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BRS FC420 RMD: transgenic carioca-seeded common bean with high yield, multiple virus resistance, and superior grain quality and architecture

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Abstract: BRS FC420 RMD is a genetically modified cultivar developed by Embrapa. It features carioca-type grains, a normal growth cycle (85–94 days), high yield potential, and excellent commercial grain quality, with very light beige coloration and light brown stripes. The plants are adapted to direct mechanized harvesting and possess multiple resistance to viruses (BCMV, BGMV, and CPMMV), making it a crucial tool for the integrated management of whitefly-transmitted viruses in bean cultivation. It is registered for planting during the rainy, dry, and winter seasons in Brazil's Central Region.

Keywords: *Phaseolus vulgaris*, plant breeding, virus resistance, yield potential.

Introduction

The common bean (*Phaseolus vulgaris* L.) is a staple food in the Brazilian diet and a significant source of protein for the population. The average per capita consumption in Brazil is approximately 13 kg per year, one of the highest worldwide and the largest among countries in the Americas (Feijão, 2023). However, in Central Brazil, a region with substantial production of common bean grains, viral diseases transmitted by the whitefly occur with greater intensity. These include the golden mosaic virus, the most aggressive disease affecting the crop, caused by the bean golden mosaic virus (BGMV), and the cowpea mild mottle virus, caused by the cowpea mild mottle virus (CPMMV) (Faria et al., 2016).

The symptoms of the golden mosaic virus include leaf yellowing, stunted plant growth, pod and grain deformation, and flower abortion. Grain yield losses can range from 40% to 100%, depending on the severity of the incidence, the sowing period, and the cultivar used (Faria et al., 2016). The peak incidence of BGMV occurs during the dry planting season (January to March), which coincides with high populations of adult whiteflies migrating from soybean crops.

Efforts to develop cultivars resis-

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tant to the golden mosaic virus began in Brazil in the 1970s under Embrapa's initiatives, alongside the search for resistance sources. However, only quantitative or partial resistance sources were identified, proving ineffective under the high disease pressure conditions observed in Central Brazil. No effective resistance was found in *Phaseolus* genotypes and accessions tested for reaction to the golden mosaic virus in the country (Faria et al., 2016; Souza et al., 2018).

To address this challenge, Embrapa developed genetically modified (GM) beans resistant to BGMV (Event Embrapa 5.1) (Bonfim et al., 2007), providing an essential tool for the integrated management of whitefly-transmitted viruses. The commercial release of this event was approved by CTNBio in 2011. However, at that time, no cultivars specifically developed for Brazil were available. The presence of this event is indicated in cultivar names by the suffix RMD (resistance to golden mosaic virus). The carioca-seeded cultivar BRS FC401 RMD, the first GM common bean cultivar developed globally, was registered in 2015 and protected in 2016. However, although it demonstrates effective resistance to BGMV, this cultivar is susceptible to CPMMV due to the susceptibility of its parent lines and exhibits a prostrate architecture, which increases harvest losses during mechanized operations (Souza et al., 2018).

Although CPMMV (cowpea mild mottle virus) was identified as pathogenic to common beans in Brazil as early as 1979 (Costa et al., 1983), its symptoms were often misinterpreted or overshadowed in the field by the more aggressive symptoms of the golden mosaic virus. Consequently, the true symptoms of CPMMV were only observed during Cultivation and Use Value (VCU) trials involving transgenic lines resistant to the golden mosaic virus (Faria et al., 2016; Souza et al., 2018). CPMMV is also transmitted by the whitefly but in a non-persistent, non-circulative manner, explaining its lower aggressiveness. Nevertheless, its occurrence has become increasingly frequent in many bean-producing regions, particularly in Central Brazil, largely due to the extensive distribution and high populations of its insect vector. The primary symptoms of CPMMV include leaf curling or wrinkling associated with vein necrosis, particularly on the abaxial surface; chlorosis or mild mosaic patterns (mottling), mainly on young leaves; stunted plant growth; and potential yield reduction depending on the resistance level of the adopted cultivar (Faria et al., 2016).

The predominant method for controlling whitefly-transmitted viral diseases has been the application of insecticides targeting the vector. However, the intensive use of the same chemical compounds, often without integration with other management techniques, has rapidly shortened the effectiveness of these insecticides. This practice has selected resistant whitefly genotypes, reducing the efficacy of most products available on the market. Reports of up to 20 insecticide applications per bean crop cycle are not uncommon (Souza et al., 2018).

