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ARTICLE



## Morphological responses, fruit yield, nutritive value and *in vitro* gas production of forage watermelon genotypes on semi-arid condition

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### ABSTRACT

This study aimed to evaluate morphological, bromatological, *in vitro* gas production and yield of forage watermelon (*Citrullus lanatus* var. *citroides*) genotypes in semi-arid condition. Seven genotypes were evaluated were BGCIA 228, BGCIA 239, Jojoba, BGCIA 228 x BGCIA239, BGCIA 228 x BGCIA Jojoba, BGCIA 239 x Jojoba and BGCIA 991. The experimental design was a complete randomized block with three replicates. The genotypes presented differences between the characteristics: fruit length ( $P = 0.01$ ), vertical diameter ( $P = 0.02$ ), peel thickness ( $P = 0.01$ ), fruit pulp thickness ( $P = 0.02$ ), transversal diameter ( $P = 0.02$ ), *in vitro* dry matter digestibility ( $P = 0.003$ ) and the latency time ( $P < 0.0001$ ). Cumulative *in vitro* gas production and gas production rate was not affected by genotypes. None of the studied genotypes had production and productivity affected. Among them, Jojoba and BGCIA 991 stood out for having heavier and longer fruits, and a higher peel thickness and pulp length.

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### KEYWORDS

*Citrullus lanatus* var. *citroides*; forage plant; semi-arid

## 1. Introduction

Ruminant production has great economic importance for the Brazilian semi-arid and others arid and semi-arid regions worldwide, but the scarcity and irregularity of rains are barriers to increase forage and animal production. So, there is a need for forage plants that can be grown under regional climatic conditions, especially considering water scarcity and high environmental temperatures (Coutinho et al. 2013).

In this condition, forage watermelon (*Citrullus lanatus* var. *citroides*), also known as horse watermelon or pig watermelon is a potential fodder crop, presenting a rapid crop cycle (90 to 120 days), propagated by seeds and besides supplying nutrients is also

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a source of water for animals, a characteristic highly appreciated in arid and semi-arid regions (Moraes et al. 2007; Mustafa and Alamin 2012).

The crude protein content (20.9%) and fat (30.1%) and the low crude fiber content (38.4%) in *C. lanatus* seeds potentiate its use as food to animals. Being an aqueous food and rich in minerals, this forage contributes to the maintenance of the rumen microbial flora, improving food digestion (Levi et al. 2013; Rodrigues and Vaz 2013).

Despite the potential advantages of this forage resource as an alternative fodder to Brazilian semi-arid and others worldwide semi-arid regions, the scarcity of information on the morphological, yield and chemical composition of forage watermelon are decisive factors for its use. In addition, there is a need to evaluate and to indicate more promising genotypes that meet the required standards of the agriculture and husbandry sector and that can be conducted under efficient and low-cost production systems (Acar et al. 2014). This information can be obtained through the evaluation of genotypes identifying potential progenitors for the insertion of important morphoagronomic characters (Upadhyaya et al. 2010).

Thus, the objective of the study was to evaluate morphological, bromatological, *in vitro* gas production and yield of forage watermelon (*Citrullus lanatus* var. *citroides*) genotypes in semi-arid condition.

## 2. Material and methods

### 2.1. Location and meteorological conditions

The experiment was conducted at the Embrapa Semi-Arid Experimental Station of Caatinga, in Petrolina-PE, Brazil (09°09'S, 40°22'W, 376 m altitude). According to Koppen classification, the climate is of the type BSW' h, characterized as a hot semi-arid region presenting two distinct seasons, a rainy with irregular rainfall and a dry without precipitation. The mean annual temperature is 26.76 °C and relative humidity 55.90%. Their study follows the principles of the Declaration of Helsinki.

### 2.2. Soil characterization

The soil in the area is classified as dystrophic red-yellow (Embrapa, 2013). Soil samples were collected in the 0–20 cm for analysis of their physical and chemical characteristics. The mineral levels were determined as described by Nogueira and Souza (2005). Chemical and physical and chemical characteristics of the soil were pH = 5.0; P (mg dm<sup>-3</sup>) = 3.28; K (cmolc dm<sup>-3</sup>) = 0.22; Na (cmolc dm<sup>-3</sup>) = 0.03; Mg (cmolc dm<sup>-3</sup>) = 0.60; Al (cmolc dm<sup>-3</sup>) = 0; H+ Al = 1.5; sum of bases (SB) = 2.5; cation exchange capacity (CEC) = 4.3, porosity (%) = 39.27; clay (g/kg) = 48.8; silt (g/kg) = 225.7; sand (g/kg) = 725.7.

