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CULTIVAR RELEASE

BRS 430 B2RF and BRS 432 B2RF: Insect-resistant and glyphosate-tolerant high-yielding cotton cultivars

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Abstract: *Transgenic cotton cultivars BRS 430 B2RF and BRS 432 B2RF, medium and late season respectively, were developed by EMBRAPA in partnership with Bahia Foundation, aiming to create a high-yielding germplasm. The Bt toxins Cry1Ac and Cry2Ab target lepidopteran pests and the CP4-EPSPS gene confers tolerance to the herbicide glyphosate.*

Key words*: Gossypium hirsutum, glyphosate tolerance, insect resistance, lint yield.*

INTRODUCTION

In Brazil, growing, commercialization and consumption of transgenic crops became legal since 2003. After that, the demand for genetically modified (GM) crops has risen significantly and currently, Brazil is the second largest GM grower globally, with 44.2 million hectares, reaching 25% of global GM crops in 2015 (James 2015). Notably, Brazil sowed the stacked insect resistance (IR) and herbicide tolerant (HT) soybean on a record 11.9 million hectares (up from 5.2 in 2014) in its third year after the launch. In cultivar development, biotechnological tools have been applied to transfer specific characteristics that would be difficult to obtain using conventional breeding (Parentoni et al. 2013).

Upland cotton (*Gossypium hirsutum* L. *latifolium* Hutch) has been cultivated in Brazilian savanna (cerrado environment) since early 1980´s (Silva Neto et al. 2016). Nowadays, more than 90% of cotton growing areas are located in this region. Genetic advances (Morello et al. 2010, Morello et al. 2012, Morello et al. 2015, Barroso et al. 2017) along with a better production system ensures high fiber yields, providing competitiveness to the Brazilian cotton in the international market. However, this tropical region is subject to high biotic stress pressure, highlighting pest damages (Bentivenha et al. 2016) and weed competition (Salgado et al. 2002). Unfortunately, pest management relied almost exclusively on regular insecticides sprays, which can led to selection for resistance in important pests and other undesirable environmental effects. In addition, the chemical control may not be efficient under less larvae exposition to chemicals, due to behavior of remaining sheltered under the cotton canopy

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(Santos 2011). Moreover, young cotton plants are not able to compete with several weeds for water and nutrients, resulting in reduced yield and contamination of the lint at harvest (Norsworthy et al. 2009). Pest and weed management increase the production costs, reducing growers´s profit. Therefore, it is imperative the development of high yielding and fiber quality cotton genotypes with transgenic IR and HT, suitable to be cultivated in Brazilian cerrado.

In parallel with the conventional cotton breeding program of the Brazilian Agricultural Research Corporation (Embrapa), a multiple transgenic trait introgression program has been implemented in order to meet the recent growers and industry demands. The complete program is being carried out in partnership with the private sector, which has provided financial and technical support through FUNDEAGRO (Fund for Cotton Agribusiness Development) and Bahia Foundation. This paper describes the development of cotton cultivars BRS 430 B2RF and BRS 432 B2RF as part of an ongoing effort to create new cotton lines and cultivars with improved yield potential and transgenic IR and HT, adapted to Brazilian cerrado.

GENETIC ORIGIN AND DEVELOPMENT

There is a high demand for commercial transgenic cotton varieties with resistance to main lepidopterans pests: *Heliothis virescens* (F.), *Pectinophora gossypiella* (Saund.), *Helicoverpa zea* (Boddie), *H. armigera* (Hubner.), *Chrysodeixis includes* (Walker) and *Spodoptera frugiperda* (J. E. Smith). Transgenic plants containing one or several genes isolated from the soil bacterias *Bacillus thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *aizawai*, were developed and are available (CTNBio 2016). The first commercial transgenic cotton cultivar was developed based on a sing transformation event (MON 531) with a vector containing a full-length synthetic cry1Ac-like Bt coding sequence driven by an enhanced 35S promoter, commercially known as Bollgard® (Perlak et al. 2001). Thus, Bollgard® cotton produces the *Cry1Ac* insecticidal protein and has been adopted broadly by growers since its commercial introduction in 1996, as it provides very effective protection from feeding damage by *H. virescens* and *P. gossypiella*, and effective against other cotton bollworm. In order to provide effective season-long control to a wide range of key lepidopteran insect pests and also provide an additional tool to delay the development of resistance, the cotton event MON 15985, commercially known as Bollgard II® (B2) was generated by re-transformation of cotton meristems of Bollgard® cotton event MON 531, variety DP 50B. A particle acceleration plant transformation procedure was used to insert the *cry2Ab2* insect coding sequence. Therefore, B2 cotton produces two proteins for effective control of the major lepidopteran insect pests of cotton. In addition, provides a more effective insect resistance management program compared to single gene products.

