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Selection of squash "Menina Brasileira" carrying the allele "Bush" with high yield potential

Mariane G Ferreira [1]; Gabriella Q de Almeida [1]; Herika P Pessoa [1]; Françoise D Dariva [1]; Felipe de O Dias [1]; Carlos Nick[1]

[1]Universidade Federal de Viçosa (UFV), Viçosa-MG, Brazil; marianegonferreira@gmail.com; gabriellaqueirozalmeida@hotmail.com; herikapaulapessoa@gmail.com; fran_dariva@hotmail.com; felipedeoliveiradias@gmail.com; carlosnickg@gmail.com

ABSTRACT

This study was performed to introduce the allele Bush of commercial cultivars with a bush growth habit into *Cucurbita moschata* accessions and select the best crossings for production potential and standard fruit shape "Menina Brasileira" through general combining ability (GCA) and specific combining ability (SCA). To determine GCA and SCA, a partial diallel analysis was performed. The parental group (group I) consisted of two cultivars containing the dwarfism bush gene, and the second group (Group II), formed by five accessions of UFV Vegetable Germplasm Bank, with high production potential and fruit shape "Menina Brasileira". We evaluated: productivity (PROD), fruit shape (FORM), growth rate until the 50th day after transplanting (CRESC), and length of the main stem on the 50th day (COMP). Significant differences were noticed for CRESC and COMP only between GCA of parents belonging to group I, in which Zapallo stood out. For PROD and FORM, diallel analysis proved to be efficient in studying GCA effects. Cultivar Piramoita (group I) and accessions BGH-4360 and BGH-5253 (group II) showed positive values for GCA, suggesting that these parents possess a higher frequency of favorable alleles for these two traits. Significance in SCA effects was also verified, highlighting the crossing Piramoita x BGH-4360, for PROD, indicating that this combination is the most promising for breeding purposes.

Keywords: *Cucurbita moschata*, plant architecture, "bush type", genetic resources, vegetable breeding.

RESUMO

Seleção de populações de abóbora menina brasileira portadoras do alelo "Bush" com alto potencial produtivo

Objetivou-se introduzir o alelo Bush, de cultivares com hábito de crescimento tipo "moita", em acessos de *Cucurbita moschata* e selecionar os melhores cruzamentos quanto ao potencial produtivo e ao formato de fruto padrão "menina brasileira" por meio da capacidade geral (CGC) e da capacidade específica (CEC) de combinação. Para determinar a CGC e a CEC foi realizada análise dialélica parcial. O grupo parental (grupo I) foi formado por duas cultivares tipo "moita", enquanto o segundo grupo (grupo II) por cinco acessos do Banco de Germoplasma de Hortaliças da UFV, com alto potencial produtivo e formato de fruto "menina brasileira". Foram avaliadas a produtividade (PROD), o formato de fruto (FORM), a taxa de crescimento até os 50 dias (CRESC) e o comprimento da rama principal aos 50 dias (COMP). Para CRESC e COMP, houve diferenças significativas apenas entre a CGC dos genitores do grupo I, onde destacou-se a cultivar Zapallo. Para as características PROD e FORM a análise dialélica mostrou-se eficiente no estudo dos efeitos de CGC. A cultivar Piramoita (grupo I) e os acessos BGH-4360 e BGH-5253 (grupo II) apresentaram valores positivos para CGC, o que indica que esses genitores possuem maior frequência de alelos favoráveis para essas duas características. Também houve significância nos efeitos da CEC, com destaque para o cruzamento Piramoita x BGH-4360, para a característica PROD, sendo essa, a combinação mais promissora e mais indicada para seguir no programa de melhoramento de abóboras.

Palavras-chave: *Cucurbita moschata*, arquitetura de planta, tipo "moita", recursos genéticos, melhoramento de hortaliças.

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Cucurbits play an important role in human nutrition mainly in tropical and subtropical regions (Glala *et al*., 2011). In Brazil, this plant is grown mainly in the Northeast region where it is used for human and animal nutrition (Aruah *et al.,* 2012). Among cucurbits, squash is considered a very expansive crop due to its indeterminate growth habit and long internodes. One single plant is able to cover an useful area ranging from 12 to 25 m^2 (Puiatti & Silva, 2005; Resende *et al*., 2013). Corroborating this statement, Wu *et al.* (2007) highlight that the species *C. moschata* sends branches which spread and can reach 15 meters long.

Few studies on genetic control of growth habit can be found in literature (Vallejo & Mosquera, 1998), however it is known that gene "Bush" is responsible for compact growth habit in this genus (Robinson *et al*., 1976). The reduction of internode results from a decrease in cell size in this region, which is a result from drastic reduction of the content of gibberellins associated with the presence of the bush gene (Lopez-Juez *et al*., 1995), which culminates in plant size reduction (Wu *et al*., 2007). The dominant homozygous bush gene promotes the compact growth of plants of the genus Cucurbita, reducing the internode length from 15 cm to 2 cm, in average (Zhang *et al*., 2012).

