Breakthroughs in the Genetics and Breeding of Capsicum and Eggplant

Torino, 2-4 September 2013

XV Meeting on Genetics and Breeding of Capsicum and Eggplant

EUCARPIA

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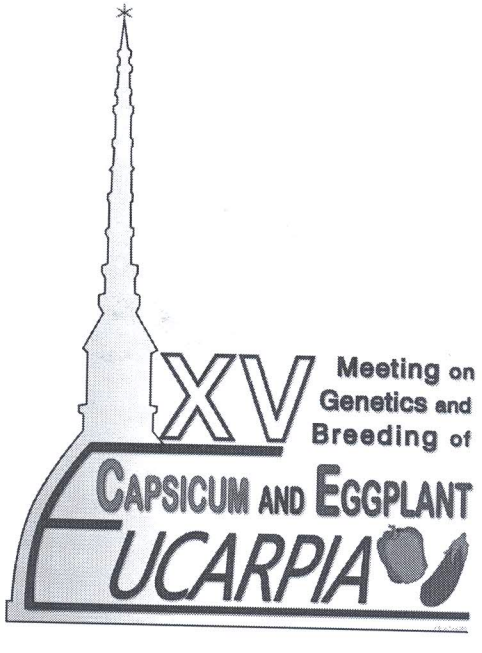
Proceedings of the
XV EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant
2-4 September 2013, Torino – Italy



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Capsicum germplasm bank maintained by EMBRAPA Vegetables, Brazil

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Abstract

Brazil is the most important center of genetic diversity for the genus *Capsicum.* Some species are considered to occur exclusively in Brazil and this emphasizes the need to adequately maintain the germplasm *in situ* and/or *ex situ.* Embrapa Vegetables' *Capsicum* germplasm bank was established in 1980 and today it has over 4,000 accessions, being the largest and most diverse *Capsicum* collection in Brazil, with domesticated species, semi-domesticated, and wild species. This bank provides the genetic base for a large breeding program implemented by Embrapa, which has released several open-pollinated (OP) and hybrid cultivars for both small-scale farmers as well as for the large entrepreneurs. The *Capsicum* breeding program has a multidisciplinary and multi-institutional team; activities include germplasm collection; conservation and documentation; morphological, molecular, cytogenetic, disease resistance, and nutritional characterization and development of inbred lines, OP, and hybrid cultivars with a focus on disease resistance. Collection expeditions have taken place in the Amazon and the Atlantic Forest (Eastern Brazil) where wild populations with high extinction risk were thoroughly sampled. During the last twelve years, 1112, 627, and around 300 accessions were characterized for morphological, molecular, and disease resistance characteristics, respectively. Knowledge and information generated by characterization have been organized in an Access software-based databank. The *Capsicum* germplasm collection has presented broad genetic variability for fruit and plant traits such as color and shape, aroma, pungency, multiple disease resistance, and plant architecture. Novel work includes the evaluation of volatiles as well as processing characteristics, the later in collaboration with the private sector. This variability has contributed to the development of new cultivars and hybrids adapted to Brazilian conditions and market niches, with superior performance.

Keywords: Variability, characterization, conservation.

Introduction

The conservation and characterization of genetic resources of *Capsicum* spp. has been strategic for obtaining superior individuals that combine desirable traits for different segments of the production chain (Ribeiro and Reifschneider, 2008). By the 1940's, the centers of origin of domesticated species were considered unlimited sources of genetic variability. The expansion of the agricultural frontier and the indiscriminate use of land, without concern for preserving the environment and genetic resources for future generations, can lead to the extinction of many wild relatives of cultivated species, including the genus *Capsicum* (Ribeiro, 2000). The possibility of a decrease in the genetic diversity within a species and related wild species has led the scientific community to advocate for the maintenance of genetic resources of different plant species. Currently, most of the public and private national and international research institutions maintain a germplasm bank or collection (Ribeiro, 2000).

Genetic resources of a domesticated species include wild species, landraces and special types, populations, inbred lines or OP varieties, obsolete varieties, and hybrids (Nass et al. 2012). Plant breeders usually appeal to the germplasm bank to generate new cultivars, which are more productive, disease resistant, with higher nutritional quality or with other characteristics of interest.

