



Potential of parents and hybrids experimental of the yellow melon

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ABSTRACT: Melon or muskmelon (*Cucumis melo* L.) is of considerable economic importance in Brazil. Nearly all the cultivars currently grown are hybrids. Heterosis has been used to advantage in the melon plant, and the use of uniform hybrids is one of the reasons for successful cultivation. The aim of the present study was to evaluate the agronomic potential of yellow melon lines and hybrids. An experiment was conducted in randomized blocks with three replications to evaluate 24 hybrids generated by crosses among three elite lines used as female parents (AF-01, AF-02, and AF-03) and eight lines used as male parents (LAM-01, LAM-02, LAM-03, LAM-04, LAM-05, LAM-06, LAM-07, and LAM-08). Yield and fruit quality traits were evaluated. Partial diallel analysis was carried out to estimate general combining ability and specific combining ability. Additive and non-additive effects were observed for the yield, mean fruit weight, and pulp thickness traits. The lines AF-02, LAM-02, and LAM-03 are the most promising as parents as they more frequently have favorable alleles. The most prominent hybrids in diallel analysis were AF-02 x LAM-02, AF-02 x LAM-03, AF-02 x LAM-04, and AF-03 x LAM-06.

Key words: *Cucumis melo* L., combining ability, heterosis, diallel.

Potencial de parentais e híbridos experimentais de melão amarelo

RESUMO: O melão amarelo (*Cucumis melo* L.) é uma hortaliça de grande importância econômica. Atualmente as cultivares plantadas, em quase sua totalidade, são híbridos. A heterose tem sido explorada no meloeiro, sendo o emprego de híbridos uniformes uma das razões do sucesso da cultura. O objetivo do presente trabalho foi avaliar o potencial agrônomo de linhagens e híbridos de melão amarelo. Foi conduzido um ensaio em blocos casualizados com três repetições para avaliar 24 híbridos gerados pelos cruzamentos entre três linhagens elites utilizadas como genitores femininos (AF-01, AF-02 e AF-03), e oito linhagens utilizadas como genitores masculinos (LAM-01; LAM-02; LAM-03; LAM-04; LAM-05; LAM-06; LAM-07 e LAM-08). Foram avaliados caracteres a produção e a qualidade do fruto. Realizou-se a análise dialélica parcial para estimação das capacidades geral e específica de combinação. Observou-se efeitos aditivos e não aditivos para os caracteres produtividade, peso médio do fruto e espessura da polpa. As linhagens AF-02, LAM-02 e LAM-03 são as mais promissoras por possuírem maior frequência de alelos favoráveis. Os híbridos de maior destaque foram AF-02 x LAM-02, AF-02 x LAM-03, AF-02 x LAM-04 e AF-03 x LAM-06.

Palavras-chave: *Cucumis melo* L., capacidade de combinação, heterose, dialélica.

INTRODUCTION

Melon (*Cucumis melo* L.) is the most economically important cucurbits in the semi-arid Northeast of Brazil. The main production areas are the Mossoró-Açu agricultural region in Rio Grande do Norte, and in the Jaguaribe Valley, Ceará. These states account for more than 85% of Brazilian production, and nearly all (>99%) of the melon exported from Brazil comes from these states (SIDRA/IBGE, 2019). The crop also has a significant social impact because it utilizes a large number of laborers throughout the year, it is estimated that the melon production chain directly and indirectly involves more than 60,000 jobs (NUNES et al., 2016).

Growing melon in the semi-arid region of Brazil has been successful mainly because of environmental conditions, such as low annual rainfall (<600 mm year⁻¹), high mean temperatures (>30 °C), and intense sunlight (2500-3000 hours of sunlight year⁻¹). In addition, the crop is developed by a production sector that uses high technology with modern techniques of irrigation, many inputs, and a sophisticated post-harvest structure for export of quality fruit to the European market. One of the most important inputs is seed from breeding programs, especially single hybrid seed (NUNES et al. 2016).

