Capsicum—An Abbreviated Compendium

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ABSTRACT. Pepper (Capsicum L.) is a major vegetable and spice crop worldwide. Global production of both fresh and dried fruit continues to increase steadily in terms of area harvested and yield. Various topics are addressed in this review, including recent additions to and clarification of Capsicum taxonomy, genetic resources of Capsicum, cytogenetic studies, the current status of our understanding of the mechanisms affecting the biosynthesis of capsaicinoids, the use of gene mutations to elucidate carotenoid biosynthetic pathways and their regulation, and recent advances in whole-genome sequencing and assembly.
Archaeological data allude to the use of *Capsicum* species (pepper, chilies) as a spice as early as 5500 BCE (Basu and De, 2003; Davenport, 1970). The crop was first introduced into Europe by Christopher Columbus during his travels in the 15th century. It later spread to countries in Africa and Asia by way of the trade routes operating at that time (Andrews, 1992). The fruit was traded initially as black pepper (*Piper nigrum* L.), a species with its own unique form of pungency but otherwise dissimilar in appearance and taxonomically unrelated to *Capsicum* (Gordo et al., 2012). For this reason, cultivated *Capsicum* acquired the common name “pepper.” Early imported cultivars were likely forms of *C. chinense* Jacq. (Scotch Bonnet, Habanero, etc.), which were favored for consumption at that time (Walsh and Hoot, 2001). The flourishing trade routes of Spain and Portugal facilitated the spread of pepper around the globe, where it was quickly adopted (Davenport, 1970) and served as a spice for those parts of the population that could not afford to purchase cinnamon (*Cinnamomum verum* J. Presl), nutmeg (*Myristica fragrans* Houtt.), or other spices that were used for seasoning and/or preserving food (Lauden, 2013).

Asia currently contributes ≈65% of the global production of pepper, whereas the Americas, Europe, Africa, and Oceania each contribute 13.3%, 11.9%, 10.1%, and 0.2%, respectively. The increasing value of the pepper crop coincides with its role in international trade [Table 1 (FAO, 2017)]. However, the genetic variability within the genus *Capsicum* manifests itself in its ability to acclimate and produce a crop in a wide range of environments (Pickersgill, 1997). This is reflected in the number of countries in which it is produced. Fresh pepper is cultivated in ≈126 countries and dried pepper in ≈70 countries [Figs. 1 and 2 (FAO, 2017)]. Although developed countries continue to be the main producers of the pepper crop, its cultivation provides an important source of income for small producers in many developing countries where production is increasing (FAO, 2017). The estimated (global) value of the fresh and dried pepper crops in 2016 was ≈$30 billion and ≈$3.8 billion, respectively (FAO, 2017).

**Taxonomy**

In the last few years, our understanding of the genus *Capsicum* and its member species has increased considerably as a result of the many published studies on the genus’ systematics, karyology, reproductive biology, and phylogeny. In the phylogenetic reconstructions of Solanaceae by Olmstead et al. (2008) and Särkinen et al. (2013), *Capsicum* and its sister taxon *Lycianthes* (Dunal) Hassl. have been recognized as the only members of the tribe Capsiceae (Solanoideae).

Progress has been made in the delimitation of some species, the addition of new species (Baral and Bosland, 2004; Barboza, 2011; Barboza and Bianchetti, 2005; Barboza et al., 2011; Bosland and González, 2000; Carrizo García et al., 2013; Nee et al., 2006), and the clarification of some nomenclatural problems (Barboza, 2011; Knapp et al., 2015). Molecular phylogenetic analyses have contributed to the understanding of species relationships (Jarret and Dang, 2004; Walsh and Hoot, 2001). However, the most comprehensive phylogeny that includes the majority of the currently recognized *Capsicum* species (based on the molecular markers *matK*, *psbA-trnH*, and

| Exporting country | Value (million $) | Production (% world total) |
|-------------------|-------------------|---------------------------|
| Spain             | 1140              | 22.4                      |
| Netherlands       | 1100              | 21.6                      |
| Mexico            | 985               | 19.4                      |
| Canada            | 353               | 6.9                       |
| USA               | 232               | 4.6                       |
| Morocco           | 155               | 3.0                       |
| Israel            | 106               | 2.1                       |
| Turkey            | 96                | 1.9                       |
| South Korea       | 91                | 1.8                       |
| China             | 82                | 1.6                       |

*Workman (2018).
species richness and therefore deserve special comments. The Bolivian, Longidentatum, Atlantic Forest, Purple Corolla, monospecific) are recognized: Andean, Caatinga, Flexuosum, America. These species (Table 2) include native to the Andes of northwestern South America and Central America. These species (Table 2) include Capsicum rhomboideum (Dunal) Kuntze, C. lanceolatum (Greenm.) Morton & Standl., C. geminifolium (Dammer) Hunz. (incl. C. scolnikianum Hunz.), C. dimorphum (Miers) Kuntze, C. lycianthoides Bitter, C. hookerianum (Miers) Kuntze, and another two to three as-yet undescribed species from Peru and Ecuador. Species in this clade are characterized by leaves strongly anisophyllous (Fig. 4A, C, and D), nongenicate pendent flowering pedicels (Fig. 4B), rotate (Fig. 4B) to campanulate (Fig. 4G) or funnel-shaped, yellow to ochre corollas (except in C. lanceolatum), orange–red or red nonpungent fruit (Fig. 4B, E, F, and G), a smooth pericarp (giant cells absent in the innermost pericarp), the presence of stone cells, mostly blackish brown seeds (G.E. Barboza, unpublished data), and by a base chromosome number \( n = x = 13 \) (Moscone et al., 2007; M.A. Scaldaferro, unpublished data). Capsicum lanceolatum is easily recognized by having only one to two axillary flowers, corollas that are white or yellowish–white with purple lines, and found growing only in Mexico and Central America (Table 2). Capsicum rhomboideum (Fig. 4B) has one of the most extensive distributions in the genus (Mexico to Peru) and is morphologically variable in its indumentum (abundance and type of hairs) but with a consistently yellow campanulate corolla and generally more than 10 axillary flowers. Capsicum dimorphum has toothless (or with two to three tiny teeth) calyx (Fig. 4F) in comparison with the three to five long subulate calyx teeth of C. geminifolium and C. lycianthoides (Fig. 4G). These latter three species have a more restrictive distribution (Colombia, Ecuador, and Peru) inhabiting the montane moist forests. Finally, C. hookerianum (Fig. 4E) is very peculiar for its calyx with 10 unequal linear teeth and is the only extra-Andean species in the clade growing in low altitudes and in drier environments. The Andean clade is strongly divergent from the rest of the genus, as evident in the most recent phylogenetic reconstruction (Carrizo García et al., 2016; G.E. Barboza, unpublished data).