Squash plants with reduced growth

habit show advantages, and, among them, the possibility of planting a larger population by area, greater earliness of plants (Maynard *et al*., 2002) and easier conducting of cultural practices and harvest. Moreover, an increase in productivity can be obtained, since in spite of the fact that the fruits are smaller, the quantity is greater (Wu *et al.*, 2007).

Studies on heterotic and combinatorial effects, which involve accessions of germplasm banks, have been carried out in relation to species breeding. This indicates accessions which can be integrated into improved populations and expand their genetic base (Machado & Miranda, 2003). Besides that, this information may allow to choose the best strategies in breeding program conduction (Azevedo *et al*., 2012; Ferreira *et al.*, 2016).

In this context, an alternative to genetic improvement is the use of a hybridization program, in order to produce new cultivars adapted to different purposes, such as small plants. In order to have a successful breeding program, knowing previously the behavior of population available in hybrid combinations is important. Thus, an analysis of the combining ability of the potential parents allows to identify those most apt to convey the desirable characteristics to the offspring (Souza *et al*., 2013). Diallel crossing systems are quite efficient to evaluate cultivars, since they can indicate the best hybrids, and also help choose the most promising parents to be used in hybridization program (Cruz *et al*., 2014).

Thus, the aim of this study was to introduce the allele "Bush", of commercial cultivars with bush growth habit, in accessions of *C. moschata* and select crossings in relation to its productive potential and standard fruit shape "menina brasileira" through general combining ability (GCA) and specific combining ability (SCA).

MATERIAL AND METHODS

Accessions and commercial cultivars were crossed in partial diallel scheme. Group I consisted of commercial cultivars Piramoita and Zapallo de Tronco, cultivars belonging to the species *C. moschata* and *C. maxima*, respectively; the second group was formed by accessions of *C. moschata*: BGH-1956, BGH-4360, BGH-5253, BGH-5621 and BGH-7663. The accessions of BGH-UFV were previously identified as plant genetic resources of high productive potential and standard fruit shape "menina brasileira". The cultivars carry the gene "Bush" in dominant homozygosity which gives them the bush growth habit. The ten hybrids F1 were obtained through manual crossings for the introgression of the "Bush" gene. The commercial cultivars were used as pollen donors, whereas the accessions were used as receptors, being the ovary shape that determines fruit type.

The experiment was carried out during 2014/2015 spring/summer harvest in Viçosa, Minas Gerais, (20°45'14''S; 42°52'53''W; 649 m altitude). According to Köppen's classification, the local climate is "Cwa", total average rainfall 1221.4 mm, with rainfall concentration in Summer.

Seeds from ten combinations obtained through the crossings were sown in expanded polystyrene, 72-cell trays, containing commercial substrate for vegetable seedling production. Seedlings were transplanted to field with two definitive leaves, approximately 20 days after sowing, in spacing 3.0x3.0 m. Hybrids and controls were placed in a randomized block design, with three replications and five plants per plot, considering useful the three central plants. Fertilization was performed according to recommendations of the 5th Approach (Ribeiro *et al*., 1999) for squash crop.

The authors analyzed the characteristics of productivity (kg ha^{-1}); fruit shape [using the grading scale: 1= globular, 2= flattened, 3= disk-shaped, 4= oblong, 5= elliptical, 6= cordiform, 7= pyriform, 8= belted, 9= elongated, 10= upper turbined, 11= crowned, 12= bottom turbined, 13= curved and, 14= crooked neck, obtained using a descriptor table for squash (MAPA, 2004)]; main stem-growth rate, 50 days after transplanting (cm day^{-1}), weekly calculated until 50 days after transplanting, and main stem growth, 50 days after transplanting (cm).

In order to evaluate hybrid combination performance, the authors compared these hybrid combinations with cultivars Sandy and Daiane, both showing the same fruit shape "menina brasileira" (Sakata Sudamerica). The comparison was done using analysis of variance and mean grouping test of Scott-Knott.

Then, the authors calculated average squares and estimates of general combining ability (GCA) and specific combining ability (SCA) in diallel analysis. The diallel analysis was performed using Method IV adapted for partial diallels, defined by Griffing (1956). So, observations were described through the statistical model:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + \varepsilon_{ij}$$

in which Y_{ij}= mean value of hybrid combination between i^{th} parent of group 1 and j^{th} parent of group 2; μ= overall mean; g_i= effect of general combining ability of i^{th} parent of group 1; g_j= effect of general combining ability of j^{th} parent of group 2; s_i= effect of specific combining ability between parents i and j, belonging to groups I and II, respectively; and ε_{ij}= mean experimental error.

