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Cowpea strains resistant to CPSMV and CABMV intended for green-grain production[1]

Linhagens de feijão-caupi resistentes ao CPSMV e ao CABMV destinadas à produção de grãos verdes

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ABSTRACT - Green cowpea is an important alternative for generating employment and income for family farmers and helps broaden production and consumption of the grain. However, biotic factors such as insects, fungi, bacteria, nematodes and viruses have hampered production, especially the Cowpea Severe Mosaic Virus (CPSMV) and Cowpea Aphid-Borne Mosaic Virus (CABMV). The most efficient control of these viruses is via the use of resistant varieties, but few varieties intended for green-grain production are resistant. As such, the aim of this study was to develop and identify superior strains of cowpea in terms of joint resistance to the CPSM and CABM viruses that show characteristics associated with green-grain production. Crosses were carried out in a greenhouse at the Agronomic Institute of Pernambuco (AIP) between the following genotypes: Sempre Verde Salgueiro (susceptible), CNC-0434 (immune to CPSMV) and TVu-966 (resistant to CABMV). From these crosses, fifty $F_{2:5}$ strains with the Sempre Verde type of grain colouring were selected, their resistance to the viruses assessed in a greenhouse, and their agronomic characteristics for green-grain production evaluated in an experiment at the Experimental Station of the AIP in Belém do São Francisco, Pernambuco. The experimental design was of completely randomised blocks, with three replications. The L300.026, L300.039, L300.040 and L300.049 strains proved to be resistant to both viruses, with a high potential for green-grain production. The Mulamba-Mock selection index facilitated the selection of superior strains that are promising for the future launch of new resistant varieties destined for the production of green grains.

Key words: *Vigna unguiculata*. Plant breeding. Resistance. Plant viruses.

RESUMO - O feijão-caupi verde é uma importante alternativa para a geração de emprego e renda para os agricultores familiares, contribuindo para expansão de sua produção e consumo. No entanto, fatores bióticos como insetos, fungos, bactérias, nematoides e vírus têm limitado sua produção, destacando-se os vírus Cowpea Severe Mosaic Virus (CPSMV) e Cowpea aphid-borne mosaic virus (CABMV). O controle mais eficiente desses vírus é por meio da utilização de variedades resistentes, porém poucas são as variedades destinadas para a produção de feijão verde que são resistentes. Dessa forma, objetivou-se desenvolver e identificar linhagens superiores de feijão-caupi quanto à resistência simultânea aos vírus CPSMV e CABMV com características associadas à produção de grãos verdes. Foram realizados cruzamentos em casa de vegetação no Instituto Agronômico de Pernambuco-IPA entre os genótipos Sempre Verde Salgueiro (suscetível), CNC-0434 (imune ao CPSMV) e TVu-966 (resistente ao CABMV). A partir dos cruzamentos foram selecionadas 50 linhagens $F_{2:5}$ com coloração do grão tipo Sempre Verde, sendo avaliada a resistência aos vírus em casa de vegetação e as suas características agronômicas para produção de grãos verdes em experimento na Estação Experimental do IPA em Belém do São Francisco-PE. O delineamento experimental foi em blocos casualizados completos, com três repetições. As linhagens L300.026, L300.039, L300.040 e L300.049 mostraram-se resistentes a ambos os vírus e possuem elevado potencial para a produção de grãos verdes; o índice de seleção de Mulamba e Mock facilitou a seleção de linhagens superiores e promissoras para futuro lançamentos de novas variedades resistentes destinadas à produção de grãos verdes.

Palavras-chave: *Vigna unguiculata*. Melhoramento vegetal. Resistência. Fitoviroses.

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INTRODUCTION

The cowpea (*Vigna unguiculata* (L.) Walp.) is a highly important species of legume, as it is a rich source of proteins, carbohydrates, vitamins, minerals, fibres and phenolic compounds (MEDEIROS *et al*., 2017). Brazil is among the largest producers in the world, with a planted area of 1,285,900 hectares in the 2019/2020 harvest, with more than 80% of this area in the northeast of the country (COMPANHIA NACIONAL DE ABASTECIMENTO, 2021).

Cowpea is consumed as boiled dry grain or cooked green grain. Although it is largely grown for the dry grain, the production and consumption of green grain has been on the increase and gaining importance (ADEWALE *et al*., 2010). The green grain is characterised by being harvested at physiological maturity, when the pods stop accumulating photosynthates, and the grain has a moisture content of around 60% to 70% (FREIRE FILHO *et al*., 2017); desired characteristics are high productivity and high green-grain production.

The possibility of growing green grain throughout the year favours production, especially during the off-season, and also increases the price (FREIRE FILHO *et al*., 2017). However, the low number of specific varieties for green-grain production, together with the damage caused by viral diseases, has resulted in large production losses.

Among the viruses that affect the cowpea, Cowpea Severe Mosaic Virus (CPSMV) and Cowpea Aphid-Borne Mosaic Virus (CABMV) are important, as they limit the productivity of many cowpea varieties in different areas of Brazil and the world, influencing the quality and quantity of the grain (AMORIM *et al*., 2021).

