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## Diallel analysis of “dedo-de-moça” pepper seeds in relation to physiological potential

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### ABSTRACT

“Dedo-de-moça” (*Capsicum baccatum* var. *pendulum*) is one of the main chili peppers grown worldwide. However, studies on genetics and breeding of this species are rare, when compared to other *Capsicum* species, mainly in relation to physiological seed quality traits. In this sense, this study aimed to evaluate the combining ability of “dedo-de-moça” chili pepper in relation to physiological seed quality traits, determine the gene effects involved in the expression of these traits, and identify promising hybrid combinations. Crosses among five parents (Hortivale, Horticeres, Brs Mari, UEL 110 and UEL 111) in a full diallel mating design, resulted in 20 F<sub>1</sub> hybrids. Griffing’s diallel model was used to estimate general and specific combining ability (GCA and SCA, respectively) as well as the reciprocal effect (RE). Wide genetic variability was observed for all evaluated traits. Significant effects of GCA, SCA and RE were also verified for all traits, showing that additive, non-additive and cytoplasmic effects are involved in the genetic control of physiological seed quality. Non-additive effects were predominant for all traits, being UEL 111 (♂) × Hortivale (♀) and Hortivale (♂) × UEL 110 (♀) considered promising hybrids for obtaining more vigorous seeds with higher germination and greater longevity.

**Keywords:** *Capsicum baccatum*, complete diallel, general and specific combining ability, reciprocal effect.

### RESUMO

#### Análise dialélica de sementes de pimenta “dedo-de-moça” em relação ao potencial fisiológico

A pimenta “dedo-de-moça” (*Capsicum baccatum* var. *pendulum*) é uma das principais pimentas cultivadas mundialmente. Todavia, são escassos estudos genéticos e de melhoramento desta quando comparados a outras espécies de *Capsicum*, principalmente, sobre a qualidade fisiológica de sementes. Nesse sentido, objetivou-se avaliar a capacidade combinatória de pimentas “dedo-de-moça” em relação à qualidade fisiológica de sementes, determinar os efeitos gênicos envolvidos na expressão dessas características, bem como identificar combinações híbridas promissoras. Os cruzamentos entre cinco genitores (Hortivale, Horticeres, Brs Mari, UEL 110 e UEL 111) em esquema de dialelo completo resultaram na formação de 20 híbridos F<sub>1</sub>. O modelo de dialelo de Griffing foi utilizado para determinar a capacidade geral e específica de combinação (CGC e CEC, respectivamente) e o efeito recíproco (ER). Foi observada ampla variabilidade genética para todas as características analisadas. Efeitos significativos para CGC, CEC e ER foram verificados para todas as características, indicando que os efeitos aditivos, não aditivos e citoplasmáticos estão envolvidos no controle genético da qualidade fisiológica de sementes. Os efeitos gênicos não aditivos foram predominantes em relação aos aditivos. As combinações híbridas UEL 111 (♂) × Hortivale (♀) e Hortivale (♂) × UEL 110 (♀) foram consideradas promissoras para obtenção de sementes mais vigorosas, com maior germinação e longevidade.

**Palavras-chave:** *Capsicum baccatum*, dialelo completo, capacidade geral e específica de combinação, efeito recíproco.

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“Dedo-de-moça” pepper (*Capsicum baccatum* var. *pendulum*) belongs to Solanaceae family. “Dedo-de-moça” is one of the main species grown in Brazil, mostly by family farmers (Rufino & Penteado, 2016). Despite its economic importance, genetic studies and breeding programs of this species are rare when compared to other *Capsicum* species, mainly studies on

physiological seed quality.

This is a perennial, shrubby plant, exploited as an annual plant, showing a wide genetic variability. This species is often found in South America. The fruits are used as natural flavoring, food dyes, cosmetics, jelly, pickled, sauces and dehydrated, in pharmacy industry, medicine and ornamental use, besides its potential for *in natura* consumption.

Pepper is consumed mainly in South and Southeast regions of Brazil (Mo *et al.*, 2015; Gomes *et al.*, 2017; Santana *et al.*, 2017).

The vegetable seed market has been growing over the past few years. However, a low supply of quality seeds can be a limiting factor for large-scale production of “dedo-de-moça” pepper (Carvalho *et al.*, 2009),

and this supply is related to several factors, such as deficient production techniques, physiological maturation stage, low yield, seed extraction difficulties, harvesting and lack of superior genotypes in the production of seeds with high physiological potential (Nascimento *et al.*, 2006).

