EMMANUEL REZENDE NAVES

CREATION OF NEW MODEL SYSTEM FOR GENETICS IN *CAPSICUM* SPP.

Thesis submitted to the Plant Physiology Graduate Program of the Universidade Federal de Viçosa as part of the requirements for obtaining the degree of *Doctor Scientiae*.

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VIÇOSA - MINAS GERAIS
2020

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
N323c
2020

Naves, Emmanuel Rezende, 1988-
Creation of new model system for genetics in *Capsicum* spp / Emmanuel Rezende Naves. – Viçosa, MG, 2020.
144 f. : il. ; 29 cm.

Inclui apêndice.
Orientador: Agustin Zsögön.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Pimenta. 2. Hibridação. 3. Heterose. 4. Produtividade. 5. Plantas - Crescimento. I. Universidade Federal de Viçosa. Departamento de Biologia Vegetal. Programa de Pós-Graduação em Fisiologia Vegetal. II. Título.

CDD 22. ed. 583.952

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APPROVED: December 21th, 2020.

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ACKNOWLEDGMENTS

Thanks to my family for their unconditional support at all times in my life.

To Universidade Federal de Viçosa, in particular the Plant Physiology program represented by Professor Fábio DaMatta.

To the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial assistance. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

To Professor Agustín for his guidance during the doctorate to contribute with my training and for the various professional advice received. Thank you!

To professor Adriano for co-guidance.

To all teachers of the plant physiology program for their patience in teaching, to contribute to my education.

To the undergraduate students Mateus and Júnio for their support in conducting the heterosis experiments.

To my friends at the Molecular Plant Physiology Laboratory, Juliene, Maria Antônia, Jessenia, Carla Bastos, Giuliana, João Victor, Bruno and Diego, my thanks for all the affection received and for the good times.

To all my friends made throughout my life, thanks for everything.

Thank you.

ABSTRACT

NAVES, Emmanuel Rezende, D.Sc., Universidade Federal de Viçosa, December, 2020. **Creation of new model system for genetics in *Capsicum* spp**. Adviser: Agustin Zsögön. Co-Adviser: Adriano Nunes Nesi.

Capsicum is a genus appreciated for its pungency, which favored the interaction with man and thus domestication. However, through crosses, pungency was removed in modern cultivars and fruit size and productivity were increased, the result of which is what we know as bell peppers. In *Capsicum* there are gaps in knowledge about phenomena related to hybridization such as heterosis, used to obtain highly productive varieties. This motivated us to perform intra and interspecific crosses with commercial cultivars of *C. annuum* and *C. chinense* to determine the extent of the phenomenon of heterosis. Intraspecific hybridization proved to be efficient in maintaining and even improving productive characteristics, while interspecific hybridization promoted extreme vegetative development to the detriment of the reproductive one. In addition, it was observed that reciprocity, compatibility and germination were dependent on the direction in which the crosses were made and the genotypes involved, as well as the influence of the pollen source on the fruit set, size and biometry of fruits. *Capsicum* fruits are not climacteric, which positions the genus favourably to create a new model system for genetics and physiology. The search for a productive cultivar, with small size and easy cultural management that can be tailored to laboratory work would represent a valuable first step. With this aim in mind, we scanned a germplasm bank for cultivars, mainly of *C. annuum*, with small size, fast cycle, prolificity and productivity with the task of representing in a reliable way, but in miniature, the productive and genetic potential of *Capsicum* found in commercial cultivars. After an initial screening of 14 *Capsicum* cultivars as candidates for the model plant, we found two cultivars of *C. annuum* here named 75 and CVO. These two cultivars were precocious in the cycle until anthesis and until the first ripe fruit, with compact size and more than adequate prolificacy. In addition, growth and reproduction were not compromised when grown under conditions of high density and root volume limitation, showing that they are able to grow in small spaces and containers, to facilitate large-scale phenotyping and thus genetic studies. Thus, we found two cultivars suitable to represent in miniature the genetic potential of *C. annuum*, to allow and facilitate the advancement of genetic studies, and in addition with characteristics inherent and exclusive to the genus *Capsicum* such as pungency. This new model could represent a valuable contribution to the genetics of non-climacteric fruit ripening and fruit metabolism.

Keywords: Plant model. Chili pepper. Hybridization. Heterosis. Yield. Plant growth.

RESUMO

NAVES, Emmanuel Rezende, D.Sc., Universidade Federal de Viçosa, dezembro de 2020. **Criação de um novo sistema modelo para genética em *Capsicum* spp.** Orientador: Agustin Zsögön. Coorientador: Adriano Nunes Nesi.

Capsicum é um gênero apreciado por sua pungência, o que favoreceu a interação com o homem e consequentemente a domesticação. No entanto, por meio de cruzamentos, a pungência foi removida em cultivares modernas e o tamanho e a produtividade dos frutos aumentaram, resultando no que conhecemos como pimentões. Em *Capsicum* existem lacunas no conhecimento sobre fenômenos relacionados à hibridização como a heterose, utilizada para a obtenção de variedades altamente produtivas. Isso nos motivou a realizar cruzamentos intra e interespecíficos com cultivares comerciais de *C. annuum* e *C. chinense* para determinar a extensão do fenômeno de heterose. A hibridização intraespecífica mostrou-se eficiente em manter e até melhorar as características produtivas, enquanto a hibridização interespecífica promoveu o desenvolvimento vegetativo extremo em detrimento do reprodutivo. Além disso, observou-se que a reciprocidade, compatibilidade e germinação foram dependentes da direção em que os cruzamentos foram feitos e dos genótipos envolvidos, bem como da influência da fonte de pólen na frutificação, tamanho e biometria dos frutos. Os frutos de *Capsicum* não são climatéricos, o que posiciona o gênero favoravelmente para criar um novo sistema modelo para genética e fisiologia. A busca por uma cultivar produtiva, de pequeno porte e de fácil manejo cultural, que possa ser adaptada ao trabalho de laboratório, representaria um valioso primeiro passo. Com este objetivo, realizamos a varredura de um banco de germoplasma de cultivares, principalmente de *C. annuum*, com tamanho pequeno, ciclo rápido, prolificidade e produtividade com a tarefa de representar de forma confiável, mas em miniatura, o potencial produtivo e genético de *Capsicum* encontrada em cultivares comerciais. Após uma triagem inicial de 14 cultivares de *Capsicum* como candidatas à planta modelo, encontramos duas cultivares de *C. annuum* aqui denominadas 75 e CVO. Essas duas cultivares foram precoces no ciclo até a antese e até o primeiro fruto maduro, com tamanho compacto e prolificidade mais do que adequada. Além disso, o crescimento e a reprodução não foram comprometidos quando cultivados em condições de alta densidade e limitação do volume radicular, mostrando que são capazes de crescer em pequenos espaços e recipientes, facilitando a fenotipagem em larga escala e, consequentemente, os estudos genéticos. Assim, encontramos duas cultivares adequadas para representar em miniatura o potencial genético de *C. annuum*, para permitir e facilitar o avanço dos estudos genéticos, além de possuir características inerentes e exclusivas ao gênero *Capsicum* como a pungência. Este novo modelo pode representar uma contribuição valiosa para a genética do amadurecimento e metabolismo de frutos não- climatéricos.

Palavras-chave: Planta modelo. Pimenta. Hibridização. Heterose. Produtividade. Crescimento de plantas.

CONTENTS

GENERAL INTRODUCTION

The ancestor of the genus *Capsicum* originates from the Andes region and with further expansion and speciation to almost all of South and Central America, in addition to the island of Galápagos (Carrizo García *et al*., 2016). With speciation time predicted at 19.1 million of years ago (Wang *et al*., 2006). The spread of *Capsicum* is an evolutionary mechanism closely related to dispersion by birds and with a specific mechanism to prevent predation provided by pungency in only mammals (Caterina *et al*., 2000; Jordt *et al*., 2002). However, our ancestors enjoyed eating peppers due to their unique property to heat up the palate (pungency), making it the oldest crop with human interaction, ,7500 Before Christ (Macneish, 1964). The activity of pungency, exclusive to *Capsicum,* is the result of the synthesis of various compounds called capsaicinoids, being a complex metabolic pathway through the various metabolic steps, responsive to varied environmental stimuli and with many trade-offs in its production (Stellari *et al*., 2010; Naves *et al*., 2019).

Capsicum breeding programs have made great strides, from small pungent red fruits dispersed by birds (Pickersgill, 1971) to large non-pungent fruits of varying colors that barely fit in one hand (sweet pepper). For this, breeders through crosses and selections use natural variability and / or mutations, for example, as the loss of pungency to make the hot peppers in sweets (Boswell, 1937). As well as the increase in productive, nutritional value, architectural characteristics of plants, organoleptics features, fruit shape and disease resistance (Gómez-García and Ochoa-Alejo, 2013; Elitzur *et al*., 2009; Joshi and Berke 2005; Hoang *et al*., 2013). The development of new cultivars through crosses between different varieties or species (hybridization) makes more use of intuition and practical observation, since the genetic basis of hybridization and its molecular mechanisms are not well defined (Evans and Fischer, 1999). Heterosis, a phenomenon resulting from hybridization, is achieved when the appropriate genetic combination between parents produces offspring that perform better than the average among parents or in relation to the average of the best parent, being described in several crops (Groszmann *et al*., 2015).

In allogamous crops such as corn, they present the genetic phenomenon of heterosis associated with inbreeding depression, in which successive self-pollinations lead to deleterious effects on productive and morphological characters in the descendants (East and Hayes, 1912); while the appropriate combination of strains that previously reached the maximum inbreeding and later crossed produce the maximum degree of heterotic effect. Allogamous cultures do not have inbreeding, but the phenomenon of heterosis has already been described in rice (Yu *et al*., 1997; Xiao *et al*., 1995), wheat (Jiang *et al*., 2017), potatoes (Krieger *et al*., 2010) and tomatoes (Gur and Zamir, 2004). *Capsicum* species are autogamous, although cross-pollination may occur; very often, they do not have an inbreeding effect when they successively self-pollinate (Allard, 1960; Raw, 2000). The heterotic effect was observed to occur in this genus in characteristics such as productivity, precocity, plant height, fruit length and fresh fruit mass (Meshram and Mukewar, 1985; Parvinder Singh *et al*., 2014) or not depending on the combination of parents used in hybridization (Rao *et al*., 2017).

The lack of a model organism in *Capsicum* makes it more difficult to reach an adequate conclusion regarding heterosis in this genus, since the results are dependent genotypes. Thus, the studies for this phenomenon are made through various crossings to find the best combinatorial ability for the characteristic of interest, without unraveling the mechanism of heterosis itself. The development and creation of a model cultivar would allow the advancement of genetics in *Capsicum* by facilitating the comparison of results, since several studies are done on the same genetic background, as is done on tomatoes, for example.

Capsicum can be used as a model for fruit ripening (Cheng *et al*., 2016) to study the expression of several genes reported for pigments, taste, smell, texture, hormonal activity and other compounds with antioxidant activity such as vitamin C, pro-vitamin A and carotenoids exclusive to this genus (Zuo *et al*., 2019; Palma *et al*., 2105; Simonne *et al*., 1997; Gómez-García and Ochoa-Alejo, 2013). In addition, the presence of an expressive metabolic pathway in producing capsaicinoid compounds (Stellari *et al*., 2010) makes it a stimulating model for the manipulation of genes in the genus or in other cultures (Naves *et al*., 2019).

A *Capsicum* cultivar with a small plant size, fast cycle and high reproduction, will allow cultivation in small spaces, with faster advances, facilitating large-scale phenotyping genetic screening studies. All of this would contribute to the promotion of *Capsicum* in various interests such as food (vegetables and spices), ornamental, medicinal, ecological and historical. In addition, all the knowledge generated in a laboratory model background can be perfectly transferred to commercial cultivars of productive interest.

REFERENCES

Allard R.W. (1960) **Principles of Plant Breeding**. John Wiley & Sons Inc, New York, pp. 485.

Boswell V.R. (1937). **Improvement and genetics of tomatoes, peppers, and eggplant**. In Yearbook of Agriculture. Washington: United States Government Printing Office, pp. 176–206.

Carrizo García C. *et al*. (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae) **Ann. Bot.**, 118, pp. 35-51.

Caterina, M.J. *et al*. (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. **Science** 288, 306–313

East E.M. and Hayes H.K. (1912) **Heterozygosis in evolution and in plant breeding**. USDA Bull. 243.

Elitzur T. *et al*. (2009) Co-ordinated regulation of flowering time, plant architecture and growth by *FASCICULATE*: the pepper orthologue of *SELF PRUNING*. **J. Exp. Bot.,** 60, pp. 869–880.

Evans L.T. and Fischer R.A. (1999) Yield potential: its definition, measurement, and significance. **Crop Sci**. 39, pp. 1544–1551.

Gómez-García M.Delr. and Ochoa-Alejo N. (2013) Biochemistry and molecular biology of carotenoid biosynthesis in chili peppers (*Capsicum* spp.). **International Journal of Molecular Sciences**, 14(9), pp. 19025–19053.

Groszmann M. *et al*. (2015) Hormone-regulated defense and stress response networks contribute to heterosis in Arabidopsis F1 hybrids. **Proceedings of the National Academy of Sciences of the United States of America**, 2, pp. 6397–6406.

Gur A. and Zamir D. (2004) Unused natural variation can lift yield barriers in plant breeding. **PLoS Biol** 2(10): e245. https://doi.org/10.1371/journal.pbio.0020245

Hoang N.H., Yang H.B., Kang B.C. (2013) Identification and inheritance of a new source of resistance against *Tomato spotted wilt virus* (*TSWV*) in *Capsicum*. **Sci. Hortic**. 161, pp. 8–14.

Jiang Y. *et al*. (2017) A quantitative genetic framework highlights the role of epistatic effects for grain-yield heterosis in bread wheat. **Nat Genet** 49, pp.1741–1746.

Jordt S.E. and Julius D. (2002) Molecular basis for species specific sensitivity to 'hot' chili peppers. **Cell** 108, pp. 421–430.

Joshi S. and Berke T. (2005) Perspectives of bell pepper breeding. **Journal of new seeds**. 6(2-3), pp. 51-74.

Krieger U., Lippman Z.B., Zamir D. (2010) The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. **Nature Genet**. 42, pp. 459–463.

Macneish R.S. (1964) Ancient Mesoamerican civilization. **Science**, 143, pp. 531-537.

Meshram L.D. and Mukewar A.M. (1986) Heterosis studies in chilli (*Capsicum annuum* L.) **Scientia Horticulturae** 28, pp. 219-225.

Naves E.R. *et al*. (2019) Capsaicinoids: pungency beyond *Capsicum*. **Trends in Plant Science** 24, pp. 109–120.

Palma J.M. *et al*. (2015). Physiology of pepper fruit and the metabolism of antioxidants: chloroplasts, mitochondria and peroxisomes. **Ann Bot**., 116(4), pp. 627-636.

Parvinder Singh *et al*. (2014) Heterosis and combining ability for earliness, plant growth, yield and fruit attributes in hot pepper (*Capsicum annuum* L.) involving genetic and cytoplasmic-genetic male sterile lines. **Scientia Horticulturae** 168, pp. 175-188.

Pickersgill B. (1971) Relationship between weedy and cultivated forms in some species of chilli peppers (genus *Capsicum*). **Evolution** 25, pp. 683–691.

Rao P.G. *et al*. (2017) Heterosis in bell pepper (*Capsicum annuum* L.) for yield and yield attributing traits. Bangladesh **J. Bot** 46(2), pp.745-750.

Raw A. (2000) Foraging behaviour of wild bees at hot pepper flowers (*Capsicum annuum*) and its possible influence on cross pollination. **Annals of Botany** 85, pp. 487-492.

Simonne A.H. *et al*. (1997) Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). **Journal of Food Composition and Analysis**, 10, pp. 299 – 311.

Stellari G.M., Mazourek M., Jahn M.M. (2010) Contrasting modes for loss of pungency between cultivated and wild species of *Capsicum*. **Heredity**, 104(5), pp. 460–471.

Wang Y. *et al*. (2006) Euchromatin and pericentromeric heterochromatin: comparative composition in the tomato genome. **Genetics** 172, pp. 2529–2540.

Xiao J. *et al*. (1995). Dominance is the major genetic-basis of heterosis in rice as revealed by QTL analysis using molecular markers. **Genetics** 140, pp.745–754.

Yu S.B. *et al.* (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. **Proc. Natl. Acad. Sci**. USA, 94: 9226.

Zuo J. *et al.* (2019) Network analysis of noncoding RNAs in pepper provides insights into fruit ripening control. **Sci Rep** 9: 8734.

CHAPTER 1

Creation of new model system for genetics in *Capsicum* spp.

Abstract

The *Capsicum* genus is composed of about 41 species with a well-resolved monophyletic classification, with many basal and other derived species in the group. Therefore, capturing the diversity from such a diverse genus and, in addition, with many ecological interactions, is not an easy task. The five domesticated species in *Capsicum* belong to the most derivative group, mainly the clade Annuum, which includes sweet peppers (*C. annuum* var. a*nnuum*). With the market and horticultural interest mainly focused on sweet peppers, nothing is more representative than the choice of a cultivar of *C. annuum* for genetic studies. Establishing a cultivar of *C. annuum* as a biological model would have the benefit of standardizing a single genetic background, offering practicality by the possibility of comparing several studies such as agronomic traits of productivity and plant architectures, improvement of nutritional compounds, qualities and size of fruits, ripening pattern and the exclusive secondary metabolism of pungency. To fulfill this aim, the cultivar of choice should have a short life cycle, grow vigorously, be prolific, produce numerous and adequately sized fruits and, fundamentally, have a small size. We have screened a series of such cultivars and found two cultivars that have potential as a laboratory model plants, with excellent characteristics that stood out, including fruit pungency, for which peppers are so well known and appreciated. We describe the process and criteria used in this selection and discuss the challenges that remain ahead to establish them as model cultivars.

Introduction

Prior to the genomics age, plants had already been used extensively as genetic and physiological model organisms: since the direct genetic studies of Gregor Mendel with peas (1865); through the tissue culture techniques for tobacco (Murashige and Skoog, 1962); up to the first instances of genetic engineering in petunia (Fraley *et al.*, 1983). Ever since the complete sequencing of the Arabidopsis genome (Genome initiative, 2000), it has become more attractive and easier to work with this species because it provides a valuable database, especially regarding the feasibility of direct (from phenotype to genotype) and reverse (from genotype to phenotype) genetics tools. Allied to this, individuals of this species have a small genome size, about 125 MB (Mega base pairs), making the association of genes with their respective functions less complex and more straightforward.

Until the advent of the next generation sequencing (NGS), efforts to sequence the full genome of an organism were hampered by financial, labor and time requirements (Vandenbussche *et al.*, 2016). Technical breakthroughs since 2000, when the first draft of the Arabidopsis sequence was published, have allowed the assembly of 181 sequenced genomes of plant species of horticultural interest (Chen *et al.*, 2019). This expanded availability in the sequence databases has challenged the position of some model organisms for large groups, to which they were initially proposed (Alfred and Baldwin, 2015). For instance, Arabidopsis for angiosperms in general: it lacks fleshy fruits, large leaves, glandular trichomes and mycorrhizal associations (Meissner *et al*., 1997).

Within this context, model organisms have emerged to explore the genetics and physiology of more particular groups. In the Solanaceae family, which comprises *Capsicum*, crop models are already well established as tobacco for tissue culture and genetic transformation (Ganapathi *et al*., 2004); potato as a reference for mechanisms related to tuberization (Xu *et al*., 2011); tomato for fresh fruits and climacteric behavior of fruit ripening (Meissner *et al*., 1997) and, more recently, the reinforcement of petunia for flower color, mycorrhization and phylogenetic studies in Asterids (Vandenbussche *et al*., 2016).

Species of the *Capsicum* genus stand out for their remarkable rich secondary metabolism. It is a genus recognized mainly for the varied capsaicinoid compounds that confer fruit pungency, a highly appreciated trait with culinary and pharmaceutical applications (Stellari *et al*., 2010). Although it is an exclusive characteristic of the genus, it does not cover all species, since the most basal representatives do not have it and some more derived species have undergone reversion to the non-pungent ancestral state (Carrizo Garcia *et al*., 2016). The genetic, physiological and evolutionary mechanisms that contributed to the emergence of pungency exclusively within the genus *Capsicum* or even the conservation of some related routes in plants within the Solanaceae family such as tomato are until now unknown. The complexity of this biochemical pathway, with the involvement of many genes, influenced by several regulatory mechanisms and responsive to abiotic and biotic stresses, makes molecular characterization and understanding difficult, so many studies will still be needed (Naves *et al*., 2019). The creation of a suitable biological model would greatly improve the feasibility and throughput of such studies.

Capsicum does not have a model cultivar, although many genetic studies have been conducted with this important culture. Many genes already discovered in different backgrounds, with their respective functions (Wang and Bosland, 2006). In addition, different research groups have been working with genetic characterization in this genus, mainly in *C. annuum*, but in different genetic backgrounds, which makes comparisons difficult between studies. Efforts have been made to characterize the genome of more species and cultivars, as characterization of the structural diversity of the genome helps discover genes and their molecular mechanisms (Chen *et al*., 2019). Thus, genome data from several cultivars or varieties of *Capsicum anuuum*, *C. chinense* and *C. baccatum* (Kim *et al*., 2014; Qin *et al*., 2014, Kim *et al*., 2017, Acquadro *et al*., 2020) has been made available.

In addition, *Capsicum* as a genetic model can foster an avenue of research for numerous other secondary compounds, such as several types of carotenoids, in addition to exclusive carotenoids of this genus such as capsanthine and capsorubine (Wahyuni *et al*., 2011). In the nutritional aspect, *Capsicum* is also an excellent source of other compounds such as provitamin A, vitamins C, E, B1, B2 (Wahyuni *et al*., 2011) and anthocyanins (Aza-

González *et al*., 2012). However, studies are lacking about genes encoding regulatory proteins and how they regulate the synthesis of these compounds, as well as the use of genetic engineering to increase the content of carotenoids (Gómez-García and Ochoa-Alejo, 2013) and other nutritional compounds.

Here, we describe all the steps to obtain a cultivar of *C. annuum* as a potential genetic model of the laboratory and all the ideal characteristics that a plant with this proposal should have. Among all the genetic variability existing in horticultural germplasm banks, we believe that there is a *Capsicum* with good characteristics such as small size, fast cycle, easy cultivation, high prolificacy and with fruits with adequate size and, in addition, to be pungent. Cultivars of *C. annuum* such as CVO and 75 are suitable for this important task.

Material and Methods

Part I: Screening of 14 *Capsicum* sp. cultivars as a potential model plant

Plant material

The Annuum clade of the *Capsicum* genus (*C. annuum*, *C. chinense*, *C. frutescens* and *C. galapagoense*) includes three of the five domesticated pepper species (*C. annuum, C. chinense*, *C. baccatum*, *C. frutescens* and *C. pubescens*) (Pickersgill, 1997). Bell pepper (*C. annuum* var. *annuum*) is considered one of the main crops for fresh consumption as a vegetable (Biswas *et al*., 2017) and *C. chinense* as a spice for its pungency and rich red color (Baruah and Lal, 2020). In addition to this clade, there are species with a large number of varieties cataloged only in USA germplasm banks, which represents a source of diversity for breeding programs, with about 3,407 accessions of *C. annuum* and 481 of *C. chinense* – (US National Plant Germplasm System, 2017).

We screened the horticultural germplasm bank (BGH) of the Federal University of Viçosa, recognized for the diversity of accessions of peppers, for seeds mainly of cultivars of *C. annuum*, and selected 13 accessions, here called: 75 (a), CVO (b), MC2-8 (c), PUR (d), 48 (e), MC3 (f), MC2 (g), 46 (h), 76 MG (i), CAO (j), WHI (k), 49 (m) and 47 (n) (Fig 1). And as a representative of the enormous diversity of *C. chinense* found in Brazil (Finger *et* *al*., 2010), cv. Biquinho (BIQ) was chosen, due to its horticultural characteristics and economic importance.

Initial screening

The initial screening consisted of selecting cultivars with a compact size, suitable for growth in small plastic pots, short life cycle, and good fruit productivity. In all, we worked with a sample number of six replicates per cultivar, grown in plastic pots with a capacity of 1.5 L filled with commercial substrate Tropstrato® plus dolomitic limestone (4 g L^{-1}) and NPK in the formulation 10-10-10 (4 g L^{-1}). The plants were grown in semi-controlled greenhouse conditions, in the spring and summer seasons between 2016 and 2017, located at the Federal University of Viçosa, Minas Gerais.

The cultivars were characterized at moment of harvest for plant size: total plant height (cm), total plant diameter (cm), height: diameter ratio, height to first flower (cm) and number of leaves until 1st flower; vegetative cycle: cycle until anthesis (days), cycle of sowing to 1st ripe fruit (days) and fruit development time (days); fruit productivity: number of fruits per plant and total fresh weight of fruits per plant (g); fruit biometry: fresh weight per fruit (g), fruit length (mm), fruit diameter (mm) and shape index fruit; and prolificacy: number of seeds per fruit, number of seeds per plant, fruit set index (%) and parthenocarpy fruit index (%).

The criterion to determine when the ripening of the first fruit was due to the change of color to the specific color of each cultivar (Fig 6), as well as the softening of the pericarp. The harvest time was determined when more than 70% of the fruits of the first fruiting cycle were ripe, and it was made at different times for each cultivar, as each one has different cycles. For fruit biometrics, at least 30 fruits were measured per plant, totaling 180 fruits per cultivar. Individual fruit was measured in fresh mass (g), diameter (mm), length (mm) and fruit shape index. For characteristics such as prolificacy, the number of seeds per fruit and the percentage of seedless fruits were estimated. Characteristics such as fruit set (%) and fruit development time were evaluated by marking at least 20 flowers per repetition, estimating how much of these marked flowers gave rise to fruits (fruit set). And for these same fruits, the time required for complete development (time from anthesis to the complete ripening of the fruit) was counted.

Part II: Behavior of *Capsicum* cultivars grown in high density combined with root limitation and grown in different pot volume / root limitation

After the initial screening of 14 *Capsicum* cultivars, three *C. annuum* cultivars (75, MC2-8 and CVO) were chosen as potential candidates as a model plant. While larger cultivars of *C. annuum* as MC2 and the cultivar of *C. chinense* BIQ were maintained for later growth and productivity tests as a benchmark of inappropriate behavior as a model plant.

Simulating high density growth conditions such as laboratory conditions or conditions required for large-scale phenotyping with reduced space and small containers, we grow the plants in sowing trays, which provide competition and development conditions imposed by the high density of plants associated with limitation root (small capacity of substrate contained in each cell). In this test, the plants were grown in two sizes of trays suitable for sowing, both with 30 cells each. A tray with capacity in each cell of 40 mL for filling with substrate. The dimensions of this tray were 20.7 × 25.2 cm, resulting in a density of one plant per 17.4 cm² (560 plants m^{-2}). In a second tray with a capacity of 120 mL per cell and dimensions of 27.5 × 32.5 cm the density was of one plant per 29.8 cm² (343 plants m^{-2}). At the time of harvest and evaluation, each plant constituted a replicate, with a total of 12 plants being quantified in the central portion of each tray, discarding the plants from the border to avoid edge effects. As control conditions of each tray at least 12 plants per cultivar were grown in a corresponding volume of substrate, however with free growth, that is, in the absence of the density treatment. Thus, for each root volume (40 or 120 mL) the effect of density was compared under growth control conditions (free standing).

For these same cultivars of *C. annuum* (75, MC2-8, MC2 and CVO) and *C. chinense* (BIQ), experiments were carried out with different volumes of substrate, to verify their growth and productivity and, with that, indicate the volume of container most suitable for cultivation. For each cultivar, five pot sizes were used with ten replicates each: 50, 150, 350, 700 and 1500 mL. All cultivars were grown in a glasshouse in semi-controlled conditions, in the spring and summer seasons, between 2017 and 2018.

Both tests (density and pot size) were repeated a second time. The standard formulation of commercial substrate Tropstrato® plus limestone (4 g L^{-1}) and N-P-K was used in formulation 10-10-10 (4 g L^{-1}) and in addition 10 g L^{-1} of controlled release Osmocote® fertilizer was used in formulation 14-14-14 (N-P-K). Due to the reduced volume of substrate, all plants were supplemented weekly with Hoagland's nutrient solution (5 mL per plant). Irrigation was carried out daily. All plants were measured at the time of harvest using parameters related to size: total plant height (cm) and total plant diameter (cm); productivity: number of fruits/plants, total weight of fresh fruit (g/plant); fruit biometry: fresh weight per fruit (g) and prolificacy: number of seeds per fruit; and the cycle was quantified: days before anthesis (days), days for the 1st ripe fruit. In addition, the total leaf area per plant was measured in a leaf area meter (LiCor-3100, Lincoln, Nebraska, USA), the total allocation of dry biomass and the partition between aerial and root biomass. The root volume was also estimated with the aid of a graduated cylinder.

Part III: Comparison between cultivars of *C. annuum* (75, MC2-8 and CVO) and the miniature model cultivar of *Solanum lycopersicum* cv. Micro-Tom

After we selected three cultivars of *C. annuum* (75, MC2-8 and CVO) as candidates for potential genetic models we carried out a comparison with the established tomato (*S. lycopersicum*) model cultivar Micro-Tom (MT), to subsidize the comparison of favorable parameters present in this cultivar. For this we evaluate these cultivars from germination to ripe fruit, as described below:

Plant material

For analysis of germination potential and average germination time, germination tests were performed, with three replicates with 30 seeds each. The seeds were sown in a commercial substrate and kept in greenhouse conditions (March, 2020). The germination evaluation was established based on the criterion of the complete emergence of the seedlings, with the count being made daily until 15 days after sowing. The calculation of the average germination time was done as established by Ranal *et al*. (2009).

For plant growth, the same substrate and fertilization protocol described in previous topics was used, however for this experiment the plants were grown in plastic pots with a capacity of 350 mL of substrate, with a total of 10 plants (repetitions) per genotype. The use of the 350 mL pot size was due to the observation of the pot volume experiment, in which these smaller cultivars did not compromise the individual fruit mass per plant. Furthermore, this is the ideal growth volume for which cv MT was bred (Meissner *et al*., 1997). The plants were grown in semi-controlled greenhouse conditions, from December 2019 to June 2020, at the Federal University of Viçosa, MG.

Characteristics such as precocity were quantified: cycle until anthesis (days), cycle of sowing to 1st fruit ripe (days), fruit development time (days). At harvest time, vegetative characteristics were measured: height to first flower (cm); total plant height (cm), total plant diameter (cm), number of leaves until 1st flower, stem diameter (mm), number of side branches, dry vegetative biomass (g), ratio shoot/root, root volume (mL), total leaf area (cm^2 $plant^{-1}$), total specific leaf area ($g\ cm^{-2}$) per plant and productive characteristics: fruit set index (%), number of fruits per plant, ripe fruits at harvest (%), fresh weight fruits ($g\ plant^{-1}$), reproductivity/vegetative dry biomass ($g\ plant^{-1}$), fresh weight per fruit (g) and number of seeds per fruit.

Part IV: Development of fruits of cultivars of *C. annuum* (75, MC2-8 and CVO) and its relation to ethylene production in different phenological phases

Plant material

For this experiment, 40 plants of each genotype (75, MC2-8 and CVO) were grown in 350 mL plastic pots with the same fertilizer formulation and cultural treatments described above. The plants were grown in greenhouse conditions during the period from January to July 2019. After the plants reached the reproductive stage, successive flower markings were made each week to obtain fruits in all phenological phases, from anthesis to the complete ripening of the fruits. For this, seven dates were sampled until the full ripening of the fruits, being 10, 20, 30, 40, 50, 60 and 70 days after anthesis (DAA).

Fruit biometrics

For each sample time, at 10, 20, 30, 40, 50, 60 and 70 (days after anthesis), at least the 20 largest fruits of each cultivar were measured. These fruits had their fresh and dry mass quantified (g), the volume measured in graduated cylinder by the water displacement method (mL), the length (mm) and the diameter measured in the most dilated portion of each fruit (mm).

Quantification of ethylene

The fruits of the cultivars in different stages were harvested at 10, 20, 30, 40, 50, 60 and 70 days after anthesis. On average, 4 fruits were harvested and incubated per Erlenmeyers flask, totaling 4 replicates per flask per genotype on each date. The volume of the flask used was dependent on the size of the fruits, with fruits in the initial stages incubated in 25 mL flasks, medium in 50 mL and many large in 125 mL. Each fruit had its fresh weight (g) and volume (mL) quantified. The flasks containing the fruits were sealed with a rubber stopper and kept in the laboratory at 25 ºC. The samples were incubated for a period of 12 hours, after which with the aid of a 10 mL syringe with a needle, the air was stirred inside the flask. After, an aliquot of air was collected with a 1 mL syringe and needle inside each flask of equal value, taking care to seal the syringe needle after collection so as not to escape the gas. Afterwards, this volume of air was injected in a gas chromatograph (Hewlett Packard 5890, Series II), according to the methodology described by Ribeiro *et al*. (2010).

In addition, the fruits harvested on each date were photographed and after the quantification of ethylene they were kept at room temperature for seven days to observe whether, depending on the phenological phase, they would change color or not. After these seven days, they were photographed again.

STATISTICAL ANALYSIS

The data in most cases were submitted to analysis of variance (ANOVA) and when significant, they were submitted to the Scott Knot test at 5% probability. For density tests in addition to ANOVA, the Scheffe's test was applied comparing the absolute values of density and density free. For the pot size assays (50, 150, 350, 700 and 1500 mL), the Genes program (Cruz, 2013), version 1990.2019.91, was used to verify which regression model was most appropriate and its significance. In addition, principal component analysis (PCA) was performed to verify the behavior/grouping of cultivars. The graphics were made by SigmaPlot software v.14.

Results and discussion

Part I: Screening of 14 cultivars as a potential laboratory model plant

Plant architecture

Among the 14 varieties, we can notice differences in various aspects such as canopy architecture, leaf and fruit morphology, as well as more or less horizontal, vertical growth habits or balance between them (Fig. 1). We found cultivars with compact size (a, b, c, d and f), medium-size (e, g, h, i, j, k), and large (l, m, and n). Despite the different size, all cultivars grew and yielded properly, showing adaptability. In addition, the architecture in *Capsicum* is different from other models in Solanaceae morphologically and in countless processes, for example, regarding meristem maturation, dichotomic forked shoot habit, flower and leaf formation and differentiation, growth in height and more pronounced secondary growth (Park *et al*., 2014; Cohen *et al*., 2014; Shleizer-Burko *et al*., 2011; Carrizo Garcia *et al*., 2016).

Life cycle

Shorter cycle plants ensure faster genetic advances, an important characteristic within the context of plant models (Meissner *et al*., 1997). In *Capsicum*, the characteristic sowing time at the opening of the first flower is associated to the appearance of the first flower, which occurs at the first node, and related to the precocity of the cultivars. We found early cultivars (WHI, 75, MC3), and late ones (BIQ, MC2-8 and MC2) (Fig. 2a). Although the genetic basis of these differences is not known for these 14 cultivars, genes related to meristematic control and floral identity have already been characterized, with some genes preventing, delaying or even anticipating flowering in *Capsicum*, as for *CaANANTHA*, *C-S* and *CaJOINTLESS*, *FASCICULATE*, *CaBLIND* (Cohen *et al*., 2014; Cohen *et al*., 2012; Elitzur *et al*., 2009; Jeifetz *et al*., 2011).

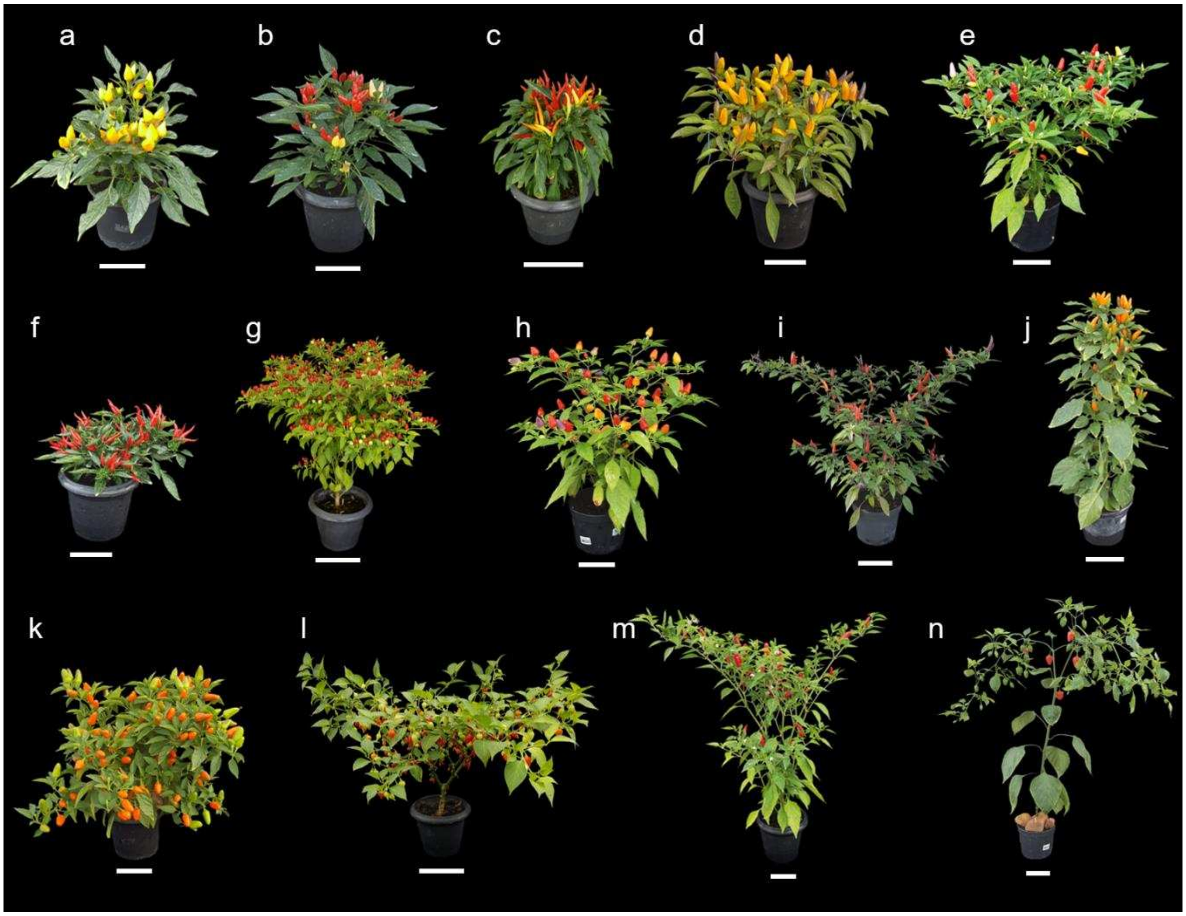


Figure 1. Morphological aspects in the full reproductive stage (more or less 150 days after sowing) of *C. annuum* plant varieties (**a**, **b**, **c**, **d**, **e**, **f**, **g**, **h**, **i**, **j**, **k**, **m** and **n**) and *C. chinense* (**l**) with potential as laboratory model plant. Name of cultivars: **a**) 75, **b**) CVO, **c**) MC2-8, **d**) PUR, **e**) 48, **f**) MC3, **g**) MC2, **h**) 46, **i**) 76 MG, **j**) CAO, **k**) WHI, **l**) BIQ **m**) 49 and **n**) 47. Scale bars 10 cm.

Early anthesis is indicative of precocity, however, it should not be considered in isolation. Characteristics such as the development time of each fruit (Fig. 2D), as well as the days required between the first open flower and the first ripe fruit (Fig. 2C), which may represent the delay of the fruit set, contribute to timeframe required for ripening of the first fruit (Fig. 2B). Therefore, cultivars such as 75, MC3, CVO, WHI and PURP accumulated characteristics that contributed to early ripening. This trait has not yet been properly defined for *Capsicum* as in tomato, where it is associated with plant architecture, flowering rate and fruit size (Gur *et al*., 2010; Wang *et al*., 2020).

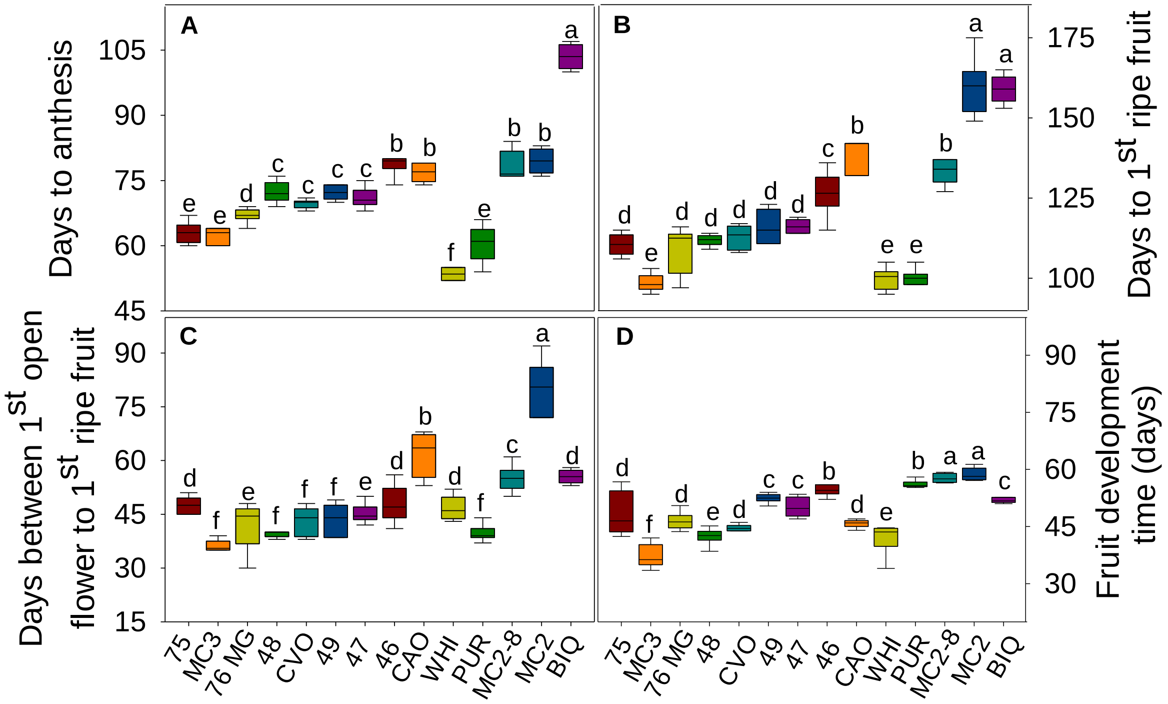


Figure 2. Variation of parameters related to the phenological cycle of thirteen cultivars of *C. annuum* (75, MC3, 76 MG, 48, CVO, 49, 47, 46, CAO, WHI, PUR, MC2-8 and MC2) and one cultivar of *C. chinense* (BIQ). **A**) Time in days of sowing to the opening of the first flower; **B**) Time in days of sowing to the ripening of the first fruit; **C**) Time in days between the opening of the first flower and the ripening of the first fruit; **D**) Time required, in days, for the complete development of the fruit (from anthesis to ripe fruit). For the graphs **A**, **B** and **C** median values, maximum amplitude and minimum of 6 repetitions per cultivar. For graph **D**, median values of maximum and minimum amplitude for on average 180 fruits per cultivar. Averages indicated by different letters differ from each other by the Scott-Knott Test at a 5% probability level.

Plant size

Plant model organisms can vary in size, depending on the purpose. As in a tomato with a larger field cultivar (M82) and a small one as a laboratory model (MT). For laboratory model plants, they are expected to be small enough to be grown in small pots, save substrate, space, facilitate transport and accommodation in environments with reduced space. In this sense, MT was proposed as a model laboratory plant (Meissner *et al*., 1997), although its initial purpose was ornamental (Scott and Harbaugh, 1989). Just as MT has a small size due to dwarfing (Marti *et al*., 2006), there are already small pepper cultivars due to mutations related to dwarfing too (Wang and Bosland, 2006), created with ornamental purposes.

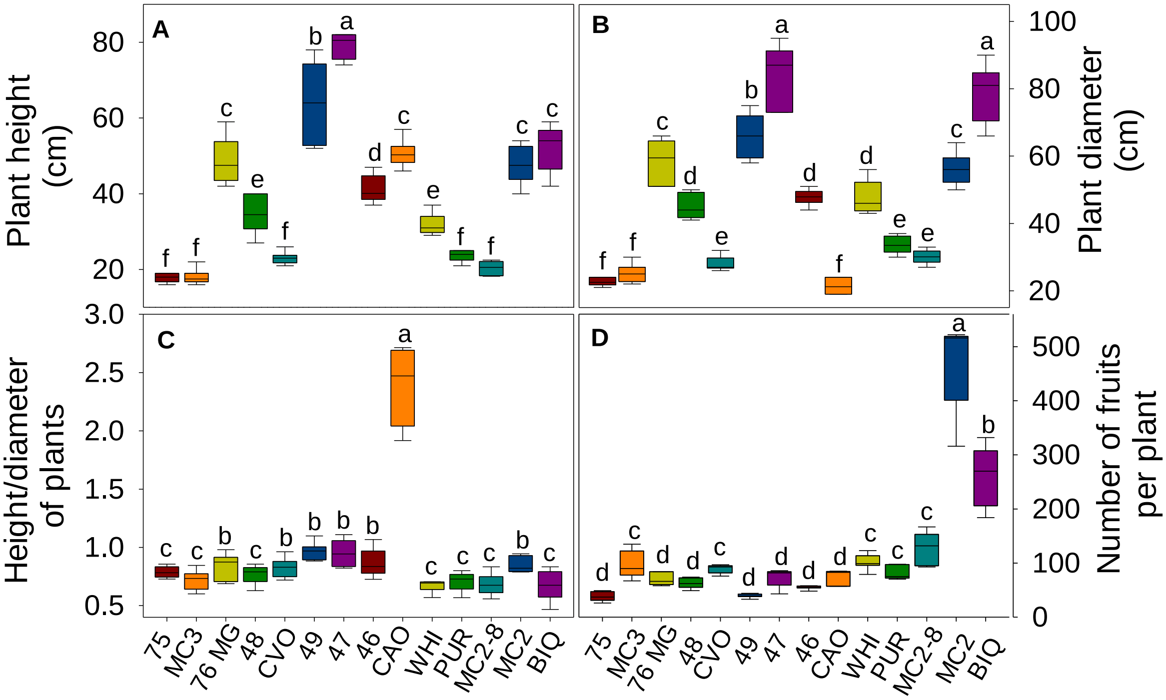


Figure 3. Variation of parameters related to the dimensions and productivity of thirteen cultivars of *C. annuum* (75, MC3, 76 MG, 48, CVO, 49, 47, 46, CAO, WHI, PUR, MC2-8 and MC2) and one cultivar of *C. chinense* (BIQ). **A**) Total height (cm) between the basal region of the stem and the apex of fruits (when erect) or vegetative meristem or leaves; **B**) Overall diameter of canopy (cm) in the wider region; **C**) Proportion between height and diameter values; **D**) Total productivity in number of fruits per plant. For the graphs **A**, **B**, **C** and **D** values of median and maximum amplitude and minimum of 6 repetitions per cultivar. Averages indicated by different letters differ from each other by the Scott-Knott Test at a 5% probability level.

The adequate proportion between vertical (height) and horizontal (diameter) growth can be measured by the ratio between these variables and with adequate values when close to one (Bird and Conner, 1999). With the exception of the CAO cultivar, all others cultivar presented an adequate proportion (Fig. 3C). Tall cultivars (Fig. 3A) and with a large diameter (Fig. 3B) were found to have a ratio close to one (Fig. 4D). Increased horizontal growth at the expense of vertical led us to disregard WHI, while higher vertical growth, compared to smaller cultivars, led us to discard PUR, despite otherwise excellent life cycle characteristics. The compact size of MC2-8 made it a strong potential model candidate, while 75, MC3, CVO increased in positive characteristics as a model plant. These potential cultivars of more compact growth (Fig. 1) have a phenotypic pattern similar to that of the *fasciculate* mutant characterized by Elitzur *et al*. (2009), in which the plants are compact with determinate growth, shorter cycle and fruits grouped in clusters.

Plant productivity and prolificacy

Cultivars such as MC2 and BIQ or stand out for the high number of fruits (Fig. 3D), fresh fruit yield per plant (Fig. 4A), number of seeds per fruit and total number of seeds per plant (Fig. 5C and D). These traits have great weight for a plant model (Meissner *et al*., 1997), so we decided to include them as potential candidates, because even though they have undesirable traits such as longer cycle and larger size, these are within an acceptable limit.