**The Atlantic Forest clade.** This clade includes 10 to 11 species endemic to the Brazilian Atlantic Forest, particularly to the coastal rainforests (Table 2): C. campylpodium Sendtn., C. cornutum (Hiern) Hunz., C. friburgense Bianch. & Barboza, C. hunzikerianum Barboza & Bianch., C. mirabile Mart. ex. Sendtn., C. pereirae Barboza & Bianch., C. recurvatum Witasek, C. schottianum Sendtn., C. villosum Sendtn., and one to two undescribed species (G.E. Barboza, unpublished data). These species are shrubs or small trees, characterized mostly by their plagiotropic habitat (Fig. 5A), geniculate pedicels at anthesis (Fig. 5B, D, and E), stellate white corollas with different color spot patterns (Fig. 5D and E), with the exception of C. friburgense (Fig. 5B) greenish–golden or yellow scarcely pungent or nonpungent fruit at maturity (Fig. 5C), alveolate pericarp (giant cells in the innermost pericarp), absence of stone cells, blackish brown seeds (G.E. Barboza, unpublished data), and a base chromosome number of \( n = x = 13 \) (Moscone et al., 2007; Pozzobon et al., 2006; M.A. Scaldaferro, unpublished data). Capsicum friburgense (Fig. 5B) is unique in the group for its campanulate entirely pink or lilac corolla and is confined to a small area in Nova Friburgo (Rio de Janeiro), Brazil. Other species in this group peculiar for their morphology or habitat are C. pereirae, which is the only species with coriaceous leaves and nongeniculate pendent pedicels at anthesis. It inhabits shady areas with high environmental humidity in southeastern Brazil [Carrizo García et al., 2013 (Table 2)]. Capsicum cornutum, C. villosum, and some populations of C. recurvatum (Fig. 5F) share a dense pubescence of long nonglandular trichomes on leaves, pedicels, and calyces, all of them restricted primarily to southeastern Brazil (Table 2). Capsicum recurvatum has 5 to 10 unequal, mainly recurved calyx teeth and stellate corollas with interior greenish
yellow spots, whereas *C. cornutum* has up to 10 long but not recurved calyx teeth, rotate-stellate white corollas with purple or maroon interior spots. *Capsicum villosum* has only five shorter calyx teeth and white corollas with purple pigmentation inside. *Capsicum mirabile* and *C. hunzikerianum* (Fig. 5D and E) are not sympatric species (Table 2) and are characterized by the glabrate indument and the purple spots in the corolla. *Capsicum mirabile* has narrow elliptic leaves and calyces with five teeth, in comparison with *C. hunzikerianum*, where the leaves are ovate and the calyx teeth vary from 6 to 10. *Capsicum campylopodium* and *C. schottianum* have toothless calyces and differ in the corolla pigmentation—the first species with yellow or golden spots in the lobes and limbs and *C. schottianum* with noticeable purple spots to absent.

In regard to the recognized monospecific clades Flexuosum (Fig. 5G and H), Longidentatum, Pubescens, and Tovarii, their placement within the genus is not yet strongly resolved (Carrizo García et al., 2016). The case of *C. pubescens* Ruiz & Pav. is particularly interesting. This species is known only as a cultigen (grown mainly in Bolivia, Peru, and northwestern Argentina) whose origin remains unclear. This and many other issues are subjects to be addressed using recent advances in DNA sequencing and data analysis that permit a broader examination of polymorphic markers across the entire genome (C. Carrizo García, unpublished data).

**Genetic Resources of Capsicum**

Genebanks are viewed as the providers of the raw materials upon which crop improvement activities often depend. Collections of pepper germplasm are available to provide seed of various fruit and plant types and taxa for crop improvement and related research activities. Two of the largest collections of *Capsicum* germplasm are those of the World Vegetable Center (WVC, Tainan, Taiwan) and the U.S. Department of Agriculture (USDA) in Griffin, GA. In addition to the WVC and the USDA, other notable collections are maintained in Costa Rica, Mexico, Brazil, The Netherlands, and Germany (Berke and Engle, 1997; Bettencourt and Kopoka, 1990).

The Asian Vegetable Development and Research Center (AVRDC (now WVC)) recognized *Capsicum* species as one of the center’s principal crop groups in 1986. At the time, the AVRDC collection held 286 accessions of *Capsicum*. In the years following, the collection rapidly expanded in size. *Capsicum* was first introduced into the U.S. National Plant Germplasm System as early as the mid-1930s, and the collection was ultimately assigned to the USDA genebank in Griffin, GA, which became operational in 1949. Maintenance activities at both locations are similar and involve regeneration in the field and greenhouse using pollination control measures. Facilities for cryopreservation are available at the WVC. Backup samples (98%) of accessions in Griffin are stored off-site at the National Laboratory for Genetic Resource Preservation in Fort Collins, CO, where cryopreservation facilities are also available.

The WVC and the USDA currently maintain active collections of 8264 and 4953 accessions, respectively (Table 3). These numbers include those accessions that are currently unclassified. In both instances, more than 99% of the classified materials are domesticated taxa that include *C. annuum* L., *C. baccatum* L., *C. chinense*, *C. frutescens* L., and *C. pubescens*. *Capsicum annuum* is the dominant taxon in both collections. *Capsicum pubescens* is only marginally represented, and crop wild relatives constitute only a fraction of a percent of the total holdings in either collection. Accessions in the WVC and the USDA collections represent materials acquired from more than 100 countries.

Characterization and evaluation data, passport data, and digital images of genebank holdings are available in the Genetic Resources Information Network (GRIN, 2018), and the WVC Genetic Resources Information System (WVC, 2018) databases. Data typically include information such as fruit and plant morphological characteristics, disease and pest resistance/susceptibility characteristics, fruit quality attributes,
photographs of plants or plant parts, etc. The diversity within Capsicum germplasm also has been examined using molecular markers and other methods (Table 4). Many published studies of these types examined a relatively small number of the accessions/populations available from the existing genebank collections, indicating that much remains to be done in terms of fully evaluating/characterizing collections, indicating that much remains to be done in terms of the accessions/populations available from the existing genebank studies of these types examined a relatively small number of markers and other approaches (Table 4). Many published studies of these types examined a relatively small number of the accessions/populations available from the existing genebank collections, indicating that much remains to be done in terms of fully evaluating/characterizing Capsicum genetic resources.

An accurate estimate of the global genetic diversity of Capsicum, or that within individual taxa, remains to be determined.

**Capsicum Cytenogenics**

Polyplody can be induced in Capsicum (Kulkarni and Borse, 2010; Kumar and Raja Rao, 2003; Pal and Ramanujam, 1939; Panda et al., 1984) but is otherwise rare. Pickersgill (1977) and Jha et al. (2012) both described what they believed to be natural tetraploids. Molecular characterization of the cultivar Dalle Khursani, a perennial shrub from West Bengal, India, confirmed it as an allotetraploid rich in GC heterochromatin (Jha et al., 2017). This cultivar differed at the cytomolecular and morphological levels from the diploid species of the C. annuum complex (C. annuum, C. chinense, and C. frutescens), leading the authors to hypothesize that it is a natural interspecific hybrid. Further analysis using fluorescent in situ hybridization (FISH) and genomic in situ hybridization is expected to facilitate the identification of the parental species (Jha et al., 2017).

