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Synthesis of a base population of Habanero pepper

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ABSTRACT

The establishment of populations with ample genetic variability and their use in breeding programs are discussed based on the *Capsicum* breeding program at Embrapa. Thirty-one accessions of "Habanero" pepper from different origins present in the *Capsicum* Active Germplasm Bank (AGB) of Embrapa Vegetables were used to establish a base population. The base population of "Habanero" was formed by an equal mixture of F_2 seeds from all crosses obtained, using a fixed weight (1 g) per cross, which is close to 150 seeds per genotype. This balanced population was introduced in the *Capsicum* AGB as CNPH 15,469. There are several possibilities to explore this variability, such as the use of base population as source of inbred lines; the use of base population for selection in specific environments; establishment of new populations from the base population, and also its use in recurrent selection programs.

Keywords: *Capsicum chinense*, pre-breeding, genetics, germplasm.

RESUMO

Síntese de uma população base de pimenta tipo Habanero

O estabelecimento de populações de base genética ampla e suas possibilidades de uso em programas de melhoramento são discutidos, baseado no programa de *Capsicum* da Embrapa. Foram utilizados 31 acessos de pimenta do grupo "Habanero" de diferentes procedências do Banco Ativo de Germoplasma (BAG) de *Capsicum* da Embrapa Hortaliças para a formação de uma população base. A população base foi formada por uma mistura equitativa de sementes F_2 de todos os cruzamentos obtidos, sendo utilizado um peso fixo (1 g), o qual equivale a cerca de 150 sementes por genótipo. Essa população balanceada foi introduzida no BAG *Capsicum* e recebeu a denominação de CNPH 15.469. Dentre as possibilidades de exploração desta variabilidade pode-se citar: o uso da população base como fonte de linhagens; manutenção da população para seleção em ambientes específicos; derivação de novas populações a partir da população base e, ainda, sua utilização em programas de seleção recorrente.

Palavras-chave: *Capsicum chinense*, pré-melhoramento, genética, germoplasma.

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The success of breeding programs depends on the genetic variability available to be used by breeders. Since breeders need to obtain promising results in a relatively short term, conditions to promptly exploit the potential variability maintained in germplasm banks are limited (Nass *et al.*, 2012). Thus, the development of base populations with wide genetic variability may stimulate the interaction among germplasm banks and breeding programs, increasing the chances of using the accessions, also because of the possibility of exploiting new combinations originating from the intercross of accessions used when forming these base populations.

Establishment of base populations involving cross of multiple parents has been used as an alternative to increase the genetic variability available for soybean (Alliprandini & Vello, 2004), rice (Guimarães & Châtel, 2005), and wheat breeding (Mackay *et al.*, 2014). The use of wild relatives of cultivated species in crosses is also an additional option (Nass & Sigrist, 2012), and a common practice since the beginning of breeding programs of several species, as surveyed by Hajjar & Hodgkin (2007). Although examples of the use of multiple parents to establish base populations are found in the literature, this procedure is not common, especially due to the difficulty of performing wide crosses, which takes time and may be even more complex due to the prevailing reproduction type.

With the advancement and reduction in cost of molecular techniques, some specifically structured populations have been established for studying the genetic basis of complex traits of agronomic interest (Cavanagh *et al.*, 2008; Rakshit *et al.*, 2012). Such populations generally use several accessions of multiple origins in their formation and are of great interest for breeding (Buckler *et al.*, 2009; Yan *et al.*, 2011; Mackay *et al.*, 2014; Huang *et al.*, 2015). Since these populations are established to enable genetic studies of different traits, the crosses are directed, which prevents wide random recombination, desirable when the goal is to exploit the maximum genetic variability among accessions available in germplasm banks. It should be highlighted that development and maintenance of populations with wide genetic base require a lot of work and

Table 1. Origin of accessions used to form the base population of Habanero pepper (origem dos acessos utilizados no desenvolvimento da população base de pimenta Habanero). Brasília, Embrapa Hortaliças, 2015.

Origin	Quantity
Brazil	**21**
Maranhão	4
Amazonas	3
Acre	2
Distrito Federal	2
Pernambuco	1
Tocantins	1
Mato Grosso do Sul	1
Commercial	5
Unidentified	2
Mexico	**2**
United States of America	**8**
Chile Pepper Institute	4
Whatcom Seed Co.	3
Reimer Seeds	1
TOTAL	**31**

Table 2. Range of variation of important traits for *Capsicum* breeding observed in the accessions used to form the base population of Habanero pepper (amplitude de variação em características de interesse para o melhoramento de *Capsicum* nos acessos utilizados no desenvolvimento da população base de pimenta Habanero). Brasília, Embrapa Hortaliças, 2015.

Trait	Maximum	Minimum
Plant height (cm)	90	46
Plant width (cm)	99	55
Fruit length (cm)	6.0	2.9
Fruit width (cm)	4.6	2.9
Fruit wall tickness (mm)	3.9	2
Fruit shape*	5	3
Capsaicin (K)	1,000	90
Vitamin C (mg/100 g)	130	54
Yield (t/ha)	48	<10
Disease resistance**	TSWV, GRSV, PepYMV, PVY, PMMoV, CMV, powdery mildew, bacterial spot, bacterial wilt, root knot nematode	

*1= Elongate (alongado), 2= Round (redondo), 3= Triangular (triangular), 4= Campanulate (campanulado), 5= Others (outros) **TSWV: Tomato Spotted Wilt Virus, GRSV: Groundnut Ringspot Virus, Pep YMV: Pepper Yellow Mosaic Virus, PepYMV: Pepper Mild Mottle Mosaic Virus, CMV: Cucumber Mosaic Virus, Powdery mildew: *Oidiopsis haplophylli*, Bacterial spot *Xanthomonas euvesicatoria* and *X. gardneri*, Bacterial wilt: *Ralstonia solanacearum*, Root knot nematode: *Meloidogyne* spp.

time.

