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Plant breeding of chili peppers (*Capsicum*, Solanaceae) – A review

[1]Henrique Kuhn Massot Padilha and [2]Rosa Lía Barbieri

[1]*Federal University of Pelotas, Crop Science Dept., Capão do Leão, RS - Brazil.*
[2]*Embrapa Temperate Agriculture, Genetic Resources Dept., Pelotas, RS - Brazil.*

Address For Correspondence:
Henrique Kuhn Massot Padilha, Federal University of Pelotas, Crop Science Dept, Faculdade de Agronomia Eliseu Maciel, S/N - CEP 96160-000, Capão do Leão, RS - Brazil.

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ABSTRACT

Background: Peppers is one of the oldest domesticated crops in the Western Hemisphere. Long before the arrival of Columbus in Americas, indigenous already used peppers as food, as war artifacts and in religious rituals. *Capsicum* genus has perfect flowers where male and female reproductive structures are in the same flower. Domesticated peppers (*Capsicum*) are diploid and predominantly perform self pollination. In comparison level, the size of *C. annuum* genome is around three to four times larger than tomato. The hot pepper genome shared highly conserved syntenic blocks with the genome of tomato, its closest relative within the Solanaceae Family. Objective: This article has reviewed the plant breeding of chili peppers (*Capsicum*, Solanaceae). Results: Genetic diversity of peppers are large, allowing alternatives to several new gene rearrangements. Thus, methodology employed will depend of the feature that aims to achieve in pepper genetic breeding. Search for the hottest pepper is one of the goals in breeding programs, even as productivity, disease and pests resistance and fruit traits. Conclusion: Pepper fruits have nutricional value, bringing benefits to consumer's health. This fact has contributed to increase the market and consumption of peppers in the world. Current studies about the complete pepper genome sequencing allows new associations between genomics and important plant traits. Usage of molecular technologies will continue to advance becoming an essential tool, combined with traditional selections and crosses techniques already established in *Capsicum* genetic breeding.

INTRODUCTION

There is a huge kinds of peppers (Solanaceae) growed worldwide with different shapes and colors. The pepper usage is as diverse as the types of *Capsicum* fruits (Albrecht *et al*., 2012) (Figure 1). Peppers are an important source of nutrients in the human diet (Shetty *et al*., 2013), and it can be consumed fresh or dried. They promote health benefits such as reducing obesity and diabetes (Vasconcelos, 2016). Pepper extracts are used in cosmetics and pharmaceuticals. Besides the use in feed as spice, peppers has also ornamental potencial when grown in pots or gardens (Bosland and Votava, 2012)

The *Capsicum* genus consists of about 30 wild species and five domesticated species: *Capsicum annuum, C. baccatum, C. chinense, C. frutescens e C. pubescens* (Dewitt and Bosland, 1996; Arimboor *et al*., 2014; Sikora and Nowaczyk, 2014). Botanically, chili peppers are classified as perennials when they are in native habitats and the multiplication is through seeds. However, they are usually grown as annual crops in parts of the world where frosting and low temperatures hinder the field production (Votava *et al*., 2005; Dewitt and Bosland, 2009). Flowers of cultivated species are hermaphrodites and show protogyny (Allard, 1960; Pickersgill, 1997). Regarding the fertilization system, domesticated *Capsicum* peppers perform self-pollination, but according to environmental conditions may occur cross-pollination (Villela *et al*., 2014). The frequency of cross-pollination

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in the field can range from just 2% to as high as 90% (Pickersgill, 1997). Fruits formed could be hot or not depending of each genotype. The pungency of hot pepper is due to the accumulation of capsaicinoids, a group of alkaloids that are unique to the *Capsicum* genus (Sun *et al*.,2015). Capsaicinoids are synthesized in the placental tissue localizaded inside of the fruits andin this same structure seeds are adhered (Wahyuni *et al*., 2013).