The most common use of FISH in Capsicum has been in efforts to identify and characterize 5S and 18S-5.8S-26S (45S) rDNA sites, although the location and copy number of other DNA sequences also can be identified using this methodology. Earlier physical mapping of the rDNA sequences in Capsicum species (Kwon and Kim, 2009; Park et al., 1999, 2000; Scaldaferro et al., 2006, 2016) detected chromosome homeologies and indicated a common ancestor of the species in the C. annuum complex, validating the species–complex proposed by Pickersgill (1991) and Zijlstra et al. (1991). FISH also was performed to verify a reciprocal translocation between chromosomes 1 and 8 in C. frutescens (Park et al., 1999, 2000; Scaldaferro et al., 2006, 2016) detected chromosome homeologies and indicated a common ancestor of the species in the C. annuum complex, validating the species–complex proposed by Pickersgill (1991) and Zijlstra et al. (1991). FISH also was performed to verify a reciprocal translocation between chromosomes 1 and 8 in C. frutescens and C. annuum (Park et al., 2014). The number and localization of rDNA sites of some Capsicum species are presented in Table 5. This table also presents genome sizes of 14 Capsicum species as estimated by flow cytometry. Small differences in DNA content were found

### Table 2. Wild relatives of the cultivated Capsicum species, recognized and/or proposed.

| Taxon | Geographic range | Chromosomes (no.) | Clade | Description reference |
|-------|------------------|-------------------|-------|-----------------------|
| C. buforum Hunz. | Br | 13 | Atlantic Forest | Hunziker, 1969 |
| C. caballeroi M. Nee | Bo | ? | Bolivian | Nee et al., 2006 |
| C. campylopodium Sendtn. | Br | 12 | Purple Corolla | Heiser and Smith, 1958 |
| C. cardenasi Heiser & Smith | Bo | 13 | Atlantic Forest | Sendtner, 1846 |
| C. caatingae Barboza & Agra | Br | 12 | Caatinga | Barboza et al., 2011 |
| C. ceratocalyx M. Nee | Bo | ? | Bolivian | Nee et al., 2006 |
| C. chacoense Hunz. | Ar, Bo, Ch, Pa | 12 | Baccatum | Hunziker, 1950 |
| C. coccineum (Rusby) Hunz. | Bo, Pe | ? | Bolivian | Hunziker, 1956 |
| C. cornutum (Hiern) Hunz. | Br | 13 | Atlantic Forest | Hunziker, 1961 |
| C. dimorphum (Miers) Kuntze | Co, Ec, Pe | ? | Andean | Hunziker, 1961 |
| C. eshbaughii Barboza | Bo | ? | Purple Corolla | Barboza, 2011 |
| C. eximium Hunz. | Ar, Bo | 12 | Purple Corolla | Hunziker, 1950 |
| C. flexuosum Sendtn. | Ar, Br, Pa | 12 | Flexuosum | Sendtner, 1846 |
| C. frihurgense Bianch. & Barboza | Br | ? | Atlantic Forest | Barboza and Bianchetti, 2005 |
| C. galapagoense Hunz. | Ec | 12 | Andean | Hunziker, 1956 |
| C. geminifolium (Dammer) Hunz. | Co, Ec, Pe | 13 | Andean | Hunziker, 1956 |
| C. hookerianum (Miers) Kuntze | Ec, Pe | ? | Hunziker, 1956 |
| C. hunzikerianum Barboza & Bianch. | Br | ? | Atlantic Forest | Barboza and Bianchetti, 2005 |
| C. lanceolatum (Greenm.) Morton & Standl. | Gu, Me | 13 | Andean | Morton and Standley, 1940 |
| C. longidentatum Agra & Barboza | Br | 12 | Longidentatum | Barboza et al., 2011 |
| C. lycanthoides Bitter | Col, Ec, Pe | ? | Bitter, 1921 |
| C. minutiflorum (Rusby) Hunz. | Br | ? | Bolivian | Hunziker, 1956 |
| C. mirabile Mart. ex. Sendtn. | Br | 13 | Atlantic Forest | Sendtner, 1846 |
| C. parvifolium Sendtn. | Br, Co, Ve | 12 | Caatinga | Sendtner, 1846 |
| C. pereirae Barboza & Bianch. | Br | 13 | Atlantic Forest | Barboza and Bianchetti, 2005 |
| C. praetermissum Heiser & P.G. Sm. | Br, Pa | 12 | Heiser and Smith, 1958 |
| C. recurvatum Witasek | Br | 13 | Atlantic Forest | Witasek JA, 1910 |
| C. rhomboideum (Dunal) Kuntze | Co, Ec, Me, Gu, Pe, Ho, Ve | 13 | Andean | Kuntze, 1891 |
| C. schottianum Sendtn. | Br | 13 | Atlantic Forest | Sendtner, 1846 |
| C. scolnikianum Hunz. | Ec, Pe | 13 | Andean | Hunziker, 1961 |
| C. tovari Eshbaugh, P.G.Sm. & Nickrent | P | 12 | Tovarii | Eshbaugh et al., 1983 |
| C. villosum Sendtn. | Br | 13 | Atlantic Forest | Sendtner, 1846 |

*Argentina (Ar), Brazil (Br), Bolivia (Bo), Chile (Ch), Colombia (Co), Cuba (Cu), Ecuador (Ec), Guatemala (Gu), Honduras (Ho), Mexico (Me), Paraguay (Pa), Peru (Pe), Venezuela (Ve).

**C. annuum** complex (**C. annuum**, **C. chinense**, and **C. frutescens**), leading the authors to hypothesize that it is a natural interspecific hybrid. Further analysis using fluorescent in situ hybridization (FISH) and genomic in situ hybridization is expected to facilitate the identification of the parental species (Jha et al., 2017).

The most common use of FISH in Capsicum has been in efforts to identify and characterize 5S and 18S-5.8S-26S (45S) rDNA sites, although the location and copy number of other DNA sequences also can be identified using this methodology. Earlier physical mapping of the rDNA sequences in Capsicum species (Kwon and Kim, 2009; Park et al., 1999, 2000; Scaldaferro et al., 2006, 2016) detected chromosome homeologies and indicated a common ancestor of the species in the C. annuum complex, validating the species–complex proposed by Pickersgill (1991) and Zijlstra et al. (1991). FISH also was performed to verify a reciprocal translocation between chromosomes 1 and 8 in C. frutescens and C. annuum (Park et al., 2014). The number and localization of rDNA sites of some Capsicum species are presented in Table 5. This table also presents genome sizes of 14 Capsicum species as estimated by flow cytometry. Small differences in DNA content were found
between analyses (within species). DNA content (2C) values varied from ≈3.91 pg in *C. rhomboideum* to 9.72 pg in *C. pubescens* (Belletti et al., 1998; Moscone et al., 2003). Analysis of DNA content and characterization of 5S and 18S-5.8S-26S (45S) rDNA sites has yet to be conducted with many *Capsicum* species.

