Review

Embrapa's *Capsicum* Breeding Program— Looking back ... into the Future

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ABSTRACT

Embrapa's Capsicum program is considered to be the largest public investment in hot pepper breeding in Brazil. The germplasm bank and associated bank-enrichment activities have been efficient in providing germplasm with variability, adaptability, yield and characteristics demanded by the breeding program since early 1980s. Moreover, the strategy adopted by the program focuses in the development of specific products demanded by the market (about 80% of the total resources) and an allocation of about 20% of the breeding efforts to explore new, higher risk opportunities. The establishment of agreements with the private sector has also been crucial for the success of the program. In the last years, Embrapa has developed and made available more than ten cultivars of different types and species: BRS Brasilândia, BRS Ema, BRS Sarakura, BRS Garça, BRS Seriema, BRS Moema, BRS Mari, BRS Juruti, BRS Nandaia, BRS Tui, and BRS Acará. Most recent efforts address the development of new, uniform, high yielding and quality, disease resistant habanero (C. chinense), calabrian and jalapeño type (C. annuum) cultivars. Additional program results include the establishment of a Capsicum knowledge e-platform, scientific and technical publications, organization of national events and training of new talents.

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Copyright © 2019 by the author(s). Licensee Hapres, London, United Kingdom. This is an open access article distributed under the terms and conditions of <u>Creative Commons Attribution</u> <u>4.0 International License</u>. **KEYWORDS:** pepper; cultivar; genetic resources; disease resistance; processing

THE BEGINNING

As pointed out by Reifschneider et al. [1] "after more than three decades of work, what actually happened and the perception of what happened merge and become one reality for those who describe it. It is impossible to specify the exact origin of a breeding program that reaches 40 years: there were certainly many factors, both personal (who is not attracted by the variability of peppers?) and professional." In the early 1980s, the Unit for Research Implementation at State Level—UEPAE of Brasilia, changed its mandate from state to national level, as a new national center of the Brazilian Agricultural Research Corporation (Embrapa), focusing on vegetable crops. Technical teams were trained, while the infrastructure was being developed. Young and enthusiastic researchers charmed by the possibilities of genetic disease resistance, received clear guidance to develop their work focusing in developing solutions to relevant problems of the Brazilian agriculture.

At that time, a disease caused by the oomycete *Phytophthora capsici*, causal agent of pepper blight, inflicted great losses in several regions of Brazil. Additionally, the pathogen was also identified as the causal agent of diseases causing significant damage in squash (*Cucurbita maxima*) fields in several states.

These two basic elements, a new institution and an important disease, more than any others, worked as catalysts for the implementation of the embryo of the *Capsicum* breeding program. Due to the relevance of the disease, surveys on losses and on the pathogen were carried out to advance knowledge on pathogen variability, compatibility groups and other important elements of the host-pathogen interaction.

Concurrently, searching for sources of resistance and chemical control tests using agrochemicals being developed at the time were initiated. Additional trials were carried out aiming at studying the effectiveness of agronomic practices on disease management, such as earthing up, in addition to chemical control.

The progress of the work, targeting fundamentally the pathosystem (bell pepper × pepper blight), confirmed the relevance of the disease, stressing the complexity that the pathogen variability lends to it. Additionally, the initiative revealed that there were other pathogens causing significant losses, such as the *Tomato Spotted Wilt Virus* (TSWV) in chili pepper, and pointed to the need for a holistic breeding program in *Capsicum* spp. In Brazil, in addition to sweet and hot peppers of the species *C. annuum, C. frutescens* (malagueta pepper), *C. chinense* (bode and murupi peppers) and *C. baccatum* (dedo-de-moça pepper) were and still are planted.

It is worth mentioning that, at the time, several public *Capsicum* breeding efforts in Brazil and in several countries, particularly in Argentina, France and the USA, further stimulated the spirit of a healthy and positive competition and cooperation. In Brazil, there were relevant examples provided by Hiroshi Nagai, at the Agronomic Institute of Campinas (IAC), and Vicente WD Casali and team, at the Federal University of Viçosa (UFV), among others.

The elements of a holistic program were met: relevant technical problems challenging the productive sector of an important genus in Brazilian horticulture; motivated, trained and expanding multidisciplinary teams; institutional encouragement and support; freedom of operate and will to face challenges. Thus, the *Capsicum* breeding program was born.

BRAZIL: A CRADLE OF Capsicum AGROBIODIVERSITY

Peppers of the genus *Capsicum* have undeniably originated in the Americas. New World natives used them long before the arrival of Europeans. Until then, they were unknown to the rest of the world and only started to be used after repeated introductions into the Old World from exploratory trips to the new continent. Today, these same peppers are consumed daily by ¹/₄ of the world's population, at least [2,3].

The first varieties of *Capsicum* peppers emerged in an area surrounded by the mountains of southern Brazil to the east, Bolivia to the west, Paraguay, and northern Argentina to the south. This region has the largest concentration of wild species in the world. In this "nuclear area", and only there, grow representatives of all five domesticated species of the genus. For thousands of years, peppers migrated from this area and spread throughout the Americas before the establishment of the first human tribes in the region [4,5].

The genus *Capsicum* includes at least 30 species and some botanical varieties, of which thirteen are concentrated in Brazil. Cultivated peppers are classified into five domesticated species according to flower and fruit characteristics: *C. annuum, C. baccatum, C. frutescens, C. chinense* and *C. pubescens.* The species *C. annuum, C. chinense* and *C. frutescens* are believed to be closely related and originated from a common ancestor. Each species was independently domesticated: *C. annuum* in Mexico, *C. chinense* in the Amazon, and *C. frutescens* in southern Central America.

