Rodriguésia 72: e01002020. 2021
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202172062

Original Paper

A reference chromosomal map of the hot chili pepper *Capsicum pubescens* cv. "locoto" (Solanaceae)

Patricia Mabel Aguilera[1,4,6,7], *Humberto Julio Debat*[2,6], *José Guillermo Seijo*[3] & *Mauro Grabiele*[1,5]

Abstract

Capsicum pubescens is a cultivated hot chili pepper, consumed in Latin American cuisine as a distinctive ingredient, and popularly known as "locoto" or "rocoto". This taxon is also an outstanding source of resistance to biotic and abiotic stresses as well as other valuable fruit traits for breeding of the worldwidely cultivated *C. annuum* and related species. In this study, the chromosome complement of *C. pubescens* cv. "locoto" (2n = 24) was deeply characterized through a sequential combination of conventional and molecular cytogenetics approaches comprising: Ag-NOR staining, heterochromatic fluorescent C-DAPI, DAPI/AMD-CMA/DA bandings, fluorescence *in situ* hybridization (FISH) of *Capsicum*-derived probes of the 5S and 18S-25S rRNA genes and different regions of spacers of the ribosomal unit, as well as telomeric probe. The markers identified were systematically combined with morphological karyotype parameters - number, size, centromeres, satellites - to produce a physical map which allowed the identification of several landmarks in each individual chromosome. The reference chromosomal map of *C. pubescens* here presented is the most comprehensively developed in *Capsicum* so far. It is envisioned that this chromosomal map will serve as a reference framework for the upcoming sequencing projects and as starting point to assist future genetic mapping of important agronomic traits.

Key words: Ag-NOR, fluorescent bandings, heterochromatin, locoto, rDNA FISH.

Resumen

Capsicum pubescens es un ají picante cultivado, consumido en Latinoamérica como ingrediente distintivo y conocido como "locoto" o "rocoto". Este taxón es un recurso sobresaliente por sus características de resistencia y tolerancia a estrés biótico/abiótico, y del fruto, que pueden ser introgresadas en programas de mejoramiento de *C. annuum* y especies relacionadas. En este estudio, se caracterizó detalladamente el complemento cromosómico de *C. pubescens* cv. "locoto" (2n = 24) a través de una combinación secuencial de herramientas citogenéticas convencionales y moleculares que incluyeron: tinción Ag-NOR, bandeos heterocromáticos fluorescentes C-DAPI, DAPI/AMD-CMA/DA, e hibridación *in situ* fluorescente (FISH) con sondas de genes de rRNA 5S y 18S-25S y diferentes regiones espaciadoras de la unidad ribosomal derivadas de *Capsicum*, y sonda telomérica. Los marcadores identificados se combinaron sistemáticamente con parámetros morfométricos del cariotipo - número, tamaño, centrómeros, satélites - para producir un mapa físico que permitió la identificación de varios caracteres de referencia en cada cromosoma individual. El mapa cromosómico de referencia de *C. pubescens* aquí presentado es el más completo hasta ahora desarrollado en *Capsicum*. Se prevé que este mapa cromosómico sirva de referencia para próximos proyectos de secuenciación y como punto de partida para asistir al mapeo genético de caracteres agronómicos importantes.

Palabras clave: Ag-NOR, bandeos fluorescentes, heterocromatina, locoto, rDNA FISH.

[1] Universidad Nacional de Misiones, Instituto de Biología Subtropical (UNaM-CONICET) and Instituto de Biotecnología Misiones, Posadas, Misiones, Argentina.

[2] Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias (INTA), Córdoba, Argentina. ORCID: <https://orcid.org/0000-0003-3056-3739>.

[3] Universidad Nacional del Nordeste, Instituto de Botánica del Nordeste (UNNE-CONICET) and Facultad de Ciencias Exactas y Naturales y Agrimensura, Corrientes, Argentina. ORCID: <https://orcid.org/0000-0001-8699-2044>.

[4] ORCID: <https://orcid.org/0000-0002-5063-9350>. [5] ORCID: <https://orcid.org/0000-0001-7386-4924>.

[6] These authors contributed equally to this work.

[7] Author for correspondence: patriciamaguilera@gmail.com

Introduction

Capsicum pubescens Ruiz et Pav. is a domesticated chili pepper found from the Central American highlands in southern Mexico to the Andean region in southern Bolivia (Eshbaugh 1979; Bosland & Votava 2012). This hot chili pepper is extensively grown in small family plots and home gardens (Bosland & Votava 2012) and is only known as a cultivated crop (Moscone *et al.* 2007). It has purple flowers, conspicuous leaf pubescence and black seeds that make it easily differentiable from any other taxa of *Capsicum* (Bosland & Votava 2012).

The highly pungent fruits of *C. pubescens* (30,000–50,000 Scoville heat units, SHUs), combining the juiciness of the bell pepper (non-pungent) with the heat of a habanero (over 100,000 SHUs), are commonly used in fresh sauces (DeWitt & Bosland 2009; Bosland & Votava 2012), and provide a valuable source of several nutritional compounds, principally provitamin A (Rodríguez-Burruezo *et al*. 2010; Rivas *et al*. 2014). *Capsicum pubescens* is a distinctive ingredient of Latin American cuisine, especially at Bolivia and Peru where it is very popular and usually known as "locoto" or "rocoto", respectively (DeWitt & Bosland 2009; Bosland & Votava 2012). Moreover, because of the particular flavour of its fruits, there is an increasing interest and demand, mostly by European speciality markets, of this hot chili pepper (Rodríguez-Burruezo *et al.* 2010).

This *Capsicum* species is an important source of disease resistance in peppers like bacterial spot of pepper (Sahin & Miller 1998) and to wilt disease (Vallejo-Gutiérrez *et al*. 2018). Also, genotypes bearing genes of resistance to tomato spotted wilt virus and to tobamoviruses were revealed in accessions of *C. pubescens* (Di Dato *et al*. 2015). In addition, antixenosis (nonpreference) against green peach aphid *Myzus persicae* infection (Bosland & Ellington 1996) as well as tolerance to waterlogging stress (Ou *et al*. 2011) have been described in this species. Therefore *C. pubescens* arose as an important source of useful genes to overcome the huge economic losses caused by a combination of diseases and abiotic stresses every year in *C. annuum*, the most consumed pepper (Barchenger *et al.* 2019).

Viable and fertile hybrids among *C. pubescens* and *C. annuum* were recently obtained opening new avenues to understand the genetic structure of the desired genetic traits and to develop molecular markers to assist and accelerate the breeding programs (Lindeman & Heidmann 2013; Phan *et al.* 2014). In this sense, the development of molecular and cytological markers and their placement onto an integrated genetic map constitutes a valuable landmark for peppers breeders in order to assist in the simultaneous selection of a particular array of genes/traits.

Essential cytological information has been produced on *C. pubescens* regarding chromosome number, size and morphology, number and position of active nucleolus organizing regions (NORs), genome size, amount, distribution and type of constitutive heterochromatin, and physical mapping of telomeric and ribosomal loci (Moscone *et al*. 1993, 1995, 1996a, 2003, 2007; Aguilera *et al*. 2016; Grabiele *et al*. 2018). However, those findings remain unintegrated in *C. pubescens* and a combined cytogenetic map that gather chromosomal landmarks from a single accession to be used as a framework is not available in this cultivated chili pepper.

In this context, the main goal of this work was to obtain a comprehensive chromosomal map of *C. pubescens* cv. "locoto", through a combination of cytogenetic approaches, allowing the identification of each chromosome and their principal landmarks. It is expected that the integrated chromosomal map developed here will become a physical reference to be integrated with future genetic linkage maps and whole-genome sequence projects of this species.

Materials and Methods

Plant material

Red, turban-shaped and pungent fruits of the domesticated *C. pubescens* Ruiz et Pav. cv. "locoto" coming from Bolivia were acquired at a marketplace in the locality of Salta, Department Capital, Province of Salta, Argentina. Plants from germinated seeds cultivated at the experimental fields of the IPAVE-INTA, in the locality of Córdoba, Argentina, were subjected to taxonomical identification according to diagnostic characters considered by Eshbaugh (1979) and a herbarium specimen was deposited at CORD (Thiers, continuously updated) under accession *E.A. Moscone 256*. The whole plant, flowers, fruits and seeds of this accession are illustrated at Figure 1a-e.

Methods

Cytological preparations

Mitotic pretreatment, fixation of root tips and chromosome preparations for subsequent banding and fluorescence *in situ* hybridization followed the Moscone *et al*. (1996a) protocol.