In Brazil, the most important *Capsicum* germplasm collection is maintained by EMBRAPA, Embrapa Vegetable Crops and Embrapa Genetic Resources and Biotechnology (Embrapa Cenargen), in Brasilia, D.F. The germplasm collection has a bit over 4,000 entries represented by OP varieties, inbred lines, hybrids, populations, and landraces of the five cultivated species, and dozens of semi-domesticated and wild species. The regeneration of this germplasm is periodically done, and this is critical for the safe conservation of Capsicum species that risk extinction. Morphological, molecular, and disease resistance characterizations are important because they identify duplicates and generate a database useful to breeders. Collection of wild species and germplasm exchanged among national and international institutions are also strategic procedures to maintain the variability of the genus.

Materials and Methods

Enrichment of germplasm bank

Enrichment of the *Capsicum* genebank was made through exchange of genotypes with national and international research institutions, by collection, and by expeditions in the Amazon and in Atlantic Forest (Eastern Brazil), where wild populations are at high risk.

Multiplication of accessions

The multiplication of *Capsicum* spp. accessions was performed simultaneously under greenhouse conditions at Embrapa Vegetables, Brasilia, Brazil. Five plants of each accession were grown using standard growing practices. Around 100 accessions were multiplied and characterized each year. Self-pollinated and O.P. seeds were harvested and kept separately. Seeds were manually extracted, washed in water, pre-dried at 32°C for 48 hours, dried in an incubator at 40°C for 48 hours, packaged in aluminum foil bag and stored in a cold storage chamber at 4°C. Around 400 accessions are maintained at -20°C in a basic collection (Colbase) by Embrapa Cenargen. A bar code system was developed for documentation, coding and control of seed stock of the germplasm bank. From 2000 to 2012, 1158 accessions including different *Capsicum* species (domesticated, semi-domesticated and wild species) were multiplied.

Morphological characterization

Morphological characterization was performed according to the *Capsicum* descriptors (IPGRI, 1995) using a total of 56 descriptors, as follows: 18 for vegetative part, 13 for inflorescence traits, 22 for fruit and 3 for seeds. Descriptors pungency, aroma and fruit position were also added because they are important for the breeding program. Different species were identified using a classification key for domesticated species, domesticated and semi-domesticated varieties of *Capsicum* genus occurring in Brazil (Ribeiro et al. 2008).

Disease resistance characterization

Around 300 *Capsicum* spp. accessions were evaluated for resistance to most important pathogens that affect pepper production in Brazil: bacterial wilt (*Ralstonia solanacearum* biovars 1 and 3), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), phytophthora root rot (*Phytophthora capsici*), Tospovirus (Groundnut Ring Spot Virus - GRSV, Tomato Spotted Wilt Virus - TSWV, Tomato Chlorotic Spot Virus - TCSV), Potyvirus (Pepper Yellow Mosaic Virus - PepYMV, Potato Virus Y - PVY) and powdery mildew (*Oidiopsis taurica*).

Molecular characterization

Molecular characterization was performed for 627 accessions using 55 RAPD (Random Amplified Polymorphic DNA) markers developed by Embrapa Cenargen (Buso et al. 2003). RAPD markers were used for the analysis of genetic similarity among accessions, and the similarity matrix was made based on Jaccard and agglomerative hierarchical analysis by UPGMA.

Documentation

Data of characterization, passport information and a digital photo of each accession have been organized in an Access software-based databank.

Results and Discussion

Brazil is an important center of diversity of the genus *Capsicum* and has the largest number of wild *Capsicum* species, which emphasizes the need to properly maintain this genetic diversity in a germplasm bank. More than 150 accessions of domesticated and semi-domesticated species were collected in the Amazonian region, and 50 accessions of wild species were collected in the Atlantic Forest. Accessions of *C. chinense* collected in the Amazonian region showed huge diversity of fruit shape, positions, color, size and degree of pungency. Many genotypes exhibited characteristics of interest and, therefore, have high potential for immediate use in the breeding program. The Amazon basin is the diversity center of *C. chinense* species (IBPGR, 1983). Three new *Capsicum* species were recently described by Barboza and Bianchetti (2005): *C. pereirae*, *C. friburgense* and *C. hunzikerianum* (Fig.1). These species are from the East coast Atlantic forest.