### 2.3. Genotypes, planting and experimental design

Seven genotypes of forage watermelon (*Citrullus lanatus* var. *citroides*) were used: BGCIA 228, BGCIA 239, Jojoba, BGCIA 228 x BGCIA239, BGCIA 228 x Jojoba, BGCIA 239 x Jojoba and BGCIA 991. The experimental design consisted of a complete randomized block in a 7 × 4 factorial arrangement with 3 replicates and 12 plants per plot. Plants were spaced 3.0 × 1.0 m between rows and plants, respectively, evaluating the four central plants of each plot.

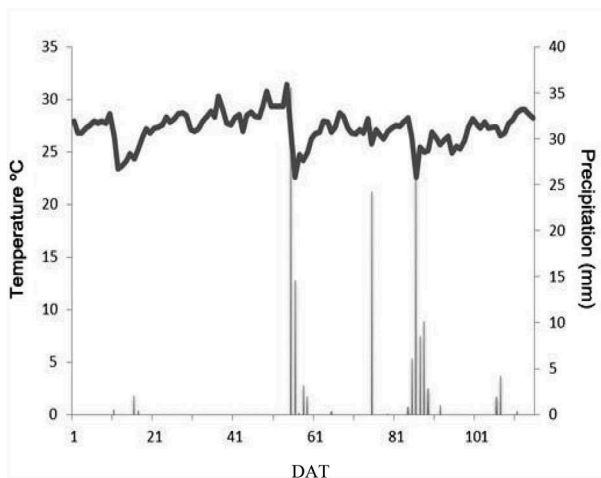
Seeds of *C. lanatus* were sown in expanded polystyrene trays with 128 cells using commercial substrate (Electric conductivity = 0.98, pH = 6.50, Calcium = 4.36, Magnesium = 3.84, Phosphorus = 1.07, Potassium = 1.15, Nitrogen = 3.65), transplanting to the field, 20 days after sowing. Twenty days after sowing, the seedlings were transplanted to the field. The climatological data during the study period was collected from the Meteorological Station of the Caatinga biome (Figure 1).

The fertilizer application was carried out following the recommendations for commercial watermelon described in the Fertilization Manual of the State of Pernambuco, applying 3 kg/pit of goat manure, 30 kg/ha of urea, 120 kg/ha of MAP and 30 kg/ha of potassium chloride (IPA, 2008). The quantity of water 20 mm of water was artificially applied simulating salvage irrigation. After 120 transplanting, fruits were harvested, and the drying of the tendril in fruit peduncle was observed, cutting the peduncle at 5 cm above calyx.

#### 2.4. Yield and morphological characteristics

The following characteristics were evaluated: number of fruits per plant (NFP); fruit weight (FW), in kg; plant yield (PY), in kg/plant, green matter productivity (GMP; kg green matter/ha) and dry matter productivity (kg dry matter/ha), considering 3.333 plants/ha.

Two fruits per plot, with similar weights, were cut longitudinally for morphological evaluations. Using a graduated scale, fruits were measured for length (FL, in cm); longitudinal diameter (LD, in cm); vertical diameter (VD, in cm); peel thickness (PT, in cm) at the floral scar area, peduncle region, and laterals at fruit ends; flesh thickness (FT, in cm) longitudinally and transversally, also at fruit ends; and transverse diameter (TD, in cm).



**Figure 1.** Meteorological data from Petrolina – PE during the experimental period in the Embrapa Semiárido. DAT = days after transplant.

## 2.5. Chemical composition

Flesh soluble solids content (°Brix) was measured by a benchtop refractometer (Abbe Mark II, model 10480 – Lucca), with automatic temperature correction (AOAC, 2016).

Fruit samples were pre-dried in an oven with forced air ventilation at 55°C for 72 h and milled in a Willey type mill with 1 mm diameter sieves. All chemical analyses were carried out using the procedures described by the AOAC (2016) for dry matter (DM, method 967.03), mineral matter (MM, method 942.05), crude protein (CP, method 981.10), and ether extract (EE, method 920.29). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed according to Van Soest et al. (1991). *In vitro* dry matter digestibility (IVDMD) was analyzed according to Silva and Queiroz (2002).