*Capsicum* disploidy (i.e., the presence of two basic chromosome numbers in the genus *Capsicum*) is an interesting feature to be more closely examined in relation to genome size evolution and species diversification. Rates of genome size evolution (not strictly genome size) have been found to be positively correlated with diversification rates in angiosperms.
The chromosome number \( x = 12 \) is dominant across all the *Capsicum* clades recognized, whereas the \( x = 13 \) taxa are restricted to two clades (Andean and Atlantic Forest). However, the latter two clades are the more speciose and include almost one-half of *Capsicum* wild species. Genome size is highly variable between *Capsicum* species (Moscone et al., 2003), although information is still lacking for many species/clades (e.g., Andean clade). Qin et al. (2014) determined that more than 81% of the *C. annuum* genome was composed of transposable elements. The activation of transposable elements can generate significant changes in the genome upon which evolutionary forces can work. This is particularly of interest in regard to these elements as promoters of genome size changes in the genus.

Species with 12 chromosome pairs have more symmetrical karyotypes than those with 13 pairs. Some data indicate that the latter group is derived from the former (Moscone et al., 1993, 1995, 1996, 2007; Pickersgill, 1991; Scaldaferrro et al., 2013; Tong and Bosland, 2003). However, the opposite scenario has also been proposed by Pozzobon et al. (2006), who hypothesized that \( x = 13 \) is the basic ancestral chromosome number of the genus and that the reduction in chromosome number is the result of the loss of the small 13th chromosome pair. The origin and fate of the 13th chromosome pair is not known. However, the occurrence of \( 2n = 13 \) taxa in clades separated by \( 2n = 12 \) taxa (Carrizo Garcia et al., 2016) suggests that the extra chromosome(s) arose and/or was lost on more than a single occasion.

It is conceivable that two \( 2n = 26 \) subgroups with asymmetrical karyotypes have arisen via centric fission. The first of these \( 2n = 26 \) groups is composed of *C. lanceolatum* and *C. rhomboideum*, which have smaller genomes (Table 5), single heterochromatic banding patterns, and one nucleolar organizer region (NOR) per haploid complement. The second group contains *C. mirabile* as the core species and also includes *C. campylododium*, *C. cornutum*, *C. friburgense*, *C. perieirae*, *C. recurvatum*, *C. schottianum*, and *C. villosum*. These taxa have larger genomes and rich heterochromatic regions with complex banding patterns. They also contain AT-rich, GC-rich, and moderately GC-rich satellite DNA in addition to one or two NORs. The 13th chromosome pair shows distinctive characteristics among the subgroups. Previous morphological characterization and geographical distribution data of some wild Brazilian species (Barboza and Bianchetti, 2005; Bianchetti, 1996; Bianchetti et al., 1999) support this theory.

**Capsaicinoids: The “Heat”**

Capsaicinoids are compounds unique to the genus *Capsicum* and are responsible for the pungency of pepper fruit (Nelson, 1919). Aside from their value as a spice (Bosland and Votava, 1999), capsaicinoids have well-established medicinal and antimicrobial properties (Emanuela et al., 2015; Khan et al., 2014; Surh et al., 2015). The image shows Atlantic Forest and Flexuosum Clade species. (A) *Capsicum campylododium*. (B) *C. friburgense*, (C) *C. schottianum*. (D, E) *C. hanzikerianum*. (F) *C. recurvatum*. (G, H) *C. flexuosum*. (Puttick et al., 2015).
and Lee, 1995) and affect seed dispersal and survival (Nabhan and Tewsksbury 2001; Tewsksbury et al., 2008). Capsaicinoids are normally synthesized and accumulated in the epidermal cells of the placental tissue of the fruit (Arce-Rodríguez and Ochoa-Alejo, 2015, 2017) but also have been detected in other tissues or organs in some cultivars (Bosland et al., 2015; Noichinda et al., 2016; Tanaka et al., 2017). Advances in the understanding of the molecular biology of the capsaicinoid biosynthetic pathway often have focused on the identification of new candidate genes, the characterization of some key previously identified genes, and the exploration of possible mechanisms of regulation.

In most pungent pepper fruit, capsaicinoids follow a characteristic accumulation pattern in which pungency is detectable at 20 d post-anthesis (DPA), reaches a peak at 30 to 40 DPA, followed by a decrease in fully mature fruit (Arce-Rodríguez and Ochoa-Alejo, 2015, 2017; Barbero et al., 2014). This is also true for genotypes that accumulate nonpungent analogs (Jarret et al., 2014). However, not all genotypes follow this pattern (Barbero et al., 2016; Nagy et al., 2015; Noichinda et al., 2016). The characteristic decrease of capsaicinoids in mature fruit has been attributed to their colocalization with peroxidases, which have a known capacity for degrading capsaicin and dihydrocapsaicin (Ruiz-Lau et al., 2011; Zamudio-Moreno et al., 2014). Alternatively, it has been attributed to the diversion of precursors and intermediaries in the phenylpropanoid pathway that are shared between capsaicinoids and lignins in the fruit (Arce-Rodríguez and Ochoa-Alejo, 2017; Díaz et al., 2004; Estrada et al., 2000). Zhang et al. (2016b) have proposed the most current model of the capsaicinoid biosynthetic pathway that identified intermediary metabolites and enzymatic steps based on both new and previously reported data (Aza-González et al., 2011; Mazourek et al., 2009; Stewart et al., 2005).

RT-qPCR and RNA-seq have revealed the differential expression of many structural genes in Capsicum fruit. The expression patterns (transcript levels) of some genes positively correlated with capsaicinoid content have been reported by Arce-Rodríguez and Ochoa-Alejo (2017), Keyhaninejad et al. (2014), Martínez-López et al. (2014), and Tanaka et al. (2017). The analysis of quantitative trait loci (QTL) also has been useful in identifying candidate genes that participate in the capsaicinoid biosynthetic pathway (Han et al., 2016, 2018). For example, Yarnes et al. (2013) identified 12 QTLs associated with capsaicinoids, six of which had been reported previously (Ben-Chaim et al., 2006). However, none of these studies found the QTL cap, which was proposed to be a regulatory or structural gene in the pathway (Blum et al., 2003). Liu et al. (2013) predicted three new structural enzymes, dihydroxyacid dehydratase, threonine deaminase, and prephenate aminotransferase, based on their RNA-seq analysis of C. frutescens fruit that compared gene expression in pericarp and placental tissues. More recently, Zhang et al. (2016b) identified 20 candidate genes involved in the capsaicinoid biosynthetic pathway using RNA-seq and digital gene expression analysis.

