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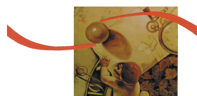

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Mapping and validation of molecular markers of genes *Dt1* and *Dt2* to determine the type of stem growth in soybean

Dorival Vicente[1*], Ivan Schuster[2], Fabiane Lazzari[2], Jonas Pedro Dolfini Paranzini[2], Marco Antonio Rott de Oliveira[2] and Cássio Egídio Cavenaghi Prete[3]

*[1]Expo Grain, Comércio de Sementes Ltda., Rua Pres. Juscelino Kubitschek, 871, 85807-440, Cascavel, Paraná, Brazil. [2]Cooperativa Central de Pesquisa Agrícola, Cascavel, Paraná, Brazil. [3]Departamento de Agronomia, Centro de Ciências Agrárias, Universidade Estadual de Londrina, Londrina, Paraná, Brazil. *Author for correspondence. E-mail: dvicente@terra.com.br*

ABSTRACT. The type of stem growth in soybean is a distinguishing feature of cultivars. The genes *Dt1* and *Dt2* affect the termination of the stem, and the types of growth are classified as determinate, semi-determinate and indeterminate. Phenotypic characterization of the type of growth is complex and is occasionally erroneously described. The objective of this study was to map and validate molecular markers to classify the type of soybean growth to facilitate the description of cultivars and genotypic selection. Two populations were used for mapping and validation of molecular markers $F_{2:3}$: T 117 (semi-determinate growth type) x Igra RA 518 RR (indeterminate growth type) and CD 235RR (determinate growth type) x Igra RA 518 RR. The study demonstrates that the association of the molecular marker with the gene *GmTFL1b* was efficient in the classification of soybean growth types. The marker sat_064 is connected to the *Dt2* gene, which is located in the Liaison Group G of the consensus map of soybeans with a recombination frequency of 19.4%. Molecular markers for *Dt1* and *Dt2* genes efficiently described the genotypes of soybean stem growth and selection.

Keywords: *Glycine max*, determinate growth, semi-determinate growth, indeterminate growth, SAM.

Mapeamento e validação de marcadores moleculares dos genes *Dt1* e *Dt2* que determinam tipo de crescimento da haste da soja

RESUMO. O tipo de crescimento da haste da soja é característica diferenciadora de cultivares. Os genes *Dt1* e *Dt2* afetam a terminação da haste, haja vista que os tipos de crescimento são classificados em determinado, semideterminado e indeterminado. A caracterização fenotípica do tipo de crescimento é complexa e, ocasionalmente, é descrito erroneamente. O objetivo deste trabalho foi mapear e validar marcadores moleculares microssatélites para classificar a soja quanto ao tipo de crescimento, buscando facilitar a descrição de cultivares e a seleção genotípica. Para mapeamento e validação dos marcadores moleculares, foram utilizadas 2 populações $F_{2:3}$: T 117 (tipo de crescimento semideterminado) x Igra RA 518 RR (tipo de crescimento indeterminado) e CD 235RR (tipo de crescimento determinado) x Igra RA 518 RR. Este estudo evidenciou a associação do marcador molecular ao gene *GmTFL1b* sendo eficiente na classificação dos tipos de crescimento em soja. O marcador sat_064 está ligado ao gene *Dt2*, localizado no Grupo de Ligação G do mapa consenso da soja, com frequência de recombinação de 19,4%. Os marcadores moleculares, para os genes *Dt1* e *Dt2,* são eficientes na descrição de genótipos quanto ao tipo de crescimento da haste da soja, bem como para serem utilizados na seleção.

Palavras-chave: *Glycine max*, crescimento determinado, crescimento semideterminado, crescimento indeterminado, SAM.

Introduction

The type of stem growth of soybean [*Glycine max* (L.) Merr.] influences several agronomic characteristics. Plants with a determined type of growth typically exhibit smaller heights with greater resistance to lodging, smaller insertion heights for the first pod and a greater number of branches per plant compared with cultivars of an undetermined growth type and similar maturity group. Lineages of determined types also present a reduced period of flowering compared with lineages of undetermined types (Bernard, 1972; Kilgore-Norquest & Sneller, 2000).

Two genes, *Dt1* and *Dt2*, affect the ending of the stem in soybean (Bernard, 1972). A recessive allele, *dt1*, and a dominant allele, *Dt2*, cause the ending of the apical growth of the stem to proceed in a rapid fashion, which diminishes the height of the plant and the number of knots. Of these genes, *dt1* exerts a much greater effect. Bernard (1972) observed this

phenotype and described it as intermediary semi-determined, which is distinct from the undetermined and determined phenotypes in hybrid populations among the undetermined and determined lineages. These semi-determined plants segregated to undetermined and determined types, indicating that *Dt1* acts as a partially dominant gene in the tested genetic base.