Smaller cultivars such as 75, MC3, CVO and MC2-8, have a much lower number of fruits than larger plants such as BIQ and MC2. This is closely related to lower support capacity of smaller plants. For example, tomato plants that grow more before the determination period are more productive (Gur *et al*., 2010). Even so, these smaller cultivars have adequate production both in number and in fresh weight of fruits per plant compared to others of the same size (Figs. 3D, 4A).

Prolific characteristics are closely related to the number of fruits produced per plant, number of seeds per fruit and the total number of seeds produced per plant. The fruit set characteristic (Fig. 5A), although it may be related to the total number of fruits produced per plant, is not predictive because it is a punctual and highly variable characteristic.

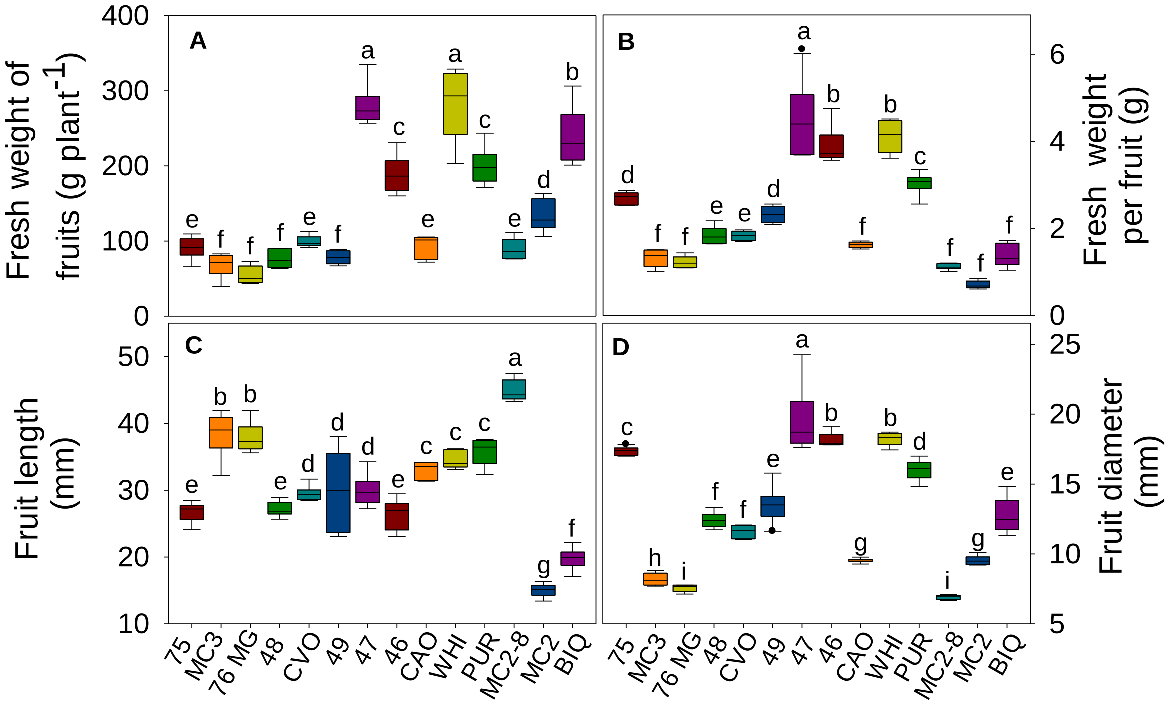


Figure 4. Variation of parameters related to fruit size and productivity in thirteen cultivars of *C. annuum* (75, MC3, 76 MG, 48, CVO, 49, 47, 46, CAO, WHI, PUR, MC2-8 and MC2) and one cultivar of *C. chinense* (BIQ). **A**) Total productivity in fresh fruit mass per plant (g); **B**) Fresh weight per fruit (g); **C**) Length between the apex and the fruit base (mm); **D**) Diameter in the most dilated portion of the fruit (mm). For the graphs **A,** values of median and maximum amplitude and minimum of 6 repetitions per cultivar. For graph **B**, **C** and **D** median values of maximum and minimum amplitude for on average 180 fruits per cultivar. Averages indicated by different letters differ from each other by the Scott-Knott Test at a 5% probability level.

Fruit set in *Capsicum* is influenced by hormones such as auxin (Robert *et al*., 2019), ethylene (Huberman *et al*., 1997) and gibberellin (Tiwari *et al*., 2012), by abiotic factors such as high temperature and cold - above 35 and below 15 - (Upreti *et al*., 2012; Mercado *et al*., 1997), for the balance and transport of sugars (Ávila Silva *et al*., 2019a, Ávila Silva *et al*., 2019b) and for the source and sink relationship (Marcelis *et al*., 2004).

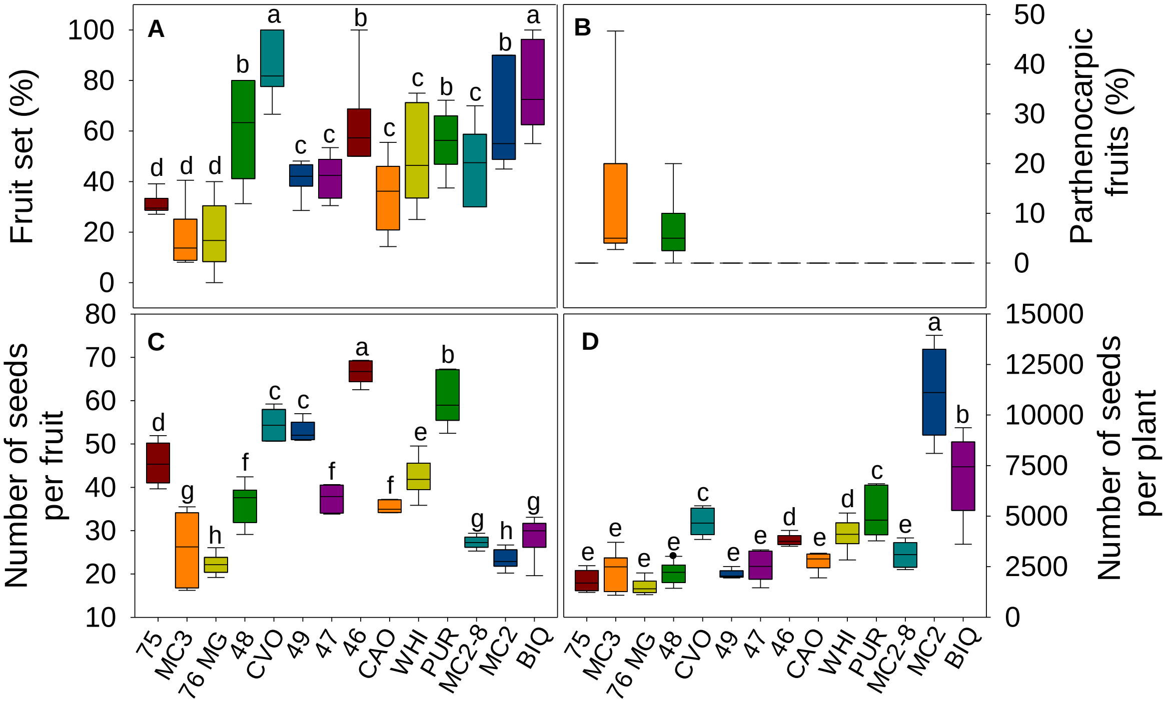


Figure 5. Variation of parameters related to prolificacy in thirteen cultivars of *C. annuum* (75, MC3, 76 MG, 48, CVO, 49, 47, 46, CAO, WHI, PUR, MC2-8 and MC2) and one cultivar of *C. chinense* (BIQ). **A**) Percentage of fruit set per plant (%); **B**) Percentage of parthenocarpy fruits per plant (%); **C**) Number of seeds per fruit; **D**) Estimated number of seeds per plant. For the graphs **A,** values of median and maximum amplitude and minimum of 6 repetitions per cultivar. For graph **B**, **C** and **D** median values of maximum and minimum amplitude for on average 180 fruits per cultivar. Averages indicated by different letters differ from each other by the Scott-Knott Test at a 5% probability level.

As noted, even though CVO had a fruit set much higher than MC3, they had equivalent number of fruits per plant (Figs. 5A and 3D). While cultivars such as MC2 and BIQ show that the high fruit set can contribute to high productivity in number of fruits per plant (Fig. 3D). Traits such as the low fruit set, associated with a high percentage of parthenocarpic fruits and, therefore, a low number of seeds were crucial in the disregard of MC3 as a potential model candidate, although there were many qualities. The sum of positive characteristics related to prolificacy or even the existence of compensation mechanisms between them, resulted in optimal to adequate total numbers of seeds produced per plant for our potential candidates as a genetic model: MC2, BIQ, CVO, 75 and MC2-8.

Fruit morphology

Screened cultivars differ in fruit morphology (Fig. 6), as expected for *C. annuum* and *C. chinense* (Naegele *et al*., 2016, Alvares-Bianchi *et al*., 2020). The size of the fruit in relation to the size of the plant can be considered small for BIQ, MC2 and large fruits for 75, CVO and MC2-8 (Fig. 6). The variation in size is well represented by the amplitude of the fresh mass (Fig. 4B), length (Fig. 4C) and diameter (Fig. 4D) of fruits. The differentiation of the diameter along the fruit (apical, median and basal portion) together with the length is responsible for the different shapes such as cylindrical for MC2-8 (Fig. 6D), spherical for MC2 (Fig. 6G) and conical fruits for 75 and CVO (Figs. 6A and B), for example.

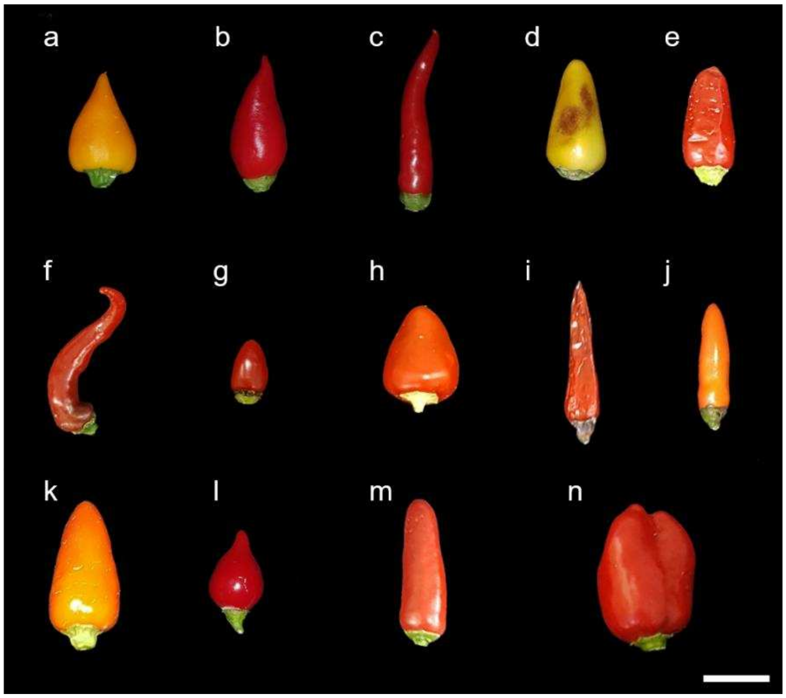


Figure 6. Variation in morphological aspects of fruits for cultivars of *C. annuum* (**a**, **b**, **c**, **d**, **e**, **f**, **g**, **h**, **i**, **j**, **k**, **m** and **n**) and *C. chinense* (**l**) with potential as laboratory model plant. Name of cultivars: **a**) 75, **b**) CVO, **c**) MC2-8, **d**) PUR, **e**) 48, **f**) MC3, **g**) MC2, **h**) 46, **i**) 76 MG, **j**) CAO, **k**) WHI, **l**) BIQ, **m**) 49 and **n**) 47. Scale bar 2 cm.

The diversity is also present in different colors, which is closely related to the degradation, synthesis and accumulation of different classes of pigments as chlorophylls, anthocyanins and carotenoids (Figs. 1 and 6). The multiplicity of fruit morphological characteristics provides excellent base material for the study of many genetics with fruit expansion and geometry (Colonna *et al.*, 2019), as well as multiple metabolic pathways for synthesis, degradation and *de novo* synthesis of several pigments (Li *et al.*, 2013; Filyushin *et al.*, 2020). Finally, we verified adequate behavior for three cultivars of *C. annuum* as candidates for the laboratory model plant, with cultivars 75, CVO, MC2-8 being chosen from the 14 cultivars of *Capsicum*. We opted to keep the larger cultivars of *C. annuum* (MC2) and the BIQ cultivar of *C. chinense* for the next stages of screening as controls for inappropriate growth behavior of cultivars as candidates for laboratory model plant.

Part IIa: Behavior of *Capsicum* cultivars grown in high density combined with root limitation

The cultivars showed a satisfactory visual appearance even when grown under extreme conditions of density and root limitation as in sowing trays (Figs. 7 and 9). Trays of this type, were developed for sowing and plant maintenance for a maximum of one month after germination and not as here, almost 6 months. The root limitation imposed by the 40 mL pot volume of the highest density tray was large. It is usual to use pots with a volume of 60 mL for the growth of *Arabidopsis* plants throughout their life cycle (Zhou *et al.*, 2017), estimated at around 60 days (Krämer, 2015). However, our goal is not to compare *Capsicum* with *Arabidopsis*, since *Capsicum* has greater complexity in life cycle in relation to *Arabidopsis* or even in comparison to tomato. Hereafter, when we speak of a 40 mL tray, we are referring to plants grown in 40 mL cells and a density of 560 plants m^{-2}, while in a 120 mL tray it refers to the growth of plants in 120 mL cells and with density of 343 plants m^{-2}. The measurement of parameters related to the entire cycle allowed the identification of different behaviors, as discussed below.

Life cycle

Characteristics such as the cycle until flowering had a relative increase for all cultivars when in a 40 mL tray, although it was of lower magnitude for MC2, CVO and MC2-8. Cultivation in a 120 mL tray also increases the BIQ cycle, which is already long, while cultivars such as MC2 with significant reduction. The cycle until the first fruit matures in a 40 mL tray cannot be determined in BIQ due to the absence of fruiting, while little has changed for the other cultivars. In 120 mL, the trend was the increase of the cycle, although again not very significantly in CVO, while only decreasing in MC2.

The reduction or increase in the time required for the first fruit to ripen was due to the influence of density and root limitation on anthesis and the time for the complete development of the first fruit. We believe that both responses, precocity as well as the prolongation of the cycle, may result from different responses of each genetic background (Anderson *et al.*, 2014) in relation to the severity of the stresses tested here. We observed that smaller cultivars (75, MC2-8 and CVO) showed a more stable behavior from the cycle until the first ripe fruit, similar results were found for MT grown in different densities and volume of containers (Meissner *et al.*, 1997).

Plant size

The perception and response to growth in trays proved to be genotype-dependent, as expected. Plants in dense environments perceive changes in photosynthetically active radiation (PAR) and ratio of red to far-red wavelengths (R:FR) and thus respond with changes in height and plant diameters (Crepy and Casal, 2015; Gruntman *et al.*, 2007).

The absence or little increase in height for BIQ, of greater natural size, exemplifies that the root restriction may have been more limiting than the density. In the opposite direction MC2, in spite of the natural height, was able to shape the height in response to the density in each tray size. Smaller plants such as CVO and 75, with mutations of determination (*fasciculate*) and dwarfism increased in height by the lengthening of the nodes in the different tray sizes (Figs. 8 and 10), being considered an avoidance response to shading.

Table 1. Relative values of increase or decrease for growth and reproduction parameters for *C. annuum* cv. (75, CVO, MC2-8 and MC2) and *C. chinense* (BIQ) grown at different densities and in pot volume compared to control conditions (free standing).

560 plants m^{-2} and 40 mL					343 plants m^{-2} and 120 mL				
75	**CVO**	**MC2-8**	**BIQ**	**MC2**	**75**	**CVO**	**MC2-8**	**BIQ**	**MC2**
Days to anthesis									
17.0 ** b	6.5 ** c	8.3 ** b	21.1 ** a	2.7 * d	-0.5 b	19.1 ** c	-10.9 ** b	32.4 ** a	-20.7 ** d
Days to 1º ripe fruit									
10.1 ** c	6.8 ** b	5.8 ** a	-100 ** a	0.47 d	24.5 ** c	6.9 ** c	23.6 ** b	24.7 ** a	-15.4 ** d
Plant height (cm)									
39.4 ** d	56.2 ** c	11.5 ** e	-1.36 a	44.0 ** b	27.3 ** d	27.7 ** c	24.6 ** d	14.5 ** a	142 ** b
Plant diameter (cm)									
14.7 ** b	7.2 c	11.3 *d	-11.0 * a	9.2 ** b	38.5 ** c	15.7 ** c	46.6 ** d	-31.4 ** a	105 ** b
Dry vegetative biomass (g plant^{-1})									
-28.4 ** c	-40.5 ** b	-46.9 ** c	-52.4 ** a	-55.7 ** b	-20.1 ** d	-0.04 c	-18.4 ** d	49.6 ** a	80.1 ** b
Root volume (mL)									
-39.6 ** c	-49.0 ** b	-43.9 ** c	39.8 ** a	-54.1 **c	-30.5 ** d	15.2 ** b	-13.0 ** d	-29.4 ** a	13.8 ** c
Ratio shoot /root									
2.8 b	-0.93 b	5.42 b	1.19 b	49.6 ** a	25.8 * b	-12.0 ** c	0.28 c	-62.1 ** c	74.4 ** a
Leaf area (cm^2 plant^{-1})									
4.86 c	-26.8 ** b	-25.6 ** c	-32.3 * a	-51.2 ** b	3.22 c	17.13 * b	-18 ** d	-60.7 ** a	-12.8 * b
Number of fruits per plant									
-20.9 * c	-53.2 ** c	-41.1 **b	-100 ** d	-39.3 ** a	-19.3 * b	-47 ** b	-58.1 ** b	-70.1 ** b	51.9 ** a
Fresh weight of fruit (g plant^{-1})									
-30.9 ** a	-51.2 ** b	-42.0 ** b	-100 **c	-40.7 ** b	-20.6 ** a	-35.2 ** b	-41.6 ** b	-89.5 ** c	14.54 a
Fresh weight per fruit (g)									
19.62 a	-6.51 b	11.62 c	-100 ** e	-4.53 d	10.5 a	24.8 ** b	27.8 ** c	-58.6 ** d	-27.7 ** e
Number of seeds per fruit									
34.8 ** a	0.22 a	-13.27 *c	-100 ** d	1.48 c	25.0 * b	58.3 ** a	-19.5 ** c	-36.7 ** c	-16.6 ** c
Number of seeds per plant									
1.68 c	-50.5 * a	-49.1 ** c	-100 ** d	-38.4 ** b	-2.88 c	-8.8 a	-66.1 ** c	-81.1 * c	27.1 * b

Comparison of growth and yield parameters between each cultivar growing in density in relation to control (free density): *, ** significant at P = 0.05 and 0.01, respectively by the Scheffe test. Lower case letters differ from absolute values between cultivars when grown in density by the Scott Knott 5% test.

Height increase is an important strategy for plants in the face of competition for light (Gruntman *et al*., 2017). In contrast, MT shows small variations in height up to the first flower, from 4 to 6 cm, in different densities and pots volume (Meissner *et al*., 1997), while MC2-8 with the same small variation in total plant height, from 13 to 15.5 cm, but in different scales.

The diameter of plants in CVO had little increase in the two tray sizes, while BIQ showed a decrease in both. BIQ naturally has indeterminate growth and a horizontal canopy, with the tray having this characteristic compromised (Figs. 8 and 10). All cultivars with determined behavior (CVO, MC2-8 and 75) had an increase in diameter in one or both tray sizes, despite the noticeable absence of lateral branching.

Figure 7. Visual aspect at the time of harvesting for cultivars of *C. annuum* (75, MC2-8, CVO, MC2) and *C. chinense* (BIQ) grown in a seeding tray with growth density of 560 plants m^{-2} and with a capacity of 40 mL of substrate/soil per cell. Scale bar 10 cm.

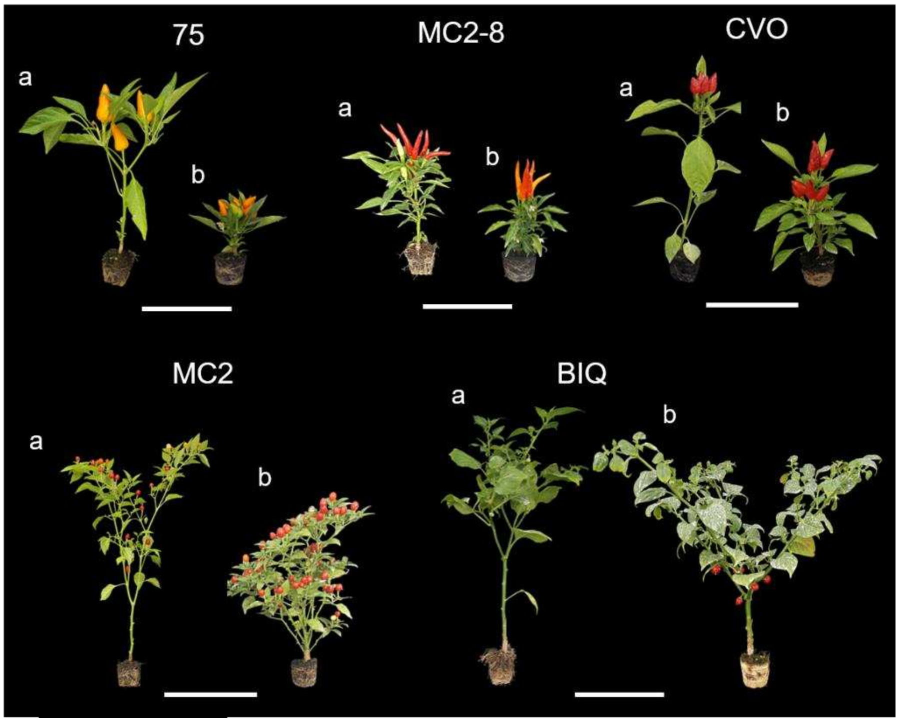


Figure 8. Details of individual plants for cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) show different behavior when grown densely (**a**. 560 plants m^{-2}) or free of density (**b**) combined with root limitation (pot volume 40 mL). Scale bars 10 cm.

We observed that for these cultivars the density increased the size of leaves and mainly petiole (Figs. 8 and 10), in addition to the interlacing without overlapping leaves (Figs. 7 and 9) and thereby relieved the effect of density. The lengthening of the petiole, as well as the optimization of leaf inclination, are important shade-avoidance responses (Gruntman *et al*., 2017).

The simulation of the competition caused by the surrounding plants in the growth in stoloniferous perennial herb, *Potentilla reptans,* through different light gradients (PAR and R:FR) showed that depending on the density and/or height of the surrounding plants, adjustments occur in vertical growth, specific leaf area and stolon length (Gruntman *et al*., 2017). *Capsicum* has a different growth habit than the remaining presenting in the authors' model, but we also observed plasticity in our cultivars with different adjustments.

Dry vegetative biomass and root volume

The decrease in vegetative biomass occurred for all cultivars in a 40 mL tray. Plants with root restriction grow less due to inhibition of photosynthesis (Poorter *et al*., 2012). In a 120 mL tray, smaller plants such as 75 and MC2-8 also decreased in biomass, while CVO was not affected. Larger cultivars, MC2 and BIQ, in density, accumulated more biomass due to greater investment in stem, making up 57 ± 1.62 and 42.55 ± 2.08 %, respectively, against 39.81 ± 0.89 and 39.65 ± 0.92 % of the total vegetative biomass in the absence of density. Behavior of this type ensures greater growth in height, in order to avoid competition imposed by density (Cipollini *et al*., 1999).

We observed that in a 40 mL tray the decrease in root volume, with the exception of BIQ. In a 120 mL tray, we observed that only the cultivars CVO and MC2 had an increase in root volume. Such behaviors are difficult to clarify, since they require complex analyzes of root biometrics. Extraordinary intent was achieved by Meissner *et al*., 1997, with tomato cultivation (MT) in a sowing tray with a density of up to 1357 plants m^{-2} and pot volume of 13 mL for 90 days. However, our pepper cultivars have been grown for a longer time, up to five months. The root growth of pepper plants is much more vigorous than MT, with a dry mass superiority of at least three times, depending on the pepper cultivar. Therefore, even our smallest tray cell size (40 mL) can be considered equally or more restrictive compared to MT.

Leaf area

Although most cultivars showed a decrease in leaf area in both tray sizes, cv. 75 was stable in both. Meanwhile, only CVO increased when in a 120 mL tray. The considerable decrease in BIQ leaf area in both tray sizes suggests that the larger size combined with the greater leafing contributed to greater self-shading, as visually observed in the absence of leaves on the main axis (Figs. 8 and 10). In MC2 in a 120 mL tray, although the plants reached a higher height, probably the smaller leaf size induced less shade, although a greater absence of leaves was also observed in the main axis.

Figure 9. Visual aspect at the time of harvesting for cultivars of *C. annuum* (75, MC2-8, CVO, MC2) and *C. chinense* (BIQ) grown in a seeding tray with growth density of 343 plants m^{-2} and with a capacity of 120 mL of substrate/soil per cell. Scale bar 10 cm.

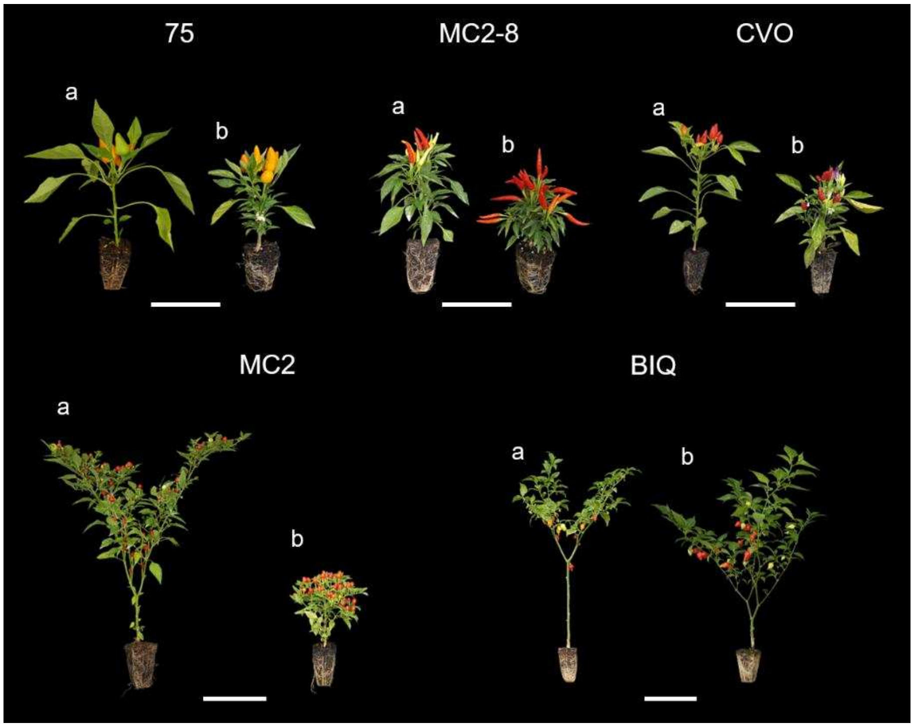


Figure 10. Details of individual plants for cultivars of cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) show different behavior when grown densely (**a**. 343 plants m^{-2}) or free of density (**b**) combined with root limitation (pot volume 120 mL). Scale bars 10 cm.

We consider the decrease in leaf area as an adaptive and mitigating mechanism of density conditions, as seen by the absence of individual mortality. A model has been proposed to explain the modulation of leaf area induced by senescence in plants in density. For this, they used as a model transgenic tobacco plants with senescence delay phenotype due to the expression of the *ISOPENTENYL TRANSFERASE* (*IPT*) gene - inducer of the highest production of cytokinin fused to the senescence promoter (Boonman *et al*., 2006). It was observed that the delay in senescence in density decreased both plant biomass and seed productivity, since the maintenance of shaded leaves, in addition to being an energy drain without increasing photosynthesis, prevented the translocation of nutrients to ensure growth and consequent developments of unshielded top leaves.

Productivity and Prolificacy

The number of fruits and fresh mass of fruits per plant in a 40 mL tray showed a decrease for all cultivars, as expected, since the limitation of root growth limits photosynthesis (Poorter *et al*., 2012). Interestingly, the decrease in fruit mass was associated with a smaller number of fruits, since the individual mass remained constant in this tray. The number of seeds per fruit was also related to productivity in number and mass of fruits per plant. The cultivars CVO and MC2 in a 40 mL tray despite stability in the number of seeds per fruit, due to the high decrease in the number of fruits per plant, there was a reduction in the number of seeds produced per plant. The increase in the number of seeds in each fruit in 75 compensated for the smaller number of fruits, which led to being the only cultivar not affected in the total production of seeds per plant in this density.

The increase in the number of seeds per fruit in a 120 mL tray for CVO and 75 coincides with the decrease in the number of fruits, but in greater size, produced per plant, which resulted in the stability of the number of seeds per plant. Incidentally, MC2-8, despite increasing the individual mass per fruit due to the production of less fruits, showed that the number of seeds per fruit and per plant were reduced by density. MC2 produces very small fruits, although the density has reduced the individual weight of each fruit, the production of a much larger number of fruits contributed to increase the fresh mass of fruits per plant. Under these conditions, MC2 shows that the greater number of fruits can compensate for the smaller number of seeds per fruit, contributing to being the only cultivar with an increase in the total of seeds per plant. As indeed observed in the plasticity in the ancestor of sweet pepper (*C. annuum* var. *glabriusculum*, chiltepín) (Hayano-Kanashiro *et al*., 2016), MC2 sharing many morphological characteristics, among them that of fruit size. We verified again the inadequacy of BIQ for growth in both types of trays (density and root limitation), either due to the lack of fruiting or the intense decrease in the number, weight per fruit and per plant and number of seeds per fruit and plant.

Cultivars such as CVO, MC2-8 and 75 are prolific even at high density (560 plants m^{-2}) and root limitation (40 mL), with a production of 695 ± 86.50, 120.5 ± 10.38 and 171.1 ± 14.87 seeds per plant, respectively. While the MT in density of 579 plants m^{-2} and pot volume of 33 mL obtained about 51 seeds per plant (Meissner *et al*., 1997). The low production of fruits and seeds in MT when grown in high densities is an obstacle for large-scale phenotyping or mutagenesis studies, which require the growth of many plants and that in the end produce enough seeds to propagate the genotype/mutant of interest (Emmanuel and Levy, 2002).

Part IIb: Behavior of *Capsicum* cultivars grown in different pot volumes

Life cycle

The parameters specified in Fig. 11 for most cultivars did not suit significantly the regression model, so they were represented in a bar graph (Supplementary Table 1). Characteristics such as the sowing cycle until anthesis showed stability in different pot volume for 75 and MC2-8, and while cultivating CVO with almost the same behavior. Interestingly, the root limitation imposed by the 50 and 150 mL pot volume in MC2 was able to further reduce the cycle until anthesis, since among the other cultivars this was the earliest. While in BIQ there was an extension of the cycle until anthesis and until the first ripe fruit when the lowest was pot volume, a fact resulting from the greatest delay in plant development. It is recognized that stressful conditions can induce or repress various genes, including genes related to flowering, making it early or late (Corrales *et al*., 2017).

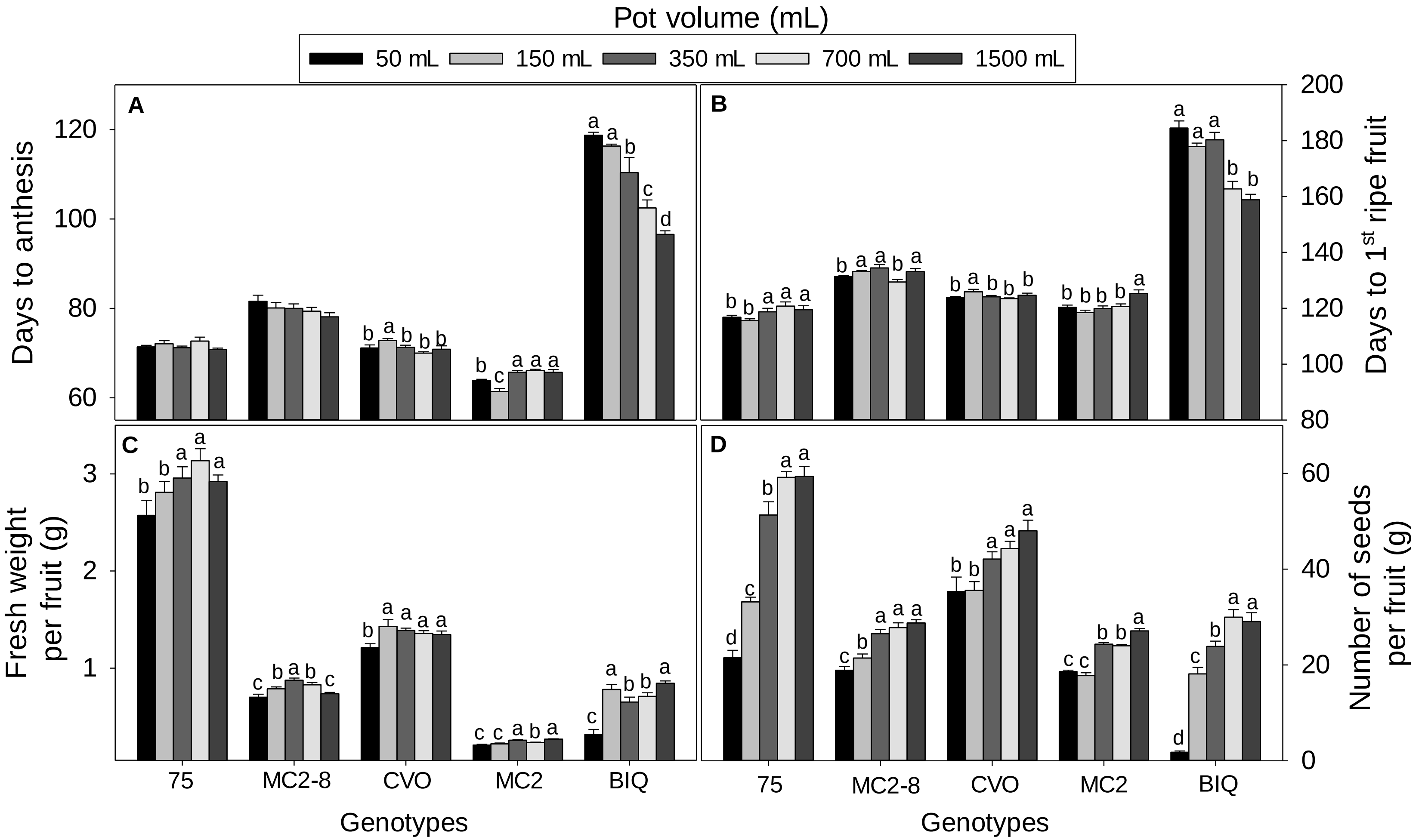


Figure 11. Behavior of cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) in parameters related to precocity (**A** and **B**), fruit size (**C**) and prolificacy (**D**). Different letters differ among the genotypes by the Scott-Knott test at a probability level of 5%.

The shortening of the cycle until the first ripe fruit was observed in almost all cultivars for the 50 mL pot volume. It is suggested that stressful conditions act on the synthesis of ABA hormone and this action on the ripening of both climacteric and non-climacteric fruits (Leng *et al*., 2014), although it has a role in pepper, this is still unclear (Hou *et al*., 2018). Interestingly, the lower development of the MC2 plant when grown in smaller pot volumes (50, 150 and 350 mL) allowed the predominance of ripe fruits, mainly in 50 and 150 mL containers (Fig. 12). This behavior suggests that the reduction of vegetative growth limits the development of new fruits and, in addition to contributing to accelerate the development of old fruits, therefore greater maturity uniformity. The CVO shows that the cycle until anthesis influences the cycle until the first ripe fruit, showing that the delay or advance of flowering, in addition to the establishment of the fruiting of the first flowers (Table 2) has relevance to the precocity until the first ripe fruit.

Fresh weight per fruit and number of seeds per fruit

Cultivation in a 350 mL pot provided the highest individual fresh fruit weight for most cultivars (Fig. 11C). The greatest stability in fresh weight per fruit occurred for 75 and CVO in pot volumes above 50 and 150 mL, respectively. Although the volume of the 350 mL pot provided larger fruit sizes for most cultivars, it did not correspond to a greater production of seeds per fruit, with the exception of CVO and MC2-8. Increasing the size of the pot volume proved to be an efficient way to increase the number of seeds per fruit for all cultivars, with 1500 mL being efficiently unanimous for all cultivars (Fig. 11D). Although 750 mL is also the case, except for the cultivar MC2. Meissner *et al*. (1997) in tomato MT, observed that the pot volumes of 200 and 465 mL led to the same individual weight per fruit, although the highest seed production per plant occurred in the highest pot volume.

The variation of the regression models applied here, with parameters with better adjustments for cubic, other quadratic or linear models (Supplementary Table 1) and the phenotypic pattern (Fig. 12) exemplify the variation in behavior of each cultivar before the progression of pot volume.

Figure 12. Root limitation in different pot volumes (from right to left: 50, 150, 350, 700 and 1500 mL) - influences vegetative growth and reproductive aspects in cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ). Scale bar 10 cm.

Cultivars such as 75, CVO and MC2-8 respond with greater growth at height in greater pot volume, although with lesser growth when compared to cultivars MC2 and BIQ (Fig. 13A). Although 75 is much smaller in size than MC2, it shows a similar progression in diameter, a fact attributed to the more upright branching of MC2 combined with the smaller size of leaves, while 75 has great length of leaves with horizontal distribution, which were considered in the measurement of plant diameter (Fig. 13B).

The greatest growth in height is not always accompanied by the greatest growth in biomass, as exemplified by MC2 (Fig. 13C). MC2 presents a progression of biomass accumulation similar to the smaller cultivar CVO, but with a predominance in leaf area, while CVO stands out for the greater progression in root volume (Fig. 14A).

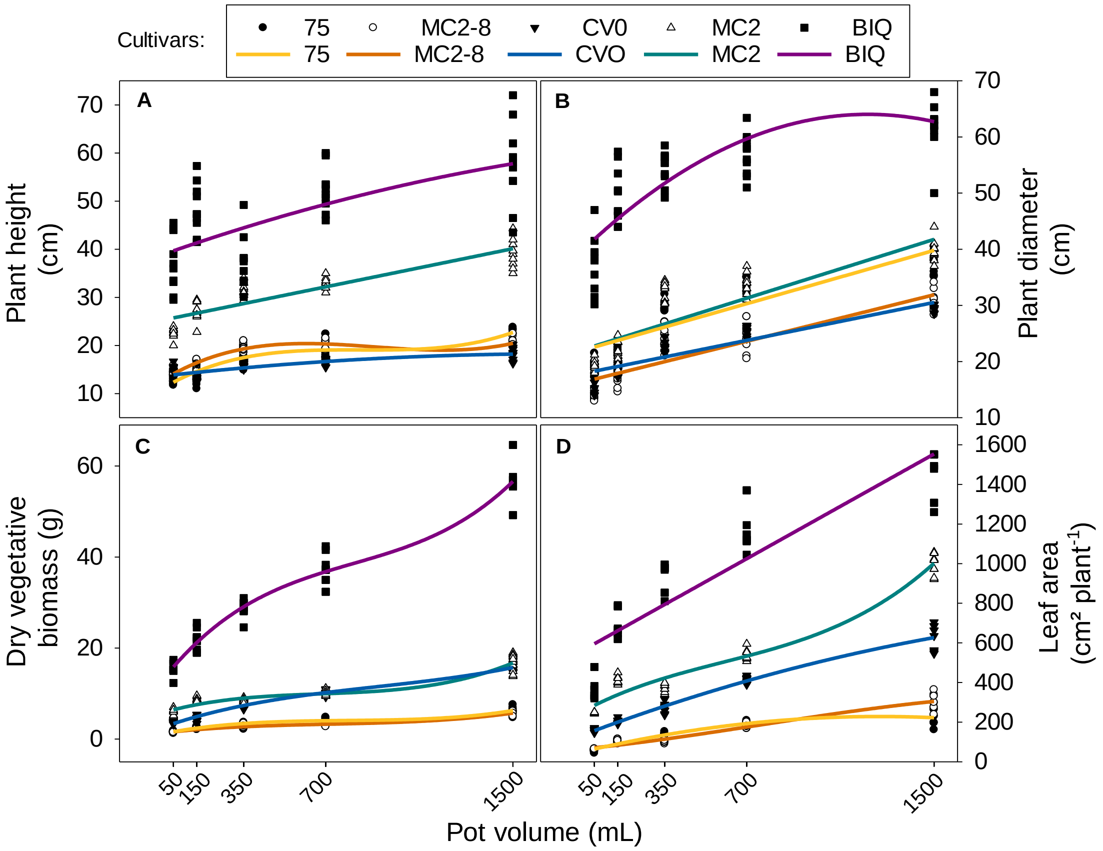


Figure 13. Better regression adjustments for different cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) grown in different pot volumes with respect to growth parameters such as height (**A**) and diameters (**B**) of plants, biomass accumulation (**C**) and leaf area per plant (**D**).

In addition, the plasticity of the lower investment in root biomass in proportion to the aerial part when in trays - high density and root limitation - for MC2 (Table 1) collaborates with the same behavior in the vessel volume. Cultivars such as MC2-8 and 75, of smaller size, showed equivalent behavior in plant height, total dry plant biomass and leaf area per plant, although with greater emphasis on 75 in the root volume in the 1500 mL pot size. This fact is attributed to the less robust MC2-8 root system, which resulted, in almost all pot volumes, in lower values of root dry mass compared to other cultivars. As noted, many pepper cultivars have natural variability in the size and efficiency of the root system (Kulkarni and Phalke, 2009). The accumulation of total vegetative biomass for all cultivars increased in greater pot volumes. In a study of meta-analyzes on various plants, from Arabidopsis to trees, grown in 5 mL containers at 1.7 L, in which, regardless of herbaceous or arboreal habit, there was a 43% increase in plant biomass when the volume of container until it reaches saturation (Poorter *et al.*, 2012).

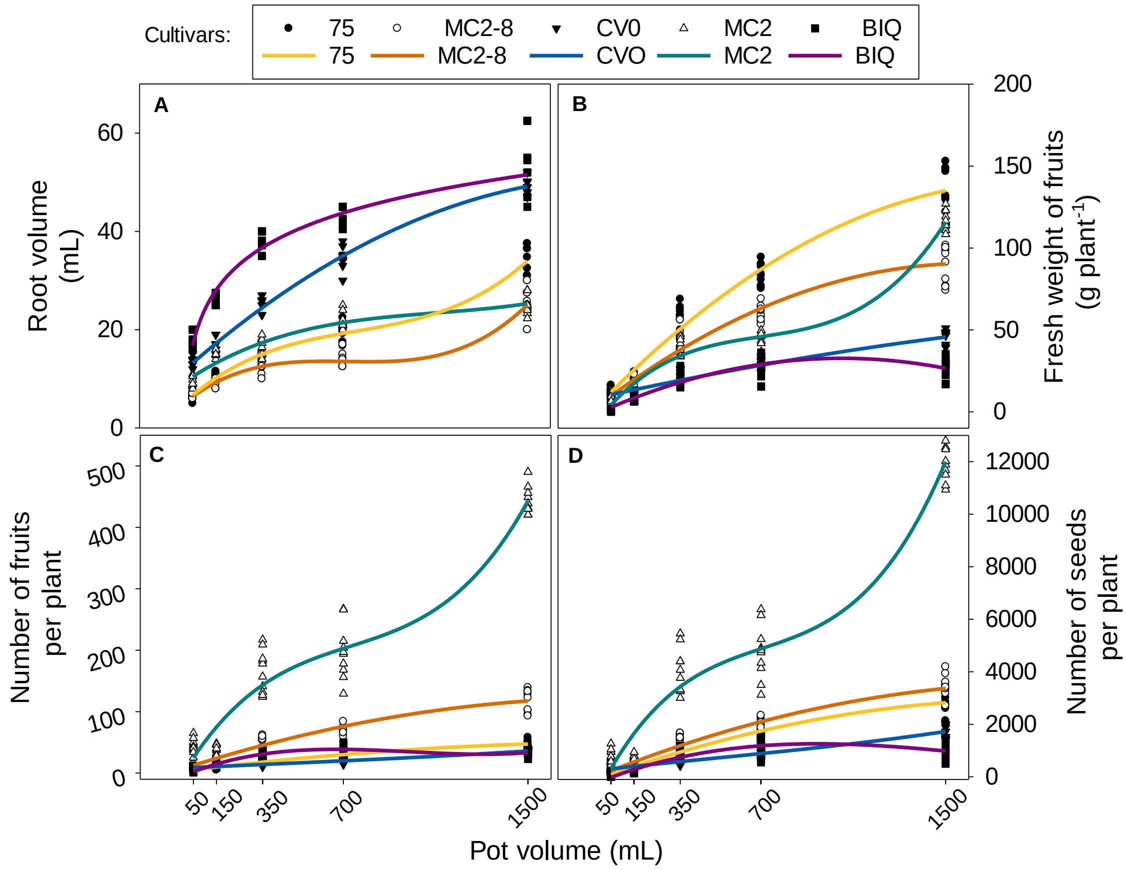


Figure 14. Best regression adjustments for different cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) grown in different pot volumes with respect to parameters such as root volume (**A**), mass productivity (**B**) and number of fruits (**C**) per plant and prolificacy (**D**).

Different insights are observed between cultivars when grown on a progressive scale of pot volume in relation to productive parameters. The cultivar 75 is characterized by the production of very large fruits in proportion to the size of the plant, which can be considered a miniature commercial pepper. In addition, it produced fruits much larger in weight than other cultivars in all pot volumes (Fig. 11C). This contributed to surpassing all cultivars in productivity for fresh mass of fruits per plant, since in greater volume of the pot there was an increase in the number of fruits, even if less when compared to the other cultivars (Figs. 14B and C). The achievement of productivity almost equivalent to MC2 to the cultivation of 75 in a volume of 1500 mL pot was a surprise, since the fruits of 75 have on average 12 times greater weight than the fruits of MC2. This fact was made possible by the cultivar MC2 having high productivity in number of fruits, with values, in pot volume of 1500 mL, in almost 10 times greater than 75 (Fig. 14C).

Similar occurred for the total number of seeds produced per plant for MC2. Although MC2 produces fewer seeds per fruit than other cultivars, it produced much more fruit. In addition, characteristics such as smaller size of fruits and seeds present in MC2 can explain the high prolificacy per plant. A model for shrub and woody angiosperms can explain what happened to MC2, which predicted associations between the smallest leaf size, but in greater numbers with the largest number of axillary meristems and, therefore, greater fruiting intensity (Dombroskie *et al*., 2016). According to this model, smaller leaf size would be associated with smaller fruit size, since small leaves have less capacity to feed the adjacent fruits, in addition, greater fertility/prolificacy would be associated with the smaller size of fruits and seeds.

The cultivar BIQ proved to be unsuitable for growth in these pot volumes, either due to the delay of the cycle, the high proportion of vegetative biomass at the expense of reproductive and the inferiority in fruit productivity (number and mass) and prolificacy in seed number by fruit and plant compared to other cultivars. MC2, on the other hand, was disregarded, despite its high prolificacy, due to its exaggerated growth in height, with compromised plant stability when in pots smaller than 1500 mL. In addition, small leaves would make measurements on devices such as the IRGA (Infrared Gas Analyzer) difficult.

It is interesting to note that the smaller size of CVO, MC2-8, and 75 cultivars determined better performance both in density and in different pot volumes. However, these cultivars retained the ability to respond in different degrees to stresses such as density combined with root restriction and growth in different pot volumes, suggesting that they can be used in physiological and genetic studies to represent plants in natural or agricultural environments.

Part III: Comparison of *C. annuum* (75, MC2-8 and CVO) cultivars with the tomato model cultivar Micro-Tom (MT)

The cultivars of *C. annuum* 75, MC2-8 and CVO show a divergent pattern between themselves and mainly when compared to MT in different vegetative, reproductive and prolificacy parameters when compared in analysis of main components (Supplementary Fig. 1). Parameters such as cycle to flowering, cycle of sowing to 1st fruit ripe, dry vegetative biomes, ratio shoot / root, fruit development time, number of fruits per plant and fresh weight per fruit were the most important in discriminating the cultivars of *Capsicum* and MT in the first component, explaining 50.9% of the data variation (Supplementary table 2). The difference between the cultivars of *Capsicum* and MT justifies the proposal of a cultivar of *C. annuum* as a new model organism within Solanaceae family. Although this differentiation is certainly not limited to vegetative, reproductive and agronomic aspects, represented here in this section. During the entire cycle of our *Capsicum* cultivars, we obtained different insights regarding about each cultivar in relation to MT cultivation, considered here only as a reference in positive characteristics, with no intention of replacing it.