The Amazon Basin is the most diverse area of *C. chinense* species. This species, very well represented by the aromatic pepper (pimenta-de-cheiro), was domesticated by the Amazonian Indians and is considered the most Brazilian peppers among domesticated species. Although fruits of the species *C. chinense* are known for their aroma, the pungency of the fruits is extremely variable, from sweet peppers such as biquinho pepper, to very pungent peppers like habanero, and to extremely pungent varieties like "Bhut Jolokia" and "Trinidad Moruga Scorpion". Among domesticated species, *C. pubescens* is the only one that does not occur in Brazil. The center of diversity of this species is Bolivia, and the best known type is the rocoto or locoto pepper.

Genetic diversity within each species is a fundamental condition for the development of new cultivars. It is necessary to know and conserve the genetic resources of the species to obtain *Capsicum* plants that combine desirable characteristics by different segments of the production chain (from producer to consumer).

Characterize to Use-Morphology and Molecular Biology at Work

Part of the genetic diversity of the genus *Capsicum* is maintained in germplasm banks by several Brazilian and International Institutions. In

Brazil, the *Capsicum* Germplasm Bank (GB), maintained by Embrapa Vegetables is the largest in South America and among the largest in the world, established almost four decades ago; currently it has about four thousand entries and holds five domesticated species and dozens of semidomesticated and wild species from different countries and different Brazilian regions [6]. This GB has served as the genetic basis for an ample genetic breeding program of Embrapa and partners in Brazil and abroad (Figure 1). In the breeding collection there are more than 30,000 lines and populations of domesticated and semi-domesticated species and dozens of cultivars of various types of spicy and low pungent peppers available to



Figure 1. Sample of genetic variability maintained in the Embrapa's Capsicum germplasm bank.

different market segments [1,7].

The use of conserved genetic resources in GBs has to be enhanced through characterization activity, adding value to conserved germplasm. Breeders depend on the availability of conserved germplasm and, fundamentally, on the quality of the information generated by results of morphological, chemical, molecular, cytogenetic characterization, among others, that enable them to make decisions about which materials should be used in breeding programs, thus boosting the use of germplasm.

Morphological characterization is usually the most accessible and economical way to quantify morphological diversity and has been widely used in *Capsicum* germplasm collections maintained by the Brazilian public sector, such as Embrapa [8–10], as well as in other public institutions. The association of morphological and molecular characterization complement each other and are able to identify phenotypic and genotypic diversity [11] resulting in an increase of knowledge, as for example in the study of the variability of *C. frutescens* accessions maintained in Embrapa's GB, representatives of the Brazilian diversity [12]. Molecular characterization of germplasm has been increasing, with decreasing costs. The use of molecular biology techniques in the program allowed a better understanding of the genetic diversity of cultivated and wild varieties of *Capsicum*. Collection expeditions in southeastern Brazil made possible the discovery of natural populations of peppers. Molecular analysis of nuclear and chloroplast DNA, including these species, confirmed the monophyletic relationship between *Capsicum* species. Wild species formed a grouping that was divided in two groups: one containing the wild species collected in the Atlantic Forest and the other containing domesticated species and their wild relatives. This was confirmed by cytogenetic studies by Pozzobon et al. [13], in which distinct chromosomal numbers were identified for wild species.

The development of microsatellite markers or Simple Sequence Repeats (SSRs) has provided an extremely efficient tool for the knowledge and use of *Capsicum* germplasm. A bit over 250 pairs of SSR primers were developed and the transferability of these markers to other species available in the *Capsicum* GB was evaluated. A total of 103 loci (87%) amplified all cultivated species and 51 loci (43%) even amplified all wild samples. This result indicated the high potential of using these markers in phylogeny, mapping and genetic improvement studies involving more than one species of *Capsicum* [14].

The transferability of microsatellite primers (SSRs) verified among *Capsicum* species allowed the characterization of *C. frutescens* and *C. chinense* collections, which enhanced the use of this germplasm. Of 185 SSR primers of *C. annuum* analyzed, 116 (62.7%) showed transferability for both species. Of these, 19 (16.37%) were polymorphic with an average of 2.89 alleles per locus for *C. frutescens* and 36 (31.03%) showed polymorphism with an average of 3.3 alleles per locus for *C. chinense*. In addition, with the analysis of the results, it was found that 17 of these *primers* can be used to analyze samples consisting of the two species studied. The lower number of alleles and the high homozygous index found in *C. frutescens* possibly reflects the low genetic variability found in this species [15].

Among the five domesticated *Capsicum* species, *C. frutescens* has the least morphological variability, according to a study of 115 *C. frutescens* accessions from the germplasm collection. This study allowed the establishment of the program's first nuclear collection, focusing on *C. frutescens* [16].

Availability of Diversity to "Instant" Breeding

Plants subjected to human selection (domesticated) undergo directed modifications, in many cases, by absolutely subjective factors such as cultural patterns and personal preferences. Thus, as a product of domestication, the human being intentionally (or not) is able to promote great morphological diversity within domesticated cultures [17,18]. In the specific case of peppers of the genus *Capsicum*, the selection is directed to the fruits and, as a result, one can observe a huge morphological diversity

in shape, size, coloration, aroma and pungency in all domesticated species. The majority of this available genetic variability has characteristics of agronomic interest, so they have high potential for immediate use in breeding programs for the development of new pepper cultivars. The program benefited from these selections and launched some pepper cultivars with high uniformity in record time, such as "BRS Seriema" and "BRS Mari".

Putting Diversity to Work for Markets

Market demands do not cease to change; markets are always looking for novelties or different niches. The most recent example of putting diversity to work for markets was the development of the BRS Tui cultivar (biquinho type), which was released by Embrapa in 2018. "BRS Tui" has non-pungent, aromatic, crisp and light-colored orange fruits that are of interest to the fresh fruit market and to the production of canned, jelly, olive oil and flavored vinegars [19]. It is noteworthy that we estimate that the largest part of the available diversity has not yet been put to use. Areas for future research include drought resistance, resistance to emerging diseases, nutraceuticals and ornamentals.