The type of growth was occasionally incorrectly classified (Bernard, 1972). This difficulty of selection – description and tendency to use undetermined and semi-determined cultivars with greater frequency in the improvement programs – broadens in a progressive manner. The type of growth is a differentiating characteristic of soybean cultivars and is a part of the minimum demands of UPOV (International Union for the Protection of New Varieties of Plants) as a descriptor of cultivars with means to protect individual property. In this regard, determined, semi-determined and undetermined growth types are used to describe cultivars (Serviço Nacional de Proteção de Cultivares [SNPC], 2012).

Furthermore, the assisted characterization by molecular markers (SAM) is a useful tool in the quick and efficient development of cultivars (Schuster & Oliveira, 2006), whereas the molecular base of the growth type was dissected by Liu et al. (2010). Based on the soybean's genome and mapping analysis, the *Dt1* gene codes the GmTFL1b protein, and the type of growth of the soybean stem is determined by variants of this gene. The *Dt1* gene was mapped with molecular markers in the GL L (Cregan et al., 1999; Liu et al., 2007; Tian et al., 2010), and the *Dt2* gene was mapped in the GL G (Muehlbauer et al., 1989; Cregan et al., 1999). These regions are currently designated as chromosomes 19 and 18, respectively (Schumutz et al., 2010).

In the beginning of the 21st century, 93% of protected soy cultivars in Brazil were of a determined growth type (Brasil, 2002). In the agricultural year of 2010/2011, among the ten soy cultivars that seeded, half presented the undetermined type of growth. Regarding the other half, which was located in the Southern region of the country, five additional seeded cultivars were classified as the undetermined growth type (Kleffmann & Partner, 2011). Facing this reality, Brazilian farmers are changing their preference of growth type and improvement programs that aim at attending to the demand to face the need for the selection, description and distinction of determined, semi-determined and undetermined types of growth. However, during the separation process of the three phenotypes, difficulty is encountered in the selection and description.

Given this difficulty, the present paper aims at mapping and validating molecular markers to classify soy according to its growth type to facilitate the description of cultivars and selection within a genetic improvement program.

Material and methods

Genetic material

For the mapping and validation of molecular markers, two $F_{2:3}$ populations were obtained according to the description below.

Population 1: the cultivar T 117 was used as a female parental (Bernard, 1972); this cultivar possesses the genotype *Dt1Dt1Dt2Dt2* (semi-determined growth type). The male parental was the Igra RA 518 RR cultivar (Igra Sementes, 2013), which possesses the genotype *Dt1Dt1dt2dt2* (undetermined growth type).

Population 2: the cultivar CD 235RR was used as a female parental; this cultivar possesses the genotype *dt1dt1dt2dt2* (determined growth type). The male parental was the Igra RA 518 RR cultivar, which possesses the genotype *Dt1Dt1dt2dt2* (undetermined growth type).

The hybridizations were made in a vegetation house in the summer of 2008/2009. However, the previous generation of the F_1 plants was made in a vegetation house in the autumn and winter of 2009, and the F_2 plants were planted in the field in the summer of 2009/2010. The F_2 plants were picked and threshed individually to obtain seeds ($F_{2:3}$). The seeds of each F_2 plant composed a $F_{2:3}$ family. These seeds were identified with the origin number of the F_2 plant. For the sowing of the $F_{2:3}$ families of each population, 60 seeds for each lineage were separated, and the remaining seeds were used for molecular analysis.

Phenotypic evaluation

The experiment was seeded in the 2010/2011 crop in the Coodetec - Agricultural Research Center Cooperative in Cascavel, Paraná State (Latitude S 24° 52' 56.9"; Longitude W 53° 32' 00.4" and altitude 690 m) in Purple Latosolic Distroferric soil. The $F_{2:3}$ lineages and cultivars were sowed in individual lines that were 4 m in length with 0.45 m spacing between the lines. For optimal results, fertilization and the control of weeds, plagues and diseases were performed according to the technical demands for the culture. Phenotypic evaluation of stem type of growth for the study and assessment of populations

was performed during plant maturation (stage R8; Fehr, Caviness, Burmood, & Pennington, 1971).

DNA extraction

For the study, ten seeds of each $F_{2:3}$ family were used for the extraction of DNA and then ground to a fine powder.

DNA was extracted from the seeds of each $F_{2:3}$ cultivar and family according to the protocol described by McDonald, Elliot and Sweeney (1994) with some modifications (Schuster, Queiroz, Teixeira, Barros, & Moreira, 2004). Approximately 50 mg of seeds were placed in 1.5-ml micro tubes. Then, 500 μL of extraction plug, which contains 200 mM Tris HCl (pH 7.5), 25 mM EDTA, 288 mM NaCl and 0.5% of SDS, was added. For a more precise maceration, a 3-mm diameter glass sphere was added in each tube, and the tube was agitated in a grinder to infuse the plant tissue. After three min. of agitation, 500 μL of the extraction plug was added followed by centrifugation at 14,000 rpm for ten min. After this process, the supernatant was transferred to a new tube, and 10 μL of K proteinase (10 mg mL^{-1}) was added for the removal of proteins. The samples were placed in a bath at 37°C for 30 min. To precipitate the DNA, 500 μL of iced isopropanol was added. After two min. of rest, the samples were centrifuged for 15 min. at 14,000 rpm. The supernatant was discarded, and the precipitate was placed in ambient temperature for 15 min. for drying. For the elimination of RNA, the precipitate was resuspended in 300 μL of TE (Tris HCl pH 7.5 and 5 M EDTA), containing 40 μg μL^{-1} RNAse A (10 mg mL^{-1}). The samples were placed in a bath at 37°C for 30 min. DNA was once again precipitated by the addition of 500 μL of iced isopropanol. After two min., the tubes were centrifuged for 15 min. at 14,000 rpm. The supernatant was discarded, and the precipitate was placed in ambient temperature for 15 min. for drying. After this period, 300 μL of TE was added to resuspend the precipitate.