Using seeds with higher physiological vigor is essential to reduce exposure time of seeds and seedlings in order to avoid weather, biological adversities, and deteriorative processes or contamination by pathogens. Also, seeds with higher physiological vigor may obtain higher yields and fruits with higher commercial and nutritional quality (Marcos-Filho, 2015).

Brazil is an important center of genetic diversity of the genus *Capsicum* having a wide range of peppers and green peppers (Costa *et al.*, 2015). This diversity has been exploited in vegetable breeding programs. This variability can be used to identify genotypes with high physiological potential of seeds in order to develop a new cultivar, or in future hybridizations. However, little is known about the genetic effects on vigor determination and seed germination of *Capsicum baccatum*.

Diallel crosses are efficient as methodology to help out in generating relevant information for breeding, such as for selecting parents for hybridization, identifying more efficient selection methods and knowledge about genetic bases which control the traits (Nascimento *et al.*, 2010).

Griffing (1956) proposed one of the main methodologies for diallel analysis, which estimates the effects of general and specific combining ability. Thus, this study aimed to evaluate combining ability of “dedo-de-moça” peppers in relation to traits of seed physiological quality, determine gene effects involved in the expression of these traits, as well as identify promising hybrid combinations.

## MATERIAL AND METHODS

Five parents of *Capsicum baccatum* var. *pendulum* were used, being three cultivars (Horticeres, Hortivale and

BRS Mari) and two accessions of the *Capsicum* spp. Germplasm Bank of Universidade Estadual de Londrina (UEL) (UEL 110 and UEL 111).

Hybridization was carried out in a greenhouse in a complete diallel scheme with inclusion of reciprocals, obtaining a total of 20 hybrid combinations. Eight plants per parent were used, and crossings were done between January and April, 2016. The flower buds of female parents were emasculated prior to anthesis and after crossed pollination the flowers were protected in paper bags to avoid contamination with undesirable pollen (Costa *et al.*, 2009). Fruits obtained from hybridization were identified, harvested at 75 days after anthesis and the seeds were extracted, washed and dried.

In order to obtain seedlings, the genotypes were sown in expanded polystyrene trays. After the first two pairs of definitive leaf appeared, the seedlings were transplanted into the experimental field of UEL (23°19'41”S, 51°12'18”W, altitude 550 m). The soil of the experimental area is classified as Red Eutroferric Latossol and climate, according to Köppen, is Cfa. Randomized complete block designs, with three replicates and six plants per plot were used. Plant spacing was 1.0×0.8 m between rows and plants. Diallel analysis was performed using Griffing's method 1 (1956), in which parents and set of the F<sub>1</sub> hybrids and reciprocals were included.

Fertilization was based on soil analysis and other cultural practices (hoeing, tutoring and sprinkler irrigation) were performed according to usual management recommendations for the crop (Figueira, 2013). Fruits were harvested between 70 and 80 days after anthesis (DAA).

Approximately 60 fruits per plot were randomly obtained. Seeds were submitted to the following evaluations:

**Dry mass of 50 seeds (MSS)** - four replicates of 50 seeds, freshly extracted from the fruit, were put in a greenhouse at 105±3°C, for 24 hours, and the results were expressed in mg seed<sup>-1</sup>.

**Germination (GER)** - seeds were sown in transparent polyethylene boxes

(11×11×3 cm), on two germination paper sheets, moistened with a solution of KNO<sub>3</sub> at 0.2% at a ratio of 2.5 times the dry paper weight. The boxes were kept in a germinator under alternating temperature of 20-30°C and eight hours of light. Evaluations were carried out on the eighth and fourteenth day after sowing, and the results were expressed in percentage of normal seedlings (Brasil, 2009).

**First germination count (PC)** - percentage of normal seedlings on the eighth day of germination test.

**Electrical conductivity (CE)** - four replicates of 50 seeds, with known masses, were used. The seeds were immersed in 25 mL distilled water and kept in a BOD incubator at 25°C, for 48 hours (Vidigal *et al.*, 2008). After this period, the electrical conductivity of each solution was determined with the aid of a conductivity meter, and the results were expressed in µS cm<sup>-1</sup> g<sup>-1</sup> of seeds.

**Germination speed index (IVG)** - seeds which emerged until the fourteenth day after sowing were counted daily (Maguire, 1962).

