

Development of Brazilian Soybean Cultivars Well Adapted to Cerrado and Rust-Tolerant

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Abstract— *The major diseases limiting soybean growth and yield must be either controlled or mitigated. Among these, soybean rust is the most harmful; in addition, Phakopsora pachyrhizi populations less susceptible to both IDM and IQe fungicides were observed. Therefore, this invention aimed at developing a new soybean cultivar rust resistant and highly productive. Generations were advanced by single seed descent method on the Capim Branco farm, Federal University of Uberlândia. Value for Cultivation and Use were carried out during a 3-year period (2010/13) in several locations, and Distinctness, Uniformity and Stability experiments for a 2-year period (2011/13) on the Capim Branco farm. Hence, we developed the novel soybean cultivar designated UFUS 7415. UFUS 7415 has shown high contents of oil (20%) and protein (40%), and high yield potential (4.300 kg ha⁻¹) even in environments with Asian rust.*

Keywords— *Cultivar release, Glycine max, Phakopsora pachyrhizi, Plant disease resistance, Soybean breeding.*

I. INTRODUCTION

Since its establishment in 1996, the Soybean Breeding Program of the Federal University of Uberlândia (UFU) has been developing and releasing new soybean cultivars, which are resistant to biotic and abiotic stresses, highly productive, and well adapted to the Brazilian states Minas Gerais (MG), Maranhão (MA), Tocantins (TO), Mato Grosso (MT), Piauí (PI) and São Paulo (SP).

The program strategy is to initiate the development of a new cultivar by the establishment of the program goals, the definition of problems and weaknesses of the current germplasm, and the definition of specific breeding objectives. Next step is the selection of germplasm that holds the traits required to meet the goals defined. The crossing between divergent parents produces the genetic variability of the specific features intended to be transferred into the new variety. The progenies from these crosses are then allowed to natural genetic segregation through generations of self-fertilization. Finally, promising breeding lines are tested and compared to appropriate standard cultivars in environments representative of the commercial target areas for three or more years; as recommended by The Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento, abbreviated MAPA).

Value for Cultivation and Use (VCU) and Distinctness, Uniformity, and Stability (DUS) assays are mandatory as part of the process to register, protect and add any new 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 must be carried out for a minimum two year to compare the performance of candidate variety with varieties already on the National Register of Cultivars. In

addition, DUS assays are carried out to provide evidence that the cultivar subject to protection is distinct from other(s) whose descriptors are well known, as well as homogeneous within generations and stable to the same traits over successive generations.

Soybeans production is dominated by Brazil and United States, which together account for nearly 80% of global exports. Brazilian soybean production is prospected to grow at 2.6% per annum, and Brazil is projected to overtake the United States as the largest soybean producer in the coming decade mainly because of its unique ability to expand the planted area [1]. However, primary factors limiting crop growth and yield must be either controlled or mitigated in order to reach this achievement. Although soybean-breeding programs have been effective solving many issues by increasing the diversity of new well-adapted crops, there are approximately 40 caused by fungi, bacteria, nematodes, and viruses already identified in Brazil, and this number continues to increase with the expansion of soybeans into new areas [2].

Among soybean diseases, the Asian-rust caused by the fungus *Phakopsora pachyrhizi* is the most harmful; losses might account more than 80% when environmental conditions are appropriate to the disease development [3]. The impact of the Asian-rust has been associated with the high specialization and variation of the pathogen concerning virulence in soybean cultivars bearing specific genes of resistance [4]. In order prevent the fungus from multiplying on the fields, the farmers are accomplishing some strategies as sanitary empty, use of cultivars with genes of resistance, sowing at the beginning of the recommended season, use of early maturity soybeans, and applications of recommended fungicides [5].

However, some populations of the *P. pachyrhizi* less sensitive to IDM fungicides were observed in 2007 [6]. In 2013-2014 were observed less sensitive populations to IQe fungicides [7]. In March 2017, the FRAC (Fungicide Resistance Action Committee) reported a lower sensitivity of *P. pachyrhizi* to ISDH fungicides in populations collected in 2015/16 and 2016/17 [8]. During the co-evolutionary race with their hosts, the pathogens evolved a vast repertoire of virulence proteins (effectors) to facilitate colonization and host infection. Many effectors are believed to directly manipulate processes within host cells by promoting the reduction of immunity, probably through their inhibitory effect on host RNA mediated defense [9].