Ag-NOR staining and Heterochromatic fluorescent banding

Ag-NOR staining to reveal the active nucleolar organizer regions (NORs) in metaphase chromosomes was carried out as described by Stack *et al*. (1991). C-DAPI fluorescent banding to reveal total heterochromatic loci (Het) - number, distribution, size - in metaphase chromosomes was carried out following the C-banding protocol of Schwarzacher *et al.* (1980) with the modification introduced by Lambrou & Ehrendorfer (2000) that includes a final staining with 4–6-diamidino-2-phenylindole (DAPI) instead of Giemsa. In addition, a multiple fluorescent banding to reveal GC and AT rich Het in metaphase chromosomes using the fluorochromes chromomycin A3 (CMA) and distamycin A (DA) preceding to DAPI and actinomycin D (AMD) was performed according to Schweizer & Ambros (1994). CMA fluorescence is enhanced in GC rich Het and it is potenciated when used in combination with DA. DAPI fluorescence is enhanced at AT rich Het. AMD increases the fluorescence of AT rich Het and decreases that of GC rich Het when used in combination with DAPI. The symbols "+", "–" or "o" are used here to designate increased, decreased or indifferent fluorescence, respectively.

Fluorescence in situ hybridization

FISH experiments to reveal telomeric and ribosomal (rDNA) 5S and 18S-25S loci in metaphase or prometaphase chromosomes were undertaken according to Moscone *et al.* (1996b). Probes were labeled by nick translation with digoxigenin-11-dUTP or biotin-11-dUTP following the manufacturer instructions (Enzo, USA). Slides preparations were subjected to RNAse and Proteinase K pretreatments, followed by steps of denaturalization, probe hybridization, blocking, probe detection by means of antibodies linked to fluorochromes [anti-digoxigenin to fluorescein (FITC) and anti-biotin to rhodamine (TRITC)] (Dako, USA), washing, and stained with DAPI alone or with DAPI and AMD. The appearance of DAPI enhanced regions subsequent to FISH procedure and DAPI counterstaining (FISH $DAPI^+$ bands) that mimic the C-banding pattern was described in Moscone *et al.* (1999); AMD in combination to DAPI in FISH was used to decrease the fluorescence of GC rich Het regions.

A probe similar to telomeric sequence of *Arabidopsis* was constructed via PCR amplification using the oligomeric primers $5'TTTAGGG3'_5$ and $5'CCCTAAA3'_5$ described by Ijdo *et al*. (1991). In addition, the 5S rDNA probe used here (Cp5S-3; 297 bp including gene and spacer regions) was obtained via PCR amplification from *C. pubescens* as detailed in Aguilera *et al*. (2016). For a comprehensive analysis of the 18S-25S rDNA loci, severeal genus specific FISH probes *i.e.* Ca25S-29, Cf18S-17, CpITS1,2/5.8S-1, CpIGS-B4rd and CpIGS-A3, that cover the entire 7.8 Kbp length of the 18S-25S rDNA unit in *C. pubescens* (Grabiele *et al.* 2012, 2018) were used. The probes Ca25S-29, Cf18S-17 and CpITS1,2/5.8S-1 target the 25S gene, 18S gene, and the 5.8S gene plus the internal transcribed spacers -ITS1 and ITS2- of the ribosomal unit, respectively. The probe CpIGS-A3 covers the entire length of the A-type IGS in *C. pubescens*, which lacks the transcription initiation site (TIS) and the regulatory elements. Meanwhile, the probe CpIGS-B4rd covers the structural regions SRIII-VI of the B-type IGS in *C. pubescens*, and includes the putative TIS and diverse regulatory elements largely conserved in Solanaceae. The later elements makes this probe partially complementary to CpIGS-A3. The probe pCp-200/33 is a short AT rich mutated version of the IGS rDNA sequence of *C. pubescens* able to discriminate among active-NOR or heterochromatinized loci (Grabiele *et al.* 2018).

Fluorescence microscopy and image acquisition

Chromosomes and nuclei were viewed and photographed with a Leica DMLB fluorescence microscope equipped with a computer assisted digital camera system. Images were captured in black and white using appropriate filters for CMA (GC rich Het), DAPI (total or AT rich Het), FITC (rDNA and telomeres) or TRITC (rDNA) excitations, respectively. Digital images were combined in Photoshop CS6 (Adobe, USA) for final processing.

Karyotype analysis and chromosomal map construction

At least ten metaphase plates CMA stained from three individuals were considered in the chromosome measurements, *i.e.* absolute (μm) and relative (RL%) chromosome length, haploid complement length (HCL; μm). Different lengths of the same arm (and band/locus, where applicable) from homologous chromosomes were combined to mean values and then represented in the idiogram. The centromeric index (C) was used to classify the

chromosomes according to Levan *et al*. (1964) in metacentric (*m*) and subtelocentric (*st*); p and q are used here to designate short and large chromosome arms, respectively. Satellite types were classified according to Battaglia (1955). Karyotype symmetry was calculated according to indexes A_1 and A_2 (Romero Zarco 1986), r > 2 and R (Stebbins 1971), AI (Paszko 2006) and also mean C. Cytological markers revealed by distinct approaches performed in *C. pubescens* cv. "locoto", *i.e.* Ag-NOR staining, C-DAPI, CMA/DA and DAPI/AMD fluorescence banding, FISH of telomeric, 5S, 18S-25S and pCp-200/33 rDNA probes, and DAPI and DAPI/AMD counterstaining subsequent to FISH, were integrated into respective idiograms. Interstitial markers were mapped using the *di* index of Greilhuber & Speta (1976); superscript "i" is used here to designate those markers associated to p or q. Finally, classical karyotype parameters - number, size, centromeres, satellites - and those repetitive loci were gathered in a chromosomal map of the species. Estimation of chromosome length in megabases (Mbp) followed to Pedrosa *et al.* (2002) and Fonseca *et al.* (2010), considering the average 1C genome size of *C. pubescens* (4420 Mbp; Moscone *et al.* 2003) and the HCL of this taxon.

Results

General karyotype features and Ag-NOR pattern

Capsicum pubescens cv. "locoto" accession EAM 256, hereafter *C. pubescens*, is a diploid based on x = 12 chili pepper with 2n = 24 chromosomes. The diploid karyotype formula was 22 *m* + 2 *st* chromosomes. The karyotype was unimodal (A_2 = 0.09; R = 1.41) and symmetrical (A_1 = 0.22; r > 2 = 0.08; mean C = 43.44) and belongs to category 2A of Stebbins and AI = 1.18 of Paszko. The haploid complement length of *C. pubescens* was 81.70 ± 5 µm with median to large chromosomes ranging from 5.50 µm (*st*; 298 Mbp) to 7.75 µm (*m*; 419 Mbp) in length, with a mean of 6.81 µm (ca. 368 Mbp). The short arms of pairs 10 (*m*) and 12 (*st*) had a terminal macrosatellite and active-NORs as revealed by Ag-NOR staining (Fig. 1f).

Heterochromatic patterns revealed by C-DAPI, CMA/DA and DAPI/AMD

Profuse heteropycnosis of chromatin in nucleus evidenced the existence of numerous heterochromatic (Het) regions in the chromosomes of *C. pubescens* (Fig. 1g). Fluorescent banding of metaphase chromosomes revealed C-DAPI$^+$ Het at the centromeres of the whole complement and at six interstitial bands, at p3^i (di = 56.86), q1^i (di = 75.00) and q3^i (di = 68.75) (Fig. 1h). C-DAPI$^+$ distal bands, 16 minor (p2, 4, 7-9, 11; q9, 10) and 24 major loci (p1, 3, 6, 10, 12; q2-6, 8, 11) were also observed. These C-DAPI$^+$ Het included the whole length of macrosatellites and a small fraction (edge of the active-NOR) of the proximal segment of the arm in pairs 10 and 12 (Fig. 1h).

To further characterize the Het regions in the chromosomes of *C. pubescens*, CMA/DA and DAPI/AMD fluorescent bandings were applied. CMA/DA$^+$ fluorescence was observed at all centromeres, and in two interstitial (p3^i) and 38 distal bands (p1-4, 6-12; q2-6, 8, 9, 11). Among the latters, 24 were major bands and included the peri-NOR (with the whole macrosatellite) of pairs 10 and 12 (Fig. 1i). Polymorphisms regarding presence or size of CMA/DA$^+$ Het among homologous chromosomes were observed at p2, q3 and q11. DAPI/AMD$^–$ fluorescence follows an identical pattern to that observed for CMA/DA$^+$ fluorescence, while DAPI/AMD$^+$ heterochromatin was observed at q1^i, q3^i and distally at q10 (Fig. 1j). CMA/DA$^+$ and DAPI/AMD$^–$ fluorescence indicate GC rich regions of chromosomes while DAPI/AMD$^+$ fluorescence revealed AT rich regions. Together, these patterns reproduce the C-DAPI banding pattern (Fig 1h).