Total carbohydrates (TC) were calculated for:  $TC = 100 - (\% CP + \% EE + \% MM)$ , and non-fiber carbohydrates (NFC) were obtained by subtracting NDF from the TC (Sniffen et al. 1992).

## 2.6 In vitro gas production

To determine *in vitro* gas production 1.0 g of sample was added in glass vials (160 mL), in which 90 mL of culture medium was added following Theodorou et al. (1994). Subsequently, 10 mL of ruminal inoculum (from rumen fistulated cattle) were added to each vial, which was kept under CO<sub>2</sub> aspersion, sealed with rubber stoppers and aluminum seals. After placing the seals, the gases generated inside each flask were discarded and placed in an oven with a constant temperature of 39°C, during the incubation period.

The pressure from the accumulated gases in the upper part of the bottles was measured using a pressure transducer (*Datalogger Universal Logger AG100*) connected to a needle (0.6 mm). The readings were measured at a higher frequency during the initial period and reduced later (2, 6, 12, 24, 48 and 72 h of incubation). From each pressure reading, the total produced by the bottles without substrate (white), referring to each sample was subtracted.

## 2.7. Statistical analysis

The data were submitted to a normality test by a univariate procedure of Univariate of Statistical Analysis System (SAS, 2015) followed by an analysis of variance and Tukey test ( $P < 0.05$ ) by the GLM procedure (*General Linear Models*). To analyze *in vitro* cumulative gas production was used non-linear regression (NLIN).

## 3. Results and discussion

The genotypes did not present difference for NFP, AFW, PY and productivity (kg GM/ha and kg DM/ha) (Table 1). The average NFP was 2.41 per plant, similar to reported by Acar et al. (2014) and Kavut et al. (2014) for FW in Turkey, ranging from 1.06 to 3.40 fruits per plant.

The NFP correlates negatively with AFW, that is, the greater NFP promotes lower AFW. This is possible due to the distribution of assimilates and nutrients is greater in plants that produce more fruits, resulting in smaller weights and sizes of fruits. However, in the present research, only the genotype BGCIA 239 x Jojoba presented NFP more than 3.50 (Table 1) and, in general, the AFW of forage watermelon genotypes were low.

**Table 1.** Yield responses of forage watermelon genotypes in Petrolina, Pernambuco, Brazil.

Genotype	Variables				
	NFP	FW	PY	GMP	DMP
Bgcia 228	2.67	1.26	3.36	11.183	736
Bgcia 239	1.83	1.36	2.48	8.265	719
Jojoba	1.42	2.36	3.35	11.165	836
Bgcia 228 x Bgcia239	2.64	0.93	2.45	8.165	680
Bgcia 228 x Jojoba	2.28	1.58	3.60	11.998	895
Bgcia 239 x Jojoba	3.56	1.22	4.34	14.465	1,136
Bgcia 991	2.50	2.25	5.62	18.731	1,446
Average	2.41	1.56	3.60	11.996	921
P	0.36	0.29	0.71	0.69	0,89
CV (%)	14.40	15.77	26.56	40.90	41.65

Average followed by the same letter in the row did not differ by Tukey ( $P > 0.05$ ) significance level. Coefficient of variation (CV); probability (P); number of fruits per plant (NFP); fruit weight (FW) (kg); plant yield (PY) (kg/planta); green matter productivity (GMP) (kg/ha); dry matter productivity (DMP) (kg/ha).

Only Jojoba and BGCIA 991 had AFW greater than 2.0 kg. According to Santos et al. (2017), the quantitative variables are strongly influenced by the environment, which may explain the low AFW. During the crop cycle the cumulative precipitation was 135 mm (Figure 1); however, in May and June (73 to 120 DAT), the average rainfall was 7.4 mm, and in this period the plant requires a larger amount of water to fruit growth and maturation.