Hybrid seeds for growing cucurbits were introduced throughout the world in the mid-1970s (GUSMINI & WEHNER, 2008). In Rio Grande

do Norte and Ceará, this began in the 1980s. The cultivars grown in the production areas are nearly all single hybrids. Exceptions are the open pollination varieties of melon, like Honey Dew (NUNES et al. 2011a). Big advantages of the use of single hybrids are high yield ($> 25 \text{ t ha}^{-1}$), higher quality ($> 11^\circ\text{Brix}$), and, above all, product uniformity, one of the main requirements of the foreign market.

The demand for hybrid seeds from producers has caused plant-breeding programs throughout the world to expend effort in obtaining increasingly high yielding hybrids that are also resistant to the main pathogens of the crop. The first step in producing hybrid melon seeds is obtaining lines with high performance themselves, with a high concentration of favorable alleles. In addition to the intrinsic performance of a line, it is important to estimate its combining ability, aiming to utilize heterosis since the main traits of economic importance, such as soluble solids content and yield, are controlled by additive and non-additive (dominance) effects (BARROS et al. 2011; FEYZIAN et al. 2009; POUYESH et al. 2017; ZALAPA et al. 2006). The main breeding programs use the elite lines of companies to study the combining ability of the most promising lines, generally obtained by the pedigree method.

Diallel crosses are one of the techniques most used by breeders to study the combining ability of lines. From the diallel analysis method proposed by GRIFFING (1956), one of the methods most used, estimates can be obtained of general and specific combining ability, information on genetic control of the traits, and an estimation of heterosis. Consequently, diallel analysis allows the breeder to identify the best hybrid combinations, both for immediate utilization in the form of F_1 hybrids and for the choice of segregating populations for obtaining lines with greater potential for generating superior hybrids. In melon, the diallel cross technique has been successfully used by various authors to choose parents and/or to study the inheritance of relevant traits (BARROS et al. 2011; FEYZIAN et al. 2009; KALB & DAVIS 1984; LIPPERT & LEGG 1972; MOHAMMADI et al. 2014; POUYESH et al. 2017).

Within the context mentioned above, the aim of the present study was to evaluate the agronomic potential of yellow melon lines and hybrids.

MATERIALS AND METHODS

Site

The study was conducted in Baraúna, RN (5°S , 37°W and 109 MASL). According to the Köppen

classification, the climate is BSw h' (CARMO FILHO et al., 1991). Soil at the location was classified as an Argissolos Vermelhos and corresponds to Ultisoil in the Soil Taxonomy (EMBRAPA, 2018). Soil chemical analysis exhibited the following characteristics: pH = 6.40, Ca = 8.50 meq/100 ml, Mg = 2.90 meq/100 ml, Al = 0.10 meq/100 ml, H+Al = 120 meq/100 ml, K = 0.79 meq/100 ml, P = 13.90 ppm, Na = 19.00 ppm, and O.M. = 1.20 ppm.

Germplasm

The lines AF-01, AF-02, and AF-03 were used as female parents and LAM-01, LAM-02, LAM-03, LAM-04, LAM-05, LAM-06, LAM-07, and LAM-08 as male parents. All are yellow melon group *inodorus* Naud. (*Cucumis melo* L.) developed by the pedigree method. The lines were manually crossed in a 3:1 proportion, i.e., one maternal line for three paternal lines, and pollination was performed by touching the stamens of the pollen donor flower on the stigma of the receiver flower.

Experimental design

The eleven parent lines described and 24 hybrids obtained from them were evaluated in a partial diallel scheme under open field conditions. A randomized complete block experimental design was used with 35 treatments (11 parents + 24 experimental hybrids) and three replications. A plot consisted of seven plants at a spacing of 2.0 x 0.40 m. A second experiment was carried out in completely randomized blocks with three replications to compare the hybrids that stood out in the diallel evaluation with five yellow melon hybrids cultivated by private farmers (4945, Goldex, Soleares, Iracema and Gladial). The plot size and cultural practices were the same applied in the first experiment.