Telomeric sequence, 5S and 18S-25S rDNA and Het patterns revealed by FISH

FISH experiments to reveal telomeric sequence distribution in metaphase chromosomes of *C. pubescens* showed the 48 expected distal loci (Fig. 1k). Further, double FISH with 5S and 18S-25S rDNA probes in metaphase chromosomes exposed a single 5S locus at p3 and widespread distal 18S-25S loci. Among the latter, 20 were constant sites (p1, 3, 6, 10, 12; q2, 4-6, 8) and six (p2; q3, 11) were polymorphic regarding presence/ausence (Fig. 1l).

To further characterize the 18S-25S rDNA loci in the metaphase chromosomes of *C. pubescens*, FISH assays were performed with probes corresponding to different parts of the 18S-25S rDNA region followed by DAPI/AMD counterstaining. The probes corresponding to the 18S and 25S rDNA genes co-localized exclusively to the 20 persistent rDNA loci so as to regions

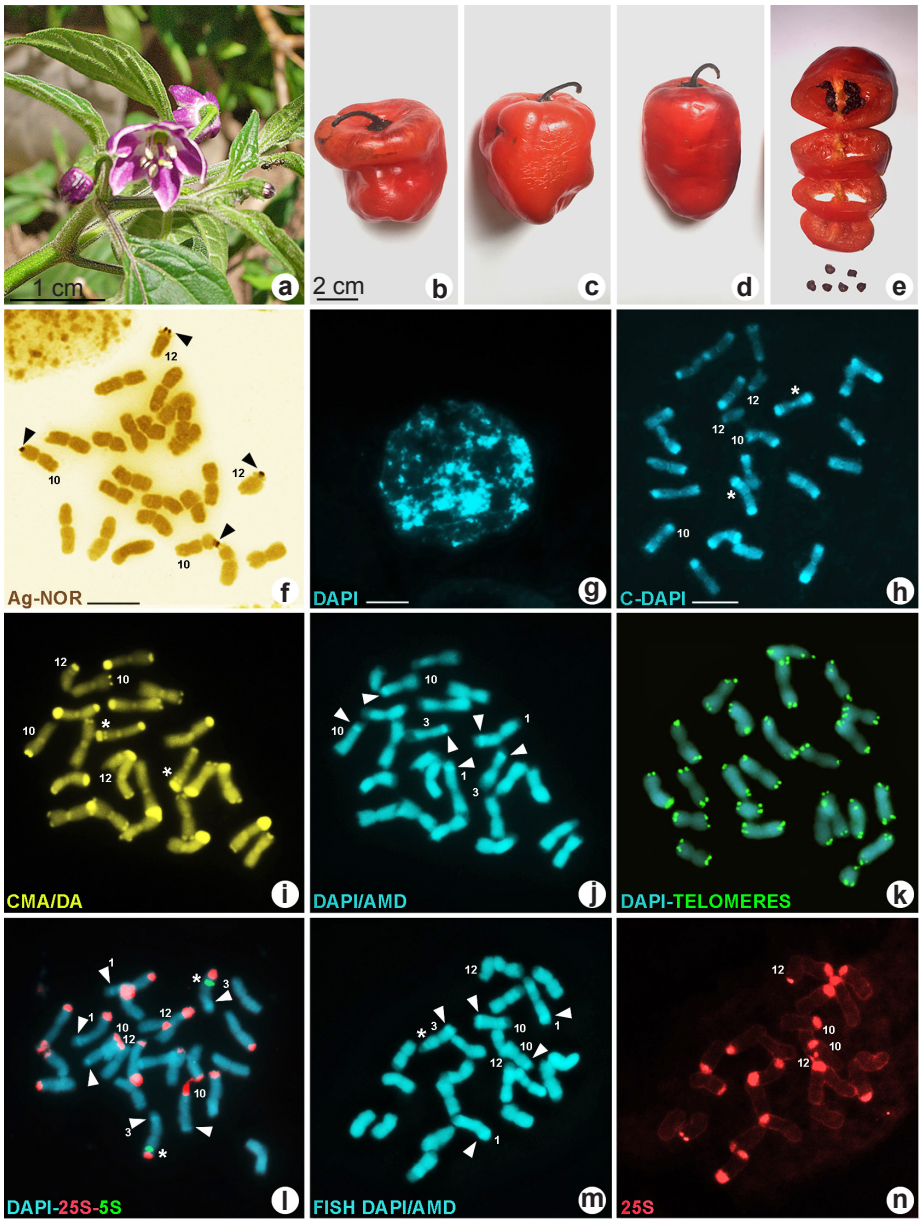

Figure 1 – a-n. General morphological features of the plant and chromosome markers of *Capsicum pubescens* – a. detail of the plant and purple flower; b-e. fruits of cv. locoto rojo and detail of its black seeds; f. Ag-NOR stained metaphase displaying the typical $2n = 24$ (arrowheads point out the satellited active-NOR pairs #10 and 12); g. DAPI stained nucleus showing heteropicnotic chromatin; h. C-DAPI fluorescent banding metaphase; note $DAPI^+$ fluorescence in all the centromeres, in six interstitial and 40 distal bands -24 major-; i. CMA/DA fluorescent banding metaphase (note CMA/DA^+ fluorescence at all the centromeres, in two interstitial and 38 distal bands -24 major-); j. same metaphase stained with DAPI/AMD (note decreased fluorescence at GC rich regions and $DAPI/AMD^+$ bands at q1, q3 and q10, respectively); k. metaphase with FISH of telomeric probe (note 48 green signals at expected sites); l. metaphase with double FISH displaying 20 distal red signals of 25S and two green interstitial signals of 5S rDNA probes (asteriks) [note FISH $DAPI^+$ bands at q1, q3 and q10 (arrowheads)]; m. DAPI/AMD counterstained metaphase subsequent to FISH; n. same metaphase with FISH of 25S rDNA probe. In (m.) note $DAPI/AMD^-$ fluorescence at all the centromeres, one interstitial and 38 distal bands -24 major-, 20 of which co-localize with red rDNA signals in (n.); also note (in m.) $DAPI/AMD^+$ bands at q1, q3 and q10 (arrowheads). Asterisks in h, i and m point out the 5S rDNA locus at p3, C-$DAPI^+$, CMA/DA^+ and $DAPI/AMD^-$, respectively. Scale bars = 10 µm; f, i-n share the scale.

with DAPI/AMD$^-$ fluorescence (Figs. 1m-n; 2a-b). The FISH experiments using CpIGS-A3 rDNA (Fig. 2c), CpIGS-B4rd rDNA (Fig. 2e) and CpITS1,2/5.8S-1 rDNA (Fig. 2f) probes followed by DAPI counterstaining revealed an identical pattern to those observed using 18S and 25S rDNA gene probes. The DAPI$^+$ bands exposed after these FISH experiments showed an identical pattern to that obtained through C-DAPI (Fig. 2d-i).

An additional characterization of the 18S-25S rDNA loci in the chromosomes of *C. pubescens* was achieved through double FISH assays with the 18S rDNA probe and the 18S-25S rDNA IGS related probe pCp-200/33. This combination discriminated active-NORs from inactive-NORs and heterochromatinized rDNA loci. From the 20 distal persistent 18S rDNA loci (p1, 3, 6, 10, 12; q2, 4-6, 8) only 16 were detected with pCp-200/33 probe. No hybridization was detected at the loci of pairs #10 and 12 (Fig. 2j-l).