CPSMV is an RNA virus of genus *Comovirus*, family Comoviridae, which can lead to large losses in production. Under natural conditions, it is spread by more than ten species of coleoptera, with *Cerotoma arcuata* (Olivier) and *Diabrotica speciosa* (Germar) being the principal vectors (BARROS *et al*., 2013). The main symptoms are local chlorotic lesions, blistering, mosaic and stunting (PIO-RIBEIRO; ASSIS FILHO; ANDRADE, 2016).

CABMV is an RNA virus of genus *Potyvirus*, family Potyviridae, widely distributed in cultivated areas (FREIRE FILHO, 2011), and is responsible for losses that can reach over 50% (MAIA *et al*., 2017). It is spread by aphids, especially the black aphid *Aphis craccivora* Koch. The symptoms are mosaic, leaf distortion, systemic chlorotic lesions, systemic necrosis and reduced plant growth (PIO-RIBEIRO; ASSIS FILHO; ANDRADE, 2016).

Prevention measures such as eliminating host plants and controlling insect vectors can be employed, but they have little effect, with the use of resistant varieties being more efficient (LIMA, 2015; ORAWU *et al*., 2013). However, there are few varieties intended for green-grain production that have joint resistance to both viruses.

To develop new varieties, it is necessary to identify superior strains using methodologies such as the Mulamba-Mock rank summation index (1978), which carries out a simultaneous selection of characteristics, classifying genotypes according to the improvement order of each characteristic, without the need to determine economic weights, or to estimate variance or covariance (CRUZ; REGAZZI; CARNEIRO, 2012).

In view of the above, the aim of this study was to develop and identify superior strains of cowpea in terms of joint resistance to the CPSM and CABM viruses with characteristics associated with green-grain production, especially maintaining the green colour of the grains once threshed.

MATERIAL AND METHODS

The crosses were carried out in a greenhouse of the Agronomic Institute of Pernambuco, in Recife, Pernambuco (PE), between March and August 2018. In order to obtain the segregating populations, crosses were made as usual between contrasting genotypes for resistance to the CPSM and CABM viruses, using Sempre Verde Salgueiro as the female parent, and CNC-0434 and TVu-966 as the male parents.

The Sempre Verde Salgueiro landrace variety was provided by the Germplasm Bank of the AIP, selected for its high productive capacity for green grain and high commercial acceptance in the state of Pernambuco, albeit susceptible to CPSMV and CABMV. The CNC-0434 variety has immune-type resistance to CPSMV, and was developed at the National Rice and Bean Research Centre of the Brazilian Agricultural Research Corporation (Embrapa). The TVu-966 strain is resistant to CABMV, and was developed by the International Institute of Tropical Agriculture (IITA).

To obtain the crosses, the parent plants were sown in beds in a greenhouse and irrigated by sprinkler system at an irrigation depth of 10 mm for 30 minutes daily. From the start of flowering, about 45 days after planting, biparental crosses were carried out: Sempre Verde Salgueiro x CNC-0434 and Sempre Verde Salgueiro x TVu-966. The crosses were made as per the method described by Zary and Miller Júnior (1982).

Two biparental crosses were obtained, originating two F_1 populations, which were sown in beds and, by self-pollination, gave two F_2 segregating populations. These were sown in pots with a capacity of 3.7 litres containing soil and cattle manure at a ratio of 3:1. Six days after sowing, each virus was inoculated separately, sprinkling a small amount of Carborundum 600 mesh (around 1 g) on the surface of the leaves, followed by rubbing the buffered plant extract (1:10) obtained by macerating 1.0 g of leaf tissue infected with each of the viruses in 9.0 mL of sodium phosphate buffer solution at a concentration of 0.01 M and pH of 7.5. A second inoculation was carried out three days later, in order to avoid possible escapes.

The inoculated plants were assessed daily by visual inspection for 40 days following the first inoculation, verifying the emergence and development of qualitative symptoms characteristic of each of the viruses (PIO-RIBEIRO; ASSIS FILHO; ANDRADE, 2016). The inoculated F_2 plants that showed no symptoms or only small local necrotic lesions were initially classified as resistant, and transplanted to beds in the greenhouse. Plants with symptoms characteristic of the viruses were considered susceptible and, as such, eliminated from both populations.

In order to obtain a single population with joint resistance to both viruses, crosses were carried out between F_2 plants resistant to CPSMV (100 plants) and F_2 plants resistant to CABMV (100 plants). The new F_1 population was sown in beds and, by self-pollination, originated the F_2 population, which underwent the same inoculation and evaluation process (10 DAI) mentioned above, this time inoculating with both viruses simultaneously. The F_2 plants resistant to both viruses were advanced to the $F_{2:5}$ generation by growing the population from one pod per plant (Single Pod Descent-SPD) in the greenhouse.

After generation advancement, 181 $F_{2:5}$ strains were obtained showing great variability in tegument colour, and evaluated, classified and selected based on the colour of the grain. Fifty $F_{2:5}$ strains with a coloured integument, subclass Sempre Verde, were selected for evaluation in the greenhouse for joint resistance to the CPSM and CABM viruses. These same strains were then evaluated for their agronomic characteristics together with green-grain production in an experiment at the AIP Experimental Station, located in Belém do São Francisco, PE (8°45’14’’ S, 38 °57’67’’ W, altitude 305m), in soil classified as a Fluvic Neosol, from November 2020 to February 2021. The parental genotypes Sempre Verde Salgueiro, CNC-0434 and TVu-966 were used as controls, with the Sempre Verde Salgueiro variety as the standard for green-grain production.

