



## Cross compatibility of domesticated hot pepper and cultivated sweet pepper

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**ABSTRACT** – This study evaluated the compatibility of crosses between *C. chinense* and *C. annuum* and the seed vigor and germination of interspecific hybrids. Twenty-one accessions of *C. chinense*, used as male parents, were crossed with the cultivar *Cascadura Ikeda* (*C. annuum*) as female parent in a completely randomized design (CRD), with three replications. Fruit setting was evaluated in 30 flowers per plot. Seed germination and hybrid vigor were analyzed using CRD with four replications and plots of 16 seeds. All interspecific crosses produced fruit, at fruit set rates between 8.9% and 40.0%. In a first count, germination ranged from 0.0% to 45.3% and in the second from 0.0% to 87.5%. The conclusion was drawn that it is possible to obtain fruit and viable seeds in interspecific crosses of *C. chinense* with *C. annuum*.

**Key words:** *Capsicum chinense*, *Capsicum annuum*, interspecific hybrids, *Capsicum* breeding.

### INTRODUCTION

Peppers and pepper fruits (*Capsicum* spp.) represent an important part of the fresh vegetable market in Brazil, and are also significant worldwide in the segment of condiments, spices and salt preserves. The genus *Capsicum* has 5 domesticated, 10 semi-domesticated and 20 wild taxa (Andrews 1984, McLeod et al. 1982, Pickersgill 1971). Brazil is a major center of diversity of the genus, where representatives at all cited levels are found. Despite being the center of origin and diversity of *Capsicum* spp., little is known about the native species, particularly *Capsicum chinense* Jacq., with great variability in the Amazon.

Hot and sweet pepper are grown by small, medium and large producers or integrated to agribusiness with

considerable socio-economic importance. Besides, the crop is a source of direct and indirect jobs. Vast plantation areas (private or in partnership) are occupied by the large agribusiness of the pepper branch where a significant number of people are employed, mainly during the harvest. The market is quite diversified, ranging from the consumption of pepper in natural form, to home-made salt preserves and the export of processed foods (Reifschneider 2000).

In the State of Amazonas, consumption and production of pepper have increased in recent years. The production process is somewhat difficult for producers, particularly with regard to the choice of the variety for planting. The available pepper varieties are not adapted to the Amazon climate, driving up

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production costs. Much of the demand is met with imports from other regions, mainly from Brasilia (Moreira et al. 2006).

Breeding of *Capsicum* spp. species started since the domestication by natives in the Americas and the group was diversified by the introduction of landraces and naturally selected genotypes in the communities. Degradation by anthropic pressure has been intense on the genus *Capsicum* over the last decades.

It is known that the domestication process increases the reproductive genetic barriers and restricts the free gene flow between species. However, a gene flow can be maintained between the improved *Capsicum* spp. species and their wild, domesticated and semi-domesticated relatives, which are carriers of genes of agronomic interest (resistance to pests and diseases etc.) (Nass et al. 2001). The Faculdade de Ciências Agrárias (FCA) of the Universidade Federal do Amazonas (UFAM) has a collection of *Capsicum* spp. accessions from the Upper Rio Negro (AM), which are being characterized with a view to the use in breeding programs and conservation of the genus (Fonseca et al. 2008).

This study aimed to investigate the compatibility of interspecific crosses between accessions of domesticated hot peppers (*C. chinense*) with cultivated sweet pepper (*C. annuum* L.).

## MATERIAL AND METHODS

To study the compatibility of domesticated hot peppers (*C. chinense*) with cultivated sweet pepper (*C. annuum*) interspecific crosses were made and subsequently the hybrid seed germination evaluated.

Interspecific crosses between *C. chinense* x *C. annuum*

The experiments were conducted in the horticulture sector of the Faculdade de Ciências Agrárias na Universidade Federal do Amazonas (FCA / UFAM) (lat 03° 06' 121''S, long 59° 58' 547''W, altitude 42 m asl), in an tunnel greenhouse (25 m long x 8 m wide).

From the hot pepper collection of FCA/UFAM, 21 *C. chinense* accessions and commercial sweet pepper cultivars Casca Dura Ikeda (*C. annuum*) were used. The fruit morphotypes of the accession were identified and characterized as proposed by Fonseca (2006) (Table 1). The seedlings were grown in polystyrene trays with 128 cells filled with substrate Plantimax HT ® and transplanted 20 days after seedling emergence to 200 mL polyethylene cups. When seedlings reached an approximate length of 10 cm they were transplanted to 5 kg bags. The soil used as substrate was previously analyzed and fertilized (Raij 1997) and during the experiment cultural treatments were performed as required by the crop.

