de resistência à ferrugem e de QTLs envolvidos na resistência à septoriose em soja. Dissertation, Universidade de São Paulo
- Garcia A, Calvo ES, Kiihl RAS, Harada A, Hiromoto DM, Vieira LGE (2008) Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. *Theor Appl Genet* 117:545–553. doi:10.1007/s00122-008-0798-z
- Goellner K, Loehrer M, Langenbach C, Conrath U, Koch E, Schaffrath U (2010) *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust. *Mol Plant Pathol* 11:169–177. doi:10.1111/J.1364-3703.2009.00589.X
- Grant D, Nelson RT, Cannon SB, Shoemaker RC (2010) SoyBase, the USDA-ARS soybean genetics and genomics database. *Nucleic Acids Res* 38:843–846. doi:10.1093/nar/gkp798
- Guamét JJ, Giannibelli MC (1996) Nuclear and cytoplasmic ‘stay-green’ mutations of soybean alter the loss of leaf soluble proteins during senescence. *Physiol Plant* 96:655–661. doi:10.1111/j.1399-3054.1996.tb00239.x
- Guamét JJ, Teeri JA, Noodén LD (1990) Effects of nuclear and cytoplasmic genes altering chlorophyll loss on gas exchange during monocarpic senescence in soybean. *Plant Cell Physiol* 31:1123–1130
- Guamét JJ, Schwartz E, Pichersky E, Noodén LD (1991) Characterization of cytoplasmic and nuclear mutations affecting chlorophyll and chlorophyll-binding proteins during senescence in soybean. *Plant Physiol* 96:227–231. doi:10.1104/pp.96.1.227
- Hartman GL, Miles MR, Frederick RD (2005) Breeding for resistance to soybean rust. *Plant Dis* 89:664–666. doi:10.1094/PD-89-0664
- Hartwig EE, Bromfield KR (1983) Relationships among three genes conferring specific resistance to rust in soybeans. *Crop Sci* 23:237–239. doi:10.2135/cropsci1983.0011183X002300020012x
- Hossain MM, Akamatsu H, Morishita M, Mori T, Yamaoka Y, Suenaga K, Soares RM, Bogado AN, Ivancovich AJG, Yamanaka N (2015) Molecular mapping of Asian soybean rust resistance in soybean landraces PI 594767A, PI 587905 and PI 416764. *Plant Pathol* 64:147–156. doi:10.1111/ppa.12226
- Hyten DL, Hartman GL, Nelson RL, Frederick RD, Concibido VC, Narvel JM, Gregan PB (2007) Map location of the *Rpp1* locus that confers resistance to soybean rust in soybean. *Crop Sci* 47:835–838. doi:10.2135/cropsci2006.07.0484
- Hyten DL, Smith JR, Frederick RD, Tucker ML, Song Q, Gregan PB (2009) Bulk segregant analysis using the GoldenGate assay to locate the *Rpp3* locus that confers resistance to soybean rust in soybean. *Crop Sci* 49:265–271. doi:10.2135/cropsci2008.08.0511
- Laperuta LDC, Arias CAA, Ribeiro AS, Rachid BF, Pierozzi PHB, Toledo JFFD, Pipolo AE, Carneiro GES (2008) New genes conferring resistance to Asian soybean rust: allelic testing for the *Rpp2* and *Rpp4* loci. *Pesqui Agropecu Bras* 43:1741–1747. doi:10.1590/S0100-204X2008001200014
- Lemos NG, Braccini AL, Abdelnoor RV, Oliveira MCN, Suenaga K, Yamanaka N (2011) Characterization of genes

- Rpp2*, *Rpp4*, and *Rpp5* for resistance to soybean rust. Euphytica 182:53–64. doi:10.1007/s10681-011-0465-3
- Li S, Smith JR, Ray JD, Frederick RD (2012) Identification of a new soybean rust resistance gene in PI 567102B. Theor Appl Genet 125:133–142. doi:10.1007/s00122-012-1821-y