Plant breeding in chili peppers – The beginning:

The *Capsicum* pepper is one of the oldest domesticated crops in the Western Hemisphere. Nowadays it is used worldwide as an important spice in different ethnic groups (Kim *et al*., 2014). Long before the arrival of Columbus in Americas (XVI century), indigenous already used chili peppers as food, as war artifacts and in religious rituals (Chiou *et al*., 2014). Domestication process occurred independently from wild species of *Capsicum* in different regions of Mexico, Central and South America (Andrews, 1984). Suppose that these wild species were part of the human diet since long before domestication (at least 7200 years ago), not only as a spice, but also as a food source (Dewitt and Bosland, 2009). The ancient civilizations believed that peppers had mystical and spiritual powers. It had great importance in Aztec, Mayan and Inca civilizations and fruits were consumed fasting in order to curry favor and please the gods (Bosland and Votava, 2012). The peppers were grown and selected for different purposes, and it undoubtedly shows that Native Americans were the first "pepper breeders" in the world. Many kinds of current cultivated peppers have been developed by them. Of course, the principle of plant breeding was slow and casual, mostly based in art of selecting individuals. However, based on principles proposed by Mendel about genetics and heredity, what was considered art became to “science”. Currently, there are many selection methods in plant breeding (including chili peppers) and choosing method depends mainly on the intended objective and plants used as parents (Greenleaf, 1986; Singh *et al*., 2014). Basically, the strategy of peppers breeder is to gather in a single cultivate, higher genetic potential to certain characteristic, as productivity, disease resistance and bioactive compounds.

Plant breeding techniques in Capsicum:

Chili peppers (*Capsicum*) are diploid and predominantly perform self pollination. *Capsicum* genus has perfect flowers (Figure 2) where male and female reproductive structures are in the same flower (Allard, 1960). They are closely related to potato, tomato, eggplant, tobacco and petunia, which are also examples of Solanaceae. Many members of this family have the same number of chromosomes (2n = 2x = 24) as well as domesticated species of peppers, although genome size varies drastically from a genus to another. Some wild species have (2n=2x=26). In comparison level, the size of *C. annuum* genome (3.48GB) is around three to four times larger than tomato size (*Solanum lycopersicum*). The average exon/intron length is 286.5 bp/541.6 bp; Number of genes is around 34.900; Total length of transposable elements 2.34 Gb (76.4%). The hot pepper genome shared highly conserved syntenic blocks with the genome of tomato, its closest relative within the Solanaceae family (Kim *et al*., 2014). There is a list of known genes can be useful to pepper breeders. The list started with 50 genes in 1965, nowadays there is the total of 292 different genes, such as *dw*-1 (dwarf, plants with 15 ot 20 cm in height), *Ef* (early flowering), *me*-2 (*Meloidogyne* spp. resistance) and others (Bosland and Votava, 2006).

The main classical methods utilized in chili pepper breeding are listed in Table 1. Mass selection, Pedigree method (or Genealogical method), Single Seed Descent - SSD method, Backcross, Recurrent Selection and Hybridization are those more utilized (Coon *et al*., 2008; Kulkarni and Phalke, 2009; Nsabiyera *et al*., 2013; Manzur *et al*., 2014). The choice of the best method or combination of them depends mainly on the type of inheritance (monogenic, oligogenic, or polygenic) from traits to be improved (Lee *et al*., 2013).

Mass selection were successufully utilized by indigenous peoples of tropical America, whereby seeds of the best plants were saved for the next growing season. This method should be used for populations with genetic variability and selected in environments where the traits express themselves and for those of high heritability (Nsbyera *et al*., 2013). The pedrigree method involves keeping records of matings and their progeny. This includes making single plant selections and self-pollination (Oliveira *et al*., 2015).

The SSD (*single seed descent* method), which does not need selection during the breeding process, is also utilized in the development of recombinant inbred lines (RILs). Advancement of generations can be performed in greenhouses (Ulhoa *et al*., 2014). Moreira *et al*. (2009) utilized this method to obtain lines resistant to bacterial spot. Recurrent selection involves selecting individuals from a population followed by intercrossing to form a new population (Singh *et al*., 2014).