**Table 1.** Characteristic of morphotypes of pepper plants of 20 accessions of *Capsicum chinense* of the *Capsicum* spp. collection of the Faculdade de Ciências Agrárias da Universidade Federal do Amazonas

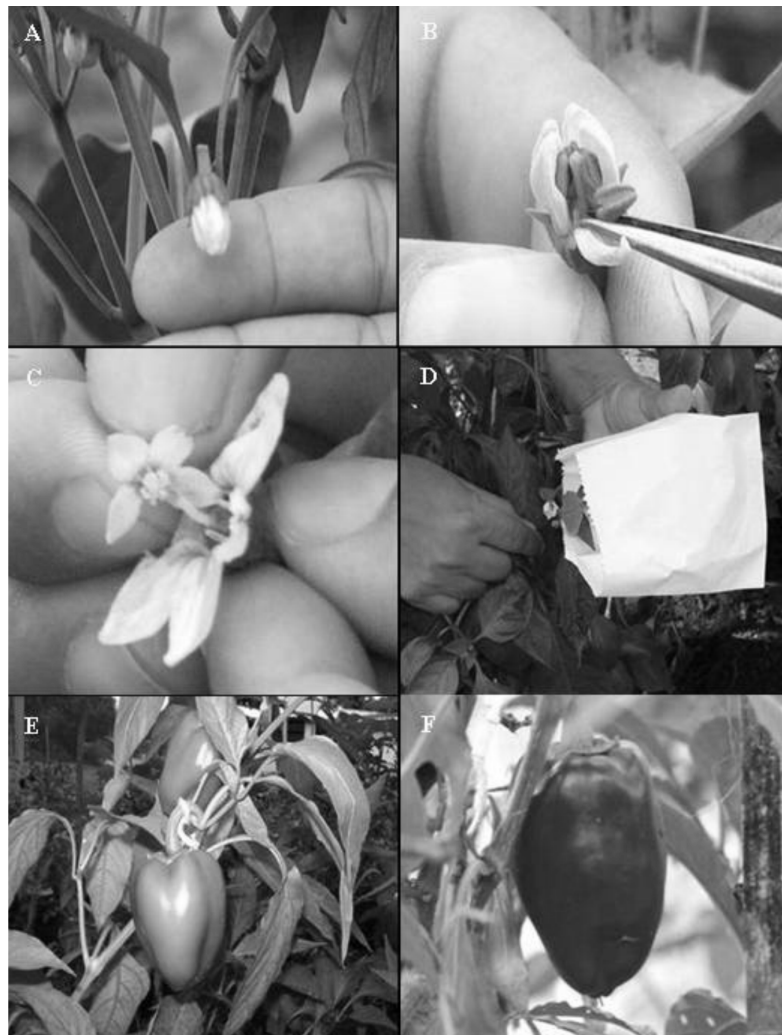
Morphotype	Fruit traits	Accessions
Olho-de-Peixe	Rounded or flat fruits with a diameter of about 1 to 2.5 cm, yellow or red colored, very tasty and highly pungent.	RIH34
Murupi	Traditionally cultivated in the northern region of Brazil, popular due to the characteristic taste and pungency. Longish fruit with a rough surface and a length between 2.0 and 9.0 cm. When ripe, the color is yellowish, yellow orange, yellow - lemon, orange, red and salmon. The flowers of this group with white anthers produce yellow fruits. Extremely pungent.	RIH05,RIH27,RIH22, RIH08 and RIH15
Dedo-de-Moça	Longish fruit of red color or deep red, when immature can have green, purple, deep purple color, about 4.0 to 8.0 cm long and 1.0 to 1.5 cm wide; pungent.	RIH10 and RIH28
Pimenta de Cheiro	Fruits with strong and characteristic taste; great variability in shape, size, color and pungency and can vary from sweet (with no pungency), to slightly or very pungent. The color can vary from yellow, yellow - orange, salmon, red and dark red when ripe. The size varies from 2.0 to 4.0 cm width by 4.0 to 7.0 cm of length.	RIH01, RIH03, RIH24, RIH31, RIH32, RIH 06, RIH25, RIH11, RIH16, RIH12 and RIH35
Pimenta de Passarinho	Small fruits, upright or hanging in triangular and longish shape. When ripe they are red and little persistent on the pedicel, therefore, readily dispersed by birds. The width can vary from 0.5 to 1.0 cm and the length from 1.5 to 3.0 cm; pungent.	RIH23
Curabiá	Longish or triangular-shaped fruits. Yellow – orange or red when mature; length of 2.0 to 4.0 cm and width of 1.0 to 2.0 cm; pungent.	RIH36

Flowers were pollinated in the rainy season, between February and September 2006, at a maximum of 33 °C, minimum of 22 °C and mean relative humidity of 88% inside the greenhouse.