Germplasm Bank—Recurrent, Laborious, Expensive, but Essential!

Conservation of genetic resources in GBs has become increasingly difficult and costly with the scarcity of financial resources and the increasing size of collections. The high number of accessions present in GBs require adequate physical structures and human and financial resources for storage, maintenance and handling. Conserved accession requires a good management program, including seed storage, seed regeneration and germplasm evaluation with a high maintenance cost [20].

To reduce these costs, maximize the efficiency of Embrapa Vegetables *Capsicum* GB and enable the use of this germplasm by the breeding program, several strategies has been adopted such as: long-term seed conservation, documentation, characterization and evaluation of genetic variability by prioritizing the main types of peppers that have characteristics of interest to pepper farmers and final consumers, association of morphological characterization and molecular markers to broaden germplasm use [12], as well as the establishment of nuclear collection [16].

THE STRATEGY

Establishment of Germplasm Bank—Traditional and Innovative Work

The introduction of *Capsicum* germplasm, via collection and exchange, has always had a prominent position in the program. After all, without exploring variability, it was obvious that it would not be possible to advance. Thus, germplasm evaluation to identify sources of resistance to

major diseases became a major task, with many interns and students from Brazil and abroad working with P. capsici. Activities were expanded to also cover resistance to viruses (TSWV) and to bacterial diseases (Xanthomonas vesicatoria, currently the Xanthomonas complex) and Ralstonia solanacearum (currently, the Ralstonia complex), and many additional students and researchers (consultants) joined the team. The work of M. Beek, now back in Wageningen, Netherlands, collecting C. baccatum and studying virus resistance as well as Jean M. Poulos (Cornell University, PhD, 1990), who developed the first Ph.D. thesis within the program, deserve to be highlighted. It is interesting to mention that some of the first *Capsicum* genotypes of the germplasm bank (among the top three in the world, along with the ARS/USDA and AVRDC/World Vegetable Center), now with more than 4000 entries, were given by fellow Embrapa researcher Josias C. Faria to one of the authors (F. Reifschneider), while still in graduate studies in the USA, working with corn in the late 1970's. Quite possibly, already a clue of the personal interest in *Capsicum*!

The program has always invested heavily in the enrichment of its germplasm bank [21] through a comprehensive strategy and received the support of several institutions for that. Students and researchers had a leading role in enriching the bank, contributing with several accessions introduced from other banks in Brazil and abroad, as well as with new accessions collected in farms, fairs and markets. The arrival of Luciano Bianchetti and Sabrina Carvalho, experts in germplasm collection, characterization and conservation, gave a great impetus to actions strengthening both the Capsicum bank and its use in breeding. Invaluable structured collecting expeditions were carried out in the Atlantic Rain Forest and in the states of Maranhão, Acre and Amazonas, the latter with the decisive and fundamental support of the Brazilian Army. It is noteworthy that technical-level students (high school equivalent) from IFET Ceres, Goiás, made a significant contribution by being requested to bring back, after their Easter break, at least one *Capsicum* genotype from a macro region in Brazil which was not, at the time, well represented in the germplasm bank; an innovative, cost-free and additional element of the strategy with excellent results!

As new accessions were collected, new species were being discovered [22], and the hard work of genotype characterization went on, producing essential information for the breeding. Currently, more than 50 morphological descriptors are used for characterization of germplasm [9]. The use breeders made of this comprehensive information allowed for the development and release of new cultivars in a short period of time. The morphological characterization, carried out in 100 to 200 genotypes per year, has been, whenever possible, complemented by agronomic and biochemical evaluation for specific groups and characteristics of interest, such as pungency, or heat (capsaicin content), vitamin C content—with a pepper accession of the habanero group reaching 129 mg/100 g [23]—and even volatiles, assessed in elite lines and cultivars released by the program

due to the importance of aroma in *C. chinense* peppers, known as scent peppers [24]. Pungency is unquestionably a characteristic of great appeal and importance for consumers, and it is also quite variable. There are bell peppers and sweet chilies ranging from 0 SHU (Scoville heat units) to extremely hot habanero peppers exceeding 500,000 SHU. As a reference, a very hot malagueta chili pepper has 150,000 SHU.

Partnerships

Since its opening, the *Capsicum* program has had valuable partnerships with national and international public and private institutions that have been and still are essential for achieving and maintaining a sustainable breeding program [1]. It is worth mentioning the involvement of researchers, teachers and students in basic and applied research activities of the University of Brasilia (UNB), Cornell University, New Mexico State University (NMSU), Wageningen University (WUR), Luiz de Queiroz College of Agriculture/University of São Paulo (ESALQ/USP), Federal University of Goiás (UFG), Norte Fluminense State University (UENF), Rural Federal University of Rio de Janeiro (UFRRJ), Faculty of Agricultural Sciences and Veterinary-Jaboticabal Campus (FCAV/UNESP) and the Federal Institute of Goiás—Campus Ceres, and the companies Fuchs Agro Brasil and Sakura-Nakaya Alimentos. The intra-institutional partnerships with Embrapa Genetic Resources and Biotechnology, Embrapa Food Agribusiness, Temperate Climate, Embrapa Embrapa Tropical Agroindustry and Embrapa Products and Markets should also be highlighted [1].

Responding to Private Sector Demand

Pioneering work models with private sector

The partnerships of the *Capsicum* program with the private sector, pioneer in Embrapa Vegetables, began in the early 1990s, first with Fuchs Agro Brasil and later with Sakura-Nakaya Alimentos. In times of volatility and scarcity of government funding for agricultural research, these partnerships have been determinant in the significant achievements of the *Capsicum* program. These achievements have socially and economically benefited different segments of the pepper production chain [1]. Research and development (R&D) partnership agreements between Embrapa and private companies have resulted in the generation of new cultivars, which are exclusively used by the private partner for an eight-year period. After this time, the cultivar moves into public domain.