The beginning of the cycle of a plant starts with germination, in this phase we observe germinative equivalence between CVO, MC2-8 and MT (Fig. 15A), and lower in cv. 75. We use new seeds and stored in the refrigerator, because the low germination in *Capsicum* it may reflect the impairment of viability during improper storage, in addition to factors such as low natural viability and dormancy mechanisms (Siri *et al.*, 2013; Alcalá-Rico *et al.*, 2019). In addition, the low germination in *Capsicum* due to dormancy can be successfully increased by simple methods such as treatment by salts, temperature or hormonal (Smith and Cobb, 1991; Hernández-Verdugo *et al.*, 2001).

The average time required for germination was considerably shorter for MT, and longer for MC2-8 (Fig. 15B). In addition, when we do the germination projection over time (Fig. 15D), we observe a high and narrow peak for MT, showing to be a very punctual event. Meanwhile, *Capsicum* cultivars showed low and large peaks. The greater width of the base of the peak offers practical significance, which is the greater distribution of germination over time for *Capsicum* in relation to tomato. Features such as germination delay due to dormancy or lack of synchrony in germination have been removed and improved, respectively, in various agricultural crops during the domestication process (Rodríguez *et al.*, 2011). In *Capsicum*, these characteristics should be improved, as it has action in the cycle.

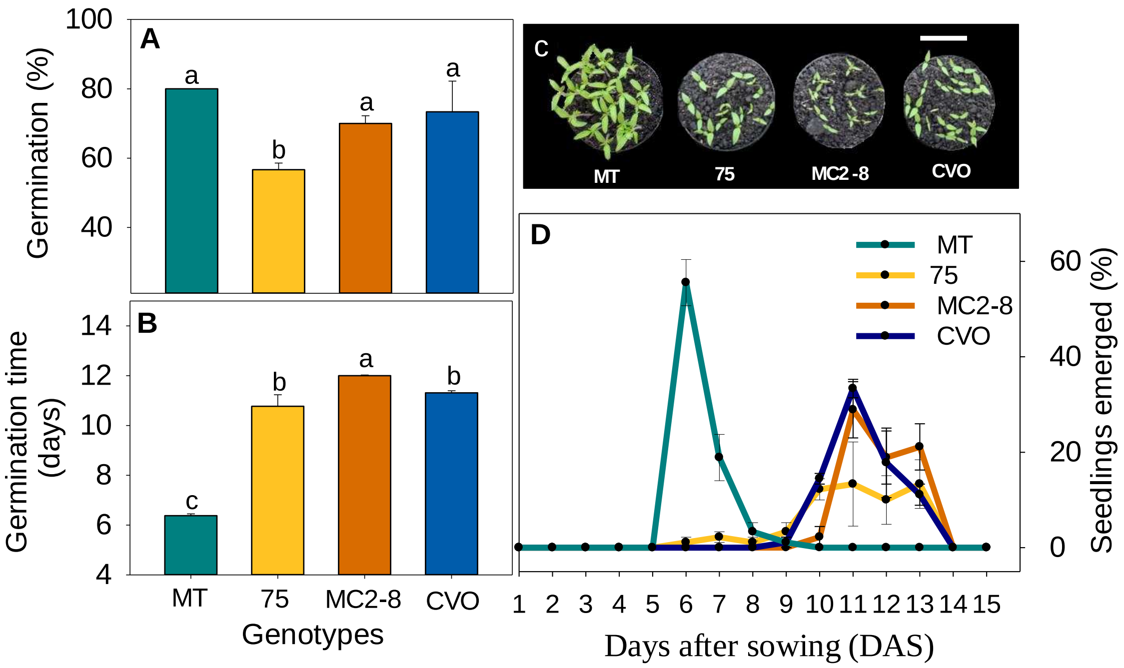


Figure 15: Parameters related to germination in different cultivars of *Capsicum annuum* (75, MC2-8 and CVO) compared to the cultivar model Micro-Tom (MT) de *Solanum lycopersicum*. **A**) Germination percentage (%); **B**) Average germination time (days); **C**) Seedlings at the stage of 13 days after sowing (scale bar represent 5 cm); **D**) Germination distribution (seedling emergence) over 15 days after sowing (DAS). Different letters differ among the genotypes by the Scott-Knott test at a probability level of 5%.

Early germination and accelerated initial growth in MT (Fig. 15C) contributed to earlier flowering, as can be seen at 45 and 60 days after sowing (Fig. 16). MT is considered to be defective in a transcription factor of the *CETS* family, with flowering repressive activity (Vicente *et al*., 2015), the same gene family of *SELF PRUNING* (*SP*). The *SP* gene has flowering repressive activity and in a non-functional state is found in the background of MT.

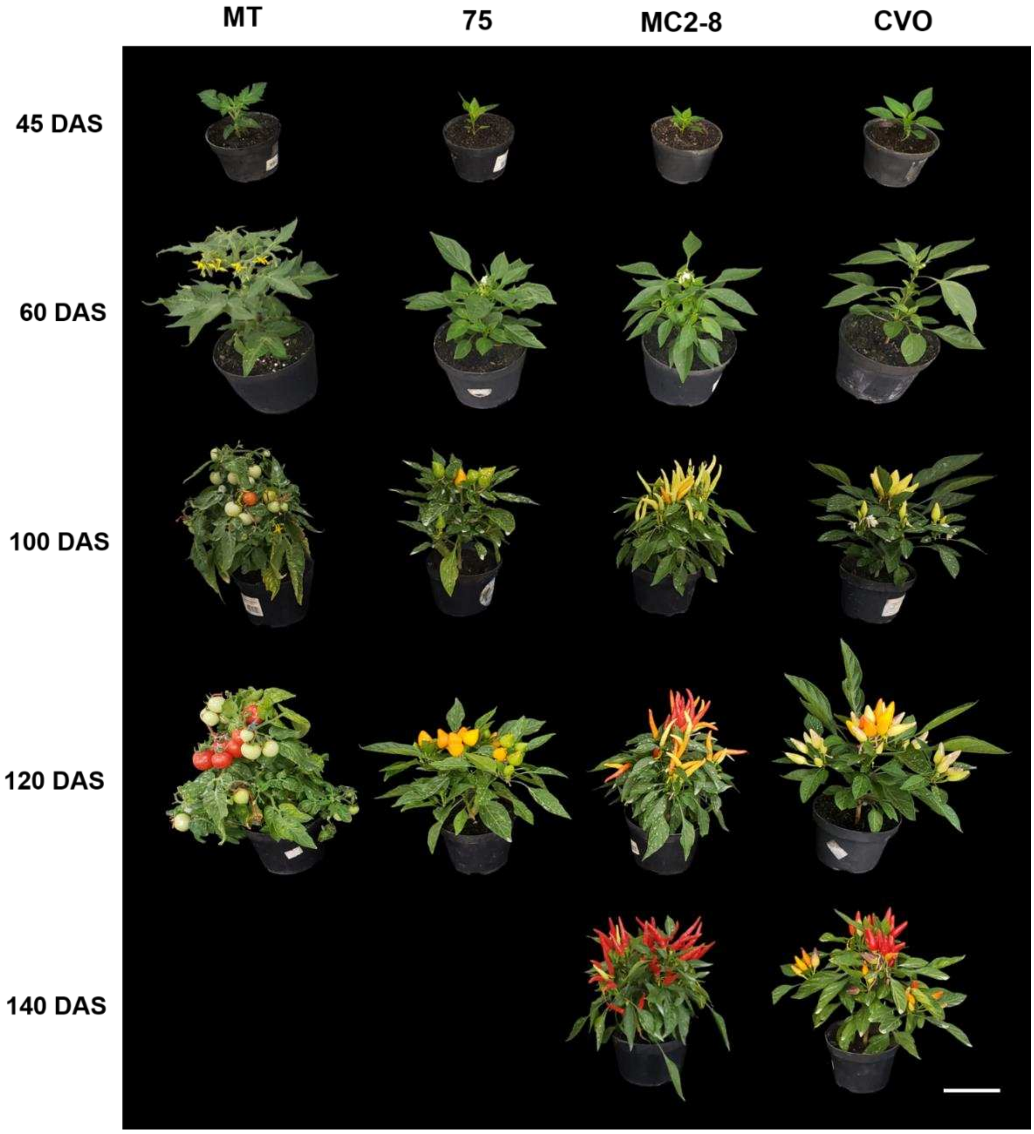


Figure 16. Visual aspects showing the different vegetative growth in different phenological phases-days after sowing (DAS) and the speed in reaching the reproductive stage (first open flower and first ripe fruit) are factors related to the precocity of the cultivars of *C. annuum* (75, MC2-8 and CVO) compared to the Micro-Tom cultivar of *Solanum lycopersicum* (MT). Scale bar 10 cm.

Although only the *sp* mutation is not considered to induce early flowering (Carmel-Goren et al., 2003), the *SP* ortholog gene in pepper has already been discovered, and the mutation is called *fasciculate* (*fa*), already known to shorten the cycle until anthesis and in the production of flowers and fruits in clusters (Elitzur *et al*., 2009). Cultivars such as 75, MC2-8 and CVO have a fasciculate phenotypic pattern, possibly the presence of this mutation in the background of these cultivars as an important factor in reducing the cycle until anthesis, but not enough to match the MT (Fig. 17). In addition, they are completely different genus plants, which certainly involve different growth mechanisms and developments. *Capsicum* cultivars showed a higher allocation of dry root biomass, with values of 33.83 ± 1.06, 28.98 ± 0.7 and 22.86 ± 1.08 % for MC2-8, CVO and 75, respectively, while only 8.02 ± 0.32 % for MT. This, in a way, would explain the faster growth of the aerial part and early anthesis in MT, while in CVO, 75 and MC2-8 the opposite occurs. In more perennialized plants, it is common to invest more in vegetative organs, mainly in roots, to the detriment of rapid or immediate reproduction as occurs in annual plants or with annual behavior (Lundgren and Marais, 2020).

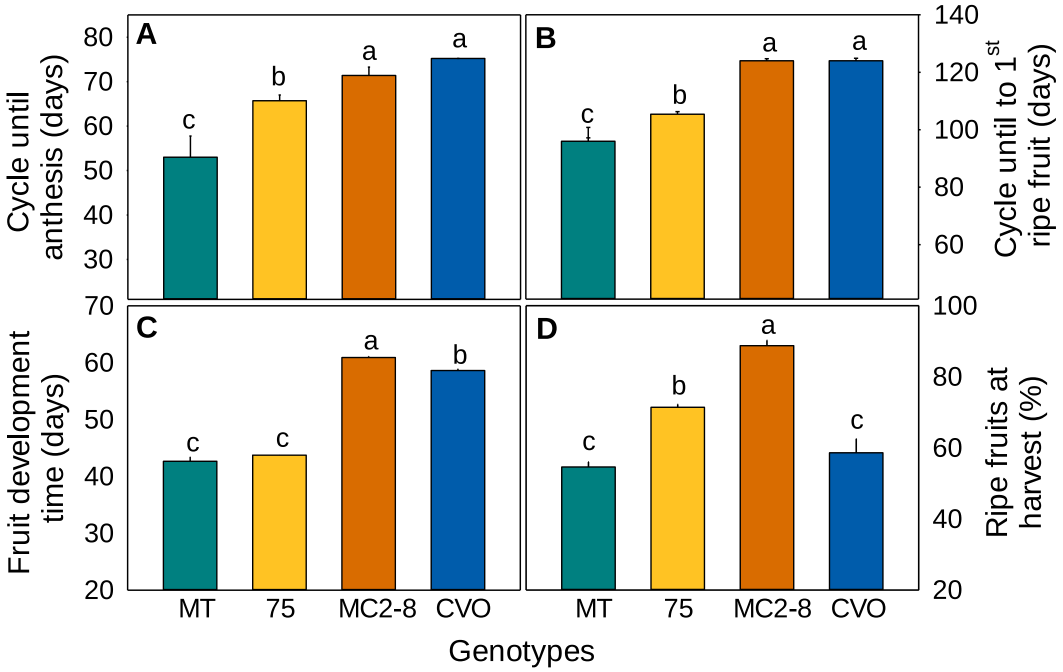


Figure 17. Parameters related to precocity in cultivars of *Capsicum annuum* (75, MC2-8 and CVO) and comparison to the Micro-Tom (MT) cultivar of *Solanum lycopersicum*. **A**) Cycle from sowing to anthesis (days); **B**) Cycle from sowing to the first ripe fruit (days); **C**) Time of development of the fruit in days (anthesis until full ripening fruit); **D**) Percentage of ripe fruits per plant at harvest for cultivars MT and 75 (130 days after sowing) and for cultivars MC2-8 and CVO (150 days after sowing). Different letters differ among the genotypes by the Scott-Knott test at a probability level of 5%.

The MT harvest was done much earlier, although most of the fruits of the side branches are green, represented here by the percentage of ripe fruits at moment harvest (Fig. 17D). Although MT has mutated the *sp* gene, which is said to cause uniform ripening of fruits in industrial tomatoes (Stevens and Rick, 1986), in MT it seems to act more on fruits from the two main consecutive inflorescences, not covering fruits obtained from the branches side. Even so, there was a need for harvesting in MT, since the fruits initially produced after ripening tend to undergo accelerated degradation due to the high-water content in the fruit (Fig. 18). While MC2-8 has a longer final cycle, the ripening process of all fruits was more uniform. Therefore, for these growing conditions, we consider that MT and the other cultivars of *Capsicum*, mainly cv. 75, may have more or less equivalent cycle.

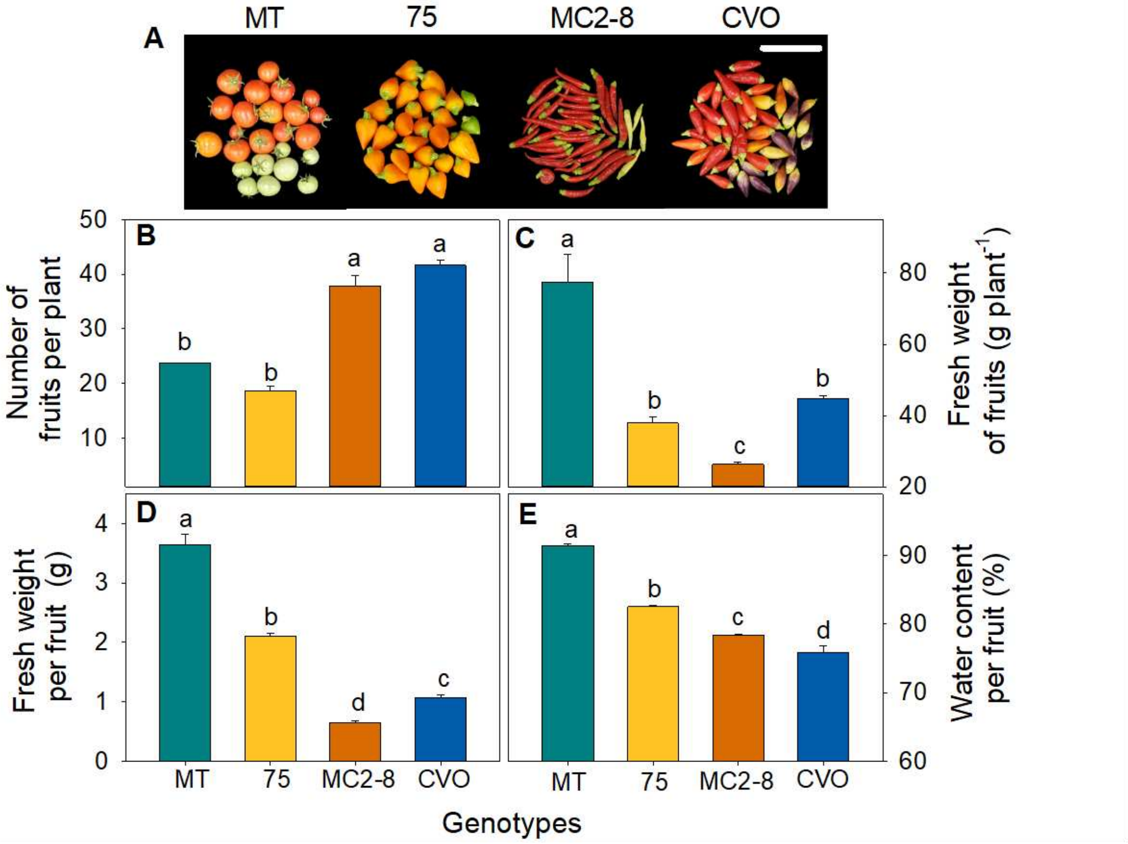


Figure 18. Parameters related to fruit yield in cultivars of *C. annuum* (75, MC2-8 and CVO) and comparison to the Micro-Tom (MT) cultivar of *S. lycopersicum*. **A**) Visual aspect of the total fruit produced per plant (Scale bar 5 cm); **B**) Total number of fruits per plant; **C**) Productivity in fresh weight of fruits per plant (g); **D**) Fresh weight per fruit (g); **E**) Relative water content per fruit (%). Different letters differ among the genotypes by the Scott-Knott test at a probability level of 5%.

The number of fruits produced by CVO and MC2-8 was higher than the values found for MT and 75 (Figs. 18A and B), however MT shows greater productivity in fresh weight of fruits per plant. This in MT is due to a smaller number of fruits per plant, but with greater individual weight; the same occurred for 75, to a lesser extent, with the trade-off between number and size of fruits already recognized (Knaap *et al*., 2014).

The cultivars of *Capsicum* 75, CVO and MC2-8, in addition to having good productivity, are good examples in terms of prolificacy parameters (Table 2), with the worst of the scenarios being compared to MT, which is considered a great reference (Meissner *et al*., 1997).

Table 2. Prolificacy parameters in cultivars of *C. annuum* (75, MC2-8 and CVO) compared to tomato cultivar MT (*Solanum lycpersicum* L.).

Traits/ Cultivars	Fruit set (%)	Nº of seeds/ fruit	Nº of seeds/ plant	Weight of seeds/ plant (g)
MT	46.7 **b**	28.3 **c**	628.4 **c**	1.50 **c**
75	46.9 **b**	51.3 **a**	967.1 **b**	3.46 **b**
MC2-8	50.8 **b**	26.5 **c**	1028.3 **b**	2.19 **c**
CVO	76.8 **a**	40.9 **b**	1691.5 **a**	8.02 **a**

Different letters differ among the cultivars by the Scott-Knott test at a probability level of 5%.

The cultivar 75, despite having a trade-off between high weight and a lower number of fruits per plant, managed to have a high production of seeds per plant due to the higher investment in the number of seeds per fruit. MC2-8 provides us with the example that even with the fruit set (%), much lower than CVO (Table 2), it can achieve equivalence in productivity in number of fruits per plant by launching many more flowers. CVO, on the other hand, shows that in the same cultivar it can be unbeatable in the production of seeds per plant, combining characteristics such as high numbers of fruits per plant and seeds per fruit. In addition, even though *Capsicum* is not a cereal crop, where the main interest is the seed, the high mass productivity of seeds per plant, as found in 75 and mainly in CVO, can be an adequate study model in Solanaceae (Table 2). It is necessary to obtain a better understanding regarding the allocation and redirection of seed reserves in proportion to the size of the fruit, explaining the trade-off between number and weight of seeds, fruit weight and domestication.

Part IV: Growth and development of fruits of cultivars of *C. annuum* (75, MC2-8 and CVO) in relation to ethylene evolution during ripening

Among many characteristics, *Capsicum*'s proposal as a model plant aims to fill the gap in the lack of an appropriate model organism to study the pattern of non-climacteric ripening of fruit. Studies for non-climacteric fruits are routinely conducted on strawberry (*Fragaria* × *ananassa*), although it is not a very appropriate model due to the complexity of its octoploid genetics (Gaston *et al*., 2020), and also because strawberry is not a fruit (berry), but an *infructescence* resulting from the development of a floral receptacle.

The potential cultivars of *Capsicum* candidates as a model plant show different colors and fruit morphology (Fig. 19), highlighting the potential also for studies with pigments and for fruit morphology. The monitoring of the development until the complete ripening of the fruits for all cultivars (70 days after anthesis - DAA) allowed us to obtain information related to growth, biomass accumulation, color change and ethylene production in different phenological phases.

Growth and development of fruits

The apex of the length occurred 20 days after anthesis, while the diameter showed an increase in values up to 30 DAA (Figs. 20 A and B; Supplementary table 3). The characteristic length of fruits is the first to be completely defined, as reported by Tadesse *et al*. (2002) and Rahman *et al*. (2014). Although, cultivars like MC2-8 show that the maximum diameter can be reached well before 30 DAA, probably for the cylindrical shape. The same earlier expansion seems to occur for the fruit volume (mL) for MC2-8, with the greatest cellular expansion coinciding with the volume expansion (Bertin and Genard, 2018).

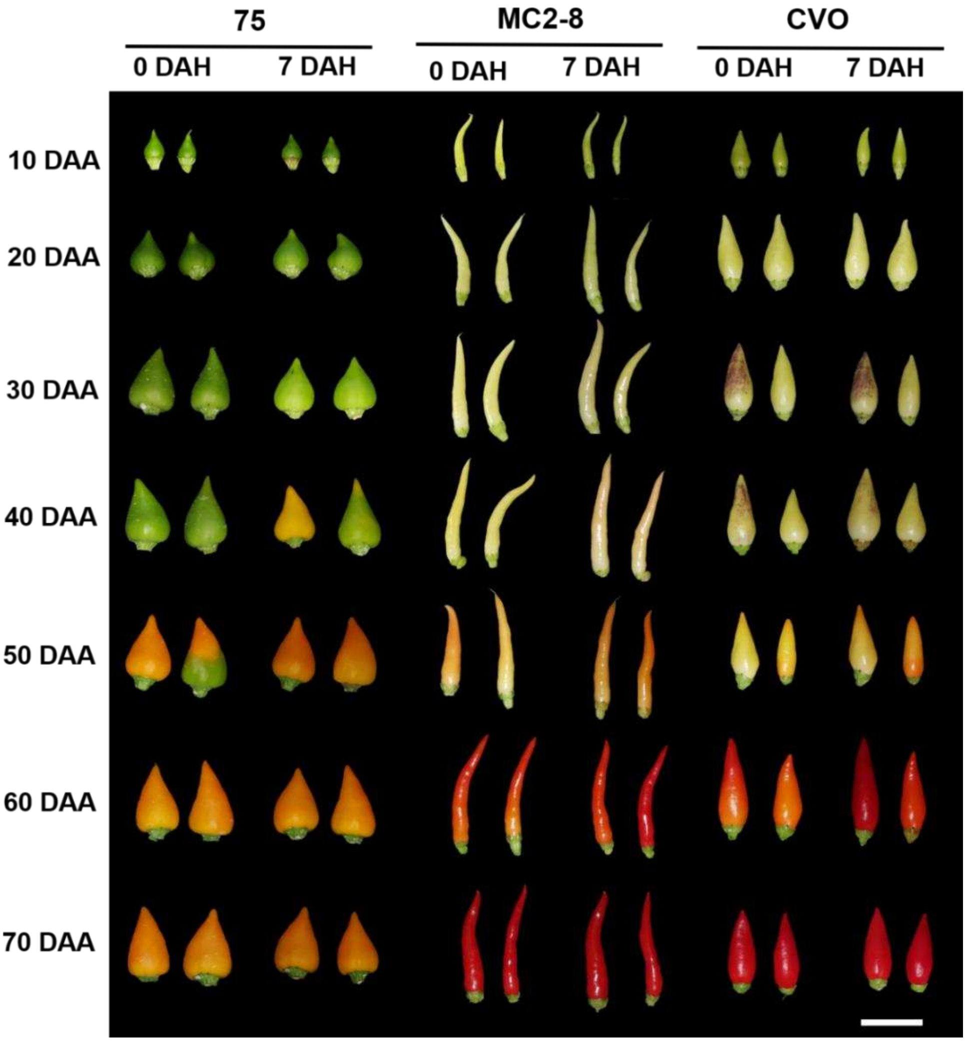


Figure 19. Visual aspects of shape, size and color in fruits of cultivars of *C. annuum* (75, MC2-8, and CVO) as candidates for model plant in different stages of development (days after anthesis-DAA) at harvest (0 days after harvest -DAH) and seven days after harvest (7 DAH). Scale bar 3 cm.

The increase in fresh weight of fruits was shown to be extended up to 40 DAA to 75, possibly attributed to the larger size of fruits. Fruit size is directly related to the number of cells and the consequent cell cycle (Lemaire-Chamley *et al*., 2005). Interestingly, the fresh weight for this cultivar starts to decrease after 50 DAA, coinciding with the post-maturation stage (Fig. 19), showing that the fruits of this cultivar as well as most cultivars are susceptible to water loss (O' Donoghue *et al*., 2018).

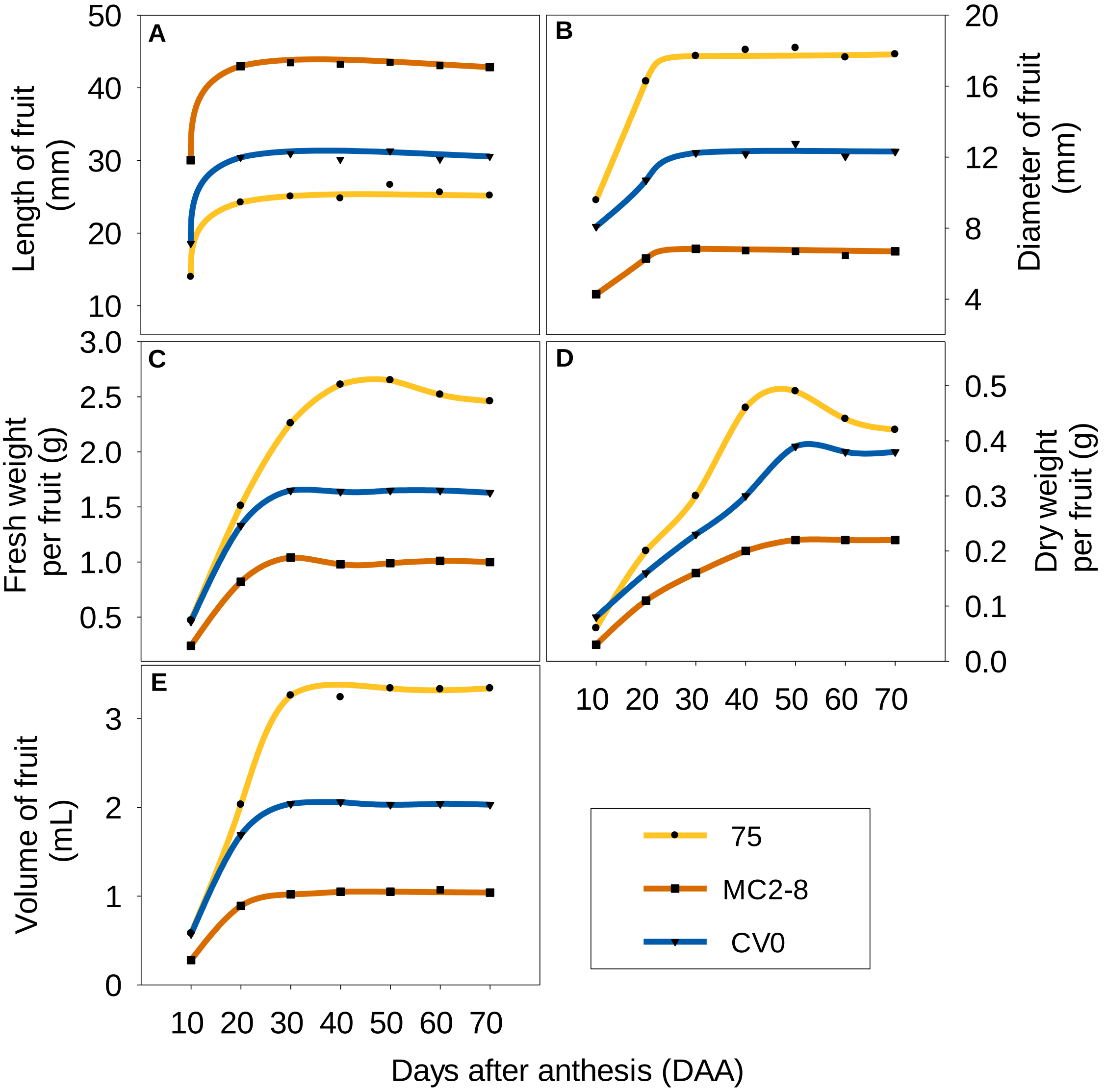


Figure 20. Biometric aspects of fruits in cultivars of *C. annuum* (75, MC2-8, and CVO) as candidates for model plants in different stages of development (days after anthesis-DAA). **A**) Fruit length between apex and base (mm); **B**) Diameter in the most dilated portion of fruits (mm); **C**) Fresh weight per fruit (g); **D**) Dry weight per fruit (g); **E**) Volume per fruit (mL). Each point on each date represents the average measure of 20 fruits per cultivar.

Water loss also occurs for the fruits of cultivars MC2-8 and CVO, however, it was not detected, since they showed late maturation and the measurement was made up to 70 DAA, not considering the post-maturation period of these cultivars. The characteristic accumulation of dry biomass per fruit reaches maximum values close to 45, 50 and 55 DAA for cultivars 75, CVO and MC2-8, respectively. We assume that these respective phases coincide with the period of maximum accumulation of seed reserves for each cultivar. In addition, the pronounced decrease in dry weight of cultivar 75 after 60 days would probably be related to the natural degradation of the fruit after being fully ripe, since this cultivar showed a high percentage of pericarp and placenta in proportion to the weight of seeds (Supplementary table 4).

Ethylene synthesis and fruit ripening

Ethylene production was detected for all *Capsicum* cultivars with the highest peak of ethylene production concomitant with the color change (Fig. 19), having no noticeable relationship with the biometric parameters of fruits (Fig. 20). Even though basal ethylene production, in some non-climacteric cultivars of *Capsicum*, has a role in promoting fruit coloring, where the inhibition of ethylene perception delays color change (O' Donoghue *et al*., 2018).

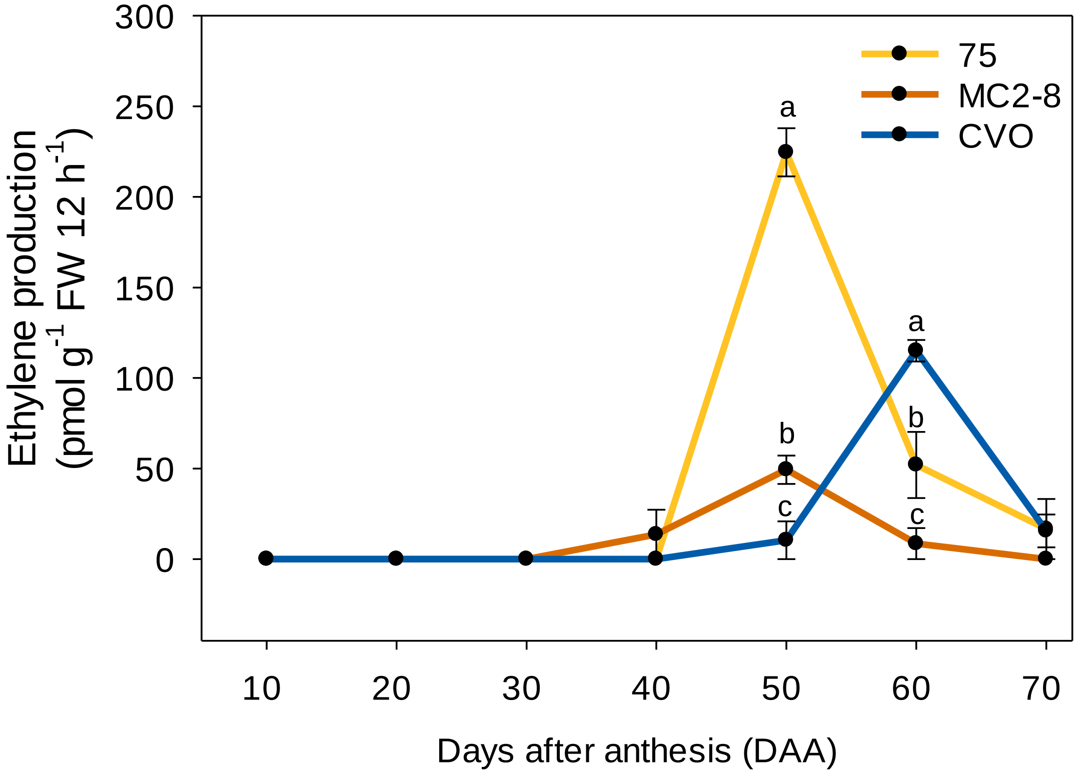


Figure 21. Ethylene production (pmol g^{-1} FW) after 12 hours of incubation in fruits of cultivars of *C. annuum* (75, MC2-8 and CVO) as candidates for model plant in different stages of fruit development (Days after anthesis-DAA). Different letters differ among the cultivars by the Scott-Knott test at a probability level of 5%. Each point on each date represents a sample number of 4 Erlenmeyer flask with 4 incubated fruits in each.

Fruits harvested before 30 DAA for 75 and MC2-8 and 40 DAA for CVO, did not show any noticeable color change after seven days after DAA harvest (Fig. 19). Between the onset of pigmentation and before complete ripening, we observed that only a few fruits of cv. 75 and MC2-8 managed to complete ripening. *Capsicum* fruits non-climacteric can complete ripening of fruits only when harvested after the Breaker stage (Aizat *et al*., 2013), the beginning of the colorimetric transition from green to ripe. With the exception of cv. 75, this was not homogeneous for MC2-8 and CVO, as not all fruits at this stage were able to fully ripen (Fig. 19).

The values of ethylene production in MT tomato in addition can go beyond 380 pmol g^{-1} FW h^{-1} (Bemer *et al*., 2012; Bodanapu *et al*., 2016). The highest value in ethylene production found for cultivar 75, with production conversion per hour was 18.72 pmol g^{-1} FW h^{-1}, showing to be far below the values found in the tomato. In *C. frutescens* cv. “Chaotianjiao 6” ethylene value of 570 pmol g^{-1} FW h^{-1} was found, in addition, to the increased expression of genes related to ethylene synthesis and signaling, which led the authors to classify it with climacteric behavior (Hou *et al*., 2018).

In preliminary tests we did not detect ethylene production when the conditions were individual fruits per bottle and with an incubation period of six hours, being only possible to detect when we put four fruits per bottle and increased the incubation period to 12 hours. Despite the fact that many of the tests involving the quantification of ethylene in pepper use a short incubation period (Lu *et al*., 1990; Biles *et al*., 1993), although the majority without ethylene detection. Hou *et al*. (2018) detected ethylene in pepper and used 10 incubated fruits per bottle; while only with the adaptation of 4 to 18 hours of incubation it allowed the detection (Gross *et al*., 1986).

The behavior of our cultivars regarding the ripening pattern was contradictory for three reasons. First, there was a peak in ethylene production and this coincided with the color transition, a characteristic behavior of climacteric fruits. Second, ethylene production values were very low, compared to another’s peppers or its closest relative tomato considered to be climacteric. Third, not all fruits in the mature green stage (for 75) or even in advanced breaker stage (MC2-8 and CVO) managed to ripen completely after seven days of harvest, this characteristic being more non-climacteric. Our *Capsicum* cultivars appear to behave closer to non-climacteric. Although, they maintain at baseline levels the auto catalytic production system of ethylene as also observed by Biles *et al*.

(1993). That is why we agree with Villavicencio *et al*. (2001) and Paul *et al*. (2012), in that *Capsicum* can be an intermediate group between climacteric or non-climacteric or with variation of genotype dependent behaviors.

Conclusion

The search in germplasm banks of pepper cultivars with good characteristics as a laboratory model plant made it possible to concentrate the screening on only 14 *Capsicum* cultivars (13 of *C. annuum* and 1 of *C. chinense*), despite the small number of cultivars allowed us observe diversity in morphological and behavioral patterns with numerous trade-offs between growth, productivity and prolificacy. In the initial screening, we observed the possibility of finding a cultivar that would serve as a model laboratory plant, although with the course of the screening and the imposition of limiting growth conditions, the number of cultivar potentials was decreasing. Adaptability tests in various density conditions combined with root restriction, as well as gradual pot volume cultivation highlighted the particular behavior of each cultivar, and within the context of a laboratory model plant, they emphasized that size was an important characteristic, to determine the inaptitude of a cultivar as a potential genetic model.

The differentiated behavior, even, in cultivars of the same size, probably sharing the same mutations related to size, shows that for genetic and physiological studies it is imperative to establish a model cultivar, in order to ensure the standardization and comparability of the results. Cultivars with CVO, MC2-8 and 75, despite the differentiation between parameters of precocity, productivity and prolificacy, in general, show to have equivalent or even superior qualities to the Micro-Tom model.

The detection of ethylene at very low levels in fruits of our pepper cultivars in comparison to the expressive climatic pattern of tomato coupled with the description in the literature of non-climacteric *Capsicum* cultivars instigates our curiosity. The need or role of ethylene and at what levels in this genus remains unclear.

Lastly, cultivars such as CVO and 75 have more conical and globose conical fruit morphology, respectively, being more plausible geometric models to study the regulation of fruit development by extrapolation and comparison with sweet pepper cultivars. In addition, they have the characteristic pungency, allowing to complement studies of this fascinating secondary metabolism. Finally, the creation of a model system in *Capsicum* is possible and necessary.

Supplementary material

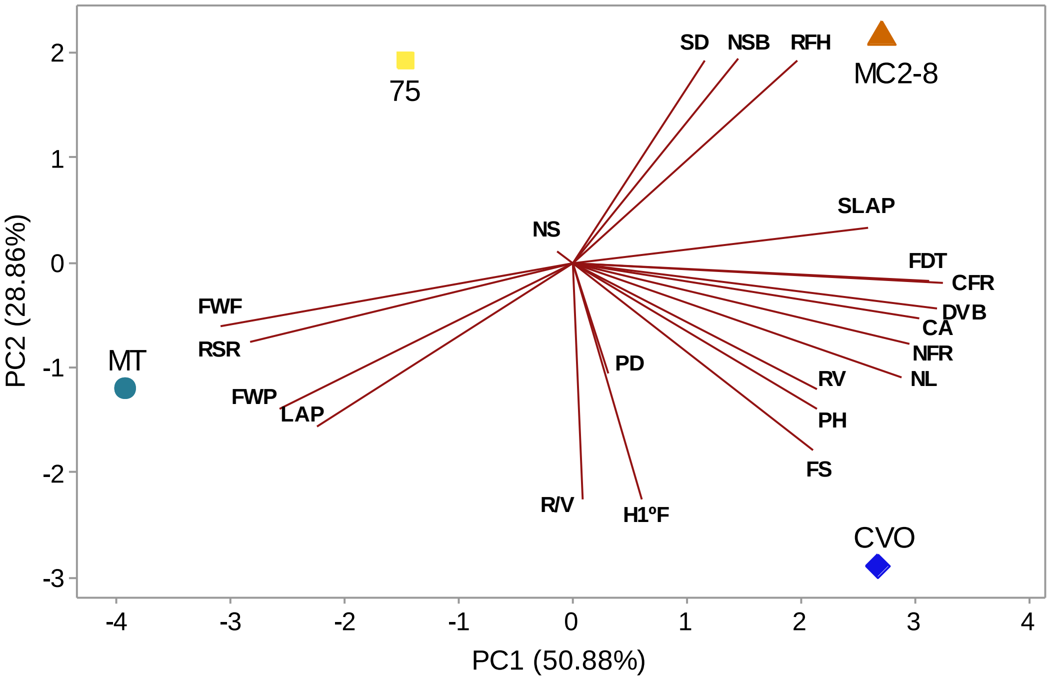
Supplementary Table 1. Best regression models for different variables when the cultivars of *C. annuum* (75, MC2-8, CVO and MC2) and *C. chinense* (BIQ) cultivated in different pot volumes (50, 150, 350, 700 and 1500 mL).

Genotypes	Equation	R^2	Sig (F) % *
	Days until anthesis		
MC2-8	$Y = 82.15 - 0.77X$	9.8	2.68
MC2	$Y = 72.7039 -14.0608X+ 5.66178X^2 - 0.6275X^3$	48.59	0
75	$Y = 71.82 - 0.06X$	0.21	100
CVO	$Y = 64.0851 + 10.7578X - 4.10511X^2 + 0.445083X^3$	22.41	0.8
BIQ	$Y = 114.1124 + 8.8955X - 4.78083X^2 + 0.459583X^3$	70.68	0
	Days to 1º ripe fruit		
MC2-8	$Y = 118.4323 + 18.9051X - 6.99815X^2 + 0.758666X^3$	17.24	3.2
MC2	$Y = 123.5132 - 4.07332X + 0.8802X^2$	37.61	0
75	$Y = 125.296 -13.5815X + 5.69607X^2 - 0.6425X^3$	22.11	0 .87
CVO	$Y = 116.5359 + 11.258X - 4.28858X^2 + 0.473333X^3$	18.27	2.46
BIQ	$Y = 178.9371 + 8.8727X - 4.57188X^2 + 0.391375X^3$	61.23	0
	Fresh weight per fruit (g)		
MC2-8	$Y = 0.5057 + 0.22258X - 0.0353X^2$	43.36	0
MC2	$Y = 0 .1453 + 0.0831X - 0.02515X^2 + 0.002666X^3$	42.51	0
75	$Y = 2.5655 - 0.125X + 0.1666X^2 - 0.025417X^3$	20.97	1.2
CVO	$Y = 0.6702 + 0.7634X - 0.24222X^2 + 0.023333X^3$	23.62	0.57
BIQ	$Y = -0.804 + 1.6136X - 0.53465X^2 + 0.055666X^3$	61.11	0
	Number of seeds per fruit		
MC2-8	$Y = 13.406 + 5.56699X - 0.493X^2$	67.63	0
MC2	$Y = 15.9181 + 1.88052X + 0.0729X^2$	74.98	0
75	$Y = -2.2928 + 24.45099X - 2.378X^2$	86.33	0
CVO	$Y = 32.1086 + 2.29821X + 0.1872X^2$	36.52	0
BIQ	$Y = -21.4752 + 28.3525X - 5.13768X^2 + 0.299749X^3$	85.9	0
	Plant height (cm)		
MC2-8	$Y = 13.2231 - 0.0505X + 1.19407X^2 - 0.179501X^3$	86.31	0
MC2	$Y = 19.0646 + 3.8783X$	89.11	0
75	$Y = 13.4564 - 1.8897X + 1.40646X^2 - 0.133509X^3$	79.99	0
CVO	$Y = 14.1066 - 0.31048X + 0.2309X^2$	62.73	0
BIQ	$Y = 40.3259 - 1.67066X + 1.0187X^2$	42.13	0
	Plant diameter (cm)		
MC2-8	$Y = 9.7001+ 4.1188X$	88.03	0

MC2	$Y = 13.5144 + 5.2698X$	89.72	0
75	$Y = 14.143 + 4.7941X$	93.91	0
CVO	$Y = 12.6821+ 3.2765X$	94.81	0
BIQ	$Y = 27.5526 + 11.96507X - 1.015X^2$	75.2	0
	Dry vegetative biomass (g)		
MC2-8	$Y = -0.9322 + 3.6057X - 1.35409X^2 + 0.179684X^3$	95.95	0
MC2	$Y = -3.3023 + 13.8118X- 5.27757X^2+ 0.663581X^3$	93.53	0
75	$Y = -1.0873 + 3.6149X - 1.17158X^2 + 0.148311X^3$	93.45	0
CVO	$Y = 2.6382 + 0.8063X + 0.02183X^2 + 0.067354X^3$	97.25	0
BIQ	$Y = 1.754 + 18.8606X - 6.08673X^2 + 0.900561X^3$	95.76	0
	Leaf area (cm² plant^{-1})		
MC2-8	$Y = -26.1383 + 133.0919X - 51.82284X^2 + 7.700509X^3$	95.58	0
MC2	$Y = -270.7075 + 791.9324X - 311.62522X^2 + 40.861908X^3$	98.57	0
75	$Y = 6.8998 + 47.96486X - 0.9053X^2$	92.19	0
CVO	$Y = 195.7422 - 61.06663X + 29.2543X^2$	96.74	0
BIQ	$Y = 130.3173 + 265.1423X$	95.01	0
	Root volume (mL)		
MC2-8	$Y = -4.7646 + 16.3024X - 6.04411X^2 + 0.793416X^3$	90.92	0
MC2	$Y = 2.7366 + 8.647X - 1.88235X^2 + 0.212899X^3$	91.53	0
75	$Y = -5.4556 + 16.9698X - 6.02465X^2 + 0.840499X^3$	96.72	0
CVO	$Y = 12.408 - 0.92001X + 1.65X^2$	98.46	0
BIQ	$\mathrm{Ln}(Y) = 2.86053 + 0.661573\,\mathrm{Ln}(X)$	94.37	0
	Ratio shoot/root		
MC2-8	$Y = 1.3171+ 0.46094X - 0.0509X^2$	35.39	0
MC2	$Y = 2.2198 + 2.9095X - 1.36808X^2 + 0.174333X^3$	51.66	0
75	$Y = 2.5325 - 0.6671X + 0.29864X^2- 0.039334X^3$	36.68	0
CVO	$Y = 4.9094 + 3.1689X - 5.69816X^{1/2} - 0.589974X^{3/2}$	32.45	0.03
BIQ	$Y = 1.2648 + 0.4708X - 0.18515X^2 + 0.015999X^3$	74.93	0
	Number of fruits per plant		
MC2-8	$Y = 3.5292 + 2.55344X + 3.9806X^2$	93.38	0
MC2	$Y = -21.5013 + 83.5355X - 32.78983X^2 + 6.873799X^3$	93.28	0
75	$Y = 3.1079 - 0.39758X + 1.8264X^2$	92.87	0
CVO	$Y = 6.43+ 4.6573X - 2.33679X^2 + 0.515833X^3$	94.01	0
BIQ	$Y = 9.4779 - 18.5938X + 14.15753X^2 - 1.90075X^3$	86.64	0
	Fresh weight of fruits per plant		
MC2-8	$Y = -2.8771 + 7.80404X + 2.144X^2$	93.05	0
MC2	$Y = -19.092 + 39.8941X - 16.54346X^2 + 2.77498X^3$	94.42	0
75	$Y = 8.0585-2.06079X+ 5.4725X^2$	96.25	0
CVO	$Y = 13.9394 - 5.26623X + 2.2992X^2$	94.45	0
BIQ	$Y = -15.1762 + 16.60916X - 1.6088X^2$	81.77	0
	Number of seeds per plants		
MC2-8	$Y = 51.8693 - 3.49282X + 131.5327X^2$	93.93	0
MC2	$Y = -1717.2605 + 3513.0949X - 1453.79818X^2 + 258.256916X^3$	93.68	0
75	$Y = -16.3744 - 36.44946X + 120.2171X^2$	91.85	0
CVO	$Y = 178.081 + 235.331X - 117.5774X^2 + 26.378583X^3$	91.6	0

BIQ	$Y = 580.2137 - 1126.924X + 629.77832X^2 - 77.68775X^3$	79.42	0

*The significance of F was obtained when less than 5%



Supplementary Figure 1. Principal component analysis (PCA) for vegetative, productive and phenological parameters for *C. annuum* cultivars (75, MC2-8 and CVO) compared for the Micro-Tom (MT) cultivar of *S. lycopersicum*. Variables: **CA**: cycle until anthesis (days); **CFR**: Cycle of sowing to 1st fruit ripe (days); **H1ºF**: Height to first flower (cm); **PH**: Total plant height (cm); **PD**: Total plant diameter (cm); **NL**: Number of leaves until 1º flower; **SD**: Stem diameter (mm); **NSB**: Number of side branches; **DVB**: Dry vegetative biomass (g/plant); **RSR**: Ratio shoot/root; **RV**: Root volume (mL); **LAP**: Total leaf area (cm^2/plant); **SLAP**: Total specific leaf area (g/cm^2)/plant); **FDT**: Fruit development time (days); **FS**: Fruit set index (%); **NFR**: Number of fruits/plant; **RFH**: Ripe fruits at harvest (%); **FWP**: Fresh weight fruits (g/plant); **R/V**: Reproductivity/vegetative dry biomass (g/plant); **FWF**: Average fresh weight per fruit (g) and **NS**: Number of seeds per fruit.