Water restriction may compromise the flowering period of the watermelon, which decreases the accumulation of dry mass (Silva et al. 2012). According to Oliveira and Bernardino (2000), in the Sertao of Pernambuco presenting average rainfall ranging from 200 to 600 mm, the productivity of the FW can vary from 10 to 60 t/ha, similar with those observed in the present research (12.0 t/ha of fruits, equivalent to 921 kg of DM/ha)

The genotypes evaluated differed for most of the evaluated characters (FL, VD, PT and TD), except for LD, indicating that genotypes present morphologically different fruits. Jojoba and BGCIA 991 presented greater FL (30.71 and 30.41 cm), respectively, in comparison to BGCIA 228. The genotypes BGCIA 228 x BGCIA 239 and BGCIA 239 x Jojoba had lower VD in relation to BGCIA 991 (Table 2). The morphological characterization of FW genotypes can contribute to the identification of genetic material and provide information to the organization of accesses in germplasm banks and can serve as a basis for genetic selection and breeding studies.

Jojoba presented greater TD in comparison to BGCIA 228 and BGCIA 239. Jojoba and BGCIA 991 had larger PT compared to BGCIA 228 and BGCIA 228 x BGCIA (Table 2). Larger PT is associated with fruit resistance during transportation and greater post-harvest durability.

Dry matter (DM), ash, neutral detergent fiber (NDF), crude protein (CP), ether extract (EE), total carbohydrates (TC), non-fibrous carbohydrates (NFC) and total soluble solids (SS, °brix) were similar to all FW genotypes evaluated (Table 3).

The high content of CP and EE in FW is promoted by significant participation of its seeds (Mustafa and Alamin 2012). In the present study, FW genotypes presented 20.45% of CP and 11.49% of EE, higher than reported by Silva et al. (2009) evaluating FW meal in lamb diet, 18.73% and 10.39% for CP and EE, respectively, which may be related with more seed participation in the fruits of present research. Ether extract and protein are essential components to determine the quality of forage, contributing to meet the energy and protein requirements of animals.

**Table 2.** Morphological characteristics of forage watermelon genotypes Petrolina, Pernambuco, Brazil.

Genotype	Fruit morphological characters (cm)					
	FL	LD	VD	PT	FFT	TD
Bgcia 228	24.72b	49.94	39.74ab	0.21a	17.23b	18.19b
Bgcia 239	26.42ab	50.17	37.74ab	0.22ab	17.32b	18.34b
Jojoba	30.71a	61.11	40.18ab	0.23a	22.19a	23.30a
Bgcia 228 x Bgcia239	24.92ab	49.77	3.34b	0.21b	17.81ab	18.72ab
Bgcia 228 x Jojoba	27.27ab	55.52	40.18ab	0.22ab	19.22ab	20.17ab
Bgcia 239 x Jojoba	26.62ab	52.64	35.81b	0.22ab	18.82ab	19.82ab
Bgcia 991	30.41a	58.80	45.76a	0.23a	20.43ab	21.53ab
Average	27.29	54.00	39.25	0.22	19.00	20.01
P	0.01	0.06	0.02	0.01	0.02	0.02
CV (%)	7.04	7.72	7.83	0.90	7.86	7.64

Average followed by the same letter in the row did not differ by Tukey ( $P > 0.05$ ) significance level. Coefficient of variation (CV); probability (P); fruit length (FL), longitudinal diameter (LD), vertical diameter (VD), peel thickness (PT), fruit flesh thickness (FFT), transversal diameter (TD).

**Table 3.** Chemical composition of forage watermelon genotypes in Petrolina, Pernambuco, Brazil.

Genotype	Chemical characteristics								
	DM	MM	NDF	EE	CP	TC	NFC	IVDMD	SS
Bgcia 228	6.59	7.80	43.16	9.62	19.09	63.49	20.33	67.62a	4.12
Bgcia 239	8.71	9.54	49.45	10.33	21.47	58.67	17.34	62.30ab	3.07
Jojoba	7.49	8.69	43.12	11.55	18.65	61.61	18.49	62.02ab	3.04
Bgcia 228 x Bgcia239	8.34	9.59	49.97	11.60	20.75	58.06	13.64	63.61ab	3.57
Bgcia 228 x Jojoba	7.46	9.90	45.69	10.94	18.76	60.39	14.71	59.61b	3.39
Bgcia 239 x Jojoba	7.86	11.40	48.61	8.74	21.34	59.55	10.94	59.77b	2.73
Bgcia 991	7.72	7.68	52.62	17.69	23.14	57.40	14.77	59.18b	3.81
Average	7.74	9.22	47.51	11.49	20.45	59.88	15.75	62.01	3.39
P	0.15	0.07	0.88	0.32	0.32	0.80	0.37	0.003	0.06
CV (%)	6.25	7.55	4.85	45.10	47.17	5.89	26.55	2.44	9.34