Traits

The traits evaluated were: yield, in t ha^{-1} ; mean fruit weight, in grams (g); number of marketable fruits per plant; pulp thickness, in millimeters (mm); total soluble solids, in $^\circ\text{Brix}$; and pulp firmness. This last measurement of pulp resistance was made using a Wagner[®] penetrometer with an 8.0 mm conical tip plunger (Fruit Pressure Tester – FT 011 Model), and results were expressed in kgf (NUNES et al. 2011a).

Statistical analysis

The data obtained were subjected to univariate analysis of variance, considering fixed treatment effects and to the Snedecor F test at 5% probability. The mean values (of the three replications)

of the parents and of the F₁s were analyzed according to the partial diallel model proposed by GERALDI & MIRANDA FILHO (1988), which is an adaptation of the model proposed by GRIFFING (1956). All the analyses were processed by the genetics and statistics computational application GENES (CRUZ, 2013).

RESULTS AND DISCUSSION

A significant effect ($P < 0.01$) of treatments was observed for all the traits evaluated (Table 1). Regarding general combining ability (GCA), in the two groups of parents, and specific combining ability (SCA), a significant effect ($P < 0.01$) was reported for yield, mean fruit weight, and pulp thickness ($P < 0.01$). In a diallel analysis, the significance of the GCA and SCA indicated the presence of additive and non-additive (dominance) effects involved in genetic control of the trait.

The GCA (additive) and SCA (non-additive) depend on the allele frequency of the parents involved in the diallel and on the level of dominance of the trait. The general combining ability effect is an indicator of the superiority of the population, in terms of frequency of the favorable genes, and of the differences between the gene frequencies of the population and the mean frequencies in the group (VIANA, 2007). This; therefore, demonstrated that results that differ among studies mainly arise from the group of parents used in the experiments. In addition, the effect of the environment and the method of analysis likewise influence the estimates and results achieved.

Concerning yield, diallel analysis studies reported additive and non-additive effects (FEYZIAN et al., 2009; MOHAMMADI et al., 2014; POUYESH et al. 2017; SHASHIKUMAR & PITCHAIMUTHU, 2016). For mean fruit weight, the results of the present study were corroborated by BARROS et al. (2011), FEYZIAN et al. (2009), KALB & DAVIS (1984), LIPPERT & LEGG (1972), POUYESH et al. (2017), SINGH & RANDHAWA (1990), and SHASHIKUMAR & PITCHAIMUTHU (2016), who reported additive and non-additive effects. For pulp thickness, BARROS et al. (2011) and POUYESH et al. (2017) observed the same results as in the present study, namely, significant additive and non-additive effects. Nevertheless, in studies carried out by KALB & DAVIS (1984), KITROONGRUANGPOO-SWANG & TOKUMASU (1992), and SINGH & RANDHAWA (1990), significant additive and non-additive effects were not detected for pulp thickness. Discordant results can be explained by the parents involved.

For the traits of number of fruits per plant, pulp firmness, and soluble solids, significant effects were not detected by the Snedecor F test in any of the sources of variation ($p > 0.05$) (Table 1). KITROONGRUANGPOO-SWANG and TOKUMASU (1992) and SINGH and RANDHAWA (1990) reported that only additive effects were present in genetic control for number of fruits; therefore, contrasting from the results of the present study. The results obtained by BARROS et al. (2011) and SHASHIKUMAR and PITCHAIMUTHU (2016) also differed from those of the present study for number of fruits per plant and pulp firmness. These authors detected significance of the additive and non-additive effects for these variables.

Table 1 - Summary of diallel analysis for six traits evaluated in a partial diallel involving two groups of yellow melon lines.