Combined chromosomal map of *C. pubescens* cv. "locoto"

The type, number and position of each repeated DNA marker found at the chromosomes of *C. pubescens* are shown in Table 1 and depicted into idiograms based on classical cytological landmarks (Fig. 3a-h). Also, all the chromosome markers detected in *C. pubescens* were assembled into a chromosomal map (Fig. 4). According to the whole information here provided, all *C. pubescens* chromosomes have the *Arabidopsis* like telomeric sequence at expected terminal loci. The centromeres of the whole complement are colonized by GC rich Het, detected here as C-DAPI$^+$, CMA/DA$^+$, DAPI/AMD$^-$, FISH DAPI/AMD$^-$ and FISH DAPI$^+$ regions. The most conspicuous feature is the heterochromatinized repeated DNA localized distally in all chromosomes arms (but p5; q1, 7, 12), and interstitially only in two pairs (p3^i; q1^i, q3^i). These repetitive loci could be classified into five different groups: 1) GC rich composed of 18S-25S rDNA that constitute the active-NORs (p10, 12); 2) GC rich Het composed of inactive 18S-25S rDNA (p1-3, 6; q2-6, 8, 11); 3) GC rich Het unrelated to rDNA (p4, 7-9, 11; q9); 4) 5S rDNA (p3^i); and 5) AT rich Het (q1^i, q3^i, q10). The first four types of heterochromatin were characterized as C-DAPI$^+$, CMA/DA$^+$, DAPI/AMD$^-$, FISH DAPI/AMD$^-$ and FISH DAPI$^+$ while the fifth type as C-DAPI$^+$, CMA/DA^o, DAPI/AMD$^+$, FISH DAPI/AMD$^+$ and FISH DAPI$^+$. The proportion of each type of heterochromatinized repeated DNA and their distribution onto chromosomes of *C. pubescens* - which sizes are measured in µm and Mbp - are shown in Table 2. As a whole, the GC rich Het composed of inactive 18S-25S rDNA was by far the major heterochromatic fraction. The Het amount varied from 7.35 to 40.43% of the chromosome size (mean 23.46%) and the mean euchromatin/total Het ratio per chromosome ranged from 1.47 to 12.60 (mean 4.29:1).

Discussion

Molecular cytogenetics can contribute significantly to a genome map through the assignment of linkage groups to physical chromosomes and markers to chromosome arms. Also, by localizing the positions of centromeres on linkage groups, by resolving the order of closely linked markers, and by confirming the physical positions of markers at the ends of linkage groups. Here we present a deep and comprehensive cytological characterization of *C. pubescens* cv. "locoto" through a combined scheme of Ag-NOR staining, heterochromatic fluorescent bandings and FISH to repetitive loci in a single accession of this chili pepper. Chromosome markers were developed mainly on the major tandemly repetitive DNA, in particular those of the large Het regions. Loci information was further combined with morphological karyotype parameters - number, size, centromeres, satellites - to produce the first reference chromosome map for this species.

General karyotype features and heterochromatin

The general karyotype features found here in *C. pubescens* cv. "locoto" are similar to those partially described previously in different reports for materials from Argentina, Bolivia, Colombia, Ecuador and Peru (Limaye & Patil 1989; Pickersgill 1991; Moscone *et al*. 1995, 1996a; Guevara *et al*. 2000). Since Het regions are the most conspicuous characters in the complements of *Capsicum* (Moscone *et al*. 1993, 1996a), in this report the traditional banding methods (Giemsa-C, CMA/DA and DAPI/AMD) were used in combination with novel approaches (as C-DAPI, FISH DAPI/AMD and FISH DAPI) to develop valuable markers for the identification of each chromosome pair in *C. pubescens* cv. "locoto". The banding patterns evidenced the existence of different types of Het regions in this domesticated chili pepper as proposed by Moscone *et al.* (1996a). Hence, highly GC rich Het coexist with AT rich Het; the former

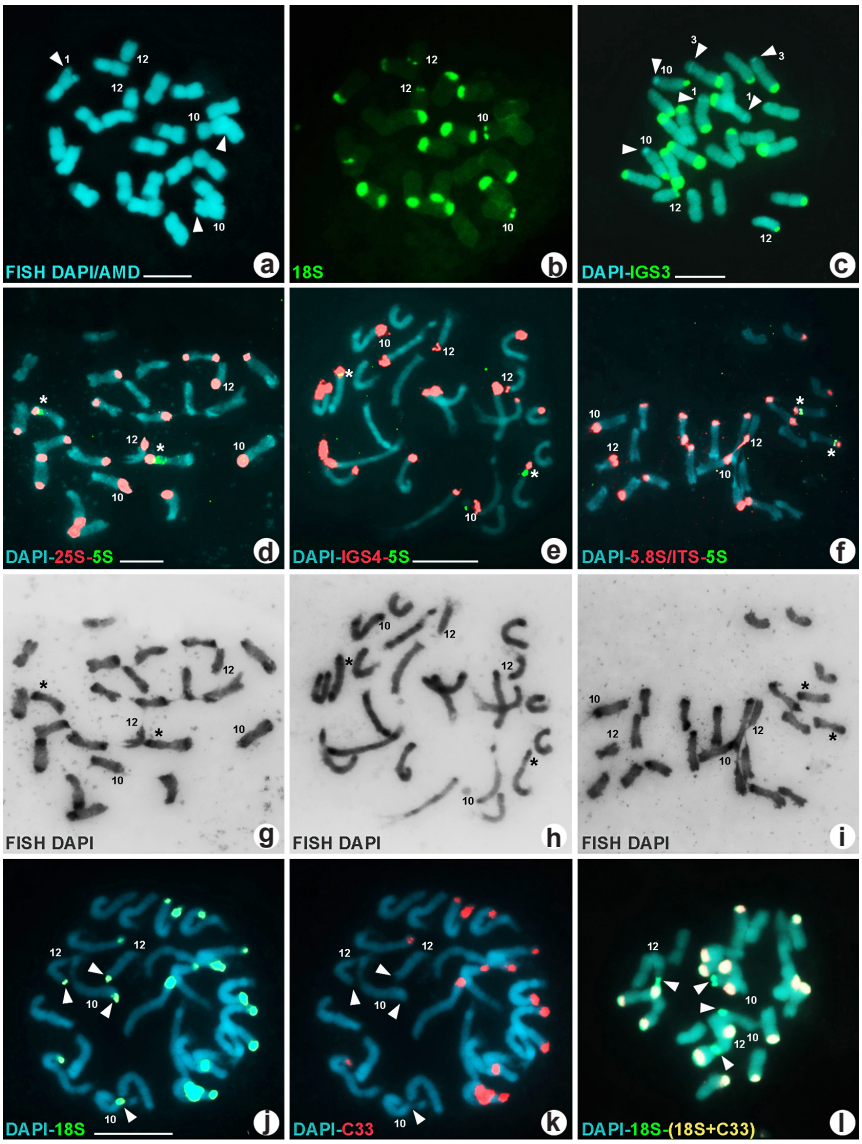

Figure 2 – a-l. Characterization of heterochromatin with homology to 18S-25S rDNA in *Capsicum pubescens* – a-b. DAPI/AMD counterstained metaphase subsequent to FISH of 18S rDNA probe; in (a) note DAPI/AMD$^-$ fluorescence at all the centromeres and to 38 distal bands -24 major-, 20 of which co-localize with green rDNA signals (in b); in addition, note in (a) DAPI/AMD$^+$ bands at q1 and q10, pointed out with arrowheads; active-NOR pairs # 10 and 12 are hereafter indicated with their respectives numbers; c. metaphase with 20 distal green signals of FISH with IGS3 (CpIGS-A3) rDNA probe counterstained with DAPI/AMD; note DAPI/AMD$^+$ bands at q1, q3 and q10 (arrowheads); d-f. double FISH of 5S rDNA probe (green) with 25S (d), IGS4 (CpIGS-B4rd) (e) and 5.8S/ITS (CpITS1,2/5.8S-1); f. rDNA probes (all the laters in red) onto metaphase (d, f) or prometaphase (e) chromosomes counterstained with DAPI; note consistently 20 distal red signals with the three probes analysed, two green interstitial signals (5S rDNA) and several FISH DAPI$^+$ bands; g-i. same metaphases as in d-f, respectively, deprived of rDNA signals and colour-inverted to highligth all the FISH DAPI$^+$ bands that mimic the C-DAPI banding pattern; note that each 18S-25S rDNA signal co-localized with a FISH DAPI$^+$ band; asterisks point out the 5S rDNA locus at p3, FISH DAPI$^+$; j-k. prometaphase with double FISH of 18S rDNA probe (green) and rDNA IGS-related probe pCp-200/33 (C33 in red), respectively; note the co-localization of 16 green and red signals, and the absence of pCp-200/33 in the active-NOR pairs #10 and 12; l. metaphase with double FISH of 18S rDNA (green) and pCp-200/33 (red) probes; note the 16 yellow signals that correspond to superimposed green and red signals of both probes and solely green signals (18S) at the active-NOR pairs #10 and 12. Arrowheads in j-l denote the 18S-25S rDNA loci at the secondary constrictions of pairs #10 and 12. Scale bars = 10 µm; a, b and l share the scale; d, f, g and i share the scale.

Table 1 – Cytogenetic markers identified in *Capsicum pubescens* cv. "locoto". Het = heterochromatinized; p = short chromosome arm; q = long chromosome arm; D = distal; I = interstitial; C = centromeric. Numbers between brackets refer to chromosome pairs. $^{+}$ = increased fluorescence; $^{-}$ = decreased fluorescence; ^{a} = according to idiogram of Fig. 4; ^{b} = sometimes fused to distal band at q3; ^{c} = all the probes of the 18S-25S rDNA assayed except pCp-200/33; ^{d} = polymorphic loci.

