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Soybean Breeding Aiming at increasing Productivity and Root-Knot Nematode Resistance

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Abstract - *In Brazil, the root-knot nematode (Pratylenchus brachyurus) has gained importance, whatever because of the damage caused to soybean crops or because of its broad dispersion and incidences in producing areas. Therefore, this invention aimed at developing a new cultivar resistant to the major soybean diseases as well as to the root-knot nematode. As a result, we developed a soybean cultivar designated UFUS 8301. Generations were advanced by the single seed descent method. Value for Cultivation and Use assays were carried out during a 3-year period (2010/13). Distinctness, uniformity, and stability experiments were carried out during a 2-year period (2011/13). We used the reproduction factor (RF) statistics to assess damage and reproductive potentials of P. Brachyurus; analysis of variance tested differences between means. We accepted the null hypothesis there was no difference between UFUS 8301 and the parameter of resistance Crotalaria spectabilis. UFUS 8301 was found distinct from any other cultivar, homogeneous to the descriptors that had identified it and stable through generations. UFUS 8301 presented 19% oil and 39% protein on the seeds, and yield (3687.5 kg ha^{-1}) above Brazilian national average.*

Keywords—Brazilian Cerrado, Glycine max, Pratylenchus brachyurus, Soybean breeding.

I. INTRODUCTION

Although Soybean [*Glycine max* (L.) Merr.] is grown primarily for grains production, its industrial uses range from feeding, production of yeasts and antibodies to manufacture of soaps and disinfectants. Concerning human consumption, this is used mainly as purified oil, in margarine, shortenings, cooking and salad oils. Besides, protein meals are dominated by soybean meal, which accounts for more than two-thirds of global protein meal production. The high-protein defatted and toasted soybean meal is used as a supplement in feed rations for livestock, such as poultry and swine, and recently also in aquaculture [1, 2].

Soybeans exports are dominated by Brazil and the United States, which together account for nearly 80% of global exports. Global soybean production has increased sharply in 2016, with the United States and Brazil registering record crops. Brazil is prospected to overtake the United States as the largest soybean producer, as more additional lands are made available, Brazilian production is expected to grow at 2.6% per annum (p.a.) compared to Argentina (2.1% p.a.) and the United States (1.0% p.a.) [2].

Brazilian soybean-breeding programs have been able to increase agricultural diversification with new well-adapted crops, bringing new lucrative opportunities for farmers. The Ministry of Agriculture is responsible for both protection and registration of new cultivars. However, to be produced and marketed in the country, it is previously required to be included in the National Register of Cultivars, regardless of whether the new variety is protected or not.

Value for Cultivation and Use (VCU) and Distinctness, Uniformity, and Stability (DUS) tests are mandatory as part of the application process to register, protect and add a new soybean cultivar on the National Register of Cultivars. VCU assays are established to assess differences in productivity, biological and chemical features and technological characteristics, resistance to pests and diseases, and other commercially important traits. These assays must be carried out for a minimum two years to compare the performance of candidate varieties with varieties already on the National Register of Cultivars. Also, The DUS assays are performed to provide evidence that the candidate cultivar subject to protection is distinct from other(s) whose descriptors are well known, also is homogeneous within generations and stable to the same traits over successive generations.

In general, soybean genetic improvement is a process of production of the variability of desired traits intending selection of superior genotypes and seeds multiplication for commercial purposes. Several selection methods have been used to identify progenies who possess the most useful combination of wanted features. Most often, these include pedigree selection, by visual screening of the best-appearing families in each generation; single-seed descent, by advancing one seed or pod from each plant the next generation to develop homozygous lines; and bulk breeding, where a population is advanced in bulk until later generations with no artificial selection, when nearly homozygous lines are selected for yield assay [3].

In the soybean-breeding program at the Federal University of Uberlândia, to develop any new cultivar, we first define our goals according to problems and weaknesses of the current germplasm. In this context, the root nematode *Pratylenchus brachyurus* has gained importance in Brazil, whatever because of damage caused to soybean crops or for its broad dispersion and high incidence in producing areas, such as Cerrado.