Demanding market-oriented partnerships

Private sector projects have been structured and coordinated to meet a demanding market. The breeding actions undertaken are highly participative, both in the selection of genotypes of interest and in the evaluation and validation of new technologies with the producer and the industry. The main objectives of the partnerships with the private sector have been the development of sweet pepper for paprika and pungent jalapeño pepper cultivar for sauces, with fruit characteristics required by the processing industry and adapted to the cultivation conditions of Central Brazil [25]. Currently, the demands have been towards green peppers for sauce, among others.

The results obtained, in addition to the experience accumulated in more than two decades of working together with the private sector, have opened new possibilities for partnerships. A successful example of the partnership between Embrapa and Fuchs Agro Brasil, the BRS Brasilândia sweet pepper hybrid for paprika, highlights the high level of capsanthin carotenoid (210 ASTA), the main component in ripe red fruits [26].

The development of BRS Sarakura jalapeño cultivar was a landmark in the Embrapa's partnership with Sakura-Nakaya Alimentos. Sakura leads the Brazilian market in the production of soy, peppers and garlic sauces, and has produced annually 2 to 3 thousand tons of "BRS Sarakura" pulp [27]. This partnership provided the sauce industry access to a raw material of better quality and uniformity, as well as higher industrial yields. It is noteworthy that the consolidation of the pepper processing industry has facilitated the interaction between the public program and the private sector.

Today, one of the major challenges of Embrapa's partnership with the private sector is the development of jalapeño pepper cultivars adapted to mechanized harvesting. The lack of rural labor to harvest fruit is one of the biggest problems faced by Brazilian pepper farmers [27] and this has limited the expansion of industry and cultivation areas (Figure 2).



Figure 2. "BRS Sarakura" jalapeño pepper harvest in Catalão, Goiás, Brazil.

Grower and industry feedback

Partnerships with private sector companies and pepper farmers have always provided essential feedback to the ongoing adjustment in the

Broad-Based Team

Aware of the need to constantly mobilize the best talents available and to strengthen/develop professional talents, the *Capsicum* program sought the establishment of partnerships in Brazil and abroad in order to conduct basic and applied research of interest and relevance to the program.

Partners in the academy, mentioned above (Partnerships) and Embrapa's in-house capacity were also explored and four Embrapa units deserve special recognition: Genetic Resources and Biotechnology, Food Technology, Temperate Agriculture and Products and Market.

More than two dozen B.Sc., M.Sc., D.Sc. and Ph.D. thesis were completed on topics ranging from the identification of disease resistant genotypes to breeding specific cultivars, as well as cultivar registration and protection. The final result of the different activities and initiatives has been the creation and consolidation of a solid national and international network, with a multidisciplinary and multi-institutional team.

With the most recent advances in genomics, IT, sensors and the like, the program faces a new and exciting challenge—to move from a biological based multidisciplinary team to a fully integrated interdisciplinary team who will integrate knowledge and methods from different disciplines to support *Capsicum* breeding and development in Brazil! Today traditional and molecular work are conducted by this broad-based team hand in hand.

The 80/20 Rule

The need to have a sustainable breeding program, capable of meeting demands in the short, medium and long term, became evident, on one hand. On the other hand, the program was concerned with innovation, offering new products of interest to the production chain, as well as new cultivars. The program strategy was progressively consolidating, gradually establishing a balance in which 80% of the efforts should be concentrated in meeting national and regional demands, while the remaining 20% should be used in exploring new opportunities, taking higher risks. Thus, risk management became a critical program element, although it was probably not identified as such in the beginning. The financial architecture implemented took and still takes into account the intrinsically disastrous variability (and escalating bureaucracy) of public funding based on single sources. Therefore, the National Council for Scientific and Technological Development (CNPq) and the private sector, particularly Fuchs Agro Brasil Ltda. And Sakura Nakaya Alimentos Ltda., were, and Sakura still is, important historical actors on this financial architecture, along with smaller, international research for development

projects, particularly those supported by the Agricultural Innovation Marketplace since 2013, having one of the authors (C. Ribeiro) as leader.

Significant impact was achieved due to two strategic decisions taken by the program: first, regular meetings held by project coordinators kept alive the flame of collaboration, especially because there were always reports of relevant results; there were also internal demands for more and better results; and the program has also benefited from external monitoring and evaluation, which has greatly contributed to its improvement. Second, the decision to focus the program on hot peppers and not sweet peppers. This decision was taken due to the Brazilian diversity on pungent *Capsicum* and also the fact that the private sector was focused primarily on the development of sweet *C. annuum*.

Focus and Priorities

Since its beginning, the program has prioritized the development of more productive cultivars that are resistant to major diseases [29].

Yield

BRS Sarakura, the first Brazilian jalapeño pepper cultivar developed by the program, has yields (~60 t/ha) which are 80–100% above those observed in open pollinated cultivars and commercial hybrids available in the country [27] and also abroad. The American open-pollinated (OP) cultivar NuMex Vaquero and the hybrid Mitla, for example, present yields of 24.4 t/ha and 22.2 t/ha, respectively [30]. "BRS Sarakura" also has a high industrial yield.

The hybrid paprika BRS Brasilândia, in addition to presenting fresh and dehydrated fruit high yields (30 t/ha and 8 t/ha), has an excellent (≥210) ASTA color standard [26] and dry matter concentration which ensures a high industrial yield when dehydrated.

In 2015, Embrapa released the first Brazilian habanero cultivars—BRS Juruti (red fruits) and BRS Nandaia (orange fruits), which serve both the fresh fruit market and the mash and pepper sauce processing industry. Both cultivars have high yield potential; BRS Juruti yields around 50 t/ha and BRS Nandaia 44 t/ha (36,000 plants/ha)[31].