Technique	Revealed marker	Number and position of loci[a]
C-DAPI fluorescent banding	Total heterochromatin^{+}	20 D (p1-4, 6-12; q2-6, 8-11); 3 I (q1; p3; q3^{b}); 12 C
CMA/DA fluorescent banding	GC-rich heterochromatin^{+}	19 D (p1-4, 6-12; q2-6, 8, 9, 11); 1 I (p3); 12 C
DAPI/AMD fluorescent banding	GC-rich heterochromatin^{-} AT-rich heterochromatin^{+}	19 D (p1-4, 6-12; q2-6, 8, 9, 11); 1 I (p3); 12 C 1 D (q10); 2 I (q1, 3)
DAPI/AMD subsequent to FISH	GC-rich heterochromatin^{-} AT-rich heterochromatin^{+}	19 D (p1-4, 6-12; q2-6, 8, 9, 11); 1 I (p3); 12 C 1 D (q10); 2 I (q1, 3)
DAPI subsequent to FISH	Total heterochromatin^{+}	20 D (p1-4, 6-12; q2-6, 8-11); 3 I (q1; p3; q3^{b}); 12 C
FISH of telomeric probe	Telomeric sequence	24 D (overall chromosome complement)
Ag-NOR staining	Active-NOR	2 D (p10, 12)
FISH of 18S-25S rDNA probes^{c}	Total 18S-25S rDNA	13 D (p1, 2^{d}, 3, 6, 10, 12; q2, 3^{d}, 4-6, 8, 11^{d})
FISH of pCp-200/33 probe	Het 18S-25S rDNA	11 D (p1, 2^{d}, 3, 6; q2, 3^{d}, 4-6, 8, 11^{d})
FISH of 5S rDNA probe	5S rDNA	1 I (p3)

is ubiquitous at centromeres and distal portions of chromosomes while the latter occurs solely at three interstitial loci. The highly GC rich Het pattern includes the peri-NOR regions and the associated macrosatellite of pairs 10 and 12, as it is usual in plants (Schweizer 1979; Guerra 2000) and, the 5S rDNA locus as well. The detection of GC rich Het at both rDNA loci was expected from its base composition and repetitiveness (Cabral *et al.* 2006; Grabiele *et al*. 2018).

The general Het pattern found here for *C. pubescens* cv. "locoto" is similar to those revealed previously in different contributions using Giemsa-C (Moscone *et al*. 1993) and CMA/DA and DAPI/AMD fluorescent bandings (Moscone *et al*. 1996a) in this species. Minor differences observed are related to the presence of AT rich regions at q1^{i} and q3^{i} and a minute GC rich block at p9, revealed before exclusively as Giemsa-C bands. Polymorphisms regarding presence or size of GC rich Het among homologous chromosomes at p2 and q3 found here confirm the findings of Moscone *et al*. (1996a), while that on q11 is a novelty. Aside of these few polymorphisms, the type, number, distribution and size of Het regions are constant features in the karyotype of this domesticated chili pepper. Thus, the integrative study done here would be applicable for accessions of *C. pubescens* from different procedences.

Distribution of telomeric and 5S rDNA loci

FISH to telomeric sequence showed a typical pattern of chromosome end distribution without ectopic localizations. This pattern is the one commonly observed in different *Capsicum* species despite their karyotypes were based on x = 12 or x = 13 (Moscone *et al*. 2007).

The observation of a single interstitial locus of 5S rDNA using a species specific probe is in agreement with previous findings in *C. pubescens* (Park *et al*. 1999; Scaldaferro *et al*. 2006) and other *Capsicum* taxa using genus specific (Aguilera *et al*. 2016) or heterologous probes (Scaldaferro *et al*. 2016). The difference in localization of the 5S locus - in this report at p3 instead of at pair 1 as was reported before (Park *et al.* 1999) - arose in the fact that the latter author used the tomato karyotype as a reference to order the chromosomes bearing rDNA loci in *Capsicum*, while here the order was established following exclusively the cytological parameters of *C. pubescens*. The chromosome

association of the 5S rDNA and 18S-25S rDNA loci observed in *C. pubescens* is a common feature to several plants (Roa & Guerra 2015).

18S-25S rDNA, NORs and Het associated patterns

The most conspicuous cytological marker in *C. pubescens* is the widespread distibution of rDNA loci featured as the major heterochromatic fraction of the complement. Understanding the complexity of 18S-25S rDNA regions detected in this species required an exhaustive analysis using different combinations of specific *Capsicum* probes that contained different parts of the rDNA sequence (Grabiele *et al.* 2018). This approach revealed that all those GC rich heterochromatic bands observed in *C. pubescens* were composed of repetitions of the whole rDNA unit. Noteworthy, the hybridization of pCp-200/33 probe to all 18S-25S rDNA loci except those at the active-NORs and their associated macrosatellites of pairs 10 and 12, revealed a structural divergence between these two types of 18S-25S rDNA in *C. pubescens*. Particularly, this is due to a 20% of nucleotide sequence difference among pCp-200/33 and its homologous region of the functional IGS (Grabiele *et al.* 2018).

Thus, the mapping of probes comprising different 18S-25S rDNA structural portions in this work and Grabiele *et al.* (2018) significantly increased the power of discrimination of chromosomes in *C. pubescens* when compared with previous reports using the wheat pTa71 probe (Park *et al*. 1999) or the *Arabidopsis* R2 probe (Scaldaferro *et al*. 2006).

Combined chromosomal map of *C. pubescens* cv. "locoto"

Variation in the spatial and quantitative distribution of the repeated DNA combined to mapping information provided by DAPI and DAPI/AMD counterstainings, C-DAPI, DAPI/AMD and CMA/DA Het bandings and Ag-NOR staining along with other morphological characteristics (*i.e.* length and arm length ratio), allowed the

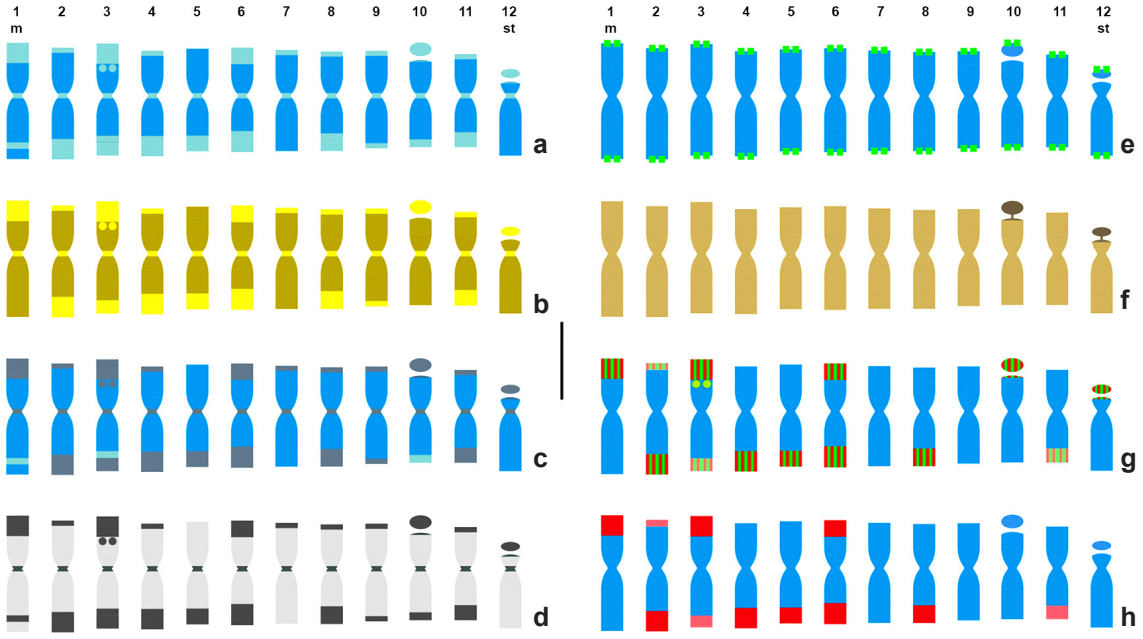

Figure 3 – a-h. Idiograms for each distinct cytological approach performed in *Capsicum pubescens* – a. C-DAPI fluorescence banding (DAPI$^+$: light blue blocks); b. CMA/DA fluorescence banding (CMA/DA$^+$: bright yellow blocks); c. DAPI/AMD fluorescence banding and DAPI/AMD counterstaining subsequent to FISH (DAPI/AMD$^-$: dark grey blocks; DAPI/AMD$^+$: light blue blocks); d. DAPI counterstaining subsequent to FISH (DAPI$^+$: dark blocks); e. FISH of telomeric probe (green dots); f. Ag-NOR staining (active-NOR: dark brown blocks); g. double FISH of 5S rDNA probe (green dots) and all the probes of the 18S-25S rDNA (red-green barred blocks) assayed except pCp-200/33; h. FISH of pCp-200/33 probe (red blocks). Polymorphic 18S-25S and pCp-200/33 rDNA loci at p2, q3 and q11 are coloured in a paler tone. Scale bar = 5 µm.

identification of each of the 12 mitotic metaphase chromosomes of *C. pubescens*. cv. "locoto". Despite pivotal chromosomal information was previuosly produced in *Capsicum* regarding active-NORs, Het and ribosomal loci (Moscone *et al.* 2007; Scaldaferro *et al.* 2013; Grabiele *et al.* 2018; Scaldaferro & Moscone 2019), the data was dispersed and remained unintegrated. The diversity and resolution of markers employed here, and the integration of repetitive loci and classical cytological landmarks generated the most complete chromosome map done for chili peppers to present.