A root-knot nematode survey in the north of Mato Grosso, in the 2016/2017 harvest and 2017-second crop, has pointed to the increasing of nematode populations numbers in all municipalities; all of 3328 samples analyzed were found positive for *Pratylenchus* sp. presence [4]. *P. brachyurus*, like many other plant-parasitic nematodes, are microscopic worms that can be damaging to many crops. Across the world, many billions of dollars have been lost due to the damaging abilities of nematodes in cash crops [5]. In the Brazilian soybean fields, *P. brachyurus* has become increasingly common. Lima et al. [6] assessed *P. brachyurus* populations throughout Tocantins state in fields grown off-season and found this pathogen on 82% of samples, with densities ranging from 23 to 20,400 nematodes per 200 cm^3 soil, or 10 g root samples.

Therefore, our goal with this invention was developing a soybean cultivar able to meet producers need as resistance against major soybean diseases, including to the root-knot nematode, in addition to high oil and protein contents along with high grain yield. As a result, we developed a new cultivar designated UFUS 8301, which presents comparative advantages over others in high productivity and *P. brachyurus* resistance.

II. MATERIALS AND METHODS

UFUS 8301 was derived from the crossing between IAC-8.2 x MG / BR 46 (Conquista) on the Capim Branco farm, located in Uberlândia, MG, Brazil; Latitude 18º 52' 94" S, Longitude 48º 20' 45" O, Altitude 835 m. Generations were advanced by the single seed descent (SSD) method. In this approach, each generation has only one single seed randomly selected from each plant, which is bulked to grow the next generation. In general, this procedure refers to planting a segregating population, harvesting a sample of one seed per plant, and using this one-seed sample to produce the next generation. Other methods as single-pod descent (SPD) and bulk methods (BM) produce redundant inbred lines that are descended from either the same F2 or F3 plant. However, single seed descent (SSD) has the advantage of minimizes the amount of genetic variability, although SSD method requires more time than SPD and BM [7].

Manual crosses produced hybrid seeds (F1). The F1s were grown on the field and allowed to self-pollinate to produce F2 seeds. One single seed from each F2 plant was collected and bulked to plant the F3 generation. F3 seeds were advanced by single seed descent (SSD) method until F6. Superior plants F6 were grown in the field, and the best lines were selected and assessed in progeny tests (F7). Progeny tests were carried out in a randomized block design, with three replications. Final yield assays were conducted in many locations for a 2-year period, and promising advanced breeding lines were thoroughly tested and compared to appropriate standards in environments representative across several places in the Brazilian states Minas Gerais (MG), Goiás (GO) Mato Grosso (MT), Bahia (BA), São Paulo (SP) and Piauí (PI) (Table 1).

Table.1: Breeding method, assays and selected features applied on developing of UFUS 8301.

[a]G/Y	Location	Method	Selected Features
F_1 2003	Capim Branco Farm MG	Bi-parental crosses	Diseases resistance and grain yield
$F_{2/3}$ 2004		[c]SSD	Diseases resistance
F_4 2005		SSD	
F_5 2005		SSD	
F_6 2006		SSD	
F_7 2006		Progeny-test	Diseases resistance and grain yield
F_8 2007		Preliminary yield assay	Diseases resistance and quantitative traits
F_9 2007/8		Intermediate yield assay	
$F_{10/11}$ 2008/10	MG, MT, BA GO	Final yield assay, [b]VCU	
$F_{11/12}$ 2010/13	MG, GO, MT, BA PI	VCU	

[a]G/Y = Genarations/Year; [b]VCU = value for cultivation and use; [c]SSD = single seed descent.

2.1 Value for Cultivation and Use (VCU)

VCU trials were carried out during a 3-year period (2010/13), in a randomized blocks design with three replications. Plot sizes were equal to 5.0 m^2. Experimental regions were labeled. The experiments in the Regions 302 (Ituverava, SP), 303 (Uberlândia, Urutaí, Itumbiara, Minas Gerais), 401 (Rondonopolis, Alto Taquari, Palmeiras de Goiás Goiás), 402 (Lucas Rio Verde, Sinop, Mato Grosso) and 403 (Porto Alegre do Norte, Mato Grosso) were carried out during 2011/12 and 2012/2013 growing seasons. Experiments in Regions 405 (Luís Eduardo Magalhaes, Bahia) and 501 (Bom Jesus, Piauí) were carried out during 2008/2009 and 2009/10 growing seasons.