Supplementary Table 2. Contribution of eigenvalues to three main axes of PCA for *Capsicum* cultivars in relation to several variables

Variables	PC1 (50.9 %)	PC2 (28.9%)	PC3 (20.3 %)
Cycle until flowering - CA	**0.285**	-0.087	0.145
Cycle of sowing to 1st fruit ripe - CFR	**0.304**	-0.033	-0.038
Height to first flower - H1ºF	0.057	**-0.371**	-0.174
Plant height - PH	0.201	**-0.288**	-0.243
Plant diameter - PD	0.029	-0.173	**0.436**
Number of leaves until 1º flower - NL	0.27	-0.179	-0.079

Stem diameter - SD	0.109	0.317	0.249
Number of side branches - NSB	0.135	0.32	-0.208
Dry vegetative biomass - DVB	**0.299**	-0.072	-0.059
Ratio shoot/root - RSR	**-0.265**	-0.125	-0.192
Root volume - RV	0.2	-0.197	**0.292**
Leaf area per plant - LAP	-0.211	**-0.257**	-0.172
Specific leaf area per plant - SLAP	0.242	0.057	**-0.289**
Fruit development time - FDT	**0.293**	-0.029	-0.136
Fruit set index - FS	0.197	**-0.296**	0.115
Number of fruits per plant - NFR	**0.277**	-0.126	-0.142
Ripe fruits at harvest - RFH	0.183	**0.319**	-0.077
Fresh weight fruits per plant - FWP	-0.241	-0.228	-0.077
Reproductivity/vegetative dry biomass - R/V	0.008	**-0.373**	0.191
Fresh weight per fruit - FWF	**-0.29**	-0.1	-0.102
Number of seeds per fruit - NS	-0.013	0.02	**0.484**

Values in bold were the ones that showed the greatest contribution in module above 0.250.

Supplementary Table 3. Biometric aspects of fruits in cultivars of *C. annuum* (75, MC2-8, and CVO) as candidates for model plants in different stages of development (days after anthesis- DAA).

Cultivars	**Days after anthesis (DAA)**						
	10	**20**	**30**	**40**	**50**	**60**	**70**
	Lenght per fruit (mm)						
75	13.9 **c C**	23.2 **b C**	25.1 **a C**	24.7 **a C**	26.6 **a C**	25.6 **a C**	25.1 **a C**
MC2-8	30.05 **b A**	43 **a A**	43.5 **a A**	42.5 **a A**	43.5 **a A**	43.1 **a A**	42.9 **a A**
CVO	18.6 **b B**	30.4 **a B**	30.9 **a B**	30.2 **a B**	31.3 **a B**	30.2 **a B**	30.6 **a B**
	Diameter per fruit (mm)						
75	9.6 **c A**	16.3 b **A**	17.7 a **A**	18 **a A**	18.2 **a A**	17.6 **a A**	17.8 **a A**
MC2-8	4.3 **c C**	6.3 **b C**	6.8 a **C**	6.7 **a C**	6.7 **a C**	6.5 **a C**	6.7 **a C**
CVO	8.1 **c B**	10.7 **b B**	12.2 **a B**	12.2 **a B**	12.8 **a B**	12 **a B**	12.3 **a B**
	Fresh weight per fruit (g)						
75	0.5 e **A**	1.5 **d A**	2.3 **c A**	2.6 **a A**	2.6 **a A**	2.5 **b A**	2.5 **b A**
MC2-8	0.2 **c B**	0.8 **b C**	1 **a C**	1 **a C**	0.99 **a C**	1 **a C**	1 **a C**
CVO	0.5 **c A**	1.3 **b B**	1.7 **a B**	1.6 **a** B	1.65 **a B**	1.6 **a B**	1.6 **a B**
	Dry weight per fruit (g)						
75	0.06 **g B**	0.2 **f A**	0.3 **e A**	0.46 **b A**	0.49 **a A**	0.44 **c A**	0.42 **d A**
MC2-8	0.03 **e C**	0.11 **d C**	0.16 **c C**	0.2 **b C**	0.22 **a C**	0.22 **a C**	0.22 a **C**
CVO	0.08 **e A**	0.16 **d B**	0.23 **c B**	0.3 **b B**	0.39 **a B**	0.38 **a B**	0.38 **a B**
	Volume per fruit (mL)						
75	0.58 c **A**	2.03 b **A**	3.26 **a A**	3.24 **a A**	3.34 **a A**	3.33 **a A**	3.33 **a A**
MC2-8	0.28 **c B**	0.89 **b C**	1.02 **a C**	1.05 **a C**	1.05 **a C**	1.07 **a C**	1.04 **a C**
CVO	0.57 c **A**	1.69 **b B**	2.04 **a B**	2.06 **a B**	2.03 **a B**	2.04 **a B**	2.03 **a B**

Capital letters within each of the date differ the cultivars from each other.

Lower case letters differ the behavior of each cultivar during the cycle (DAA) by the Scott Knott test at 5%.

Supplementary Table 4. Dry biomass partition between seeds, pericarp and placenta in different cultivars of *C. annuum* (75, MC2-8 and CVO) as candidates as a model plant.

Cultivars	Total dry biomass per fruit (g)	dry fruit biomass partition (%)	
		Seeds	Pericarp + placenta
75	0.52 **a**	36.25 **b B**	63.75 **b A**
MC2-8	0.24 **c**	26.64 **c B**	73.36 **a A**
CVO	0.45 **b**	43.02 **a B**	56.97 **c A**

Different lowercase letters differ among the cultivars, while capital letters differ the dry biomass partition within each cultivar by the Scott-Knott test at a probability level of 5%.

References

Acquadro A. *et al*. (2020) Whole genome resequencing of four italian sweet pepper landraces provides insights on sequence variation in genes of agronomic value. **Sci Rep** 10: 9189. https://doi.org/10.1038/s41598-020-66053-2

Aizat W.M. *et al*. (2013) Characterization of ethylene pathway components in non-climacteric *Capsicum*. **BMC Plant Biol**, 13, 191. https://doi.org/10.1186/1471-2229-13-191

Alcalá-Rico J.S.G.J. *et al.* (2019) Seed physiological potential of *Capsicum annuum* var. *glabriusculum* genotypes and their answers to pre-germination treatments. **Agronomy**, 9: 325.

Alfred J. and Baldwin I.T. (2015) The Natural History of Model Organisms: New opportunities at the wild frontier. **eLife**, DOI: 10.7554/eLife.06956

Alvares-Bianchi P. *et al.* (2020) Biomorphological characterization of Brazilian *Capsicum Chinense* Jacq. germplasm. **Agronomy**,10: 447.

Anderson J.T., Lee C.R., Mitchell-Olds T. (2014) Strong selection genome-wide enhances fitness trade-offs across environments and episodes of selection. **Evolution**, 68(1), pp. 16-31. doi:10.1111/evo.12259.

Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. **Nature**, 408, pp. 796–815.

Ávila Silva L. *et al*. (2019a). Nitrogen differentially modulates photosynthesis, carbon allocation and yield related traits in two contrasting *Capsicum chinense* cultivars. **Plant Science**, 283, pp. 224-237.

Ávila Silva L. *et al*. (2019b). Source strength modulates fruit set by starch turnover and export of both sucrose and amino acids in pepper. **Plant Cell Physiol**, 60, pp. 2319–2330.

Aza-González C., Núñez-Palenius H.G., Ochoa-Alejo N. (2012) Molecular biology of chili pepper anthocyanin biosynthesis. **J. Mex. Chem. Soc.**, 56, pp. 93–98.

Baruah J. and Lal M. (2020) ***Capsicum chinense*** **Jacq.: Ethnobotany, Bioactivity and Future Prospects.** In: Singh B. (eds) Botanical Leads for Drug Discovery. Springer, Singapore. https://doi.org/10.1007/978-981-15-5917-4_16

Bemer M. *et al*. (2012) The Tomato FRUITFULL Homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. **Plant Cell**, 24, pp. 4437–4451.

Bertin N. and Génard M. (2018) Tomato quality as influenced by preharvest factors. **Sci. Hort.** 233, pp. 264-276. doi.org/10.1016/j.scienta.2018.01.056

Biles C.L. *et al*. (1993) Morphological and physiological changes during maturation of New Mexican type peppers. **J. Soc. Hort. Sci.,** 118, pp. 476–480.

Bird R.E. and Conner J.L. (1999) Container grown azalea response to sumagic sprays. **Proc. Southern Nursery Assn. Res. Conf**., 44, pp. 274–276.

Biswas T. *et al*. (2018) "**An Overview of the U.S. Bell Pepper Industry**". EDIS 2018. https://doi.org/10.32473/edis-fe1028-2017

Bodanapu R. *et al*. (2016) Nitric oxide overproduction in tomato *shr* mutant shifts metabolic profiles and suppresses fruit growth and ripening. **Front. Plant. Sci**., 7: 1714.

Boonman A. *et al*. (2006) Functional significance of shade-induced leaf senescence in dense canopies: an experimental test using transgenic tobacco. **Am. Nat.**, 168, pp. 597-607.

Carmel-Goren L. *et al*. (2003) The *SELF-PRUNING* gene family in tomato. **Plant Mol Biol**., 52, pp. 1215–1222.

Chen F.L.X. *et al*. (2019) Genome sequences of horticultural plants: past, present, and future. **Hortic Res,** 6: 112. https://doi.org/10.1038/s41438-019-0195-6

Cheng Y. *et al*. (2016) Putative WRKYs associated with regulation of fruit ripening revealed by detailed expression analysis of the WRKY gene family in pepper. **Sci Rep**, 6: 39000.

Cipollini D.F. and Schultz J.C. (1999) Exploring cost constraints on stem elongation in plants using phenotypic manipulation. **The American Naturalist**, 153:2, pp. 236–242.

Cohen O. *et al*. (2012) *CaJOINTLESS* is a MADS-box gene involved in suppression of vegetative growth in all shoot meristems in pepper. **J. Exp. Bot**., 63, pp. 4947–4957.

Cohen O. *et al*. (2014) *Capsicum annuum S* (*CaS*) promotes reproductive transition and is required for flower formation in pepper (*Capsicum annuum*). **New Phytol,** 202, pp. 1014-1023

Colonna V. *et al*. (2019) Genomic diversity and novel genome-wide association with fruit morphology in *Capsicum*, from 746k polymorphic sites. **Sci Rep**, 9, 10067.

Corrales A.R. *et al*. (2017) Multifaceted role of cycling Dof Factor 3 (CDF3) in the regulation of flowering time and abiotic stress responses in Arabidopsis. **Plant Cell Environ**, 40:5, pp. 748–764.

Crepy M.A. and Casal J.J. (2015) Photoreceptor-mediated kin recognition in plants. **New Phytol,** 205, pp. 329–338. https://doi.org/10.1111/nph.13040

Cruz C.D. (2013) Genes - a software package for analysis in experimental statistics and quantitative genetics. A**gronomy**, 353, pp. 271-276.

Dombroskie S.L. *et al*. (2016). Leafing intensity and the fruit size/number trade-off in woody angiosperms. **J Ecol**, 104, pp. 1759–1767.

Elitzur T. *et al*. (2009) Co-ordinated regulation of flowering time, plant architecture and growth by *FASCICULATE*: the pepper orthologue of SELF PRUNING. **J Exp Bot**, 60, pp. 869–880.

Emmanuel E. and Levy A.A. (2002) Tomato mutants as tools for functional genomics. **Curr Opin Plant Biol**, 5, pp. 112-117.

Filyushin M.A. *et al*. (2020) Dependence of pepper fruit colour on basic pigments ratio and expression pattern of carotenoid and anthocyanin biosynthesis genes. **Russ J Plant Physiol**, 67, pp. 1054–1062.

Finger F.L. *et al*. (2010) Genetic diversity of *Capsicum chinense* (Solanaceae) accessions based on molecular markers and morphological and agronomic traits. **Genet Mol Res**, 9, pp.1852–1864.

Fraley R.T. *et al*. (1983) "Expression of bacterial genes in plant cells". **Proc. Natl. Acad. Sci. U.S.A.**, 80, pp. 4803–4807.

Ganapathi T.R. *et al*. (2004) Tobacco (*Nicotiana tabacum* L.) – A model system for tissue culture interventions and genetic engineering. **Indian J Biotechnol,** 3, pp. 171-184.

Gaston A. *et al*. (2019) Applying the Solanaceae strategies to strawberry crop improvement. **Trends Plant Sci,** https://doi.org/10.1016/j.tplants.2019.10.003

Gross K.C. *et al*. (1986) Biochemical changes associated with the ripening of hot pepper fruit. **Physiol Plant**, 66, pp. 31-36.

Gruntman M. *et al*. (2017) Decision-making in plants under competition. **Nat Commun** 21;8(1), pp.2235.

Gur A. *et al*. (2010) hi2-1, A QTL which improves harvest index, earliness and alters metabolite accumulation of processing tomatoes. **Theor Appl Genet,** 121, pp. 1587–1599.

Hayano-Kanashiro C. *et al*. (2016) Wild pepper *Capsicum annuum* L. var. *glabriusculum*: taxonomy, plant morphology, distribution, genetic diversity, genome sequencing, and phytochemical compounds. **Crop Sci**, 56, pp. 1–11.

Hernández-Verdugo S. *et al*. (2001) Differentiation in seed germination among populations of *Capsicum annuum* along a latitudinal gradient in Mexico. **Plant Ecol,** 155, pp. 245–257.

Hou B. *et al*. (2018) Characterization of the hot pepper (*Capsicum frutescens*) fruit ripening regulated by ethylene and ABA. **BMC Plant Biol**, 18, pp. 162.

Huberman M. *et al*. (1997) Role of ethylene biosynthesis and auxin content and transport in high temperature-induced abscission of pepper reproductive organs. **J Plant Grow Regul**, 16, pp. 129–135.

Jeifetz D. *et al*. (2011) *CaBLIND* regulates axillary meristem initiation and transition to flowering in pepper. **Planta**, 234, pp. 1227–1236.

Kim S. *et al*. (2017) New reference genome sequences of hot pepper reveal the massive evolution of plant disease-resistance genes by retroduplication. **Genome Biol,** 18: 210.

Knaap E. van der *et al*. (2014) What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape. **Front Plant**, Sci 5: 227.

Krämer U. (2015) Planting molecular functions in an ecological context with *Arabidopsis thaliana*. **eLife** 4: e06100.

Kulkarni M. and Phalke S. (2009) Evaluating variability of root size system and its constitutive traits in hot pepper (*Capsicum annuum* L.) under water stress. **Sci Hortic,** 120, pp. 159–166.

Lemaire-Chamley M. *et al*. (2005) Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. **Plant Physiology**, 139:2, pp. 750-769; DOI: 10.1104/pp.105.063719

Leng P. *et al*. (2014) The role of abscisic acid in fruit ripening and responses to abiotic stress. **J Exp Bot**, 65, pp. 4577-4588.

Li Z. *et al*. (2013) A further analysis of the relationship between yellow ripe fruit color and the capsanthin-capsorubin synthase gene in pepper (*Capsicum* sp.) indicated a new mutant variant in *C. annuum* and a tandem repeat structure in promoter region. **PLoS ONE**, 8. https://doi.org/10.1371/journal.pone.0061996

Lu G. *et al*. (1990) ‘Changjiao’ hot peppers are non-climacteric. **HortSci**, 25:7, pp. 807-807.

Lundgren M.R. and Marais Des D.L. (2020) Life history variation as a model for understanding trade-offs in plant–environment interactions. **Curr Biol**, 30, pp.180–189.

Marcelis L.F.M. *et al*. (2004) Flower and fruit abortion in sweet pepper in relation to source and sink strength. **J Exp Bot**, 55, pp. 2261–2268.

Meissner R. *et al*. (1997) A new model system for tomato genetics. **Plant J**, 12:6, pp. 1465-1472.

Mercado J.Á. *et al.* (1997) Effects of low temperature on pepper pollen morphology and fertility: Evidence of cold induced exine alterations. **J Hort Sci,** 72:2, pp. 317-326.

Naegele R.P. *et al*. (2016) Genetic diversity, population structure, and heritability of fruit traits in *Capsicum annuum*. **PLoSONE**, 11(7): e0156969. doi:10.1371/journal.pone.0156969

Naves E.R. *et al*. (2019) Capsaicinoids: pungency beyond *Capsicum*. **Trends Plant Sci,** 24:2, pp. 109–120.

O’ Donoghue E.M. *et al*. (2018) Sweet *Capsicum*: post-harvest physiology and technologies. **New Zealand J Crop Hort Sci**, 46:4, pp. 269-297.

Park S.J. *et al.* (2014) Meristem maturation and inflorescence architecture—lessons from the Solanaceae. **Curr Opin Plant Biol**, 17, pp. 70–77.

Paul V. *et al.* (2012) The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview**. J Food Sci Technol**, 49:1, pp. 1–21.

Pickersgill B. (1997) Genetic Resources and Breeding of *Capsicum* spp. **Euphytica**, 96, pp. 129-133.

Poorter H. et al. (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. **Funct. Plant Biol**., 39, pp. 839-850.

Qin *et al*. (2014) Whole-genome sequencing of cultivated and wild peppersprovides insights into *Capsicum* domestication and specialization. **ProcNatl Acad Sci U.S.A.**, 111, pp. 5135–5140.

Rahman M.A. *et al*. (2014) Changes in physicochemical attributes of sweet pepper (*Capsicum annuum* L.) during fruit growth and development. **Bangladesh J Agric Res,** 39, pp. 373-383.

Ranal M. *et al*. (2009) Calculating germination measurements and organizing spreadsheets. **Rev. bras. Bot.**, 32:4, pp. 849-855.

Ribeiro D.M. *et al*. (2010) Dormancy breakage of *Stylosanthes humilis* seeds by aluminium. **Seed Sci Res**, 20:3, pp. 145–152.

Robert H.S. (2019) Molecular communication for coordinated seed and fruit development: What can we learn from auxin and sugars? **Int J Mol Sci,** 20: 936. doi: 10.3390/ijms20040936

Rodríguez M.V. *et al*. (2011) Challenges facing seed banks and agriculture in relation to seed quality. In: Kermode A. (eds) Seed Dormancy. **Methods in Molecular Biology** (Methods and Protocols), vol 773. Humana Press.

Scott J.W. and Harbaugh B.K. (1989) **Micro-Tom: a miniature dwarf tomato**. Agricultural Experiment Station, Institute of Food and Agricultural Sciences, University of Florida, Circular, S-370, pp.1–6.

Shleizer-Burko S. *et al*. (2011) Dynamic growth program regulated by *LANCEOLATE* enables flexible leaf patterning. **Development**, 138, pp. 695-704.

Siri B. *et al*. (2013) Improvement of quality, membrane integrity and antioxidant systems in sweet pepper (*Capsicum annuum* L.) seeds affected by osmopriming. **Aust J Crop Sci,** 7, pp. 2068–2073.

Smith P.T. and Cobb B. (1991) Accelerated germination of pepper seed by priming with salt solutions and water. **HortScience HortSci**, 26, pp. 417-419.

Stellari G.M. *et al*. (2010) Contrasting modes for loss of pungency between cultivated and wild species of *Capsicum*. **Heredity**, 104, pp. 460–471.

Stevens M.A. and Rick C.M. (1986) **Genetics and breeding**. InJAtherton,J Rudich, eds, The Tomato Crop: A Scientific Basis for Improvement. Chapman & Hall, London, pp. 35–100.

Stewart C. *et al*. (2005) The *Pun1* gene for pungency in pepper encodes a putative acyltransferase. **Plant J**, 42, pp. 675–688.

Tadesse T. *et al*. (2002) Changes in physicochemical attributes of sweet pepper cv. Domino during fruit growth and development. **Sci. Hortic**., 93:2, pp. 91-103.

Tiwari A. *et al*. (2012) Auxin-induced fruit set in *Capsicum annuum* L. requires downstream gibberellin biosynthesis. **J Plant Growth Regul**, 31, pp. 570–578.

U.S. National Plant Germplasm System. 2017. Published on the Internet: **https://www.ars-grin.gov/npgs/cgc_reports/pepper_vulnerability_statement_2017.pdf**.

Upreti K.K. *et al*. (2012). Floral abscission in *Capsicum* under high temperature: role of endogenous hormones and polyamines. **Indian J Plant Physiol**, 17:3, pp. 207-214.

Vandenbussche M. *et al*. (2016) Petunia, your next supermodel? **Front Plant Sci**, 7:72.

Vicente M.H. *et al*. (2015) Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*). **J Plant Physiol,** 177, pp. 11-19.

Villavicencio L.E. *et al*. (2001) Ethylene and carbon dioxide concentrations in attached fruits of pepper cultivars during ripening. **Sci Horticul**, 91, pp. 17-24.

Wahyuni Y. *et al*. (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: variation in health-related compounds and implications for breeding. **Phytochem**, 72, pp. 1358–1370.

Wang D. and Bosland P. (2006) The genes of *Capsicum*. **HortSci**, 41, pp. 1169–1187.

Wang T. *et al*. (2020) Phenotypic diversity and genome-wide association mapping of earliness-related traits in cultivated tomato (*Solanum lycopersicum* L.). **Sci Horticul,** 264: 109194.

Xu X. *et al*. (2011) Genome sequence and analysis of the tuber crop potato. **Nature,** 475, pp. 189–195.

Zhou Y. *et al*. (2017) Atmospheric CO2 alters resistance of Arabidopsis to *Pseudomonas syringae* by affecting abscisic acid accumulation and stomatal responsiveness to coronatine. **Front Plant Sci**, 8: 700.

CHAPTER 2

New perspectives of heterotic action in *Capsicum* crosses

Abstract

Capsicum has been appreciated worldwide as a culinary component since Colombus' journey, widespread as an alternative culinary component to black pepper. *Capsicum* fruits have other functions than cooking, but recurrently related to pungency. Like recreational use in competitions to eat the world's hottest peppers, medicinal use for pain and weight loss and as a protective weapon used in the form of spray. The number of species described in this genus has been growing showing that much still needs to be explored botanically in this genus. Here, we use two cultivars of *Capsicum annuum* (IKE and JAL) and two cultivars of *C. chinense* (HAB and BIQ) to perform crosses in all possible combinations, in order to assess F1 performance in terms of germination, growth and development, productivity, fruit morphology, fertility and heterotic potential. Crossings carried out between the same species (intraspecific crossings), we observed at least the maintenance of high productive potential of the hybrids, although with plants with less vigor. Crossbreeding between different species (interspecific crossings) provided extreme vegetative growth, but with impaired fertility and, therefore, productivity depending on the genotypes involved in the crossings. We obtained insights into the hybridization phenomenon by regulating seed size, vegetative growth, biomass accumulation and allocation, parental effect and in addition to interfering with fertility with action on fruit characteristics such as size, biometrics, organoleptic and nutritional properties. With this study we open new perspectives and challenges that must be explored and overcome to improve the genetic improvement in this genus.

Introduction

In *Capsicum*, 41 species have been described (Barboza *et al*., 2020), of which only five (*C. annuum*, *C. chinense*, *C. baccatum*, *C. frutescens* and *C. pubescens*) are considered domesticated. The number of species in the genus *Capsicum* is low in relation to that found in other large genera, and they have wild species with different chromosome numbers in relation to cultivated species of *Capsicum* (Carrizo García *et al*., 2016). This differentiation in chromosome number precludes gene exchange by conventional methods between these species, making access to this genetic pool impractical for breeding. Even the cultivated species, belonging to the most derived group within the phylogenetic classification, despite sharing chromosome number and being more genetically closely related, have varying degrees of incompatibility, restricted to species belonging to the same clade (Pickersgill, 1991; Gupta and Tsuchyva, 1992; Martins *et al*., 2015). Allied to this, studies with the cultivated species of *Capsicum* show recalcitrance regarding *in vitro* regeneration and genetic transformation (Ochoa-Alejo and Ramirez-Malagon, 2001; Kothari *et al*., 2010), making it difficult to use molecular tools for understanding of genes and genetic improvement. Therefore, the employability of various crosses to investigate the best combinations of genetic characteristics is a plausible tool for breeding purposes for this genus. In contrast to the low number of species, a large number of varieties are found within each domesticated species of *Capsicum*, mainly in *C. annuum* and *C. chinense*, and for this reason it constitutes a fertile field for such endeavors.

The intimate and long-standing contact of *Capsicum* with man’s artificial selection resulted in strong genetic and phenotypic changes over time (Gonzávez-Jara *et al*., 2011). The large, non-pungent bell peppers fruits (*C. annuum*) found in the markets are almost nothing like their ancestors, which harbor tiny red fruits scattered by birds (Pickersgill, 1971). *Capsicum* breeding programs for food have several aspects, which go beyond increasing fruit size. There is the global improvement for colored, standardized fruits, increased firmness and thickness of pericarp, flavor, presence of sugars, pungency and to increase yield, resistance to diseases and pests (Rêgo *et al*., 2012; Joshi and Berke, 2008). *Capsicum* fruits have numerous secondary compounds, which, in addition to providing color and different organoleptic characteristics, have recognized nutritional properties (Matsufuji *et al*., 1998; Kim *et al*., 2009). Among others, they are considered one of the richest fruits in vitamin C (Palma *et al*., 2015). The increase in nutritional compounds is modest, either due to the slowness of conventional breeding programs with results below expectations (Gómez García *et al*., 2013), or because *Capsicum* is already a good source for many of them.

Capsaicinoids, the secondary compounds that provide *Capsicum* fruits with their characteristic pungency are synthesized in the fruit placenta through a complex biochemical pathway, with the participation of various organelles (Mazourek *et al*., 2009). In addition to imparting the 'heat' of pungent pepper fruits, they are clinically recognized for pain treatments (Spiller *et al*., 2008), antioxidant activity (Bogusz *et al*., 2017) and potentially considered to have antitumoral activity (Chapa-Oliver and Mejía-Tenient, 2016). The color in *Capsicum* fruits is due to the synthesis and accumulation of various compounds, among them carotenoids with provitamin A and antioxidant activity (Simonne *et al*., 1997). In addition, red fruits mainly contain capsorubin and capsanthin-5,6-epoxide, which are exclusive *Capsicum* pigments; while yellow or orange colored fruits have α and β-carotene, zeaxanthin, lutein and β-cryptoxanthin pigments (Gómez-García and Ochoa-Alejo, 2013). There are improvement programs aimed at the creation of increasingly pungent cultivars, with the emergence of more pungent cultivars being reported in *Guinness World Records* with some frequency. The diversity of coloring in *Capsicum* fruits is also explored in breeding programs aimed at attracting the consumer market, although the presence of certain colors are associated with certain pigments with greater or lesser nutritional value (Wahyuni *et al*., 2011).

The various breeding programs in *Capsicum* were based on crosses followed by directional selection, and is commonly called hybridization. The practical results in *Capsicum* show that the cultivation of hybrids instead of varieties can increase productivity, precocity, plant height, fruit length, fruit mass (Madhavi Reddy, 2010; Meshram and Mukewar, 1985; Parvinder Singh *et al*., 2014), depending on the combination of parents used in the cross (Rao *et al*., 2017). Hybridization has been used as a practical tool in the improvement of various cultures for decades, even though its genetic mechanisms are not yet fully understood (Evans and Fischer, 1999). The initial description of the increase in vegetative, productive and growth vigor when crossing plants is remote to the 18th century (Chen *et al*., 2013; Goff, 2011), and can be considered the first scientific report of heterosis. The occurrence of the heterotic phenomenon can be observed by the better performance in growth, productivity or adaptability of hybrids in relation to parental (Chen *et al*., 2013), although it is safer to define the occurrence of heterosis only when the performance of hybrids is superior to the performance of the best parental (Whaley, 1944).

In spite of its relevance to agriculture, the heterotic phenomenon still intrigues due to its many peculiarities and no consensus has yet been reached about the mechanistic basis of its operation. It does not occur among all crosses, nor for all crops or when it occurs it does not happen for all productive or vegetative characteristics, and it is common to increase one characteristic at the expense of others. Instead, it depends on the adequate combinatorial ability between the parental genotypes, and it is reported to be more pronounced in proportion to the genetic distance between the parents (Offermann and Peterhansel, 2014). However, the presence of epigenetic distance, that is, only the differentiation as to the pattern of gene expression among parents, even in genetically close parents, seems to be important in the manifestation of heterosis (Groszmann *et al*., 2011; Groszmann *et al*., 2014).

But what is the mechanism or genetic basis responsible for heterosis? The most reasonable answer would be that it depends. Heterosis can be manifested through different types of genetic inheritance, depending on the culture and / or the type of traits analyzed. There are references to the phenomenon of heterosis through the action of epistatic (Yu *et al*., 1997) and dominant (Xiao *et al*., 1995) genes in rice, the epistatic action for growth parameters in Arabidopsis (Melchinger *et al*., 2007), dominance (Zhang *et al*., 2019) and DNA methylation (Nakamura and Hosaka, 2010) in potatoes and the super-dominance genes in tomato productivity (Krieger *et al*., 2010). So, as several genetic mechanisms can lead to heterosis, in practical terms it can be synthesized as a result of the appearance of a new favorable pattern of expression of genes arising from hybridization (Lippman and Zamir, 2006).

Cultures such as corn (*Zea mays*) show a high degree of heterosis after crossing of strains that artificially suffered the phenomenon of inbreeding depression through successive self-pollinations, since as naturally it is an allogamous species (East and Hayes, 1912). Unlike corn, the genus *Capsicum* does not show inbreeding effect when self-pollinated successively. In part, because it is considered an autogamous crop (Allard,

1960) even though cross-pollination can be very frequent in field conditions depending on the presence of bees (Raw, 2000). As noted by Friedman (2015), the consequences of hybridization and therefore the promotion of heterosis depend on the evolutionary history of plants and also on the selection system obtained during the domestication process.

For breeding purposes, both intraspecific and interspecific hybridization can be used. The first is more usual for the production of highly productive hybrids of both tomato (Gur and Zamir, 2004) and *Capsicum* (Bosland and Votava, 2012). The second, on the other hand, offers a greater challenge due to the incompatibility barriers that have arisen with the evolution and separation of species that make its realization more complex and with deleterious effects. Nevertheless, it has been used for the incorporation of resistance genes from *C. chinense* to *Tomato spotted wilt virus* in *C. annuum*, for example (Ngog *et al*., 2013), with the recovery of the productive characteristics of the parent of interest through successive backcrosses.

The difficulty in using interspecific hybridization in both tomatoes and *Capsicum* is due to the high degree of the phenomenon of gametophytic or sporophytic incompatibility, where the crossability and F_1 viability and fertility depend on which species is used as donor or recipient of pollen (Peralta *et al*., 2008; Pickersgill, 1997). Based on reciprocal crossings, it is possible to verify in which direction the incompatibility exists. In addition, it allows the study of genetic inheritance of the extrachromosomal type, also called mitochondrial and chloroplast cytoplasmic inheritance.

It is clear that within *Capsicum* improvement programs there are many questions and gaps that need to be answered or better clarified. The understanding of mechanisms related to heterosis and the relationship with the genetic distance associated with the type of hybridization (intra or interspecific), with the incompatibility mechanisms and with the presence or absence of extrachromosomal inheritance could serve as a guide for the creation of new cultivars, for example, to increase the size and shape, flavor, pungency and nutraceutical properties of the fruit or even to promote more vigorous vegetative characteristics for existing cultivars. From this, in this work we seek to outline the perspectives of inter and intraspecific hybridization, in addition to genetic reciprocity, within our study as tools of genetic gain in this fascinating culture.

Material and methods

Plant material and growth conditions

The experiments were conducted in semi-controlled greenhouse conditions located at the Federal University of Viçosa, between August 2018 and December 2019. For productivity analysis, each *Capsicum* hybrid and its respective parents (controls) were grown until harvest (when 70% of the fruits reached the ripeness state) in 7.5 L pots containing commercial substrate Tropstrato® and irrigated daily, with a total of 10 plants per genotype. The commercial substrate was enriched with an amount of 4.5 g. L^{-1} of NPK fertilizer (4-14-8) and 4 g. L^{-1} of dolomitic limestone during planting and complementary fertilizations were carried out as required by the plants. During the fruit formation phase, foliar fertilization was performed with calcium nitrate to assist in fruit development.

Crosses and obtention of F_1 hybrids

Commercial cultivars of *Capsicum annuum* and *C. chinense* were chosen to obtain F_1 hybrids and estimate heterosis. The choice of these two species to compose the hybridization was made by the representativeness of the species they have and also by their economic importance. For *Capsicum annuum* the cultivars "Cascadura Ikeda" (IKE) and "Jalapeño" (JAL) were used. For *C. chinense*, the chosen cultivars were “Habanero” (HAB) and “Biquinho” (BIQ). The seeds of each cultivar were obtained commercially from Topseed® (São Paulo, Brazil), and were grown for four generations under controlled self-pollination system to guarantee varietal purity. These species and cultivars have numerous contrasting characteristics, among them: plant architecture, productivity in number and weight, size and morphology of fruits, in addition to the presence or absence of secondary metabolites responsible for pungency: JAL and HAB are hot peppers, whereas IKE and BIQ are sweet. The crosses were performed in a diallel arrangement, totaling 12 combinations. The methodology to carry out the crossings consisted of emasculating the flower buds of the mother plant 1-3 days before anthesis, usually in the morning, when the pollen transfer was made. The floral bud was then covered with aluminum foil to prevent contamination of exogenous pollen.

Seed germination test

For the seed germination test, 100 seeds were sown per replicate, with a total of four replicates. The seeds were pre-treated with sodium hypochlorite at a concentration of 30 % and subsequently washed with plenty of deionized water before sowing. Afterwards they were sown in plastic pots containing commercial substrate Tropstrato® and kept in greenhouse conditions in the summer. The total number of emerged plants was counted 30 days after the sowing date and the germination percentage was estimated.

Plant growth determinations

30 days after sowing, 15 days after emergence, 30 plants were individually transplanted into each cell in a sowing tray with a capacity of 125 mL per cell. The first evaluation of growth parameters was made 45 days after sowing (timepoint 1, T1) with 10 plants for the quantification of plant height (height of soil base up to leaf apex), canopy diameter, stem diameter, number of leaves and the leaf area and root, stem and leaves dry mass. Another 10 had their stem diameters, number of leaves, soil base height up to the leaf apex and canopy diameter, and were subsequently transplanted individually to pots with a Tropstrato® commercial substrate with volume of 1.5 L. These were measured at 90 days after sowing (pre-flowering stage-T2), with all parameters as measured at T1.

For time point 3 (T3), 10 plants that were transplanted 30 days after seeding in plastic containers with a capacity of 7.5 L containing Tropstrato commercial substrate were measured at harvest. For the T3 plants, all the parameters evaluated in T1 and T2 were determined. In addition, phenological characteristics related to precocity were evaluated, such as the time in days required between sowing until anthesis and sowing until the first ripe fruit. At the time of harvest, productivity was determined as number and in total fresh weight of fruits per plant. The angle formed between the first ten branches was measured in addition to the total number of sympodial units per plant.

Fertility Estimation

The fertility component can influence several aspects of hybridization, from the difficulty in crossing due to incompatibility between parents and problems of germination, development and fruiting of the resulting F_1. Therefore, we measured it as fruit set (%), size of fruit, number of seeds per fruit and germination rate, since isolated analyses may incur errors.

The compatibility between the parents, as well as the compatibility between the F_1 hybrids and their respective parents was measured through crosses in all possible combinations. For this purpose, an average of 25 flowers per repetition was crossed, with four repetitions, totaling 100 flowers per cross. As a control condition for each cross, 100 flowers were also marked, however, they were self-pollinated. The fresh weight, length, diameter and number of seeds per fruit were estimated for the 12 largest fruits among all produced from each controlled cross.

The average mass (g) of 100 F_1 seeds was also quantified (n = 6) and germination tests were performed to verify the relationship between seeds production, number, size and germination with fertility.

Determination of soluble solids content (º Brix)

Six fully ripe fruits per plant were harvested, totaling 60 fruits per genotype. The ºBrix was determined through the digital bench refractometer model RTD-45 (Konica, Minolta®). The analyses were performed after calibrating the refractometer with distilled water and obtaining the zero, after which the collector part was wiped clean, then the pericarp was squeezed in order to extract enough liquid to cover the prismatic surface of the digital refractometer.

Fruit biometry

For fruit biometrics, Tomato Analyzer® version 4.0 computer software was used, as described by Brewer *et al.*, (2006). 12 major representative mature fruits were sampled within each parental and hybrid genotype. In addition, as interspecific hybrids showed different morphology and exorbitant increase in fruit size when they received pollen from parents, we decided to include them in these analyzes. The fruits were cut longitudinally and were digitized in a scanner brand Hp Scanjet G2410. In some cases, the fruits had the biometric contours adjusted manually in order to correct points of non-detection in the image. 32 biometric parameters were measured, such as: perimeter (cm), area (cm^2), width mid-heigh (cm), maximum width (cm), height mid-width (cm), maximum height (cm), curved height (cm), fruit shape index external I, fruit shape index external II, curved fruit shape index, proximal fruit blockiness, distal fruit blockiness, fruit shape triangle, ellipsoid, circular, rectangular, obovoid, ovoid, v. asymmetry (cm), h. asymmetry.ob (cm), h. asymmetry.ov (cm), width widest, eccentry, proximal eccentricity, distal eccentricity, fruit shape index internal, eccentricity area index, lobedness degree, pepper pericarp area (cm²), pepper pericarp area ratio, pepper pericarp thickness (cm) and pepper pericarp thickness ratio.

Experimental design and data analysis

The experiments were carried out in a completely randomized design, always with the parents being cultivated together with their hybrids in their respective growing season. The data were subjected to analysis of variance and after found the significance and in most cases the Scott-Knott test at 5% probability was used. Heterosis calculations were performed for the main parameters evaluated, using the following equations:

Absolute Mid-Parental Heterosis (AMPH) = $F1- \overline{P}$

Absolute Best Parental Heterosis (ABPH) = $F1- Pmax$,

Where, F1: F1 progeny performance; $\overline{P}$: Average performance between parents and Pmax: Average performance of the best parent. When the crosses took place reciprocally, the F1 performance value was considered as the average value between them.

In addition, Heterosis relative values were calculated as:

Relative Mid-Parental Heterosis (rMPH) = $(AMPH / \overline{P}) * 100$

Relative Best Parental Heterosis (rBPH) = $(ABPH / Pmax) * 100$

To measure the reciprocal effect at the crosses between HAB × JAL, JAL × IKE and HAB × BIQ, it was done through the difference in performance between the hybrids (HAB × JAL) - (JAL × HAB) and so on for the others. The absolute values of heterosis and reciprocity were subjected to analysis of variance and subsequently subjected to Scheffe's test in order to verify the significance of each value individually.

Results

Plant growth and hybridization

The plants used in the crosses have contrasting characteristics in the vegetative and reproductive aspects (Fig. 1). The cultivars of *C. annuum* (Cascadura Ikeda and Jalapeño) have a more upright canopy architecture and large fruits. Although they are more upright, in their cultivation the conduction system that supports the growth of vegetative branches and fruits is necessary. Its numerous and large fruits and fragile stems are responsible for this requirement. Ikeda has non-pungent fruits for consumption like sweet peppers, while Jalapeño is a cultivar recognized for its pungency. The cultivars of *C. chinense* (Biquinho and Habanero), in an opposite way, show predominantly horizontal canopy architecture, with more vigorous stems and in addition with smaller and / or less weight of fruits. Both are consumed as spice, while Biquinho is appreciated for its non-pungency, Habanero is for its high pungency.

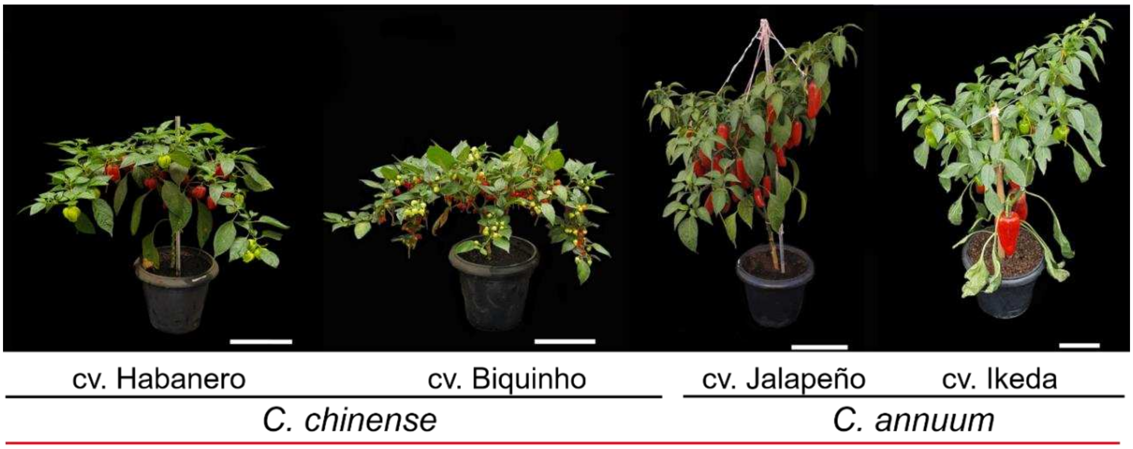


Figure 1. Pepper plants used in diallel crosses showing different vegetative architecture and fruit morphology in full reproductive stage (180 days after sowing). From left to right: *C. chinense* cv. Habanero, *C. chinense* cv. Biquinho, *C. annuum* cv. Jalapeño and *C. annuum* cv. Ikeda. Scale bars 15 cm.

The contrasts regarding these innumerable aspects were fundamental, because in addition to facilitating the observation of the contribution of each parental cultivar to each F_1 hybrid, they allowed the investigation of favorable characteristics for the development of new cultivars. In addition, the cross between these parents resulted in hybrids with completely different characteristics in relation to the parents (Fig. 2). The intraspecific hybridization between *C. annuum* showed to be more moderate in vegetative characteristics, with predominance of intermediate characteristics among its parents, although dry vegetative mass for JAL × IKE and its reciprocal counterpart IKE × JAL with values lower than the parents (Table 1). The same occurred for the hybrids between *C. chinense*, with a decrease in vegetative biomass and plant height in relation to their parents, although characteristics such as leaf area with increase only for HAB × BIQ and plant diameter increased for both hybrids.

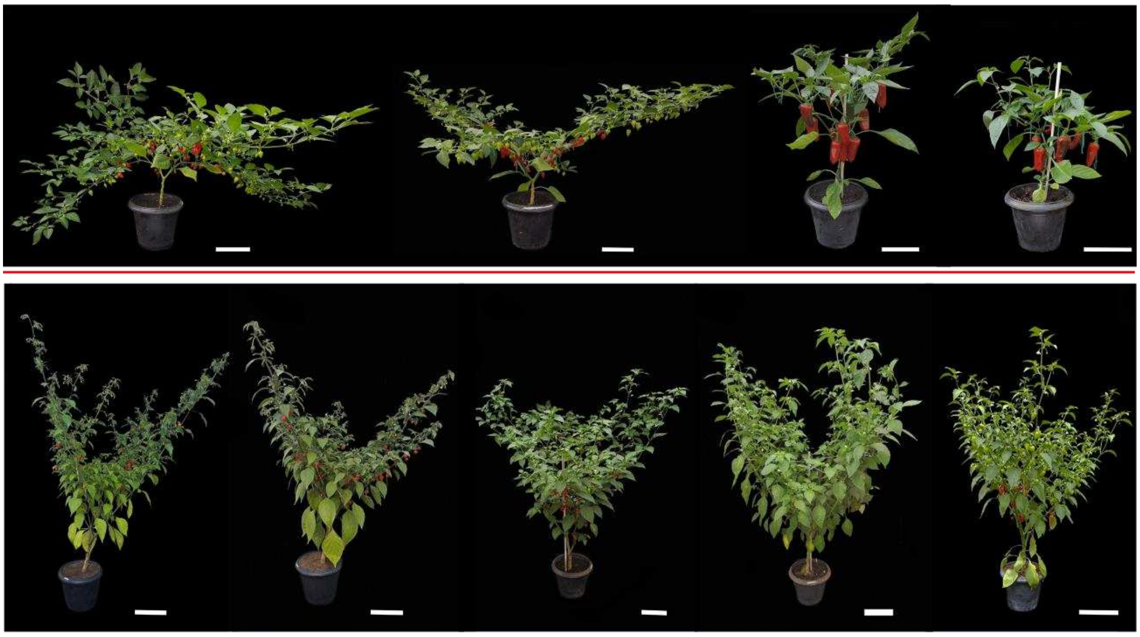


Figure 2: Representative F_1 hybrid plants from intraspecific (top) and interspecific (bottom) crosses at 180 days after sowing. Top from left to right: *C. chinense* cv. Habanero × *C. chinense* cv. Biquinho (HAB x BIQ), *C. chinense* cv. Biquinho × *C. chinense* cv. Habanero (BIQ × HAB), *C. annuum* cv. Jalapeño × *C. annuum* cv. Ikeda (JAL × IKE), *C. annuum* cv. Ikeda × *C. annuum* cv. Jalapeño (IKE × JAL). Bottom from left to right: *C. chinense* cv. Habanero × *C. annuum* cv. Jalapeño (HAB × JAL), *C. annuum* cv. Jalapeño × *C. chinense* cv. Habanero (JAL × HAB), *C. annuum* cv. Jalapeño × *C. chinense* cv. Biquinho (JAL x BIQ), *C. annuum* cv. Ikeda × *C. chinense* cv. Habanero (IKE × HAB) and *C. annuum* cv. Ikeda × *C. chinense* cv. Biquinho (IKE × BIQ). Scale bars 15 cm.

The interspecific hybridization shows the non-occurrence of all possible combinations of crosses between our cultivars of *C. anuuum* and *C. chinense*, mainly when *C. chinense* as a mother plant, due to problems inherent to the fertility of this type of crossing, reported in later topics. Contrary to parental and intraspecific hybrids, we saw that interspecific hybrids showed increased vegetative development, with greater vertical (height) and horizontal (diameter) growth, greater leaf area accumulation, resulting in greater dry vegetative biomass at the time of harvest for all these hybrids (Table 1, Fig 2).

Table 1. Parental (top), intraspecific (middle) and interspecific (bottom) hybrids differs in morphological parameters and yield at harvest moment -180 days after sowing.

Genotypes	Plant height (cm)	Plant diameter (cm)	Leaf area (m^2 $plant^{-1}$)	Dry vegetative mass (g $plant^{-1}$)
Parental				
HAB	61.0 ± 1.6 **g**	93.3 ± 1.5 **f**	0.736 ± 0.02 **e**	142.35 ± 0.96 **e**
BIQ	64.6 ± 1.3 **g**	100.6 ± 3.2 **e**	0.784 ± 0.02 **e**	141.46 ± 2.33 **e**
JAL	69.6 ± 1.7 **g**	69.3 ± 1.4 **h**	0.271± 0.01 **h**	54.62 ± 1.29 **h**
IKE	82.5 ± 1.9 **f**	60.7 ± 1.0 **i**	0.549 ± 0.01 **f**	78.75 ± 0.64 **g**
Intraspecific hybrids				
HAB × BIQ	42.6 ± 2.0 **h**	174.0 ± 2.7 **a**	0.995 ± 0,02 **c**	105.2 ± 5.4 **f**
BIQ × HAB	41.8 ± 1.8 **h**	153.6 ± 3.9 **c**	0.811 ± 0.02 **e**	111.7 ± 39 **f**
JAL × IKE	72.5 ± 2.7 **g**	79.1 ± 0.9 **g**	0.354 ± 0.005 **g**	48.8 ± 0.2 **h**
IKE × JAL	65.8 ± 2.1 **g**	70.4 ± 2.1 **h**	0.336 ± 0.01 **g**	33.4 ± 0.6 **i**
Interspecific hybrids				
HAB × JAL	140.3 ± 4.4 **c**	127.0 ± 2.8 **d**	0.890 ± 0.03 **d**	482.8 ± 14.8 **a**
JAL × HAB	110.0 ± 3.9 **e**	107.9 ± 2.57 **e**	0.766 ± 0.014 **e**	328.3 ± 5.25 **b**
JAL × BIQ	130.4 ± 5.1 **d**	127.9 ± 2.94 **d**	0.946 ± 0.025 **c**	188.2 ± 5.3 **d**
IKE × HAB	154.8 ± 3.5 **b**	165.4 ± 4.4 **b**	1.547 ± 0.050 **a**	235.4 ± 3.7 **c**
IKE × BIQ	175.8 ± 1.8 **a**	176.7 ± 3.7 **a**	1.253 ± 0.022 **b**	480.0 ± 6.28 **a**

Different letters within each column differentiate the genotypes from each other statistically using the Scott-Knot test at 5%.

The type of hybridization showed different results in relation to vegetative parameters, with the intraspecific hybrids with characteristics more similar to the parental ones, while the interspecific hybrids in the opposite way. *Capsicum* is recognized and appreciated for its fruits, so there is nothing more reasonable to verify the action of the type of hybridization in the partition between reproductive characteristics (dry weight of fruits) in proportion to the total dry biomass per plant, which is recognized as the harvest index (Fig. 3). The type of hybridization again showed to discriminate the genotypes. Intraspecific hybrids between JAL and IKE did not differ in relation to parental ones, with values of 0.47 ± 0.02 for JAL × IKE and 0.5 ± 0.03 for IKE × JAL. While hybrids between HAB and BIQ with intermediate values between parental ones, with 0.44 ± 0.01 for HAB × BIQ and 0.42 ± 0.02 for BIQ × HAB.