The experiment was carried out in a randomised block design, with 53 treatments comprising 50 $F_{2:5}$ strains and three controls: CNC-0434, TVu-966 and Sempre Verde Salgueiro, with three replications. Each experimental plot was represented by one row, 5.0 m in length, with a spacing between plants of 0.25 m and 0.80 m between rows, with four seeds sown per hole, leaving two plants after thinning.

Ploughing and harrowing were carried out 30 days before planting, while furrowing and marking the holes was performed when planting. Soil fertilisation followed the recommendation handbook for the crop, based on the chemical analysis of the soil. Cultivation was by sprinkler irrigation system, at an irrigation depth of 10 mm h^{-1} for one hour, and irrigating every two days. Invasive plants, diseases and pests were controlled as necessary. Harvesting took place whenever there were pods around physiological maturity.

The evaluations were made based on the descriptors proposed by Bioversity International (2007), with the following quantitative characteristics being evaluated: number of days to the start of flowering (NDSF) – the interval from planting to the appearance of the first flower; number of days to physiological maturation (NDPM) – the interval from planting until ripe for harvesting the green grains; green pod length (GPL) – the mean length of ten green pods taken at random; weight of 10 green pods (W10GP) – the weight of the ten green pods under evaluation; grain weight of 10 green pods (GW10GP) – the weight of the grains from the ten pods under evaluation; number of grains per green pod (NGGP) – the mean number of grains in the 10 pods under evaluation; weight of 100 green grains (W100GG) – the mean value of three replications of the weight of 100 green grains; green-grain index (GGI%) – the ratio between the weight of the grains from ten green pods and the weight of the ten green pods; and green-grain production (PROD) – the total green-grain production per hectare. Also evaluated were the following qualitative characteristics: plant size (PS); ease of green-pod threshing (EGPT) and cultivation and usage value (CUV).

The PS, EGPT and CUV characteristics were evaluated using a rating scale, as follows: PS – 1 = erect, 2 = semi-erect, 3 = semi-prostrate, and 4 = prostrate; EGPT – 1 = very difficult to thresh, 2 = difficult, 3 = easy, and 4 = very easy; and CUV – 1 = a strain with no commercial characteristics, 2 = with few commercial characteristics, 3 = with most commercial characteristics, 4 = with all commercial characteristics, and 5 = with excellent commercial characteristics.

The data were submitted to analysis of variance and the mean values grouped by Scott-Knott test at 5% probability for all the quantitative descriptors under evaluation. The Mulamba-Mock rank summation index (1978) was then used to identify the superior strains from the quantitative characteristics evaluated, and the selection determined in descending order for NDSF and NDPM, and in ascending order for the other characteristics. Each analysis was carried out using the GENES software (CRUZ, 2013).

RESULTS AND DISCUSSION

After evaluating the 50 $F_{2:5}$ strains for resistance to CPSMV + CABMV, it was possible to identify 27 strains that were resistant to both viruses (Table 1).

According to the F-test in the analysis of variance, all the evaluated characteristics showed a significant difference at a level of 1% ($p < 0.01$), showing there to be variability between the strains under study (Table 2).

Table 1 - Symptoms presented by the 50 $F_{2:5}$ strains evaluated in the greenhouse for joint resistance to the Cowpea Severe Mosaic Virus (CPSMV) and the Cowpea Aphid-Borne Mosaic Virus (CABMV). Recife, PE, 2021

Strain	CPSMV + CABMV	
	Symptoms	Response
L300.001	Ms, Nc, Lr	S
L300.002	Ms, Nc, Lr	S
L300.003	Ns	R
L300.004	Ns	R
L300.005	Ns	R
L300.006	Lc, M, Nc, Lr	S
L300.007	Lc, Ms	R
L300.008	Ns	R
L300.009	Ms	S
L300.010	Bl, Lc, Ms, Da, Nc, Lr	S
L300.011	Lc, Ms, Nc, Da, Lr	S
L300.012	Bl, Lc, Ms, Da, Nc, Lr	S
L300.013	Ns	R
L300.014	Ms	S
L300.015	Ms	S
L300.016	Bl, Lc, Ms, Nc, Lr	S
L300.017	Ns	R
L300.018	Bl, Lc, Ms, Da, Dp, Nc, Lr	S
L300.019	Ns	R
L300.020	Ns	R
L300.021	Ns	R
L300.022	Lc, Ms, Nc	S
L300.023	Ml	R
L300.024	Bl, Lc, Ms	S
L300.025	Ns	R
L300.026	Ns	R
L300.027	Ns	R
L300.028	Ns	R
L300.029	Ns	R
L300.030	Ns	R
L300.031	Lc, Ms, Nc	S
L300.032	Ns	R
L300.033	Ns	R
L300.034	Ns	R
L300.035	Ns	R
L300.036	Ml	R
L300.037	Ml	R