We have assessed agronomic traits: number of days to flowering, plant height at flowering, number of days to maturity, plant height at maturity, first pod height, number of nodes on the main stem at maturity, number of pods with one, two and three seeds per plant, total number of pods per plant, number of seeds per pod and grain yield. We computed the number of days from the emergence to maturation when 95% of pods were dried; and the vegetative cycle by the number of days from emergence to flowering (50% of flowering plants); maturity was reported in the first day on which 95% of the pods turned brown. We measured first pod insertion hight from the soil level to the insertion of the first pod. We have assessed pod dehiscence using a scale ranging from 0 (no dehiscence) to 10 (complete dehiscence), and lodging resistance from 1 (no lodging) to 5 (all plants are prostrate) [8].

We assessed yielding per useful plots area to evaluate the productivity of cultivars and lineages; standardized to 13% of humidity and transformed into kilograms per hectare [9]. Oil and protein were assessed by NIR spectrophotometry and results were reported on an as is percentage basis.

Data obtained were assessed by analysis of variance considering the randomized block design model $Y_{ij} = \mu + G_i + B_j + E_{ij}$, where: Y_{ij} = observed value of the *i*-th genotype in the *j*-th block; μ = general mean; G_i = effect of the *i*-th genotype ($i = 1, 2, ..., g$); B_j = effect of the *j*-th block ($j = 1, 2, ..., b$); E_{ij} = experimental error.

The Scott-Knott test grouped means at 5% probability level. Experiments that had presented Coefficients of Variation higher than 20% were not computed in the yielding by region analysis.

We have assessed UFUS 8301 resistance, on the field and greenhouse conditions, regarding the diseases caused by the pathogens: *Xanthomonas axonopodis* pv. *Glycines*, *Pseudomonas syringae* pv. *glycinea*, *Cercospora sojina*, *Phialophora gregata*, VMCS, *Microsphaera diffusa*, *Diaporthe phaseolorum* f. sp. *meridionalis*, *Fusarium solani*, and nematodes *Pratylenchus brachyurus*, *Meloidogyne incognita*, *Meloidogyne javanica*, and *Heterodera glycines*.

2.2 *Pratylenchus brachyurus* resistance

We evaluated UFUS 8301 *P. brachyurus* resistance in a completely randomized design, with three replicates. The experiment was carried out from November 2013 to February 2014. A pure population of *P. brachyurus* inoculum was multiplied and maintained at greenhouse conditions in soybean plants. We have extracted *P. brachyurus* isolates from soybean roots through the method proposed by Coolen & D'Herde [10].

To assess damage and reproductive potentials of *P. Brachyurus* populations, we have measured the reproduction factor (RF). This statistics has been widely used in nematological studies to define resistance and susceptibility of plants to nematodes. According to Taylor & Sasser [11], since the reproductive ability on a given host is directly related to resistance nematode reproduction that can be used to measure root-knot nematode resistance. The FR is the ratio, R = final nematode population/initial nematode population, where $R \leq 1$ indicates an inefficient host [12]. In our experiment, the RF was assessed 30, 60 and 90 days after inoculation. BRS MT Pintado was setting as the parameter of susceptibility, and *Crotalaria spectabilis* as the parameter of resistance. Santos et al. [13] have shown that the best moment for assessing the FR of soybean genotypes under greenhouse conditions range from 75 to 90 days afterward inoculation with *P. brachyurus*.

Analysis of variance (ANOVA) was used to test the differences between RF means. The null hypothesis stated there was no difference between the observed values and the expected value given by *C. spectabilis*, which is a poor-host *Pratylenchus* spp. The use of *C. spectabilis* in succession or rotation with soybean has been the most effective measure for the control of root-knot and root-lesion nematodes in infested areas [13, 14]. RF means were grouped by Scott-Knott at 5 % of probability level to find out the homogeneous groups, whenever the situation had led to a significant *F*-test [16].