The DNA concentration in each sample was estimated using a spectrophotometer (Nanodrop1000) to assess absorbance at 260 nm; each absorbance unit corresponded to 50 μg mL^{-1} double-stranded DNA (Sambrook, Fritsch, & Maniatis, 1989). The DNA of each sample was diluted in TE to a working concentration of 5 ng μL^{-1}.

The quality of the DNA was evaluated in a 0.8% agarose gel containing 0.02 μL mL^{-1} ethidium bromide.

Dt1 gene amplification

For *Dt1* gene amplification, we used the primers *TFL1b-pro-F* (5´-CCATGCTTAATCGGCATCACT-3´) and *TFL1b-pro-R* (5´-GGTGGTGGCATAGTTTAATT-3´), which generate 410-bp (base pairs) fragments. These primers were designed to detect a single nucleotide polymorphism (SNP) in the promoter of the *Dt1* gene (Liu et al., 2010). To validate the efficiency of this marker, plants with determined and undetermined growth types were used. The marker was subsequently assessed in the two CD 235RR x Igra RA 518 RR (145 lineages $F_{2:3}$) and T 117 x Igra RA 518 RR (90 lineages $F_{2:3}$) populations.

For polymerase chain reaction (PCR), the following reagents were added for a final volume of 20 μL: 2 mM $MgCl_2$, plug 1x (50 μM Tris, 10 μM HCl), 300 μM dNTPs, 0.2 μM primer (sense and antisense), 1 unit of Taq DNA polymerase, and 50 ng of DNA. PCR was performed in a Veriti thermocycler (Applied Biosystems). The following program was used: 94°C for 5 min.; 45 cycles at 94°C for 1 min., 55°C for 1 min., 72°C for 1 min.; and a final extension at 72°C for 10 min. The products of the amplification were submitted to electrophoresis in a 2% agarose gel. The gel was stained with ethidium bromide, and the products were revealed under ultraviolet light in a Vilber Lourmat photo documentation device (Marne La valle, FR).

Next, 10 μL of the obtained PCR products were submitted to digestion with the *NdeI* enzyme (New England) according to the manufacturer's instructions. The reaction proceeded in a 37°C bath for 3 hours and 50 min. and then submitted to 2% agarose gel electrophoresis.

Dt2 gene mapping

DNA was extracted from ten semi-determined growth type samples and ten undetermined growth type samples derived from the hybrids obtained from T 117 x Igra RA518 RR, all of which contained the *Dt1* gene. The sample comprised 10 seeds from each $F_{2:3}$ lineage. For each type of growth, two DNA bulk preparations were made from five samples each and used as patterns for the T 117 (semi-determined *Dt2Dt2*) and Igra RA518 RR (undetermined *dt2dt2*) cultivars.

To obtain candidate markers for the selection of the *Dt2* gene, ten micro-satellite primers from the G connection of soy (chromosome 18) (Sat_038, Sat_064, Sat_168, Sat_358, Satt070, Satt115, Satt130, Satt288, Satt340, Satt372) (Cregan et al., 1999) were used. DNA was amplified using the PCR conditions

previously described and visualized in 2% agarose gels.

The primers that identified polymorphism among the bulk of DNA with contrasting growth types were considered as candidates and used individually for the amplification of the ten samples of each growth type that formed the bulks.

PCR was performed in a Veriti thermocycler (Applied Biosystems) programed follows: 94ºC for 3 min.; 35 cycles at 94ºC for 30 s, 55ºC for 30 s, and 72ºC for 45 s; and a final extension at 72ºC for 20 min. The products of the amplification were dyed with a charging plug (bromophenol blue) and submitted to denaturation at 95ºC for 4 min. The fragments were separated by electrophoresis in a 6% denaturing polyacrylamide gel and dyed with 0.1% silver nitrate for fragment visualization.

Evaluation of the efficiency of selection with molecular marker for growth type in soybean

To validate the candidate marker, these methods were applied to the populations derived from the crossings between CD 235RR x Igra RA 518 RR and T 117 x Igra RA 518 RR. For the evaluation, 145 and 90 $F_{2:3}$ lineages of each crossing were used. The association of the marker with the phenotype was evaluated using the χ^2 test with contingency tables containing phenotypic and molecular data. The analysis was conducted using the Genes Program (Cruz, 2013).