Continuation Table 1

L300.038	Ms	S
L300.039	Ml	R
L300.040	Lc, Ml	R
L300.041	Bl, Lc, Ms	S
L300.042	Bl, Ms	S
L300.043	Ms, Nc	S
L300.044	Ns	R
L300.045	Lc, Ms, Nc	S
L300.046	Bl, Lc, Ms, Nc	S
L300.047	Lc, Ms, Lr	S
L300.048	Lc, Ms, Nc, Lr	S
L300.049	Ml, Nc	R
L300.050	Bl, Lc, Ms, Nc, Lr	S
CNC-0434	Bl, Lc, Ms, Nc	S
TVu-966	Lc, Ms, Nc	S
S. Verde S.	Bl, Lc, Ms, Da, Dp, Nc, Lr	S

[1] Lc- chlorotic lesion; Bl- blistering; M-mosaic; Ml- light mosaic; Ms- severe mosaic; Da - Apical death; Dp - plant death; Nc - systemic necrosis; Lr - leaf reduction; Ns - no symptoms; R - resistant and S – susceptible

Table 2 - Summary of the analysis of variance relative to the characteristics evaluated in 50 $F_{2:5}$ strains of green cowpea in the district of Belém do São Francisco, PE, 2021

SV	DF	Mean Square								
		NDSF (days)	NDPM (days)	GPL (cm)	W10GP (g)	GW10GP (g)	NGGP (g)	W100GG (g)	GGI (%)	PROD (Kg ha^{-1})
Blocks	2	22.04	13.33	0.05	72.30	57.68	0.92	66.59	18.36	353850.79
Genotypes	52	16.6**	46.3**	12.2**	1055**	125.7**	5.6**	78.8**	108.1**	1643082**
Residual	104	6.83	26.06	1.01	131.80	21.98	1.42	10.49	25.92	409766.15
Mean		46.41	69.42	17.76	93.80	47.44	14.26	34.79	51.74	2751.37
CV(%)		5.63	7.35	5.66	12.23	9.88	8.35	9.31	9.84	23.27

[1] NDSF - number of days to the start of flowering; NDPM - number of days to physiological maturation; GPL- green pod length; W10GP - weight of ten green pods; GW10GP - weight of the grain of ten green pods; NGGP - number of green grains per pod; W100GG - weight of one hundred green grains; GGI - grain index; and PROD - green-grain production per hectare. [2] (**) Significant at a level of 1% probability by F-test

The coefficients of variation for the variables under analysis were below the maximum acceptable limits and agree with those seen in other studies with cowpea intended for green-grain production (MELO *et al*., 2021; SANTOS *et al*., 2018; SILVA *et al*., 2013; SOUSA *et al*., 2015).

From the Scott-Knott clustering test at 5% probability, groups of mean values were seen, in which the strains that had mean values with no statistical difference were grouped in the same group for each characteristic under evaluation (Table 3).

The number of days to the start of flowering (NDSF) ranged from 40 in the L300.034 strain to 52 in L300.025, with a mean of 46.4 days. A similar result was found by Silva *et al*. (2013) who, evaluating different cowpea cultivars for green-grain production under irrigated conditions in the district of Serra Talhada, PE, found a mean of 46.9 days with a range of 43.5 to 55.2 days to the start of flowering. According to the Scott-Knott test, two groups were formed, where 21 strains had early to normal flowering and 29 strains began flowering after more than 45 days. Precocity is one of the factors that make it possible to grow up to three cycles per year using an irrigation system; in addition, the plants are less exposed to biotic and abiotic factors that may compromise their ability to express their productive characteristics (OLIVEIRA *et al*., 2016).

Table 3 - Mean values resulting from applying the Scott–Knott test to the variables number of days to the start of flowering (NDSF), number of days to physiological maturation (NDPM), green pod length (GPL), weight of ten green pods (W10GP), weight of the grain of ten green pods (GW10GP), number of green grains per pod (NGGP), weight of one hundred green grains (W100GG), green grain index (GIV%), and green-grain production per hectare (PROD), obtained from the evaluation of 50 $F_{2:5}$ strains of green cowpea in the district of Belém do São Francisco, PE, 2021