Somatic chromosome karyotypes provide a physical basis for analysis and comparison of genomes (Chowdhary *et al.* 1998; Fonseca *et al.* 2010). The physical map of repetitive loci and classical landmarks of *C. pubescens* cv. "locoto" presented here is based on uniformly condensed mitotic metaphase chromosomes. These framework maps reach the double purpose to precisely

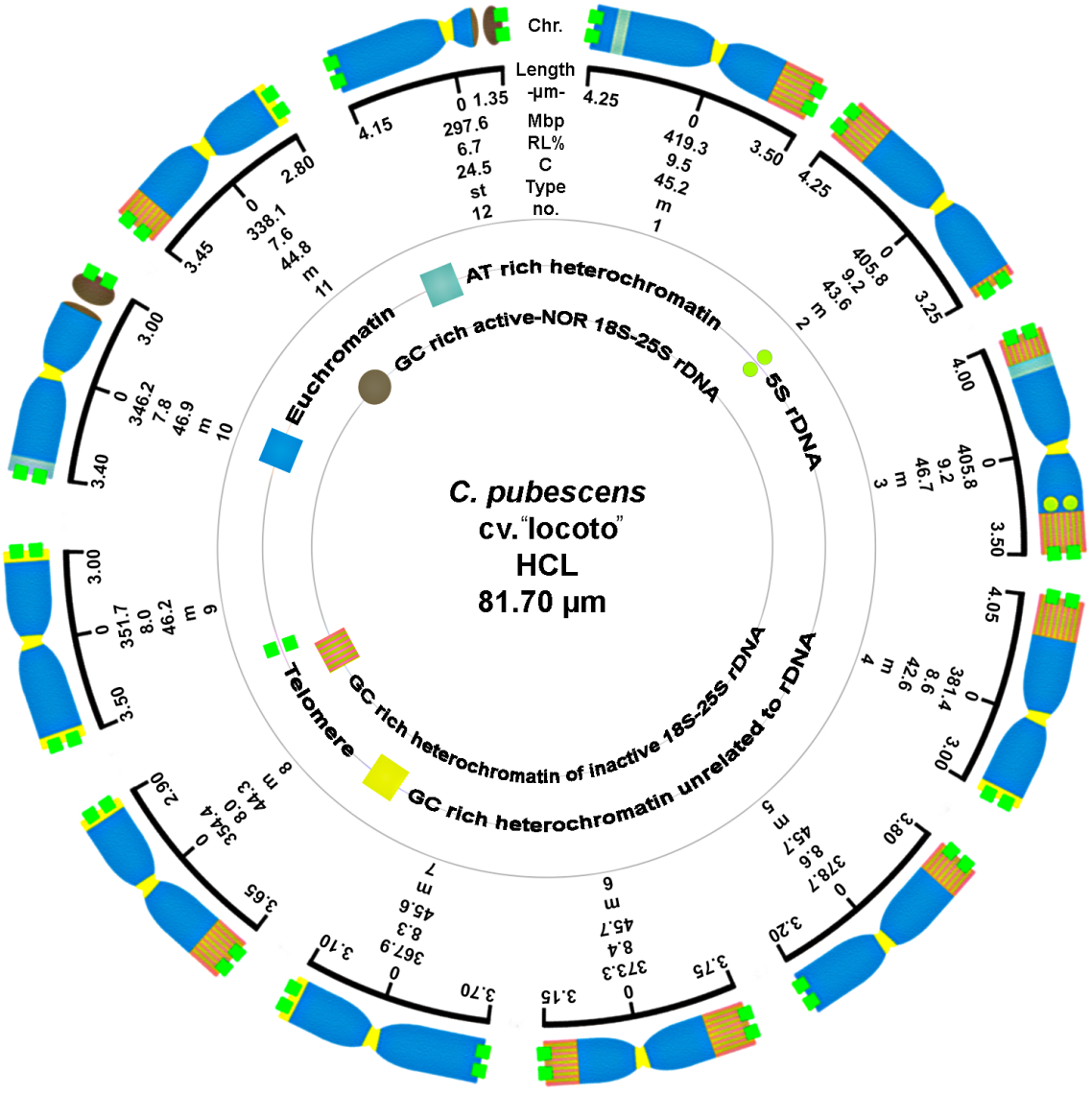

Figure 4 – Combined chromosomal map of repetitive loci and classical cytological landmarks in *Capsicum pubescens*. Short chromosome arms are clockwise oriented. HCL = haploid complement length; RL% = relative length of chromosomes expressed as percentage; C = centromeric index. Size of each chromosome length (centromere-telomere) is expressed in µm. Estimated whole chromosome length is expressed in Mbp.

Table 2 – Heterochromatin amount, type and distribution in somatic chromosomes of *Capsicum pubescens* cv. "locoto". Chr = chromosome; Het = heterochromatin; a = values are expressed as percentage of the chromosome size; b = includes all types of Het detected with different fluorochromes.

Chr	Size µm	Size Mbp	GC rich active-NOR 18S-25S rDNA[a]	GC rich Het of inactive 18S-25S rDNA[a]	GC rich Het unrelated to rDNA[a]	5S rDNA[a]	AT rich Het[a]	Total Het[ab]	Euchromatin/ Het[b] ratio
1	7.75	419.28	-	17.68	-	-	3.87	24.77	3.04
2	7.50	405.75	-	23.60	-	-	-	26.93	2.71
3	7.50	405.75	-	27.20	-	3.33	4.00	37.87	1.64
4	7.05	381.41	-	19.72	3.55	-	-	26.81	2.73
5	7.00	378.70	-	13.71	-	-	-	17.29	4.79
6	6.90	373.29	-	36.81	-	-	-	40.43	1.47
7	6.80	367.88	-	-	3.68	-	-	7.35	12.60
8	6.55	354.36	-	18.93	3.82	-	-	26.56	2.76
9	6.50	351.65	-	-	7.69	-	-	11.54	7.67
10	6.40	346.24	12.97	-	-	-	5.47	22.34	3.48
11	6.25	338.13	-	16.00	4.00	-	-	24.00	3.17
12	5.50	297.55	11.09	-	-	-	-	15.64	5.40

localize markers and estimate chromosome length in megabases, allowing to correlate cytological information with genetic linkage groups and genome sequences up to a chromosome-scale organization, overcoming the repetitive loci issues (Pedrosa *et al.* 2002; Fonseca *et al.* 2010; Belser *et al.* 2018). Despite high-density genetic maps and whole-genome sequences projects were done in few domesticated *Capsicum* species (Wu *et al.* 2009; Wu & Tanksley 2010; Moulin *et al.* 2015; Kim *et al.* 2014, 2017; Qin *et al.* 2014; Ahn *et al.* 2018), such initiatives in *C. pubescens* are still awaited. The chromosomal map presented here will serve as a reference framework for the upcoming sequencing projects and a starting point to assist future genetic mapping studies necessary to the design of breeding strategies in this important chili pepper.

Acknowledgements

This study was funded by the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-Argentina), UNaM PICT 2014-3328 Préstamo BID Nº AR-L 1181. FISH experiments were done at Instituto de Botánica del Nordeste (IBONE-UNNE-CONICET) with the finantial support of PICT 2012 1875 (ANPCyT-Argentina). This contribution is dedicated to the memory of PhD Daniel A. Ducasse.