Transcriptome analysis in Capsicum has been used to examine gene expression during plant and fruit development (Li et al., 2016; Martínez-López et al., 2014) as well as

Table 3. Genetic resources of Capsicum species (domesticated, wild, and unclassified) maintained by the World Vegetable Center (WVC, Tainan, Taiwan) and the U.S. Department of Agriculture (USDA, Washington, DC).

| Category          | Taxon                                      | Accessions (no.) |
|-------------------|--------------------------------------------|------------------|
| Domesticated      | C. annuum L.                              | 5489             |
|                   | C. baccatum L.                            | 388              |
|                   | C. chinense Jacq.                         | 505              |
|                   | C. frutescens L.                          | 741              |
|                   | C. pubescens Ruiz & Pav.                 | 30               |
|                   | Subtotal                                  | 7153             |
| Wild              | C. chacoense Heiser & P.G. Sm.            | 25               |
|                   | C. praetermissum                          | 9                |
|                   | Subtotal                                  | 1111             |
| Miscellaneous     | 10                                         | 12               |
| Unclassified      | 1076                                       | 277              |
| Total             | 8264                                       | 4953             |

Table 4. Reports on the characterization and/or evaluation of Capsicum germplasm.

| Principle objective/result                                      | Reference                           |
|----------------------------------------------------------------|-------------------------------------|
| Evaluation of chemical profile and antioxidant activity in C. annuum cultivars | Loizzo et al., 2015                |
| Phenotyping of Capsicum species from Bolivia and Peru           | van Zonneveld et al., 2015          |
| Orthologous analysis of C. annuum cultivars                     | Ahn et al., 2016                    |
| AFLP analysis of 71 Brazilian C. chinense accessions            | Baba et al., 2016                   |
| Variation in vitamin content in Capsicum species                | Kantar et al., 2016                 |
| Profiling of carotenoids in 27 paprika (C. annuum) lines        | Kim et al., 2016a                   |
| Selection of a Capsicum core collection                         | Lee et al., 2016                    |
| Genetic diversity and population structure in C. annuum        | Naegle et al., 2016                 |
| Genome-wide divergence in C. baccatum                          | Nimmakayala et al., 2016            |
| Vitamin C content and reducing sugars in 123 C. baccatum genotypes | Perla et al., 2016                  |
| SSR analysis of 26 landrace-derived inbred lines               | Rivera et al., 2016                 |
| Diversity and population structure of Eritrean pepper          | Saleh et al., 2016                  |
| Screening Capsicum species for pharmacological properties      | Shaimaa et al., 2016                |
| Variation in chemical composition of oleoresin from different cultivars | Sricharoen et al., 2017            |
| SNP discovery and population structure analysis in C. annuum   | Taranto et al., 2016                |
| SSR analysis of 372 C. chinense cultivars and landraces of C. annuum | Zhang et al., 2016a                 |
| Variation in chemical composition of C. annuum from Tunisia    | Lahbibi et al., 2017                |
| Genetic diversity and population structure in C. chinense      | Moreira et al., 2018                |

AFLP = amplified fragment length polymorphism; SSR = simple sequence repeat; SNP = single-nucleotide polymorphism.
capsaicinoid biosynthesis. Kim et al. (2014) and Qin et al. (2014) studied the orthologous genes of the capsaicinoid pathway via transcriptomic analyses to detect differential gene expression in pepper and tomato (Solanum lycopersicum L.). Significant differences in the expression levels of BCAT, Kas, and AT3 were found, indicating that these are key genes in the pathway (Kim et al., 2014). Qin et al. (2014) analyzed the genome of pungent pepper and proposed that as many as 51 gene families were involved in the capsaicinoid biosynthetic pathway. Orthologous genes in tomato, potato (Solanum tuberosum L.), and arabidopsis (Arabidopsis thaliana) were found, indicating that these are key genes in the pathway (Kim et al., 2014). Qin et al. (2014) analyzed the genome of pungent pepper and proposed that as many as 51 gene families were involved in the capsaicinoid biosynthetic pathway. Orthologous genes in tomato, potato (Solanum tuberosum L.), and arabidopsis (Arabidopsis thaliana) L. Heynh.) were also described (Qin et al., 2014).

Few of the many genes reported to be involved in capsaicinoid biosynthesis have been characterized. Virus-induced gene silencing (VIGS) was used to demonstrate the participation of Kas, Cont, pAmt, and AT3 in the pathway (Abraham-Juárez et al., 2008; Arce-Rodríguez and Ochoa-Alejo, 2015; Stewart et al., 2005). The silencing of these genes decreased capsaicinoid production. Gururaj et al. (2012) transformed Nicotiana tabacum L. callus cultures with the pAmt gene and generated transgenic callus lines with the capacity to produce vanillylamine. They also obtained transformed callus cultures of C. frutescens containing a pAmt-antisense binary vector and observed a significant reduction in vanillylamine. The purified pAmt enzyme exhibited the biochemical activity of vanillin transaminase (Weber et al., 2014). Ogawa et al. (2015) generated antiPun1 antibodies and used them to antagonize endogenous AT3 activity. The addition of antiPun1 antibodies to the in vitro assay of de novo capsaicinoid synthesis (using protoplasts from placental tissue of a pungent pepper line) inhibited the synthesis of capsaicin.

A variety of studies have proposed AT3 as a key regulator of the capsaicinoid biosynthetic pathway. Nonfunctional AT3 alleles are responsible for the nonpungency of some pepper cultivars (Stellari et al., 2010; Stewart et al., 2005, 2007). The association mapping of 94 accessions of C. annuum revealed the presence of six single-nucleotide polymorphisms (SNPs) in AT3 that were associated with principle metabolites in the capsaicin pathway (Reddy et al., 2014). In addition, AT3 silencing not only decreased the capsaicinoid content in pungent pepper fruit, it also reduced the expression of other capsaicinoid structural genes (BCAT, Kas, Acl, and pAmt), possibly through a negative regulatory mechanism at the transcriptional level via the accumulation of intermediates or precursors (Arce-Rodríguez and Ochoa-Alejo, 2015). The final product of the capsaicinoid biosynthetic pathway, capsaicin, suppresses the expression of AT3, Pal, Kas, and pAmt genes, indicating negative feedback regulation (Kim et al., 2009).

The loss of function of pAmt is associated with a loss of pungency in pungent pepper fruit. Lang et al. (2009) reported a T insertion in the pAmt gene that produced a stop codon (TGA) that prevents its normal translation in the cultivar CH-19 Sweet. A different pAmt allele, important for its enzymatic activity, was identified in the cultivar Himo, which has a SNP (T → C) substitution in the coding region of the protein that results in one amino acid substitution of cysteine by arginine in the pyrodoxal 5-phosphate binding domain (Tanaka et al., 2010a).