For the crosses 21 accessions of hot pepper (*C. chinense*) were used as male parents and the commercial sweet pepper (*C. annuum*) cultivar Cascadura Ikeda as female parent, in a completely randomized design with 21 treatments and three replications. Each experimental plot consisted of three *C. annuum* plants, where treatments were applied to 30 randomly collected flowers, with a total of 90 pollinated flowers per treatment. Ten plants of each *C. chinense* accession were grown for pollen collection. During the experiment,

the efficiency of the emasculatation process was evaluated in a control where 90 *C. annuum* buds were emasculated and then covered.

Controlled pollination was carried out in emasculated flower buds prior to anther dehiscence, as recommended by George (1999). Buds were emasculated on the day before pollination with tweezers, in the late afternoon, and protected with paper bags to prevent undesirable pollen contamination. On the morning of the following day, after 10:00 pm, when pepper flowers release pollen, the newly opened anthers were rubbed directly on the stigma of the flowers to perform pollination. Thereafter, the flowers were protected for a period of three days and the crosses labeled on the flower stalk (Figure 1).



**Figure 1.** Procedure of flower pollination of *Capsicum annuum* and established fruits: flower bud before emasculatation (A), emasculatation (B), pollination (C), bud protection after pollination (D), green fruit (E) and ripe fruit (F)

The dropping of reproductive structures (flowers and immature fruit) was monitored daily. When mature, fruits were harvested and seeds extracted manually and dried in the shade for a period of three to five days and then stored at 12 °C and 14% humidity.

Variance analysis was carried out with the data of percentage of established fruits in relation to the number of pollinated flowers, using software GENES (Cruz 2006) and the mean test (Tukey's test) was applied at 5% probability.

### Seed vigor and germination in hot pepper crossed with cultivated sweet pepper

The experiment was conducted at the seed laboratory of FCA/UFAM, from November to December 2006.

A completely randomized design was used with 41 treatments (20 hybrids, 20 *C. chinense* accessions and cultivar Cascadura Ikeda) with four replications. The experimental plot consisted of 16 seeds. Before germinating, seeds were treated with the fungicide Thiophanate Methyl group Benzimidazoles (0.5 g 500 mL<sup>-1</sup> water for 3 minutes).

The seeds were placed in Petri dishes on 2 sheets of germitest paper moistened with distilled water. The plates were placed in a BOD growth chamber at 28 °C, and evaluated 7 and 14 days after the start of the experiment. Seven days after sowing seed vigor was estimated and after 14 days, the germination itself. Seeds were considered germinated when they originated normal seedlings, according to the Rules for Seed Analysis - RAS (Brazil 1992).

Germination data were subjected to analysis of variance and test of means. Vigor and germination of hybrid seeds were compared with the parents (t test, P <0.05) and also among hybrids (Tukey, P <0.05). The tests were performed using software GENES (Cruz 2006).

## RESULTS AND DISCUSSION

Interspecific crosses between *C. chinense* x *C. annuum*

All accessions produced fruits, the overall mean was 22.64%, with a minimum of 8.9% (RIH01 x CA) and a maximum of 40.0% (RIH 22 x CA). Mean fruit set values are shown in Table 2. The analysis of variance showed that effect of treatments on fruit set was significant at 5% probability, indicating no reproductive barrier that

would prevent the establishment of fruits and seeds between accessions of the different morphotypes evaluated. These results differ from those presented by Campos et al. (2005), where hybrid combinations of *C. chinense* and *C. annuum* produced either no fruits or fruits with no viable seeds, as observed by Pickersgill (1993) as well.