Backcross is used particularly for traits controlled by one or few genes, which involves selection of individual plants and successive crosses to a recurrent parent (Prakash *et al*., 2014; Boslandand Votava, 2012). Hybridization is an important factor in evolution of plants as a source of new genetic combinations and as a mechanism of speciation. Hybridization and pedigree breeding with simple selection methods can be used to improve most traits controlled by both additive and non-additive genes (Nsabiyera *et al*., 2013; Moreno *et al*., 2015).

Fig. 1: Genetic diversity in chili pepper fruits from *Capsicum* Genebank of Embrapa Temperate Agriculture. Photo: Henrique Padilha

Fig. 2: Chili pepper flower (*Capsicum baccatum*) and its reproductives structures (stigma and anthers). Photo: Henrique Padilha and Juliana Villela.

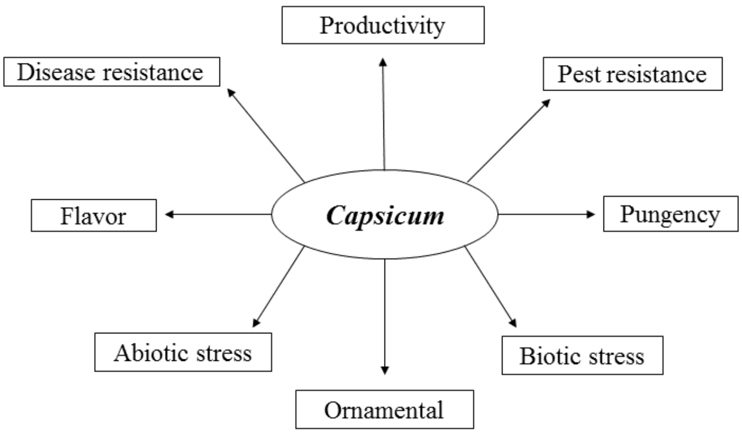

Fig. 3: Main goals of chili peppers breeding programs.

Table 1: Main classical techniques used in *Capsicum* breeding programs.

Technique name	Principle	References
Mass selection	Seeds of the best plants were saved for the next growing season; oldest method	Nsabyera *et al*., 2013.
Pedigree method	Keeping records of matings and their progeny. This includes making single plant selelections and self-pollination	Oliveira *et al*., 2015.
SSD (Single seed descent)	This method involves the advance of generations without selection, it is also utilized in the development of recombinant inbred lines	Ulhoa *et al*., 2014.
Recurrent selection	Selecting individuals from a population followed by intercrossing to form a new population	Singh *et al*., 2014.
Backcross	Used particularly for traits controlled by one or few genes, which involves selection of individual plants and successive crosses to a recurrent parente	Prakash *et al*., 2014.
Hybridization	Genes of one specie or variety move into another through the process of crossing	Moreno *et al*., 2015.

Its possible to make introgressive hybridization, where genes of one species move into another through the process of interspecific hybridization followed by successive backcrosses to one of the parentes. For example, at AVRDC – The World Vegetable Center - Taiwan, resistance from *C. chinense* PBC932 to *Colletotrichum* was successfully transferred to *C. annuum* progressive lines through conventional backcrossing (Suwor *et al*., 2015). Depending on the purpose, we can use the most appropriate method to make a new plant variety.

To protect a new plant variety is necessary to carry out DUS (Distinctness, Uniformity and Stability) tests. DUS testing for *Capsicum* spp. is based on 48 descriptors, which involve qualitative and quantitative traits, observed from germination to fruit harvest. This is applicable to all countries, members of the UPOV (Union Internationale pour la Protection des Obtentions Végétales), of which Brazil is a signatory (Pimenta *et al*., 2016).

