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# Heterosis in okra hybrids obtained by hybridization of two methods: traditional and experimental

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## ABSTRACT

High heterosis values can be obtained using hybrid okra seeds. However, despite high heterotic values, it is very difficult to produce hybrid okra seeds. The objective of this study was to evaluate an experimental methodology of hybridization. Two different methods of hybridization were used: traditional manual hybridization with complete emasculation followed by hand pollination (artificial) and, an experimental methodology, performing manual hybridization with incomplete emasculation followed by entomophilous natural pollination between two inbred lines (UFU-QB-040D and UFU-QB-107G).These inbred lines were obtained from three selection cycles. Three hybrid types were obtained from the two hybridization methods: F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G). The resulting hybrid seeds and their parents were evaluated via an experiment set up in a randomized block design with four repetitions. We evaluated fruit production per plant, fruits per plant, number of branchess per plant, superiority of hybrids relative to parents (h) and heterosis (%). At the end of the cycle, morphological characterizations was done of the leaves (pubescence, length and width) and fruit (length, diameter and shape). Heterosis resulting from the incomplete emasculation followed by natural insect pollination was similar to that from the traditional hybrid method, being a viable alternative for the production of hybrid okra seeds without losses in heterosis.

**Keywords:** *Abelmoschus esculentus*, hybridization, heterosis, pollination.

## RESUMO

**Heterose em híbridos de quiabo obtidos por dois métodos de hibridação: tradicional e experimental**

Altos valores de heterose podem ser obtidos utilizando sementes híbridas de quiabo. Apesar dos altos valores heteróticos há grande dificuldade para produzir sementes híbridas de quiabo. O objetivo deste trabalho foi avaliar uma metodologia experimental de hibridação para produção de sementes híbridas de quiabo. Foram utilizadas duas metodologias de hibridação: hibridação tradicional, manual com emasculação completa seguida por polinização manual (artificial) e uma metodologia experimental, realizando hibridação manual com emasculação incompleta seguida por polinização entomófila natural, entre as duas linhagens endogâmicas (UFU-QB-040D e UFU-QB-107G). Essas linhagens endogâmicas foram obtidas após três ciclos de seleção. Utilizando as duas metodologias de hibridação foram obtidos três tipos de híbridos: F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) e F1 IHM(UFU-QB-040D x UFU-QB-107G). De posse das sementes dos híbridos e dos parentais foi realizado um experimento em delineamento em blocos casualizados com quatro repetições. Foram avaliadas a produção de frutos por planta, número de frutos por planta, número de hastes por planta, superioridade dos híbridos em relação aos pais (h) e heterose (%). No final do ciclo, foram realizadas a caracterização morfológico de folhas (pilosidade, comprimento e largura) e de frutos (comprimento, diâmetro e formato). Pode-se concluir que, utilizando a emasculação incompleta com polinização entomófila para produção de sementes híbridas de quiabo, foram obtidos valores similares de heterose quando comparado com a hibridação pelo método tradicional, indicando uma nova alternativa de produção de sementes híbridas de quiabo sem qualquer prejuízo no efeito heterótico dos híbridos.

**Palavras-chave:** *Abelmoschus esculentus*, hibridação, heterose, polinização.

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Okra (*Abelmoschus esculentus*) belongs to the Malvaceae family and originates from Africa. The species performs well in tropical regions and is especially well adapted to the tropical regions of Brazil. Santa Cruz 47 is the cultivar most commonly used by Brazilian growers. Currently, varieties predominate over hybrid seeds in fruit production. Nevertheless, the estimated use of hybrid okra seeds rose 29% from 2010 to 2011 (ABCSEM, 2012).

Improving productivity is one of the main goals of crop breeding programs. Hybrid seeds are extensively used because they provide one of the fastest ways to increase productivity (Paterniani, 1974). Okra heterosis has already been exploited for decades (Sing & Singh, 1978). Current research has also shown the heterotic potential of hybrid okra seeds in targeting greater productivity (Mehta *et al*., 2007; Weerasekara *et al*., 2007; Jaiprakashnarayan *et al*., 2008; Jindal *et al*., 2009). Hybrid seeds are more expensive than varieties; however, hybrid plants are more productive and produce greater income (Medagam *et al*., 2012).