In the population derived from the crossing of CD 235RR x Igra RA 518 RR, the efficiency of selection with the *Dt1* gene marker was evaluated once segregation occurred exclusively for this gene. In the population obtained from the T 117 x Igra RA 518 RR crossing, the efficiency of the *Dt2* gene marker was evaluated for the population exclusively segregated for this gene.

With the data obtained from the crossing of T 117 x Igra RA 518 RR, the frequency of recombination of the marker was calculated using the GQMOL program (Cruz; Schuster, 2006). The parameters of the F_2 population were determined using co-dominant markers and an estimate method for maximum analytic verisimilitude with an LOD minimum score of 3.0 for the significance of re-combination.

Evaluation of cultivars using *Dt1* and *Dt2* gene markers

DNA was extracted from 22 soy cultivars according to the method of McDonald et al. (1994) with modifications (Schuster et al., 2004). DNA was also extracted from samples of seeds from four individual plants of each cultivar that presented more than one pod in the apex of the stem, with the hypothesis that these plants possess the *Dt2* gene or that they were heterozygote for the *Dt1* gene. In total, 36 samples, which are identified in Table 1, amplified the *Dt1* and *Dt2* gene markers. The phenotypic information of stem growth type, which is presented in Table 1, is described by the products of the respective cultivars. The samples were amplified with markers for the *Dt1* and *Dt2* gene as described previously.

Table 1. Cultivars of soy and their respective stem growth type phenotypes as described by the obtainers.

Genotype	PPhenotype	Source
CD 201	Determined	Cooperativa Central de Pesquisa Agrícola (Coodetec, 2006)
TP 50981 (T 117 x Igra RA 518 RR)[1]	Semi-determined	Germoplasma Coodetec
NK412113	Undetermined	Reunião de Pesquisa de Soja da Região Central do Brasil (RPSRCB, 2003)
BRS 283	Undetermined	Empresa Brasileira de Pesquisa Agropecuária (Embrapa, 2010)
CD 207	Determined	Coodetec (2006)
CD 2630RR	Undetermined	Cooperativa Central de Pesquisa Agrícola (Coodetec, 2013)
CD 204	Determined	Coodetec (2006)
FTS CAMPO MOURÃO RR	Semi-determined	Fehr, Caviness, Burmood and Pennington (1971)
CD 202	Determined	Coodetec (2006)
BMX Apolo RR	Undetermined	Brasmax (2011)
CD 203	Determined	Coodetec (2006)
ANTA 82	Semi-determined	Tropical Melhoramento e Genética (TMG, 2013)
NK 7059RR	Undetermined	Syngenta Seeds (2013)
CD 219RR	Determined	Coodetec (2006)
BMX Ativa RR	Determined	Brasmax (2011)
GB 874RR	Determined	Girassol Agrícola (2013)
CD 211	Determined	Coodetec (2006)
CD 206RR	Determined	Cooperativa Central de Pesquisa Agrícola (Coodetec, 2011)
NK 7059RR	Undetermined[2]	Syngenta Seeds (2013)
BMX Apolo RR	Undetermined[2]	Brasmax (2011)
NA 5909RR	Undetermined[2]	Nideira Sementes (2013)
BMX Potência RR	Undetermined[2]	Brasmax (2011)
T 117	Semi-determined	Bernard (1972)
Igra 518 RR	Undetermined	Igra Sementes (2013)

[1]Experimental lineage. [2]Plant with more than one pod in the apex of the stem.

Results and discussion

To validate the selection with molecular markers for the *Dt1* gene in soybean, the primers *TFL1b-pro-F* and *TFL1b-pro-R* (Liu et al., 2010) were used. These primers amplify a 410-bp fragment of genomic DNA in lineages with determined and undetermined growth. Only the fragments that amplify the *Dt1* allele are digested into 217- and 193-bp fragments with *NdeI* enzyme.

Thus, we demonstrated that the evaluated plants (Figure 1) exhibited the expected DNA profile. The cultivars T 117, Igra RA 518 RR, BMX Apolo RR, NK 7059RR and plants 1 through 6 exhibit the *Dt1Dt1* genotype. The cultivars CD 214RR and CD 206RR exhibit the determined growth type and the *dt1dt1* genotype.

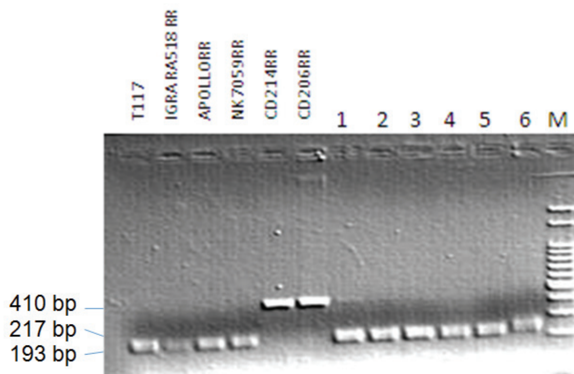

Figure 1. Amplification with GmTFL1b-pro marker and digestion with *NdeI* enzyme. Plants with a semi-determined T 117 growth type and plants with an undetermined growth type: Igra RA 518 RR, BMX Apolo RR, and NK 7059 RR. Plants with a determined growth type: CD 214RR, CD 206RR. Plants 1 through 6 are obtained from a population that results from the crossing T 117 x Igra RA 518 RR, for which the phenotype is undetermined. M: 100-bp molecular weight marker.