**Table 2.** Mean percentage of fruits obtained from 21 crosses between *C. annuum* and *C. chinense*

Hybrid	Established fruits (%)
RIH01 x CA	8.88
RIH06 x CA	11.11
RIH08 x CA	13.33
RIH34 x CA	16.66
RIH36 x CA	16.66
RIH10 x CA	17.77
RIH12 x CA	18.89
RIH11 x CA	20.00
RIH27 x CA	21.11
RIH15 x CA	22.22
RIH27 x CA	22.22
RIH05 x CA	24.44
RIH03 x CA	25.55
RIH31 x CA	26.66
RIH28 x CA	26.66
RIH35 x CA	26.66
RIH32 x CA	27.77
RIH24 x CA	27.77
RIH16 x CA	30.00
RIH23 x CA	31.11
RIH 22 x CA	40.00
Mean	22.64

The mean fruit set values observed here (8.9% to 40.0%) were lower than reported by Ribeiro and Melo (2005) for the cross between genotypes of *C. chinense* with *C. annuum* with an establishment rate of 73.5% to 100%, using *C. chinense* as female parent and 87.5% to 91.5% in reciprocal crosses. Saccardo and Ramulu (1977) reported a fruit set ranging from 70% to 76% in crosses between *C. chinense* and *C. annuum* and from 7 to 14 viable seeds per fruit. Souza (1987) reported fruit set rates from 20.00% to 92.31% in interspecific hybrids between *C. chinense* and *C. annuum*, using *C. annuum* as female parent and 0.00 to 100.00% in the reciprocal crosses. In general, the studies confirm the feasibility

of interspecific hybridization between the species *C. annuum* and *C. chinense*. The differences observed are most likely due to genetic differences between the plants and the procedures of pollination used.

### Hybrid seed vigor and germination

Seeds were collected from fruits of the crosses and fruits of the parents for an analysis of vigor and germination. The mean values of seed vigor and germination for parents and for hybrids were compared by the t test ( $P < 0.05$ ) (Tables 3 and 4) and the hybrid data were compared by the analysis of variance and Tukey test (Table 5)

Seeds from 20 interspecific hybrids were analyzed with an unbalanced representation of morphotypes (4 murupi, 11 pimenta-de-cheiro, 2 dedo de moça, 1 pimenta de passarinho, 1 curabiá and 1 olho de peixe). Due to this distribution it was not possible to compare the influence of the morphotypes on the results. Only in

the case of morphotype curabiá, represented by only one accession (RIH36), we observed that seeds did not germinate until the seventh day. In morphotype murupi seeds of three of the four evaluated crosses germinated, in pimenta-de-cheiro 6 of 11 and in dedo de moça one of two. In the morphotypes pimenta de passarinho and olho de peixe, only one accession of each morphotype was used and the seeds of the crosses did germinate (Table 5).

Among the hybrid seeds the vigor ranged from 1.56% to 45.31%; the hybrids with extreme values were those derived from pimenta-de-cheiro, with the highest representation in the experiment. In *C. chinense* accessions seed vigor ranged from 1.56% (RIH28, dedo de moça) to 57.81% (RIH23, pimenta de passarinho). The seed vigor of sweet pepper cultivar Cascadura Ikeda was 46.87%. Souza (1987) examined the vigor of hybrid parents and of *C. chinense* and *C. annuum*, and reported changes in vigor from 73% to 97% in the parents and from 2 to 96% in hybrids.

**Table 3.** Mean of seed vigor, in percent, of the interspecific hybrids (HI) between *Capsicum chinense* (PMA) and *Capsicum annuum* (PMO) accessions and their parents

Morphotype	Hybrid	Vigor (%)			Mean comparison (t test)		
		HI	PMA	PMO	HI x PMA	HI x PMO	PMA x PMO
Murupi	RIH05 x CA	14.06	21.87	46.87	is	*	is
	RIH27 x CA	0	15.62	46.87	-	-	-
	RIH08 x CA	37.5	54.68	46.87	is	is	is
	RIH15 x CA	18.75	26.56	46.87	is	*	*
Pimenta de cheiro	RIH01 x CA	0	15.62	46.87	-	-	-
	RIH03 x CA	0	28.12	46.87	-	-	-
	RIH24 x CA	3.12	3.12	46.87	is	*	*
	RIH31 x CA	0	28.12	46.87	is	*	is
	RIH32 x CA	9.37	35.93	46.87	*	*	*
	RIH06 x CA	1.56	4.68	46.87	is	*	*
	RIH25 x CA	17.8	1.56	46.87	is	*	*
	RIH11 x CA	0	9.37	46.87	-	-	-
	RIH16 x CA	45.31	34.37	46.87	is	is	is
	RIH12 x CA	0	48.3	46.87	-	-	-
RIH35 x CA	3.12	17.18	46.87	is	*	*	
Dedo de moça	RIH10 x CA	0	0	46.87	-	-	-
	RIH28 x CA	4.68	1.56	46.87	*	*	*
Pimenta passarinho	RIH23 x CA	4.68	57.81	46.87	*	*	*
Curabiá	RIH36 x CA	0	34.37	46.87	-	-	-
Olho de peixe	RIH34 x CA	6.25	31.25	46.87	*	*	*