Transgenic tolerance to herbicide glyphosate was introduced into cotton plants and transgenic cultivars have been commercially released since 1997 in USA. This herbicide inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) in the shikimate acid pathway of plants. As consequence, the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan is inhibited, causing plant death (Pline et al. 2003). The transgenic cotton event, MON 88913, was generated by an Agrobacterium-mediated transformation of cotton cells, commercially known as Roundup Ready Flex® (RF). Broader spectrum of weed control and flexibility for timing herbicide application in HT cultivars are considered advantageous over conventional weed management by growers

Cultivars BRS 430 B2RF and BRS 432 B2RF were developed through backcross breeding using BRS 372 as the recurrent parent, followed by the pedigree selection method, to incorporate the events MON 15985 and MON 88913. BRS 372 (Cacique INTA x CNPA ITA 90) has good fiber quality, high fiber yield, adaptation to central Brazilian cerrado and resistance to ramularia leaf spot (Silva et al. 2017). The donor, DP 164 B2RF {DP 565/3*[DP 565 x (Cocker 312 RF x DP 50 B2)]} is a high-yielding cotton cultivar; however, it has a medium lint percent (37.8%) and is susceptible to blue disease, caused by *Cotton Leafroll Dwarf Virus*, and bacterial blight, caused by *Xanthomonas citri* subsp. *malvacearum*.

BRS 430 B2RF and BRS 432 B2RF were originated from a biparental cross followed by three backcrosses involving cotton cultivars BRS 372 and DP 164 B2RF (BRS 372/4*DP 164 B2RF). Hybridizations were performed in Santa Helena de Goiás, GO in 2011, and BC_1, BC_2 and BC_3, crosses were performed in Embrapa's greenhouse in Santo Antônio de Goiás, GO from 2012 to 2013. BC_3F_1 and BC_3F_2 plants were grown and selfed in the nethouses located at Embrapa. From BC_3F_2 generation, only homozygous plants for the Bollgard II® and Roundup Ready Flex® genes were selected to progeny rows tests. Qualitative expression of transgenic proteins (Cry1Ac, Cry2Ab and CP4 EPSPS) were tested individually by commercial immunochromatographic strip test (lateral flow membrane strip test) according to manufacturer's recommendations (Envirologix Inc.). The selection of homozygous plants (for each transgenic events) was carried out

in a specific real-time PCR assay.

A total of 168 progeny rows ($BC_3F_{2:3}$) were evaluated in 2013/2014 season in Santo Antônio de Goiás, GO. Nine individual progeny rows were selected based on apparent yield potential, high volume instrument fiber properties, disease resistance (bacterial blight and blue disease), and overall plant conformation. The progenies CNPA GO 2014 30 B2RF and CNPA GO 2014 32 B2RF were grown during 2014 off-season in a controlled environment, and evaluated in replicated tests at three locations in 2014/2015 season. Thus, BRS 430 B2RF (CNPA GO 2014 30 B2RF) and BRS 432 B2RF (CNPA GO 2014 32 B2RF) were treated as pure lines. BRS 430 B2RF and BRS 432 B2RF were sent to irrigated experimental field in Barbalha, CE for seed increase during the 2015 off-season. Afterwards, eight trials were evaluated across the states of Bahia, Goiás, Distrito Federal, Piauí, Mato Grosso do Sul, and Mato Grosso, in the 2015/2016 season.

PERFORMANCE CHARACTERISTICS

BRS 430 B2RF and BRS 432 B2RF are picker-type upland cotton with different growth habits, when grown at São Desidério, BA (lat 12° 05' 33" S, long 45° 48' 08" W, alt 776 m asl). BRS 430 B2RF is medium size with mature plant height ranging from 110 to 120 cm, while plants of BRS 432 B2RF are medium/tall (120 to 135 cm), when 35 to 50 g of the active ingredient of growth regulator (mepiquat chloride and chlormequat chloride) are applied. BRS 430 B2RF is a midseason to early maturity and BRS 432 B2RF is mid-full season. Both cultivars have trichome on leaves and on the main stem. Leaves are normal-shaped, glanded and nectaried. Bracts have 7 to 12 lobes in BRS 430 B2RF and more than 12 lobes in BRS 432 B2RF. First reproductive branch is generally inserted on the fifth node, and branches have oblique angle insertion. Flowers from plants of both cultivars have cream-colored petals, anthers, and pollen. Full-size green bolls are longer than their width and are broader in the middle. Bolls have five locules; however, four locules may occasionally occur. Open bolls resist shattering, but are not storm proof, and thus are suitable for picker harvesting. Produced lint and fuzz are white in color.