Despite the superior genetic potential

of hybrid okra seeds, seed production remains an obstacle. Okra is considered 100% self-pollinated (Malerbo-Souza *et al*., 2001) with high outcrossing rates that vary from 18.75% (Purewal & Rhandhawa, 1947) to as much as 42.2% (Mitidieri & Vencovsky, 1974), provided by frequent visits from insects, especially *Apis melífera* (Mitidieri & Vencovsky, 1974; Malerbo-Souza *et al*., 2001). This dynamic reproductive behavior means that female flowers need to be protected immediately after emasculation to avoid contamination. Some studies show that gametocides can induce male sterility (Deepak *et al*., 2007), which would reduce the cost of producing hybrid okra seeds and eliminate the need to emasculate and protect flowers. Nevertheless, gametocides use is still undefined, uncommon, and may be toxic to plants, which results in reduced seed yield. In fact, there is little research on economical ways of producing hybrid okra seeds. A traditional method is currently used in Brazil for large scale production of hybrid okra seeds. The method consists of emasculation of the female parent followed by manual crosses, leading to high production costs (Nascimento, 2014). Higher costs have limited greater exploitation of the heterotic potential of okra hybrids and reduced seed availability for growers.

The objective of this study was to evaluate an experimental methodology of hybridization for production of hybrid seeds of okra.

## MATERIAL AND METHODS

The experiment was carried out at a university experimental station in Monte Carmelo, Brazil (EE da UFU, Monte Carmelo-MG) (18º42'43"S, 47º29'56"W, elevation of 873 m). The regional climate is humid temperate with hot summers and dry winters.

The genetic material consisted of inbred lines (UFU-QB-040D and UFU-QB-107G) obtained after three selection cycles from the okra germplasm bank at the Monte Carmelo campus of the Federal University of Uberlandia and maintained at LAGEN-UFU (laboratory for the analysis of seeds and plant genetic resources).

Two inbred lines (UFU-QB-040D and UFU-QB-107G) were sown on May 13, 2013 and used for a fourth selfing to produce hybrid seeds. Polystyrene trays (200 cells) were filled with a commercial coconut fiber substrate and used to produce 200 seedlings for each line. The seedlings were planted in the field 35 days after sowing.

The plants were distributed 1:1 in twenty rows with 20 plants per row. The plants were spaced 1.2x0.8 m, covering a total area of 384 $m^2$. Both inbred lines started flowering approximately 65 days after transplanting. Hybridization commenced at the peak of flowering for both lines, 92 days after transplanting. This strategy guarantees okra seeds of higher physiological quality than seeds produced from the initial phase (Purquerio *et al*., 2010). *Apis melifera* was the most common insect present during flowering. During this step, two different hybridization methods were used (traditional and new) on the two inbred lines. Traditional hybridization method (THM) consisted of emasculating the female parent flowers to expose the flower stigma before anthesis and eliminate the presence of pollen. Next, pollen from the male parent was moved by hand to the stigma of the previously emasculated female flower. After pollination, the female flowers were protected with Kraft paper to avoid contamination from insect pollination (Nascimento, 2014). This process resulted in the THM F1 hybrid (UFU-QB-040D x UFU-QB-107G). The new hybridization method (IHM) consisted of emasculating the female flowers and then allowing pollination to occur via insects that were naturally present at the experiment site. The number of steps was reduced in this process by eliminating the need to protect the emasculated female flowers or collect male flowers. This new method was adopted because of the results obtained by Malerbo-Souza *et al*. (2001), which showed that cross pollination of unprotected okra flowers without any kind of emasculation produced fruits that were 65% heavier, 26% wider and 28.3% longer. Two reciprocal hybrids [F1 IHM (UFU-QB-107G x UFU-QB-040D) and F1 IHM (UFU-QB-040D x UFU-QB-107G)] were obtained using the new methodology.

Fifteen flowers were emasculated from the female parent for both methods (THM and IHM). Originated fruits of hybridization were harvested when they reached physiological maturity and then the seeds were extracted separately according to hybridization method (THM and IHM). After extraction, the seeds were placed in pre-labeled Kraft paper bags and stored. Just after the first experiment, another experiment was conducted using the hybrid seeds originated from the first experiment (F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D), F1 IHM(UFU-QB-040D x UFU-QB-107G) with their parents (seeds from the inbred lines). This experiment was set up in randomized blocks design (RBD) with four replications and used the following statistical model: $Y_{ij} = \mu + b_j + t_i + e_{ij}$

The five treatments were sown on November 21, 2013: F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D), F1 IHM(UFU-QB-040D x UFU-QB-107G), including both inbred lines used in the hybridizations.