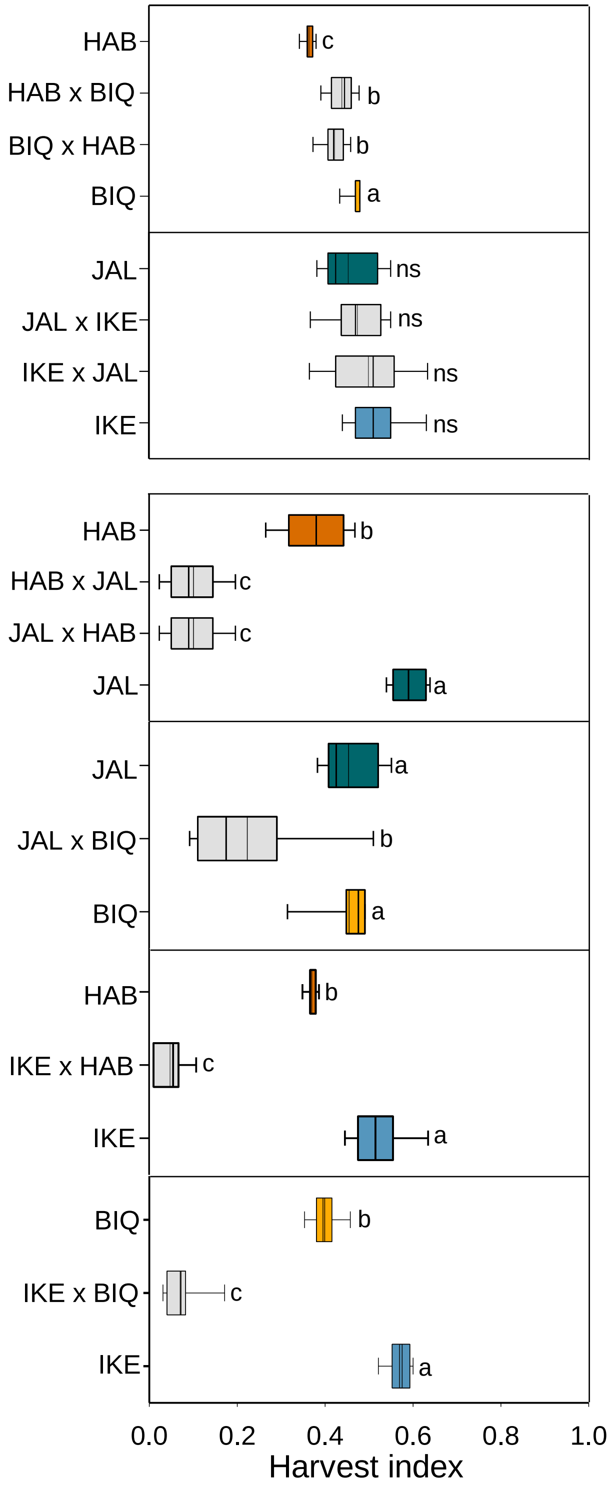


Figure 3. Harvest index between intraspecific hybrids (top) and interspecific hybrids (bottom) with their respective parents. Different letters within each biomass partition differ statistically by the 5% Scott Knot test.

The interspecific hybrids were unanimous in values lower than their respective parents, with the lowest harvest index for the hybrid IKE × HAB with 0.05 ± 0.02 and the highest for the hybrid JAL × BIQ with 0.19 ± 0.04, showing that the proportion of reproductive dry biomass is very low in relation to total biomass. The higher accumulation in dry vegetative biomass (Table 1) is not accompanied by the increase in dry reproductive biomass as observed by the low harvest index for interspecific hybridization. Then, we measure the percentage of preferential carbon allocation (dry matter) between the different vegetative (root, stem, leaf) and reproductive (fruit) organs to understand the relationship between the type of hybridization and the destination of carbon (Fig. 4). Carbon allocation is a key trait controlling yield in crops. The cultivars of *C. annuum* (JAL and IKE) partitioned the highest proportion of biomass to fruits (>50%), followed by stems, leaves and roots. *C. chinense* cultivars, on the other hand, partitioned proportionally less biomass to fruits (37 -43 %) and more to stems, leaves and roots. Intraspecific hybrids followed a similar pattern as their parents, however, the partitioning to fruits was reduced in *C. annuum* hybrids. Notably, in JAL × IKE F_1 plants, >20% of the biomass was found in roots, in contrast to 10% in the reciprocal IKE × JAL hybrids. The viable interspecific hybrids consistently partitioned around half of their biomass to stems and between 15% and 25% to roots. This resulted in severely reduced biomass partition (<20%) to fruits and, consequently, a low harvest index in the interspecific hybrids, compared to the intraspecific hybrids and the parentals (Fig. 3). The type of hybridization was decisive in the partition of biomass between vegetative and reproductive parts.

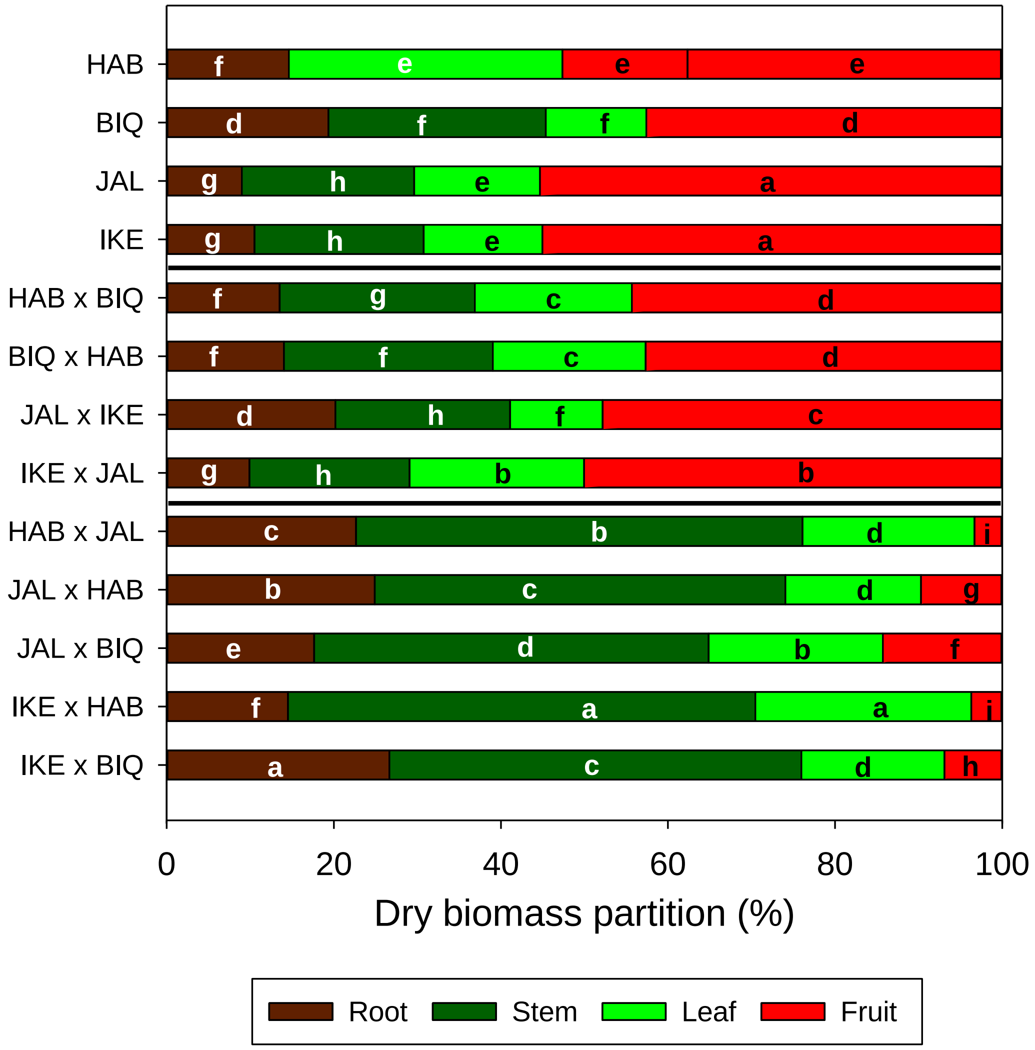


Figure 4. Biomass partitioning in intra- and interspecific crosses of *Capsicum*. Parental genotypes: *C. chinense* cv. Habanero (HAB), C. *chinense* cv. Biquinho (BIQ), *C. annuum* cv. Ikeda (IKE) and *C. annuum* cv. Jalapeño (JAL). Different letters within each biomass partition differ statistically by the 5% Scott Knot test.

Germination, seed size and viability

Germination and its related variables are determinants of success in the initial establishment of individuals, which is why it is very important for crops. The heaviest seeds among the parental genotypes were observed for the cultivars of *C. annuum*, with 0.72 ± 0.007 grams for JAL, then IKE with 0.68 ± 0.006 g. While *C. chinense* has seeds with lower weight compared to *C. annuum*, with 0.52 ± 0.004 g for HAB and 0.25 ± 0.004 g for BIQ (Fig 5, a). Unexpectedly, we observed that hybridization between parents to obtain F1 hybrid seeds showed a change in seed weight compared to self-pollination treatments, showing that pollen is important in determining the weight of seeds in *Capsicum*. The change in seed size occurred for both intraspecific and interspecific crosses. Obtaining hybrids with HAB as the mother plant and BIQ as the father did not show any difference in seed size in relation to the self-pollination treatment with HAB. Meanwhile, obtaining the reciprocal hybrid BIQ × HAB, showed that when BIQ receives pollen from HAB, the seed size is greatly increased for 0.393 ± 0.002 g in relation to the self-pollination BIQ control. For both parents (HAB and BIQ) when they received pollen from *C. annuum* (JAL and IKE) there was a decrease in the weight of seeds in relation to the self-pollination controls. Contrary to what happened to the cultivars of *C. chinense*, the cultivars of *C. annuum* when receiving interspecific pollen show a higher weight of seeds in relation to self-pollination controls, with the exception of IKE when receiving BIQ pollen, with seed weight equivalent to IKE when in self-pollination. For the crosses between cultivars of *C. annuum* we observed that variable seed size. When JAL received pollen from IKE, we obtained a decrease (0.623 ± 0.026 g) in the weight of seeds compared to the control, while IKE when pollinated with pollen from JAL produced seeds with greater weight, with 0.848 ± 0.008 g.

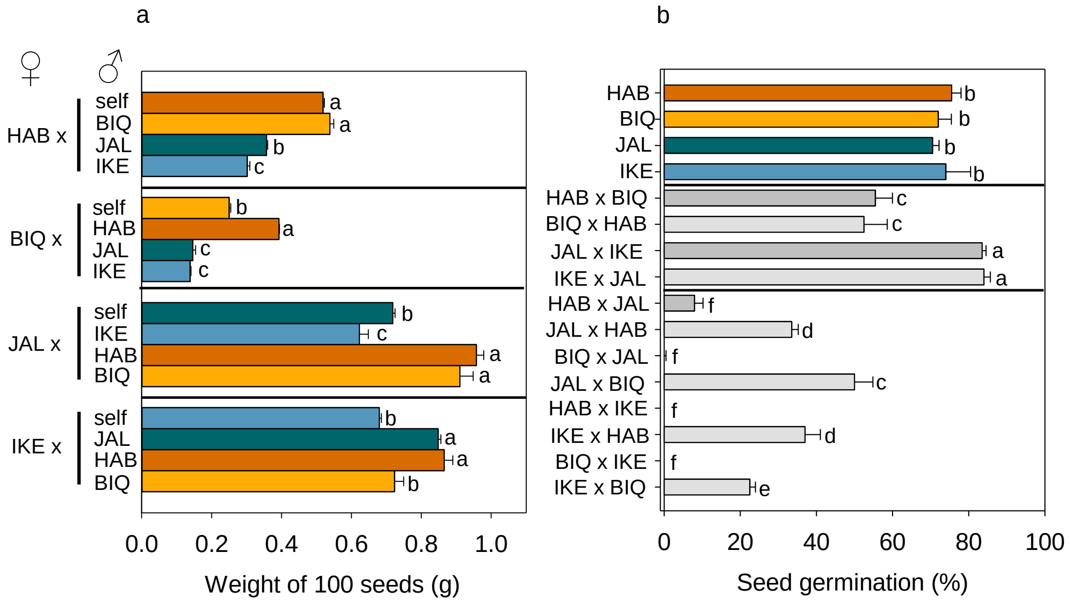


Figure 5. Seed weight (Panel a) and germination (Panel b) in parental and in intra- and interspecific *Capsicum* hybrids. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Different letters differ by Scott Knott's 5% test.

The seeds of the parents germinated with their normal, expected proportion of > 70%. The germination of intraspecific hybrids between JAL and IKE was superior to their parents, with values above 80%. While the germination of intraspecific hybrids among *C. chinense* with germinability less than 60 %. For both intraspecific hybrids, germinability did not differ between the reciprocal counterparts. Interspecific hybrids, on the other hand, had a severe impairment on seed germination, quite evident when compared to intraspecific hybrids and parental genotypes (Fig. 5, b). However, we observed unexpected results such as the equivalent germination of the interspecific hybrid JAL × BIQ in relation to the intraspecific reciprocal hybrids between HAB and BIQ. In fact, the lower weight of seeds for interspecific crosses with *C. chinense* as the mother plant, with the exception of HAB × JAL, did not show any germination. While, the greater or lesser weight of seeds for the other crossings with viable seeds did not show any influence with germinability due to the low correlation of Pearson (-0.067) and not significant (0.827) between the weight of seeds and germination.

Initial plant growth

The parent cultivars of *C. annuum* and *C. chinense* used to obtain F_1 hybrids show moderate vertical and horizontal growth as seen in the harvest stage at 180 days after sowing (Fig. 1). The combination of these parents when crossed resulted in F1 with different characteristics, with great vegetative development almost exclusively for interspecific hybrids (Table 1; Fig. 2, bottom). So, the question remains whether the greatest growth of these hybrids is also maintained during the stages of vegetative development such as initial growth (45 days after sowing) or in the moments preceding anthesis (90 days after sowing).

45 days after sowing is a physiological phase of initial growth with close proximity to the germination phase. The analysis of growth parameters such as plant height (Fig. 6), diameter (Fig. 7), vegetative dry biomass (Fig. 8) and leaf area (Fig. 9) indicate the superiority of the hybrids in relation to the parents, or at least in comparison to one of the parents. In addition, all interspecific hybrids were superior to their respective parental in relation to dry vegetative biomass. For the other parameters measured here, with the exception of the hybrid IKE × BIQ for the variables height and diameter of plants and for the height of the hybrid HAB × JAL, the other interspecific hybrids maintained

superior performance. In the case where it was possible to obtain reciprocal hybrids, for all intraspecific crosses, and only for interspecific crosses involving JAL and HAB, we note the differentiation of a hybrid in relation to its reciprocal counterpart. As the greatest performance of the IKE × JAL and JAL × HAB hybrids in plant height, HAB × BIQ, IKE × JAL and JAL × HAB in plant diameter, HAB × BIQ, IKE × JAL and JAL × HAB in dry vegetative biomass and HAB × BIQ, IKE × JAL and JAL × HAB in leaf area in relation to each reciprocal counterpart.

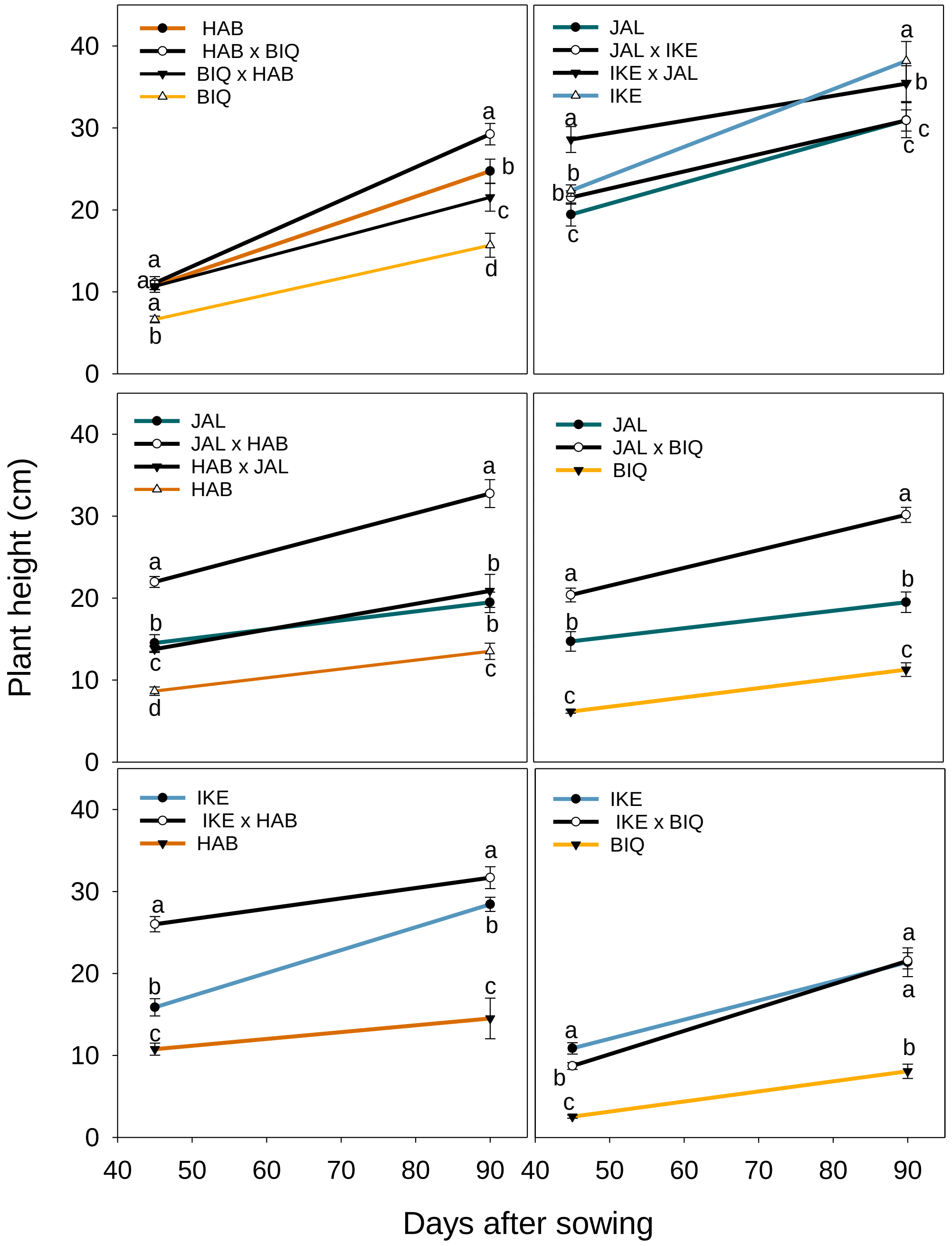


Figure 6. Initial growth in height (cm) at 45 and 90 days after sowing resulting from intraspecific (Top) and interspecific (Bottom) hybridization compared to each parent. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Different letters differ by Scott Knott's 5% test.

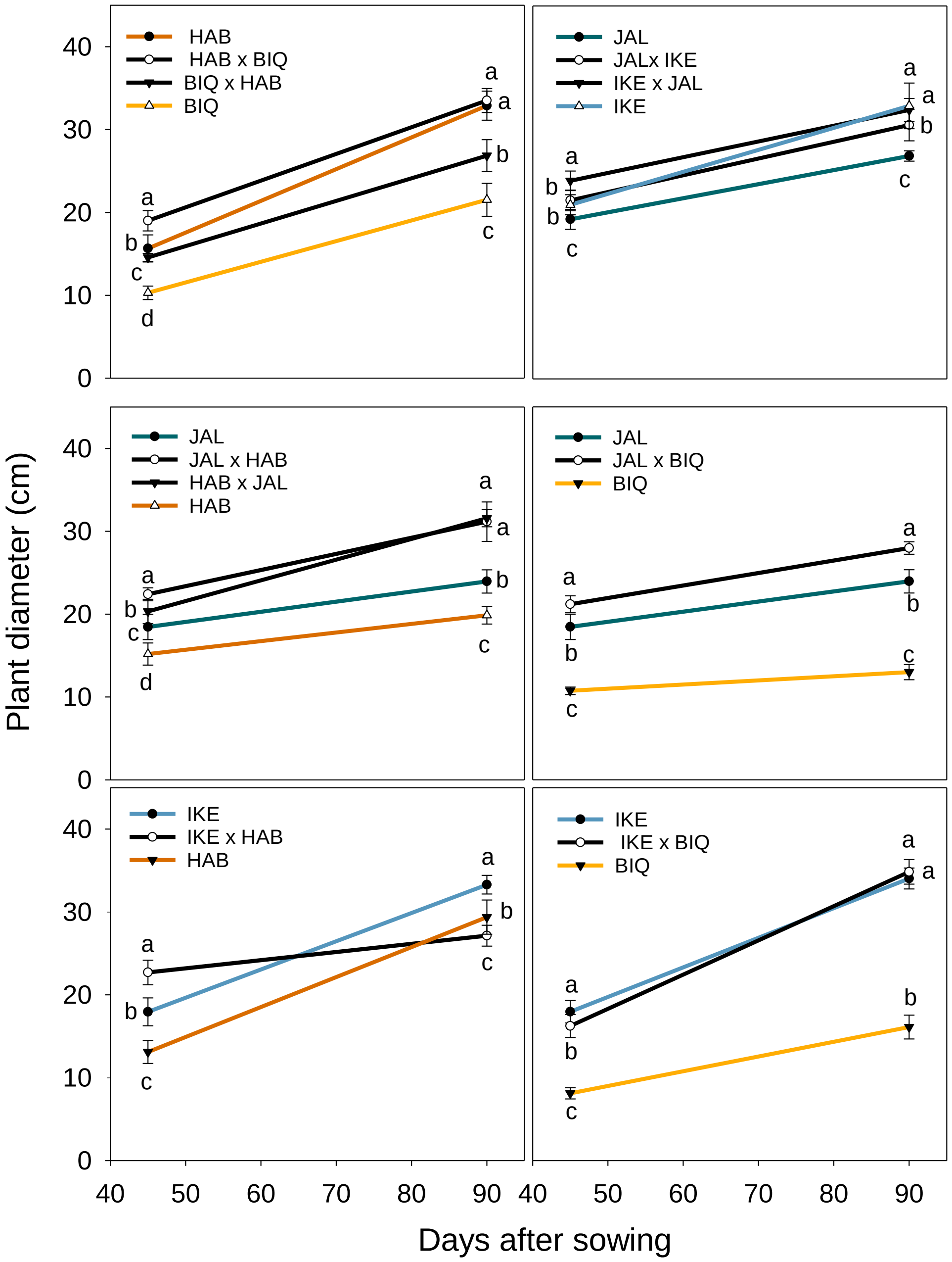


Figure 7. Initial growth in diameter (cm) at 45 and 90 days after sowing resulting from intraspecific (Top) and interspecific (Bottom) hybridization compared to each parent. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Different letters differ by Scott Knott's 5% test.

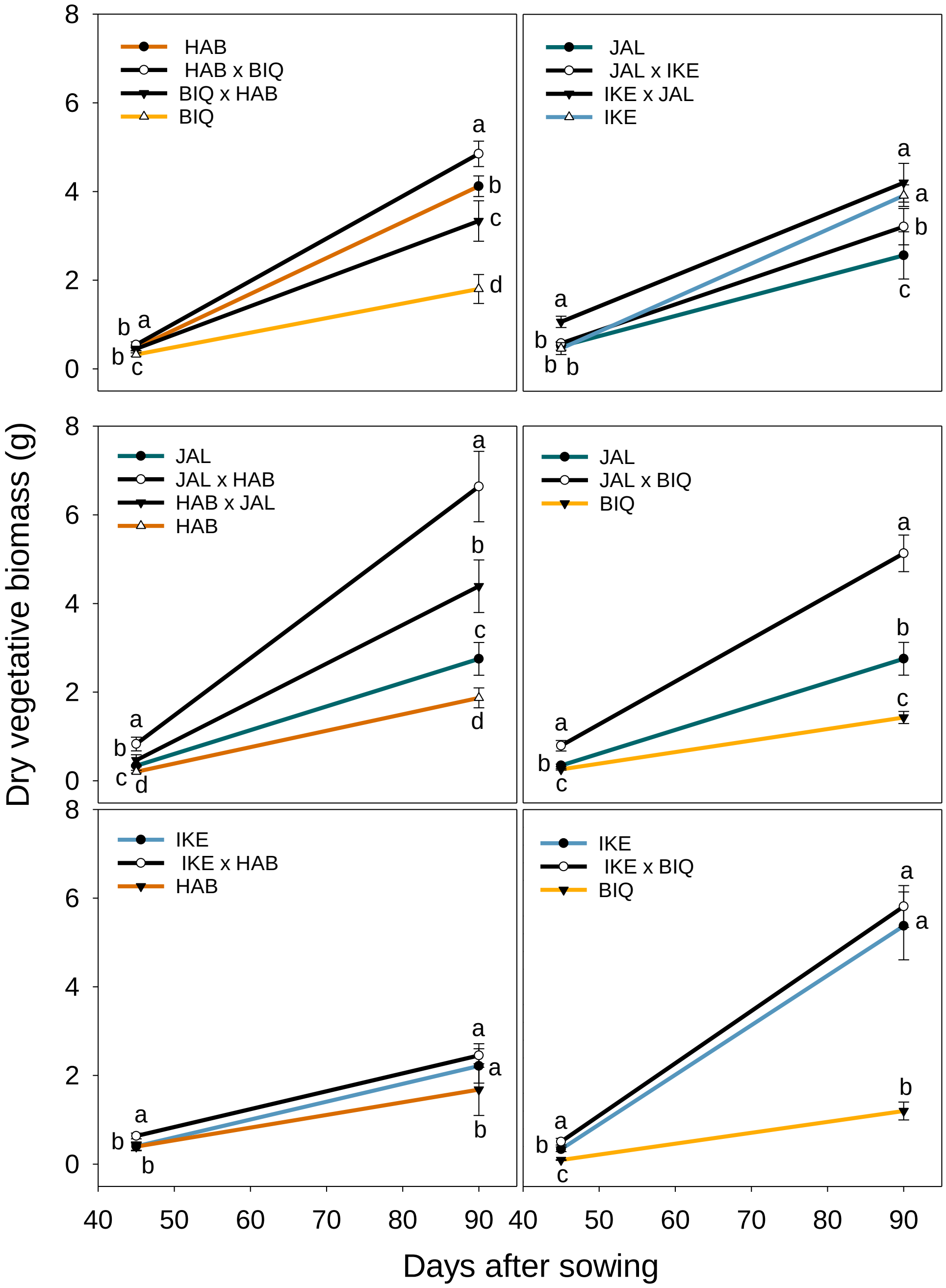


Figure 8. Initial growth in dry biomass per plant (g) at 45 and 90 days after sowing resulting from intraspecific (Top) and interspecific (Bottom) hybridization compared to each parent. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Different letters differ by Scott Knott's 5% test.

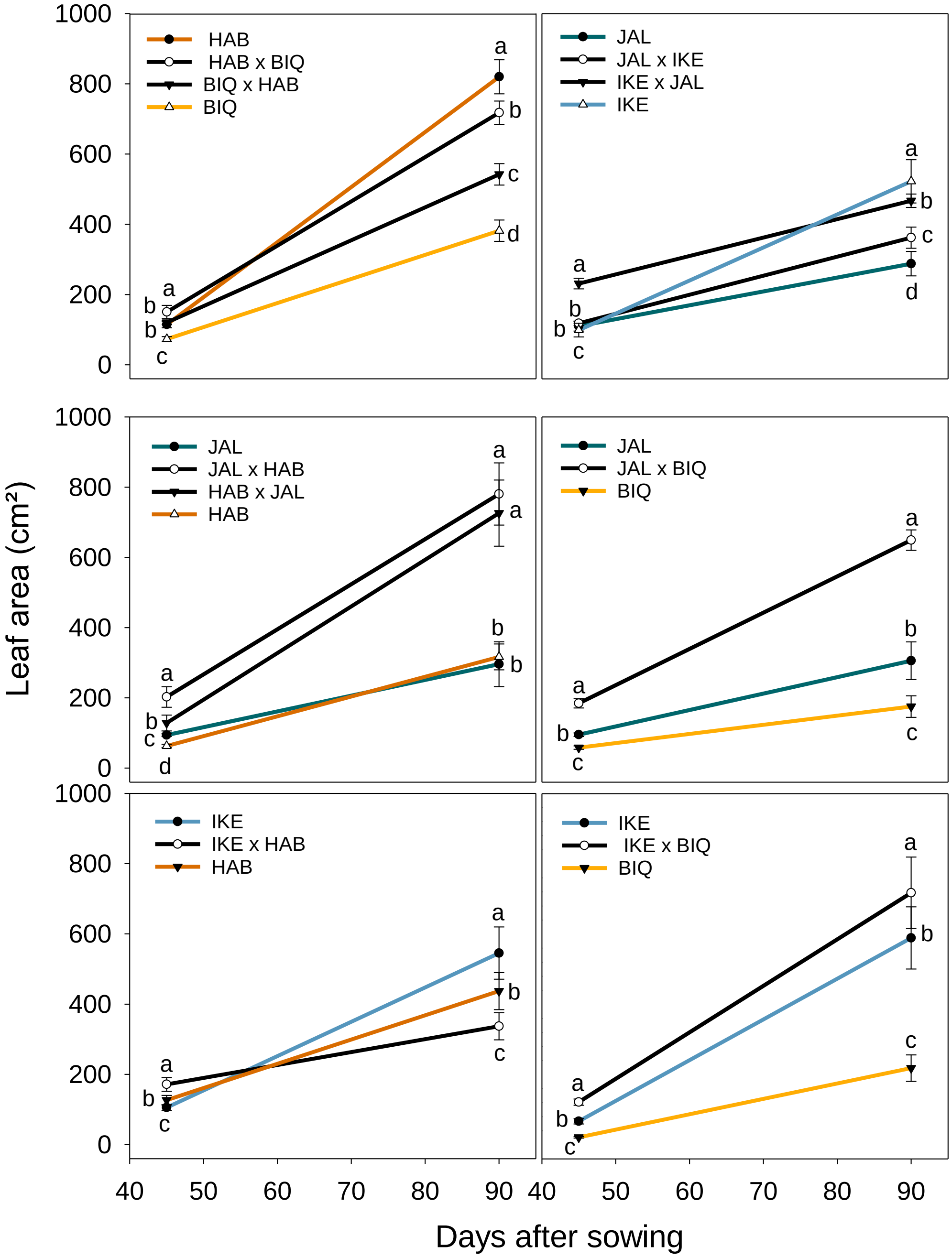


Figure 9. Initial growth in leaf area (cm²) at 45 and 90 days after sowing resulting from intraspecific (Top) and interspecific (Bottom) hybridization compared to each parent. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Different letters differ by Scott Knott's 5% test.

At 90 days after sowing, a phenological phase characterized by the transition from vegetative to reproductive, we observed a change in the growth parameters of some individuals. For hybrids involving the Ikeda cultivar, a slowdown in growth was observed in comparison to the IKE parental (Fig. 6 to 9). The same occurred for the other hybrids such as HAB × BIQ, being overcome by the parental HAB in leaf area and with this parental reaching the same diameter. In this phase, it was remarkable to observe inferior performance of the BIQ × HAB hybrid compared to its reciprocal counterpart, maintaining superiority only in relation to the parental BIQ for all variables. The same occurred for the JAL × IKE hybrid. Although HAB × JAL maintains height much lower than its reciprocal counterpart, it already acquires equivalence in plant diameter and leaf area (Figs. 6, 7, 9).

The inferior performance of the BIQ × HAB hybrid was maintained in relation to its reciprocal counterpart, with the superiority only in relation to the parental BIQ for all variables. Similar occurred with the JAL × IKE hybrid in relation to the parental JAL. BIQ × HAB showed growth loss in parameters with height, dry vegetative biomass and area in relation to parental HAB. Meanwhile, JAL × IKE in this phase is surpassed by parental JAL in height and parental IKE in diameter, vegetative biomass and leaf area. Although HAB × JAL maintains a much smaller height than its reciprocal counterpart, it already acquires equivalence in plant diameter and leaf area (Figs. 6, 7, 9). In addition, we see the reciprocal hybrids with different phenotypic / visual characteristics (Figs. 10, 11).

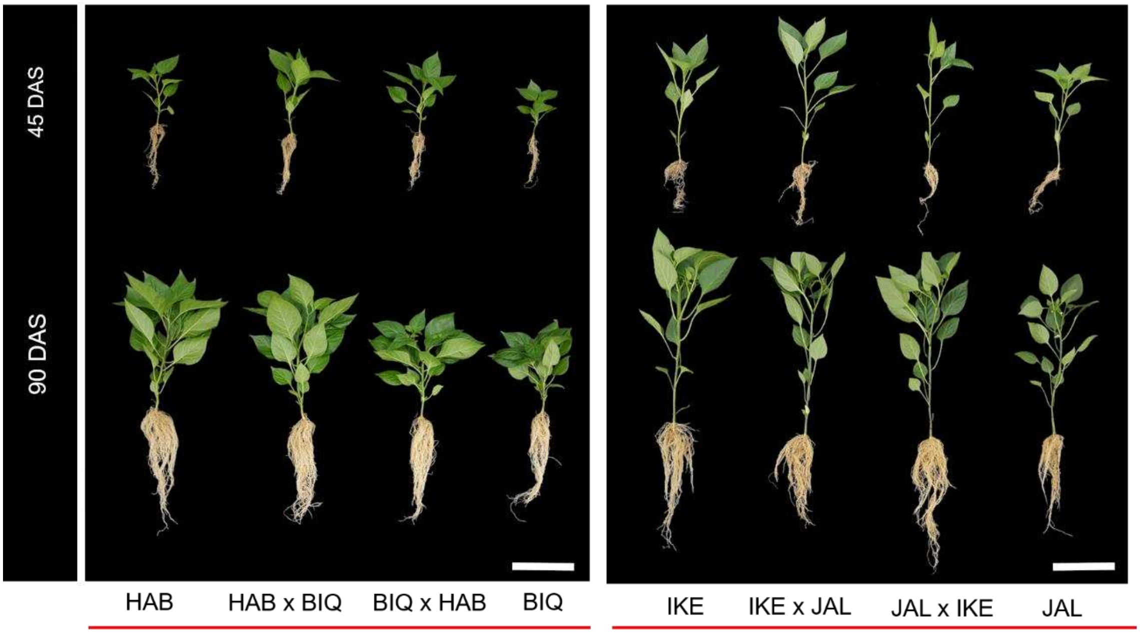


Figure 10: Vegetative aspect of intraspecific hybrids between *Capsicum chinense* (left) and *Capsicum annuum* (right) with their respective parents at 45 and 90 days after sowing (45 and 90 DAS). Left panel, from left to right: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Habanero × *C. chinense* cv. Biquinho (HAB × BIQ), *C. chinense* cv.

Biquinho × *C. chinense* cv. Habanero (BIQ × HAB), *C. chinense* cv. Biquinho (BIQ). Right panel, from left to right: *C. annuum* cv. Ikeda (IKE), *C. annuum* cv. Ikeda × *C. annuum* cv. Jalapeño (IKE × JAL), *C. annuum* cv. Jalapeño × *C. annuum* cv. Ikeda (JAL × IKE), *C. annuum* cv. Jalapeño (JAL). Scale bars 15 cm.

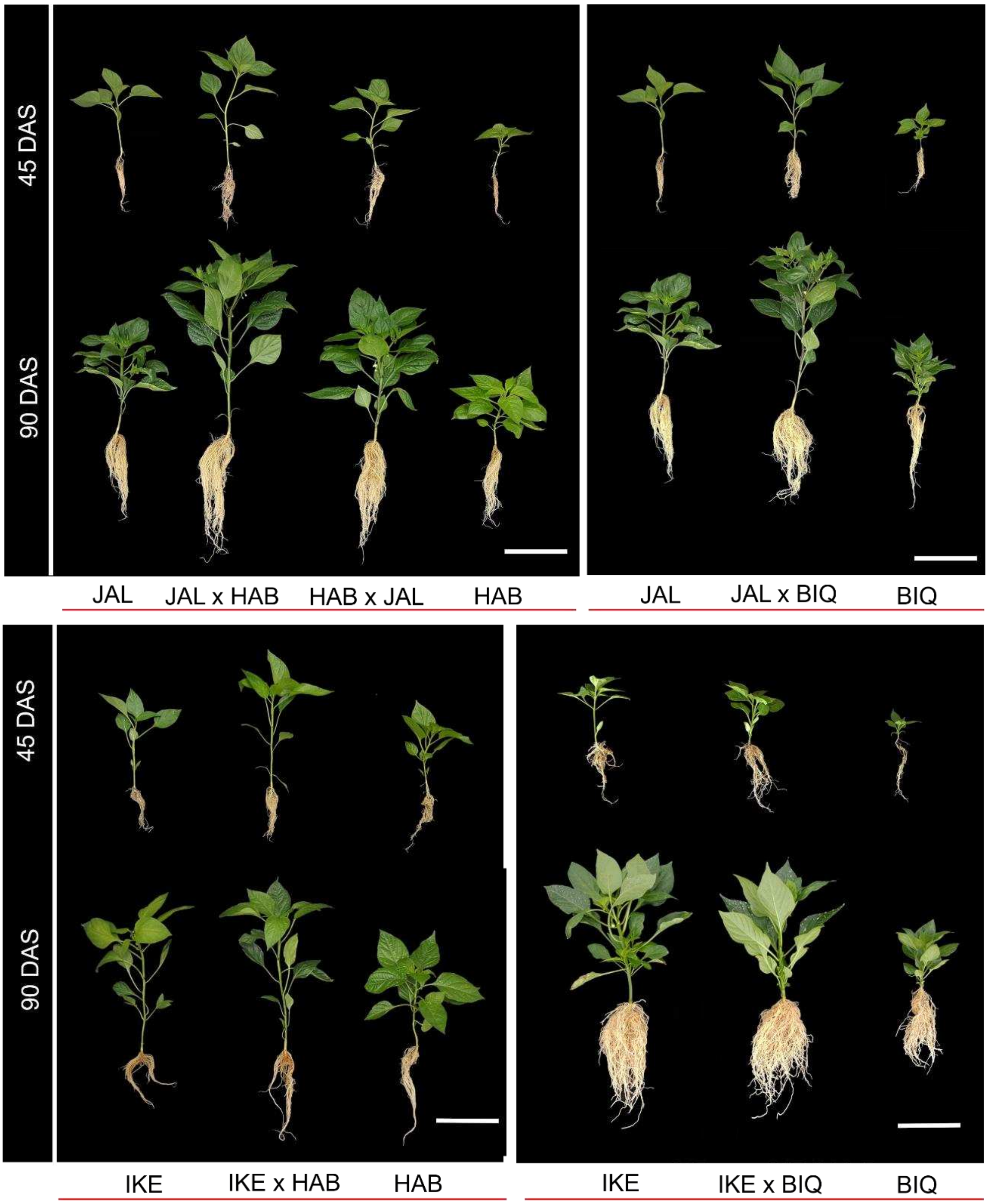
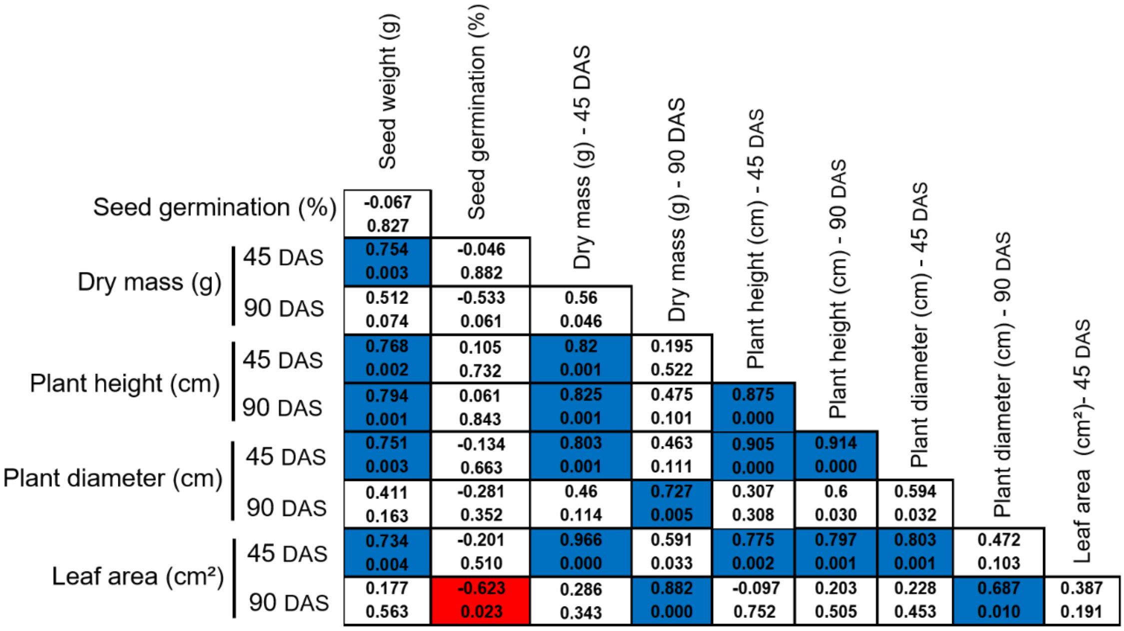


Figure 11: Vegetative aspect of interspecific hybrids between *Capsicum annuum* and *C. chinense* with their respective parents at 45 and 90 days after sowing (45 and 90 DAS). Top left, from left to right: *C. annuum* cv. Jalapeño (JAL), *C. annuum* cv. Jalapeño x *C. chinense* cv. Habanero (JAL × HAB), *C. chinense* cv. Habanero × *C. annuum* cv. Jalapeño

(HAB × JAL), *C. chinense* cv. Habanero. Top right, from left to right: *C. annuum* cv. Jalapeño (JAL), *C. annuum* cv. Jalapeño × *C. chinense* cv. Biquinho (JAL × BIQ), *C. chinense* cv. Biquinho (BIQ). Bottom left, from left for right: *C. annuum* cv. Ikeda (IKE), *C. annuum* cv Ikeda × *C. chinense* cv. Habanero (IKE × HAB), *C. chinense* cv. Habanero (HAB). Bottom right, from left to right: *C. annuum* cv. Ikeda (IKE), *C. annuum* cv. Ikeda × *C. chinense* cv. Biquinho (IKE × BIQ), *C. chinense* cv. Biquinho (BIQ). Scale bars 15 cm.

We observed differentiation in the growth pattern in interspecific, intraspecific hybrids and also in reciprocal hybrids in both phenological phases, 45 and 90 days after sowing. We observed the hybridization action on the seed weight (Fig. 5 a) and for many crops the greater seed weight has a recognized relationship with the greater vigor / initial growth, we decided to investigate the relationship between the seed weight and the initial growth in *Capsicum*. Almost all viable F_1 seeds of the non-reciprocal hybrids have a higher seed weight in relation to their respective parents (Fig. 5 a), with the exception of IKE × BIQ hybrid. As for the reciprocal hybrids, they have higher seed mass (JAL × HAB), only slightly higher (HAB × BIQ) or even lower (BIQ × HAB, JAL × IKE, HAB × JAL) than some of their parents or reciprocal counterpart. For these reciprocal hybrids, the ones that showed the highest growth in this phase (JAL × HAB, IKE × JAL, HAB × BIQ) were precisely those with the highest seed mass. As predicted, the relationship between seed weight and initial growth was confirmed in Pearson's correlation, mainly at 45 days after sowing (DAS) as for dry mass, plant height, plant diameter and leaf area (Fig. 12). This correlation between the weight of the seeds and the growth parameters certainly we consider as a factor that contributes to the greatest growth.



		Seed weight (g)	Seed germination (%)	Dry mass (g) - 45 DAS	Dry mass (g) - 90 DAS	Plant height (cm) - 45 DAS	Plant height (cm) - 90 DAS	Plant diameter (cm) - 45 DAS	Plant diameter (cm) - 90 DAS	Leaf area (cm^2)- 45 DAS
Seed germination (%)		-0.067 0.827								
Dry mass (g)	45 DAS	0.754 0.003	-0.046 0.882							
	90 DAS	0.512 0.074	-0.533 0.061	0.56 0.046						
Plant height (cm)	45 DAS	0.768 0.002	0.105 0.732	0.82 0.001	0.195 0.522					
	90 DAS	0.794 0.001	0.061 0.843	0.825 0.001	0.475 0.101	0.875 0.000				
Plant diameter (cm)	45 DAS	0.751 0.003	-0.134 0.663	0.803 0.001	0.463 0.111	0.905 0.000	0.914 0.000			
	90 DAS	0.411 0.163	-0.281 0.352	0.46 0.114	0.727 0.005	0.307 0.308	0.6 0.030	0.594 0.032		
Leaf area (cm^2)	45 DAS	0.734 0.004	-0.201 0.510	0.966 0.000	0.591 0.033	0.775 0.002	0.797 0.001	0.803 0.001	0.472 0.103	
	90 DAS	0.177 0.563	-0.623 0.023	0.286 0.343	0.882 0.000	-0.097 0.752	0.203 0.505	0.228 0.453	0.687 0.010	0.387 0.191

Figure 12. Correlation analysis between phenotypic traits in *Capsicum* genotypes. Pearson's correlation values and their respective levels of significance (bottom) between seed size and initial growth parameters at 45 and 90 days after sowing (45 and 90 DAS). Parameters: Seed weight (g), seed germination (%), vegetative dry mass at 45 and 90 days after sowing (DAS), plant height of plants at 45 and 90 days after sowing (DAS), plant diameter of plants at 45 and 90 days after sowing (DAS), leaf area at 45 and 90 days after sowing (DAS). The blue and red boxes show positive and negative correlation values, respectively, greater than 60% with significance - p value less than 5%.

In addition, we did not observe any positive correlation between seed germination and any growth parameter, only a negative correlation with the leaf area at 90 DAS. In fact, most interspecific hybrids have higher leaf area values at 90 DAS and these same hybrids have low germinability (Fig. 5 b). Height, diameter and leaf area at 45 DAS correlated with dry mass at 45 DAS, showing that they are positively correlated parameters. Meanwhile, only the plant diameter and leaf area at 90 DAS positively correlated with dry mass at 90 DAS. Parameters such as plant height at 90 DAS and plan diameter and leaf area at 45 DAS were positively influenced by plant height at 45 DAS. While the plant diameter and leaf area at 45 DAS are positively related to the plant height at 90 DAS. The plant diameter at 45 has a positive correlation with the leaf area at 45 DAS, and likewise the plant diameter at 90 DAS with respect to the leaf area up to 90 DAS.

Therefore, the higher initial growth of hybrids, mainly at 45 DAS, was related to the higher seed weight. However, in the phase 90 DAS the advantage of the initial vigor promoted by the larger seed size at 45 DAS begins to lose importance, as neither dry mass, plant diameter or leaf area are correlated with seed weight anymore (Fig. 12). Hybrids with larger seed sizes such as IKE × BIQ, IKE × HAB, IKE × JAL, JAL × IKE, at 90 DAS, slow growth in relation to IKE, even having seeds with greater weight in relation to this parent. The same occurred for the hybrid HAB × JAL, even with seeds smaller than their reciprocal counterpart (JAL × HAB), it already acquires equivalence in parameters such as leaf area and plant diameter.

Fertility

Fertility is important as an agronomic parameter and has an influence on parameters such as fruit set and, therefore, action on yield. The fact that parental cultivars are commercial and therefore with fertility and recognized fruit morphology allowed them to be a safe reference for these characteristics. Intraspecific hybridization resulted in different fruit morphology in relation to parental ones, as expected, and in addition showed sufficient seed production, which we consider as a satisfactory parameter indicative of fertility (Fig. 13, Supplementary Fig. 1). In contrast, the strange fruit morphology of the interspecific hybrids of *Capsicum* combined with the almost complete absence of seeds led us to investigate the action of fertility in interfering with fruit biometric parameters.