The molecular basis of the growth type was determined by Liu et al. (2010). The authors isolated two pea orthologs (*Pisum sativum*), *GmTFL1a* and *GmTFL1b*. Based on the soy genome and mapping analysis, it was suggested that the *Dt1* gene encodes the *GmTFL1b* protein and that the type of soy stem growth is determined by variations in this gene.

The *dt1dt1* genotype is responsible for the determined growth type, whereas the *Dt1Dt1* genotype results in the undetermined growth type. *Dt1dt1* causes a semi-determined phenotype (Bernard, 1972).

The contrasting bulks were amplified, and the marker (considered candidate) used in this assessment was sat_064, which results in polymorphisms between the bulks and consistency with the parenting results.

The sat_064 marker was used to individually amplify all the plants that formed the bulks (Figure 2). A possible segregation among the samples was observed.

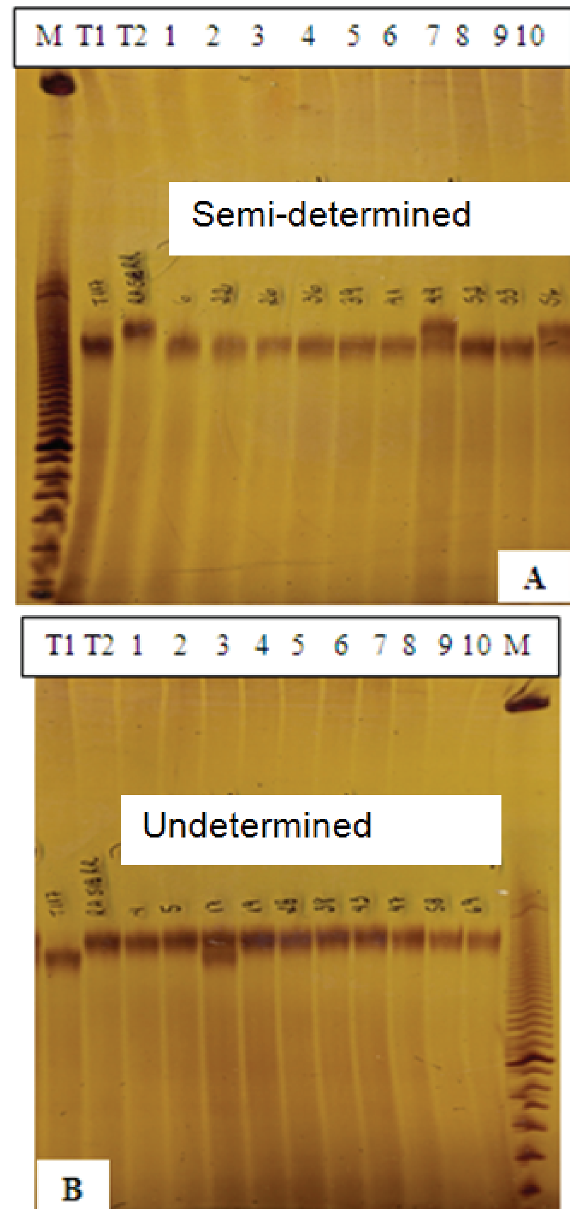

Figure 2. Amplification with the sat_064 marker. M: 100-bp molecular weight marker; T1: Control 1 – Cultivar T 117, which exhibits a semi-determined growth type as conferred by the *Dt2* gene; T2: Control 2 – Cultivar Igra RA 518 RR, which exhibits an undetermined growth type and does not possess the *Dt2* gene; A: Plants with a semi-determined growth type derived from crossing T 117 x Igra RA 518 RR; B: Plants with an undetermined growth type derived from crossing T 117 x Igra RA 518 RR.

All the plants derived from crossing CD 235RR x Igra RA 518 RR and T 117 x Igra RA 518 RR were analyzed with molecular marker for *Dt1* and *Dt2*.

The association test between the phenotypic and molecular data obtained from the CD 235RR x Igra RA 518 RR population were significant given that the phenotypic and molecular data are not independent (Table 2). However, various different results between the phenotypic and molecular evaluation were analyzed. This result was not expected; once the marker is used within the gene in the promoting area, there should be no risk for genetic recombination. In the field evaluations, some difficulty was encountered in distinguishing the plants according to the growth type based on the phenotype, which has led to erroneous classifications. In this case, we attribute the observed inconsistencies to errors in phenotype determination. Thus, the maker used for this analysis can be a very efficient tool to avoid such errors.