To address this emerging challenge, Embrapa's bean breeding program has continued its efforts to develop second-generation GM common bean lines. These lines feature carioca beans with the commercial quality standards demanded by the Brazilian market, high productivity potential, production stability, adaptability to direct mechanized harvesting, and multiple virus resistances (including common mosaic, golden mosaic, and CPMMV). This new cultivar will represent another critical tool for the integrated management of whitefly-transmitted viral diseases in bean crops. It is intended to complement other equally important measures, e.g., ad-

herence to crop-free periods and optimal planting seasons, monitoring of insect vectors, chemical control, and field infestation level management.

Breeding methods

The cultivar BRS Sublime was used as a donor parent for resistance to the cowpea mild mottle virus (CPMMV) and other desirable agronomic traits, e.g., upright architecture and very light beige seed color. The CNFCT 16206 line served as the donor of the transgene present in Embrapa Event 5.1, which provides immunity to Bean golden mosaic virus (BGMV) and resistance to the bean common mosaic virus (BCMV), although being susceptible to CPMMV. The presence of the transgene/Embrapa Event 5.1 was identified using the STS molecular marker associated with the *ahas* gene. Resistance conferred by gene *I* to BCMV was identified using the SCAR SW13 marker and SNP marker ss715648456. Amplification of these loci was performed through gene-specific PCR or RT-PCR (Silva et al., 2022).

Artificial crosses, including initial simple crosses, backcrosses, and final simple crosses, were conducted in greenhouses at Embrapa Rice and Beans (Embrapa Arroz e Feijão) in Santo Antônio de Goiás (GO) between 2014 and 2016. In the initial crosses, line CNFCT 16206 was consistently used as the male parent. During all backcrossing cycles (RC), the presence of the transgene was verified using event-specific PCR (*ahas* marker). The genetic similarity of RC_3F_1 plants with the recurrent parent was determined through molecular fingerprinting analysis using microsatellite markers. From the RC_3F_2 and RC_4F_2 generations, subsequent generations were advanced using the Single Seed Descent (SSD) method, leading to RC3F5 and RC4F4 generations. At these stages, plants were harvested individually and advanced to $RC_3F5_{:8}$ and $RC_4F_{4:7}$ generations. Superior lines were then selected based on the presence of the marker linked to the *ahas* gene/Embrapa Event 5.1, progeny testing, and greater genetic similarity to the recurrent parent (Silva et al., 2022). Crosses were also performed between the BRS Sublime cultivar and RC_3F_1 plants (BRS Estilo × CNFCT 16206) with greater genetic proximity to BRS Estilo. The resulting F_1 plants were tested for the transgene using event-specific PCR (AHA marker). From the F_2 generation onward, subsequent generations were advanced using the SSD method to obtain F_3 and F_4 generations. At these stages, plants were harvested individually and advanced to $F_{3:7}$ and $F_{4:7}$ generations. Superior lines were selected based on the presence of the marker linked to the *ahas* gene/Embrapa Event 5.1, progeny testing, and greater genetic similarity to the BRS Sublime parent (Silva et al., 2022).

Lines developed from the two populations mentioned above were evaluated in a field trial at Embrapa Rice and Beans (Embrapa Arroz e Feijão) during the 2016 winter planting season to identify superior lines for CPMMV resistance. Out of 477 lines evaluated, 39 were selected as resistant. These lines were subsequently reassessed in two experiments conducted during the 2016 rainy season and the 2017 dry season, also at Embrapa Rice and Beans (Embrapa Arroz e Feijão), to evaluate the agronomic performance, particularly grain yield and visual grain quality (VGQ) (Silva et al., 2022). The control cultivars BRS FC401 RMD, BRS Estilo, and BRS FC402 were used in all trials. Lines were considered resistant if they scored ≤ 3.0 for virus severity, assessed visually using a 1-to-9 scale, where 1 indicates plants or plots without visible symptoms, and 9 indicates plants with 60–100% infected tissues or plots with 80–100% symptomatic plants. Grain yield was measured in $kg.ha^{-1}$ at

13% grain moisture. VGQ was evaluated on a 1-to-5 scale, where 1 corresponds to grains with all desirable attributes for the carioca class (very light beige color, light brown stripes, and matte finish), and 5 corresponds to grains completely outside the carioca standard.