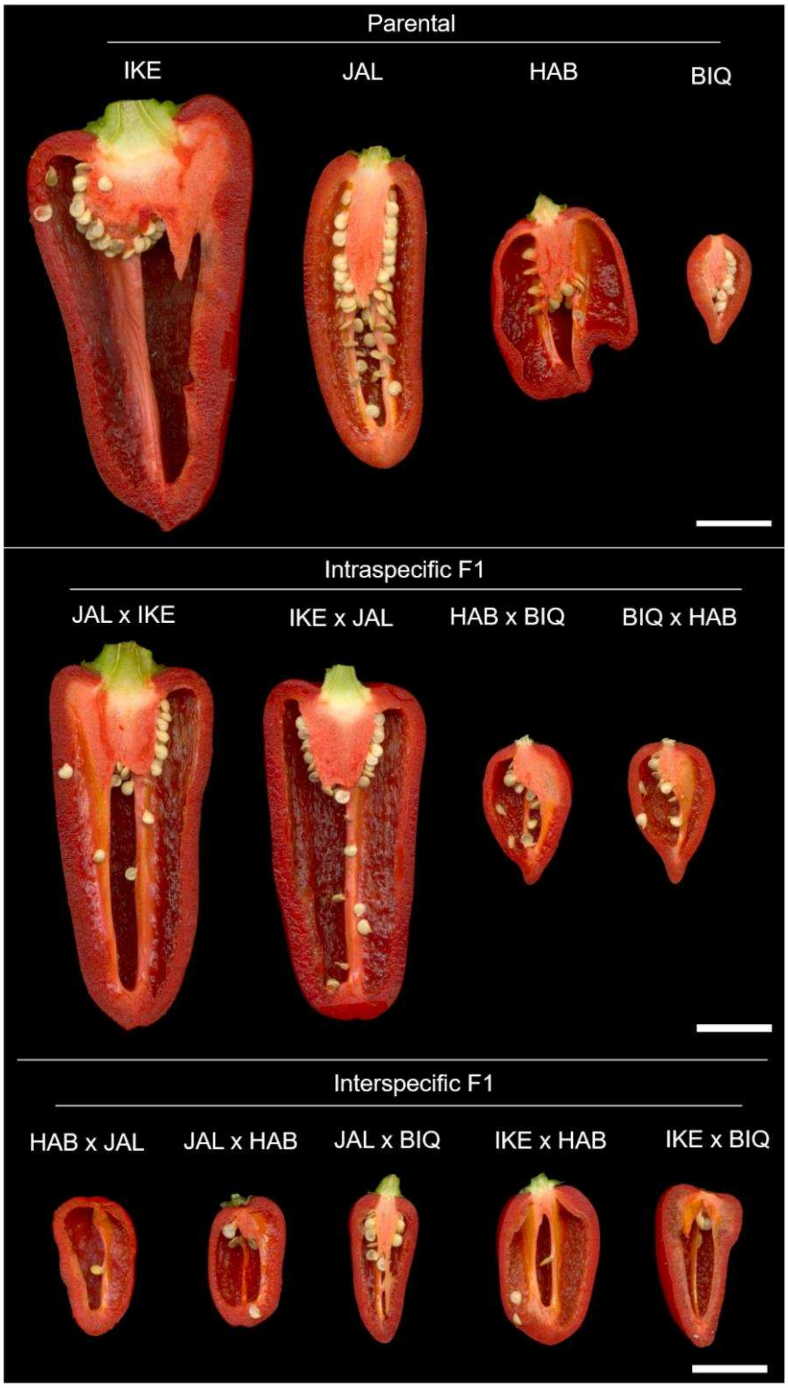


Figure 13. Representative longitudinal sections showing fruit morphometry and the number of seeds for parental lines (Top), intraspecific hybrids (middle) and interspecific hybrids (bottom) of *Capsicum*. Top, from left to right: *C. annuum* cv. Ikeda (IKE), *C. annuum* cv. Jalapeño (JAL), *C. chinense* cv. Habanero (HAB) and *C. chinense* cv. Biquinho (BIQ). Scale bars 2 cm.

Among the interspecific hybrids, JAL × BIQ showed the least compromise of fruit set and number of seeds per fruit (Fig. 13 and Supplementary Fig. 1) when self-pollinated, with about 23.4 ± 1.48% fruit set and 13.17 ± 1.17 seeds per fruit, respectively. However, these values are below those expected when compared to the averages between their parents, with 42.11 ± 2.59 % of fruit set and 132.4 ± 2.10 number of seeds for JAL, while BIQ with 43.08 ± 3.24% of fruit set and 59.25 ± 1.96 number of seeds per fruit.

We observed an increase in the fruit set of interspecific hybrids when pollinating with pollen from the parents, showing that the pollens of these hybrids are not suitable for establishing the fruit set (Fig. 14). In addition, as proof, parental plants when pollinated with pollen from interspecific hybrids showed an intense decrease in the fruit set (Fig. 15, left). Pollen quality in addition to contributing to the appropriate fruit set has an action on fresh weight per fruit as observed by increasing fruit weight for interspecific hybrids when pollinated with parental pollen or weight reduction when parental plants were pollinated with pollen from interspecific hybrids (Fig. 14 middle and Fig. 15 middle). The increment of the fruit set when the interspecific hybrids received pollens from the parents was of the magnitude of ca. 75.55%. And unexpectedly, there was an increase in the fruit set for intraspecific hybrids when receiving pollen from the parents, considering that it did not result in an increase of the seed set for them (Fig. 14 right). This suggests that hybrid plants, regardless of the type of hybridization, may have their fruit set increased by exogenous pollen. In addition, as an influencing factor for differentiating the fresh weight per fruit when receiving different parental pollens, we decided to investigate the relationship with the number of seeds produced per fruit. However, the number of seeds produced by backcrosses was almost equivalent in interspecific hybrids, which did not explain the difference in fruit weight. And the number of seeds per fruit also had little relation to the greater or lesser weight of fruits for most intraspecific hybrids when receiving different parental pollens (Fig. 14).

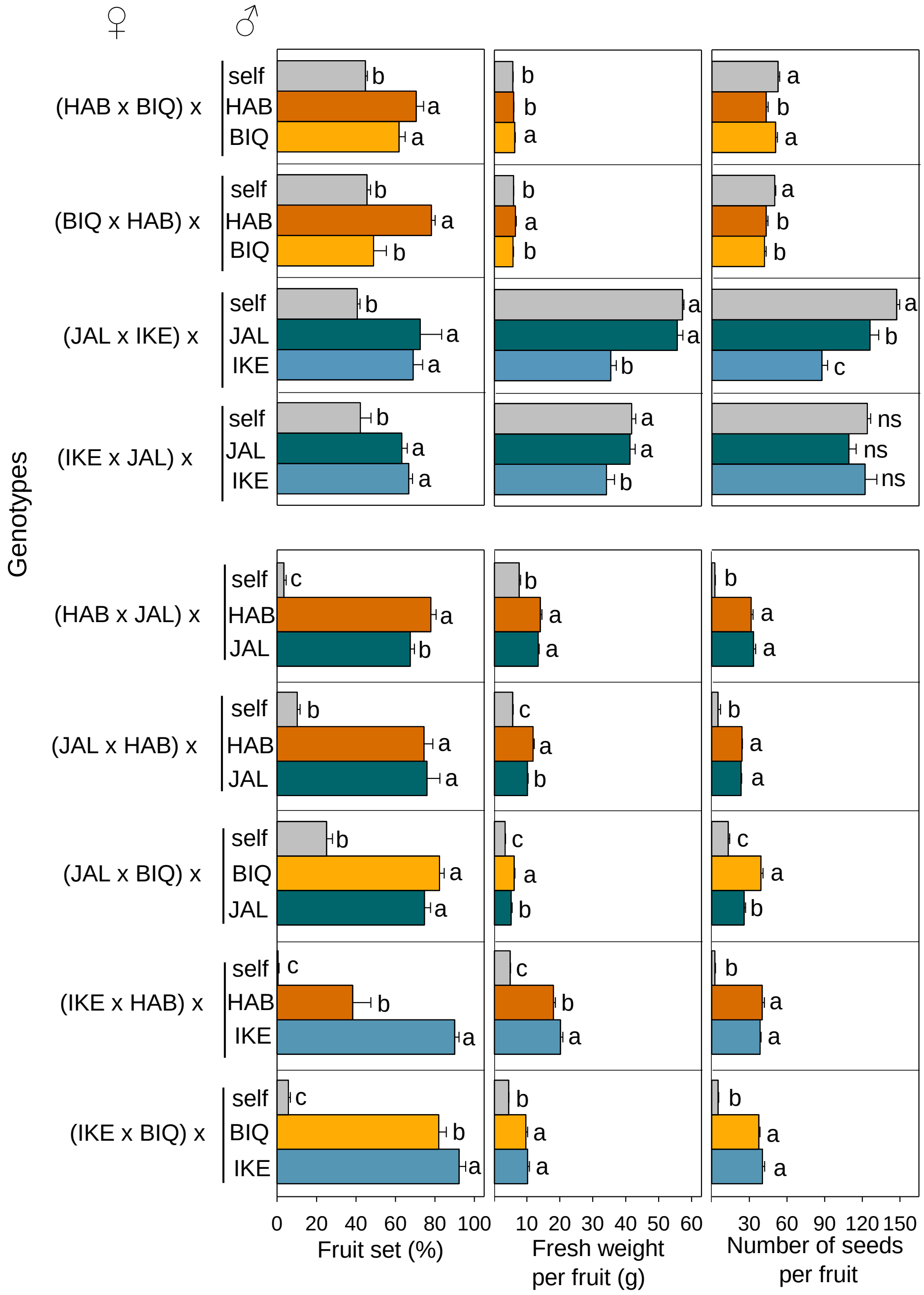


Figure 14. The pollen source has an effect on prolificacy parameters (fruit set and number of seeds per fruit) and fruit weight in intraspecific (Top) and interspecific hybrids (Bottom) of *Capsicum*. *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.

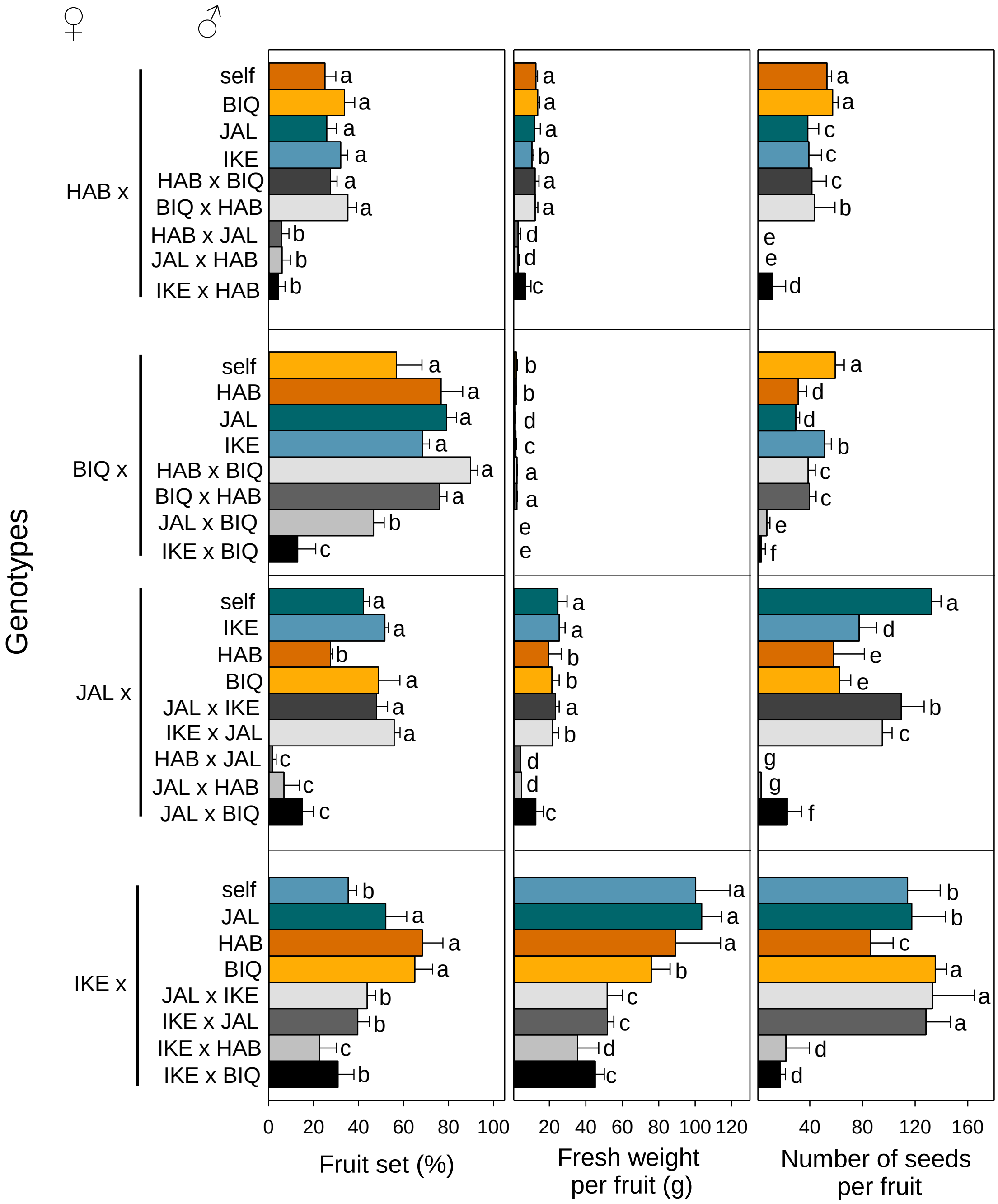


Figure 15. The pollen source has an effect on prolificacy parameters (fruit set and number of seeds per fruit) and fruit weight of *Capsicum* parents used in crosses. *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.

We observed that in addition to interspecific hybridization, compromising fertility (fruit and seed set) in *Capsicum* has action in aspects such as fresh weight and fruit biometrics (Figs. 14, 16). Most fruits produced by interspecific hybrids when naturally self-pollinated have fruits with few or no seeds, showing that fruit production for interspecific hybrids, despite being reduced, can occur even without proper fertility. Especially for interspecific hybrids, the pollination of flowers with parental pollen, and therefore with recognized fertility, has shown to influence beyond size in parameters such as fruit morphology.

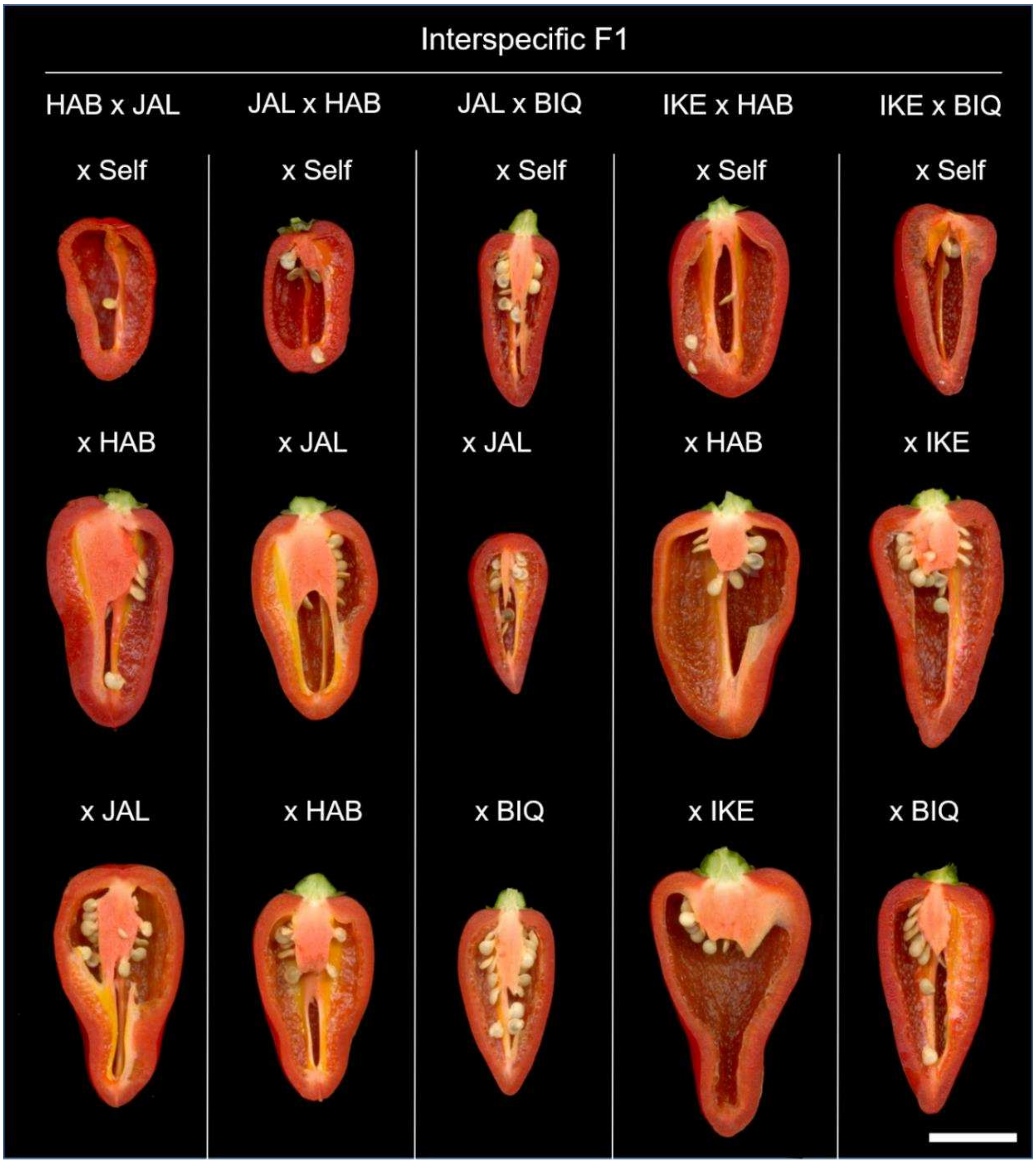


Figure 16. Longitudinal section showing fruit morphometry and the amount of seeds produced by half a fruit for interspecific *Capsicum* hybrids. In general, self-pollination (self) contributes to small fruits with few seeds. However, the backcrosses with pollen from parents increases the size and number of seeds per fruit. Parental genotypes: *C. chinense* cv. Habanero (HAB), C. *chinense* cv. Biquinho (BIQ), *C. annuum* cv. Ikeda (IKE) and *C. annuum* cv. Jalapeño (JAL). Interspecific hybrids: *C. chinense* cv. Habanero × *C. annuum* cv. Jalapeño (HAB × JAL), *C. annuum* cv. Jalapeño × *C. chinense* cv.

Habanero (JAL × HAB), *C. annuum* cv. Jalapeño × *C. chinense* cv. Biquinho (JAL ×BIQ), *C. annuum* cv. Ikeda × *C. chinense* cv. Habanero (IKE × HAB) and *C. annuum* cv. Ikeda × *C. chinense* cv. Biquinho (IKE × BIQ). Scale bar 2 cm.

The non-viability of pollen as observed in interspecific hybrids when self-pollinated or for parental fruits when pollinated with interspecific pollen had a modifying action on the length and diameter of fruits and on the fruit shape index (Supplementary Figs. 2 and 3). In addition to pollen fertility, the type of pollen had an action on fruit morphology. Different parental pollens, and therefore with recognized fertility, when used to pollinate interspecific hybrids have been shown to have an effect on the morphological differentiation of fruits. As observed visually for interspecific hybrids (Fig. 16) and in parameters such as diameter and length of the fruit and fruit shape index (Supplementary Fig. 2). This morphological differentiation in these parameters when pollinating with different pollens was observed to a lesser extent for intraspecific hybrids (Supplementary Fig. 2) and also in parental plants (Supplementary Fig. 3). For a more detailed analysis, in addition to the length and diameter of fruits, we measured in the Tomato Analyzer software a total of 32 biometric parameters of fruits for interspecific hybrids. When we plot these 32 parameters in analysis of the main components, we once again confirm the effect of pollen on the discrimination of the morphology of fruits of the same genotype (hybrid) when receiving pollen different from yours (Fig. 17A).

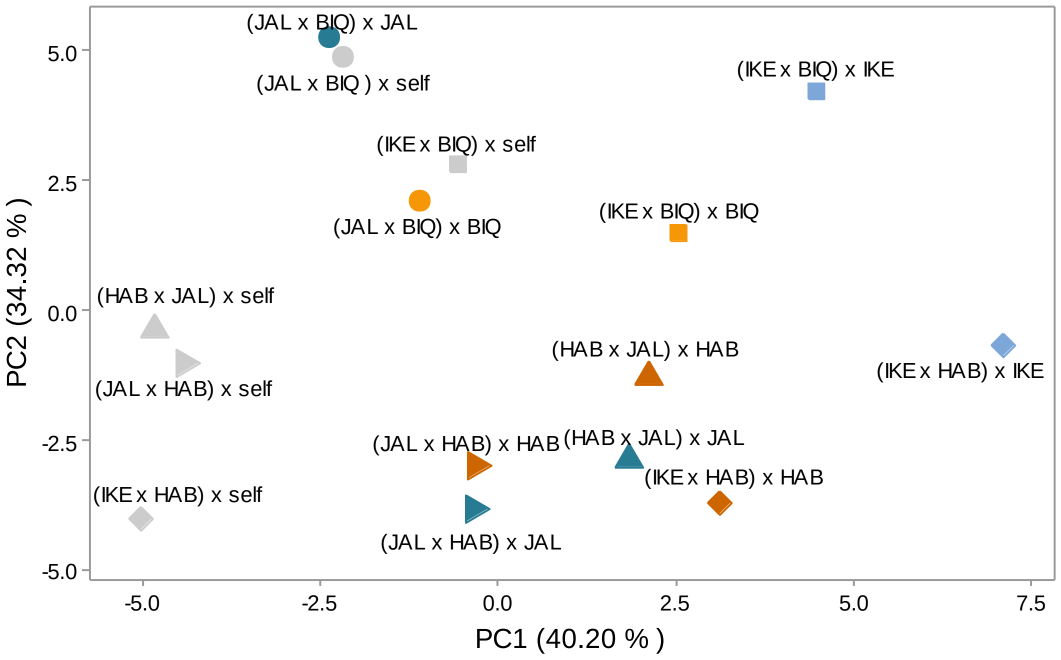


Figure 17A. The pollen source contributes to alter the fruit morphology of interspecific hybrids. Parental plants: *Capsicum annuum* cv. Jalapeño (JAL), *C. annuum* cv. Ikeda (IKE); *Capsicum chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ); and their interspecific (IKE × BIQ; IKE × HAB; JAL × BIQ; JAL × HAB; HAB × JAL) hybrids. **Note:** The genotype inside the parentheses represents the interspecific hybrids as the female parent source of the fruit, while the name after the × symbol represents the genotype used as the pollen source.

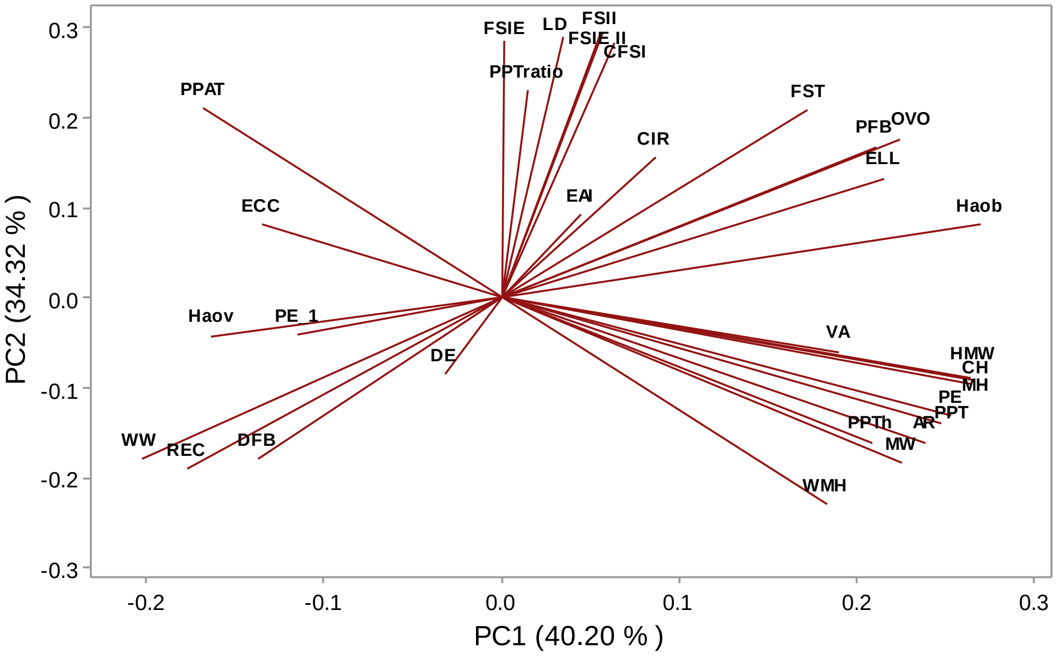


Figure 17B. Component loadings with respect to fruit biometry and its relationship with source of pollen origin. **PE:** Perimeter; **AR:** area; **WMH**: width mid-heigh; **MW:** Maximum width; **HMW**: Height mid-width; **MH:** Maximum height; **CH**: curved height; **FSIE**: fruit shape index external I; **FSIE II**: Fruit shape index external II; **CFSI**: Curved fruit shape index; **PFB**: Proximal fruit blockiness; **DFB**: Distal fruit blockiness; **FST**: fruit shape triangle; **ELL:** Ellipsoid; **CIR**: Circular; **REC**: Rectangular; **OBO**: Obovoid; **OVO:** Ovoid; **VA**: V. asymmetry; **Haob**: H. asymmetry.ob; **Haov**: H. asymmetry.ov; **WW**: Width widest; **ECC:** Eccentry; **PE_1**: Proximal eccentricity; **DE**: Distal Eccentricity; **FSII**: Fruit shape index internal; **EAI**: Eccentricity area index; **LD**: Lobedness degree; **PPT**: Pepper pericarp area; **PPAT**: Pepper pericarp area ratio; **PPTh**: Pepper pericarp thickness; **PPTratio**: Pepper pericarp thickness ratio.

The first component explained 40.20% of the data variation, with parameters with greater eigenvalues, in module above 0.2, for perimeter, area, maximum width, height mid-width, maximum height, proximal fruit blockiness, curved height, ellipsoid, ovoid, H. asymmetry.ov, pepper pericarp area, pepper pericarp thickness and width widest (Fig.

17B, Table 2). Interestingly, all interspecific hybrids when self-pollinated were grouped to the left of the zero point, and therefore with negative values for PC1. Different hybrids such as IKE × BIQ and IKE × HAB when they received pollen from IKE were shown to increase in components for PC1 because they were positioned more to the right of the origin axis and therefore with greater positive values for PC1.

PC2 contributed slightly less than PC1 in explaining the variation in the data, with 34.32%. The parameters that contributed most were width mid-height, fruit shape index external I, fruit shape index external II, curved fruit shape index, fruit shape triangle, fruit shape index internal, lobedness degree, pepper pericarp area ratio and pepper pericarp thickness ratio (Fig. 17B, Table 2). Interestingly, regardless of the pollen source, component 2 separates very well the interspecific hybrids that have *C. chinense* HAB in their composition at the bottom and therefore with negative values (Fig. 17A). While, at the top of PC2 and therefore with positive values, we saw the distribution of hybrids that have *C. chinense* BIQ in their composition. PC1 and PC2 together explain the major part, about 74%, of the variation in the shape of the fruits in relation to the pollen source. We observed reciprocal hybrids such as HAB × JAL and JAL × HAB to group together when self-pollinating, but they distanced themselves when they received different pollen sources. In contrast, we observed a cluster of the hybrid JAL × BIQ when self-pollinated close to JAL × BIQ when pollinated with JAL pollen, while JAL × BIQ when pollinated with BIQ distancing from both and with greater approximation as to the shape with IKE × BIQ when self-pollinated. In turn, the interspecific hybrid JAL × HAB would not differ in fruit form by component 1 when pollinated by HAB or JAL, with differentiation only by component 2, with more negative values of JAL × HAB when pollinated with JAL than when pollinated with HAB. The third component explains only 8.5% of the variance. Parameters with eigenvalues with values in module above 0.2 such as eccentricity area index, distal fruit blockiness, H. asymmetry.ob, distal eccentricity, V. asymmetry, rectangular and eccentry. For PC4 it was 4.7% important in the variation of data, with greater importance for parameters such as eccentry, circular, pepper pericarp thickness ratio, proximal eccentricity, ellipsoid, distal eccentricity, V. asymmetry and H. asymmetry.ob.

We made a heatmap for each of the 32 variables for each genotype when receiving different pollen to yours (Fig. 18). As noted in the analysis of main components (Fig.

17A, Fig. 17B), that interspecific hybrids have differentiated fruit shapes when self-pollinated compared to backcrosses with pollens from parents, represented here by different colors and letters (Fig. 18). All interspecific hybrids, with the exception of JAL × HAB, showed to differ in biometric parameters of fruits when receiving different parental pollens. The detection of differences by normal statistical test was not efficient in differentiating the fruit form for the JAL × HAB genotype when crossed with parental pollens. They were differentiated when in principal component analysis and only by the characteristics of the second component as seen by the positioning of differentiated only when viewed from the perspective of PC2 (Fig. 17A).

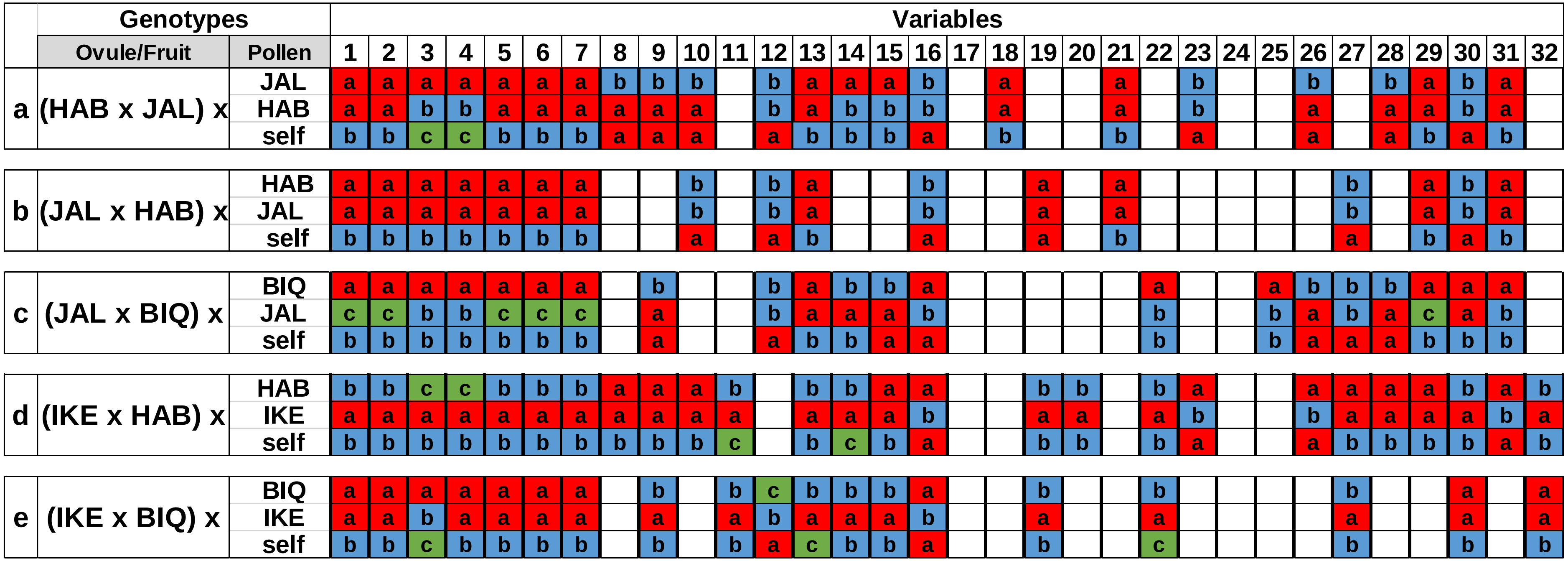
Table 2. Contribution of eigenvalues to the four main axes of PCA for intraspecific hybrids in biometric parameters of fruits according to the influence of the pollen source

Biometric parameters	PC1 (40.2 %)	PC2 (34.3 %)	PC3 (8.5 %)	PC4 (4.7%)
Perimeter – PE	**0.253**	-0.132	-0.016	0.026
Area – AR	**0.238**	-0.161	0.028	0.075
Width mid-height - WMH	0.183	**-0.229**	0.062	0.041
Maximum width - MW	**0.226**	-0.184	-0.002	0.037
Height mid-width - HWM	**0.264**	-0.09	0.032	0.052
Maximum height - MH	**0.265**	-0.095	-0.008	0.016
Curved height - CH	**0.265**	-0.095	-0.008	0.016
Fruit shape index external I - FSIE	0.002	**0.287**	0.034	0.023
Fruit shape index external II - FSIEII	0.056	**0.294**	-0.039	0.035
Curved fruit shape index - CFSI	0.063	**0.285**	-0.111	-0.043
Proximal fruit blockiness - PFB	**0.211**	0.169	-0.196	0.035
Distal fruit blockiness - DFB	-0.137	-0.18	**-0.371**	0.074
Fruit shape triangle - FST	0.172	**0.208**	0.191	-0.006
Ellipsoid - ELL	**0.215**	0.134	-0.113	**-0.288**
Circular - CIR	0.086	0.156	0.026	**-0.386**
Rectangular - REC	-0.177	-0.19	**-0.232**	-0.041
Obovoid - OBO	*	*	*	*
Ovoid - OVO	**0.224**	0.177	-0.024	0.002
V. asymmetry - VA	0.19	-0.06	**-0.251**	**0.282**
H. asymmetry.ob - Haob	-0.164	-0.042	**-0.359**	**-0.212**
H. asymmetry.ov - Haov	**0.27**	0.082	-0.006	0.015
Width widest - WW	**-0.203**	-0.179	0.16	-0.027
Eccentry - ECC	-0.135	0.082	**0.225**	**0.508**
Proximal eccentricity - PE_1	-0.115	-0.04	-0.17	**0.331**
Distal eccentricity - DE	-0.032	-0.085	**0.297**	**-0.286**

Fruit shape index internal - FSII	0.056	**0.294**	-0.028	0.042
Eccentricity area index - EAI	0.045	0.092	**-0.544**	-0.019
Lobedness degree - LD	0.034	**0.29**	-0.041	0.032
Pepper pericarp area - PPT	**0.247**	-0.139	0.001	0.024
Pepper pericarp area ratio - PPAT	-0.169	**0.211**	0.048	**-**0.138
Pepper pericarp thickness - PPTh	**0.209**	-0.16	0.088	-0.055
Pepper pericarp thickness ratio - PPTratio	0.015	**0.231**	0.071	**0.378**

* The data for the variable Obovoid do not differ from zero for the different genotypes, not allowing its inclusion in the analysis of the main component.

Figure 18: The fruits of interspecific hybrids (shown in parentheses) show variation in biometric aspects of fruits (1-32) depending on the genotype and or the pollen source. The red 🟥 , blue 🟦 and green 🟩 boxes represent statistically values of a, b and c by the Scott Knott test by % 5, respectively. White ☐ boxes do not differ statistically from each other.



	Genotypes		Variables																															
	Ovule/Fruit	Pollen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
a	(HAB x JAL) x	JAL	a	a	a	a	a	a	a	b	b	b		b	a	a	a	b		a			a		b			b		b	a	b	a	
		HAB	a	a	b	b	a	a	a	a	a	a		b	a	b	b	b		a			a		b			a		a	a	b	a	
		self	b	b	c	c	b	b	b	a	a	a		a	b	b	b	a		b			b		a			a		a	b	a	b	
b	(JAL x HAB) x	HAB	a	a	a	a	a	a	a			b		b	a			b			a		a						b		a	b	a	
		JAL	a	a	a	a	a	a	a			b		b	a			b			a		a						b		a	b	a	
		self	b	b	b	b	b	b	b			a		a	b			a			a		b						a		b	a	b	
c	(JAL x BIQ) x	BIQ	a	a	a	a	a	a	a		b			b	a	b	b	a						a			a	b	b	b	a	a	a	
		JAL	c	c	b	b	c	c	c		a			b	a	a	a	b						b			b	a	b	a	c	a	b	
		self	b	b	b	b	b	b	b		a			a	b	b	a	a						b			b	a	a	a	b	b	b	
d	(IKE x HAB) x	HAB	b	b	c	c	b	b	b	a	a	a	b		b	b	a	a			b	b		b	a			a	a	a	a	b	a	b
		IKE	a	a	a	a	a	a	a	a	a	a	a		a	a	a	b			a	a		a	b			b	a	a	a	a	b	a
		self	b	b	b	b	b	b	b	b	b	b	c		b	c	b	a			b	b		b	a			a	b	b	b	b	a	b
e	(IKE x BIQ) x	BIQ	a	a	a	a	a	a	a		b		b	c	b	b	b	a			b			b					b			a		a
		IKE	a	a	b	a	a	a	a		a		a	b	a	a	a	b			a			a					a			a		a
		self	b	b	c	b	b	b	b		b		b	a	c	b	b	a			b			c					b			b		b

1: Perimeter (cm); **2:** Area (cm^2); **3**: width mid-heigh (cm); **4:** Maximum Width (cm); **5**: Height mid-width (cm); **6:** Maximum height (cm); **7**: curved height (cm); **8**: fruit shape index external I; **9**: Fruit shape index external II; **10**: Curved fruit shape index; **11**: Proximal fruit blockiness; **12**: Distal fruit blockiness; **13**: Fruit shape triangle; **14:** Ellipsoid; **15**: Circular; **16**: Rectangular; **17**: Obovoid; **18:** Ovoid; **19**: V. asymmetry (cm); **20**: H. asymmetry.ob (cm); **21**: H. asymmetry.ov (cm); **22**: Width widest; **23:** Eccentry; **24**: Proximal eccentricity; **25**: Distal eccentricity; **26**: Fruit shape index internal; **27**: Eccentricity area index; **28**: Lobedness degree; **29**: Pepper pericarp area (cm^2); **30**: Pepper pericarp area ratio; **31**: Pepper pericarp thickness (cm); **32**: Pepper pericarp thickness ratio.

Fruit morphology

The morphology of the fruit encompasses several parameters related to the shape of fruits and all are related to the acceptability and preference of the consumer market. The measurement of 32 biometric parameters of fruits using the Tomato Analyzer software allowed us to compare the fruit shape and their relationship with hybridization. Plotting many parameters, as measured here, into main analysis components facilitates data analysis and reduction of dimensionality and thus identifies possible relationships in morphological parameters. For the distribution of 32 morphological parameters in PCA analysis among parental, intraspecific and interspecific hybrids, we observed the division into two main components, with PC1 explaining 54.04 % of the data variation and PC2 with 18.61%. PC1 was efficient in separating fruit genotypes by their size and related parameters. With large fruits on the right such as the parents of *C. annuum* (IKE and JAL) with their reciprocal intraspecific hybrids IKE × JAL and JAL × IKE. Fruits of intermediate size for interspecific hybrids at the center such as IKE × BIQ, IKE × HAB and HAB × JAL (Fig. 19A). Meanwhile, smaller fruits on the left, mainly for reciprocal intraspecific hybrids HAB × BIQ and BIQ × HAB and for parental BIQ of *C. chinense*.

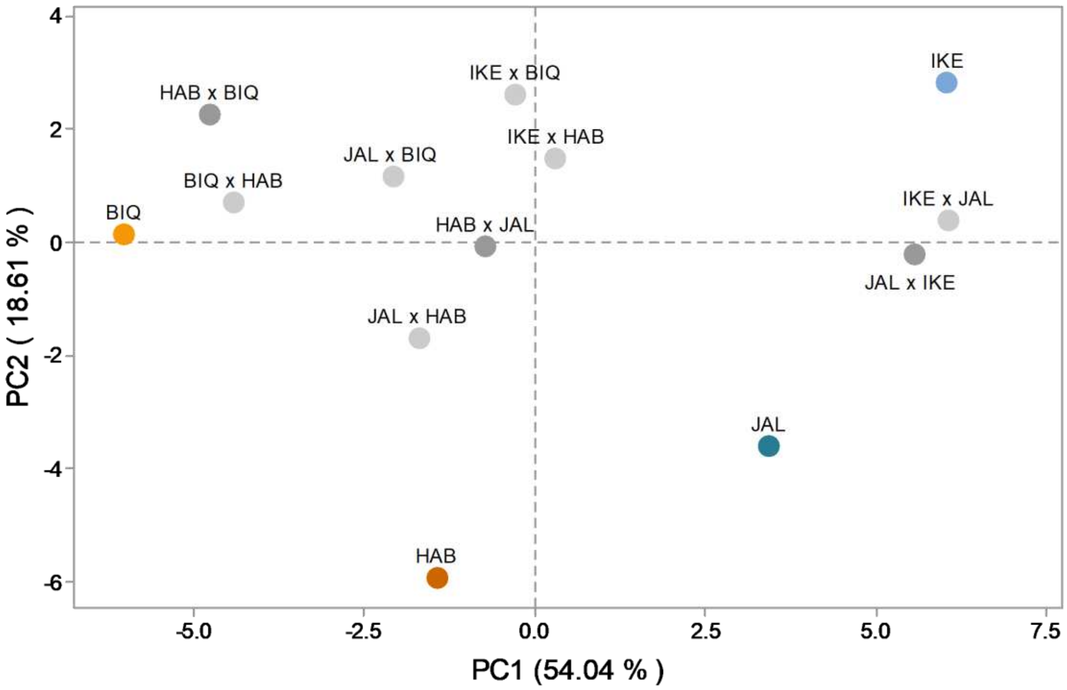


Figure 19A: Component loadings in grouping of main components in 32 biometric parameters of fruits in intra and interspecific hybrids shows to receive differential influence of the fruit morphology of their parents. Parental plants: *C. chinense* cv.

Habanero (HAB), *C. chinense* cv. Biquinho (BIQ); *C. annuum* cv. Jalapeño (JAL), *C. annuum* cv. Ikeda (IKE).

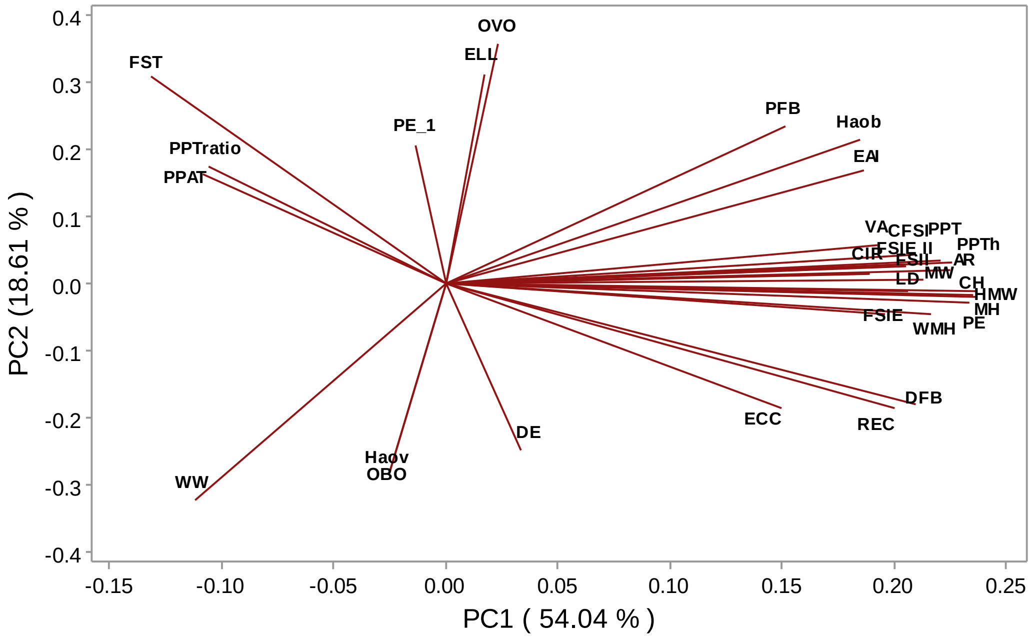


Figure 19B: Component loadings in grouping of main components in 32 biometric parameters of fruits in intra and interspecific hybrids shows to receive differential influence of the fruit morphology of their parents. **PE:** Perimeter; **AR:** area; **WMH**: Width mid-heigh; **MW:** Maximum Width; **HMW**: Height mid-width; **MH:** Maximum height; **CH**: curved height; **FSIE**: Fruit shape index external I; **FSIE II**: Fruit shape index external II; **CFSI**: Curved fruit shape index; **PFB**: Proximal fruit Blockiness; **DFB**: Distal fruit blockiness; **FST**: Fruit shape triangle; **ELL:** Ellipsoid; **CIR**: Circular; **REC**: Rectangular; **OBO**: Obovoid; **OVO:** Ovoid; **VA**: V. asymmetry; **Haob**: H. asymmetry.ob; **Haov**: H. asymmetry.ov; **WW**: Width widest; **ECC:** Eccentry; **PE_1**: Proximal eccentricity; **DE**: Distal eccentricity; **FSII**: Fruit shape index internal; **EAI**: Eccentricity area index; **LD**: Lobedness degree; **PPT**: Pepper pericarp area; **PPAT**: Pepper pericarp area ratio; **PPTh**: Pepper pericarp thickness; **PPTratio**: Pepper pericarp thickness ratio.

For PC1, many variables had similar contributions as shown by the overlapping vector arrows on the right (Fig. 19B) and with eigenvectors above 0.2 (Table 3), although parameters such as ellipsoid, ovoid, distal eccentricity, H. asymmetry.ov and obovoid contributed almost nothing. PC2 explains a small variation in data compared to PC1. The main variables related to PC2 with a module contribution above 0.2 were related to asymmetry of fruits such as ovoid, obovoid, h. asymmetry.ov and width widest. Parameters reported with blockiness as proximal fruit blockiness and fruit shape triangle, others with homogeneity (Ellipsoid) and internal eccentricity (Distal eccentricity) to a lesser extent (Table 3). Parental genotypes such as JAL and mainly HAB had a more pronounced and negative contribution from PC2 (Fig. 19A). While, in a positive contribution, the intraspecific hybrid HAB × BIQ, the interspecific hybrid IKE × BIQ and the parental IKE were highlighted. The parental BIQ genotypes, interspecific hybrid HAB × JAL and intraspecific reciprocal hybrids JAL × IKE and IKE × JAL had intermediate values and, therefore, close to zero for the main component 2. The third component contributed 10.7% of data variance (Table 3). Parameters such as fruit shape index external I, fruit shape index external II, circular, curved fruit shape index, eccentry, distal eccentricity, fruit shape index internal and lobedness degree were the ones that contributed most positively, both width mid-height, maximum width, v. asymmetry and proximal eccentricity with negative contribution. The fourth component had a contribution of 7.6% in the variance, with emphasis on proximal eccentricity with eigenvectors of 0.447 and also pepper pericarp thickness ratio and pepper pericarp area ratio with positive contribution. Parameters such as ellipsoid, proximal fruit blockiness and ovoid had a negative contribution to this component, with values below -0.2.

Table 3. Eigenvalues contribution of four main PCA axes for biometric parameters of fruits for parental and interspecific and intraspecific hybrids of *Capsicum*.