STRAIN	NDSF (days)	NDPM (days)	GPL (cm)	W10GP (g)	GW10GP (g)	NGGP (g)	W100GG (g)	GIV% (%)	PROD (Kg ha^{-1})
L300.001	47.7 A	68.3 B	18.4 C	96.0 C	50.0 B	15.6 A	33.3 D	51.9 B	1382.0 C
L300.002	45.0 B	63.7 B	14.5 E	53.0 E	30.7 D	14.9 A	28.3 E	57.9 A	3770.3 A
L300.003	47.0 A	67.3 B	15.9 D	66.0 E	42.7 C	16.1 A	30.0 E	64.5 A	3090.6 A
L300.004	49.0 A	76.33 A	16.8 D	85.0 D	42.0 C	11.1 B	37.7 C	48.9 B	1218.0 C
L300.005	42.3 B	73.3 A	17.4 D	75.0 D	42.0 C	13.2 B	33.3 D	56.2 A	1617.0 C
L300.006	47.0 A	69.7 A	16.7 D	94.0 C	54.0 B	16.0 A	34.0 D	57.5 A	2849.6 A
L300.007	45.7 B	66.3 B	16.0 D	65.0 E	38.7 D	15.3 A	25.3 E	62.3 A	3561.0 A
L300.008	45.7 B	65.0 B	16.0 D	84.0 D	49.3 B	16.1 A	32.7 D	58.8 A	2276.3 B
L300.009	46.3 A	71.3 A	15.7 D	70.0 E	40.7 C	12.7 B	34.0 D	58.6 A	2594.3 B
L300.010	41.7 B	72.0 A	16.1 D	83.0 D	45.3 C	12.7 B	41.0 C	54.5 A	3391.0 A
L300.011	49.0 A	66.7 B	14.4 E	104.0 C	52.0 B	12.7 B	41.7 C	50.0 B	2747.0 B
L300.012	44.3 B	66.7 B	17.8 C	91.0 D	48.0 B	13.4 B	38.0 C	53.4 A	1958.0 C
L300.013	43.3 B	70.7 A	14.7 E	51.3 E	32.7 D	11.6 B	28.0 E	63.6 A	3296.3 A
L300.014	48.7 A	73.0 A	18.9 C	97.0 C	49.3 B	15.3 A	32.7 D	51.2 B	1693.6 C
L300.015	45.3 B	66.7 B	15.8 D	91.0 D	50.7 B	14.6 A	35.3 D	55.9 A	3256.3 A
L300.016	48.0 A	70.7 A	18.2 C	95.0 C	48.7 B	15.4 A	31.3 D	51.5 B	2327.3 B
L300.017	46.7 A	71.0 A	17.6 C	116.0 B	49.3 B	14.7 A	34.0 D	42.6 C	2363.6 B
L300.018	45.7 B	64.7 B	18.2 C	84.0 D	47.3 B	14.8 A	31.3 D	56.3 A	4223.0 A
L300.019	48.7 A	70.7 A	17.5 D	121.0 B	50.7 B	12.3 B	41.3 C	42.3 C	2520.3 B
L300.020	47.7 A	78.7 A	13.3 E	116.0 B	48.7 B	12.1 B	38.3 C	41.9 C	2635.0 B
L300.021	44.0 B	75.3 A	15.7 D	117.0 B	58.7 B	12.8 B	46.7 B	50.2 B	2200.3 B
L300.022	49.0 A	70.33 A	22.4 A	133.3 A	52.7 B	14.6 A	37.3 C	39.6 C	3272.0 A
L300.023	43.0 B	71.3 A	17.9 C	90.1 D	42.7 C	13.3 B	32.7 D	47.1 B	1808.0 C
L300.024	45.0 B	74.3 A	19.1 C	111.0 B	44.0 C	11.9 B	36.7 C	39.9 C	1730.6 C
L300.025	52 A	74.7 A	19.1 C	105.2 C	52.7 B	13.3 B	36.0 C	49.9 B	2554.6 B
L300.026	45.3 B	69.7 A	20.3 B	103.0 C	51.3 B	16.4 A	32.0 D	50.0 B	2442.3 B
L300.027	46.0 B	63.3 B	20.4 B	99.1 C	50.7 B	16.0 A	33.3 D	50.9 B	3052.6 A
L300.028	48.7 A	71.7 A	15.0 E	76.3 D	39.3 D	14.1 B	28.7 E	52.0 B	2630.0 B
L300.029	46.3 A	68.3 B	21.8 A	98.2 C	41.3 C	13.1 B	31.3 D	43.1 C	2962.0 A
L300.030	47.0 A	72.3 A	17.9 C	75.0 D	37.3 D	13.1 B	28.7 E	50.8 B	2701.7 B
L300.031	48.7 A	64.0 B	18.9 C	100.1 C	48.7 B	14.2 A	34.7 D	48.9 B	3511.0 A
L300.032	49.3 A	69.3 A	10.0 E	87.0 D	44.0 C	13.8 B	32.7 D	51.0 B	2620.0 B
L300.033	45.0 B	68.3 B	18.0 C	93.0 C	45.3 C	15.3 A	30.0 E	49.2 B	2862.0 A
L300.034	40.0 B	68.7 B	16.7 D	76.1 D	42.7 C	15.2 A	29.3 E	56.1 A	3388.0 A
L300.035	45.0 B	64.3 B	17.4 D	88.2 D	44.0 C	14.8 A	30.7 E	50.4 B	2711.3 B
L300.036	49.0 A	73.0 A	17.8 C	76.3 D	46.0 C	15.7 A	30.7 E	60.5 A	2602.3 B
L300.037	47.7 A	70.7 A	16.6 D	64.0 E	38.0 D	13.4 B	29.3 E	59.8 A	3608.0 A
L300.038	43.0 B	70.0 A	17.2 D	89.0 D	49.3 B	14.9 A	33.3 D	55.2 A	2459.3 B
L300.039	49.0 A	69.3 A	23.1 A	102.1 C	53.3 B	15.6 A	36.7 C	52.5 A	3700.6 A
L300.040	44.3 B	62.0 B	18.4 C	99.0 C	47.3 B	13.8 B	33.7 D	47.6 B	3226.0 A
L300.041	49.3 A	77.3 A	16.2 D	99.0 C	56.7 B	15.4 A	37.3 C	57.2 A	2763.3 B
L300.042	47.7 A	73.3 A	15.8 D	107.0 C	49.3 B	12.8 B	40.7 C	45.9 C	1953.7 C
L300.043	47.0 A	67.0 B	18.5 C	93.0 C	49.3 B	13.7 B	36.0 C	53.4 A	3325.0 A