Table 2. Contingency table with molecular and phenotypic data obtained in the analysis of $F_{2:3}$ CD 235RR and Igra RA 518 RR populations.

CD 235RR x Igra RA 518 RR		Molecular Analysis: Determined	Segregating *Dt1*	Undetermined	Total
Phenotypic Analysis	Determined	23	3	0	26
	Segregating *Dt1*	5	52	5	62
	Undetermined	5	16	36	57
	Total	33	71	41	145

(χ^2 = 128.88; GL = 4; P(%) = 0.001).

The obtainer of cultivar CD 235RR described its stem growth type as semi-determined (Cooperativa Central de Pesquisa Agrícola [Coodetec], 2011). In this case, this cultivar possessed the *Dt1Dt1 Dt2Dt2* genotype. In addition, the crossing with 'Igra RA 518 RR' should not result in segregation of the *Dt1* gene. As a result, determined lineages in the progeny of this crossing should not be observed. The evaluation of the progeny of the hybrid CD 235RR x Igra RA 518 RR revealed F_2 determined, undetermined and semi-determined plants (which segregated in the F_3 generation). Based on these results, we conclude that the cultivar CD 235RR presents the *dt1dt1* genotype and a determined growth type.

In the progeny of the crossing T 117 x Igra RA 518 RR, we expected 100% of the plants to be homozygous for the *Dt1* gene and both parental factors (T 117 x Igra RA 518 RR) present in this gene. All the analyzed plants exhibited the *Dt1* gene in the molecular evaluation, which confirms the efficiency of the molecular marker used in this assessment. Moreover, no plants were classified as a determined type in the phenotypic analysis.

Two types of growth were expected in the offspring of the crossing T 117 x Igra RA 518 RR: undetermined and semi-determined. The T 117 cultivar harbors the *Dt2* gene and an undetermined growth type, whereas the Igra RA 518 RR cultivar exhibits an undetermined type.

For cultivars of determined and semi-determined growth, the terminal gem transforms into a terminal inflorescence. In contrast, for cultivars of undetermined growth, this gem does not form, and the stem continues to develop, even after the beginning of the florescence (Müller, 1981). In general, the cultivars are characterized as a determined type and are rarely characterized as semi-determined.

The association analysis between the phenotypic and molecular data obtained from the T 117 x Igra RA 518 RR population was also highly significant, and the distribution of phenotypic and molecular data was not independent (Table 3). This result indicates that the marker sat_064 is associated with the *Dt2* gene.

Table 3. Contingency table with molecular and phenotypic data obtained in the analysis of the $F_{2:3}$ population obtained from the crossing of T 117 and Igra RA 518 RR.

T 117 x Igra RA 518 RR		Molecular Analysis: Semi-determined	Undetermined	Segregating *Dt2*	Total
Phenotypic Analysis	Semi-determined	13	2	8	23
	Undetermined	0	12	4	16
	Segregating *Dt2*	7	8	36	51
	Total	20	22	48	90

(χ^2= 44.79; GL = 4; P(%) = 0.001).

The frequency of recombination obtained between the sat_064 marker and the *Dt2* gene was 19.4% with a LOD score of 8.19. It is possible that this estimate was increased compared with the actual frequency of recombination between the gene and the marker due to the difficulty in classifying the plants phenotypically as semi-determined types. According to the work of Bernard (1972), the progeny tests occasionally indicated that semi-determined or undetermined plants were incorrectly classified. In Table 3, the increased number of mistakes between phenotypic and molecular data is observed in classes wherein the plants were phenotypically classified as semi-determined type and segregating.

To evaluate the efficiency of the markers, 22 soybean varieties were evaluated using the markers for the *Dt1* and *Dt2* genes.

As noted in Figure 3, all the materials corresponded to that described by the obtainers (Table 1) with regard to the genotype and the presence or absence of the *Dt1* gene. The only exception was the cultivar FTS Campo Mourão RR, which was described by the obtainer as a semi-determined growth type; however, the *Dt1* gene is not present this genotype.

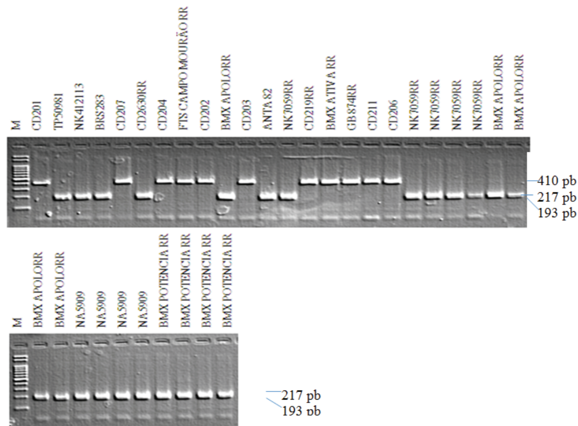

Figure 3. Amplification of DNA in soy cultures using the GmTFL1b-pro marker and digestion with *NdeI* enzyme. M: 100-bp molecular weight marker.