2.3 Distinctness, uniformity and stability (DUS)

DUS experiments were carried for a 2-year period (2011/13) on the *Capim Branco* farm, latitude 18º 52' 94" S, longitude 48º 20' 45" O, Altitude 835 m, under conditions ensuring normal development of plants. For differentiation, characteristics contained in the official descriptor of the species/genus were used. As required by the Ministry of Agriculture and Food Supplies (MAPA) for completion of the detailed technical report, in each assay was included 300 plants, with three replicates. Distinguishability and stability were assessed in 20 plants. We evaluated the descriptors hypocotyl color, type of growth, pubescence color, flower color, pod color, the shape of the seed, integument color, and peroxidase reaction.

III. RESULTS AND DISCUSSION

We found UFUS 8301 distinct from any other cultivar, homogeneous to the descriptors that had identified it, as well stable through successive generations. The number of atypical plants found in the DUS experiments was equal to 3. Conquista was found the most similar cultivar to UFUS 8301; therefore, it was used for differentiation purposes; traits that differentiate them both are described in Table 2.

Table.2: Most similar cultivar to UFUS 8301 and characteristics that differentiate them both.

Differentiating features	**Features expression on MG / BR 46 Conquista**	**Features expression on UFUS 8301**
Anthocyanin pigments	Present	Absent
Type of growth	Determinate	Determinate
Flower color	Purple	Wight
Pubescence	Brown	Light brown
Hilum color	Black	Medium brown
Plant size	Medium	Medium
Pratylenchus brachyurus	Susceptive	Resistant
Meloidogyne incognita	Resistant	Susceptive
Meloidogyne javanica	Resistant	Susceptive

UFUS 8301 presented determined growth and lodging resistance. Excessive vegetative growth might lead to lodging. Soybean lodging can reduce yield potential and increase harvest losses as lodged plants are more difficult to cut and gather into the combine [17]. Buzzello et al. [18] found a negative correlation between lodging and grain yield, and a positive correlation between plant height and lodging. UFUS 8301 has also shown to be pod dehiscence resistant. Pod dehiscence (shattering) is a significant source of yield loss of mechanically harvested soybeans. Harvesting shattering-susceptible soybean varieties in dry weather conditions can lead to seed losses of 50 to 100% [19].

Pereira Júnior et al. [20] described standard values of insertion of the first pod greater than 14 cm. However, according to Almeida et al. [21], the first pod insertion height should be higher than 10 cm because it is desirable to mechanical harvesting since that would avoid losses of uncollected pods due to its low insertion height. UFUS 8301 first pod height ranged from 10 to 11 cm (Table 3). Val et al. [22] assessed agronomic traits of 30 soybean genotypes in Jaboticabal, SP, 2012/2013 harvest, and observed first pod heights ranging from 5.40 to 20.73 cm; in particular, for Conquista, the most similar cultivar to UFUS 8301, that was equal to 13.07 cm.

Table.3: Average results of agronomic traits assessed in UFUS 8301.

[a]Reg	[b]DF	[c]DM	[d]PH	[e]FPH	100-seed weight (g)
302	45	129	71	10	14
401	45	122	70	10	14
402	46	118	70	11	14
403	44	119	69	11	14
405	45	120	65	10	15
501	42	121	67	10	14

[a] Experiments in Regions 302; 303; 401; 402; 403 were carried out in during 2011/12 and 2012/2013 growing seasons. Experiments in Regions 405 and 501 were carried out in during 2008/2009 and 2009/10 growing seasons. [b] NDF = Days to 50% flowering; [c] NDM = Days to maturity; [d] PH = Plant height at maturity (cm); [e] FPH = First pod height (cm).

Cordeiro Júnior et al. [23] studying the behavior of 30 soybean cultivars in Sao Paulo Northern, 2016/17 harvest, observed plant heights at maturity ranging from 47.92 to 88.75 cm. Arantes et al. [24] described features and use recommendations of soybeans indicated to the central region of Brazil; Conquista average plant height was reported to be equal to 80 cm. Another study performed in Jaboticabal, SP, 2012/2013 harvest, assessed agronomic traits assessed of 30 soybean genotypes, plant heights at maturity were reported ranging from 55.67 to 108.13 cm, Conquista average plant height was equal to 94.20 cm [22]. We found UFUS 8301 averages plant height ranging from 65 to 71 cm (Table 3).