**Table 4.** Mean of seed germination, in percent, of the interspecific hybrids (HI) between *Capsicum chinense* (PMA) and *Capsicum annuum* (PMO) accessions and their parents

Morphotype	Hybrid	Germination (%)			Mean comparison		
		HI	PMA	PMO	HI x PMA	HI x PMO	PMA x PMO
Murupi	RIH05 x CA	81.25	35.93	53.12	*	*	is
	RIH27 x CA	0	64.06	53.12	-	-	-
	RIH08 x CA	62.5	57.81	53.12	is	is	is
	RIH15 x CA	29.68	29.68	53.12	*	is	*
Pimenta de cheiro	RIH01 x CA	0	31.25	53.12	-	-	-
	RIH03 x CA	0	31.12	53.12	-	-	-
	RIH24 x CA	4.68	15.62	53.12	is	*	*
	RIH31 x CA	0	37.5	53.12	*	*	is
	RIH32 x CA	79.68	46.87	53.12	*	*	is
	RIH06 x CA	67.18	6.25	53.12	is	*	*
	RIH25 x CA	76.56	26.56	53.12	*	is	is
	RIH11 x CA	0	54.68	53.12	-	-	-
	RIH16 x CA	60.93	50	53.12	*	is	is
	RIH12 x CA	0	62.5	53.12	-	-	-
Dedo de moça	RIH35 x CA	87.5	43.75	53.12	*	is	*
	RIH10 x CA	0	0	53.12	-	-	-
Pimenta passarinho	RIH28 x CA	57.81	1.56	53.12	*	is	*
	RIH23 x CA	34.37	78.12	53.12	*	*	*
Curabiá	RIH36 x CA	67.18	53.12	53.12	*	*	is
Olho de peixe	RIH34 x CA	7.81	82.81	53.12	*	*	*

**Table 5.** Means of seed vigor and germination, in percent, of the interspecific hybrids compared by the Tukey test

<i>C. chinense</i> x <i>C. annuum</i>	Vigor (%) <sup>1</sup>		Germination (%) <sup>1</sup>	
RIH16 x CA	45.30	a	60.93	a b
RIH08 x CA	37.50	a b	62.50	a b
RIH15 x CA	18.75	b	67.18	a b
RIH25 x CA	17.18	c	76.56	a b
RIH28 x CA	14.06	c	57.81	a b
RIH05 x CA	14.06	c	81.25	a b
RIH32 x CA	9.37	c	79.68	a b
RIH34 x CA	6.25	c	7.81	c d
RIH23 x CA	4.68	c	34.37	b c d
RIH24 x CA	3.12	c	4.68	d
RIH35 x CA	3.12	c	87.50	a
RIH06 x CA	1.56	c	1.56	d
RIH36 x CA	0.00	c	67.18	a b
RIH31 x CA	10.93	c	0.00	d
RIH12 x CA	0.00	c	0.00	d
RIH10 x CA	0.00	c	0.00	d
RIH27 x CA	0.00	c	0.00	d
RIH01 x CA	0.00	c	0.00	d
RIH03 x CA	0.00	c	0.00	d
RIH11 x CA	0.00	c	0.00	d

<sup>1</sup>Means followed by the same letter in a column do not differ significantly from each other by the Tukey test (P<0.05)

In 8 of the 12 crosses in which the seeds germinated the vigor did not differ statistically from the seeds of hot peppers parents. Only in the cross using accession RIH28 the vigor exceeded that of hot pepper; in the other cases, values were lower. But in comparison with the sweet pepper cultivar (46.87%) the vigor of hybrid seeds was lower; in only two crosses the difference was statistically insignificant. The vigor of sweet pepper seeds exceeded that of nine hot pepper accessions and did not differ from three. In general, we can conclude that the seed vigor of the hybrids is close to that of hot peppers, which is in turn lower than that of the sweet pepper cultivar used.