**Accelerated aging test (EA)** - 1.0 g of seeds was uniformly distributed on a screen tray attached to gerbox-type plastic boxes, with 40 mL distilled water at the bottom. The seeds were kept in an incubator at 42°C, for 96 hours (Bhering *et al.*, 2006). Afterwards, 4 replicates of 50 seeds of each treatment of the five varieties were put to germinate, following the method described for germination test. A single evaluation was performed on the fourteenth day and the percentage of normal seedlings was computed.

Data were submitted to variance analysis (p<0.05) and, then, estimates of general and specific combining ability and reciprocal effect were obtained using Griffing diallel analysis model (1956) to estimate the general (GCA) and specific (SCA) combining ability and reciprocal effect. The genetic-statistical analyses were carried out using Genes software (Cruz, 2016).

## RESULTS AND DISCUSSION

Through variance analyses, the

authors verified a significant effect ( $p < 0.05$ ) for genotypes, for all evaluated traits (Table 1). The accurate selection showed values superior to 0.90 for all traits, resulting in greater credibility in the selection of superior genotypes and higher-experimental quality (Table 1).

Unfolding the sum of genotype squares to sum of squares of the general (GCA) and specific (SCA) combining ability, as well as reciprocal effect, was significant for all traits, indicating that additive, non-additive and cytoplasmatic genetic effects participated in the gene control of seed physiological potential of “dedo-de-moça” pepper.

This is an important fact, since this is an autogamous species, in which superior values for these traits can be fixed over successive generations of self-fertilization, obtaining inbred lines superior than those already existing (Rodrigues *et al.*, 2012). In addition, the GCA ( $\phi_g$ )/SCA ( $\phi_s$ ) ratio showed values below one, which indicates predominance of non-additive effects over additive effects (Table 1) (Nascimento *et al.*, 2010).

Barroso *et al.* (2017) evaluated seed germination percentage and growth and development of morphological traits of  $F_{1s}$  hybrid seedlings by crossing different

pepper parents of the genus *Capsicum*, and reported that these traits were controlled by additive and non-additive effects, and, as in this study, with a predominance of dominance effects related to SCA. Aiswarya *et al.* (2020) verified the GCA ( $\phi_g$ )/SCA ( $\phi_s$ ) ratio less than one for morpho-agronomic traits, such as plant height, number of primary branches per plant, days to flowering, fruit length and circumference, yield of fruits and seeds, and for percentages of capsaicin, oleoresin and ascorbic acid in a study of complete diallel with different *Capsicum annuum* genotypes.

The highest estimates of GCA effects for the traits first germination count (PC), germination speed index (IVG) seed dry mass (MSS) were obtained using the parent UEL 110 (11.66%; 0.70; and 0.008 mg, respectively), followed by the parent UEL 111 (8.14%; 0.58; and 0.008 mg, respectively) (Table 2). Parent UEL 111 also showed higher estimates of GCA for germination percentage (GER) and accelerated aging (EA), 9.80% and 13.19%, respectively.

According to Marcos Filho (2015), seed physiological quality is determined as the ability of seeds to perform their vital functions, such as germination, vigor and longevity, which directly

affect the crop in field conditions. Thus, parents UEL 110 and UEL 111 show high potential for breeding programs aiming to improve attributes related to seed physiological potential of “dedo-de-moça” pepper.

Moreover, using high-quality pepper seeds at the beginning of planting results in strong, vigorous, well-developed seedlings, being able to establish under different edaphoclimatic conditions, with greater emergence speed and plant growth. As a consequence, the plant in the field will show less problems with weed incidence, need for herbicides, reseeding and others, ensuring greater performance and productivity (Nakada *et al.*, 2011).

However, preponderance of non-additive effects in this study indicates the possibility of hybrid vigor exploration, as mentioned by Barroso *et al.* (2017) and Aiswarya *et al.* (2020), since  $F_1$  hybrids have been increasingly used commercially (Jeeatid *et al.*, 2018).

Using estimates of SCA effects, we verified negative effects for hybrids UEL 111  $\times$  UEL 110 and Horticerres  $\times$  Hortivale for EA, PC, GER, IVG and MSS, suggesting that these hybridizations are unfavorable for these traits (Table 2). On the other

**Table 1.** Estimates of the mean squares of genotypes, general combining ability (GCA), specific combining ability (SCA), reciprocal effect and the quadratic components of CGA and SCA of electrical conductivity (CE), accelerated aging (EA), first count (PC), germination (GER), germination speed index (IVG) and seed dry mass (MSS) evaluated in *Capsicum baccatum* var. *pendulum*. Londrina, UEL, 2019.