SV	df	MS (Traits)					
		YIELD	MFW	NMF	PT	SS	PF
(Treatments)	(34)	185.95**	0.39**	0.67**	28.58**	1.75**	0.47**
GCA (Group I)	2	1148.88**	72.82**	1.04 ^{ns}	39.99**	0.74 ^{ns}	0.13 ^{ns}
GCA (Group II)	7	254.65**	185.28**	0.58 ^{ns}	31.459**	3.47 ^{ns}	0.68 ^{ns}
SCA (I x II)	24	93.08**	349.42**	0.46 ^{ns}	13.23**	1.401 ^{ns}	0.32 ^{ns}
Groups	1	8.10 ^{ns}	8937.83**	5.52 ^{ns}	354.12**	0.14 ^{ns}	3.36 ^{ns}
Error	68	53.90	0.02	0.13	4.76	0.52	0.05
C.V. (%)		23.49	8.32	28.32	4.70	5.74	8.13
Mean		30.92	1.70	1.28	46.41	12.	2.83

** , * : Significant by the Snedecor F test ($P < 0.01$), ^{ns}: not significant. YIELD: yield in t ha⁻¹; MFW: mean fruit weight, in g; NMF: number of marketable fruits per plant; PT: pulp thickness in the equatorial region of the fruit, in cm; SS: total soluble solids, in °Brix; PF: pulp firmness, in kgf.

There are controversies in relation to genetic control for soluble solids: Barros et al. (2011), Kalb and Davis (1984), Shashikumar and Pitchaimuthu (2016), and Singh and Randhawa (1990) reported additive and non-additive effects, whereas Lippert and Legg (1972) detected only the additive effect as significant.

The estimates of GCA of the parent lines of the two groups involved in the partial diallel are shown in table 2. For yield, in the first group, the AF-02 line stood out, whereas the lines LAM-02, LAM-03, and LAM-06 stood out in the second group.

For mean fruit weight, lines AF-02 and AF-03, belonging to the first group, showed the highest estimates of GCA. In the second group, the lines LAM-03, LAM-02, and LAM-08 had the highest magnitudes for GCA.

Lines AF-02 and AF-01 had the highest estimates of GCA for pulp thickness in the first group, whereas line AF-03 had a negative estimate. In the second group, in decreasing order, lines LAM-07, LAM-01, LAM-03, and LAM-02 had positive estimates, whereas lines LAM-08, LAM-04, LAM-05, and LAM-06 had negative estimates (Table 2).

The GCA indicates the frequency of favorable alleles present in parents according to the trait. When the item of interest is the highest mean value for the trait, high and positive values of GCA indicate greater frequency of favorable alleles.

However, when the lowest mean value of the trait is beneficial for the genotype, high and negative values also indicated the greater frequency of favorable alleles. Thus, considering that in yellow melon, greater yield, fruit with thick pulp, and medium to large size (1600 to 2300 g) are desirable traits, line AF-02 in the first group and LAM-02 and LAM-03 in the second group have a greater concentration of favorable alleles for these traits (Table 2). These lines contributed to obtain segregating populations with higher mean values in derived lines (FERREIRA et al., 2004).

In table 3 are the estimates of SCA and heterosis of the traits in which a significant effect of SCA was detected in diallel analysis. The magnitude of SCA indicated the degree of complementation of the parents of a cross. Its significance shows heterogeneous performance among the parents; consequently, it is not possible to predict their behavior based only on the GCA because interactions occur among the parents as a result of divergence in the loci with dominance.

For yield, the combinations that stood out, in decreasing order, were AF-03 x LAM-06, AF-02 x LAM-04, AF-02 x LAM-02, and AF-02 x LAM-03. For mean fruit weight, the crosses with the highest estimates for SCA, in decreasing order, were AF-02 x LAM-03, AF-02 x LAM-02, AF-02 x LAM-04, and AF-03 x LAM-06. The line AF-02 was among the highest estimates of SCA for both traits (Table 3).

Table 2 - Estimates of general combining ability (GCA) for yield, mean fruit weight, and pulp thickness in a partial diallel with two groups of yellow melon lines.

Parent	-----GCA (Traits)-----		
	YIELD	MFW	PT
AF-01	-4.19	-1.64	0.61
AF-02	6.43	0.89	0.61
AF-03	-2.27	0.75	-1.22
DP (g _i)	0.99	0.19	0.29
LAM-01	-0.30	0.46	1.13
LAM-02	4.12	2.08	0.51
LAM-03	2.59	4.99	1.11
LAM-04	-3.86	-0.73	-1.46
LAM-05	-1.07	-4.35	-0.36
LAM-06	3.93	-0.61	-0.26
LAM-07	0.04	-3.24	1.26
LAM-08	-5.45	1.39	-1.94
SD (g _i)	1.49	0.29	0.44

YIELD: yield, in t ha⁻¹; MFW: mean fruit weight, in g; PT: pulp thickness in the equatorial region of the fruit, in cm. SD: standard deviation.