Seeds of 13 (65%) of the 20 crosses germinated; among these the germination rate varied from 4.68% (RIH24 x CA) to 87.5% (RIH35 x CA), both pimenta-de-cheiro morphotypes. In *C. chinense* accessions the seed germination rate ranged from 1.56% (RIH28, dedo de moça) to 82.10% (RIH34, olho de peixe). The seed germination rate of the pepper cultivar Cascadura Ikeda was 53.12%. Souza (1987) observed germination rates of interspecific hybrids between *C. chinense* and *C. annuum* ranging from 0% to 97%, whereas Casali (1970) reported lower rates, ranging from 0 to 6.6%, when *C. chinense* was used as male parent, and failure when it was used as female parent.

In the 13 interspecific crosses in which the seeds germinated, the means of 10 were statistically different from the hot pepper parents, eight higher and two lower. In comparison with the sweet pepper cultivar, the mean of six interspecific crosses did not differ statistically from the parent. Of the significantly different ones four had higher and three lower means than the parents.

A significant effect of hybrids was stated in the analysis of variance of the traits seed vigor and germination. Means were compared by the Tukey test (Table 5). It was found that there was greater discrimination of the accessions for the trait seed germination. The mean overall effect of seed germination was 9.7% and 37.18%.

The highest mean for seed vigor was observed in cross RIH16 x CA (45.3%), which did not differ statistically from the mean of cross RIH08 x CA (37.5%) which in turn is statistically not different from the mean of cross RIH15 x CA (18.75%). The other means were lower than these crosses and did not differ from each other.

Mean seed germination ranged from 0% to 87.5%, and the values were higher than the vigor, with exception of cross RIH06 x CA, with the same values of vigor and germination, and of cross RIH31 x CA, with a vigor of 10%, but 0% germination in the evaluation four days after seeds had been left to germinate. The incidence of chromosomal disharmony in hybrids may undermine the initial seedling development and may lead to death or paralysis of the seedling development. This phase of cell differentiation is critical, when many genes responsible for the production of proteins essential for plant development (formation of tissues and chlorophyll) are triggered, often simultaneously (Souza, 1987). Therefore, germination was evaluated 14 days after seeds had been placed to germinate, so normal germinated seedlings could be identified, according to the RAS.

Casali (1970) reported germination rates ranging from 0% to 6.6% in crosses using *C. chinense* as female parent and *C. annuum* as male and between 0% and 47% when *C. chinense* was used as male parent; higher values were obtained here. Due to the large intraspecific variation found in *C. chinense* variations between results from different experiments are expected, although one should also consider the efficiency of the pollination procedure.

The pollen viability of interspecific hybrids was not evaluated, but seedlings obtained from the germination test were taken to the field and backcrossed with the sweet pepper cultivar Cascadura Ikeda; for all backcrosses at least one progeny was obtained to continue the breeding program.

## CONCLUSIONS

The interspecific cross between *Capsicum annuum* and *Capsicum chinense* is possible, and can obtain viable seeds and fruits, although results vary according to the genotype used.

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# Compatibilidade no cruzamento entre pimentas domesticadas e pimentão cultivado

**RESUMO** - Nesse trabalho foi avaliada a compatibilidade do cruzamento entre *C. chinense* e *C. annuum* e o vigor e a germinação das sementes dos híbridos interespecíficos. Foram cruzados vinte e um acessos de *C. chinense*, usados como genitores masculinos, com a variedade Casca dura Ikeda (*C. annuum*), genitor feminino. Foi utilizado o delineamento inteiramente casualizado (DIC), três repetições e 30 flores por parcelas avaliando-se o vingamento de frutos. A germinação e o vigor das sementes dos híbridos obtidos foram analisados utilizando o DIC com quatro repetições e parcelas de 16 sementes. Todos os cruzamentos interespecíficos realizados produziram frutos, com taxas de vingamento variando de 8,9 % a 40,0 %. Na primeira contagem obteve-se a germinação variando de 0,0 % a 45,3 % e a segunda contagem os valores de germinação estavam entre 0,0 % a 87,5 %. Assim pode-se concluir que é possível a obtenção de frutos e sementes viáveis no cruzamento interespecífico *C. chinense* e *C. annuum*.

**Palavras-chave:** *Capsicum chinense*, *Capsicum annuum*, Híbridos interespecíficos, Melhoramento de *Capsicum*.

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