FV	GL	Mean square <sup>1/</sup>					
		CE <sup>2/</sup>	EA	PC	GER	IVG	MSS
Genotypes	(24)	568.77**	303.63**	207.14**	188.60**	10.24**	51.39**
GCA	4	519.89**	474.55**	522.44**	432.30**	23.74**	48.47**
SCA	10	257.04**	297.28**	132.93**	131.48**	7.37**	30.69**
Reciprocal	10	900.04**	241.60**	155.22**	148.24**	7.70**	73.25**
Residual	75	27.07	19.12	15.08	14.45	0.84	51.39
Average		385.18	52.66	57.44	72.90	44.4	0.0132
Accuracy		0.95	0.96	0.94	0.92	0.93	0.90
<b>Quadratic components</b>							
$\hat{\phi}_{ge}$		123.05	113.86	126.84	104.46	0.572	0.001
$\hat{\phi}_{se}$		574.91	695.35	294.63	292.47	1.632	0.005
$\hat{\phi}_{ge}/\hat{\phi}_{se}$		0.21	0.16	0.43	0.36	0.35	0.200

<sup>1/</sup>\*\* = significant at 1% probability by F test. <sup>2/</sup>CE=  $\mu\text{S cm}^{-1}\text{g}^{-1}$  of seeds; EA= %; PC= %; GER= %; MSS= mg.

hand, hybrids UEL 111 × Hortivale and Hortivale × UEL 110, showing greater effects of SCA for PC, GER and IVG, respectively, provided seed hybrids with greater vigor and germination, considering these traits favorable and essential to produce high physiological potential seeds.

According to Hallauer *et al.* (2010) SCA effects manifest in relation to dominance effects and differences in

allele frequencies of parents for the loci involved in the control of a given character.

Hybrids Hortivale × BRS Mari, UEL 111 × Hortivale, BRS Mari × UEL 110, Hortivale × BRS Mari, UEL 111 × BRS Mari and UEL 111 × Hortivale, respectively, showed higher values of SCA for EA, considering that this trait is related to storage capacity, resulting in a lower deterioration speed (Table 2)

(Marcos-Filho, 2015).

The effect of reciprocal crosses was significant for all evaluated traits (Table 1), which demonstrated the importance of cyto-nuclear interactions in these traits. The genomes of cellular organelles containing DNA (mitochondria and chloroplasts) play an important role in genotypic variation; this genome has been underestimated, though (Roux *et al.*, 2016).

**Table 2.** Estimates of general combining ability ( $\bar{g}_{ij}$ ), specific combining ability ( $\bar{s}_{ij}$ ) and reciprocal effects ( $r_{ij}$ ) for electrical conductivity (CE), accelerated aging (EA), first count (PC), germination (GER), germination speed index (IVG) and seed dry mass (MSS) evaluated in *Capsicum baccatum* var. *pendulum*. Londrina, UEL, 2019.