Table 3 - Estimate of specific combining ability (SCA) and heterosis of yield, mean fruit weight, and pulp thickness in a partial diallel with two groups of yellow melon lines.

Cross	-----SCA-----			-----Heterosis-----		
	YIELD	MFW	PT	YIELD	MFW	PT
AF-01 x LAM-01	1.40	-7.518	-0.104	0.04	-8.98	-2.82
AF-01 x LAM-02	-4.06	-0.805	-1.685	-5.11	-3.11	-5.42
AF-01 x LAM-03	1.84	-5.520	-1.086	1.67	-6.07	-4.01
AF-01 x LAM-04	-0.33	-4.255	0.685	-1.84	-5.98	-0.50
AF-01 x LAM-05	-2.54	-5.775	-1.478	-4.12	-6.83	-5.17
AF-01 x LAM-06	-2.23	1.818	1.286	-1.51	-0.79	1.49
AF-01 x LAM-07	-1.23	-5.613	-0.377	-4.26	-7.27	-2.51
AF-01 x LAM-08	1.44	-4.118	-2.368	-1.97	-7.30	-8.70
AF-02 x LAM-01	-0.43	5.016	-1.979	2.32	13.40	1.09
AF-02 x LAM-02	5.65	11.999	2.038	8.72	20.65	10.88
AF-02 x LAM-03	4.60	16.814	2.837	8.55	22.59	12.66
AF-02 x LAM-04	10.60	10.939	3.678	13.21	19.06	15.29
AF-02 x LAM-05	-0.25	7.089	1.786	2.29	17.19	10.41
AF-02 x LAM-06	-4.27	7.883	0.210	0.57	19.60	7.80
AF-02 x LAM-07	-3.83	4.922	0.687	-2.74	14.85	7.93
AF-02 x LAM-08	-1.32	5.546	0.696	-0.61	13.37	6.43
AF-03 x LAM-01	-0.73	-1.633	2.048	-1.91	-0.53	6.46
AF-03 x LAM-02	-0.06	1.279	0.997	-0.92	3.53	5.01
AF-03 x LAM-03	-1.42	-2.365	-0.134	-1.41	0.90	2.59
AF-03 x LAM-04	-10.59	-0.911	-2.892	-11.91	1.31	-3.84
AF-03 x LAM-05	2.20	5.439	0.874	0.82	5.25	4.73
AF-03 x LAM-06	15.06	9.233	1.308	15.97	9.26	6.70
AF-03 x LAM-07	-1.35	3.072	1.385	-4.19	3.16	5.99
AF-03 x LAM-08	-8.05	-7.163	-0.006	-11.27	-3.86	1.10
SD (s _{ii})	3.34	0.644	0.991	r 0.93**	0.94**	0.88**
SD (s _{ij})	2.68	0.516	0.795			
SD (s _{ij})	3.76	0.725	1.116			

**Significant by the Mantel test ($p < 0.01$). YIELD: yield, in $t\ ha^{-1}$; MFW: mean fruit weight, in g; PT: pulp thickness in the equatorial region of the fruit, in cm. SD: standard deviation.

The highest estimates of SCA for pulp thickness were reported in the crosses AF-02 x LAM-04, AF-02 x LAM-03, AF-03 x LAM-01, and AF-02 x LAM-02 (Table 3). The lines AF-02 and AF-03 were present in four of the ten highest estimates.