As soybean is a photoperiod-sensitive and self-pollinated species, days to flowering and maturity, duration of flowering-to-maturity and plant height are crucial for soybean adaptability and yield [25]. The photoperiod influences soybean cultivars causing changes in behavior depending on the latitude, i.e., in Brazil, Arantes et al. [24] observed Conquista reaches maturity of about 130 days in Uberaba, MG (latitude 19 ° S); but in Sorriso, MT (latitude 15 ° S), it reached maturity at 110 days. UFUS 8301 has shown the life cycle of 118 to 129 days. Soybean flowering period is relatively extensive (ranging from 30 to 40 days) and overlaps with the formation of pods and seeds, which makes it resist short periods of drought during flowering [26]. Sudhanshu et al. [27] found that both days to 50% flowering and plant height have a direct effect on reducing seed yield. UFUS 8301 has shown 42 to 46 days to 50% flowering (Table 3).

Soybean yielding depends on numerous traits contributing to production, which might have their action linked. Therefore, the selection practiced on one characteristic may simultaneously bring change in the other related feature. Path coefficient analysis has shown that seed yield/plant is a positive and significant association with biological yield, pods per plant and 100-seed weight, indicating that an intense selection for these characters will improve seed yield in soybean. Among these traits, 100 seed-weight exhibited the highest positive direct effect on seed yield [26]. The average weight of 100 seeds may vary depending on the sowing season and locality [24]; UFUS 8301 average 100-seed weight ranged from 15g to 14g (Table 3).

UFUS 8301 advantage to the producers was confirmed by comparing its productivity over other materials (Table 4). Besides, crop average national productivity estimated by the CONAB [28], from 2011 to 2013, recorded 2220.95 kg / ha^{1}; the average productivity of UFUS 8301 in the same period was 3687.5 kg ha^{-1} representing a productivity increase of 1.66 times.

Table.4: Production comparative results (kg ha^{-1}) of UFUS 8301 and cultivars witness.

[a]Regions [b](CV %)		302 (12)	303 (13)	401 (12)
	UFUS 8301	3700	3660	3660
Witnesses	*BRS 262*	3670	3550	3560
	M SOY 8001	3650	3590	3700
	Emgopa 316	3590	3630	3680
Regions (CV %)		401 (12)	402 (11)	403 (13)
	UFUS 8301	3730	3700	3680
Witnesses	*BRSMG 752S*	3650	3600	3760
	Emgopa 316	3700	3550	3540
	MSoy 8001	3750	3650	3670
Regions (CV %)		405 (11)	501 (12)	
	UFUS 8301	3700	3670	-
Witnesses	*Sambaiba*	3670	3600	-
	MSoy 8914	3550	3656	-
	MSoy 8866	3650	3650	-

[a] Experiments in Regions 302; 303; 401; 402; 403 were carried out in during 2011/12 and 2012/2013 growing seasons. Experiments in Regions 405 and 501 were carried out in during 2008/2009 and 2009/10 growing seasons. [b] CV = Coefficient of Variation.

Modern soybean is one of the world's most important crops mainly because of its high protein (40%) and oil (20%) content [29]. However, soybean oil and protein content in the seeds are under polygenic genetic control, hence subject to environmental effects. We found 19% oil content and 39% protein content UFUS 8301 seeds (Table 5). In the study performed in 2010/2011 harvest, in the central region of Brazil, Conquista seeds were reported to hold 42.70 % protein, and 19.70 % oil content [24].

Table.5: Oil and protein contents percentage found contents in the seeds of UFUS 8301 and the cultivars witness.

[a]Reg	**UFUS 8301**		**Witnesses**					
			BRS 262		*M Soy 8001*		*Emgopa 316*	
	[b]O	[c]P	O	P	O	P	O	P
302	18	38	19	39	18	38	-	-
303	19	39	18	39	19	38	-	-
401	18	39	18	38	18	38	18	38
			BRSMG 752S		*Emgopa 316*		*MSoy 8001*	
401	18	39	18	38	18	38	-	-
402	19	38	18	39	19	38	18	39
403	18	39	19	39	18	39	19	39
			Sambaiba		*MSoy 8914*		*MSoy 8866*	
405	19	39	19	39	18	39	18	39
501	18	38	18	38	19	38	19	38

[a] Reg = Experiments in Regions 302; 303; 401; 402; 403 were carried out in during 2011/12 and 2012/2013 growing seasons. Experiments in Regions 405 and 501 were carried out in during 2008/2009 and 2009/10 growing seasons; [b] O = Oil; [c] P = Protein.