Before transplanting, the soil was plowed and harrowed twice and analyzed and corrected for specific crop requirements. The characteristics of the soil were: pH ($H_2O$)= 5.9, available P= 30.1 mg/$dm^3$, K= 85 mg/$dm^3$, $Ca^{+2}$= 2.8 cmolc/$dm^3$, Mg= 1.0 cmolc/$dm^3$, exchangeable H $^+$Al= 3.40 cmolc/$dm^3$, organic matter = 4.2 dag/kg, clay (%)= 30, Al= 0.0 cmolc/$dm^3$, CEC pH 7.0 = 7.42 cmolc/$dm^3$, Sat CEC pH 7.0 for bases = 54.0%; Effective sat CEC for Al= 0.0, Cu= 2.3 mg/$dm^3$, Zn= 8.6 mgdm$^3$ and Mn= 6.6 mg/$dm^3$. Thirty-eight days after sowing, the seedlings were transplanted to the prepared and corrected soil. Twelve plants were planted per plot and spaced 1 m between plants and 1.5 m between rows (18 $m^2$/plot). Thus, the entire experiment covered 360 $m^2$. Crop treatments (insecticides, fungicides, weeding and irrigation) were used as recommended for okra (Filgueira, 2008).

The fruits were harvested every three days from February 2, 2014 until June 27, 2014, totalling 41 harvests. The following agronomic characteristics were determined: production per plant (g/plant), number of fruits/plant and average number of branches/plant.

**Production per plant (g/plant):** obtained from the sum of all samples harvested from the plot divided by the number of plants in the plot. A precision scale (four decimal places) was used.

**Number of fruits/plant:** obtained from the sum of all harvested fruits from the plot divided by the number of plants in the plot.

**Average number of branches/plant:** The branches were counted after the last harvest of the experiment.

The hybrid combinations were evaluated, resulting from the hybrid seed production methods (THM and IHM), by estimating the superiority of the hybrids in relation to parents [h=F1-(P1+P2)/2] and heterosis (%). We used the expression proposed by Fehr (1987) to evaluate the heterosis.

$$Heterosis\ (\%) = \frac{hybrid\ average - parent\ average}{parent\ average} . 100$$

After the last harvest (218 days after sowing), we obtained the morphological characterizations of the leaves and fruit of the parents (UFU-QB-040D and UFU-QB-107G) and hybrids [F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G)]. The leaves were characterized by its pubescence (1= sparse, 2= intermediate and, 3= dense pubescence), length (mm) and width (mm). The fruits were evaluated by length at harvest (LH), diameter (mm) and fruit shape (FS), which was scored from 1 to 5 (1= strongly angular, 2= somewhat angular, 3= angular, 4= cylindrical with few angles or ridges and 5= cylindrical without angles or ridges). Ten plants per plot were evaluated.

Data for each of the agronomic characters were submitted to analysis of variance (F test). Means were compared by the Tukey test ($p<0.05$) using the GENES (Cruz, 1997) software. Orthogonal contrasts [(hybrids versus strains), (THM versus IHM) using the Scheffé test ($p<0.01$)] were used to strengthen the *h* and *h*% values and the heterotic potential of the new method of producing hybrid okra seeds (IHM) relative to the traditional hybrid method (THM).

## RESULTS AND DISCUSSION

Leaf and fruit morphology (Table 1) and agronomic assessments (production per plant, number of fruits per plant and number of branches per plant) had significant differences between genotypes (UFU-QB-040D, UFU-QB-107G, F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G)] (Table 2).

Significant differences were not observed for the leaf pubescence, but the leaf length of the UFU-QB-040D and UFU-QB-107G parents, which were directly involved in obtaining the hybrids, were significantly different (22.1 and 17 mm respectively). The leaf length (21.7 mm) of the F1 IHM(UFU-QB-107G x UFU-QB-040D) hybrid did not differ significantly from its male parent. Leaf width was similar and possibly transmitted between the UFU-QB-040D parent and the F1 IHM(UFU-QB-107G x UFU-QB-040D) hybrid. Compact okra plants (i.e. plants with smaller leaves) are more highly valued by breeders because compactness facilitates harvesting (Carvalho *et al*., 2003; Melo & Vilela, 2005) and reduces the costs of labor, inputs, and especially plant protection products (Oliveira *et al*., 1995).