Therefore, our goal with this invention was developing a new soybean cultivar to meet producer requirements as disease resistance, high yielding, and high oil and protein contents. As a result, we developed a new soybean cultivar called UFUS 7415, which presents comparative

advantages over others in high productivity and good tolerance to *P. pachyrhizi*.

II. MATERIAL AND METHODS

To develop this new cultivar we chose breeding and selection methods based on the heritability of traits intending to be improved. Therefore, we derived UFUS 7415 from the double-crosses [(UFV-16 x Liderança) x (BR 95015308 x UFV-18)], carried out on the Capim Branco farm, Uberlândia, MG, Brazil; Latitude 18° 52' 94" S, Longitude 48° 20' 45" O, Altitude 835 m (Table 1). By manual crosses, we produced the hybrid seed (F1). The F1s were then grown on the field and allowed to self-pollinate to produce the F2 seeds. One single seed from each F2 plant was collected and bulked to grow the F3 generation. We then, advanced the F3 seeds by single seed descent (SSD) method until F6. Other methods as single-pod descent (SPD) and bulk methods (BM) produce redundant inbred lines, which are descended from either F2 or F3 same plants. However, single seed descent (SSD) has the advantage of minimizes the amount of genetic variability, although SSD method requires more time to process the seed than SPD or BM [10].

We grow superior plants F6 on the field, and the best lines were selected and assessed in progeny tests (F7). We carried out the final yield assays 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 locations in the Brazilian states Minas Gerais (MG), Goiás (GO) and Mato Grosso (MT) (Table 1).

Table.1: Breeding procedures applied to develop of UFUS 7415, preliminary and standard yield trials.

Place	Generation (Yr.)	Method	Selected Traits
	F1 (2003)	Double-cross	Selective breeding
	F2 (2004)	Bulk	-
	F3 (2004)		
Capim Branco Farm	F4-F5 (2005)	^a SSD	Disease resistance
	F6 (2006)		
	F7 (2006)	Progeny-test	
	F8 (2007)	Preliminary yield assay	Disease resistance, quantitative traits
	F9 (2007/08)	Intermediate yield assay	
^c Several cities	F10 - F11 (2008/10)	Final yield assay	

^dSeveral areas F11 - F12 (2010/13) ^bVCU

^aSSD = single seed descent method; ^bVCU = value for cultivation and use; ^cSeveral cities: Uberlândia (MG), Alto Taquari (MT), Campo Alegre, Goiatuba and Palmeiras de Goiás (GO); ^dSeveral areas in the states: MG = Minas Gerais, GO = Goiás, MT = Mato Grosso.

The VCU trials were carried out on a randomized blocks design with three replications during a 3-year period (2010/13). Plot sizes were equal to 5.0 m². We have labeled regions as Region 302 (Ituverava, SP), 303 (Uberlândia, Urutaí, Itumbiara, MG), 401 (Rondonópolis, Alto Taquari, Palmeiras de Goiás Goiás), 402 (Lucas Rio Verde, Sinop, MT) and 403 (Porto Alegre do Norte, MT). The 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 were assessed. We had computed the number of days from the emergence to maturation when 95% of the pods were found dried; vegetative cycle accounted for the number of days from emergence to flowering (50% of flowering plants), and maturity on the first day in which 95% of the pods turned brown. We measured the height of the first pod from the soil level to the insertion of the first pod. We assessed pod dehiscence using a scale ranging from 0 (no dehiscence) to 10 (complete dehiscence); and lodging resistance with a scale from 1 (no lodging) to 5 (all plants are prostrate) [11].

Cultivars and lineages productive performance was assessed per useful plots; standardized to 13% of humidity and transformed into kilograms per hectare [12]. NIR spectrophotometry measured oil and protein.