Continuation Table 3

L300.044	44.7 B	69.0 A	17.4 D	94.0 C	45.7 C	13.7 B	34.3 D	48.8 B	2327.0 B
L300.045	46.7 A	73.3 A	19.3 C	136.1 A	56.0 B	12.9 B	44.7 B	41.3 C	1471.3 C
L300.046	47.3 A	70.0 A	19.7 C	112.1 B	49.3 B	12.1 B	42.7 B	45.1 C	1496.3 C
L300.047	45.0 B	62.7 B	18.9 C	121.2 B	54.7 B	15.4 A	39.0 C	45.3 C	3818.0 A
L300.048	47.3 A	68.7 B	18.3 C	99.2 C	49.3 B	14.0 B	36.0 C	50.7 B	3047.6 A
L300.049	48.0 A	68.3 B	17.9 C	101.1 C	53.3 B	14.0 B	38.7 C	52.7 A	3230.7 A
L300.050	46.7 A	70.3 A	17.8 C	85.1 D	46.7 B	14.9 A	32.7 D	54.6 A	2764.0 B
CNC-0434	43.7 B	63.3 B	16.6 D	95.0 C	52.0 B	15.9 A	36.7 C	54.9 A	2870.3 A
Tvu-966	50.3 A	70.3 A	18.6 C	77.0 D	42.0 C	15.6 A	27.3 E	54.5 A	3246.6 A
Sempre Verde S.	44.3 B	62.0 B	22.1 A	134.0 A	68.0 A	15.6 A	51.7 A	50.7 B	4695.0 A

[1] Mean values followed by the same letters in a column do not differ between groups at a level of 5% probability by the Scott-Knott clustering test

The number of days to physiological maturation (NDPM) ranged from 62 in the L300.040 strain to 78.6 in L300.020, with the strains classified as early and medium-early cycle, respectively. Two groups were also formed for this characteristic, the first comprising strains with an early cycle, and classified as such for their cycle of 61 to 70 days, which included the Sempre Verde Salgueiro variety, with 62 days, and a further 21 strains; the second group was formed of strains with a medium-early cycle, and included 29 strains, classified for their cycle of 71 to 80 days. According to Rocha *et al.* (2021), medium-cycle cultivars are more suitable for a small producer who is able to carry out two or more manual harvests. For cultivating large areas, the use of two or more cultivars with different cycles is recommended, so as to stagger maturation and avoid losses. Despite some strains showing early flowering, this does not mean that physiological maturation for harvesting will also be early, as the time for grain filling varies according to the number and weight of the grains, as well as the size of the pod.

The green pod length (GPL) ranged from 13.3 cm in the L300.020 strain to 23.1 cm in L300.039, with a mean of 17.7 cm. The mean length was less than that found by Ramos *et al*. (2015) who, evaluating the potential for green-grain production of twenty cowpea genotypes from Embrapa Meio-Norte under similar experimental conditions, found a mean of 18.5 cm. However, the highest mean value was 19.9 cm, far below that seen in the present study, where it was possible to verify the formation of five groups, two of them formed by strains with mean values greater than 18.5 cm, reaching values of 21.8 cm in L300.029, 22.4 cm in L300.022 and 23.1 cm in L300.039, thereby forming the group with the greatest mean value together with the Sempre Verde Salgueiro variety, at 22 cm. These results meet the preference of the green-grain market for large pods longer than 18 cm (FREIRE FILHO *et al*., 2005).

The weight of ten green pods (W10GP) ranged from 51.3 g in the L300.013 strain to 136.0 g in L300.045, with a mean of 93.8g, forming five groups, two of them (A and B) should be highlighted as they included nine strains with a W10GP greater than 110.0 g, similar to the value of the Sempre Verde Salgueiro variety (134 g). These results are superior to those of Silva *et al.* (2013), who found values of less than 52 g. Another fairly important characteristic related to pod weight is the weight of the grain of ten pods (GW10GP), varying from 30.6 g in the L300.002 strain to 58.6 g in L300.021, with a mean of 47.4 g. Four groups were formed in the mean-value grouping test, where the strain with the highest GW10P in group B, L300.021 (58.6 g), was around ten grams less than the Sempre Verde Salgueiro variety (68.0 g), enough for this variety to be classified in a separate group.

The number of green grains per pod (NGGP) varied from 11.1 in the L300.004 strain to 16.4 in L300.026, with a mean of 14.2 grains per pod. Two groups were formed, with emphasis on group A, in which each strain had a mean value of more than 14 grains per pod and was grouped together with the Sempre Verde Salgueiro variety (15.5 grains per pod). Most of the strains with the highest number of grains per pod also had a high value for pod length, there being a probable positive correlation between both characteristics. The results are similar to those found by Ramos *et al*. (2014), who evaluated green-grain production in the BRS Guariba and BRS Paraguaçu varieties under different water regimes and found values of 15 and 12 grains per pod, respectively, for an irrigation depth of 125% of the ETo. Sousa *et al*. (2015) evaluated 16 cowpea genotypes from the Active Germplasm Bank and the Cowpea Breeding Program of Embrapa Meio-Norte for their potential on the green-grain and green-pod market, finding mean values ranging from 13.1 to 16.7 grains per pod.