Disease resistance

The identification of disease resistance sources and the introgression of these genes in cultivars of different types of peppers (*Capsicum* spp.) has become a recurring task of the program since its beginning. Over the past twenty years, a significant number of OP cultivars and multi-disease resistance hybrids developed by Embrapa Vegetables were made available to the market.

Cultivar BRS Moema (biquinho type, red fruits) has resistance to the *Meloidogyne javanica* and to *Pepper yellow mosaic virus* (PepYMV); "BRS Mari" (dedo-de-moça type) has also high resistance to PepYMV and

intermediate resistance to powdery mildew (*Oidiopsis haplophylli*) and bacterial spot (*Xanthomonas* spp.), while "BRS Seriema" (bode type) is resistant to *M. incognita*, with a low incidence of several viruses under field conditions (*Groundnut ring spot virus*—GRSV, TSWV and *Tomato chlorotic spot virus*—TCSV). BRS Tui cultivar is resistant to *M. incognita*, with intermediate resistance to mildew and bacterial spot, and field tolerance to GRSV, TCSV, PepYMV and *Potato virus* Y (PVY) viruses [19].

"BRS Juruti" has shown field resistance to TSWV, PepYMV and PVY, intermediate resistance to bacterial spot and wilt, resistance to powdery mildew and *M. incognita*; while "BRS Nandaia" is highly resistant to mildew, resistant to *M. javanica* and *M. enterolobii*, bacterial wilt and spot, and field resistant to PepYMV. "BRS Sarakura" has been shown to be resistant to *M. javanica* and *M. incognita* and to bacterial spot [25].

BRS Acará, a hybrid rootstock developed for bell peppers released by Embrapa in 2018, has multiple resistance to the main soil pathogens that affect pepper: *Ralstonia, P. capsici, M. javanica* and *M. incognita,* in addition to resistance to PepYMV and PVY.

Breeding for specific niches—the pepper groups

The wide diversity of *Capsicum* pepper types and species is a challenge for the breeding program, which has taken into account the demands of growers and the processing industry, while observing the new niches and opportunities, as the market demand different varietal types such as calabresa, pimenta-de-cheiro, murupi and ornamental peppers.

Peppers from the calabresa (calabrian) group (*C. annuum*), mainly used for processing in the form of dehydrated flakes with seeds, have elongated fruits and fine pulp [28,32]. Two lines (Figure 3), soon to be released, were selected based on plant yield (0.6–1.0 kg/plant), earliness, absence of lateral shoots, and low heat (~5000 SHU).

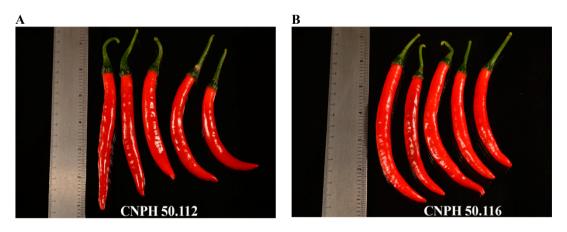


Figure 3. Fruit of the selected calabrian lines CNPH 50.112 (**A**) and CNPH 50.116 (**B**) for processing as dehydrated flakes.

Peppers from the murupi and pimenta-de-cheiro groups are very common in northern Brazil, but are gaining space in the South-Central markets of the country due to the aroma and flavor of their fruits. Murupi (*C. chinense*), besides being aromatic, is very spicy, and there is a strong interest by the food industry to use its fruits to add flavor and aroma to its products. Pimenta-de-cheiro (*C. chinense*) usually has low heat and its fruits are consumed green (immature) and fresh, adding flavor and aroma to several dishes of the Brazilian cuisine. Breeding work has begun on these two groups.

Another niche that has been gaining space in the Brazilian market is the ornamental pepper. The *Capsicum* GB is rich in genotypes with variegated leaves, small plants and fruits of intense colors, which contrast with the foliage and that have different colors depending on the stage of fruit maturation [33]. The main interest of this new line of the ornamental program is to explore the beauty of the pepper fruits concurrently with their use in cooking. The Brazilian ornamental plant sector mobilized over US\$ 1.2 billion in 2018 (http://www.ebc.com.br/ibraflor), and the Distrito Federal, where the program is located, has a per capita ornamental plant consumption that is over 100% the national average.

Beyond Yield—Quality as Key Criterion for Selection

Sweet and pungent *Capsicum* fruits are important sources of natural antioxidants, vitamin C, carotenoids (pro-vitamin A), B-complex vitamins and vitamin E. *Capsicum* spp. Elite lines and cultivars from the program are being characterized mainly in terms of capsaicinoids, vitamin C and total carotenoid contents [23,34]. In addition to the analysis, the sensory profiles and volatile compounds of different *C. chinense* varietal groups are being determined for the identification of genotypes rich in aroma and flavor [24].

Pungency is a feature of great appeal and importance to pepper consumers, and in addition to being quite variable, it is directly associated with the type or group of peppers. "BRS Nandaia" and "BRS Juruti" are highly pungent (200,000 SHU and 260,000 SHU, respectively) and have high levels of vitamin C (above 120 mg/100 g of fruit). These cultivars have been developed to meet both the fresh fruit market as well the pepper paste and sauce industry [31]. "BRS Moema" and "BRS Tui" have pungencies ranging from 100 to 270 SHU [19].

STRATEGIC RESULTS AND PROGRAM MARKETING

There are numerous results achieved that ended up giving visibility to the program, directly and indirectly impacting the *Capsicum* production chain in the country.