In the Analysis of Variance (ANOVA), we considered 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; B_j = effect of the j -th block; E_{ij} = experimental error. We grouped the means by the Scott-Knott test at 5% probability level. Significance tests regarding genotype x environment (GxA) interactions were performed using the model $Y_{ijk} = \mu + g_i + b/a_{jk} + a_j + g_{aij} + e_{ijk}$. Where: y_{ijk} = trait value observed for the i -th genotype in the j -th environment in the k -th block; μ = overall mean; g_i = effect of the i -th genotype; b/a_{jk} = effect of the k -th block within the j -th environment; a_j = effect of j -th environment; g_{aij} = effect of genotype-environment interaction; and e_{ij} = effect of experimental error. We do not have included the experiments with

Coefficients of Variation (CV) higher than 20% in the analysis of yielding by region. Statistical analysis of data was made through the software GENES [13].

UFUS 7415 resistance was assessed 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*.

We assessed soybean rust severity and lesion type in experiments carried out on the *Capim Branco* farm in the 2016/17 growing seasons. Experiments were made up of fourteen soybean genotypes assessed in a randomized complete block design with three replicates; no chemical was used for preventive the Asian-rust. We applied Godoy's diagrammatic scale to assess rust severity [14]. We performed three evaluations per week for three weeks, and the mean of five plants per plot was used to calculate the area under disease progress curve or AUDPC. We included both, TMG 801 and BRSGO 7560 in the experiments as parameters of resistance.

DUS experiments were carried out over a 2-year period (2011/13) under conditions ensuring normal development of plants on the *Capim Branco* farm, latitude 18° 52' 94" S, longitude 48° 20' 45" O, altitude 835 m. We utilized characteristics included in the official descriptor of the species/genus with the purpose of differentiation regarding other cultivars. As required by the MAPA to completion of the technical report, 300 plants with three replicates made up each assay, and we assessed distinguishability and stability in 20 plants. The descriptors hypocotyl color, type of growth, pubescence color, flower color, pod color, the shape of the seed, integument color and peroxidase reaction were evaluated.

III. RESULTS

UFUS 7415 was found distinct from any other cultivar, homogeneous to the descriptors that had identified it, as well stable through successive generations. MSoy 6101 was the most similar cultivar to UFUS 7415; thus, it was used for differentiation purposes; traits that differentiate them both are in Table 2.

Table.2: Most similar cultivar to UFUS 7415 and characteristics that differentiate them both.

Differentiating features	Features expression MSoy 6101	Features expression UFUS 7415
Anthocyanin pigments	Absent	Present

Type of growth	Determinate	Semi Determinate	05		b	
Flower color	Wight	Purple	UFUS 1117-06	19.7 a	257.3 a	2656.74 a
Pubescence	Brown	Light brown	UFUS 1117-07	16.8 a	91.6 b	2368.32 a
Hilum color	Black	Imperfect black	UFUS 1117-08	18.8 a	136.3 b	2523.92 a
Plant size	Medium/height	Medium	UFUS 1117-09	20.5 a	163.6 b	2599.65 a
Hypocotyl Color	Green	Purple	UFUS 1117-10	13.5 b	64.3 b	2283.12 b
Fertility	High	Low	UFUS 1117-11	14.3 b	114.0 b	1040.67 b
<i>Meloidogyne incognita</i>	Susceptive	Susceptive				466.78 a
<i>Meloidogyne javanica</i>	Susceptive	Susceptive				

We found UFUS 7415 resistant to the virus VMCS (soybean mosaic virus) and bacterium *X. axonopodis* pv. *glycines* (bacterial pustule) and *P. syringae* pv. *glycinea* (bacterial blight). UFUS 7415 was also resistant to frogeye leaf spot (*C. sojina*), brown stem rot (*P. gregata*), stem canker (*D. phaseolorum* f. sp. *meridionalis*), fusarium root rot (*F. solani*) and powdery mildew (*M. diffusa*).

UFUS 7415 was found susceptible regarding the root-knot nematodes *M. incognita* and *M. javanica* (Table 2).

3.1 Soybean cultivars performance in the presence of *Phakopsora pachyrhizi*

As shown in Table 3 UFUS 7415 was found among the most productive genotypes under natural infection by *P. pachyrhizi*. It did not differ statistically from both parameters of resistance TMG 801 and BRSGO 7560.