To obtain contribution of additive (GCA) and dominance (SCA) effects GCA the authors calculated coefficients of determination (R^2) of each trait, through ratio between the sum of squares of GCA and SCA and the sum of squares of treatments, according to the methodology proposed by Ramalho *et al*. (2012). The genetic analyses were carried out using statistical software Genes, v 3.1 (Cruz, 2013).

RESULTS AND DISCUSSION

Significant effect between cultivars, for all traits, was noticed; this fact shows genetic variation between the cultivars for these traits. Significant differences for GCA were observed only between cultivars Piramoita and Zapallo Tronco in relation to growth rate up to 50 days and main stem size at

50 days, and no difference was noticed among accessions. Pandey *et al.* (2010) reported that information related to GCA effects of parents is essential, since they successfully help predict genetic potential of crossings.

Evaluating plant growth habit in all field crossings, the authors noticed that the plants grew in an indeterminate form, sending stems throughout their growth period, mainly after flowering. This phenomenon occurred due to the presence of bush gene in heterozygosity (*Bubu*) in F1 generation, promoting a reversal of dominance and therefore the plants send stems in an indeterminate form, as related by several authors who worked with species of the genus *Curcubita* (Robinson *et al.*, 1976).

The authors verified significant effects for GCA and SCA ($p<0.01$ and $p<0.05$ respectively) for productivity, showing that besides additive, the presence of non additive gene action was significant among loci related to this trait, since SCA does not show significance in absence of dominance (Vencovsky & Barriga, 1992).

For fruit shape, GCA for groups I and II were significant. SCA can be used to show the best crossings through genetic complementarity among the evaluated parents, since it depends on loci with dominance and/or epistasis effects (Lalla *et al.*, 2010). However, in the absence of significance for SCA, a promising combination observing only the estimates of GCA may be suggested (Ramalho *et al.*, 2012).

Significant effect of SCA for productivity also highlights different degrees of complementation among individuals belonging to two groups, where specific hybrid combinations show a difference in the expected phenotypic performance based only on GCA effects. The authors verified, through determination coefficient, a predominance of additive gene effects, since R^2 values for GCA are always higher than R^2 values for SCA, with R^2 values for GCA of 89.2; 92.8; 72.4; 68.7 and SCA of 10.8; 7.2; 27.6 and 31.3 for fruit shape, productivity, growth rate up to 50 days and main stem size at 50 days, respectively.

As a matter of fact, if we take ‘productivity’ as an example, whereas GCA values (Table 1) in group II ranged from –2531.26 to +8835.77, that means, an amplitude of 11367.03, SCA values (Table 2) ranged from -2505.75 to +2505.75, an amplitude of only 5011.35. The same happens to fruit shape, showing GCA of amplitude 2.0 whereas SCA of only 1.9. Significance of GCA shows that additive gene effects are involved in genetic control of the trait. So, the existence of additive gene effects for evaluated traits allows us to assume the possibility of obtaining new cultivars from segregating populations through crossings with tested parents as suggested by Carvalho *et al.* (1999).

Cultivar Piramoita belonging to group I and the accessions BGH-4360 and BGH-5253 of group II stood out for productivity and fruit shape, since they showed positive estimates of *gi* (Table 1). Accession BGH-4360 showed the highest estimate for productivity and BGH-5253 the highest estimate for fruit shape. The authors highlight that the accession BGH-5621 of group II showed positive estimate for fruit shape, despite presenting a negative estimate for productivity.

Values relatively high for *gi* showed that the accessions and cultivar Piramoita showed high frequency of favorable alleles for the evaluated traits (Vencovsky & Barriga, 1992). Thus, these accessions and this cultivar can be used in intercrosses with individuals belonging to different heterotic groups, aiming to form new populations with higher concentration of favorable alleles, allowing to provide new superior individuals when compared to the current ones (Cruz *et al.*, 2014).

Hybrid combinations associated with higher estimates of ***Sij*** (Table 2) in relation to productivity were those resulting from the crossings between BGH-4360 x Piramoita (***Sij***= 2505.75) and BGH-5253 x Zapallo Tronco (***Sij***= 2465.11). The two parents of combination BGH-4360 x Piramoita showed positive estimates of GCA, thus, the authors believe that this combination has a high degree of allelic complementation. Hybrid combinations BGH-7663 x Zapallo Tronco (***Sij***= 1482.15), BGH-5621 x Piramoita (***Sij***= 700.69) and BGH-1956 x Piramoita (***Sij***= 740.81) involved parents with negative estimates of GCA and, considering the combinations mentioned above, also showed positive estimates of ***Sij*** for productivity. However, the magnitude of SCA was relatively lower for the combinations mentioned above, suggesting the occurrence of an average degree of complementation between the mentioned accesses and cultivars (Table 2).