References

Aguilera PM, Debat HJ, Scaldaferro MA, Martí DA & Grabiele M (2016) FISH-mapping of the 5S rDNA locus in chili peppers (*Capsicum*-Solanaceae). Anais da Academia Brasileira de Ciências 88: 117-125. DOI: 10.1590/0001-37652301620140616

Ahn Y-K, Manivannan A, Karna S, Jun T-H, Yang E-Y, Choi S, Kim J-H, Kim D-S & Lee ES (2018) Whole genome resequencing of *Capsicum baccatum* and *Capsicum annuum* to discover single nucleotide polymorphism related to powdery mildew resistance. Scientific Reports 8: 5188. DOI: 10.1038/s41598-018-23279-5

Barchenger DW, Naresh P & Kumar S (2019) Genetic resources of *Capsicum. In*: Ramchiary N & Kole C (eds.) The *Capsicum* genome. Springer, Cham. Pp. 9-23. DOI: 10.1007/978-3-319-97217-6

Battaglia E (1955) Chromosome morphology and terminology. Caryologia 8: 179-187. DOI: 10.1080/00087114.1955.10797556

Belser C, Istace B, Denis E, Dubarry M, Baurens FC, Falentin C, Genete M, Berrabah W, Chèvre AM, Delourme R, Deniot G, Denoeud F, Duffé P, Engelen

S, Lemainque A, Manzanares-Dauleux M, Martin G, Morice J, Noel B, Vekemans X, D'Hont A, Rousseau-Gueutin M, Barbe V, Cruaud C, Wincker P & Aury JM (2018) Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps. Nature Plants 4: 879-887. DOI: 10.1038/s41477-018-0289-4

Bosland PW & Ellington JJ (1996) Comparison of *Capsicum annuum* and *C. pubescens* for antixenosis as a means of aphid resistance. Hortscience 31: 1017-1018. DOI: 10.21273/HORTSCI.31.6.1017

Bosland PW & Votava EJ (2012) Peppers: vegetable and spice capsicums. 2nd ed. Crop production science in horticulture series 22. CABI, Wallingford. 248p.

Cabral JS, Pessoa Félix L & Guerra M (2006) Heterochromatin diversity and its co-localization with 5S and 45S rDNA sites in chromosomes of four *Maxillaria* species (Orchidaceae). Genetics and Molecular Biology 29: 659-664. DOI: 10.1590/S1415-47572006000400015

Chowdhary BP, Raudsepp T, Fronicke L & Scherthan H (1998) Emerging patterns of comparative genome organization in some mammalian species as revealed by Zoo-FISH. Genome Research 8: 577-589. DOI: 10.1101/gr.8.6.577

DeWitt D & Bosland PW (2009) The complete chile pepper book. A gardener's guide to choosing, growing, preserving and cooking. Timber Press, London. 336p.

Di Dato F, Parisi M, Cardi T & Tripodi P (2015) Genetic diversity and assessment of markers linked to resistance and pungency genes in *Capsicum* germplasm. Euphytica 204: 103-119. DOI: 10.1007/s10681-014-1345-4

Eshbaugh WH (1979) Biosystematic and evolutionary study of the *Capsicum pubescens* complex. National Geographic Society Research Reports, 1970 Projects: 143-162.

Fonseca A, Ferreira J, Santos TRB, Mosiolek M, Bellucci E, Kami J, Gepts P, Geffroy V, Schweizer D, Santos KGB & Pedrosa-Harand A (2010) Cytogenetic map of common bean (*Phaseolus vulgaris* L.). Chromosome Research 18: 487-502. DOI: 10.1007/s10577-010-9129-8

Grabiele M, Debat HJ, Moscone EA & Ducasse DA (2012) 25S-18S rDNA IGS of *Capsicum*: molecular structure and comparison. Plant Systematics and Evolution 298: 313-321. DOI: 10.1007/s00606-011-0546-8

Grabiele M, Debat HJ, Scaldaferro MA, Aguilera PM, Moscone EA, Seijo GJ & Ducasse DA (2018) Highly GC-rich heterochromatin in chili peppers (*Capsicum*-Solanaceae): a cytogenetic and molecular characterization. Scientia Horticulturae 238: 391-399. DOI: 10.1016/j.scienta.2018.04.060

Greilhuber J & Speta F (1976) C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica*, and *Puschkinia* (Liliaceae). Plant Systematics and Evolution 126: 149-188. DOI: 10.1007/BF00981669

Guerra M (2000) Patterns of heterochromatin distribution in plant chromosomes. Genetics and Molecular Biology 23: 1029-1041. DOI: 10.1590/S1415-47572000000400049

Guevara M, Siles M & Bracamonte O (2000) Análisis cariotípico de *Capsicum pubescens* R&P (Solanaceae) "rocoto". Revista Peruana de Biología 7: 134-141. DOI: 10.15381/rpb.v7i2.6816

Ijdo JW, Wells RA, Baldini A & Reeders ST (1991) Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. Nucleic Acids Research 19: 4780. DOI: 10.1093/nar/19.17.4780

Kim S, Park M, Yeom S-I, Kim Y-M, Lee JM, Lee H-A, Seo E, Choi J, Cheong K, Kim K-T, Jung K, Lee G-W, Oh S-K, Bae C, Kim S-B, Lee H-Y, Kim S-Y, Kim M-S, Kang B-C, Jo YD, Yang H-B, Jeong H-J, Kang W-H, Kwon J-K, Shin C, Lim JY, Park JH, Huh JH, Kim J-S, Kim B-D, Cohen O, Paran I, Suh MC, Lee SB, Kim Y-K, Shin Y, Noh S-J, Park J, Seo YS, Kwon S-Y, Kim HA, Park JM, Kim H-J, Choi S-B, Bosland PW, Reeves G, Jo SH, Lee B-W, Cho H-T, Choi H-S, Lee M-S, Yu Y, Choi YD, Park B-S, van Deynze A, Ashrafi H, Hill T, Kim WT, Pai H-S, Ahn HK, Yeam I, Giovannoni JJ, Rose JKC, Sørensen I, Lee S-J, Kim RW, Choi I-Y, Choi B-S, Lim J-S, Lee Y-H & Choi D (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nature Genetics 46: 270-278. DOI: 10.1038/ng.2877

Kim S, Park J, Yeom S-I, Kim Y-M, Seo E, Kim K-T, Kim M-S, Lee JM, Cheong K, Shin HS, Kim S-B, Han K, Lee J, Park M, Lee H-A, Lee H-Y, Lee Y, Oh S, Lee JH, Choi E, Choi E, Lee SE, Jeon J, Kim H, Choi G, Song H, Lee JK, Lee S-C, Kwon J-K, Lee H-Y, Koo N, Hong Y, Kim RW, Kang W-H, Huh JH, Kang B-C, Yang T-J, Lee Y-H, Bennetzen JL & Choi D (2017) New reference genome sequences of hot pepper reveal the massive evolution of plant disease-resistance genes by retroduplication. Genome Biology 18: 210. DOI: 10.1186/s13059-017-1341-9

Lambrou M & Ehrendorfer F (2000) DAPI-banded karyotypes and hybrid compatibility in diploid and tetraploid taxa of *Achillea millefolium* Agg. Botanikertagung Jena, 17–22 September 2001, Tagungsband. 207p.

Levan A, Fredga K & Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220. DOI: 10.1111/j.1601-5223.1964.tb01953.x

Limaye VA & Patil VP (1989) Karyomorphological studies in the genus *Capsicum* Linn. Cytologia 54: 455-463. DOI: 10.1508/cytologia.54.455

Lindeman W & Heidmann IA (2012) Method for transferring one or more genetic traits from a plant of the purple-flowered *Capsicum* species to a plant of the white-flowered *Capsicum* species. International Patent Classification A01H 5/08. Available at

<https://patentimages.storage.googleapis.com/72/5b/f2/672ebfb1bfa2e5/AU2005337085B2.pdf>. Access on 02 April 2020.