Table.3: Yield-performance of 14 soybean genotypes under natural infection by *Phakopsora pachyrhizi* on the Capim Branco farm, Uberlândia, MG, Brazil. 2016/17 growing season.

Genotypes	^a NPG	^b NPA	Yield (kg ha ⁻¹)	^c AUDPC
UFUS1117-01	17.3 a	125.3 b	2283.12 a	237.77 b
TMG 801	18.0 a	122.3 b	2805.11 a	191.57 b
UFUS 1117-02	16.5 a	64.0 b	1638.52 b	287.23 a
BRSGO 7560	12.1 b	32.0 b	3468.56 a	94.78 b
UFUS Riqueza	19.5 a	161.0 b	2200.95 a	320.60 a
UFUS 1117-03	18.8 a	266.0 a	1392.39 b	395.73 a
UFUS 7415	17.4 a	83.0 b	2385.66 a	285.60 a
UFUS 1117-	12.5 b	84.6 b	1378.72	396.90 a

^aNPG: number of pods with grains; ^bNPA: number of aborted pods; ^cAUDPC: area under the disease progress curve. Means followed by the same vertical letters belong to the same group, by the Scott-Knott test, at 5% level of probability.

3.2 Yielding and agronomic traits

UFUS 7415 presented determined growth; it is also resistant to the lodging and the pod dehiscence. Plant height at maturity ranging, days to 50% flowering, life cycle and 100-seed weight are in Table 4.

Table.4: Average results of UFUS 7415 agronomic traits and yielding assessed during a 3-year period (2010/13).

Regions	^a DF	^b DM	^c PH	^d FPH	100-seed weight (g)
302	47	116	68	10	15
303	43	109	66	10	14
401	48	114	78	11	15
402	45	112	77	10	14
403	42	110	75	10	15

^aDF = Number of days to 50% flowering; ^bDM = Number of days to maturity; ^cPH = Plant height at maturity (cm); ^dFPH = First pod insertion height (cm).

Productivity was assessed by comparison of grain yield of UFUS 7415 with other standard cultivars, by region, locality and year. Table 5 shows grain yield (kg ha⁻¹) average results of the data assessed during a 3-year period.

Table.5: Comparative grain yield (kg ha⁻¹) results assessed during a 3-year period (2010/13).

Regions	^a (CV %)	302 (13)	303 (14)	401 (12)
<i>UFUS 7415</i>		3300	3400	3650
<i>BRSMG 752S</i>		3150	3500	3450
<i>Witnesses Emgopa 316</i>		3200	3200	3200
<i>M SOY 6101</i>		3350	3350	3750
Regions (CV %)		401	402	403

	(12)	(12)	(13)
UFUS 7415	3600	3700	3600
BRSMG 752S	3550	3660	3550
Witnesses M SOY 6101	3330	3750	3640
M SOY 8001	3650	3650	3450

^aCV = Coefficient of variation

Table 6 shows two-year average assessments of UFU 7415 seeds quality and industrial yield compared with other standard cultivars, according to the region where tests were performed.

Table.6: Percentage of protein and oil content found in the UFUS 7415 seeds in evaluations performed during a 3-year period (2010/13).

^a Reg.	UFUS 7415		Witnesses					
	Oil	^a Pr	BRSMG 752S		M SOY 6101		Emgopa 316	
302	18	39	19	39	18	39	-	-
303	19	38	18	39	19	38	-	-
401	19	39	18	38	18	39	-	-
			BRSMG 752S		M SOY 6101		M SOY 8001	
401	18	39	18	39	18	38	19	38
402	19	38	18	39	19	38	19	38
403	19	39	19	39	18	37	18	39

^aPr = protein.

IV. DISCUSSION

Although UFUS 7415 did differ from both resistant checks TMG 801 and BRSGO 7560 regarding the AUDPC, it was found among the most productive genotypes under natural infection by *P. pachyrhizi* (Table 3). UFUS 7415 yielding (2385.66) did not differ statistically from both parameters of resistance TMG 801 (2805.11) and BRSGO 7560 (3468.56); however, all genotypes have shown averages below the national average yield (2998 kg ha⁻¹) [15]. Genotypes TMG 801, BRSGO 7560, UFUS 7415 and UFUS 1117-07 were found with high general adaptability.