Concerning heterosis, in terms of yield, the highest estimates were observed in the crosses AF-03 x LAM-06, AF-02 x LAM-04, AF-02 x LAM-02, and AF-02 x LAM-03 (Table 3). In regard to mean fruit weight, the highest estimates of heterosis were observed in the crosses AF-02 x LAM-03, AF-02 x LAM-02, AF-02 x LAM-01, AF-02 x LAM-04, and AF-02 x LAM-05. The line AF-02 was involved in the eight highest estimates of heterosis. Regarding pulp thickness, the cross with greatest heterosis were AF-02 x LAM-04, AF-02 x LAM-03, AF-02 x LAM-

02, and AF-02 x LAM-05. The line AF-02 was in the six highest estimates of heterosis.

Positive heterosis indicated that the hybrid combination is better than the mean of the parents. However, the magnitude and the sign (positive or negative) of heterosis depend on the parents and on the trait that is being studied. Monforte et al. (2005) crossed a 'Pele de Sapo' melon line with thirteen accessions and found positive, negative, or null heteroses for mean fruit weight. Barros et al. (2011) reported results for mean fruit weight and pulp thickness similar to those of the present study. These authors used parents belonging to different botanical groups. In this study the obtained hybrids belong to the group *inodorus* Naud. just like your parents lines.

According to Cruz & Vencovsky (1989), when breeders choose parents or hybrids in diallel crosses, they should select the cross with high SCA, and at least one of the parents should have a high estimate of GCA. The idea was to join wide variability (genetic divergence and heterosis) and high frequency of favorable alleles (LYNCH & WALSH, 1998). These characteristics make it possible to obtain high performance lines. In addition, the mean of the cross and of the parents themselves are important for choosing the best hybrid combination. Thus, the crosses that fulfill the conditions suggested by Cruz & Vencovsky (1989) are mainly those that involve the elite line AF-02 as parent, especially the crosses AF-02 x LAM-02, AF-02 x LAM-03, and AF-02 x LAM-04, as well as the cross AF-03 x LAM-06 (Table 3). The lines with the highest frequency of favorable alleles (highest GCA) in their respective groups were AF-02, LAM-2, and LAM-03 (Table 2).

The hybrids that most stand out in the diallel (AF-02 x LAM-02, AF-02 x LAM-03, and AF-02 x LAM-04 plus AF-03 x LAM-06) exhibited performance similar to the main hybrids grown and were exported by the productive sector when compared in a trial under field conditions (Table 4).

An ideal yellow melon hybrid should have high yield ($>25 \text{ t ha}^{-1}$), fruit size acceptable to the foreign market (1.5 to 2.6 kg), good pulp thickness ($>4.5 \text{ cm}$), high pulp firmness ($>3.0 \text{ kgf}$), and high soluble solids content (12.0°Brix) (NUNES et al.

2016b). All the hybrids that stood out in the diallel gathered together these characteristics since they have mean yields above the mean in the Northeast region; the number of fruits per plant was within the standard acceptable to the export market (>1.5 fruits/plant); they have sufficient pulp firmness to bear up under sea transport and arrive in good condition for sale in European supermarkets; and they have soluble solids above the minimum required for yellow melon (ALVES et al. 2000).

Furthermore, these hybrids have excellent appearance regarding peel and pulp color and elliptical fruit shape, as desired for yellow melon (Figure 1). For these reasons, they are promising for use in melon cultivation under the conditions of the Brazilian semi-arid region. These hybrids will be evaluated under different growing conditions in coming years to confirm the results of the present study.

CONCLUSION

The lines AF-02, LAM-02, and LAM-03 are most promising for use as parents since they have higher frequency of favorable alleles. The hybrids that most stood out in diallel analysis were AF-02 x LAM-02, AF-02 x LAM-03, AF-02 x LAM-04, and AF-03 x LAM-06, and they are promising for use in melon cultivation under the conditions of the Brazilian semi-arid region.

Table 4 - Mean of five traits evaluated in experimental and commercial hybrids of yellow melon.