- Luquez VM, Guiamét JJ (2001) Effects of the ‘Stay Green’ genotype *GGd1d1d2d2* on leaf gas exchange, dry matter accumulation and seed yield in soybean (*Glycine max* L. Merr.). Ann Bot 87:313–318. doi:10.1006/anbo.2000.1324
- Luquez VM, Guiamét JJ (2002) The stay green mutations *d1* and *d2* increase water stress susceptibility in soybeans. J Exp Bot 53:1421–1428. doi:10.1093/jexbot/53.373.1421
- Nakano M, Yamada T, Masuda Y, Sato Y, Kobayashi H, Ueda H, Morita R, Nishimura M, Kitamura K, Kusaba M (2014) A green-cotyledon/stay-green mutant exemplifies the ancient whole-genome duplications in soybean. Plant Cell Physiol 55:1763–1771. doi:10.1093/pcp/pcu107
- Oliveira ACB, Godoy CV, Martins MC (2005) Avaliação da tolerância de cultivares de soja à ferrugem asiática no Oeste da Bahia. Fitopatol Bras 30:658–662
- Ott A, Yang Y, Bhattacharyya M, Horner HT, Palmer RG, Sandhu D (2013) Molecular mapping of *D1*, *D2* and *ms5* revealed linkage between the cotyledon color locus *D2* and the male-sterile locus *ms5* in soybean. Plants 2:441–454. doi:10.3390/plants2030441
- Ribeiro AS, Moreira JUV, Pierozzi PHB, Rachid BF, De Toledo JFF, Arias CAA, Soares RM, Godoy CV (2007) Genetic control of Asian rust in soybean. Euphytica 15:15–25. doi:10.1007/s10681-007-9404-8
- Silva DCG, Yamanaka N, Brogin RL, Arias CAA, Nepomuceno AL, Di Mauro AO, Pereira SS, Nogueira LM, Passianotto ALL, Abdelnoor RV (2008) Molecular mapping of two loci that confer resistance to Asian rust in soybean. Theor Appl Genet 117:57–63. doi:10.1007/s00122-008-0752-0
- Woodworth CM (1921) Inheritance of cotyledon, seed-coat, hilum and pubescence colors in soy-beans. Genetics 6:487–553
- Yamanaka N, Yamaoka Y, Kato M, Lemos NG, Passianotto ALL, Santos JVM, Benitez ER, Abdelnoor RV, Soares RM, Suenaga K (2010) Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. Trop Plant Pathol 35:153–162. doi:10.1590/S1982-56762010000300003
- Yamanaka N, Lemos NG, Akamatsu H, Yamaoka Y, Silva DCG, Passianotto ALL, Abdelnoor RV, Soares RM, Suenaga K (2011) Soybean breeding materials useful for resistance to soybean rust in Brazil. JARQ 45:385–395
- Yamanaka N, Akamatsu H, Yamaoka (2013a) Laboratory manual for studies on soybean rust resistance. JIRCAS website. http://www.jircas.affrc.go.jp/english/manual/soybean_rust/soybean_rust.html. Accessed 1 December 2013
- Yamanaka N, Lemos NG, Uno M, Akamatsu H, Yamaoka Y, Abdelnoor RV, Braccini AL, Suenaga K (2013b) Resistance to Asian soybean rust in soybean lines with the pyramided three *Rpp* genes. Crop Breed Appl Biotechnol 13:75–82. doi:10.1590/S1984-70332013000100009
- Yorinori JT (2008) Soybean germplasm with resistance and tolerance to Asian rust and screening methods. In: Kudo H, Suenaga K, Soares RM, Toledo A (eds) JIRCAS Working Report No. 58: Facing the challenge of soybean rust in South America. JIRCAS, Tsukuba, pp 70–87
- Young LD, Ross JP (1978) Resistance evaluation and inheritance of a nonchlorotic response to brown spot of soybean. Crop Sci 18:1075–1077. doi:10.2135/cropsci1978.0011183X001800060043x