Therefore, UFUS 7415 is highly productive and well adapted even to environments with natural populations of *P. pachyrhizi*. Among soybean diseases, rust is the most harmful; losses can reach more than 80% when environmental conditions are conducive to the development of this disease [3]. Soybean rust genetic resistance is a complex issue since the fungus presents a sizeable genetic variability and so, over the years, materials have lost resistance. Hence, cultivars moderately susceptible such as UFUS 7415 are an excellent alternative to environments with rust.

Arabidopsis is non-host resistant (NHR) to *P. pachyrhizi* and has been used as a model to study the basis of genetic resistance to *P. pachyrhizi* [16, 17]. *Arabidopsis* rust resistance comprises different layers of defense; epidermal penetration resistance requires functional genes PEN1, PEN2 and PEN3, while post-invasion resistance in the mesophyll depends on the combined functionality of PEN2, PAD4, and SAG101. Other genetic components of post-invasion resistance remain elusive in *Arabidopsis* [18].

UFUS 7415 present desirable characteristics such as determined growth and lodging resistance; excessive vegetative growth might lead to lodging reducing yield potential, and increasing harvest losses as lodged plants are more difficult to cut and gather into the combine [19]. Buzzello et al. [20], found a negative correlation between lodging and grain yield, and a positive correlation between plant height and lodging. Besides, UFUS 7415 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% [21].

UFUS 7415 displayed average of 10 cm height for the first pod insertion. Pereira Júnior et al. [22], described standard values greater than 14 cm to the insertion of the first pod. However, according to Almeida et al. [23] at least 10 cm height is enough to avoid losses of uncollected pods due to low insertion height.

UFUS 7415 plant height at maturity ranging from 66 to 78 cm, and days to 50% flowering from 42 to 48 (Table 4). A study performed in Jaboticabal, SP, 2012/2013 harvest, assessed agronomic the traits of 30 soybean genotypes and plant heights at maturity were reported ranging from 55.67 to 108.13 cm [24]. Soybean flowering period is relatively extended (ranging from 30 to 40 days) and overlaps with the formation of pods and seeds, which makes it resistant to short periods of drought during flowering [25]. Sudhanshu et al. [26], found days to 50% flowering and plant height have the negative and direct effect on seed yield.

UFUS 7415 100-seed weight oscillated from 15g to 14g (Table 4); the average weight of 100 seeds may vary depending on the sowing season and locality [27]. UFUS 7415 grain yield (3300 to 3700 kg ha⁻¹) was above national average production (Table 5), which ranges from 2.5 to 3 thousand kg ha⁻¹ in the last ten years [15]. Yielding depend on numerous traits, which might have their action linked. Therefore, the selection practiced on one trait may simultaneously bring change in the other related trait. Path coefficient analysis has shown that seed yield/plant shows positive and significant association with biological yield, pods per plant, and 100-seed weight;

indicating that an intense selection for these characters might improve seed yield in soybean. Among these traits, 100 seed-weight exhibited the highest positive direct effect on seed yield [26].

Soybean seeds are an essential world's source of vegetable oil and protein meal, being widely used to feed animals like poultry, swine, and cattle. Soybean oil is composed of saturated, monounsaturated and polyunsaturated fatty acids. It has a typical composition of 11% palmitic, 4% stearic, 25% oleic, 50% linoleic and 9% linolenic fatty acid content according to the Economic Implications of Modified Soybean Traits Summary Report, Iowa Soybean Promotion Board and American Soybean Association Special Report 92S [28]. UFUS 7415 seeds presented 20% oil and 40% protein contents (Table 6).

V. CONCLUSION

We have developed a new non-GMO (non-genetically modified organism) soybean well adapted to MT, MG and GO. Population size 250.000 to 310.000 Plants/ha; recommended cropping from October 20 to December 10 in growing seasons; yielding potential 4.300 kg ha⁻¹.

UFUS 7415 aspects that represent comparative advantages over others cultivars are the high oil and protein contents and the high seed yielding, even in environments with *P. pachyrhizi*.

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