Only UFU-QB-107G presented shorter fruits than average value (Table 1). Conversely, mean fruit diameter (21.5 mm) of the same strain (UFU-QB-107G) was significantly greater than that of the other genotypes. There were no significant differences in fruit shape between the UFU-QB-107G parent and the three experimental hybrids [F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G)]. These fruits tended to be cylindrical and significantly different from the angular UFU-QB-040D parent (Table 1). An understanding of the morphological characteristics of parents (male and female) and the consequent hybrids is useful in plant breeding. Hybridization can be more confidently confirmed by analyzing a

**Table 1.** Morphological characterization of leaves and fruit from okra genotypes. Monte Carmelo, UFU, 2014.

| Genotypes* | Leaf** | | | Fruit** | | |
|---|---|---|---|---|---|---|
| | Pubescence | Length | Width | Length | Diameter | Shape |
| UFU-QB-040D | 1.7 a | 22.1 a | 20.7 a | 124.2 a | 11.7 d | 2.8 b |
| UFU-QB-107G | 1.6 a | 17.0 b | 15.3 c | 105.4 b | 21.5 a | 4.7 a |
| F1 THM(UFU-QB-040D x UFU-QB-107G) | 2.9 a | 20.1 ab | 17.5 b | 120.0 a | 16.1 c | 4.3 a |
| F1 IHM(UFU-QB-107G x UFU-QB-040D) | 2.9 a | 21.7 a | 20.1 a | 121.0 a | 18.5 b | 4.4 a |
| F1 IHM(UFU-QB-040D x UFU-QB-107G | 3.0 a | 19.6 ab | 17.0 bc | 123.1 a | 18.1 b | 4.9 a |
| CV (%) | 23.4 | 8.8 | 4.8 | 1.8 | 3.8 | 12.2 |
| Overall averages | 2.4 | 20.1 | 18.1 | 118.7 | 17.2 | 4.2 |

*Means followed by the same letter within a column do not differ significantly (Tukey, p= 5%); **Description of evaluations: pubescence (1= sparse, 2= intermediate and, 3= dense pubescence); length and width (mm); Fruit length at harvest, diameter and shape scored from 1 to 5 (1= strongly angular, 2= somewhat angular, 3= angular, 4= cylindrical with few angles or ridges and 5= cylindrical without angles or ridges).

**Table 2.** Agronomic evaluation of heterosis in okra genotypes depending on hybridization type. Monte Carmelo, UFU, 2014.

| Genotypes[1] | Production/plant | | | Number of fruits/plant | | | NS |
|---|---|---|---|---|---|---|---|
| | PP (g) | Heterosis | Heterosis (%) | NFP | Heterosis | Heterosis (%) | |
| T1= F1 IHM (UFU-QB-107G x UFU-QB-040D) | 9985.0 a | 4845.2 | 94.2 | 741.2 a | 375.2 | 102.5 | 18.0 c |
| T2= F1 IHM (UFU-QB-040D x UFU-QB-107G) | 8278.7 ab | 3138.9 | 61.0 | 673.7 b | 307.7 | 84.0 | 27.7 ab |
| T3= F1 THM (UFU-QB-040D x UFU-QB-107G) | 10157.5 a | 5017.7 | 97.6 | 781.5 a | 415.5 | 113.5 | 22.7 bc |
| T4= UFU-QB-040D | 6657.3 c | - | - | 471.0 c | - | - | 30.5 a |
| T5= UFU-QB-107G | 3622.2 d | - | - | 261.0 d | - | - | 22.0 bc |
| CVs (%) | 18.2 | | | 18.0 | | | 11.4 |
| **Contrasts (y)** | **Contrasts of interest** | | | | | | |
| C1=[(T1+T2+T3)/3] - [(T4+T5)/2] | 4333.9** | | | 366.1** | | | |
| C2=[(T1+T2+T3)/3] - (T4) | 2816.4* | | | 261.1** | | | |
| C3=[(T1+T2+T3)/3] - (T5) | 5851.5** | | | 471.1** | | | |
| C4=(T1) - [(T4+T5)/2] | 4845.2** | | | 375.2** | | | |
| C5=(T2) - [(T4+T5)/2] | 3138.9* | | | 310.7** | | | |
| C6=(T3) - [(T4+T5)/2] | 5017.7** | | | 415.5** | | | |

[1]Means followed by lowercase letters within the same column do not differ significantly (Tukey, p=0.05); **= significant at p= 0,01, *p= significant at p=0,05 and ns= not significant (Scheffé test); PP= production per plant (g), NFP= number of fruits per plant, NS= number of branches per plant; Heterosis = superiority of hybrid relative to parents and, Heterosis (%)= percentage of heterosis.

combination of these morphological characteristics from parents and hybrids. Pedrosa *et al*. (1983) stated that the morphological characterization of okra genotypes is needed to take advantage of characteristics that are highly correlated with productivity. With this information, heterosis in okra can be exploited regardless of the method used to obtain hybrid sees.