Average followed by the same letter in the row did not differ by Tukey ( $P > 0.05$ ) significance level. Coefficient of variation (CV); probability (P); dry matter (DM, in %fresh matter); mineral matter (MM, in %dry matter); neutral detergent fiber (NDF, in %dry matter); ether extract (EE, in %dry matter); crude protein (CP, in %dry matter); total carbohydrates (TC, in %dry matter); non-fibrous carbohydrates (NFC, in %dry matter); *in vitro* dry matter digestibility (IVDMD, in %dry matter) soluble solids (SS, in °Brix).

Total soluble solids are commonly referred to as °Brix and increases during fruit maturation. In this research, there were no differences in TSS for FW genotypes (Table 3). The °Brix indicates the amount of the solids that are dissolved in the water in food (Chitarra and Chitarra 2005). In the present study, °Brix ranged from 4.12 to 2.73, indicating a low amount of rapidly fermentable saccharide.

The fruits of FW genotypes evaluated had high IVDMD and low NDF content (Table 3). In the present study, IVDMD differed between genotypes. BGCIA 228 presented higher IVDMD in relation to BGCIA 228 x Jojoba, BGCIA 239 x Jojoba and BGCIA 991.

In general, IVDMD found in the present research is lower than reported by Santos et al. (2017), who obtained 74.86% for IVDMD of the FW fruits, possibly due to genetic material. BGCIA 228 presented greater IVDMD compared to BGCIA 228 x Jojoba, BGCIA 239 x Jojoba and BGCIA 991. Although there was no significant difference in bromatological composition of genotypes, BGCIA 228 presented 43.16% of NDF, 63.49% of TC and 20.33% of NFC, while on average BGCIA 228 x Jojoba, BGCIA 228 x Jojoba and BGCIA 991 had 48.97%, 59.11% and 13.47% of NDF, TC and NFC, respectively. High digestibility contributes to greater intake and dry matter utilization by the animal.

**Table 4.** Gas *in vitro* production (mL/g) of forage watermelon genotypes.

Genotype	Incubation time (hour)						Kd (%h)	L (h)
	2	6	12	24	48	72		
Bgcia 228	6.11	10.91	27.85	79.30	110.38	118.60	0.084	4.35d
Bgcia 239	6.84	11.59	27.04	78.99	117.83	128.51	0.080	5.80b
Jojoba	7.25	12.60	33.66	88.32	128.34	139.03	0.079	7.43a
Bgcia 228 x Bgcia239	6.63	12.29	36.20	98.92	134.63	143.31	0.097	7.52a
Bgcia 228 x Jojoba	5.45	10.81	38.93	99.43	129.64	136.43	0.106	4.59cd
Bgcia 239 x Jojoba	6.28	11.67	38.70	97.05	128.36	138.17	0.100	5.51bc
Bgcia 991	6.12	11.89	0.51	0.73	121.74	129.99	0.089	5.50bc
SEM	0.33	38.83	1.98	0.07	4.01	3.67	0.04	0.24
P	0.54	92.69	3.54	0.13	0.06	0.17	0.71	<0.0001

Average followed by the same letter in the row did not differ by Tukey ( $P > 0.05$ ) significance level. SEM = standard error medium; P = probability. Kd (%/h) – gas production rate; L (h) = latency.

*In vitro* gas production of FW was not different for the genotypes evaluated (2 h to 48 h) and also a 72 h cumulative gas production was not affected by genotypes (Table 4).

*In vitro* gas production during 2 to 72 h of FW indicates that the fruits of the genotypes evaluated had a similar amount of fermentative substrates. The greater amount of gas produced is related to carbohydrate fermentation, which presents high correlation. The high content of the soluble fraction constitutes an energy substrate of fast fermentation for the microorganisms, promoting high final gas volume.

The gas production rate was not affected by genotypes, on average presenting 0.090 mL/g. The latency time (L) was higher for BGCIA 228 x BGCIA 239 and Jojoba in comparison to the others. The low latency time is due to the physicochemical constituents as a soluble fraction to fast fermentation by microorganisms, facilitating the adhesion processes and substrate colonization increasing carbohydrate fermentation, reducing the latency period.

## Disclosure statement

No potential conflict of interest was reported by the authors.


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