Continuity

The *Capsicum* program has nearly four decades of uninterrupted existence, reacting and adapting to market changes, which were not few. The need to have partnerships with the private sector, focusing on innovation, was recognized from the beginning of the program. The continuity of the program, set in a public institution, depends more than ever on its financial sustainability with the participation of the private sector, the expansion of motivated and trained multidisciplinary teams, and to keep the focus on the new challenges of *Capsicum* research.

Impact

The main impact of the breeding program so far has been "BRS Sarakura", which is responsible for more than 50% of all sauce produced in Brazil. The most prominent long-term impact remains the training of human resources, in different fields of knowledge. The establishment of an e-platform for knowledge management and communication between pepper productive chain actors, under the coordination of the program, was essential for improving information exchange in a continental country, as well as significantly reinforcing the program's visibility. Created in 2008, the e-platform based on a Google group is mainly composed of farmers and processing companies (http://groups.google.com/group/capsicums), with 400 members.

Well Trained, Committed, Field-Support Staff

Program success is also due to the effort and dedication of Embrapa's technicians, and laboratory and field assistants, committed to their daily tasks and well-trained in carrying out their activities, such as crossing, selfing, inoculation of pathogens to evaluate resistance, crop management in field and in greenhouse trials.

High Quality Final Product (Seeds)

Before the *Capsicum* program was consolidated as the largest public investment in pepper and bell pepper breeding in the country, there were virtually no improved pepper seeds in the market. The focus of private breeding programs was and still is the development of bell peppers hybrids, leaving hot peppers mostly aside. Most farmers produce their own hot pepper seeds, and this increases the risk of low uniformity, seedcarrying pathogens and the like. Most cultivars developed by program are being marketed by private seed companies. Embrapa offers the cultivars to the private sector through a bidding system, offering high quality seeds. The improved seed continues to be a main carrier of new technology and innovation to farmers.

Network in Expansion—Young Talents

Since its beginning, the program has sought to expand the *Capsicum* R&D network in Brazil and abroad through the strengthening and/or development of young talents in different areas of knowledge. Some of these talented professionals are currently working in Brazil and abroad,

in the public and private sectors, including non-biological areas, significantly expanding the network and its reach.

Public and Private Resources

Partnerships established through large multi-annual projects supported by Prodetab [35], Fuchs Agro Brasil Ltda., Sakura Nakaya Alimentos Ltda., Agricultural Innovation MarketPlace and National Council for Scientific and Technological Development (CNPq), were essential for significant advances in *Capsicum* research, as well as in the quantity and quality of the results obtained. Within the bureaucracy and limits imposed by Embrapa, the program maintained its flexibility, meeting private sector demands, and, at the same time keeping its deliveries of national public goods.

Events

In 2004, the first National Meeting of *Capsicum* pepper Agribusiness was organized in Brasília, a milestone of the program, whose main objective was to bring together different segments of the Brazilian production chain to exchange information, knowledge and experiences, survey of demands, identification of problems, dissemination of new technologies, as well as the search for better integration among the different pepper professionals. In 2011, the fourth event was organized in Monte Carmelo, Minas Gerais, with strong support from the municipality, which stands out regionally in the production of peppers. In total, around 1000 professionals linked to peppers participated.

Books, Scientific, Technical Publications, Thesis, Brochures

The first books on *Capsicum* written in Portuguese [36–38], were edited and published by program researchers. The book "*Capsicum*: pimentas e pimentões no Brasil" [37] was included in the commemorative publications for the 500th anniversary of the discovery of Brazil and it won the prestigious Jabuti award of Brazilian literature [1]; the book on pepper management "Pimentas *Capsicum*" [38] received the 11th Jorge Salim Prize, textbooks category.

In addition, dozens of scientific articles and book chapters [7] have been published in Brazil and abroad, as well as technical leaflets, research bulletins, and similar publications covering various aspects, from historical approaches [5] to evolutionary questions, with morphological aspects and molecular studies [15,39]. More than two dozen theses (B.Sc., M.Sc., D.Sc. and PhD) on different topics of interest to the program were also completed [1].

CNPq group leadership

In 1998, the *Capsicum* Research Group was established within the National Council for Scientific and Technological Development (CNPq)

(<u>http://lattes.cnpq.br/web/dgp</u>), which contains information on human resources (researchers, students and technicians); ongoing research topics; knowledge areas; involved sectors; scientific, technological and artistic productions and partnerships between groups and institutions.

INTERNATIONAL LINKAGES SUPPORT PROGRAM BENCHMARKING

Research and development actions in conjunction with international institutions were also important for the growth and maturity of the *Capsicum* program. From 2007 to 2011, the main author developed her PhD thesis at New Mexico State University (NMSU), seeking new knowledge and techniques to employ in Embrapa's program [40]. NMSU's pepper breeding program is considered to be the longest in the world, and similar to Embrapa's program, it has focused on disease resistance (*P. capsici*), industrial processing and mechanized harvesting. More recently, from 2013 to 2018, the program at Embrapa supported three international research and development projects with African countries (Nigeria, Togo and Uganda), funded by the Agricultural Innovation Platform-MKTPlace.

Working with public and private institutions outside Brazil is important because it allows the benchmarking of the Brazilian program, in addition to the obvious advantages for all partners to exchange knowledge and germplasm.

KEY PROGRAM PRODUCTS

Knowledge

The involvement of a multidisciplinary and multi-institutional team with vast knowledge and experience, accumulated over years of research with the crop, has made the program a national reference for different segments of the *Capsicum* production chain. In addition, the program has contributed to the expansion of knowledge in several areas and this has been made available to the national and international scientific community through the publications [1,29,31,38,39,41,42].

Cultivars and Hybrids

The *Capsicum* program has contributed significantly with several nonpungent and pungent pepper cultivars and hybrids to the Brazilian market, enabling the expansion of production and the occupation of market niches, focusing on multiple disease resistance and the best nutritional quality of the new materials. Cultivars released by the program include: BRS Ema, BRS Sarakura, BRS Garça, BRS Seriema, BRS Moema, BRS Mari, BRS Juruti, BRS Nandaia, BRS Tui and hybrids such as BRS Brasilândia and BRS Acará (Table 1)[1,19,25,29,31,41].