Significant differences in production per plant (PP) and number of fruits per plant were observed in the different hybrids regardless of hybridization method (THM and IHM) (Table 2). Production per plant (9985.0, 8278.7 and 10,157.5 g/plant) did not differ significantly between the experimental hybrids F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G). Production per plant (6657.3 and 3622.2 g) from the parents (UFU-QB-040D and UFU-QB-107G) was lower than that of the F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G) hybrids. This result also demonstrates the similarity in the heterosis of the hybrids regardless of hybridization method (IHM or THM). Number of fruits per plant (NFP) was also similar between the newly proposed hybridization method for okra (IHM) and the traditional method (THM) (Table 2). Furthermore, prolificacy (number of fruits per plant) was not statistically different between F1 IHM (UFU-QB-107G x UFU-QB-040D) and F1 THM (UFU-QB-040D x UFU-QB-107G), which had been hybridized by IHM and THM respectively (Tukey, $p<0.05$). Singh & Singh (1978) observed genotype expression in number of fruits per plant and productivity per plant and found that there were genes with no additive effect and that most of these genes were dominant and epistatic. Some reports show that the number of fruits per plant is related to the action of other types of gene actions such as epistatic (additive x additive) that allow transgressive segregation (Kulkarni *et al*., 1978). In fact, the hybrids obtained in this study showed superiority to their parents regardless of the hybrid seed production method (Table 2).

The number of branches per plant was statistically different between the genotypes evaluated in our study. The parent UFU-QB-040D and the hybrid F1 IHM (UFU-QB-040D x UFU-QB-107G) had similar number of branches (30.5 and 27.7), but differed from the other genotypes. There were no significant differences between the hybrids based on hybridization method, whether by the traditional method [F1 THM (UFU-QB-040D x UFU-QB-107G) and F1 IHM (UFU-QB-040D x UFU-QB-107G)] or by the new method.

Similarities in PP and NFP resulting from two methods of producing hybrid okra seeds (IHM and THM) were confirmed by $h$ and $h\%$ estimates. Satisfactory increases were obtained regardless of seed hybridization method (IHM versus THM).

The $h$ and $h\%$ values for PP as a function of hybrid seed production method were 4845.2, 3138.9 and 5017.7g ($h$) and 94.2, 61.0 and 97.6% ($h\%$) for the hybrids [F1 THM(UFU-QB-040D x UFU-QB-107G), F1 IHM(UFU-QB-107G x UFU-QB-040D) and F1 IHM(UFU-QB-040D x UFU-QB-107G)], respectively (Table 2). Similarly, heterotic effects were also found for $h$ of NFP (375.2, 307.7 and 415.5 fruits, respectively) and $h\%$ (102.5, 84.0 and 113.52, respectively).

Orthogonal contrasts showed similarities between the okra seed hybridization methods (IHM and THM). Thus, the traditional and more labor intensive okra seed hybridization method (THM) can be reliably replaced by the less labor intensive method (IHM) without any loss in heterosis. An analysis of C1 (hybrids vs parents) showed the superiority of the hybrids to the parents in terms of production per plant and number of fruits per plant (4,333.9 g and 366.1 fruits, respectively) (Scheffe, $p<0.01$) (Table 2). C2 (hybrids *vs* UFU-QB-040D) and C3 (hybrids *vs* UFU-QB-107G) contrasts also reinforced the heterotic advantages of okra hybrids over parents. These advantages have already been shown by others (Mehta *et al*., 2007; Weerasekara *et al*., 2007; Jaiprakashnarayan *et al*., 2008; Jindal *et al*., 2009) and even under different tropical conditions in Brazil (Paiva & Costa, 1994).

Other contrasts were created, C4 [F1 (IHM UFU-QB-107G x UFU-QB-040D) *vs* parents], C5 [F1 IHM (UFU-QB-040D x UFU-QB-107G) *vs* parents] and C6 [F1 THM (UFU-QB-040D x UFU-QB-107G) *vs* parents], to confirm the individual superiority of each hybrid over their respective parents. Not only did the C4, C5 and C6 contrasts show the superiority of these three hybrids, regardless of seed hybridization method, they also demonstrated the heterosis of the hybrids and the use of new method of producing hybrid okra seeds. These results reflect the $h$ and $h\%$ results obtained between the hybrids (Table 1).

Heterosis resulting from the incomplete emasculation followed by natural insect pollination was similar to that from the traditional hybrid method, being a viable alternative for the production of hybrid okra seeds without losses in heterosis.

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