Biometric parameters	PC1 (53.4 %)	PC2 (18.6 %)	PC3 (10.7 %)	PC4 (7.6%)
Perimeter - PE	**0.233**	-0.026	-0.112	0.053
Area – AR	**0.225**	0.021	-0.164	0.043
Width mid-height - WMH	**0.217**	-0.045	**-0.216**	-0.027
Maximum width - MW	**0.213**	0.008	**-0.236**	-0.061
Height mid-width - HMW	**0.237**	-0.018	-0.071	0.063
Maximum height - MH	**0.236**	-0.011	-0.084	0.059
Curved height - CH	**0.235**	-0.016	-0.093	0.064
Fruit shape index external I - FSIE	0.196	-0.043	**0.257**	0.189
Fruit shape index external II - FSIE II	**0.205**	0.028	**0.244**	0.104
Curved fruit shape index - CFSI	**0.209**	0.045	**0.21**	0.122
Proximal fruit blockiness - PFB	0.151	**0.236**	0.095	**-0.28**
Distal fruit blockiness - DFB	**0.209**	-0.179	-0.018	-0.047
Fruit shape triangle - FST	-0.132	**0.311**	0.063	-0.047
Ellipsoid - ELL	0.017	**0.314**	0.091	**-0.337**
Circular - CIR	0.189	0.017	**0.275**	0.002
Rectangular - REC	**0.200**	-0.186	0.045	0.049

Obovoid - OBO	-0.025	**-0.284**	-0.176	-0.196
Ovoid – OVO	0.023	**0.358**	0.059	**-0.257**
V. asymmetry - VA	0.192	0.058	**-0.278**	-0.053
H. asymmetry.ob - Haob	0.184	**0.216**	-0.085	-0.196
H. asymmetry.ov - Haov	-0.025	**-0.284**	-0.176	-0.165
Width widest - WW	-0.112	**-0.322**	0.029	0.133
Eccentry - ECC	0.15	-0.186	**0.278**	-0.011
Proximal eccentricity – PE_1	-0.014	**0.208**	**-0.235**	**0.447**
Distal eccentricity - DE	0.034	**-0.247**	**0.279**	**-0.288**
Fruit shape index internal - FSII	**0.205**	0.029	**0.246**	0.102
Eccentricity area index - EAI	0.187	0.169	-0.027	-0.141
Lobedness degree - LD	**0.206**	-0.01	**0.245**	0.139
Pepper pericarp area - PPT	**0.22**	0.036	-0.166	0.056
Pepper pericarp area ratio - PPAR	-0.109	0.166	0.184	**0.262**
Pepper pericarp thickness - PPTh	**0.226**	0.032	-0.135	0.043
Pepper pericarp thickness ratio - PPTratio	-0.106	0.175	-0.081	**0.35**

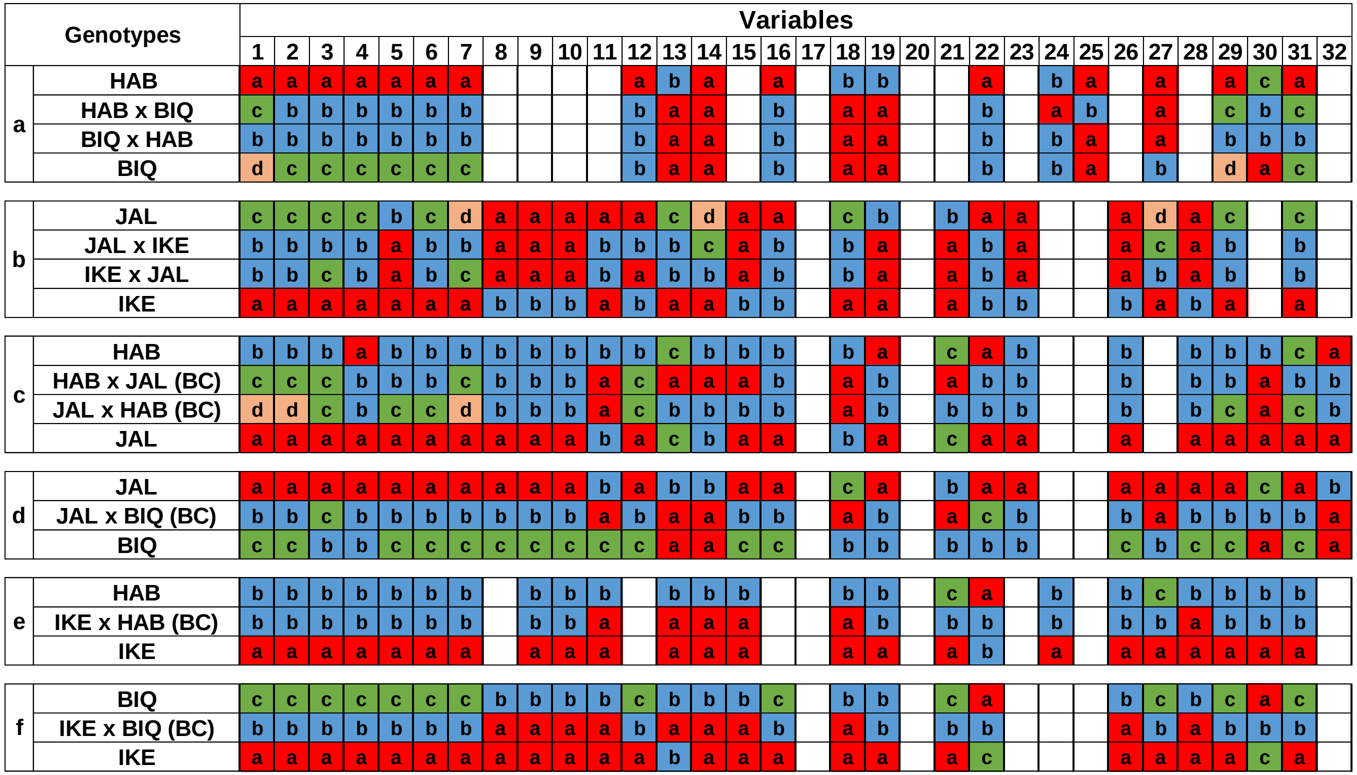
The degree of morphological differentiation depended on the type of hybridization and the parents involved (Fig. 20). We represent interspecific hybrids with biometric values obtained by the average of the fruits obtained with different parental pollens, in order to dilute the influence of the pollen. Interspecific hybrids involving the parental HAB show greater transmissibility of biometric characteristics. IKE × HAB fruits showed 15 biometric characteristics of HAB versus 7 of IKE. JAL × HAB with 9 HAB characteristics and only one JAL characteristic. While, HAB × JAL with 10 characteristics of HAB and 2 of JAL. Hybrids with JAL in their composition have very little characteristics, with JAL × BIQ, JAL × HAB and HAB × JAL having in number 1 (27), 1 (30) and 2 (15, 30) characteristics of this parent. Interestingly, hybrids between IKE show to be influenced by another parent, since IKE × BIQ has more characteristics of IKE (9 against 1), while IKE × HAB shows it in a smaller proportion (7 against 15). Similar occurred for hybrids with BIQ, with a predominance of BIQ characteristics only when together with JAL (JAL × BIQ).

Contrary to the low expression of JAL characteristics in interspecific hybridization, the intraspecific hybrids involving this cultivar showed to have a balance between the characteristics of parents, but with a prevalence of JAL characteristics. IKE × JAL has 9 characteristics of JAL against 5 of IKE and JAL × IKE with 7 characteristics of JAL and 6 of IKE. The opposite occurred for intraspecific hybrids of HAB. Both HAB

× BIQ and BIQ × HAB have only one characteristic of HAB (27), while they share the same 6 characteristics of BIQ.

With the exception of IKE × HAB, in which the fruits showed only 2 new characteristics (Fig. 20). IKE × BIQ, JAL × BIQ, JAL × HAB and HAB × JAL showed 16, 21, 16 and 15 new characteristics, respectively. Intra-specific hybrids such as IKE × JAL, JAL × IKE, HAB × BIQ and BIQ × HAB presented new fruit morphometric patterns in 12, 13, 11 and 10 characteristics, respectively. In addition, we observed the effect of the parent-of-origin in differentiating the fruit morphology in reciprocal crossings, depending on the direction of the crossings, represented here in figure 19 by the different colors and letters in the 32 biometric parameters of fruits. The parental-effect-of-origin for interspecific hybrids was more pronounced, with 11 divergent parameters and 16 biometric parameters of fruits shared between the hybrids JAL × HAB and HAB × JAL. Meanwhile, the intraspecific hybrids IKE × JAL and JAL × IKE presented 5 divergent and 20 shared parameters. The same occurred for the reciprocal hybrids HAB × BIQ and BIQ × HAB, with only 5 divergent and 15 shared parameters.

Figure 20. The variation of biometric characteristics of fruits (1-32) between parents and their respective F1 hybrids is variable, showing different characteristics even among reciprocal hybrids. The red 🟥 , blue 🟦, green 🟩 and pink 🟧 boxes represent statistically values of a, b, c and d by the Scott Knott test by % 5, respectively. White boxes do not differ statistically.



Genotypes		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
a	HAB	a	a	a	a	a	a	a					a	b	a		a		b	b			a		b	a		a		a	c	a	
	HAB x BIQ	c	b	b	b	b	b	b					b	a	a		b		a	a			b		a	b		a		c	b	c	
	BIQ x HAB	b	b	b	b	b	b	b					b	a	a		b		a	a			b		b	a		a		b	b	b	
	BIQ	d	c	c	c	c	c	c					b	a	a		b		a	a			b		b	a		b		d	a	c	
b	JAL	c	c	c	c	b	c	d	a	a	a	a	a	c	d	a	a		c	b		b	a	a			a	d	a	c		c	
	JAL x IKE	b	b	b	b	a	b	b	a	a	a	b	b	b	c	a	b		b	a		a	b	a			a	c	a	b		b	
	IKE x JAL	b	b	c	b	a	b	c	a	a	a	b	a	b	b	a	b		b	a		a	b	a			a	b	a	b		b	
	IKE	a	a	a	a	a	a	a	b	b	b	a	b	a	a	b	b		a	a		a	b	b			b	a	b	a		a	
c	HAB	b	b	b	a	b	b	b	b	b	b	b	b	c	b	b	b		b	a		c	a	b			b		b	b	b	c	a
	HAB x JAL (BC)	c	c	c	b	b	b	c	b	b	b	a	c	a	a	a	b		a	b		a	b	b			b		b	b	a	b	b
	JAL x HAB (BC)	d	d	c	b	c	c	d	b	b	b	a	c	b	b	b	b		a	b		b	b	b			b		b	c	a	c	b
	JAL	a	a	a	a	a	a	a	a	a	a	b	a	c	b	a	a		b	a		c	a	a			a		a	a	a	a	a
d	JAL	a	a	a	a	a	a	a	a	a	a	b	a	b	b	a	a		c	a		b	a	a			a	a	a	a	c	a	b
	JAL x BIQ (BC)	b	b	c	b	b	b	b	b	b	b	a	b	a	a	b	b		a	b		a	c	b			b	a	b	b	b	b	a
	BIQ	c	c	b	b	c	c	c	c	c	c	c	c	a	a	c	c		b	b		b	b	b			c	b	c	c	a	c	a
e	HAB	b	b	b	b	b	b	b		b	b	b		b	b	b			b	b		c	a		b		b	c	b	b	b	b	
	IKE x HAB (BC)	b	b	b	b	b	b	b		b	b	a		a	a	a			a	b		b	b		b		b	b	a	b	b	b	
	IKE	a	a	a	a	a	a	a		a	a	a		a	a	a			a	a		a	b		a		a	a	a	a	a	a	
f	BIQ	c	c	c	c	c	c	c	b	b	b	b	c	b	b	b	c		b	b		c	a				b	c	b	c	a	c	
	IKE x BIQ (BC)	b	b	b	b	b	b	b	a	a	a	a	b	a	a	a	b		a	b		b	b				a	b	a	b	b	b	
	IKE	a	a	a	a	a	a	a	a	a	a	a	a	b	a	a	a		a	a		a	c				a	a	a	a	c	a	

1: Perimeter (cm); **2:** Area (cm²); **3**: Width mid-heigh (cm); **4:** Maximum Width (cm); **5**: Height mid-width (cm); **6:** Maximum height (cm); **7**: Curved height (cm); **8**: Fruit shape index external I; **9**: Fruit shape index external II; **10**: Curved fruit shape index; **11**: Proximal fruit blockiness; **12**: Distal fruit blockiness; **13**: Fruit shape triangle; **14:** Ellipsoid; **15**: Circular; **16**: Rectangular; **17**: Obovoid; **18:** Ovoid; **19**: V. asymmetry (cm); **20**: H. asymmetry.ob (cm); **21**: H. asymmetry.ov (cm); **22**: Width widest; **23:** Eccentry; **24**: Proximal eccentricity; **25**: Distal eccentricity; **26**: Fruit shape index internal; **27**: Eccentricity area index; **28**: Lobedness degree; **29**: Pepper pericarp area (cm²); **30**: Pepper pericarp area ratio; **31**: Pepper pericarp thickness (cm); **32**: Pepper pericarp thickness.

Productive Parameters

The cultivars of *C. annuum*, IKE and JAL, produce few fruits, of great size and weight, mainly IKE. Whereas, *C. chinense* cv. Biquinho (BIQ) with numerous small fruits, *C. chinense* Habanero (HAB) has a fruit production lower than BIQ and higher than IKE and JAL, while the weight is much higher than BIQ, but also much lower than IKE and JAL (Figs. 21, 22). For the characteristic individual weight of fruits, the intraspecific hybrids were unanimous in show intermediate weight, while for the interspecific hybrids they presented weight in much lower than the fruits of the lower weight parental (HAB × JAL, JAL × HAB and IKE × HAB), or in maximum equivalents (JAL × BIQ and IKE × BIQ) (Fig. 22, left).

The production in number of fruits per plant was almost unanimous in intermediate characteristics for the F_1 hybrids in relation to the parental ones, regardless of the type of hybridization. The smallest contrast between the number of fruits between the parents resulted in hybrids with values closer to the parents, even if lower (JAL × IKE, IKE × JAL) or even higher (JAL × HAB), showing that the crossing of parents with number of fruits less contrasting is the best method to increase or maintain this characteristic.

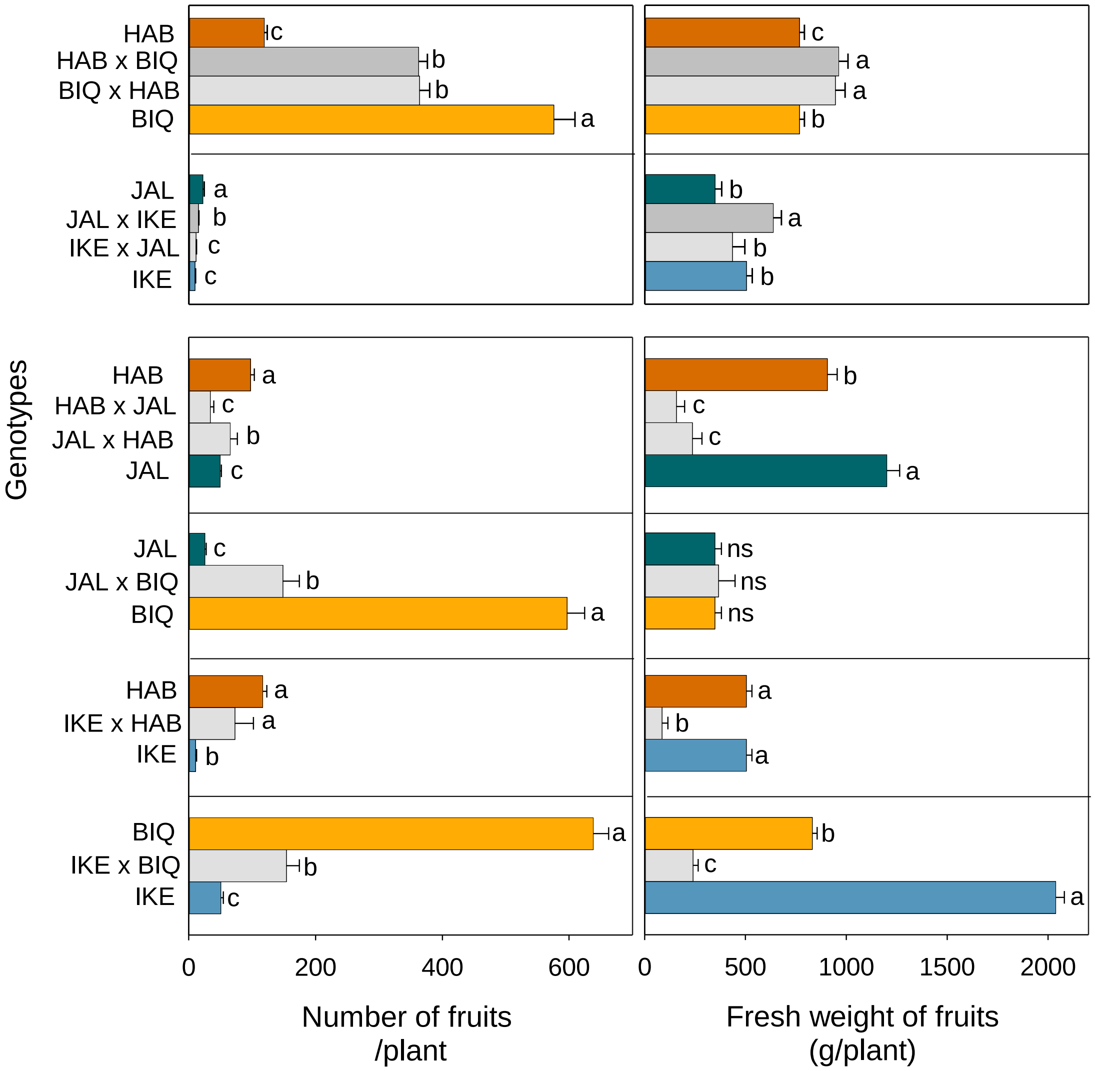


Figure 21: Agronomic parameters in *Capsicum* intraspecific (Top) and interspecific (Bottom) hybrids with their respective parents for the variables Number of fruits/plant and Total fresh fruit weight/plant. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test. ns: non-significant.

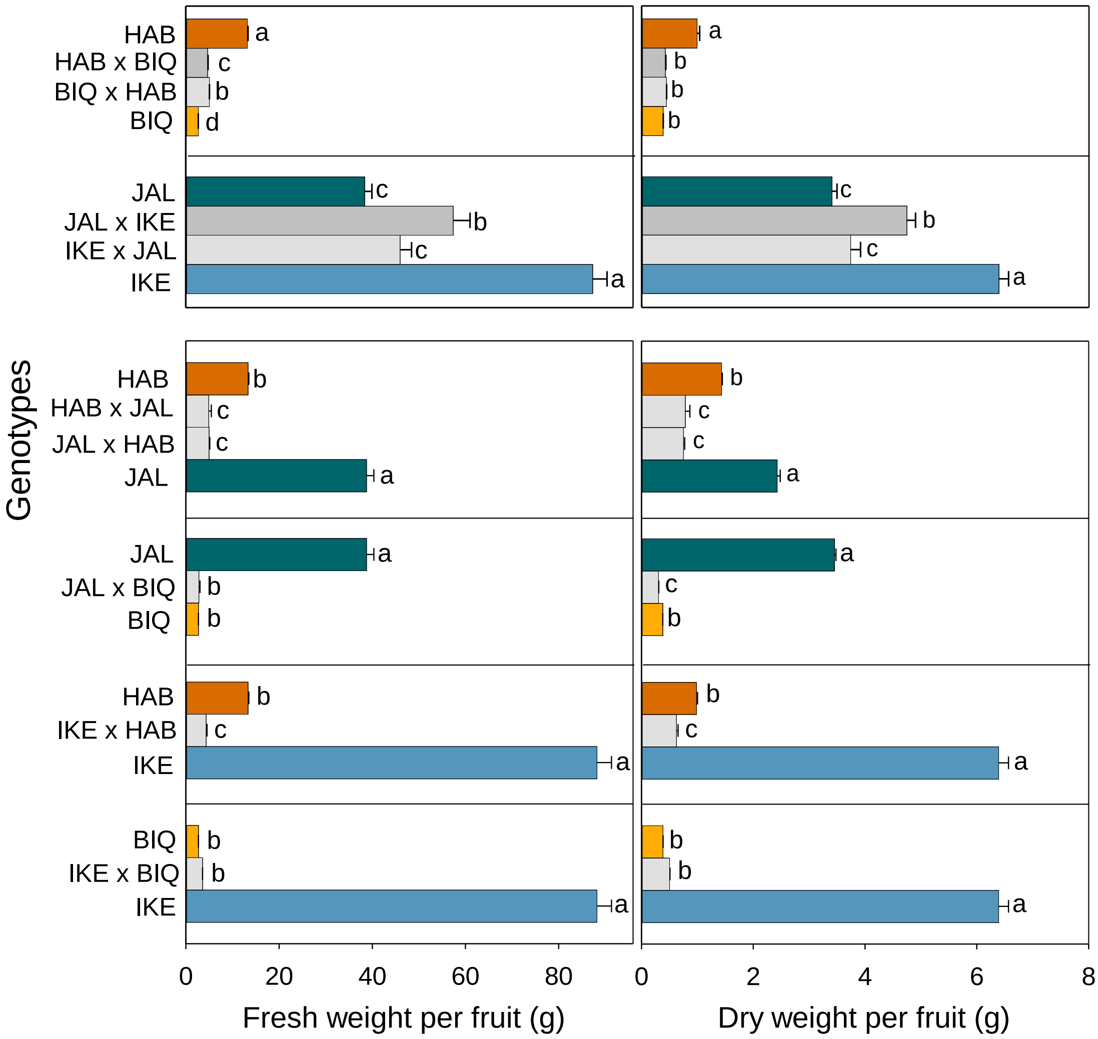


Figure 22: Agronomic parameters for *Capsicum* intraspecific (Top) and interspecific (Bottom) hybrids with their respective parents for the variables Fresh weight per fruit (g) and Dry weight per fruit (g). Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.

The productivity component in number of fruits per plant is the result of the interaction of variables such as number of fruits per plant with the individual weight of each fruit. Intraspecific hybrids such as HAB × BIQ and BIQ × HAB show to be superior to parental in mass fruit productivity per plant by jointly increasing the individual fruit weight compared to BIQ and the number of fruits per plant in relation to HAB. The same happened with the JAL × IKE hybrid. Interspecific hybrids show the opposite, failing to overcome parental productivity. Which shows that for these hybrids that the interaction between number of fruits and individual mass was not satisfactory enough to surpass their parents in productivity in fresh weight of fruits per plant.

The plotting of the production parameters in principal component analysis (PCA) explains the characteristics of the F_1 hybrids (Supplementary Fig. 4). The first component explained much of the data variation, with 53.7%. For this component, variables with positive eigenvalues greater than 0.3 were fresh weight per fruit, dry weight per fruit, fruit length, fruit diameter and number of seeds per fruit (Supplementary Table 1). Genotypes with higher values for PC1, and therefore, with greater importance for this variable, were in increasing order IKE × JAL, JAL, JAL × IKE and IKE, with PC1 efficient in separating them from the other genotypes. PC2 explained 31.4% of the data variation. Genotypes with the highest values for this component, in ascending order, were JAL × BIQ, IKE × BIQ, IKE × HAB, JAL × HAB and HAB × JAL. This component was positively associated with Brix, which means that genotypes with high Brix were highlighted in this component. While, negatively for the number of fruits per plant, fresh weight of fruits per plant and fruit set, that is, genotypes with prominence for PC2, located at the top left, were negatively associated with these parameters. In addition, the pairing of the interspecific hybrids for Brix was evident, as confirmed by the larger ones by the higher values in relation to the intraspecific hybrids or even the parental cultivars (Supplementary Fig. 5). The third component (PC3) explained only 9.6% of the data variation, with greater positive eigenvalues for fruit diameter and negative for fruit shape index.

Parental-of-origin effect

In the productive parameters, analyzed here, we observed different actions, apparently unrelated to the type of hybridization (inter or intraspecific) (Table 4). We found no parental-of-origin effect on any productive characteristics evaluated at the time of harvest between the hybrids HAB × BIQ and BIQ × HAB. For the intraspecific hybrids between JAL and IKE, we see a positive reciprocal effect for JAL × IKE over IKE × JAL in parameters such as dry vegetative biomass, fresh weight per fruit and number of seeds per fruit. Interestingly, characteristics such as number of seeds per fruit are higher in JAL, fresh weight per fruit is higher in IKE, while dry vegetative biomass are equivalent in both parents. The hybrid IKE × JAL showed a superior parental-of-origin effect on characteristics such as number of sympodial units and Brix, these characteristics being superior in the parental IKE. The interspecific hybrid HAB × JAL had a significant effect on JAL × HAB in all characteristics such as total plant height, total plant diameter, dry vegetative biomass (g plant^{-1}) and fresh weight per fruit (g). The parental HAB has superior characteristics in plant diameter and dry vegetative biomass while JAL in plant height and fresh weight per fruit.

Table 4. Parental-of-origin effect for twelve traits in two intraspecific and one interspecific crosses of *Capsicum* in F$_1$ generation.

Variables	Hybrids		
	Intraspecific		Interspecific
	HAB^a x BIQ^b	**JAL^a x IKE^b**	**HAB^a x JAL^b**
Precocity until anthesis (days)	-1.30	0.00	-0.40
Precocity until 1° ripe fruit (days)	-2.50	2.10	5.20
Plant height (cm)	0.10	6.50	28.90 **
Plant diameter (cm)	11.40	12.40	21.30 **
Number of sympodial units	29.10	-43.50 **	97.70
Dry vegetative biomass (g plant^{-1})	0.05	11.06 *	127.28 **
Fruit set (%)	-3.07	0.80	-5.60
Number of fruits per plant	-4.90	2.40	-39.60
Fresh weight of fruits per plant	-16.46	157.02	-66.46
Fresh weight per fruit (g)	-0.18	15.49 **	2.01 **
Number of seeds per fruit	2.75	26.59 **	-2.50
°Brix	0.25	-0.73 **	0.340

*, ** significant at P = 0.05 and 0.01, respectively by the Scheffe test.
a, b=P1, P2, respectively**.**

Heterosis determinations

The analysis of the hybrids from the heterotic point of view demonstrates the ability of the hybrids to be superior or not to the parental ones, being the rMPH (Relative-mid-parental heterosis) as the heterosis of the F1 hybrid as to values between the mean of the parents, while rBPH (Relative best-parental heterosis) refers to heterosis on the performance of the best parental.

Flowering precocity and the first ripe fruit had a positive heterotic effect for Relative mid-parental heterosis (rMPH) as well as for best-parental heterosis (rBPH) only for intraspecific hybrids (Table 5). While precocity to anthesis occurred only with positive values referring to rMPH for one interspecific hybrid IKE × BIQ. For this hybrid, both types of heterosis (rMPH and rBPH) were positive for precocity up to the first ripe fruit.

Table 5: Relative mid-parental heterosis (rMPH) and best-parental heterosis (rBPH) for twelve traits in two intraspecific and four interspecific hybrids of *Capsicum*.

Traits	Hybrids					
	Intraspecific		Interspecific			
	HAB x BIQ	**JAL x IKE**	**HAB x JAL**	**JAL x BIQ**	**IKE x HAB**	**IKE x BIQ**
Time to anthesis (days)						
rMPH	21.07 **	12.85 **	-3.03	2.62	5.15	9.37 **
rBPH	19.64 **	10.91 *	-7.85	-3.96	-0.88	-5.46 **
Time to first ripe fruit (days)						
rMPH	17.22 **	10.64 **	-10.94 **	5.7	-0.62	6.05 **
rBPH	14.13 **	7.00 *	-14.71 **	2.97	-5.94	5.06 *
Plant height (cm)						
rMPH	-28.63 **	0.69	91.86 **	102.48 **	129.72 **	138.05 **
rBPH	-31.82 **	-8.09	81.66 **	100.6 **	99.62 **	115.48 **
Plant diameter (cm)						
rMPH	61.07 **	12.44 *	34.2 **	52.15 **	126.07 **	106.48 **
rBPH	53.73 **	4.34	17.69 **	28.19 **	85.99 **	63.79 **
Number of sympodial units						
rMPH	-6.49	-3.41	262.28 **	70.79 **	257.37 **	206.84 **
rBPH	-12.59	-19.49 *	113.19 **	0.29	132.41 **	93.53 **
Dry vegetative biomass (g plant^{-1})						
rMPH	-17.98 **	-19.18	259.58 **	118.81 **	131.26 **	248.56 **
rBPH	-19.85 **	-19.73	171.09 **	59.08 **	59.7 **	188.58 **
Fruit set (%)						
rMPH	32.57 *	2.11	-70.73 **	-45.27 *	-98.97 **	-83.71 **
rBPH	-8.40	-22.09	-78.04 **	-46.28 **	-99.13 **	-87.41 **
Number of fruits per plant						
rMPH	3.28	-21.58	-22.94	-41.76 **	14.51	-55.29 **
rBPH	-37.1 *	-45.11 **	-41.8 **	-69.97 **	-37.58	-75.88 **
Fresh weight of fruits per plant (g)						
rMPH	37.34 **	12.11	-78.13 **	63.33	-81.7 **	-80.71 **
rBPH	22.55 **	-4.35	-81.48 **	36.42	-83.3 **	-86.63 **
Fresh weight per fruit (g)						
rMPH	-20.98 **	-14.23 *	-64.36 **	-74.86 **	-91.54 **	-97.28 **
rBPH	-54.69 **	-53.35 **	-73.06 **	-86.49 **	-95.25 **	-95.71 **
Number of seeds per fruit						
rMPH	-2.8	17.24 **	-95.87 **	-86.26 **	-96.91 **	-94.04 **
rBPH	-7.95	9.26	-97.11 **	-90.06 **	-97.74 **	-95.48 **
°Brix						
rMPH	-7.21 **	3.9 *	31.42 **	34.14 **	16.06 **	31.26 **
rBPH	-17.19**	1.54	19.85 **	30.96 **	7.99	25.3 **

*, ** significant at P = 0.05 and 0.01, respectively by the Scheffe test.

The significance test for the variables rMPH and rBPH was performed based on the absolute values of MPH and BPH.

Hybrids of HAB × BIQ and BIQ × HAB showed a decrease in vegetative biomass and high heterosis in productivity of fresh fruit mass, even with low heterosis for number of fruits, only rMPH, and negative for individual fruit mass. This fact can be explained by the discrepant performance of the parents. HAB has low fruit set and high individual fruit weight, whereas BIQ has high fruit set and number of fruits per plant, but with small individual fruit size (Supplementary Fig. 1, Figs. 21 and 22). When hybridized (HAB × BIQ), fruit size was increased in relation to BIQ, the number of fruits and fruit set in relation to HAB, contributing to expressive values in mass productivity per plant.

Hybrids between IKE and JAL were highly productive, but with a median value between parents and no significant heterosis in fruit set, fruit number and fresh weight of fruits per plant, except for negative values for fresh weight per fruit. This fact can be explained by the breadth of mass to fruit mass among the parents. IKE has fruits with great weight, on average of 102 ± 6.10 mg, JAL with 24 ± 1.46 mg while JAL × IKE with 57 ± 0.4 mg (Fig. 22).

As for interspecific hybrids, negative heterosis was unanimous for variables such as fruit set, fresh weight per fruit and number of seeds per fruit (Table 5). For some of these hybrids there was no significance in the values of heterosis because they are very close to the variation found between the average of parents, being considered here as neutral (not significant) heterosis, even with values far from zero. This occurred in HAB × JAL for numbers of fruits per plant and in JAL × BIQ for fresh mass of fruits per plant, therefore considerable in view of the magnitude of negative values for the other hybrids. In addition, interspecific hybrids showed a great increase in organoleptic characteristics of fruits such as Brix (Table 5, Supplementary Fig. 5). However, it must be considered that these hybrids had a low reproductive sink (fruits), both in number and in total and individual mass of fruits per plant (Figs. 21, 22), in relation to high vegetative biomass (Table 1).

From a heterosis point of view, intraspecific hybridization decreased the height and increased plant diameter in HAB × BIQ; and modestly the diameter in JAL × IKE (rMPH only) (Table 5). On the contrary, parameters like these, as well as the number of sympodial units were shown to be very high in both types of heterosis (rMPH and rBPH) for most interspecific hybrids. In addition to the high accumulation of biomass vegetative dryness per plant (Table 1). It is clear that the interspecific hybrids studied here naturally grow more, in addition, the initial growth data corroborate this fact.

Discussion

Hybridization action on viability, germination, and seed size

Many characteristics can serve as an indicator of seed germination, such as visuals, weight, biochemical tests and germination tests. Seeds from non-viable crosses in *Capsicum* may have blackened spots (Hundal and Dhall, 2005), small size and low weight (empty seeds due to the absence of endosperm and/or embryo) (Tong and Bosland, 2003) or even have normal characteristics (Tanksley and Iglesias-Olivas, 1984). In fact, non-viable crosses had less seed weight, but we also observed a cross that produced germinable F_1 seeds, even if low, with much less weight (Fig. 5a). This same cross (HAB × JAL) resulted from the hybridization of *C. chinense* cv. Habanero as a mother plant and *C. annuum* cv. Jalapeño as a male parent, and the other crosses with *C. chinense* as a female parent and *C. annuum* as a pollen donor were not viable (Fig. 5b). It is reported that the formation of interspecific hybrids with *C. chinense* as a female parent is rare (Tanksley and Iglesias-Olivas, 1984; Martins *et al*., 2015). Interspecific hybridization is expected to occur depending on the direction of the parent used as a parent or pollen donor, that is, they occur satisfactorily in only one direction (Prestes and Goulart, 1995). A rare crossing like this must be investigated thoroughly, allowing the understanding of molecular and genetic mechanisms that generally restrict crossability between *Capsicum* species.

The change in seed size within the same female parent when receiving different pollen sources is an unprecedented observation for the genus, although higher seed weight and seed embryo size have been reported in crosses of *Capsicum* (Popova and Mihailov, 1978). The specific size of seeds can be controlled by the fruit in which they are housed (maternal effect) (Denny, 1992) or controlled by the signaling of the female parent and also with zygotic influence (Li and Li, 2015), depending on the species. There is a theory that predicts seed size by maternal and paternal influence. In it, the greater or lesser size of the seeds and their reserves would be a tug of war between the action of factors derived from the paternal inheritance to provide greater reserves and thus greater chances of survival of their descendants in opposition to maternal factors to prevent or balance this action (Moore, 1991). In addition, depending on the crossing or the direction that it is taken, it can increase genes related to cell proliferation, contributing to the increase of the endosperm and seed size as observed in Arabidopsis (Lu *et al*., 2012). Therefore, it is plausible that with the change in the zygotic content in our different crossings it would result in a change in weight / size. The size of the seed embryo is considered to depend mainly on the number of eggs fertilized per fruit (Whaley, 1944), and therefore with a connection between the number of seeds produced and their size (Li and Li, 2015). Although, we did not observe a correlation between the number of seeds produced by each fruit in different crosses with the size of seeds.

With the exception of non-viable seeds, there was no relationship between seed size and germinability (Fig. 12). In particular, most *Capsicum* interspecific hybrids have compromised germination (Martins *et al*., 2015). In this type of hybridization, the occurrence of post-zygotic barriers of incompatibility with the formation of seeds with absence of endosperm and / or with embryo degeneration is common (Monteiro *et al*., 2011). In all crosses to obtain interspecific hybrids, seeds were formed, demonstrating the unanimity of the presence of post-zygotic barriers. As observed by the greater compromise in the germinability of interspecific hybrids in relation to intraspecific and parental hybrids (Fig. 5b). Despite the magnitude of post-zygotic barriers being more prevalent in interspecific hybrids, it does not occur for all of these hybrids, proving to be a dependent genotype phenomenon. As observed by the equivalent germination of an interspecific hybrid to that of intraspecific hybrids. Unusual characteristics such as this make us highlight *Capsicum* as a diverse genus in physiological characteristics

Intraspecific and intraspecific hybridizations differ in plant growth

The evaluation in three phenological phases allowed us to show the superiority of growth at 45 days after sowing (DAS) for all hybrids in comparison to parental ones, in addition when it was possible to produce reciprocal hybrids, there was the best performance of some hybrids over the others as for JAL × HAB, IKE × JAL and HAB × BIQ (Figs. 6 to 11). This can first suggest the action of different genetic inheritance in these hybrids and therefore different results regarding growth. In fact, this may have had an influence, but we believe that the driving force for this performance differentiation between reciprocal counterparts and greater performance of interspecific hybrids in relation to parental ones was also due to the size / weight of the seeds (Fig. 12).

In fact, at 45 DAS, the highest performance for most growth parameters measured for both interspecific hybrids and for reciprocal and / or intraspecific hybrids and even the parental were associated with the highest seed weight (Fig. 12). In the process of domestication of species there is an increase in the search for greater seed size, often showing a relationship with the vigor of initial growth (Doganlar *et al*., 2000). Ashby (1932) showed that the difference in vigor in growth in reciprocal hybrids in maize occurred due to the difference in embryo size, since the different mother plants naturally have different seed sizes. In our crosses to obtain reciprocal hybrids with different performance between them, we did not discard the potential effect of embryo size as a driving force, however, we discard the isolated maternal effect as a seed size controller. Our results show that the seeds generated in the same mother plant, but originated from different crosses, due to the effect of different pollen grains had different weights (Fig. 5a).

The relationship between hybridization combined with reciprocity in *Capsicum* with seed size and initial vigor is unprecedented. This behavior can have agronomic and ecological consequences. First, in order to obtain reciprocal crosses with more vigorous seedlings in the initial phase, it is important to note in which direction of the crossing they provide seeds with greater weight. Second, it is common for *Capsicum* crosses to occur spontaneously in natural environments (Raw, 2000). If this relationship between hybridization and seed size it is recurrent for crosses between other species and in natural populations, they could certainly result in successful initial establishment, avoidance of competition and propelling of hybrid individuals even though there may be compromised germination in some crosses.

In the phenological phase (90 DAS) we see hybrids with equivalence or already surpassed by some of their parents or reciprocal counterpart in some growth parameters

(Figs. 6 to 11), showing that the greater weight of seeds is important for the initial vegetative vigor, however, other genetic and physiological characteristics are conditions to maintain or increase growth. As noted, that the largest seed size in tetraploid tomatoes is able to maintain the initial vigor in just 11 days after sowing (Fabergé, 1936). The size of seeds is important for the initial vigor, but not primordial, since weeds are able to suppress the growth of various crops, even though the seed size is much smaller, as they have a high relative growth rate of part of the area and low investment in the root system (Seibert and Pearce, 1993). The change in growth patterns in different hybrids and parents up to 90 days is indicative of plasticity regarding growth in *Capsicum*. Such behavior is common to occur in plants with a longer or perennialized cycle, in which growth occurs in cycles depending on the environmental conditions combined with the phenological pattern of the species (Lundgren and Des Marais, 2020).

The central role of fertility in hybridization

We found *in vivo* that the interspecific hybrids have fertility-related problems, since the fruit set parameters and production in number of seeds per fruit were very affected, in addition, this happened for the parental plants when we pollinated with pollen of interspecific hybrids (Figs. 14, 15). In interspecific crosses *Capsicum* is usual male sterility, with greater or lesser extent depending on the genotypes used in the crosses (Shifriss, 1997). As observed by the viability of pollen grains between hybrids resulting from the crossing between accessions of *C. annuum* and *C. chinense* varied in magnitude from 13 to 87% (Monteiro *et al*., 2011; Kumar *et al*., 1987; Lanteri and Pickersgill, 1993). JAL × BIQ among the other interspecific hybrids was the least affected by parameters such as fruit set and number of seeds per fruit. JAL × BIQ shows that even with reduced fruit set and a minimum necessary seed production per fruit resulted in at least yield equivalent to both parents. Therefore, in *Capsicum* we cannot make any generalizations and mainly about interspecific hybridization drastically affect fertility and productivity. Studies show that incompatibility can even occur between intraspecific crosses, with a strong dependence on genotype (Monteiro *et al*., 2011; Nascimento *et al*., 2015).

We observed unprecedented attributes when carrying out several crosses or backcrosses to check the fertility of F_1 hybrids. In fact, interspecific hybrids demonstrate problems related to fertility and their pollination with parent pollen was an effective method of restoring fertility (set of fruits and number of seeds per fruit) (Fig. 14). However, we did not expect such a sharp increase in the fruit set for these hybrids. The expressive increase in the fruit set may have been related to the low yield (sink) that these hybrids have in relation to the high leaf area, which represents a great capacity for source. The trade-off between sink and source has an influence on the fruit set in *Capsicum* (Ávila Silva *et al*., 2019a; Ávila Silva *et al*., 2019b). However, this increase in the fruit set occurred for almost all intraspecific hybrids when they received pollens from their parental (Fig. 14). Such an unexpected response, since intraspecific hybrids are highly productive and had no problems related to fertility. In addition, we would expect that only fertility had an influence on the fresh fruit weight for interspecific hybrids, but we observed this same fact for intraspecific hybrids and for some parents (Figs. 14, 15), which we suggest as an exogenous pollen action.

Similar results were observed in mandarin, in which, in addition to the pollen having different effects depending on the cultivar in parameters such as fruit set and number of seeds per fruit; had fruit size and organoleptic characteristics of fruits (Wallace and Lee, 1999), which the authors attributed to a xenia effect. The concept adopted by Darwin (1896), regarding xenia, despite not having defined that term, is any effect of the foreign pollen grain on the mother plant, which generally includes mainly in fruit characteristics such as size, shape and organoleptic characteristics, in characteristics of the seeds (integument color, shape and weight) in addition to characteristics such as embryo sizes and endosperm (Denney, 1992).

We observed this same phenomenon for many biometric characteristics of fruits for interspecific hybrids (Figs. 17A and 17B), intraspecific hybrids (Supplementary Fig. 2) and parental (Supplementary Fig. 3) and also for F_1 seed weight obtained by crossing between parents (Fig. 5a). It is plausible that the xenia effect on seeds may cause morphological changes in fruits, since differentiated genetic material in embryos can induce different contents of hormones related to fruit growth such as auxin, cytokines and gibberellins (Swingle, 1928; Denney, 1992). In fact, the relationship between auxins present in tissues in seeds and their requirement for their formation and fruit may presuppose a coordinated signaling mechanism between this hormone and the establishment of fruits as the preferred sugar drain (Robert *et al*., 2019). The importance of redirecting sugars (sucrose) to flowers in establishing fruit set and fruit development in *Capsicum* has already been determined (Ávila Silva *et al*., 2019a, Ávila Silva *et al*., 2019b). These facts demonstrate that the mechanisms involved in fertility, formation and fruit development in *Capsicum* are more complex and specific than previously described.

Fruit Morphology

The morphology of fruits in *Capsicum* proved to be slightly to highly influenced for the intra and interspecific hybrids, respectively, by the pollen source with action in parameters such as length and diameters of fruits and fruit shape index (Supplementary Fig. 2). The same occurred with the fruits of the parents when the flowers received different sources of pollen (Supplementary Fig. 3). The relationship between fertility and fruit morphology would explain the change in fruit morphology for interspecific hybrids, however little does it for intraspecific or parental hybrids. Therefore, we disregard the different pollen sources for intraspecific and parental hybrids for detailed analyzes of fruit morphology, one that does not have fertility problems reported with fruit morphology when self-pollinated. In contrast, due to the aberrant fruit morphology of the interspecific hybrids when self-pollinated, we measure these hybrids with biometric values obtained by the average of the fruits obtained when pollinated with different parental pollens, in order to dilute the pollen influence of each parental.

Here we see how the biometric parameters of the fruit is strongly influenced by the parents involved in the composition and depending on the type of hybridization (intra or interspecific). In addition, we observed that the PCA groupings of the interspecific hybrids were distant and concentrated with intermediate values in relation to the other parental and intraspecific hybrids (Fig. 19A). This is certainly due mainly to the intermediate values in biometric parameters of fruits for interspecific hybrids, since their respective parents had very contrasting characteristics in this sense. Thus, we observed that interspecific hybridization was more striking in the emergence of different biometric characteristics of fruits in relation to both parental (Fig. 20). In addition, intraspecific hybrids between IKE and JAL showed fruit shape much closer to IKE, mainly IKE × JAL, for component 1, which explained most of the data variation (Fig. 19A). In the same way, the intraspecific hybrids between HAB and BIQ, showed to receive greater influence of biometric parameters of the parental BIQ by the positioning closer by the component PC1. The influence of crosses on biometric parameters of fruits is very variable and seems to be influenced depending on the genotype involved and / or the evaluated characteristics (Santos *et al*., 2014; Rego *et al*., 2009).

In addition, we also observed the effect of the parent of origin in providing biometric differences in fruits depending on the direction of crossing more pronounced for reciprocal interspecific hybrids. Although it is not safe to delimit even when these differences were properly parental in origin or artifacts of greater or lesser differentiation from parental pollen source used to obtain fruits with normal morphological characteristics (Fig. 20). The difference in biometric parameters depending on the direction of cross-breeding also occurred for fruits between reciprocal intraspecific hybrids, but to a lesser extent, as seen by the shorter distance between these hybrids and their reciprocal counterparts in relation to the PC1 axis (Fig. 19A). In reciprocal crosses with 8 parental lines of *C. baccatum* resulting in a total of 56 F1 hybrids, the presence of maternal effects on fruit characteristics was observed (Rêgo *et al*., 2009). Reciprocity is already recognized for affecting biometric parameters of fruits; however, our study is a pioneer in *Capsicum* when using phenotyping through this Tomato Analyzer software as a tool for detecting these differences.

Capsicum is a genus recognized and appreciated by the consumer market for its diversity in fruit morphometric parameters (colors and shapes). Notably, this diversity of shapes and colors tends to be greater in domesticated plants than in wild plants (Tripodi and Greco, 2018). However, in Brazil there is a great diversity of accessions of *C. chinense* collected in domestic crops or in natural populations in several states, which have a high diversity in fruit formats and growth parameters (Lannes *et al*., 2007; Bianchi *et al*., 2020; Rosado-Souza *et al*., 2015). The reason why such high diversity exists is not well understood; however, we do not rule out the occurrence of crossings, even if sporadic, as the driving source of this variability. Since in natural populations, both genotypic variant individuals and cross-pollination can occur. We observed that even in our reciprocal crosses, within the same species, but between different cultivars, depending on the direction that occurs, they generated subtle changes in biometric patterns of fruits and in growth patterns, but that over the generations could be amplified and contribute to differentiation into new morphological types or even ecotypes. In addition, dispersion by birds over long distances would be a factor in isolating these new individuals and fixing these new characteristics.

The hybridization influences production parameters and the allocation of biomass

Between intraspecific and interspecific hybridization, we observed differences in the appearance of plants at the time of harvest (Fig. 2). If during the initial growth (45 or 90 days after sowing) we saw an interspecific hybrid with some growth parameter similar to a parental (Fig. 6 to 9, and Fig. 11), at the end of the cycle, up to 180 DAS, these hybrids were much more superior in height, diameter of plants and dry vegetative biomass (Table 1, Fig. 2). In fact, interspecific hybrids grow more, however we have seen changes in the patterns of biomass allocation between vegetative and reproductive organs depending on the type of hybridization. Interestingly, the largest accumulations of vegetative biomass were for interspecific hybrids and allocated in stem and then in root or leaf depending on the hybrid (Fig. 4). In contrast to intraspecific hybrids, which have a high allocation in reproductive biomass and to a lesser extent in vegetative biomass, converging with the common sense of domestication in plants. Plants that have high proportion of mass allocation to reproductive tissued to the detriment of vegetative tissues can be considered more improved (Gepts, 2004). In fact, breeders working on bell pepper and tomatoes have used intraspecific hybridization to generate high yielding varieties. As a trade-off, these crops naturally have less allocation of biomass in stems and roots, and in addition to depletion due to excess production leads to increased susceptibility to diseases and pests and, thereby, compromises the vigor of perennials. Interspecific hybridization would be a way to introduce vigorous characteristics such as stem and root or even to gain an understanding of related mechanisms and genes related to greater vegetative vigor. It is worth mentioning that there are already lines of research seeking to reverse the programmed seasonality of many cultures, rethinking the trade-off between perennialization, annuality, productivity and sustainability (Lundgren and Des Marais, 2020).

Intra or interspecific hybridizations differ in terms of production parameters. Part of this is due to the fact that the parental cultivars used as matrices to obtain the crosses present contrasting productive characteristics in parameters such as productivity in number (Fig. 21, left) and individual fresh weight per fruit (Fig. 22, left), mainly between the cultivars *C. annuum* and *C. chinense*. In fruit individual fresh weight, we would expect at least intermediate characteristics for the F1 hybrids. In fact, this has occurred for intraspecific hybrids, but for interspecific hybrids it was far below expectations. The reason for this is due to the fertility problems reported for these hybrids, in which they reduced the size of the fruits far below their genetic potential, as reported in previous topics. When these hybrids were backcrossed with pollen from the parents, we saw that the individual fresh weight (Fig. 14, middle) of fruits acquired intermediate characteristics in relation to their parents.

Yield in number of fruits per plant tended to intermediate values in relation to parental for both intraspecific and interspecific hybrids (Fig 21, left). In tomato, the relationship between the largest number of flowers and productivity in number of fruits is known (Semel *et al*., 2016), as long as it has an adequate fruit set. We consider that our interspecific hybrids had productivity in number of fruits below the genetic potential. Even though these hybrids have a fruit set in much lower than the parental ones, they managed to achieve productivity in intermediate number among the parental ones, showing for this reason a high investment in number of flowers.

As expected, we did not obtain large values of yield in weight of fruit per plant for interspecific hybrids, except for a hybrid with values equivalent to their parents (JAL × BIQ). In *Capsicum* hybridization, the fruit yield weight component is mainly related to the production of number of fruits per plant (Abu *et al*., 2019), although in addition to the number the individual weight of each fruit is also important (Aditika *et al*., 2020). For this reason, only one interspecific hybrid that had less impairment of fertility was the only one to acquire similarity to its parents. In addition, we observed that the lack of fertility, despite not being an impediment to fruit set, although decreasing it a lot, leads to a decrease in fruit weight and size (Supplementary Fig. 2). For this reason, we obtained only an increase in productivity in weight per plant for intraspecific hybrids (Fig. 21 right). The crossing between high-performance cultivars of *C. annuum* made it possible to obtain an increase in yield (Aditika *et al*., 2020). Likewise, our parental cultivars of both *C. annuum* and *C. chinense* used as parent plants for hybridization were commercial and highly productive cultivars. Thus, we believe that the increase in productivity would also be possible for interspecific hybrids, as long as there were no inherent fertility problems of this type of crossing.

The parental-of-origin effect is dependent on the genotype or variables

When obtaining reciprocal crosses, we can observe or not the occurrence of the parental-of-origin phenomenon (Table 4), in which due to mechanisms of different genetic inheritance depending on which genotype is the male or female parent. The unequal expression of genes inherited from parents can confer different expression patterns with different associated phenotypes, depending on whether the inheritance is maternal or paternal (Schnable and Springer, 2013). For the productive parameters, we observed that the parental-of-origin effect is manifested in some genotypes, occurring both in intraspecific and intraspecific hybrids. Interspecific hybridization is difficult in *Capsicum* (Tanksley and Iglesias-Olivas, 1984; Martins *et al.*, 2015), even more the occurrence of reciprocal hybrids. For this reason, we obtained only a reciprocal interspecific hybrid, and therefore we cannot judge whether the parental-of-origin effect has a greater occurrence depending on the type of hybridization.