Cultivar	Pepper Type	Species	Release Year	Register Number *	Protection Certificate **
Tico	Bell pepper	Capsicum annuum	1989	-	-
BRS Brasilândia	Paprika	C. annuum	2007	20290	-
BRS Ema	Jalapeño	С. аппиит	2008	22491	-
BRS Mari	Dedo-de-Moça	C. baccatum	2008	22492	-
BRS Moema	Biquinho	C. chinense	2008	22493	-
BRS Seriema	Bode	C. chinense	2010	26546	-
BRS Garça	Jalapeño	С. аппиит	2011	22896	20100117
BRS Sarakura	Jalapeño	С. аппиит	2011	22897	20100118
BRS Juruti	Habanero	C. chinense	2015	32010	20150221
BRS Nandaia	Habanero	C. chinense	2016	32009	20160050
BRS Tui	Biquinho	C. chinense	2018	36495	20180165
BRS Acará	Rootstock	C. annuum	2019	36504	_ (*)

Table 1. Cultivars of different pepper types (*Capsicum* spp.) developed by Embrapa's breeding program over the last thirty years.

* RNC—National Cultivar Register (Ministry of Agriculture, Livestock and Food Supply-MAPA);

** National Service of Plant Variety Protection (SNPC-MAPA);

(*) Hybrids are not protected, only their parental line.

Inbred Lines with R

The most relevant inbred lines developed and/or selected by the program include "CNPH 148" (resistant to root rot caused by *P. capsici*), "CNPH 703" (resistant to various species of *Xanthomonas*) and "CNPH 679" (tospovirus resistant), that have been used by public and private breeding programs in Brazil and abroad [29,43,44].

Broad Based Populations—The Habanero Example

In 2011, a habanero-based pepper population with broad genetic variability was established and will be the source for the selection of new cultivars over the next 20 to 30 years [42]. Three habanero lines were selected from this base population which has high variability for relevant traits such as fruit shape, fruit color, aroma and capsaicin content were selected for high yield, high pungency (>300,000 SHU) and color (yellow and red mature fruit). Capsaicin content among the selected habanero lines (Figure 4) varied from 4500 (CNPH 15.744) to 500,000 + SHU (CNPH 15.749).



Figure 4. Fruit of the selected habanero lines CNPH 15.740 (**A**), CNPH 15.744 (**B**) and CNPH 15.749 (**C**) of Embrapa's breeding program.

Strengthening Promising Talents at Multiple Levels with Diverse Focus

Research

Several students from different levels of education participated in the program and were mentored or co-advised by researchers, encouraging and consolidating talented professionals such as Jean Poulos and Caroline Wagner, who work abroad, and Arildo Rego, who worked in Brazil in the private sector as a plant breeder. This effort continues and promising students as Lucas Gomes and Renato Soares are now developing their PhD thesis.

Project management

Some trainees (Rodrigo Ferraz, Ana Glaucia Heirinch and Thiago Silva) collaborated with the management of the program, besides the biological research work, and ended up dedicating themselves to the management area, which has been expanding in the country.

CHALLENGES—LINKING PRODUCTS TO MARKET

Bureaucracy and Legislation

The approval and internalization in Embrapa of the Innovation Law (LEI No 13.243/2016) positively impacted research actions of the institution, which began to have a new dynamic and greater impact on scientific development. This law regulates and creates the contractual tools that enable the mobilization of efforts and results obtained by genetic breeding conducted in the public sphere for private enterprises operating in the country. The idea behind the Innovation Law is to generate and/or stimulate an atmosphere of synergism and the establishment of strategic partnerships between public institutions and private sector companies, resulting in faster availability of improved genotypes for Brazilian agribusiness.

It is important to highlight that actions for cultivar registration and protection comply with a complex set of laws and norms, essential for the partnership with the private sector. However, this legislation has required a great deal from the program due to the multiple stages, slow pace and complexity of the processes [41,45]. The first two cultivars of *Capsicum* protected in Brazil were BRS Garça and BRS Sarakura in 2010 (<u>http://extranet.agricultura.gov.br/php/snpc/cultivarweb/cultivares protegidas.php?</u>).

Risk Aversion—Moving out of the Comfort Zone

The biggest risk that exists in any breeding program is the eventual rejection of new cultivars by seed companies, farmers or the processing industry. Because the architecture of the *Capsicum* program has been designed to keep breeding activities and end users close, this risk of rejection has been minimized. The work with growers and processing industry, and their demands, constantly keeps program researchers out of their comfort zones.

To reduce these risks, it is also important to identify the right niche for public research with *Capsicum*, and to innovate by creating new niches, especially in a period of major changes in the Brazilian and global agribusiness, and in consumer behavior and preferences.

CHALLENGES AHEAD

Continuity at Risk, Limited Public Resources

The biggest risk to the continuity of the program is an eventual change of priorities for Embrapa. The lack and/or limitation of public financial resources to invest in research could lead to a drastic reduction in the number of crops researched by the institution.

Furthermore, Embrapa has a considerable part of its staff reaching retirement age. The non-replacement of these employees, coupled with limited funding for scholarships, also compromises the continuity of the program.

Public resources are increasingly scarce in Brazil and the *Capsicum* program leadership foresaw this scenario in the early 1990s. As a result, the program has been designed to interact permanently with the private sector, which has complemented the public resources allocated to the program.

Market Changes and Globalization

The program has had to reinvent itself at times in reaction to partnerships changes, seeking new private sector linkages employees not to lose its sustainability. A remarkable moment of change was the interruption of the partnership with Fuchs Agro Brasil, whose main research focus was paprika breeding, due to the transfer of the company to China. Later, the challenge shifted to the development of jalapeño pepper for the sauce industry.