The weight of one hundred green grains (W100GG) ranged from 25.3 g in the L300.007 strain to 46.6 g in L300.021, with a mean of 34.8 g. Five groups were formed, with emphasis on groups B and C, in which 20 strains had a mean value for W100GG greater than 36 g. The greatest value found in the present study was 46.6 g in L300.021, higher than the greatest mean value found by Ramos *et al*. (2015) of 32.8 g in the MNC03-725F-3 strain; by Santos *et al*. (2018) of 21.9 g in the BRS Novaera cultivar; and by Melo *et al*. (2021) of 44.28 g in the BRS Tumucumaque cultivar. The L300.021 (46.7 g), L300.045 (44.7 g) and L300.046 (42.7 g) strains, which comprised group B, had the closest value for W100GG to that in the Sempre Verde Salgueiro variety of 51.7 g, considered the standard for green-grain production in the region.

The green-grain index (*GGI%*) varied from 39.6% in the L300.022 strain to 64.4% in L300.003, with a mean of 51.7%. Three groups were formed: 10 strains forming group C, with a grain index of less than 50%; 18 strains forming group B, with a grain index of around 50%; and a further 21 strains, including the Sempre Verde Salgueiro variety, forming group A, which had a green-grain index greater than 53%. Some strains had a value above 60%, a better result than that found by Ramos *et al*. (2015), Rocha *et al*. (2012) and Silva *et al*. (2013), with a GGI% of 51.1%, 52.6% and 58.7%, respectively. The use of irrigation affords the highest ratio between the weight of the green grains and the weight of the empty pod. According to Freire Filho *et al*. (2005), this characteristic is an important parameter in selecting cultivars for green-grain production, as it helps in measuring plant efficiency in allocating photoassimilates to the grain.

Green-grain production per hectare (PROD) ranged from 1,2018 Kg ha^{-1} in the L300.004 strain to 4,695 Kg ha^{-1} in the Sempre Verde Salgueiro variety, with a mean of 2,751.4 Kg ha^{-1}. Three groups were formed, with 10 strains forming group C, the group with the worst productive performance and productivity less than 2,000 Kg ha^{-1}; 19 strains forming group B, where productivity ranged from 2,000 to 2,700 Kg ha^{-1}; and finally 21 strains, including the Sempre Verde Salgueiro variety, forming group A, the group with the best productivity, where 18 strains produced over 3,000 Kg ha^{-1}, and the L300.018 strain produced 4,223 Kg ha^{-1}. The mean value and values for best productivity found in the present study are higher than those found by Silva *et al*. (2013): 1,353.23 Kg ha^{-1} and 2,552.7 Kg ha^{-1}; Ramos *et al*. (2015): 1,398.75 Kg ha^{-1} and 2,182 Kg ha^{-1}; Sousa *et al*. (2015): 2,390 Kg ha^{-1} and 3,322 Kg ha^{-1}; Santos *et al*. (2018): 1,134.8 Kg ha^{-1} and 1,488.45 Kg ha^{-1}; and Melo *et al*. (2021): 731 Kg ha^{-1} and 1,951.70 Kg ha^{-1}. However, the mean value in the present study was lower than that found by Freitas *et al*. (2016), who obtained a mean productivity of 2,827 Kg green grains ha^{-1} during the first half of 2013 when evaluating 12 landrace varieties irrigated by sprinkler, where the greatest productivity was 2,990 Kg ha^{-1}; however, the number and variability of the strains used in the present study can explain this difference in the mean value.

As for the qualitative characteristics, plant size (PS) varied between semi-erect (10%), semi-prostrate (24%) and prostrate (66%). According to Rocha *et al*. (2012), for small areas and for intercropping, preference is given to the use of semi-prostrate cultivars with medium to long branches, which is the case for 12 of the strains under evaluation. For technified or irrigated crops, the preference is to use more-erect cultivars with short branches that allow the use of mechanised harvesting. Despite there being no upright strain, five semi-upright strains were found, which may allow for greater population density, possibly contributing to an increase in the productivity of the area.

From evaluating the ease of green-pod threshing (EGPT), strains were observed that had the following characteristics: very difficult to thresh (8%), difficult to thresh (24%), easy to thresh (54%) and very easy to thresh (14%). This characteristic is important, because the more difficult it is to open the green pods, the more time and more effort is required for threshing, whether manual or mechanical. According to Rocha *et al*. (2006), traders prefer genotypes that are easy to thresh, with good post-harvest conservation. In this respect, the strains that stood out the most were L300.001, L300.010, L300.024, L300.032, L300.039, L300.041 and L300.048.