Pepper flowers are complete, they have calyx, corolla, and male and female sex organs. Thus, the hybrid plants can be produced using manual emasculation technique and subsequent pollination or using the system of male sterility (Nsabiyera *et al*., 2013). The manual emasculation technique is relatively easy due the distinction of sex organs. Technique consists in removing male structure (anthers - which contains pollen) and permanence of female structure (stigma). Crossings are run by the breeder, putting extracted pollen of the plant used as a parent on flower stigma of the plant used as mother (Singh *et al*., 2014). Before this transaction, flower that will receive pollen is emasculated to avoid stigma contamination with own pollen. Cross flower is covered (eg.with aluminum foil) to prevent insects action contaminated with foreign pollen, and its identified with label. However, seeds production of hybrid peppers originated from manual emasculation techniques has high cost. An alternative to solv this problem is the use of male sterility.

The male sterility in peppers can be found naturally, as spontaneous mutants or by action of mutagenic agents. There are several genes that determine male sterility and it can be genetic or cytoplasmic type. More than 20 genes have been found for genetic male sterility (GMS). Shifriss and Frankel (1969) were in charge for naming the first male genetic sterility gene (*ms*-1), found spontaneously in the mutant *C. annuum* cv. All Big (Wang and Bosland, 2006). The cytoplasmic male sterility (CMS) in peppers was found by Peterson (1958) in *C. annuum* PI 164835 in India. Nowadays, Aulakh *et al*. (2016) identified and localized the *ms*-10 gene at chromossome 1 from *Capscium* genome. Thus, use of male sterile lines as parents can be an alternative for cost reduction (Singh *et al*., 2014; Aulakh *et al*., 2016). Male-sterility, genic and CMS sources are widely used on both experimental and commercial bases in the hybrid seed industry. The CMS system is the dominant one (Shifriss *et al*., 1997).

Goals of chili peppers genetic breeding:

Variability and genetic diversity of peppers are wide, allowing alternatives to several new gene rearrangements (Figure 1). On that basis, the methodology employed will depend of the feature that aims to achieve in pepper genetic breeding (Padilha *et al*., 2015). The main objectives of peppers genetic breeding are characteristics such as productivity, disease and pests resistance, fruit characteristics (bioactive compounds, fruit color, pungency, flavor), and abiotic stresses (drought, salinity) (Figure 3)(Manzur *et al*., 2014).

There are people collectors and "addicts" to peppers scattered all over the world, and all of them are constantly searching for the most burnt pepper that overcomes previous already launched. Thus, the search for the hottest pepper is one of the goals in breeding programs. For profit, and with aid of advertising to attract "peppers lovers", breeding companies use techniques to obtain hybrid increasingly hot (Baruah *et al*., 2014). In 1992 "*Habanero*" pepper (*Capsicum chinense*) was the most stinging pepper in the world with around 200,000 SHU (Scoville unit for measuring pungency) (Bosland, 1992). Currently, there are several cultivars known as "nuclear peppers", all belonging to the species *C. chinense*, reaching over 1,000,000 SHU. Examples of this type of pepper cultivars: Bhut Jolokia, Trinidad 7-pot Jonah, Trinidad Scorpion, Naga Morich, Trinidad Moranas, Trinidad Moruga Scorpion (Bosland and Baral 2007; Bosland *et al*., 2012; Dagnoko *et al*., 2013; Arimboor *et al*., 2014). The last cultivar launched was "Carolina Reaper" (*C. chinense*), developed by the American company PuckerButt Pepper in South Carolina. This cultivar exceeds 1,569,300 SHU, being recognized the hottest pepper in the world by Guinness Book (http://www.guinnessworldrecords.com/world-records/hottest-chili).