With globalization, Brazilian companies had to reorganize themselves to better serve internal and external consumers, with better quality products and competitive prices. To reach new markets, the private sector began to invest in innovation and quality in search of new market niches, demanding from the agricultural sector the production of different pepper types. The international market also has its doors open for products based on typical Brazilian peppers.

The growing US and European demand for habanero mash is of relevance to the Brazilian productive sector. Brazilian agribusiness is interested in supplying part of this demand with high quality products and competitive prices; however, it needs cultivars well adapted to the country. Embrapa Vegetables' breeding program has made a significant effort to develop habanero cultivars with high yield and fruit quality.

New Consumers Demanding New Experiences

The Brazilian market has offered a wide range of pepper-based products, which also include more elaborate lines known as "gourmet" products such as jellies, chocolates, beers, flavored olive oils and vinegars, barbecue sauce, sauces with different heat levels mixed with unusual ingredients (bacon, whiskey, Cola soda and passion fruit), blends of different pepper types, with different colors, flavors and aromas (sauces, preserves, dehydrated), dehydrated and smoked jalapeño pepper (Chipotle), as well as Asian sauces (like Sriracha).

The "gourmet" products niche serves mainly to demands of young consumers (25–35 years old) with high purchasing power, interested in diversity of flavors and new taste experiences. We must be aware of the demands of society as a whole, providing the possibility of new experiences for everyone, always focusing on quality.

Among the current domestic market demands two deserve to be highlighted: the development of cultivars adapted to organic production, as well as the demand for fresh vegetables produced by local farmers, whose sales occur directly to consumers at farmers' markets. The trend is to stimulate more local sustainable food production and the traceability of fresh and processed products. New niches demand fresh fruits and sauces with high nutritional quality, in addition to cultivars of varietal groups that have not yet been explored by the *Capsicum* program (malagueta, "cumari-do-Pará", among others).

Shifts in Labor—Machines Needed!

Today, the biggest challenge of growing peppers is the lack of labor for harvesting, which has required a major new effort from the program to develop cultivars adapted to the mechanical harvesting of fruits, both at micro and macro production scales. Shortage of harvesting labor in Central Brazil has caused significant losses, which has economically affected pepper growers and the processing industry [27]. In 2013, Sakura and Embrapa established a new project for the development of jalapeño cultivars adapted to mechanical harvesting. Jalapeño plant selection for mechanical harvesting has been mainly based on plant height (>60 cm), height of first bifurcation (>16 cm), erect plant growth habit, earliness, high yield (>1 kg/plant), fruit pungency (>30,000 SHU) and concentrated fruit set. Four lines (CNPH 30.375; CNPH 30.777; CNPH 30.607 and CNPH 30.609) have been selected so far (Figure 5).



Figure 5. Fruit of selected jalapeño lines CNPH 30.375 (**A**), CNPH 30.377 (**B**), CNPH 30.607 (**C**) and CNPH 30.609 (**D**) of Embrapa's breeding program for mechanical harvesting.

Major Challenge Is the Speed of Change!

There is a clear need to establish a continuous flow in the breeding program to allow rapid responses to the constant pressures arising from the emergence and/or introduction into the country of new pathogens and/or new pathogen variants, many of them endemic in tropical and subtropical regions. In this regard, there are several historical examples indicating that the early identification of these new pathogens and the anticipation of genetic improvement actions aiming to identify sources of resistance may ensure the viability of entire agribusiness segments [1].

It is also necessary to closely monitor the dynamics of the market and to be prepared with the changing habits and preferences of consumers, which occur very quickly today and guide the interests of the productive sector. Certainly the biggest challenge today and ahead is the speed of change.

IMPLEMENTING THE STRATEGY—LESSONS LEARNED

As presented below, there are possibly two—institutional and personal—major sets of lessons learned during the decades of organization and implementation of the program that started with a focus on breeding, but turned out to be much broader. Undoubtedly, strong leadership, team work, diversity of funding sources and continuity (in good and bad times) are noteworthy pillars that deserve to be highlighted.

- a. **Institutional Lessons:** focus on the solution of real problems of the production and processing chain, pursuit of the best science couples with technological changes leading to innovation and significant partnerships, preservation of a flexible funding basket, development and delivery of relevant and impacting results, permanent concern with productivity metrics, persistence, and leadership Nelson Mandela's way (*"It is better to lead from behind and to put others in front, especially when you celebrate victory, when nice things occur. You take the front line when there is danger. Then people will appreciate your leadership"*).
- b. **Personal Lessons:** to have an ideal, not to fear challenges, to constantly stay outside of the "comfort zone", to think outside the box; to believe in the team, to foster talents, to value partners and partnerships, to persevere, to be cautiously optimistic, and to believe that dreams come true—if you work hard!

A BIRD'S EYE VIEW OF THE FUTURE

The scientific advances in genomics and related fields, and the increase in interdisciplinary research presents a huge array of opportunities for any breeding program which makes the present and near future an exciting period to do breeding work. From CRISPR technology to edit genomes to mobile-based apps and drones a breeder can easily see many possibilities to advance the program to new levels!

Unfortunately, a high level of uncertainty about the future of this breeding program is warranted due to the recent financial crisis in Brazil, as well as macro and micro institutional changes taking place. The ongoing discussion on the role, focus and size of public institutions, including that of a major research institution like Embrapa, the links and balance between public and private efforts supporting agricultural growth and development, and similar issues are extremely relevant to the future of public breeding programs. It would be naïve not to expect a significant impact! Optimistically, the foundation and network that has been established in these past decades will be sufficiently strong and flexible to adjust to the changes ahead. And challenges and changes are now observed on an almost daily basis. The speed of change shall be the eternal challenge!

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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