The first flower appears at about 50 to 55 (BRS 430 B2RF) or 55 to 60 (BRS 432 B2RF) days after emergence (DAE), and the first boll opens at about 110 to 120 DAE, at 776 meters asl. (Table 3). In these environmental conditions, and using harvest-aid chemicals, BRS 430 B2RF total harvest was carried out at 160 DAE and BRS 432 B2RF, at 175 DAE.

BRS 430 B2RF and BRS 432 B2RF are resistant to bacterial blight [caused by *Xanthomonas citri* subsp. *malvacearum*] and to cotton blue disease [caused by *Cotton leafroll dwarf virus*- CLRDV], moderately susceptible to ramularia leaf spot [caused by *Ramularia areola*], and susceptible to ramulosis [caused by *Colletotrichum gossypii* var. *cephalosporioides*] (Table 3). Fungicide foliar sprays are necessary to avoid losses caused by ramularia leaf spot. Resistance to bacterial blight and blue disease were measured at field conditions and confirmed by genotyping a sample of seeds for the presence of molecular markers CIR 246, linked to B_{12} resistance to bacterial blight gene, and DC20027 linked to *Cdb* resistance gene to cotton blue disease (Xiao et al. 2010, Fang et al. 2010). There was no incidence of cotton blue disease symptoms or bacterial blight on plants evaluated in two independent assays with no control of virus vector (*Aphis gossypii*). Severity

Table 1. Means of cottonseed yield (CY), lint percentage (LP), and lint yield (LY) of the cotton cultivars BRS 430 B2RF and FM 980 GLT (control), in eight field performance trials in 2015/2016 season.

Counties/State	BRS 430 B2RF			FM 980 GLT (control)			PCC†		CV*
	CY (kg ha^{-1})	LP (%)	LY (kg ha^{-1})	CY (kg ha^{-1})	LP (%)	LY (kg ha^{-1})	CY	LY	
Luís Eduardo Magalhães/BA	4782	40.0	1913	4452	44.4	1977	7.4	-3.2	10.7
São Desidério/BA	4277	41.3	1766	3681	43.4	1598	16.2	10.6	11.4
Santo Antônio de Goiás/GO	5519	40.1	2213	5034	44.8	2255	9.6	-1.9	9.0
Planaltina/DF	4535	40.0	1814	3774	44.3	1672	20.2	8.5	13.1
Sorriso/MT	4476	39.8	1781	3899	43.5	1696	14.8	5.0	16.2
Campo Verde/MT	6432	40.0	2573	6294	45.6	2870	2.2	-10.4	6.3
Chapadão do Sul/MS	3266	41.1	1342	2646	40.2	1064	23.4	26.2	15.4
Uruçuí/PI	3362	41.0	1378	2898	46.3	1342	16.0	02.7	10.8
Mean	4581	40.4	1848	4085	44.1	1809	13.7	4.7	

* CV - Coefficient of variation (%) for cottonseed yield (kg ha^{-1}); † PCC - Percentage in comparison to the control (increase or decrease)

of ramularia leaf spot was determined based on foliar lesion area grade scale varying from 1 (no symptoms) to 5 (highly susceptible) in two field assays with no fungicide application. Resistance to cotton ramulosis was assessed in a greenhouse assay artificially inoculated with *Colletotrichum gossypii* var. *cephalosporioides,* as proposed by Cia et al. (2002).

Averaged across the eight field performance trials of cultivation value and use (VCU) in central and northeastern Brazil in 2015/2016, BRS 430 B2RF and BRS 432 B2RF presented 4.7 and 3.5% more lint yield than check (FM 980 GLT), respectively. It is worth to highlight that the lint percentage was higher in FM 980 GLT; however, raw cotton yield in BRS 430 B2RF and BRS 432 B2RF was higher than FM 980 GLT, reflecting in higher fiber yield (Tables 1 and 2). High Volume Instrument (HVI) measurements reveal desirable physical attributes of a medium fiber length for both new cultivars: micronaire reading 4.4; fiber length (UHML) 30.5 mm (BRS 430 B2RF) and 29.9 mm (BRS 432 B2RF); relative strength 30.7 gf tex^{-1} (BRS 430 B2RF), and 30.2 gf tex^{-1} (BRS 432 B2RF); reflectance 80.1% (BRS 430 B2RF), and 80.0 (BRS 432 B2RF); yellowness (+b) 7.6 (BRS 430 B2RF), and 7.7 (BRS 432 B2RF); short fiber index, 6.8 (BRS 430 B2RF), and 7.2 (BRS 432 B2RF) (Table 3).