The chili peppers have a ornamental potential due their different plants sizes, fruits colors and shapes and especially because they have a double purpose, when grown in pots or in gardens. The use of ornamental peppers for decoration or consumption adds value to this product, increasing the financial return to the producer. The New Mexico State University has a long pepper genetic breeding program. In this program, cultivars are developed for ornamental purposes, mainly to being grown in pots (Coon *et al*., 2008). These plants are selected for agronomic characteristics such as more compact plant growth, erect fruits spread over the plant, high fruit persistence in plant, growth ability in pots, leaves with attractive colors and early germination. Currently ornamental peppers have a great acceptance in consumer market. European countries such as Germany had used widely these plants to decorate environments and conduct research to better understand the development and physiological functioning of this species to increasingly improve ornamental aspects (Hoffmann *et al*., 2015).

There are studies, mainly in countries suffering from excess salinity in the soil, that seek identification of genotypes tolerant to abiotic stress (Afzal *et al*., 2014). In general, there is a concern about the negative effect of environmental stresses regarding to crops development. According to FAO (2010), 20% of agricultural land in the world are strongly affected by soil salinity. This is a limiting factor to productivity of many crops, including pepper. This issue may continue to increase by the deficient irrigation practices, widespread fertilizers usage, high evaporation conditions and the recurrence of drought (Urrea-Lópes *et al*., 2014).

Anthracnose, fungal disease caused by *Colletotricum* genus, originates losses in *Capsicum* peppers production, both before and after harvest, in countries around the world such as China, South Korea, United States and Brazil (Mahasuk *et al*., 2013). Breeding programs have been developing methods that aimed to identify and incorporate resistance genes in peppers cultivars (Pereira *et al*., 2011). However, understanding about resistance genes control and inheritance for anthracnose is not clearly yet. Sun *et al*. (2015) suggests different kinds of genes (QTLs) that control resistance to immature and mature fruits. Park *et al*. (2012) evaluating healthy and deseased fruits (*Capsicum annuum*) by chromatography, identified molecules acted as phytoalexin and helped in response against the fungus. Silva *et al*. (2014) found accessions of *C. baccatum* var. *pendulum* species promising to be introduced in breeding programs (UENF 1718 e UENF 1797). Two SNP maps were constructed from two chili populations including *Capsicumannuum* 'Bangchang' x *C. chinense* 'PBC932', and *C. baccatum* 'PBC80' x 'CA1316', aiming to identify QTLs for anthracnose resistance. The validated SNPs will be greatly beneficial to select for the anthracnose resistance in chili breeding programs (Mahasuk *et al*., 2016).

Conclusion and prospects for genetic breeding in peppers:

According to FAO (2016), value generated from global production of peppers was 14.4 billion dollars, approximately forty times greater than that obtained in 1980. In 2013, nearly 200 million and 33 million tonnes of green and dry peppers, respectively, were produced worldwide. These number include chile, bell, and specialty-type peppers. Clearly, market and consumption of peppers still growing due mainly to pepper fruits nutritional value (Kim *et al*., 2014). Genetic breeding of peppers has followed this increase to satisfy consumer needs. The variability and genetic diversity of peppers are wide, allowing alternatives to several new gene rearrangements. Techniques and studies about biotechnology and molecular biology have advanced in many ways, including vegetal area and pepper research. It has contributed significantly to better understanding about evolutionary plants systems, pathways of secondary metabolites production, protein synthesis and specific genes identification for different traits (Wang and Bosland, 2006; Josine *et al*., 2011; Koeda *et al*., 2014). Molecular markers are used to characterize the genetic structure of individuals and populations. Plant breeders also use it as a tool in selection process, via marker-assisted selection (Bosland and Votava, 2012). Kim *et al*., (2014) provides more detailed information of relationship and evolution about more than 20 alkaloids involved in process that gives pungency solely in *Capsicum* genus. Furthermore, the complete pepper genome sequencing allows advancement of new associations between genomic studies and important characteristics such as fruit size, fruit production, pungency, abiotic stress tolerance, nutritional content and disease resistance. Thus, the usage of new technologies will continue to advance becoming an essential tool, combined with traditional techniques of selections and crosses already established in *Capsicum* peppers genetic breeding.

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