Genotype	Mean (Traits)					
	YIELD	MFW	NMF	PT	PF	SS
-----Hybrid-----						
AF-02 x LAM-02	42.78	2.07c	1.65a	4.96a	2.39b	12.39b
AF-02 x LAM-03	36.68	2.28b	1.29b	5.10a	2.78b	12.91b
AF-02 x LAM-04	33.26	1.75d	1.52a	4.93a	3.68a	12.32b
AF-03 x LAM-06	57.30	1.65d	1.31b	4.63b	2.53b	13.90a
-----Control-----						
4945	36.54	1.98c	1.48a	4.85a	2.45b	12.68b
Goldex	28.07	1.89c	1.19b	5.13a	2.57b	13.75a
Soleares	30.61	1.69d	1.45a	4.46a	2.43b	13.35a
Iracema	37.44	2.39b	1.25b	5.15a	2.16b	11.79b
Gladial	43.34	2.60a	1.33b	5.31a	2.41b	13.26a

Mean values followed by the same lowercase letter belong to the same group according to Scott-Knott (1974). YIELD: yield, in t ha^{-1} ; MFW: mean fruit weight, in g; NMF: number of marketable fruits per plant; PT: pulp thickness in the equatorial region of the fruit, in cm; SS: total soluble solids, in $^\circ\text{Brix}$; PF: pulp firmness, in kgf.

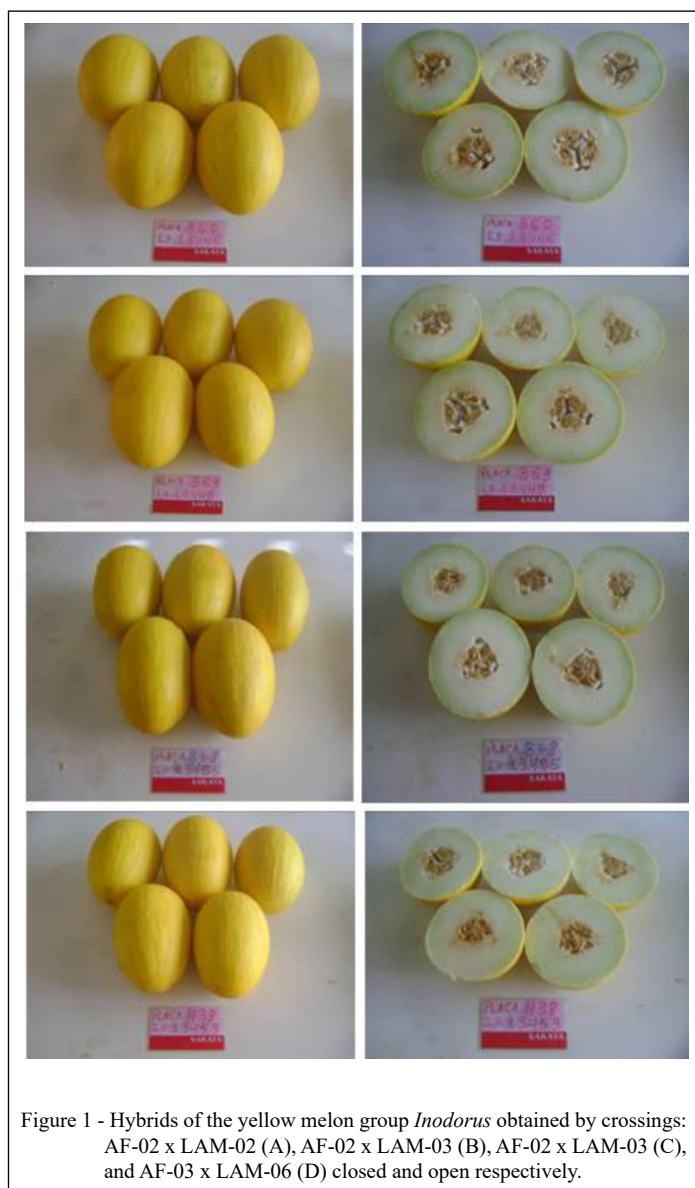


Figure 1 - Hybrids of the yellow melon group *Inodorus* obtained by crossings: AF-02 x LAM-02 (A), AF-02 x LAM-03 (B), AF-02 x LAM-03 (C), and AF-03 x LAM-06 (D) closed and open respectively.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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