In the past twelve years, 1158 accessions of the *Capsicum* germplasm bank of Embrapa Vegetable Crops were multiplied by self-pollination and from these, 1112 were characterized morphologically. Out of these, 432 were classified as *C. annuum* L. var. *annuum*, 118 as *C. baccatum* var. *pendulum* (Wild.) Eshbaugh, 428 as *C. chinense* Jacquin, 101 as *C. frutescens* L., 1 accession as *C. pubescens* Ruiz & Pavon, 28 accessions as semi-domesticated and 4 accessions as wild species (Table 1). Morphological characterization has allowed the identification of similar accessions and genetic variability within each species. The use of morphological descriptors has also been crucial to the registration and protection of cultivars, for the establishment of a core collection, development of base populations, besides adding value to conserved germplasm that could be used in the breeding program.

Molecular markers have been useful tools in assessing the genetic diversity of germplasm banks. The genetic similarity analysis performed on 627 accessions organized these accessions into four major groups, subdivided into species by genetic similarity (Buso et al. 2003). Accessions of the cultivated species *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* were grouped according to their respective classification. Groups of *C. chinense* and *C. frutescens* were closer to the group of *C. annuum* than accessions of *C. baccatum*. Wild species have formed a separate group with about 30% similarity with cultivated species. The classification of most wild accessions corroborates the morphological classification, i.e., *C. flexuosum* was closest to cultivated species.

For disease resistance, 328 accessions were evaluated for resistance to *Ralstonia solanacearum*, 329 for *Xanthomonas campestris* pv. *vesicatoria*, 307 for *Phytophthora capsici*, 273 for *Leveillula taurica* and 399 for different virus (Tospovirus and Potyvirus) (Table 1). Sources of resistance or tolerance to *Ralstonia solanacearum* (Lopes and Quezado-Duval, 2001; Lopes and Boiteux, 2004) and to *Xanthomonas campestris* pv. *vesicatoria* (Lopes and Quezado-Duval, 2001) and Phytophthora root rot (Ribeiro et al. 2003) were identified in accessions of domesticated species including *C. annuum*. Accessions of *C. frutescens* had good levels of resistance to viruses (Lima et al. 2011). Accessions with resistance to one or more pathogens and from different species of *Capsicum* were successfully incorporated to the breeding program. In the past twenty years, a significant number of OP and hybrid cultivars were released: a bell pepper with resistance to *Cercospora* (cultivar Tico), three OP cultivars of typically Brazilian hot peppers (BRS Seriema, BRS Mari and BRS Moema) and three Jalapeño cultivars developed in partnership with processing companies (BRS Ema, BRS Garça, BRS Sarakura) (Reifschneider and Ribeiro, 2012). Moreover, several lines resistant to different pathogens were made available to the national and international research institutions. Examples include: CNPH 148 (resistant to *Phytophthora* root rot), CNPH 703

(resistant to several species of *Xanthomonas* spp; Poulos et al. 1991) and CNPH 679 (resistant to Tospovirus), which have been used by public and private breeding programs in Brazil and abroad.

The information generated by the characterization has been organized in an Access software-based databank (Fig. 2). The germplasm presents broad genetic variability for plant and fruit traits such as plant architecture, multiple disease resistance, color, shape, aroma, and pungency.The availability of characterization data (morphological, molecular and disease resistance) in a database has facilitated the access to the genetic diversity maintained in the *Capsicum* genebank by breeders and to select desirable genotypes for the breeding program. This documentation is available on the website http://www.cnph.embrapa.br/paginas/servicos/banco_germoplasma_capsicum.htm.

Novel research lines have been incorporated to the *Capsicum* program like characterization for volatile compounds related to aroma, vitamin C, oleoresins, anthocyanin and antioxidant activity in the fruits. A new project has been initiated and focuses on germplasm characterization for resistance to arthropods and nematodes and the establishment of a core collection of *C. frutescens*.

Table 1. Number of accessions of Embrapa Vegetables' *Capsicum* germplasm bank conserved and with morphological, molecular and disease resistance characterization (2000 to 2012).