Parent	General combining ability					
	CE <sup>2/</sup>	EA	PC	GER	IVG	MSS
UEL 111	-6.71	13.19	8.14	9.80	0.58	0.008
Hortivale	20.64	7.22	4.36	-2.40	0.24	-0.010
Hortivale	-35.05	-15.09	-14.27	-16.30	-1.17	-0.009
BRS Mari	-29.91	-0.81	-9.89	0.90	-0.36	0.003
UEL 110	51.03	-4.51	11.66	8.00	0.70	0.008
DP ( $\bar{g}_i - \bar{g}_j$ )	11.63	3.09	2.75	2.68	0.21	0.001
Hybrids	Specific combining ability					
UEL 111 × Hortivale	25.59	12.19	0.32	5.45	-0.29	0.082
UEL 111 × Hortivale	44.14	0.61	17.19	19.85	1.713	0.076
UEL 111 × BRS Mari	-46.68	2.96	5.19	-3.10	-0.32	-0.055
UEL 111 × UEL 110	24.08	-20.72	-7.49	-12.70	-0.69	-0.030
Hortivale × Hortivale	-124.64	-6.54	-16.54	-19.70	-1.14	-0.379
Hortivale × BRS Mari	46.92	5.69	21.09	8.60	1.59	-0.073
Hortivale × UEL 110	74.43	-13.24	0.79	3.25	0.08	0.139
Hortivale × BRS Mari	48.87	30.61	-5.66	-5.00	-0.68	0.154
Hortivale × UEL 110	-11.20	-4.19	10.42	19.15	0.89	0.036
BRS Mari × UEL 110	-9.46	9.79	2.04	-2.80	-0.27	-0.049
DP ( $\bar{s}_{ij} - \bar{s}_{kl}$ )	20.15	5.36	4.76	4.65	0.36	0.031
Hybrids <sup>1/</sup> ♂ × ♀	Reciprocal effect					
UEL 111 × Hortivale	-48.50*	-6.25 <sup>ns</sup>	0.25 <sup>ns</sup>	-0.75 <sup>ns</sup>	-0.26 <sup>ns</sup>	0.088*
UEL 111 × Hortivale	25.30 <sup>ns</sup>	18.12*	-5.00 <sup>ns</sup>	-2.75 <sup>ns</sup>	-0.29 <sup>ns</sup>	-0.079*
UEL 111 × BRS Mari	-49.11*	14.00*	-12.38*	-7.00 <sup>ns</sup>	-0.92*	0.115*
UEL 111 × UEL 110	-16.78 <sup>ns</sup>	-19.62*	-6.25 <sup>ns</sup>	-9.50*	-0.60 <sup>ns</sup>	0.067*
Hortivale × Hortivale	263.75*	38.25*	31.00 <sup>ns</sup>	34.50*	2.39 <sup>ns</sup>	0.749*
Hortivale × BRS Mari	-51.56*	0.25 <sup>ns</sup>	-7.50 <sup>ns</sup>	-6.50 <sup>ns</sup>	-0.69*	-0.035 <sup>ns</sup>
Hortivale × UEL 110	-93.17*	-14.37*	1.75 <sup>ns</sup>	1.75 <sup>ns</sup>	0.43 <sup>ns</sup>	-0.025 <sup>ns</sup>
Hortivale × BRS Mari	72.20*	14.87*	-17.62*	-16.00*	-0.62 <sup>ns</sup>	0.052 <sup>ns</sup>
Hortivale × UEL 110	-31.02 <sup>ns</sup>	7.875 <sup>ns</sup>	13.75*	12.25*	0.79*	0.005 <sup>ns</sup>
BRS Mari × UEL 110	140.50*	10.87*	14.25*	8.00 <sup>ns</sup>	0.96*	-0.079*
DP ( $\bar{r}_{ii} - \bar{r}_{kl}$ )	26.01	6.91	6.14	-0.75	-0.26	0.040

<sup>1</sup>ns and \* = not significant and significant at 5% probability by t test, respectively. <sup>2</sup>CE=  $\mu\text{S cm}^{-1}\text{g}^{-1}$  of seeds; EA= %; PC= %; GER= %; MSS= mg.

Barroso *et al.* (2017) reported the presence of reciprocal effect in diallel with peppers of the genus *Capsicum*. According to these authors, the underlying genetic basis of the reciprocal differences for the *in vitro* response of germination and early development of pepper seedlings can be related to cytoplasmic factors, such as mtDNA, physiological characteristics of maternal plants or segregation of nuclear factors from the maternal parent.

Considering the two more promising hybrid combinations (UEL 111 × Hortivale and Hortivale × UEL 110), based on SCA, hybrid UEL 111 × Hortivale presented significant reciprocal effect for EA and MSS, showing positive and advantageous effects for EA, when the genotype Hortivale is fixed as a female parent. The hybrid Hortivale × UEL 110 showed gains in vigor (positive effects in PC) and in germination, using its reciprocal with genotype UEL 110 as female parent (Table 2).

Despite being significant, GCA, SCA and reciprocal effects on electrical conductivity (EC) (Tables 1 and 2) are little related to other important traits, such as accelerated aging, first germination count, germination speed and percentage of normal seedlings. Considering the above, this analysis showed to be little informative for selecting parents to obtain seeds with high physiological potential.

We concluded that the physiological potential of *Capsicum baccatum* var. *pendulum* seeds is governed by additive, non-additive and cytoplasmic genetic effects. The preponderance of non-additive effects indicates that the exploitation of hybrid vigor can be considered a relevant strategy to obtain superior genotypes. The hybrids UEL 111 (♂) × Hortivale (♀) and Hortivale (♂) × UEL 110 (♀) were considered promising for obtaining more vigorous seeds, with greater germination and longevity.

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