Moscone EA, Lambrou M, Hunziker AT & Ehrendorfer F (1993) Giemsa C-banded karyotypes in *Capsicum* (Solanaceae). Plant Systematics and Evolution 186: 213-229. DOI: 10.1007/BF00940799

Moscone EA, Loidl J, Ehrendorfer F & Hunziker AT (1995) Analysis of active nucleolus organizing regions in *Capsicum* (Solanaceae) by silver staining. American Journal of Botany 82: 276-287. DOI: 10.1002/j.1537-2197.1995.tb11495.x

Moscone EA, Lambrou M & Ehrendorfer F (1996a) Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). Plant Systematics and Evolution 202: 37-62. DOI: 10.1007/BF00985817

Moscone EA, Matzke MA & Matzke AJM (1996b) The use of combined FISH/GISH in conjunction with DAPI counterstaining to identify chromosomes containing transgene inserts in amphidiploid tobacco. Chromosoma 105: 231-236. DOI: 10.1007/BF02528771

Moscone EA, Klein F, Lambrou M, Fuchs J & Schweizer D (1999) Quantitative karyotyping and dual-color FISH mapping of 5S and 18S-25S rDNA probes in the cultivated *Phaseolus* species (Leguminosae). Genome 42: 1224-1233. DOI: 10.1139/g99-070

Moscone EA, Baranyi M, Ebert I, Greilhuber J, Ehrendorfer F & Hunziker A (2003) Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by flow cytometry and Feulgen densitometry. Annals of Botany 92: 21-29. DOI: 10.1093/aob/mcg105

Moscone EA, Scaldaferro MA, Grabiele M, Cecchini NM, Sánchez García Y, Jarret R, Daviña JR, Ducasse DA, Barboza GE & Ehrendorfer F (2007) The evolution of chili peppers (*Capsicum*-Solanaceae): a cytogenetic perspective. Acta Horticulturae 745: 137-169. DOI: 10.17660/ActaHortic.2007.745.5

Moulin MM, Rodrigues R, Bento CS, Gonçalves LSA, Santos JO, Sudré CP & Viana AP (2015) Genetic dissection of agronomic traits in *Capsicum baccatum* var. Pendulum. Genetics and Molecular Research 14: 2122-2132. DOI: 10.4238/2015.March.20.23

Ou LJ, Dai XZ, Zhang ZQ & Zou XX (2011) Responses of pepper to waterlogging stress. Photosynthetica 49: 339-345. DOI: 10.1007/s11099-011-0043-x

Park YK, Kim BD, Kim BS, Armstrong KC & Kim NS (1999) Karyotyping of the chromosomes and physical mapping of the 5S rRNA and 18S-26S rRNA gene families in five different species in *Capsicum*. Genes & Genetic Systems 74: 149-157. DOI: doi.org/10.1266/ggs.74.149

Paszko B (2006) A critical review and a new proposal of karyotype asymmetry indices. Plant Systematics and Evolution 258: 39-48. DOI: 10.1007/s00606-005-0389-2

Pedrosa A, Sandal N, Stougaard J, Schweizer D & Bachmair A (2002) Chromosomal map of the model legume *Lotus japonicus*. Genetics 161: 1661-1672.

Phan C, Kao J, Berke T & Jones C (2014) Hybrid pepper plants resulting from a cross between *C. annuum* and *C. pubescens*. United States Patent No. US 8,802,939 B2. Available at <https://patentimages.storage.googleapis.com/73/da/b6/9eb32c3256ab4b/US8802939.pdf>. Access on 02 April 2020.

Pickersgill B (1991) Cytogenetics and evolution of *Capsicum* L. *In*: Gupta PK & Tsuchiya T (eds.) Chromosome engineering in plants: genetics, breeding, evolution, Part B. Elsevier, Amsterdam. Pp. 139-160.

Qin C, Yu C, Shen Y, Fang X, Chen L, Min J, Cheng J, Zhao S, Xu M, Luo Y, Yang Y, Wu Z, Mao L, Wu H, Ling-Hu C, Zhou H, Lin H, González-Morales S, Trejo-Saavedra DL, Tian H, Tang X, Zhao M, Huang Z, Zhou A, Yao X, Cui J, Li W, Chen Z, Feng Y, Niu Y, Bi S, Yang X, Li W, Cai H, Luo X, Montes-Hernández S, Leyva-González MA, Xiong Z, He X, Bai L, Tan S, Tang X, Liu D, Liu J, Zhang S, Chen M, Zhang L, Zhang L, Zhang Y, Liao W, Zhang Y, Wang M, Lv X, Wen B, Liu H, Luan H, Zhang Y, Yang S, Wang X, Xu J, Li X, Li S, Wang J, Palloix A, Bosland PW, Li Y, Krogh A, Rivera-Bustamante RF, Herrera-Estrella L, Yin Y, Yu J, Hu K & Zhang Z (2014) Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. Proceedings of the National Academy of Sciences of the United States of America 111: 5135-5140. DOI: 10.1073/pnas.1400975111

Rivas M, Vignale D, Ordoñez RM, Zampini IC, Alberto MR, Sayago JE & Isla MI (2014) Nutraceutical properties and toxicity studies of flour obtained from *Capsicum pubescens* fruits and its comparison with "Locoto" commercial powder. Food and Nutrition Sciences 5: 715-724. DOI: 10.4236/fns.2014.58081

Roa F & Guerra M (2015) Non-random distribution of 5S rDNA sites and its association with 45S rDNA in plant chromosomes. Cytogenetic & Genome Research 146: 243-249. DOI: 10.1159/000440930

Rodríguez-Burruezo A, González-Mas MC & Nuez F (2010) Carotenoid composition and vitamin A value in ají (*Capsicum baccatum* L.) and Rocoto (*C. pubescens* R. & P.), 2 pepper species from the Andean region. Journal of Food Science 75: 446-453. DOI: 10.1111/j.1750-3841.2010.01795.x

Romero Zarco C (1986) A new method for estimating karyotype asymmetry. Taxon 35: 526-530. DOI: 10.2307/1221906

Sahin F & Miller SA (1998) Resistance in *Capsicum pubescens* to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. Plant Disease 82: 794-799. DOI: 10.1094/PDIS.1998.82.7.794

Scaldaferro MA, Seijo JG, Acosta MC, Barboza GE, Ducasse DA & Moscone EA (2006) Genomic characterization of the germplasm in peppers (*Capsicum*-Solanaceae) by fluorescent in situ hybridization. Plant Science (Sofia) 43: 291-297.

Scaldaferro MA, Grabiele M & Moscone EA (2013) Heterochromatin type, amount and distribution in wild species of chili peppers (*Capsicum*, Solanaceae). Genetic Resources and Crop Evolution 60: 693-709. DOI: 10.1007/s10722-012-9867-x

Scaldaferro MA, Romero da-Cruz MV, Ceccini NM & Moscone EA (2016) FISH and AgNor-mapping of the 45S and 5S rRNA genes in wild and cultivated C*apsicum* species (Solananceae). Genome 59: 95-113. DOI: 10.1139/gen-2015-0099

Scaldaferro MA & Moscone EA (2019) Cytology and DNA content variation of *Capsicum* genomes. *In*: Ramchiary N & Kole C (eds.) The *Capsicum* genome. Springer, Cham. Pp. 57-84. DOI: 10.1007/978-3-319-97217-6

Schwarzacher T, Ambros P & Schweizer D (1980) Application of Giemsa banding to orchid karyotype analysis. Plant Systematics and Evolution 134: 293-297. DOI: 10.1007/BF00986805

Schweizer D (1979) Fluorescent chromosome banding in plants: applications, mechanisms, and implications for chromosome structure. *In*: Davies DR & Hopwood DA (eds.) Proceedings of the Fourth John Innes Symposium. John Innes Charity, Norwich. Pp. 61-72.

Schweizer D & Ambros PF (1994) Chromosome banding. Stain combinations for specific regions. *In:* Gosden JR (ed.) Methods in molecular biology 29. Chromosome analysis protocols. Humana Press, Totowa. Pp. 97-112.

Stack SM, Herickhoff L, Sherman J & Anderson L (1991) Staining plant cells with silver. I. The salt-nylon technique. Biotechnic & Histochemistry 66: 69-78. DOI: 10.3109/10520299109110553

Stebbins GL (1971) Chromosomal evolution in higher plants. Addison-Wesley, Reading (Massachusetts). Pp. 220.

Thiers B [continuously updated] Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available at <http://sweetgum.nybg.org/science/ih/>. Access on 29 May 2020.

Vallejo-Gutiérrez AJ, Mejía-Carranza J, García-Velasco R & Ramírez-Gerardo MG (2018) Response of *Capsicum pubescens* genotypes to damage caused by the fungal wilt complex. Revista Mexicana de Fitopatología 37: 50-70. DOI: 10.18781/R.MEX.FIT.1809-3

Wu FN, Eannetta NT, Xu YM, Durrett R, Mazourek M, Jahn MM & Tanksley SD (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*. Theoretical and Applied Genetics 118: 1279-1293. DOI: 10.1007/s00122-009-0980-y

Wu F & Tanksley SD (2010) Chromosomal evolution in the plant family Solanaceae. BMC Genomics 11: 182. DOI: 10.1186/1471-2164-11-182

Area Editor: Dr. Leandro Giacomin
Received in June 04, 2020. Accepted in August 28, 2020.