In Figure 4, all of the genotypes corresponded to that described by the obtainers (Table 1). The only exception is the cultivars FTS Campo Mourão RR, which the obtainer described as semi-determined growth; however, the *Dt2* gene is not present. In addition, the *Dt2* gene was not noted in the cultivar Anta 82 during molecular analysis. This result diverges from the obtainer's description of the cultivars NK 7059RR, BMX Apolo RR, BMX Potência RR and NA 5909RR. These cultivars were classified as semi-determined types, and the genotype should possess the *Dt2* gene because they presented a plant with more than one pod in the apex of the stem. In the four analyses of the individual plant of each cultivar, the presence of the *Dt2* gene was not confirmed, validating and confirming the information from the obtainers that indicated that these cultivars are homogeneous in regards to the absence of the *Dt2* gene.

According to the obtained results, the cultivar FTS Campo Mourão RR has a *dt1dt1dt2dt2* genotype and determined growth. This result is inconsistent with the obtainer's description of semi-determined growth and the *Dt1Dt1Dt2Dt2* genotype.

'Anta 82,' which is described as having a semi-determined growth type, demonstrated the presence of the *Dt1* gene and absence of the *Dt2* gene through the use of molecular markers. This result indicates that this cultivar presents a genotype that confers a phenotype of undetermined growth. This result is substantiated by information obtained in 2011 and never published by the Coodetec. A hybrid of Anta 82 cultivar (a female parental and male parental CD 216 and M-SOY 6101 with undetermined growth type) resulted in F_2 populations wherein all of the plants presented an undetermined growth type, indicating that 'Anta 82' exhibits an undetermined growth type.

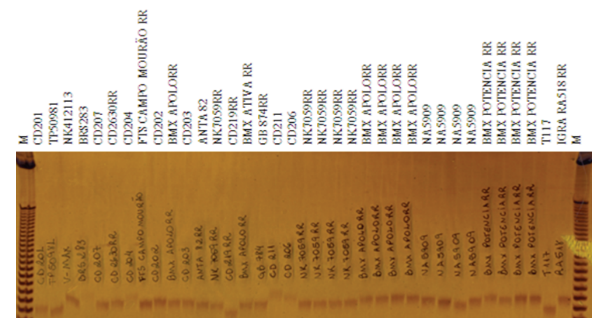

Figure 4. Amplification of soy cultivars' DNA with the sat_064 marker. M: 100-bp molecular weight marker.

The Dt1 gene was absent from and the Dt2 gene was present in CD 201 and CD 219RR cultivars. This phenotype confers a determined growth type and confirms the description of the obtainer. OC 4-Iguaçu*5 and W20 are the parental stains of CD 201; OC 94-2062 and CO 2131 are the parental strains of CD 219RR. The progenitor CO 2131 possesses the CP4-EPSPS gene, which confers tolerance to glyphosate (RPRCB, 2005). This result is supported by unpublished information by Coodetec: the OC 94-2062 lineage was the originator of the CD 211 cultivar, which possesses the dt1dt1dt2dt2 genotype. The probable origin of the Dt2 gene in the CD 219RR cultivar is CO 2131, which configures itself in a cross between [OC 95-3585 X OC 95(4)-3355 X H 5566RR]. Here, OC 95-3585 and OC 95(4)-3355 are brother lineages of OC 95(4)-2422 and are responsible for the origin of the CD 201 cultivar. The origin of the Dt2 gene in CD 201 and CD 219RR cultivars likely involves the common ancestor Ocepar 4- Iguaçu.

Conclusion

The association of molecular markers with the *GmTFL1b* gene is highly effective for the classification of soybean growth.

The sat_064 marker is associated with the *Dt2* gene, which is located in Connection Group G of the joint soybean map and exhibits a recombination frequency of 19.4%.

The molecular markers for *Dt1* and *Dt2* genes are efficient in the description of genotypes with regard to soybean stem growth and can be used in the selection of the improvement program.

References

Bernard, R. L. (1972). Two genes affecting stem termination in soybean. *Crop Science, 12*, 235-239.

Brasil. (2002). Ministério da Agricultura, Pecuária e Abastecimento. Serviço Nacional de Proteção de Cultivares. *Catálogo de cultivares protegidas de soja (Glycine max. (L.) Merrill)*. Brasília, DF: Mapa.

Brasmax. (2011). *Produtos 2012 – Região Sul*. Londrina, PR: Brasmax Genética Ltda.

Cooperativa Central de Pesquisa Agrícola. (2006). *Guia de produtos 2006*. Cascavel, PR: Coodetec.

Cooperativa Central de Pesquisa Agrícola. (2011). *Guia de produtos Sul – trigo, soja, milho 2011*. Cascavel, PR: Coodetec.

Cooperativa Central de Pesquisa Agrícola. (2013). *Catálogo de produtos 2013 Brasil – Sul e Paraguai*. Cascavel, PR: Coodetec.