Table 2. Means of cottonseed yield (CY), lint percentage (LP), and lint yield (LY) of the cotton cultivars BRS 432 B2RF and FM 980 GLT (check), in eight field performance trials in 2015/2016 season.

Counties/State	BRS 432 B2RF			FM 980 GLT (check)			PCC†		CV*
	CY (kg ha^{-1})	LP (%)	LY (kg ha^{-1})	CY (kg ha^{-1})	LP (%)	LY (kg ha^{-1})	CY	LY	
Luís Eduardo Magalhães/BA	4737	40.5	1918	4452	44.4	1977	6.4	-2.9	10.7
São Desidério/BA	4079	41.2	1680	3681	43.4	1598	10.8	5.2	11.4
Santo Antônio de Goiás/GO	5256	39.7	2087	5034	44.8	2255	4.4	-7.5	9.0
Planaltina/DF	3960	41.0	1624	3774	44.3	1672	4.9	-2.9	13.1
Sorriso/MT	4920	41.0	2017	3899	43.5	1696	26.2	18.9	16.2
Campo Verde/MT	6234	41.3	2575	6294	45.6	2870	-1.0	-10.3	6.3
Chapadão do Sul/MS	3084	41.8	1289	2646	40.2	1064	16.6	21.2	15.4
Uruçuí/PI	3393	42.1	1428	2898	46.3	1342	17.1	6.5	10.8
Mean	4458	41.1	1827	4085	44.1	1809	10.7	3.5	

* CV - Coefficient of variation (%) for cottonseed yield (kg.ha^{-1}); † PCC - Percentage in comparison to the control (increase or decrease)

Table 3. Agronomic traits and fiber quality of BRS 430 B2RF, BRS 432 B2RF, BRS 372 (check), and FM 980 GLT (check).

Traits	BRS 430 B2RF	BRS 432 B2RF	BRS 372	FM 980 GLT
First flower (DAE)*	50-55	55-60	60-65	60-65
First open boll (DAE)*	110-120	110-120	115-125	115-125
Boll weight (g) [a]	5.3	5.2	6.0	5.1
Fiber length (UHML) (mm) [a]	30.5	29.9	30.0	30.2
Uniformity index (ML/UHML - %) [a]	84.1	84.0	84.3	84.1
Strength HVI (gf.tex^{-1}) [a]	30.7	30.2	30.6	30.3
Micronaire reading [a]	4.4	4.4	4.6	4.5
Elongation (%) [a]	6.3	6.7	6.3	6.2
Reflectance – Rd (%) [a]	81.0	80.0	80.9	80.2
Yellowness (+ b) [a]	7.6	7.7	7.7	7.7
Short fiber index (%) [a]	6.8	7.2	7.0	6.7
Cotton leafroll dwarf virus (CLRDV) [b]	0.0	0.0	0.0	0.0
Atypical form of *CLRDV* [cd]	3.0	2.0	-	3.0
Ramularia leaf spot [d]	2.5	3.0	1.0	3.0
Bacterial blight [d]	1.0	1.0	1.0	1.0
Ramulosis [e]	0.42	0.51	1.0 [f]	0.57

* Data recorded in São Desidério – BA (lat 12° 05' 33" S, long 45° 48' 08" W, alt 776 m asl).
[a] Means from eight field trials in 2015/2016 season, in several environments (States of Goiás, Bahia, Mato Grosso, Mato Grosso do Sul, Piauí, and Distrito Federal); [b] Incidence (%) of plants with cotton blue disease symptoms - data from two assays with no control of virus vector (*Aphis gossypii*), confirmed by SNP marker (Fang et al. 2010); [c] Symptoms of Atypical form of *CLRDV,* according to Silva et al. 2015; [d] Disease severity (grades from 1 = resistant to 5 = highly susceptible) - data from two assays with no fungicide application. For bacterial blight, resistance was confirmed by using SNP marker (Xiao et al. 2010); [e] Relative disease index, where 1.0 is resistant and 0 is susceptible (Cia et al. 2002) – data from plants inoculated artificially with *Colletotrichum gossypii* var. *cephalosporioides*; [f] Cotton cultivar IMA 1318 was used as a resistant/partial resistant check.

SEED MAINTENANCE AND DISTRIBUTION

BRS 430 B2RF and BRS 432 B2RF are catalogued in the Ministry of Agriculture, Livestock and Food Supply under the numbers 36252 and 36251, respectively. Basic seeds are produced by Embrapa Products and Markets (Embrapa SPM). Bahia Foundation, working in partnership with Embrapa, is responsible for the certified seed production.

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