For fruit shape, the most favorable hybrid combination was BGH-1956 x Piramoita (***Sij***= 0.97), which showed the highest positive estimate of ***Sij***, even having a parent belonging to group II with negative estimate of GCA for this trait. This fact suggests that this crossing shows high degree of allelic complementation between parents. Similar result was observed for combination BGH-5253 x Zapallo Tronco (***Sij***= 0.81), in which parent of group I also presented negative estimate

Table 1. Estimates of general combining ability for fruit shape, productivity, growth rate of the main stem until the 50th day after transplanting and length of the main stem at the end of the 50th day. Viçosa, UFV, 2015.

Parent	Fruit shape	Productivity	Growth rate	Stem length
Zapallo Tronco	-1.58	-2956.4	-0.63	-43.92
Piramoita	1.58	2956.4	0.63	43.92
DP (gi)	0.19	545.49	0.16	11.82
BGH-7663	-0.30	-2531.26	0.08	-2.62
BGH-5621	0.20	-8017.18	-0.51	-48.55
BGH-1956	-0.97	-1027.92	0.09	5.80
BGH-5253	1.03	2740.59	0.66	51.60
BGH-4360	0.32	8835.77	-0.32	-6.23
DP (gj)	0.38	790.99	0.32	23.64

Table 2. Estimates of specific combining ability for fruit shape, productivity, growth rate of the main stem until the 50th day after transplanting and length of the main stem at the end of the 50th day. Viçosa, UFV, 2015.

Treatments	Fruit shape	Productivity	Growth rate	Stem length
BGH-7663 X Zapallo Tronco	-0.30	1482.15	-0.24	-19.36
BGH-7663 X Piramoita	0.30	-1482.15	0.24	19.36
BGH-5621 X Zapallo Tronco	0.20	-700.69	0.20	4.58
BGH-5621 X Piramoita	-0.20	700.69	-0.20	-4.58
BGH-1956 X Zapallo Tronco	-0.97	-740.81	-0.29	-26.28
BGH-1956 X Piramoita	0.97	740.81	0.29	26.28
BGH-5253 X Zapallo Tronco	0.81	2465.11	-0.46	-31.53
BGH-5253 X Piramoita	-0.81	-2485.11	0.46	31.53
BGH-4360 X Zapallo Tronco	-0.26	-2505.75	0.79	72.58
BGH-4360 X Piramoita	0.26	2505.75	-0.79	-72.58
DP(Sij)	0.38	790.99	0.32	23.65

of GCA for fruit shape (Tables 1 and 2).

The authors used the average test for evaluated traits in order to compare the agronomic performance of hybrid combinations with the performance of commercial cultivars. This test is necessary due to the fact that when diallel crossings are performed, positive values of GCA and SCA can only show that the parent or crossing is superior to the average of other tested parents or crossings in that experiment. These values do not show if a parent or hybrid combination has superior or similar agronomic performance when compared to commercial cultivars, though.

Hybrid combinations and two commercial cultivars showed significant differences among each other for productivity and main stem size at 50 days. The authors verified that the crossings with accession BGH-4360 showed higher productivities, overcoming the productivity of commercial cultivars Sandy and Dayane. This indicates a great productive potential of this accession, confirmed by the positive values of GCA.

Hybrids from crossings BGH-5253 x Piramoita, BGH-7663 x Piramoita, BGH-1956 x Piramoita and BGH-5253 x Zapallo Tronco also showed significant values for productivity, overcoming two controls. In general, the crossings with cultivar Piramoita showed higher productivity average in relation to crossings with Zapallo Tronco, when compared with the same accessions, confirming the presence of favorable alleles related to positive value of general combining ability.

For main stem size at 50 days, the authors noticed emission of stems after flowering in all hybrid combinations. Although significant differences between treatments were verified, the results for growth rate of the main stem are not conclusive in this study. More combinations for generation F2, for a possible dominant homozygous bush gene, are necessary.

The authors concluded that dominant bush allele was introduced into plants of generation F1. Partial diallel analysis showed to be efficient in the study on effects both for parents GCA and SCA of hybrid combinations. The combination BGH-4360 x Piramoita was promising among the evaluated combinations, presenting positive estimates of SCA for productivity, as well as averages higher than the ones of the two controls. Thus, this combination is the most indicated to form a basic population for squash breeding program. The accessions BGH-4360 and BGH-5253 showed positive values of GCA for fruit shape and productivity being recommended to continue being parents in a breeding program.

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