The manifestation of the parental-of-origin effect for our crosses shows to occur depending on the analyzed treatments, although for characteristics such as accumulation of vegetative biomass and fresh weight per fruit occurred in two reciprocal hybrids (Table 4). Results like these serve as a guide for breeding programs, since depending on the direction of the crossing, plants with greater vigor or larger fruit sizes can be obtained. The HAB parental has higher vegetative biomass, which resulted in a positive maternal parent effect of HAB × JAL performance over JAL × HAB. The opposite happened for the hybrids involving JAL × IKE and IKE × JAL, in which the parent of greater vegetative biomass (IKE) resulted in greater performance when as a male parent for the hybrid JAL × IKE. The maternal effect was verified for Arabidopsis for vegetative biomass during initial growth, in which F_1 having C24 as the maternal plant accumulated greater biomass than its reciprocal counterpart with Col-0 (Chen, 2013).

For the individual weight per fruit, we observed that the combinations of the hybrids that had the highest fruit weight were obtained only when the parental with the highest individual fruit weight was the male parent (HAB × JAL and JAL × IKE). The higher performance in height and diameter of the hybrid HAB × JAL over JAL × HAB is difficult to judge, since the parental JAL has a higher height, while the HAB has a larger diameter. However, we believe that the highest value in height and diameter for this hybrid is due to the greater growth it has, since the greater growth in height can be followed by the greater horizontal growth due to the dichasially forked shoot present in *Capsicum*.

Characteristics such as Brix, number of sympodial units and number of seeds were shown to be positively influenced by the female parent, since they were higher for the hybrids between IKE and JAL when the parent with greater prominence was used as a maternal plant. Given these results, it is difficult to judge which parents have influence on certain parameters, since the results showed here have variation. Other studies on *Capsicum* have shown parental-of-origin effect on growth parameters (Rego *et al*., 2012; Nascimento *et al*., 2015), however, little is known on whether this effect is predominantly maternal, paternal or both.

The reciprocal crosses between HAB and BIQ did not show any parental-of-origin effect on productive parameters. In fact, the hybrids between HAB and BIQ showed more homogeneous phenotypic characteristics at the end of the cycle. However, these hybrids show differentiated initial growth parameters as well as differentiated phenotypic characteristics, which we can consider as a parental-of-origin effect, but only visible in the initial development phase. In addition, we saw a parental-of-origin effect on biometric parameters of fruits (Fig. 20).

Heterosis is governed by the type of hybridization

Discussing heterosis is a complicated task, since despite being easily measured and observed in practical and visual terms, it is a mechanism that involves genetic and physiological bases that are still poorly understood. Traits such as duration of the life cycle are an important agronomic parameter and can be used as a selection criterion for precocity, that is, how fast a plant can reach and complete the reproductive stage, represented here by the number of days to anthesis and to first ripe fruit, respectively. The precocity until anthesis occurred for all intraspecific crosses, with positive values for both types of heterosis, while only one specific hybrid obtained precocity only for relative mid-parental heterosis (Table 5). In intraspecific hybridization with 66 F_1 hybrids, heterosis was not obtained on the best parent for early flowering, this characteristic being more common than the opposite (Parvinder *et al*., 2014). The precocity until the first ripe fruit was observed for the same hybrids that achieved early flowering, showing that early flowering can contribute to an early harvest. Although, early harvesting has been shown to occur for *Capsicum* without the need for early flowering (Parvinder *et al*., 2014).

The high values of heteroses found for interspecific hybrids demonstrate the potential of this type of hybridization in increasing characteristics such as plant height and diameters. In contrast, the intraspecific hybridization contributed to decrease the height or with maximum values equivalent to parental (Table 5). In obtaining various intraspecific hybrids, both an increase in height and diameter as well as a decrease were observed (Parvinder *et al*., 2014) show that there is no consensus, with characteristics depending on the genotypes involved in the hybridization. Interestingly, for our intraspecific hybrids between HAB and BIQ, an increase in plant diameter without an increase in height was obtained, for these hybrids hybridization resulted in greater angle values between branches, further enhancing more horizontal growth already observed in the parental. For these hybrids, the greatest growth in diameter did not result in a greater number of sympodial units. While the slight increase in diameter between JAL and IKE hybrids resulted in a decrease in performance in relation to the best parent. The greatest vertical or horizontal growth without an increase in the number of sympodial units is indicative of the change in the length of each sympodial unit.

The highest accumulation of vegetative biomass was significant for interspecific hybrids with relative best-parental heterosis values at least greater than 60% (Table 5). While for intraspecific hybrids without heterosis or with negative heterosis. The superiority of biomass is associated with greater carbon gain and one of the factors related to this is photosynthesis, sometimes unrelated by the increase in photosynthetic rate per se, but by the increase in leaf area, resulting in increased photosynthesis per plant (Fujimoto *et al*., 2012; Offermann and Peterhansel, 2014). In fact, this certainly happened with our interspecific hybrids, with a significant heterotic increase in leaf area per plant.

The greater growth and accumulation of vegetative biomass does not correspond equally to the increase in productive parameters. As seen by the opposite proportion, in which the reduction of vegetative growth allowed the increase in the productivity of rice and wheat contributing to the green revolution (Hedden, 2003). Our interspecific hybrids accumulated much more vegetative biomass (Table 1), but in contrast with the absence of heterosis or with negative heterosis for the number and mass production of fruits per plant (Table 5). However, we do not believe that vegetative heterosis is excluding reproductive heterosis, but impaired fertility may have been responsible for the low yield of these hybrids (Supplementary Fig. 1, right). Low fertility, in addition to compromising the fruit set and also the number of seeds for interspecific hybrids, causing the low number of fruits per plant, decreases the fruit weight (Fig. 21 left, 22 left). Interspecific tomato hybrids show great vegetative vigor (Moyle and Graham, 2005); however, they have low productive potential due to the low fertility resulting from sterility due to the action of harmful recessive genes (Semel *et al.*, 2006). In the absence of sterility, the increase in vegetative growth can also contribute to productivity. In tomato when the lowest expression of the flowering-promoting gene *SINGLE FLOWER TRUSS* (*SFT*), when in heterozygosity, in addition to the absence of repressive response the *SELF PRUNING* (*sp* / *sp*) causes an increase in vegetative growth and also in the number of flowers promoting an appropriate balance between reproductive and vegetative, with subtle anticipation in flowering and increased fruit production (Jiang *et al.*, 2013).

The heterosis for Brix occurred for all interspecific hybrids, although only subtly for intraspecific hybrids between IKE and JAL and with negative values for hybrids between HAB and BIQ (Table 5). We do not rule out the potential of interspecific hybridization in increasing the Brix, however the trade-off of this characteristic with others such as productivity in number and in mass is recognized. In non-domesticated plants with small fruits, low productivity and abundant vegetative biomass, high Brix is common, as in *Solanum pimpinellifolium* (Zsögön *et al.*, 2018). For our interspecific hybrids, low productivity in number and weight of fruits per plant as well as individual weight per fruit was common, mainly in proportion to high vegetative biomass. Brix in tomatoes has an inverse proportion to fruit yield and size (Zsögön *et al.*, 2017). Which makes us suggest that for interspecific hybrids it has a high proportion of sources for few sinks, which may have contributed to the greater accumulation of sugars and soluble solids in the fruits. The imbalance of Brix with productive parameters such as productivity, which is highly required for domesticated crops, requires other tools to improve Brix. Therefore, is recommended that the Brix characteristic should be increased in commercial tomatoes cultivars using genomic tools (Zsögön *et al.*, 2018) or introduced through crosses with wild plants and with successive backcrosses with parents to recover productive characteristics (Liu *et al.*, 2016).

Conclusion

Capsicum breeding through hybridization has enormous potential, however many gaps in basic knowledge need to be explored. In addition, both inter and intraspecific hybridization have the potential to be used as tools for genetic improvement, each with its potential and limitations. Intraspecific hybridization proved to be able to maintain or even increase productive parameters, without compromising fertility and related parameters. However, it proved to be inappropriate to transmit adequate vegetative characteristics. Hybrids between *C. annuum*, studied here, showed a decrease in vegetative characteristics, resulting in plants susceptible to vegetative overturning or breaking of branches in view of the high productive load, which is why staking in cultivation is essential. The hybrids between *C. chinense*, on the other hand, showed extreme horizontal growth, many times the branches touched the ground, sometimes reminding the behavior of their relative tomato. Of course, often the way of cultivation and driving can be adapted to the crop (*i.e.* tomato salad and tomato for sauce), however, in agriculture there is a demand for plants that are suitable for a particular cultivation system (*i.e.* densified cultivation) and mainly mechanization.

Interspecific hybridization, on the other hand, proved to be positive in implementing in many vigorous vegetative characteristics, with abundance in root system and vigorous stems, to the detriment of aspects of productivity. If the problem of the highly productive cultivars of *C. annuum* (*i.e.* usually resulting from intraspecific hybridization) were only the root system and problems associated with it, it would be easy to suggest interspecific hybridization as a methodology for creating vigorous rootstocks, however the problem goes further. Interspecific hybridization could somehow circumvent problems such as fragile stems and roots, lack of vigorous growth, short production cycle and high susceptibility to diseases. Resolving impasses like these are necessary for the establishment of a more sustainable agriculture and less dependent on pesticides and inputs. The challenge to implement this type of tool is the fertility aspect, which compromises the productivity of these hybrids.

The effect of reciprocal crosses shows relevance within this genus *Capsicum* both in vegetative and biometric aspects of fruits, however with behavior depending on the characteristics to be evaluated and / or the parents involved. Another would be the magnitude of the xenia effect here relative at first hand, its genetic and molecular bases and all its interactions.

Notwithstanding, in our study, even with a small number of species and cultivars, we can observe unique features. Like the normal germination and growth of an interspecific F_1 hybrid with *C. chinense* as mother plant (HAB × JAL), a pungent F_1 hybrid (IKE × BIQ) resulting from the crossing between two non-pungent parents and an interspecific hybrid with productivity equivalent to and reasonable seed production (JAL × BIQ). In practical terms this means that among the enormous genetic variability of cultivars and accessions of *Capsicum*, there are probably possible combinations that minimize problems related to fertility and that result in an appropriate balance between vegetative and reproductive growth.

In addition, we saw that in relation to biometric parameters of fruits, the crossing combinations used here were not efficient in increasing in a heterotic way the size and weight of fruits. This was due to the fact that parents have very contrasting characteristics in these aspects. Changes of this type would in no way compromise the *Capsicum* market, as it is appreciated for its diversity in fruit characteristics, including size and shapes. Besides, what would be the ideal fruit size? The consumer tastes evolve, whereas bigger fruits were previously preferred, more recently smaller fruits (*i.e.* cherry tomatoes or small watermelons) have been sought. Our study lays the groundwork for future developments along these lines.

References

Abu Ngozi E. *et al*. (2019) Evaluation of the parental and hybrid lines-Heterosis and other genetic parameters among *Capsicum annuum* genotypes and their hybrids. J. **Plant Breed. Crop Sci.** 11(1), 11-16.

Aditika *et al*. (2020) Heterotic potential, potence ratio, combining ability and genetic control of quality and yield traits in bell pepper under net-house conditions of NW Himalayas. **Agric Res** 9, 526–535.

Allard R.W. (1960) **Principles of Plant Breeding**. John Wiley & Sons Inc, New York, p: 485

Ashby E. (1932) Studies in the inheritance of physiological characters. I'I. Further experiments upon the basis of hybrid vigour and upon the inheritance of efficiency index and respiration rate in maize. **Ann. Bot**. 46, 1007-1032.

Ávila Silva L. *et al*. (2019a) Nitrogen differentially modulates photosynthesis, carbon allocation and yield related traits in two contrasting *Capsicum chinense* cultivars. **Plant Science**. doi:10.1016/j.plantsci.2019.02.014.

Ávila Silva L. *et al*. (2019b) Source strength modulates fruit set by starch turnover and export of both sucrose and amino acids in pepper. **Plant and Cell Physiology** 60, 10, 2319–2330. doi.org/10.1093/pcp/pcz128.

Barboza G.E. *et al.* (2020) *Capsicum carassense* (Solanaceae), a new species from the Brazilian Atlantic Forest**. Phytokeys** 140: 125-138.

Bianchi P.A. *et al*. (2020) Biomorphological characterization of Brazilian *Capsicum chinense* Jacq. germplasm. **Agronomy** 2020, 10(3), 447; https://doi.org/10.3390/agronomy10030447.

Bogusz S. *et al.* (2017) Brazilian *Capsicum* peppers: capsaicinoid content and antioxidant activity. **Journal of the Science of Food and Agriculture** 98(1), 217–224. doi:10.1002/jsfa.8459.

Bosland P.W. and Baral Jit B. (2007) 'Bhut Jolokia'—The World's Hottest Known Chile Pepper is a Putative Naturally Occurring Interspecific Hybrid. **Hortscience** 42(2):222–224.

Bosland P.W. and Votava E.J. (2012) **Peppers: vegetable and spice capsicums**, Crop production science in horticulture series, 2nd edition Wallingford: CAB International, 230 p.

Brewer M.T. *et al.* (2006) Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. **Plant Physiology** 141(1), 15–25.

Carrizo García C. *et al*. (2013) Wild capsicums: identification and in situ analysis of Brazilian species. In: Lanteri S Rotino GL, eds. **Breakthroughs in the genetics and breeding of *Capsicum* and eggplant**. Turin: Eucarpia, 205–213.

Carrizo García C. *et al*. (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum,* Solanaceae) **Ann. Bot**. 118, pp. 35-51.

Castellón-Martínez É. *et al.* (2012) Consumption preferences of pepper (*Capsicum annuum* L.) landraces in the central valleys of Oaxaca, México. **Revista Fitotecnia Mexicana** 35, 27-35.

Chapa-Oliver A.M. and Mejía-Teniente L. (2016) Capsaicin: From Plants to a Cancer-Suppressing Agent. **Molecules** 21, 931.

Chen Z.J. (2013) Genomic and epigenetic insights into the molecular bases of heterosis. **Nature Reviews Genetics** 14, p. 471–482.

Cruz C.D. (2013) Genes - a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy** 35(3), 271-276.

Darwin C. (1896) **The variation of animals and plants under domestication**, vol. 1. D. Apple-ton, New York. p. 360-361, 427-435.

Denney J.O. (1992) Xenia Includes Metaxenia. **Hortscience** 27(7), 722-728.

do Rêgo E.R. *et al.* (2009) A diallel study of yield components and fruit quality in chilli pepper (*Capsicum baccatum*). **Euphytica** 168 275–287. https://doi.org/10.1007/s10681-009-9947-y

Doganlar S. *et al.* (2000) The genetic basis of seed-weight variation: tomato as a model system. **Theor Appl Genet** 100:1267–1273.

East E.M. and Hayes H.K. (1912) **Heterozygosis in evolution and in plant breeding**. USDA Bull. 243.

Evans L.T. and Fischer R.A. (1999) Yield potential: its definition, measurement, and significance. **Crop Sci**. 39, 1544–1551.

Fabergé A.C. (1936) The physiological consequences of polyploidy. I. Growth and size in the tomato. **Jour. Genet.** 33, 365-382.

Fridman E. (2015) Consequences of hybridization and heterozygosity on plant vigor and phenotypic stability. **Plant Science** 232, 35-40.

Fujimoto R. *et al.*, (2012) Heterosis of Arabidopsis hybrids between C24 and Col is associated with increased photosynthesis capacity. **Proc Natl Acad Sci U S A**. 1,109(18):7109-14. doi: 10.1073/pnas.1204464109.

Gepts P. (2004) Crop domestication as a long-term selection experiment. **Plant Breed. Rev.** 24, 1–44.

Goff S.A. (2011) Unifying theory for general multigenic heterosis: energy efficiency, protein metabolism, and implications for molecular breeding. **The New Phytologist** 189, p. 923–937.

Gómez-García M. del. R. and Ochoa-Alejo N. (2013) Biochemistry and molecular Biology of carotenoid biosynthesis in chili peppers (*Capsicum* spp.). **International Journal of Molecular Sciences** 14(9), 19025–19053.

González-Jara P. *et al.* (2011) Impact of human management on the genetic variation of wild pepper, *Capsicum annuum* var. *glabriusculum*. **PLoS One** 6: e28715.

González-Pérez S. *et al.* (2014) New Insights into *Capsicum* spp. relatedness and the diversification process of *Capsicum annuum* in Spain. **PLoS ONE** 9(12): e116276.

Groszmann M. *et al.* (2011) Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor. **Proc Natl Aca Sci USA** 108, 2617-2622.

Groszmann M. *et al.* (2014) Intraspecific Arabidopsis hybrids show different patterns of heterosis despite the close relatedness of parental genomes. **Plant Physiol** 166, 265-280.

Gupta P.K. and Tsuchiya T. (1991) **Chromosome Engineering in Plants. Genetics, Breeding, Evolution. Amsterdam**: Elsevier, Part A. 639 p. Part B. 630 p.

Gur A. and Zamir D. (2004) Unused natural variation can lift yield barriers in plant breeding. **PLoS Biol** 2(10): e245.

Hedden P. (2003) The genes of the Green Revolution. **Trends Genet** 19, 5-9.

Hoang N.H. *et al*., (2013) Identification and inheritance of a new source of resistance against *Tomato spotted wilt virus* (*TSWV*) in *Capsicum*. **Sci. Hortic**. 161:8–14.

Hundal J.S. and Dhall R.K (2005) Breeding for hybrid hot pepper. **Journal of New Seeds** 6:2-3, 31-50.

Jiang K. *et al.* (2013) Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. **PLoS Genet** 9(12): e1004043. https://doi.org/10.1371/journal.pgen.1004043.

Joshi S. and Berke T. (2005) Perspectives of Bell Pepper Breeding. **Journal of New Seeds** 6(2), 51-74. DOI: 10.1300/J153v06n02_04.

Kim S. *et al*. (2009) Analysis, bioavailability, and potential healthy effects of capsanthin, natural red pigment from *Capsicum* spp. **Food Rev**. Int. 25, 198–213.

Kothari S.L. *et al*. (2010) Chilli peppers - A review on tissue culture and transgenesis. **Biotechnology Advances** 28(1), 35–48.

Krieger U. *et al*. (2010) The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. **Nature Genet**. 42, 459–463.

Kumar A.O. *et al.* (1987) Cytogenetic studies of the hybrids of *Capsicum annuum* with *C. chinense* and *C. baccatum*. **Theoretical and Applied Genetics** 74, 242-246.

Lannes S.D. *et al.* (2007) Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. **Sci Hortic** 112, 266–270.

Lanteri S. and Pickersgill B. (1993) Chromosomal structural changes in *Capsicum annuum* L. and *C. chinense* Jacq. **Euphytica** 67, 155-160.

Li N. and Li Y. (2015) Maternal control of seed size in plants**. J. Exp. Bot**. 66, 1087-1097.

Lippman Z.B. and Zamir D. (2006) Heterosis: revisiting the magic. **TRENDS in Genetics** 23, 2, 60-66

Liu Z. *et al.* (2016) Identification of a *Solanum pennellii* chromosome 4 fruit flavor and nutritional quality-associated metabolite QTL. **Front. Plant** Sci.7:1671.

Lu J *et al*. (2012) Maternal siRNAs as regulators of parental genome imbalance and gene expression in endosperm of Arabidopsis seeds. **Proc. Natl Acad. Sci. USA** 109, 5529-5534.

Lundgren M.R. and Des Marais D.L. (2020). Life history variation as a model for understanding trade-offs in plant–environment interactions. **Current Biology** 30, R180–R189.

MacNeish R.S. (1964) Ancient Mesoamerican civilization. **Science** 143:531-537.

Madhavi R.K. (2010) **Hybrid development for yield and quality**. R. Kumar, A.B. Rai, M. Rai, H.P. Singh (Eds.), Advances in Chilli Research, Indian Institute of Vegetable Research-Studium Press, India (2010), pp. 145-152.

Maillard M.N. *et al*. **(**1997) Analysis of eleven capsaicinoids by reversed-phase high performance liquid chromatography. **Flavour and fragrance journal** 12, 409-413.

Martins K.C. *et al.* (2015) Crossability and evaluation of incompatibility barriers in crosses between *Capsicum* species. **Crop Breed. Appl. Biotechnol**. [online], 15, 3, 139-145.

Matsufuji H. *et al.* (1998) Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). **J. Agric. Food Chem** 46, 3468–3472.

Mazourek M. *et al.* (2009) A dynamic interface for capsaicinoid systems biology. **Plant Physiology** 150, 1806–21.

Melchinger A.E. *et al.* (2007) Genetic basis of heterosis for growth-related traits in Arabidopsis investigated by testcross progenies of near-isogenic lines reveals a significant role of epistasis. **Genetics** 177, 1827–1837.

Meshram L.D. and Mukewar A.M. (1986) Heterosis studies in chilli (*Capsicum annuum* L.) **Scientia Horticulturae** 28, 219-225.

Monteiro C.E.da.S. *et al.* (2011) Reproductive characterization of interspecific hybrids among *Capsicum* species. **Crop Breeding and Applied Biotechnology** 11, 241-249.

Moore T. and Haig D. (1991) Genomic imprinting in mammalian development: A parental tug-of-war. **Trends Genetics** 7, 45–49.

Moses M. and Umaharan P. (2012) Genetic structure and phylogenetic relationships of *Capsicum chinense*. **J. Amer. Soc. Hort. Sci**.137(4), 250-262.

Moyle L.C. and Graham E.B. (2005) Genetics of hybrid incompatibility between *Lycopersicon esculentum* and *L. hirsutum*. **Genetics** 169, 355-373.

Nakamura S. and Hosaka K. (2010) DNA methylation in diploid inbred lines of potatoes and its possible role in the regulation of heterosis. **Theor Appl Genet** 120:205–214.

Nascimento N.F.F. *et al.* (2015) Intraspecific cross-compatibility in ornamental pepper. **Acta Hortic** 1087, 339–344.

Ochoa-Alejo N. and Ramirez-Malagon R. (2001) *In vitro* chili pepper biotechnology. **In Vitro Cell. Dev. Biol.-Plant** 37, 701–729. https://doi.org/10.1007/s11627-001-0121-z.

Offermann S. and Peterhansel C. (2014) Can we learn from heterosis and epigenetics to improve photosynthesis? **Curr Opin Plant Biol**. 19, 105-110.

Palma J.M. *et al.* (2015) Physiology of pepper fruit and the metabolism of antioxidants: chloroplasts, mitochondria and peroxisomes. **Ann Bot.** 116(4):627-636. doi:10.1093/aob/mcv121.

Parvinder S. *et al.* (2014) Heterosis and combining ability for earliness, plant growth, yield and fruit attributes in hot pepper (*Capsicum annuum* L.) involving genetic and cytoplasmic-genetic male sterile lines. **Scientia Horticulturae** 168, 175-188.

Peralta I.P. *et al*., (2008) Taxonomy of wild tomatoes and their relatives (Solanum sect. Lycoersicoides, sect Juglandifolia, sect. Lycopersicon: Solanaceae. **Systematic Botany Monographs** 84: 1-186.

Pickersgill B. (1971) Relationship between weedy and cultivated forms in some species of chilli peppers (genus *Capsicum*). **Evolution** 25: 683–691.

Pickersgill B. (1997) Genetic Resources and Breeding of *Capsicum* spp. **Euphytica** 96, 129-133.

Popova D. and Mihailov L. (1978) Biological basis of the hybrid seed production in peppers (*Capsicum annuum* L.). **Acta Horticulturae** 83, 83–88. doi:10.17660/actahortic.1978.83.11.

Prestes A.M. and Goulart L.R. (1995) Transferência de resistência a doenças de espécies silvestres para espécies cultivadas. **Revisão Anual de Patologia de Plantas** 3, 315-363.

Rao P.G. *et al.* (2017) Heterosis in bell pepper (*Capsicum annuum* l.) for yield and yield attributing traits. **Bangladesh J. Bot** 46(2),745-750.

Raw A. (2000) Foraging behavior of wild bees at hot pepper flowers (*Capsicum annuum*) and its possible influence on cross pollination. **Annals of Botany** 85, 487-492.

Reboud X. and Zeyl C. (1994) Organelle inheritance in plants. **Heredity** 72, 132-140.

Rêgo E.R. *et al*. (2009). A diallel study of yield components and fruit quality in chilli pepper (*Capsicum baccatum*). **Euphytica** 168, 275-287.

Rêgo E.R. *et al.* (2012a) Analysis of diallel cross for some vegetative traits in chili pepper. **Acta Horticuture** 937, 297-304

Rêgo E.R. *et al*. (2012b) **Consumption of pepper in Brazil and its implications on nutrition and health of humans and animals.** In: Peppers: Nutrition, Consumption and Health. New York, Nova Science Publishers. p.159-170.

Robert H.S. (2019) Molecular communication for coordinated seed and fruit development: What can we learn from auxin and sugars? **Int. J. Mol. Sci**. 20:936. doi: 10.3390/ijms20040936.

Rosado-Souza L. *et al.* (2015) Exploring natural variation of photosynthetic, primary metabolism and growth parameters in a large panel of *Capsicum chinense* accessions. **Planta** 242, 677 –691.

Salvador A.R. (2015) **Aspectos metabólicos em frutos de acessos contrastantes de *Capsicum chinense* ao longo do desenvolvimento**.36 f. Dissertação de mestrado em Fisiologia Vegetal, Universidade Federal de Viçosa, Viçosa-MG.

Santos R.M.C. *et al.* (2014) Epistasis and inheritance of plant habit and fruit quality traits in ornamental pepper (*Capsicum annuum* L.). **Genet. Mol. Res.** 13 (4): 8876-8887.

Schnable P.S. and Springer N.M. (2013) Progress toward understanding heterosis in crop plants. **Ann. Rev. Plant Biol.** 64, 71-88.

Seibert A.C. and Pearce R.B. (1993) Growth analysis of weed and crop species with reference to seed weight. **Weed Science** 41:52-56.

Semel Y. *et al.* (2006) Overdominant QTL for yield and fitness in tomato. **Proc. Natl. Acad. Sci. U. S. A.** 103, 12981-12986.

Shifriss C. (1997) Male sterility in pepper (*Capsicum annuum* L.). **Euphytica** 93, 83-88.

Simonne A.H. *et al.* (1997) Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). **Journal of Food Composition and Analysis** 10, 299 – 311.

Singh P. *et al.* (2014) Heterosis and combining ability for earliness, plant growth, yield and fruit attributes in hot pepper (*Capsicum annuum* L.) involving genetic and cytoplasmic-genetic male sterile lines. **Scientia Horticulturae** 168, 175-188.

Spiller F. *et al*., (2008) Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan- and antigen-induced inflammation. **J Pharm Phar- macol**. 60: 473–478.

Subodh J. and Terry B. (2005) Perspectives of bell pepper breeding. **Journal of New Seeds** 6:2-3, 51-74, DOI: 10.1300/J153v06n02_04.

Swingle W.T. (1928) Metaxenia in the date palm possibly a hormone action by the embryo or endosperm. **J. Hered.** 19:257-268.

Tanksley S.D. and Iglesias-Olivas J. (1984) Inheritance and transfer of multiple-flower character from *Capsicum chinense* into *Capsicum annuum*. **Euphytica** 33, 769-777.

Tong N. and Bosland P.W. (2003) Observations on interspecific compatibility and meiotic chromosome behavior of *Capsicum buforum* and *C. lanceolatum*. **Genetic Resources and Crop Evolution** 50, 193–199.

Tripodi P. and Greco B. (2018) Large scale phenotyping provides insight into the diversity of vegetative and reproductive organs in a wide collection of wild and domesticated peppers (*Capsicum* spp.). **Plants** 7, 103. doi:10.3390/plants7040103.

WahyunI Y. *et al.* (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. **Phytochemistry** 72 (11–12), 1358–1370.

Wallace H.M. and Lee L.S. (1999) Pollen source, fruit set and xenia in mandarins. **The Journal of Horticultural Science and Biotechnology** 74, 1, 82-86.

Whaley W.G. (1944) Heterosis. **Bot. Rev**. 10**,** 461–498. https://doi.org/10.1007/BF02861125.

Xiao J. *et al.* (1995) Dominance is the major genetic-basis of heterosis in rice as revealed by QTL analysis using molecular markers. **Genetics** 140, 745–754.

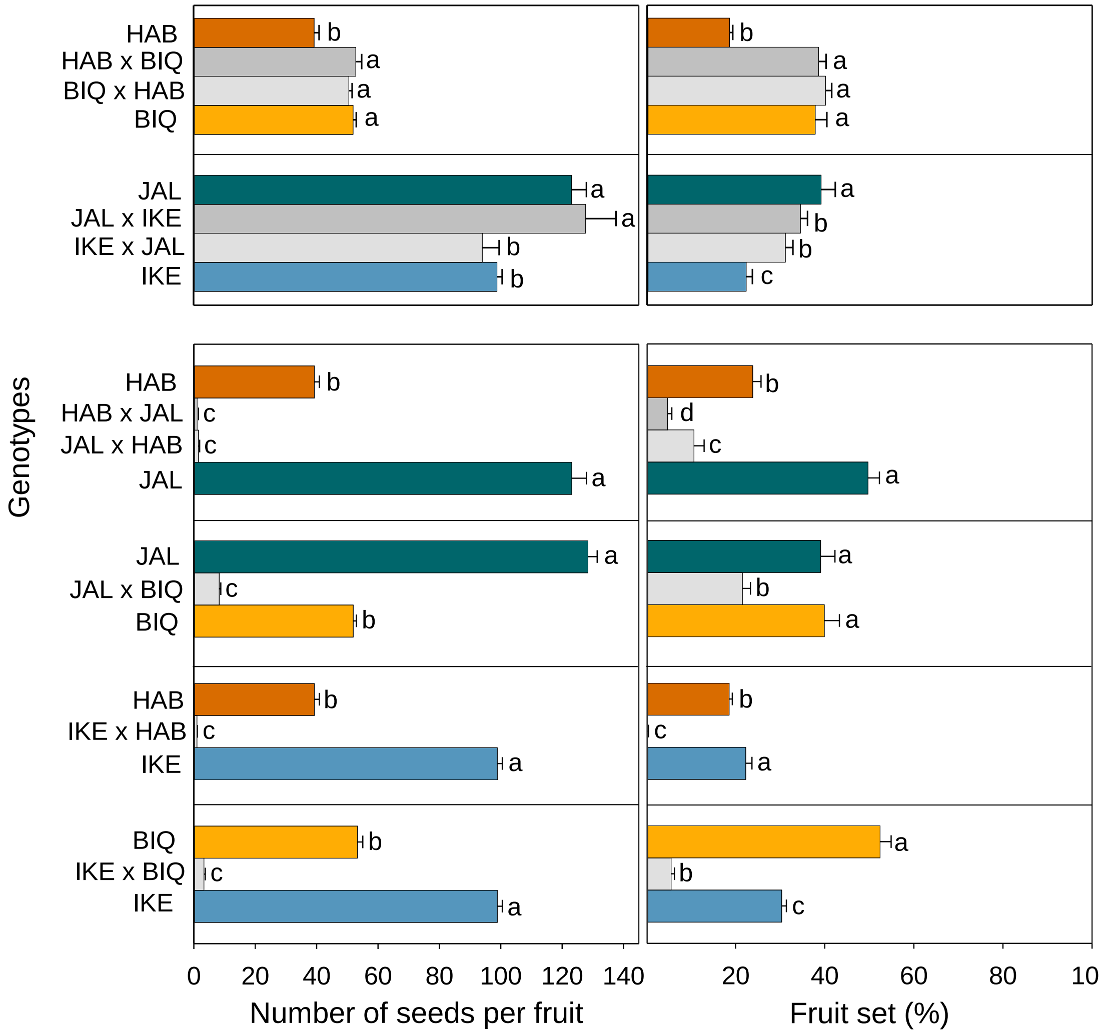
Yu S.B. *et al.* (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. **Proc. Natl. Acad. Sci. USA** 94: 9226.

Zhang C. *et al.* (2019) The genetic basis of inbreeding depression in potato. **Nat Genet** 51, 374–378. https://doi.org/10.1038/s41588-018-0319-1.

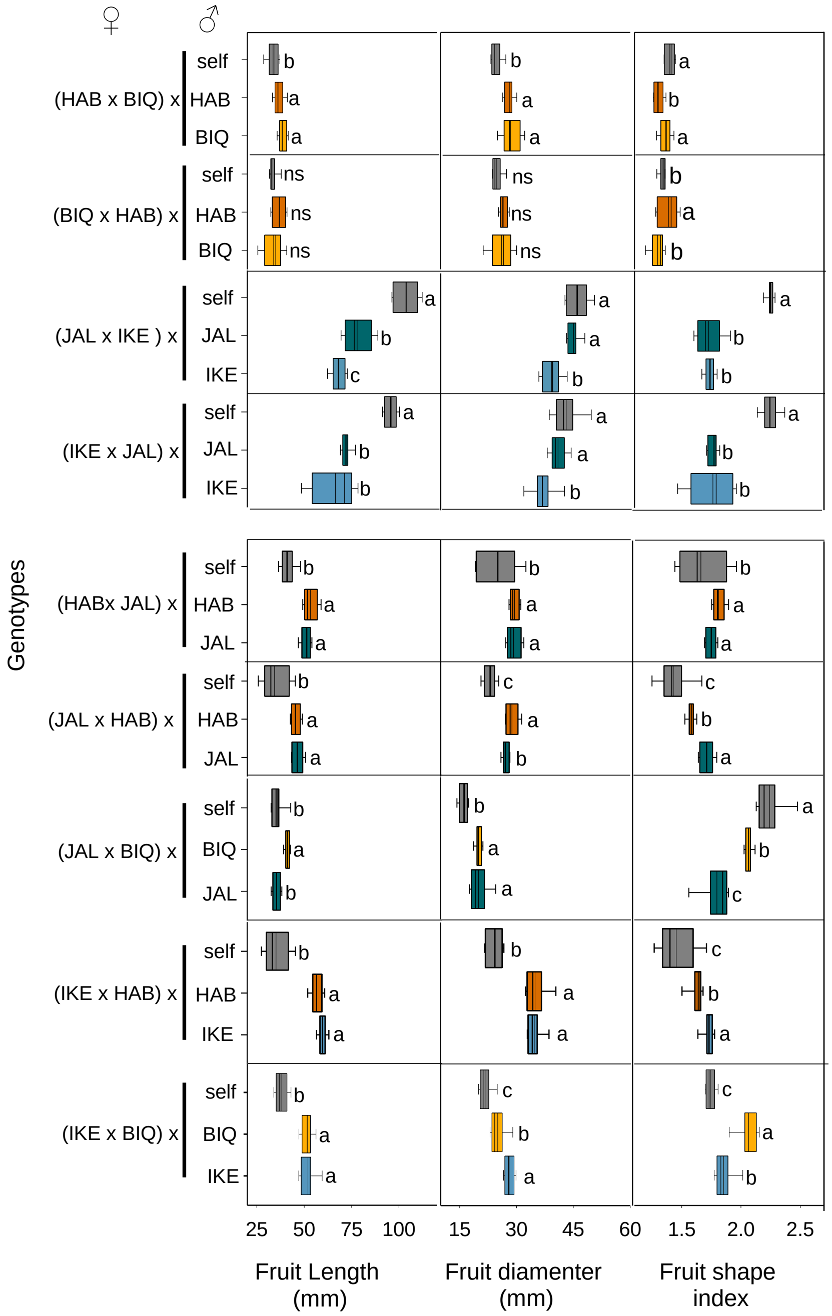
Zsögön A. *et al.* (2017) Genome editing as a tool to achieve the crop ideotype and de novo domestication of wild relatives: case study in tomato. **Plant Science** 256: 120–130.

Zsögön A. *et al.* (2018) De novo domestication of wild tomato using genome editing. **Nature Biotechnology** 36, 1211–1216.

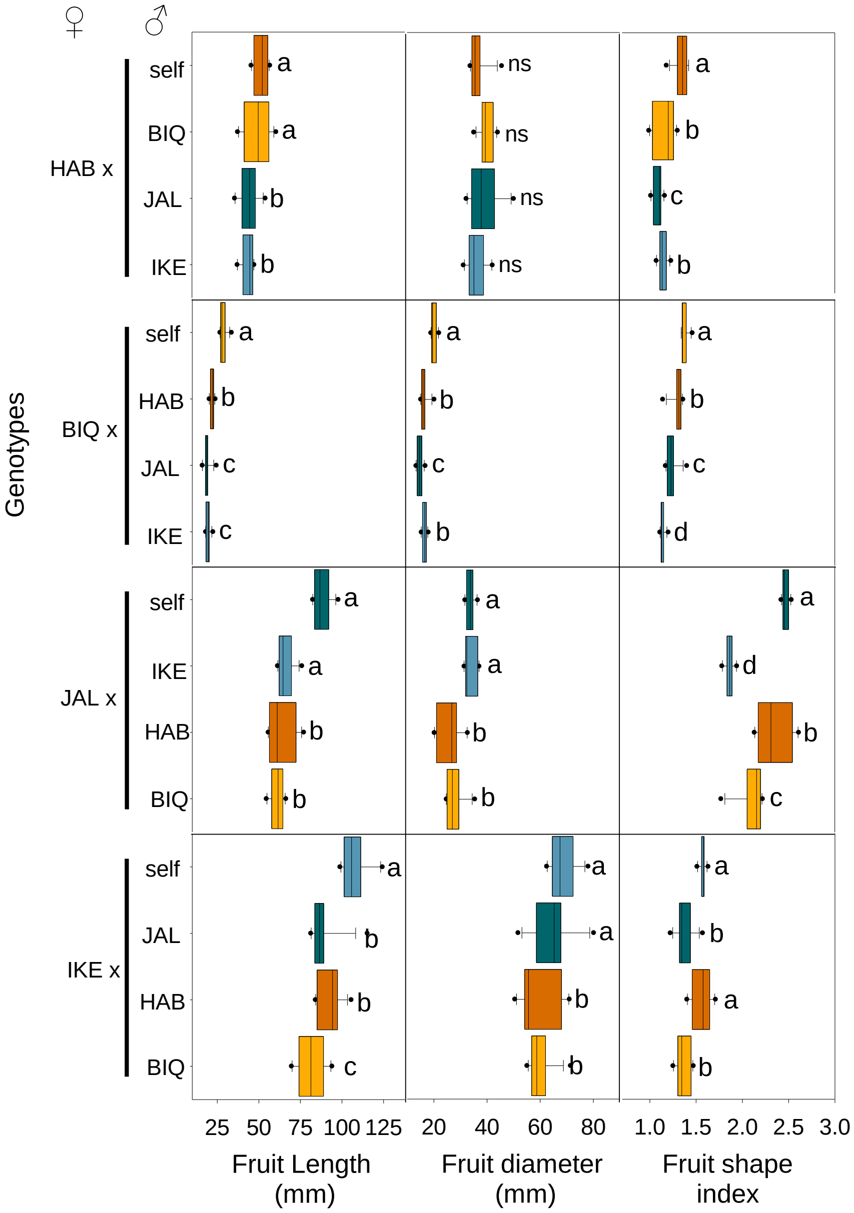
Supplementary material



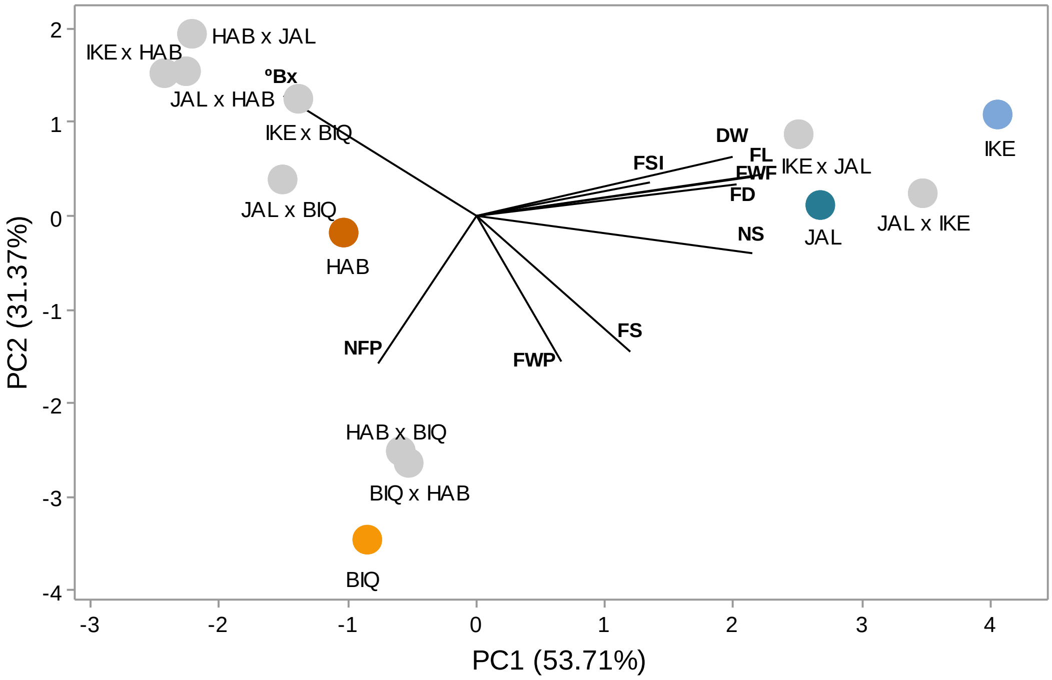
Supplementary Figure 1: Agronomic parameters *Capsicum*'s intraspecific (Top) and interspecific (Bottom) hybrids with their respective parents for the variables Number of seeds per fruit and Fruit set (%). Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.



Supplementary Figure 2: The pollen source acts on biometric parameters such as fruit length (mm), fruit diameter (mm) and fruit shape index on intraspecific (Top) and on interspecific (Bottom) *Capsicum* hybrids. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ); *C. annuum* cv. Jalapeño (JAL), *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.



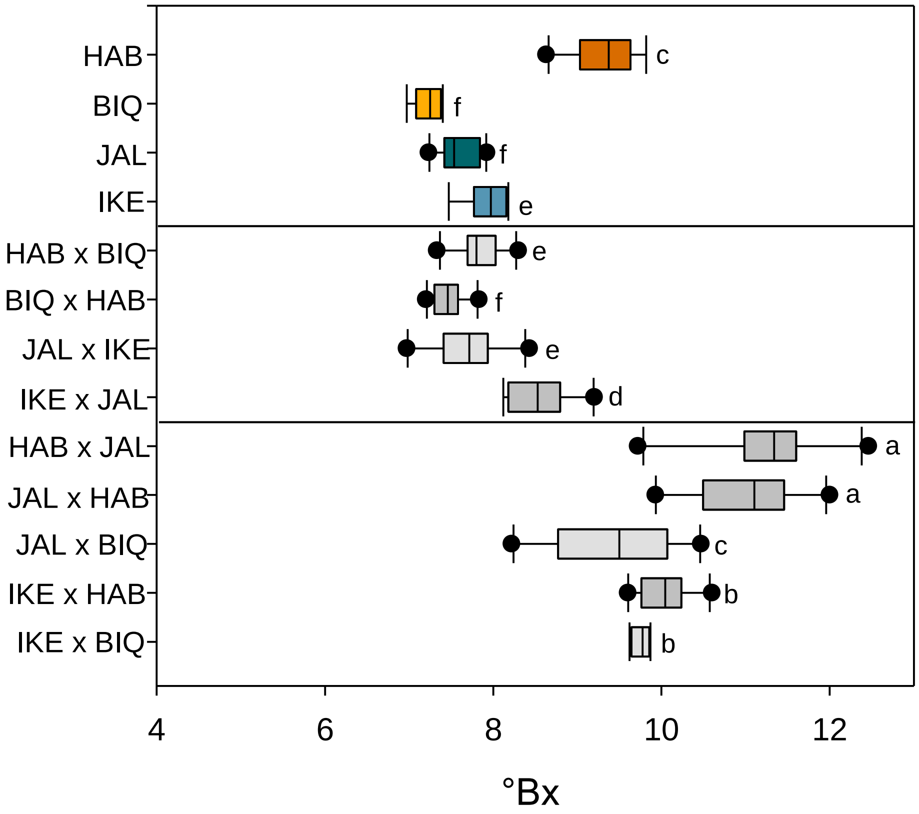
Supplementary Figure 3. The pollen source also acts on biometric parameters of fruits such as fruit length (mm), fruit diameter (mm) and fruit shape index in parental plants. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.



Supplementary Figure 4: Principal component analysis biplot for productive characteristics for intraspecific and interspecific hybrids and parental cultivars of *Capsicum*. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). **°Bx**: Brix; **FWF**: Fresh weight per fruit (g); **DW**: Dry weight per fruit (g); **FL**: Fruit length (mm); **FD**: Fruit diameter (mm); **FSI**: Fruit shape index (Fruit length/ Fruit diameter); **NS**: Number of seeds per fruit; **FS**: Fruit set (%); **FWP**: Fresh weight of fruits per plant (g); **NFP**: Number of fruits per plant.

Supplementary Table 1. Eigenvalues contribution for 4 PCA axes for productive parameters for parents, intra and interspecific hybrids.

Productives parameters	PC1 (53.7%)	PC2 (31.4%)	PC3 (9.6%)
°Brix -°Bx	-0.281	**0.408**	0.073
Fresh weight per fruit - FWF	**0.403**	0.135	0.221
Dry weight per fruit - DW	**0.37**	0.203	0.157
Fruit length - FL	**0.413**	0.138	-0.085
Fruit diameter - FD	**0.376**	0.11	**0.434**
Fruit shape index - FSI	0.252	0.113	**-0.789**
Number of seeds per fruit - NS	**0.401**	-0.129	-0.12
Fruit set - FS	0.224	**-0.46**	-0.163
Fresh weight of fruits per plant -FWP	0.121	**-0.496**	0.25
Number of fruits per plant - NFP	-0.144	**-0.506**	0.009



Supplementary Figure 5. Brix (ºBX) of fruits of parental (Top), intraspecific (middle) and interspecific (Bottom) hybrids of *Capsicum* compared to their parents. Parental plants: *C. chinense* cv. Habanero (HAB), *C. chinense* cv. Biquinho (BIQ), *C. annuum* cv. Jalapeño (JAL) and *C. annuum* cv. Ikeda (IKE). Each box corresponds to an independent experiment submitted to the Scott Knott 5% test.

CONCLUDING REMARKS

Both chapters provide valuable information about the *Capsicum* genus, reinforcing the need to create the model laboratory system. We observed adequate characteristics of cycle, vigor, productivity and prolificacy for two cultivars of *Capsicum annuum*, called 75 and CVO. These cultivars are small in size, allowing them to be grown under conditions of extreme density and root limitation provided by cultivation in a sowing tray or cultivation in small pots. In addition, even under stress these cultivars produced and grew properly without exaggerated responses regarding the cycle, size and parameters of prolificacy. Characteristics associated with fruits in these cultivars such as large size, the presence of pigments such as carotenoids, ripening of fruits probably associated with the intermediate pattern between climacteric and non-climacteric and in addition the presence of secondary metabolism for pungency make these cultivars able to serve as organisms' model in various studies.

The action of heterosis between crosses of commercial cultivars of *C. annuum* and *C. chinense* was demonstrated mainly in vegetative aspects such as accumulation of vegetative biomass, vigorous growth and high plant sizes in interspecific hybrids. We provide enough information to support the fact that germination, fertility, productivity and prolificacy in interspecific hybrids were compromised, and the backcross with pollens from the parents restored characteristics such as fruit set and seed production by fruit. The response of productivity in fresh mass of fruits in intraspecific hybrids shows that heterosis is possible, but depending on the combination between parents. Characteristics involving reciprocity were also found, but with occurrence depending on the measured variable and the genotype involved.

In addition, reciprocal crosses have also been shown to influence some biometric parameters of fruits. In an unprecedented way, we observed the influence of the foreign pollen source on characteristics such as seed size in a mother plant between crosses with parents. In hybrids, especially in interspecific, we can judge that the exogenous pollen source has the ability to alter biometric parameters of fruits. These diverse behaviors found in *Capsicum* need to be better elucidated, in order to understand at the molecular and genetic level how heterosis occurs in *Capsicum* and the different phenomena involved.

Appendix

Publications associated with this thesis:

1. Zsögön A, Čermák, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas D, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. **Nature Biotechnology** 36: 1211-1216

2. Naves ER, Silva LA, Sulpice R, Araújo WL, Nunes-Nesi A, Peres LEP, Zsögön A (2019) Capsaicinoids: pungency beyond Capsicum. **Trends in Plant Science** 24: 109-120