The 39 lines selected as superior for virus response (BCMV, BGMV, and CPMMV), yield, and VGQ in field experiments were further evaluated in a greenhouse for reaction to CPMMV using artificial inoculation (isolate CPMMV: BR: GO: 14 — GenBank MK202583). These lines were also subjected to molecular characterization using markers linked to gene *I* (SW13) and the *ahas* gene/Embrapa Event 5.1, which are associated with genetic control of resistance to BCMV and CPMMV, respectively. The aim was to perform progeny tests for resistance to the three viruses and identify homozygous lines with multiple resistance to BCMV, BGMV, and CPMMV. This strategy led to the selection of eight lines, designated as elite RMD bean lines and coded as "CNFCT."

These eight elite lines, including CNFCT 19119, were evaluated alongside two control cultivars (BRS Estilo and BRS FC401 RMD) in Value for Cultivation and Use (VCU) trials conducted from 2017 to 2021. The trials used a randomized block design with three replicates and plots consisting of four 4-meter rows. A total of 37 valid VCU experiments, meeting Embrapa's experimental quality standards, were conducted in the following planting seasons: winter 2017 (one environment), rainy season 2017 (two environments), dry season 2018 (one environment), winter 2018 (two environments), rainy season 2018 (six environments), dry season 2019 (five environments), rainy season 2019 (three environments), dry season 2020 (five environments), winter 2020 (two environments), dry season 2021 (five environments), and rainy season 2021 (five environments). These trials took place across seven municipalities: Santo Antônio de Goiás-GO, Brazabrantes-GO, Cristalina-GO, Brasília-DF, Planaltina-DF, Itaberá-SP, and Ponta Grossa-PR. Additionally, two DUS (Distinctness, Uniformity, and Stability) trials necessary for the morphophysiological characterization of promising lines and the protection of the new cultivar were conducted during the winter planting seasons of 2017 and 2018. In all VCU and DUS trials, the relevant actions outlined in the Responsible Management Program for RMD Technology were implemented, monitored, and audited.

In the VCU experiments, various grain traits were assessed: yield ($kg.ha^{-1}$ at 13% grain moisture) in 36 environments; sieve 12 yield (4.5 mm) in 25 environments; 100-seed weight (g) in 31 environments; cooking time (minutes) in two environments; and protein concentration (%) in two environments. Plant traits were also evaluated using a 1-to-9 scale, where 1 represented a completely favorable phenotype and 9 represented a completely unfavorable phenotype (Melo, 2009). These traits included plant architecture (25 environments), lodging resistance (28 environments), and disease response, such as angular leaf spot (*Pseudocercospora griseola*) in 12 environments, anthracnose (*Colletotrichum lindemuthianum*) in six environments, and viral diseases (BCMV, BGMV, and CPMMV) in seven environments.

Grain yield was measured in $kg.ha^{-1}$ and adjusted to 13% grain moisture. The sieve yield was measured as follows: a 300 g sample was taken from each plot and sieved using a 4.5 mm oblong-hole sieve. The seeds retained on the sieve were weighed, and the retained weight was divided by the initial sample weight. A 100-seed sample was then taken from the retained seeds to calculate 100-seed

weight. Cooking time was determined using a Mattson cooker, and protein concentration was analyzed by determining nitrogen content via the micro Kjeldahl method.

Agronomic characteristics and commercial and nutritional grain quality

Based on the combined analysis of data from 37 valid VCU experiments, line CNFCT 19119 stood out and was selected as a new cultivar, named BRS FC420 RMD. This cultivar features carioca-type grains with high commercial quality, high grain yield potential, plants adapted to direct mechanized harvesting, and multiple virus resistance (BCMV, BGMV, and CPMMV). Compared to the reference cultivar BRS FC401 RMD — the only carioca-grain cultivar resistant to golden mosaic virus currently developed and available on the market — BRS FC420 RMD demonstrated similar performance in grain yield (2,114 kg.ha^{-1} vs. 2,134 kg.ha^{-1} for BRS FC420 RMD and BRS FC401 RMD, respectively), resistance to BCMV and BGMV (average score of 1.0), cooking time (27 minutes), and protein concentration (22%), as shown in Tables 1 and 2. However, BRS FC420 RMD showed superior performance in commercial grain quality (Table 3), including VGQ scores (2.3 vs. 3.1), 100-seed weight (26.6 g vs. 25.7 g), and sieve yield for sieve 12 (81% vs. 71%). Additionally, it outperformed BRS FC401 RMD in plant architecture (5.0 vs. 6.4) and lodging resistance (4.7 vs. 6.6), making it better suited for direct mechanized harvesting. It also exhibited improved resistance to angular leaf spot (3.7 vs. 4.2). For CPMMV resistance, BRS FC420 RMD was resistant (average score 3.0), whereas BRS FC401 RMD, as expected, was susceptible (average score 5.4). Conversely, BRS FC420 RMD showed inferior performance in resistance to anthracnose (average score 4.0) compared to the control BRS FC401 RMD (average score 1.3).