Embrapa Vegetables develops, for more than three decades, a successful *Capsicum* breeding program. The success of this program is based on the collection maintained in the *Capsicum* Active Germplasm Bank (AGB), currently with more than 4,000 accessions of several species. This collection was initiated in 1980 and it is composed of the domesticated species *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*, besides semi-domesticated and wild species. The *Capsicum* breeding program has a multidisciplinary team, from several institutions, carrying out germplasm collection, conservation, documentation, characterization, and development of populations, inbred lines, cultivars and hybrids of interest to the market. More recently, the program has included an additional strategy to strengthen its work on populations, with the development of base populations of two pepper types of interest to Brazilian agriculture: the traditional "Malagueta" and a sort of a newcomer to the Brazilian market, the "Habanero".

"Habanero" is among the most pungent peppers in the world. This pepper is an important ingredient in the cuisine of countries such as Mexico, China, Thailand, and South Korea. The demand for this type of pepper has been increasing in Brazil. In order to better meet the Brazilian market demand, Embrapa Vegetables' *Capsicum* breeding program has concentrated efforts on developing more productive and uniform genotypes with better nutritional quality and resistance to diseases both in "Malagueta" (*C. frutescens*) and in "Habanero" peppers (*C. chinense*).

With the perspectives of future demands for "Habanero" peppers, this letter reports on the establishment of a base population with wide genetic base and the possibilities it opens for *Capsicum* breeding programs and highlights the advantages of such an approach for vegetable breeding in Brazil and elsewhere.

Using the variability: the devil is in the details

Embrapa Vegetables *Capsicum* AGB maintains 542 entries of *C. chinense*, from which 31 accessions of "Habanero" pepper from different origins were used to develop the base population (Table 1).

Hand pollinations of emasculated flowers were carried out using a mixture of pollen from all the accessions. After pollination, flowers were covered with aluminum foil to avoid contamination by unwanted pollen. Eighty-one F_1 hybrids were obtained, which were harvested in the second semester of 2010. In 2011, the advance of generation F_1 to F_2 was carried out. At this stage, the main purpose was the production of a large number of seeds per plant. Harvest was performed periodically according to maturation of fruits. From five to ten fruits per genotype were harvested, followed by extraction of seeds.

The base population of "Habanero" was formed by an equal mixture of F_2 seeds from all hybrids obtained, using a fixed weight (1 g), which is close to 150 seeds per genotype. This balanced base population was introduced in the *Capsicum* AGB of Embrapa Vegetables and received the identification number CNPH 15,469.

A success story needs to continue

The *Capsicum* AGB of Embrapa Vegetables provides material with high genetic variability for its breeding program. This has guaranteed the success of the program, evidenced by releases of hot pepper cultivars which are widely used by producers, including a *C. annuum* Jalapeño pepper, BRS Sarakura, which has consistently yielded ca. 60 t/ha in growers' fields, for several years. Recently, efforts in developing new "Habanero" cultivars have yielded good results of interest to the Brazilian and North American/ European Community markets due the growing demand in the latter. Noteworthy are the first two Brazilian-bred Habanero-type peppers produced by Embrapa (BRS Juruti RNC 32019, protected 20 May 2015, and BRS Nandaia, RNC 32009). This experience in establishing a base population to explore more fully the variability of *C. chinense* is part of this major breeding effort.

The accessions used to form the base population showed high variability (Table 2). It should be pointed out that previously Embrapa has made limited efforts in the exploitation of *C. chinense* variability in Brazil: Ulhoa *et al*. (2010) reported on morphologic characterization of 23 accessions of "Habanero" pepper under field conditions in two environments, Brasília-DF and Catalão-GO. The authors observed great variability among the accessions, mainly associated to color, shape, productivity, size and cycle. Accessions CNPH 15,031, CNPH 15,037, and CNPH 15,045 were considered promising for characteristics of plant, fruit, and yield. Teodoro *et al*. (2013) characterized 22 accessions of "Habanero" pepper maintained in a greenhouse in relation to vitamin C content. A great variability among accessions was observed, considering that vitamin C contents ranged from 54 to 130 mg/100 g, with an average of 98±24 mg/100g. The recommended daily levels of vitamin C are from 75 to 90 mg (Chen *et al.*, 2003).

Aiming to exploit the wide genetic variability present in the population CNPH 15,469, the *Capsicum* breeding program has different possibilities, such as:

a) Selection of specific characteristics of interest to the markets through immediate extraction of promising inbred lines, generating more adapted and productive hot pepper cultivars of Habanero-type;

b) Use this population in recurrent selection programs according to an increased frequency of favorable alleles, procedure that will ensure continuity of the breeding program with perspective of synthesizing materials with great interest to farmers;

c) Use of these materials as a subject for genetic studies aiming to a better understanding of specific traits through molecular analysis;

d) Use of base population for assisting in the structuring and establishment of nuclear collections, including specific characteristics of interest;

e) A case study on the methodology for establishing the base population as a mechanism to exploit wide genetic variability available in germplasm banks.

In this sense, the establishment of base populations, as exemplified by this effort with "Habanero" pepper, provides possibilities of developing new cultivars for pepper agribusiness, besides providing support and strengthening of Embrapa Vegetables' *Capsicum* breeding program, as well as for other national and international institutions via germplasm exchange.

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