Similarly, in the cultivar Belize Sweet, the presence of a 5-bp insertion (TGGGC) in the pAmt gene led to a frameshift mutation that inhibited capsaicinoid biosynthesis (Tanaka et al., 2010b). Park et al. (2015) reported a 12-bp deletion (TCTGCTGGTCTC) in exon seven, and a SNP in exon 14 of the pAmt gene.
Expression patterns of the transcription factor genes *Erf*, *Jerf*, and *CaMYB31* were positively correlated with the capsaicinoid content and proposed as candidate genes regulating the capsaicinoid biosynthetic pathway (Arce-Rodríguez and Ochoa-Alejo, 2017; Keyhaninejad et al., 2014). In addition, a function study of *CaMYB31* by VIGS showed strong evidence of its participation as a regulator of capsaicinoid biosynthesis. Silencing it caused a reduction in capsaicin and dihydrocapsaicin contents and diminished expression levels of most of the capsaicinoid structural genes in fruit of the cultivar Tampiqueno 74 (Arce-Rodríguez and Ochoa-Alejo, 2017). QTL and genome-wide association studies recently revealed several SNPs in the structural genes *Fat* (acyl-ACP thioesterase), 4CL (4-coumaroyl-CoA ligase), CSE (cafeoyl shikimate esterase), *Ca4H*, and *pAmt*, indicating that they also participate in the regulation of capsaicinoid content (Han et al., 2018).

*Capsicum* genotypes respond differentially to environmental and cultural factors in regard to capsaicinoid biosynthesis (Gurung et al., 2011, 2012; Meckelmann et al., 2015; Wahyuni et al., 2011). Examples are noted in Table 6 and include temperature, light, water stress, nitrogen, and phytohormones. However, some genotypes do appear to show less of a response than others to environmental influences (Gurung et al., 2012).

**Table 6. Environmental factors effecting capsaicinoid biosynthesis/accumulation in *Capsicum* fruit.**

| Effector          | Result                                                                 | Reference                                      |
|-------------------|------------------------------------------------------------------------|------------------------------------------------|
| Temperature       | Positive correlation between elevated temperature and capsaicin content  | Rahman and Inden, 2012                         |
|                   | Negative correlation between temperature and capsaicin content          | Gurung et al., 2012                            |
|                   | *Kas* and *pAmt* display negative response to high temperature         | Arce-Rodríguez and Ochoa-Alejo, 2017          |
| Light             | LED enhances capsaicin content when compared with fluorescent           | Gangadhar et al., 2012                         |
|                   | Expression of *Kas*, *pAmt*, and *CaMYB31* greater in light (vs. dark) | Arce-Rodríguez and Ochoa-Alejo, 2017          |
| Water stress      | Negative effect larger on mildly pungent vs. pungent cultivars          | Ruiz-Lau et al., 2011; Zamudio-Moreno et al., 2014 |
|                   | Water stress increased capsaicinoids in fruit of *C. chinense*          |                                                |
|                   | Activities of enzymes PAL, Ca4H, and AT3 increase under drought conditions | Phimchan et al., 2012, 2014                    |
|                   | Peroxidase enzyme activity decreased under drought conditions           | Zamudio-Moreno et al., 2014                    |
|                   | Differential effect of water stress on *C. chinense* cultivars          | Jeeatid et al., 2018                           |
| Nitrogen          | Nitrate accumulation in fruit positively correlated with increased capsaicinoids | Monforte-González et al., 2010; Rahman and Inden, 2012 |
| Phytohormones     | Capsaicinoid induction (in vitro) requires primary ammonia assimilation | del Ancona-Escalante et al., 2013              |
|                   | Positive effect of salicylic acid, and positive or negative effect of jasmonic acid on the capsaicinoid biosynthetic pathway | Gutiérrez-Carbajal et al., 2010; Altúzar-Molina et al., 2011; Rodas-Junco et al., 2013 |
|                   | GA$_3$ and IAA effect expression of *Kas*, *pAmt* and *CaMYB31*         | Arce-Rodríguez and Ochoa-Alejo, 2017          |

LED = light-emitting diode; PAL = phenylalanine ammonia lyase; GA$_3$ = gibberellic acid; IAA = idole-3-acetic acid.
Table 7. Examples of mutations affecting fruit color in Capsicum.

| Gene                                      | Coloration/cause                                           | Reference            |
|-------------------------------------------|------------------------------------------------------------|----------------------|
| Capsanthin caprorubin synthase<sup>a</sup> | Yellow ripe fruit color/two-point mutations and a tandem repeat. | Li et al., 2013     |
| Phytosynthese<sup>b</sup>                 | Orange ripe fruit color/ccs-3 allele due to premature stop codon. | Rodriguez-Urib et al., 2011 |
|                                           | Orange ripe fruit color/three different SNPs.              | Kim et al., 2017b    |
| Phytoene synthase<sup>c</sup>             | Orange ripe fruit color/point mutation in Psy that generates a frame shift and premature translation in the recessive allele c2. | Kim et al., 2010     |
| B-carotene hydroxylase<sup>d</sup>        | Orange fruit from X-ray–induced mutant (red progenitor)/impaired gene activity due to 3′ terminal region mutation. | Petrov et al., 2013  |
| B-carotene hydroxylase 2<sup>e</sup>      | Orange fruit from EMS-induced mutant (red progenitor)/point mutation leads to B-carotene accumulation. | Borovsky et al., 2013 |
| Arabidopsis pseudo response Regulator2-like<sup>f</sup> | Immature fruit color and color intensity/base change results in a stop codon in white-fruited lines. | Pan et al., 2013     |
| Golden2 ortholog<sup>g</sup>              | Immature fruit chlorophyll content altered/alternation of chloroplast compartment size. | Brand et al., 2014   |

<sup>a</sup>Catalyzes conversion of antheraxanthin and violaxanthin into capsanthin and capsorubin, respectively.
<sup>b</sup>Catalyzes the formation of phytol from prephytolone diphyosphate.
<sup>c</sup>Involves in xanthophyll biosynthesis.
<sup>d</sup>Produces β-carotene and zeaxanthin.
<sup>e</sup>Involves in xanthophyll biosynthesis.
<sup>f</sup>Transcriptional activator; regulates chloroplast development.
SNP = single-nucleotide polymorphism; EMS = ethyl methanethiol.

Caps, other factors, such as normal expression levels of this gene during fruit color development and the existence and normal expression of additional genes such as Psy, lycopene-β-cyclase (Lycb), and β-carotene hydroxylase (CrtZ), are necessary for red coloration (Tian et al., 2015). Kim et al. (2017b) described a Ccs mutant with a nonsense mutation due resulting in a single-base insertion in the coding region. Fruit of this mutant lacked the major carotenoid capsanthin but accumulated β-carotene, lutein, and zeaxanthin in greater concentrations than the normal red-fruited cultivar. Orange peppers carrying the Ccs mutation can be nutritionally superior to other orange-fruited cultivars due to elevated levels of carotenoids (Kim et al., 2017b). Now, only the Ccs’ promoter region has been examined (Gómez-García and Ochoa-Alejo, 2013; Li et al., 2013).