Species	Conservation	Characterization						
		Morphol.	Mol.	Disease resistance				
				RS	XCV	PC	LT	Viruses
C. annuum L. var. *annuum*	432	432	354	207	210	209	206	208
C. baccatum var. *pendulum* (Wild.) Eshbaugh	118	118	62	39	37	38	9	37
C. chinense Jacquin	428	428	96	57	57	35	36	76
C. frutescens L.	101	101	101	24	24	24	21	59
C. pubescens Ruiz & Pavon	1	1	-	-	-	-	-	-
C. annuum var. *glabriusculum* (Dunal) Heiser & Pickersgill	10	10	1	1	1	1	1	1
C. baccatum L. var. *baccatum*	11	11	-	-	-	-	-	11
C. baccatum L. var. *praetermissum* (Heiser & Smith) Hunziker	7	7	3	-	-	-	-	7
C. flexuosum Sendtner	1		1	-	-	-	-	-
C. villosum Sendtner var. *villosum*	4		4	-	-	-	-	-
C. dusenii Bitter	2		2	-	-	-	-	-
C. buforum Hunziker	2		2	-	-	-	-	-
C. parviflorum Sendtner	1	1	-	-	-	-	-	-
C. campylopodium Sendtner	1	-	1	-	-	-	-	-
C. chacoense Hunziker	15	-	-	-	-	-	-	-
C. hunzikerianum Barboza & Bianchetti	1	1	-	-	-	-	-	-
C. friburgense Bianchetti & Barboza	1	1	-	-	-	-		-
C. pereirae Barboza & Bianchetti	1	1	-	-	-	-	-	

RS= *Ralstonia solanacearum* biovares 1 e 3 , XCV= *Xanthomonas campestris* pv. *vesicatoria*, PC= *Phytophthora capsici*, LT= *Leveillula taurica*, Viruses= Potyvirus (Pepper Yellow Mosaic Virus - PepYMV, Potato Virus Y - PVY), Tospovirus (Groundnut Ring Spot Virus - GRSV, Tomato Spotted Wilt Virus - TSWV, Tomato Chlorotic Spot Virus - TCSV).

Figure 1. Three new species identified.

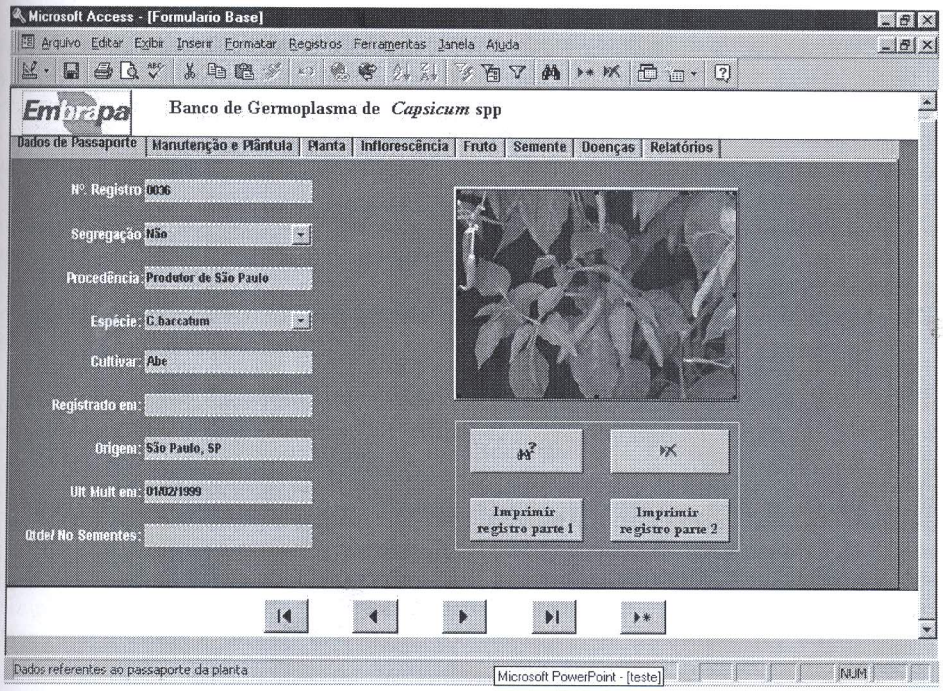

Figure 2. Characterized *Capsicum* germplasm is organized in an Access software-based databank.

Acknowledgements

The authors wish to thank CNPq, the Brazilian National Research and Development Council; and the Brazilian network of pepper researchers (Grupo *Capsicum*) from the public and private sectors for their invaluable support.

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