Cregan, P. B., Bush, A. L., Shoemaker, R. C., Lark, K. G., Kahler, A. L., Kaya, N., … Specht, J. E. (1999). An integrated genetic linkage map of the soybean genome. *Crop Science, 39*, 1464-1490.

Cruz, C. D. (2013). Genes - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy, 35*(3), 271-276.

Cruz, C. D., & Schuster, I. (2006). *GQMOL: aplicativo computacional para análise de dados moleculares e de suas associações com caracteres quantitativos. Versão 9.1*. Viçosa, MG: UFV.

Empresa Brasileira de Pesquisa Agropecuária. (2010). *Cultivares de soja – regiões sul e central do Brasil 2010/2011*. Londrina, PR: Embrapa.

Fehr, W. R., Caviness, C. E., Burmood, D. T., & Pennington, J. S. (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merril. *Crop Science, 11*, 929-931.

Girassol Agrícola. (2013). *GB 874RR*. Recuperado de http://www.girassolagricola.com.br/?page_id=825

Igra Sementes. (2013). *Igra RA 518 RR*. Recuperado de http://www.igrasemillas.com/

Kilgore-Norquest, L., & Sneller, C. H. (2000). Effect of stem termination on soybean traits in southern U.S production systems. *Crop Science, 40*, 83-90.

Kleffmann & Partner. (2011). *Comércio e assessoria mercadológica e representação. Amis seed soybean 2010/2011*. Campinas, SP. Recuperado de http://www.portalklff.com.br/

Liu, B., Fujita, T., Yan, Z.-H., Sakamoto, S., Xu, D., & Abe, J. (2007). QTL mapping of domestication-related traits in soybean (*Glycine max*). *Annals of Botany, 100*, 1027-1038.

Liu, B., Watanabe, S., Uchiyama, T., Kong, F., Kanazawa, A., Xia, Z., ... Abe, J. (2010). The soybean stem growth habit gene *Dt1* is an ortholog of Arabidopsis terminal flower1. *Plant Physiology, 153*, 198-210.

McDonald, M. D., Elliot, L. J., & Sweeney, P. A. (1994). DNA extraction from dry seeds for RAPD analyses in varietal identification studies. *Seed Science and Technology, 22*(1), 171-176.

Muehlbauer, G. J., Staswick, P. E., Specht, J. E., Graef, G. L., Shoemaker, R. C., & Keim, P. (1989). RFLP mapping using near-isogenic lines in the soybean [*Glycine max* (L.) Merr.]. *Theoretical and Applied Genetics, 81*, 189-198.

Müller, L. Taxionomia e morfologia. (1981) In: S. Miyasaka, & J. C. Medina (Eds.), *A Soja no Brasil* (Cap. 4, p. 65-104). São Paulo, SP: ITAL.

Nideira Sementes. (2013). *NA 5909 RR*. Recuperado de http://www.niderasementes.com.br/produtodetalhe.aspx?id=95

Reunião de Pesquisa de Soja da Região Central do Brasil (RPSRCB). (2003). *Resumos*. Uberaba, MG: Embrapa.

Reunião de Pesquisa de Soja da Região Central do Brasil (RPSRCB). (2005). *Resumos*. Cornélio Procópio, PR: Embrapa.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning a laboratory manual* (2nd ed.). New York, NYC: Cold Spring Harbor Laboratory Press.

Schumutz, J., Cannon, S. B., Schulueter, J., Ma, J., Mitros, T., Nelson, W., ... Jackson, S. A. (2010). Genome sequence of the palaeopolyploid soybean. *Nature, 463*, 178-183.

Schuster, I., & Oliveira, M. A. R. (2006). Biotecnologia aplicada ao melhoramento de plantas. In V. Carpentieri-Pípolo (Ed.), *Biotecnologia na agricultura: aplicações e biossegurança* (p. 213-237). Cascavel, PR: Coodetec.

Schuster, I., Queiroz, V. T., Teixeira, A. I., Barros, E. G., & Moreira, M. A. (2004). Determinação da pureza varietal de sementes de soja com o auxílio de marcadores moleculares microssatélites. *Pesquisa Agropecuária Brasileira, 39*, 247-25.

Serviço Nacional de Proteção de Cultivares. (2012). Ministério da Agricultura, Pecuária e Abastecimento. *Instruções de DHE e tabela de descritores mínimos*. Recuperado de http://www.agricultura.gov.br/

Syngenta Seeds. (2013). *V-Max RR (NK 7059RR)*. Recuperado de http://www.syngenta.com/country/br/pt/produtosemarcas/sementes/grandesculturas/soja/Documents/sementes_soja.pdf

Tian, Z., Wang, X., Lee, R., Li, Y., Specht, J. E., Nelson, L. R., … Ma, J. (2010). Artificial selection for determinate growth habit in soybean. *PNAS, 107*, 8563-8568.

Tropical Melhoramento e Genética. (2013). *Anta 82*. Recuperado de http://www.tmg.agr.br/cultivares/soja/anta82

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