The gene C2 encodes Psy. Psy is a rate-limiting enzyme in the carotenoid biosynthetic pathway (Kim et al., 2010). A point mutation was identified in a recessive allele (c2) in a C. annuum × C. chinensis recombinant inbred line that resulted in both a frame shift and a premature translation termination. This indicated that the orange coloration of the fruit was due to the impaired activity of Psy (Kim et al., 2010).

Orange coloration also can occur as the result of induced mutation. An ethyl methane sulfate–induced, orange-fruited mutant was identified using a red-fruited cultivar as the progenitor. The mutant had a unique pattern of carotenoid accumulation when compared with the wild type in that it accumulated β-carotene. A point mutation was identified in c2 in the orange-fruited mutants. Thus, this gene was considered to be responsible for the orange mutation (Borovsky et al., 2013). An additional orange-fruited induced mutant (of) with elevated levels of β-carotene accumulation was developed via X-ray mutagenesis of dry seeds of a red-fruited genotype. This mutation was later termed the “orange-fruited” trait and was found to be in the 3′-terminal region of CrtZ (Daskalov, 1986; Daskalov and Poulos, 1994; Petrov et al., 2013). This mutation was transferred into several cultivars that subsequently displayed enhanced β-carotene levels at maturity. The elevated levels of β-carotene in the fruit had no detrimental effect on the concentrations of essential minerals (Tomlekova et al., 2017).

Evidence indicates that the pepper ortholog of the tomato AFR2-like gene acts as a regulator of fruit color and intensity. Upon sequencing genes derived from a wild-type and from a white parental line, a single-nucleotide base change resulting in a stop codon was detected. This SNP was associated with both immature fruit color and intensity (Pan et al., 2013).

Multiple gene interactions, in relation to changes in fruit color, were studied in detached fruit using VIGS. The silencing of a single or multiple genes differentially affected fruit color. When Ccs was silenced, the fruit color was yellow due to decreased capsanthin synthesis. Capsanthin synthesis was also reduced in the Psy-silenced fruit, although it was greater when compared with the Ccs-silenced fruit, and the resulting fruit color was orange. Fruit-carrying constructs of TRV-Lycb or TRV-CrtZ showed yellow coloration. When applied together, the resulting fruit phenotype was yellow in all silencing combinations, indicating that yellow and orange coloration are not the result of a single Ccs gene but are also dependent on the interactions of other genes (Tian et al., 2014). All these studies indicated that the regulation of the pathways is more complex than previously proposed.

A VIGS approach was successfully employed in the silencing of the R2R3-MYB transcription factor in Capsicum species (Kim et al., 2017a). Silencing of the MYB also altered MYC and WD40 transcript levels in the CaMYB-silenced leaves. The expression of flavonoid pathway-related genes was also altered in the silenced plants (Zhang et al., 2015). Similar results were found when Tobacco rattle virus constructs were used for VIGS in Capsicum eximium Hunz. When plants were agro-infected.
with TRV2-MYB and TRV2-WD40 constructs, the accumulation of anthocyanins was reduced when compared with the control. This reduction included both the structural and the transcription factor genes. Plants transformed with TVR2-MYB constructs exhibited decreased expression of the structural genes CHS, CHI, F3’5’H, DFR, and 3GT, whereas there was no decrease in the level of F3H. Those infected with the TRV2-WD40 construct displayed reductions in CHS, F3H, F3’5’H, DFR, and 3GT (but not in CHI) in their fruit (Aguilar-Barragán and Ochoa-Alejo, 2014).

Brand et al. (2014) reported two QTLs associated with variations in fruit pigmentation. QTL pc8.1 and pc10.1 both acted to regulate immature fruit chlorophyll content. QTL pc8.1 also affected the carotenoid content of the mature fruit, although its effect was not consistent in subsequent generations. QTL pc8.1 affected the color intensity via an increase in chloroplast compartment size. In addition to chlorophyll, the level of chlorophyll-associated tocopherols and carotenoids also were elevated (Brand et al., 2012). In a later study, the pepper ortholog of the tomato GOLDEN2 gene was found to govern QTL pc10.1, which also affects the natural variation of the immature fruit chlorophyll content by altering chloroplast compartment size (Brand et al., 2014; Kilcrease et al., 2013).

**Capsicum Genomics**

Adoption of advanced technologies in crops that command large funding pools and have small, less-complicated genomes such as maize (Zea mays L.), soybean [Glycine max (L.) Merr.], and rice (Oryza sativa L.) has largely been rapid and straightforward. The rice genome was sequenced to a high quality in 2002 (Goff et al., 2002; Yu et al., 2002), maize in 2009 (Schnable et al., 2009), and soybean in 2010 (Schmutz et al., 2010). In contrast, for small-market specialty crops, particularly those with larger complex genomes such as Capsicum, advances in genomics have lagged behind. Pepper has the largest estimated genome size within the Solanaceae group at 3.25 to 3.48 Gbp (Fig. 6), depending on genotype (Bombarely et al., 2016; Hirakawa et al., 2014; Moscone et al., 2003), although this value is likely to change as the genomes of additional taxa are analyzed.

Over the past few decades, sequencing technologies have both decreased in price as well as increased in sophistication. This allowed for multiple sequencing efforts in pepper to be accomplished, with the first draft genome sequences becoming available in 2014 (Kim et al., 2014; Qin et al., 2014). Two efforts led to the availability of multiple cultivated *C. annuum* draft genomes for the cultivars CM334, Zunla-1, and a wild progenitor [C. annuum var. glabriusculum (Dunal) Heiser & Pickersgill], as well as resequencing of the cultivars Perennial and Dempsey. A draft genome for *C. chinense* also was produced (Kim et al., 2017c). These initial draft genomes used short-read sequencing technologies with multiple insert libraries and generated assemblies with similar statistics (Tables 8 and 9) such as the N50. Initial studies also determined that 76% to 81% of the pepper genome was composed of transposable elements, primarily long terminal repeat elements, and that Gypsy elements were the main cause of genome expansion (Kim et al., 2014). Comparing annotations, all assemblies showed *C. annuum* to have approximately 35,000 genes, which is consistent with the findings in other members of the Solanaceae.