Table 1. Mean seed yield of the transgenic common bean cultivar BRS FC420 RMD compared to control cultivars in final field trials carried out from 2017 to 2021.

Growing region	Crop season	BRS FC420 RMD (kg ha^{-1})	Control cultivars (kg ha^{-1})	Number of environments
I[a]	Rainy	2890	2780	5
	Dry	1856	1691	3
	Overall	2503	2372	8
II[b]	Rainy	2078	2108	11
	Dry	1714	1708	13
	Winter	2607	2888	5
	Overall	2006	2063	29
Overall[c]	Rainy	2349	2324	16
	Dry	1740	1705	16
	Winter	2607	2888	5
	Overall	2114	2127	37

[a]Region I: RS, SC, PR, MS, and SP; [b]Region II: ES, RJ, GO/DF, MG, MT, TO, BA, and MA; [c]Overall: regions I and II; Control cultivars: BRS FC401 RMD and BRS Estilo.

Table 2. Seed traits of the common bean cultivar BRS FC420 RMD compared to the control cultivar BRS FC401 RMD, in final field trials carried out from 2017 to 2021.

Cultivar	Cooking time (minutes)	Protein content (%)	100 seed weight (g)[a]	Sieve yield (%)[a]	CQG[b]
BRS FC420 RMD	27	22	26,6	81	2,3
BRS FC401 RMD	27	22	25,7	71	3,1

[a] Estimates obtained in field trials without chemical control of diseases and using a sieve with mesh no. 11 (4.25 mm).

[b] Commercial quality of grains: scale 1-to-5 (better-to-worse).

Table 3. Agronomic traits and disease resistance of the common bean cultivar BRS FC420 RMD compared to the control cultivar BRS FC401 RMD, in final field trials carried out from 2017 to 2021.

Cultivar	Crop cycle[a]	Plant architecture	Disease resistance[b]			
			ANT	BCMV	BGMV	CPMMV
BRS FC420 RMD	N	Upright	S	R	R	R
BRS FC401 RMD	N	Prostrate	MR	R	R	S

[a] N – Normal cycle (85-95 days). [b] ANT – Anthracnose (Colletotrichum lindemutianum), BCMV – Bean common mosaic virus, BGMV – Bean golden mosaic virus, CPMMV – Cowpea mild mottle virus; R – Resistant (score 1), MR – Moderately resistant (scores 2 and 3), MS – Moderately susceptible (scores 4 to 6), and S – Susceptible (scores 7 to 9).

These results highlight the superiority of BRS FC420 RMD over BRS FC401 RMD in critical traits demanded by the production sector. Its grain color is much lighter, with paler stripes, and its upright plant architecture allows for direct mechanized harvesting. The introduction of this new cultivar to the market will reestablish beans as a viable planting option in regions with high whitefly incidence and, consequently, high virus pressure, which had rendered bean cultivation unfeasible during the second cropping season (dry season). Furthermore, it is expected to significantly reduce the number of insecticide applications per crop cycle, which has reached up to 15 applications per season, thereby lowering production costs (Paula et al., 2015).

Seed production

BRS FC420 RMD was officially registered as a new carioca-seeded common bean cultivar in the Registro Nacional de Cultivares (National Cultivar Registry) of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA; Brazilian Ministry of Agriculture, Livestock, and Food Supply) in 2023 under registration number 53056. The process for cultivar protection is currently underway through the Serviço Nacional de Proteção de Cultivares (National Cultivar Protection Service), also managed by MAPA. Embrapa is responsible for the production of basic seeds for this cultivar.

Conclusions

BRS FC420 RMD is a carioca-grain cultivar with a normal cycle (85–94 days), high commercial quality, and high grain yield potential. The plants are adapted to direct mechanized harvesting and possess multiple virus resistance (BCMV, BGMV, and CPMMV). It is registered for planting during the rainy, dry, and winter seasons in Region II (Goiás, Federal District, Mato Grosso, Tocantins, Maranhão, Bahia, Espírito Santo, and Rio de Janeiro).

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