An evaluation of the cultivation and usage value (CUV) resulted in strains with no commercial characteristics (22%), with few commercial characteristics (46%), with most commercial characteristics (22%), with all commercial characteristics (6%), and with excellent commercial characteristics (4%). Andrade *et al*. (2010), evaluating the genetic parameters for green grain in the cowpea, saw a significant positive phenotypic and genotypic correlation of the CUV with the pod weight, grain weight, grain index and one-hundred-grain weight, showing the possibility of selecting the genotype by means of the phenotype.

By applying the Mulamba-Mock rank summation index (1978), it was seen that, in general, the characteristics under evaluation showed satisfactory gains, with a total of 51.9% (Table 4). Oliveira *et al*. (2017) and Rodrigues *et al*. (2017) also found satisfactory progress from the simultaneous selection of characteristics in the cowpea using the Mulamba-Mock rank summation index.

The number of days to the start of flowering (NDSF) and number of days to physiological maturation (NDPM) showed a negative gain, which is of interest for obtaining new cultivars with a shorter production cycle. However, the grain index (GGI%) also showed a negative gain of 0.3%; this was due to the strains selected by rank summation showing no superiority for this characteristic. This highlights the need to carry out selection based on several characteristics and not just one, which can result in cultivars failing on the market (RAMALHO *et al*., 2012).

According to the Mulamba-Mock selection index, the most promising strains for green-grain production under the evaluated conditions were L300.047, L300.027, L300.039, L300.049, L300.006, L300.026, L300.043 and L300.040, with L300.026, L300.039, L300.040 and L300.049 showing joint resistance to CPSMV and CABMV, with the others resistant to CPSMV only (Table 5).

Despite not having been selected by the selection index, the L300.008, L300.017, L300.020, L300.021, L300.025 and L300.036 strains show joint resistance to the viruses and have good agronomic characteristics compared to the Sempre Verde standard, and can be used in future crosses.

Table 4 - Mean value of the original population (Xo), mean value of the selected population (Xs), narrow-sense heritability (h_r), and selection gain (GS) based on the Mulamba-Mock rank summation selection index in 50 $F_{2:5}$ strains of green cowpea in the district of Belém do São Francisco, PE, 2021

VARIABLE	Xo	Xs	h^2%	GS	GS%
NDSF	46.41	45.97	58.97	-0.26	-0.57
NDPM	69.42	65.73	43.70	-1.61	-2.32
GPL	17.76	19.4	91.69	1.50	8.46
W10GP	93.86	104.1	87.51	8.96	9.55
GW10GP	47.44	53.4	82.51	4.91	10.37
NGGP	14.26	15.26	74.53	0.75	5.24
W100GG	34.78	37.17	86.68	2.06	5.93
GGI%	51.73	51.53	76.01	-0.15	-0.3
PROD	2751.38	3321.03	75.06	427.59	15.54
TOTAL GAIN				443.75	51.9

[1] NDSF – number of days to the start of flowering; NDPM - number of days to physiological maturation; GPL - green pod length; W10GP - weight of ten green pods; GW10GP - weight of the grain of ten green pods; NGGP - number of green grains per pod; W100GG - weight of one hundred green grains; GGI% - grain index; and PROD - green-grain production per hectare

Table 5 – Mean values of the characteristics of $F_{2:5}$ strains of green cowpea selected for selection gain based on the Mulamba-Mock rank summation index, in the district of Belém do São Francisco, PE, 2021

Strain	NDSF (days)	NDPM (days)	GPL (cm)	W10GP (g)	GW10GP (g)	NGGP (g)	W100GG (g)	GGI (%)	PROD (Kg ha^{-1})
L300.047	45.0	62.7	18.9	121.2	54.7	15.4	39.0	45.3	3818.0
L300.027	46.0	63.3	20.4	99.1	50.7	16.0	33.3	50.9	3052.6
L300.039	49.0	69.3	23.13	102.1	53.3	15.6	36.7	52.5	3700.6
L300.049	48.0	68.3	17.9	101.1	53.3	14.0	38.7	52.7	3230.7
L300.006	47.0	69.7	16.7	94.0	54.0	16.0	34.0	57.5	2849.6
L300.026	45.3	69.7	20.3	103.0	51.3	16.4	32.0	50.0	2442.3
L300.043	47.0	67.0	18.5	93.0	49.3	13.7	36.0	53.4	3325.0
L300.040	44.3	62.0	18.43	99.0	47.3	13.8	33.7	47.6	3226.0
Salgueiro	44.3	62.0	22.1	134.0	68.0	15.6	51.7	50.7	4695.0

[1] NDSF – number of days to the start of flowering; NDPM - number of days to physiological maturation; GPL - green pod length; W10GP - weight of ten green pods; GW10GP - weight of the grain of ten green pods; NGGP - number of green grains per pod; W100GG - weight of one hundred green grains; GGI% - grain index; and PROD - green-grain production per hectare

CONCLUSIONS

The L300.026, L300.039, L300.040 and L300.049 strains were resistant to the Severe Cowpea Mosaic Virus and the Aphid-Transmitted Cowpea Mosaic Virus, and have a high potential for green-grain production. The Mulamba-Mock selection index facilitated the selection of superior strains by the use of joint characteristics, making possible future launches of new varieties for green-grain production with resistance to both viruses.

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