The diseases are among main factors limiting the increase of soybean yield. In recent years, many soybean diseases and plagues have already been reported for the crop; however, its incidence and severity depend on factors such as climate, cultivars, pathogen inoculum potential, soil structure and fertility, plant vigor, among others [30]. We found UFUS 8301 resistant to the virus VMCS (soybean mosaic virus), and to bacterium *X. axonopodis* pv. *glycines* (bacterial pustule) and *P. syringae* pv. *glycinea* (bacterial blight). Bacterial pustule and bacterial blight can occur in all soybean-producing regions. However, most of the cultivars in use are genetic resistance to these bacteria; therefore, nowadays, these are considered minor diseases problem [30]. Also, UFUS 8301 was resistant to *C. sojina* (frogeye leaf spot) and *P. gregata*. In Brazil, some *C. sojina* breeds have already been detected; fortunately, most soybean cultivars in use are genetic resistance to these breeds. However *P. gregata*, the fungus that causes the disease known as brown stem rot can be severe in soils with reduced fertility and when temperature and moisture are favorable for soybean seed development [30].

UFUS 8301 was also resistant to *D. phaseolorum* f. sp. *meridionalis* (stem canker) and *F. solani* (fusarium root rot). Stem canker is a fungus adapted to regions with higher temperature; all Brazilian soybean cultivars currently in use are resistant to this pathogen. The fusarium-root-rot damages vary and depend on the intensity of the inoculum and environmental conditions; however, the decline in yield in some soybean producing regions might be evident [30]. We also found UFUS 8301 moderate resistant to powdery mildew. The fungus *M. diffusa*, which is common in plants grown in greenhouses, causes this disease; however, the vast amount of spores formed on the leaf surface lately is easily disseminated by the wind, and this disease is gaining economic importance, leading to the need for chemical control in many soy-producing regions [30].

Regarding the root-knot nematodes *M. incognita* and *M. javanica*, UFUS 7401 have not shown resistance; however, it has proved to be unfavorable or poorly adapted to the *P. brachyurus* reproduction. There was no significate difference, by the Scott Knott test at 5% probability, between UFUS 8301 and *C. spectabilis* (Table 6).

Parameter of Resistance	**Cultivars**	**RF**
Crotalaria spectabilis **FR** = 1.14 b	UFUS Carajás	12.78 a
	UFUS Mineira	11.70 a
	UFUS 105	7.16 a
	Pintado	6.98 a
	UFUS Vila Rica	5.02 b
	BR 46 (Conquista)	3.70 b
	UFUS 119	3.68 b
	UFUS 32	3.56 b
	UFUS Guará	3.50 b
	UFUS 37	2.84 b
	UFUS 8301	2.56 b
	UFUS 7401	1.70 b
	UFUS 8401	1.54 b
	UFUS 6901	1.40 b
[b]CV (%)		25.80

[a]Averages followed by the same letter constitute a homogeneous group by the Scott Knott test at 5% probability; [b] CV = Coefficient of Variation.

There has been considerable progress in our knowledge of microorganisms and push-pull plants (e.g., *C. spectabilis*) that contribute to the biocontrol of nematodes [31]. According to Monteiro [32], despite *Crotalaria* species are generally unfavorable or poorly adapted to the reproduction of *Pratylenchus* spp., some breeds can multiply or remain in these plants as these reactions vary as species and their populations in both plants, and parasites. Therefore, this might explain our *Crotalaria* RF results superior to one (Table 6).

IV. CONCLUSION

We developed a new soybean cultivar with high productivity and resistant to *P. brachyurus*; that aspects represent comparative advantages over others cultivars current in use in Brazil. UFUS 8301 has been included in the National Registry of Cultivars (RNC) under *P. brachyurus* resistant status, register number 33899. Seed production has begun in by the Federal University of Uberlândia (UFU), Uberlândia – MG 2014, in compliance with the regulations of MAPA. Recommended cropping from October 20 to December 10 in growing seasons in Minas Gerais, Mato Grosso, Bahia, Piauí, São Paulo and Goiás states. Ideal population density is 240 to 270 thousand plants per hectare. Suggested production systems are no-till and conventional tillage.

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