Initial efforts spurred a rapid proliferation of genome-wide analyses to characterize gene families, including some of the efforts described previously (Guo et al., 2015), development of markers for mapping disease resistance and other traits (Cheng et al., 2016; Devran et al., 2015; Han et al., 2018; Hill et al., 2013, 2015; Hulse-Kemp et al., 2016; Kang et al., 2016), and experiments with technologies for reducing the costs associated with the integration of genomics into breeding programs (Taranto et al., 2016). The draft sequences also allowed for the structuring of reference data sets such as the identification of a reference gene set for normalization of future qPCR studies (Cheng et al., 2017). Genes that were identified and implicated in important phenotypes can be validated and targeted for additional studies through genetic transformation and genetic engineering (Cardi et al., 2017). The full application of these studies and their integration moving forward into breeding programs are dependent on the quality of the genome sequences. The studies highlighted the different advantages and shortcomings of the currently available draft sequences (Gapper et al., 2014).

Additional efforts recently have been undertaken to provide a greater-quality version of the *C. annuum* cultivar CM334 genome (Kim et al., 2014), the development of an additional *C. chinense* draft sequence, and a genome of an additional species (*C. baccatum*) using traditional Illumina short-read sequencing.
Complex plant genome using a C. annuum information. This approach was used to test the technology on chromosome end sequencing, which retains inherent physical reads that are fundamentally similar to bacterial artificial (Weisenfeld et al., 2017). This technology produces linked gene R3a et al. (2011) found this to be the case with the known resistance can sometimes be misordered or completely unresolved. Xu traditional short-read sequencing technologies, these regions highly repetitive regions (Ellis et al., 2000). Due to the nature of important genes for disease resistance are typically found in retrotransposons. This emphasizes the importance of resolving the large complex repetitive regions of the genome, as retroduplication and subsequent neofunctionalization of disease resistance genes in the Solanaceae using the greater-quality genome sequences. The authors (Kim et al., 2017c) found that a large portion of the overall genes (≈4% to 10%) appeared to have originated from long terminal repeat retrotransposons. This emphasizes the importance of resolving these regions can sometimes be misordered or completely unresolved. Xu et al. (2011) found this to be the case with the known resistance gene R3a of potato.

To overcome some of the shortfalls associated with traditional short-read sequencing technologies, third-generation sequencing technologies have focused on sequencing longer fragments to attempt to ameliorate difficulties associated with genome assembly and the inherently repetitive regions present in most genomes (Chin et al., 2016). One example is the Chromium Linked-Read technology by 10× genomics (Weisenfeld et al., 2017). This technology produces linked reads that are fundamentally similar to bacterial artificial chromosome end sequencing, which retains inherent physical information. This approach was used to test the technology on a complex plant genome using a C. annuum F1 hybrid (cultivar CM 334 × Bell) (Hulse-Kemp et al., 2018). This approach provided an improvement in contig and scaffold sizes in the assembly when compared with previous efforts using only short-read technologies (Table 8). Although completely phased molecules were not obtained, the technology was able to successfully generate representatives of each of the two parental haplotypes containing important genes with structural differences. This was demonstrated with the PUN1 gene, which is responsible for pungency as discussed previously, where both the serrano-type (cultivar CM 334) and the bell-type representatives of the sequence were obtained.

The recent efforts by Kim et al. (2017c) and Hulse-Kemp et al. (2018) emphasize the need for the continued improvement of the currently available genome resources, as there are inherent shortcomings in each of the used technologies. To move Capsicum genomics forward, a special effort must be directed toward integrating these resources and improving them to ensure that regions of particular interest to breeders, such as disease resistance loci, which are likely to be located in particularly problematic regions of the genome assemblies, are improved and are not omitted from the reference genomes. Breeder use of the reference genomes and associated forthcoming technologies are dependent on the quality of the references available and applicability will only continue to improve with improvement of the resources.

One such resource that has been developed to allow validation across the Solanaceae is the SOL Genomics Network database (Fernandez-Pozo et al., 2015). Although this database provides broad applicability to evolutionary studies, a more thorough and targeted database specifically for pepper has recently been developed, the PepperHub (Liu et al., 2017). This effort is the first step in centralizing Capsicum data with the goal of empowering “omics”-level studies and incorporating multilevel experiments. As multiple genomes and improved versions of genomes are now becoming available in pepper, breeders can start to use genomics-based methods to identify traits of interest. Now and in the future, adequate reference genomes will enable the study of complex biological questions associated with crop improvement and move breeding and related research programs forward.

### Conclusion

Progress has been made in conserving and characterizing genetic diversity in Capsicum, although many taxa remain unrepresented or underrepresented in the available genebanks. No taxon-wide assessment of diversity has yet been undertaken. Field studies supported by molecular and cytological/karyological analyses continue to provide new information that

| Organellar Genome | Taxon | Reference |
|-------------------|-------|-----------|
| Mitochondria      | C. annuum (CMS + normal) | Jo et al., 2014 |

CMS = cytoplasmic male sterile.

| Chloroplast | Taxon | Reference |
|-------------|-------|-----------|
| C. annuum   | Jo et al., 2011 |
| C. annuum var. glabriusculum | Raveendar et al., 2015 |
| C. annuum var. glabriusculum | Zeng et al., 2014 |
| C. baccatum | Kim et al., 2016b |
| C. frutescens | Shim et al., 2016 |
| C. chinense | Park et al., 2016 |
| C. chinense | Raveendar et al., 2017 |

Table 8. Statistics for the available Capsicum draft and reference genomes.

| Species | Line/cultivar | N50 size | Total size (Gb) | Assembled (%) | Version | Reference |
|---------|---------------|----------|----------------|---------------|---------|-----------|
| C. annuum | CM 334       | 2.47     | 3.06           | 88            | 1.0     | Kim et al., 2014 |
| C. annuum | CM334        | 2.08     | 3.06           | 88            | 1.6     | Kim et al., 2017c |
| C. annuum | CM 334       | 2.08     | 3.06           | 88            | 2.0     | Kim et al., 2017c |
| C. chinense | PI 159236   | 3.3      | 3.00           | 94            | 1.2     | Kim et al., 2017c |
| C. baccatum | PBC81       | 2        | 3.20           | 82            | 1.2     | Kim et al., 2017c |
| C. annuum | Zunla        | 1.23     | 3.35           | 103           | 2.0     | Qin et al., 2014 |
| C. annuum var. glabriusculum | 0.45 | 3.48 | 113 | 2.0 | Qin et al., 2014 |
| C. annuum F1 – CM 334 × Bell | 3.69 | 3.21 | 92 | 1.0 | Hulse-Kemp et al., 2018 |

Table 9. Sequenced organelle genomes of Capsicum species with corresponding reference.
advances the understanding of broad taxonomic and genetic relationships. Mechanisms governing the genetic regulation of the pathways involved in capsaicinoid and pigment synthesis remain unclear. Further research targeting regulatory elements and post-transcriptional regulation is needed. Advances in technology have resulted in the current availability of sequenced *Capsicum* genomes. The potential of this information to augment crop improvement activities is substantial, given adequate resources to employ this information and to improve and expand upon it.

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