Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Marlon Garcia Paitan, Maricielo Postillos-Flores, Luis Rojas Vasquez, Maria Siles Vallejos, Alberto López Sotomayor (2022). Nuclear DNA content and comparative FISH mapping of the 5s and 45s rDNA in wild and cultivated populations of *Physalis peruviana* L. *Caryologia* 75(3): 123-134. doi: 10.36253/caryologia-1728

Received: July 03, 2022

Accepted: November 29, 2022

Published: April 5, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Nuclear DNA content and comparative FISH mapping of the 5s and 45s rDNA in wild and cultivated populations of *Physalis peruviana* L.

Marlon Garcia Paitan*, Maricielo Postillos-Flores, Luis Rojas Vasquez, Maria Siles Vallejos, Alberto López Sotomayor

Research Group on Genetic Resources (RecGen), Faculty of Biological Sciences, Universidad Nacional Mayor de San Marcos, Lima, Peru
*Corresponding author. E-mail: marlon.garcia@unmsm.edu.pe

Abstract. *Physalis peruviana* L. often known as goldenberry, has increased its commercial growth in the international market in recent years due to its nutritional value and antioxidant potential. This situation has enabled countries such as Peru to increase their production in order to meet the global demand. However, investigations about the genetic diversity of cultivated and wild populations of goldenberry are still in their early stages. FISH mapping of 5s and 45s rDNA loci and flow cytometry estimation of nuclear DNA content were used to assess genetic differences between wild and cultivated goldenberry populations from Ayacucho and Cajamarca. The majority of metaphases had six 5s rDNA sites for all populations and two and four 45s rDNA sites for the cultivated and wild populations, respectively. We were able to characterize nine different types of chromosomes based on their morphology, fluorescence, rDNA location, and conservation across populations by analyzing the chromosomes that contained rDNA. Furthermore, cultivated populations had more nuclear DNA (13.262±0.087 pg) than wild populations (12.955±0.086 pg). The results show genetic differences between wild and cultivated populations of goldenberry at molecular cytogenetic level as well as in genome size. These findings establish a precedent for future cytogenetic and genomic studies in goldenberry populations, enabling future breeding programs.

Keywords: Goldenberry, FISH, rDNA, chromosomes, flow cytometry.

1. INTRODUCTION

Physalis peruviana L., also known in Latin America as "aguaymanto," "uchuva," or "uvilla," is a plant in the Solanaceae family that originated as well as diversified in the Andes Mountains. It produces orange-yellow berry-shaped fruits covered by a calyx. These fruits are high in vitamin C, carotenoids, phenolic compounds with antioxidant precursors, and withanolides, which have anticancer and antitumor properties (Singh et al. 2019). Because of these characteristics and the increased international demand for organic food, the production of *Physalis peruviana* L. in Peru has quickly grown in recent years, establishing it as the second-largest producer of goldenberry

in Latin America. However, the amount of goldenberry production compared to Colombia, the leading producer in Latin America, and other countries outside the American continent remains limited (Sierra y Selva Exportadora 2021). To increase commercial competitiveness, genetic improvement programs that increase fruit quality and production must be implemented, and our understanding of the genetic diversity of *Physalis peruviana* L. in cultivated and wild populations will determine the success of these programs. Cytogenetic studies provide a foundation for the characterization of germplasm resources and offer guidance in selecting the parental cultivars for plant breeding programs (Herrera 2007). Furthermore, for future complex genomic projects such as cloning of genes of interest or genome sequencing, genome size data is essential to design effective projects (Doležel et al. 2007). Most cytogenetic studies in *Physalis peruviana* L. have used classical staining techniques to determine the chromosome number, where 2n=48 predominates (Rodríguez and Bueno 2006; Sánchez 2014; Liberato et al. 2014; Trevisani et al. 2018), while other studies report mixoploid plants (Sánchez 2014), and somatic aneuploidy (Carbajal et al. 2021). Karyotypic formulas of some populations have been previously reported (Azeez and Faluyi 2019; Carbajal et al. 2021). However, since goldenberry chromosomes are small, numerous, and have similar morphology, the karyotype determination and its analysis are considered limited when classical staining techniques are used.

FISH is a molecular cytogenetic technique for mapping specific sequences on chromosomes by hybridizing probes with their complementary sequences. 5s and 45s ribosomal DNA (rDNA) probes have been widely used to perform cytogenetic characterization and to understand intraspecific and interspecific evolutionary relationships at chromosomal level by determining the position and number of signals on metaphase chromosomes, even in species with numerous and small chromosomes (Herrera 2007). This is due to the nature of rDNA sequences, which are tandemly repeated DNA regions in the genome with high transcription rates and a predisposition for unequal recombination, which makes them inherently unstable and prone to variation in copy number and location in the genome (Salim and Gerton 2019). Previously, the number of 5s rDNA signals in two cultivated populations from the same region has been studied at the molecular cytogenetic level in *Physalis peruviana* L. (Siles et al. 2021). However, no studies have been conducted to analyze the number and position of the 5s and 45s rDNA sites to determine if there are any chromosomal differences between wild and cultivated populations as a consequence of the domestication of the species.

Plant genome size varies enormously and is considered a biodiversity trait that provides valuable information for systematic and evolutionary studies (Pellicer et al. 2018). It is also useful for determining the genetic characterization of germplasm and plant identification because of these characteristics (Jedrzejczyk and Rewers 2020). Flow cytometry is a quick and easy way to estimate nuclear DNA content, making it a powerful tool for accelerating selection processes in plant breeding and providing critical data for sequencing projects (Ciprian-Salcedo et al. 2020).

Given the foregoing, the goals of this study were to characterize and analyze the genetic differences between wild and cultivated populations of *Physalis peruviana* L. from the regions of Ayacucho and Cajamarca through the mapping of 5s and 45s rDNA by FISH, and to estimate the nuclear DNA content using flow cytometry. These two approaches will set a precedent as potential tools for programs to improve this genetic resource in Peru and, as a result, improve the international competitiveness of Peru against other producing countries.

2. METHODS AND MATERIALS

2.1 Plant material and preparation of metaphase chromosomes

Seeds were collected from wild and cultivated populations of ripe goldenberry fruits in the Cajamarca and Ayacucho regions (Figure 1). Plant material was transported to the Genetics Laboratory of the Universidad Nacional Mayor de San Marcos (UNMSM) and correctly identified as *Physalis peruviana* L. by the UNMSM Natural History Museum. (Certificates 224-USM-2015 and 001-USM-NHN -2022).

We modified the protocols described by Aguilera et al. (2016), Aliyeva-Schnorr et al. (2015), and Carbajal et al. (2021) in order to obtain mitotic metaphase chromosomes. Goldenberry seeds were germinated, and roots with an approximate size of 4 mm were treated with 0.03 percent colchicine for 80 minutes at room temperature before being submerged in distilled water for 60 minutes at 37 °C and fixed in an ethanol solution: acetic acid (3:1) at -20 °C for at least one day. Subsequently, the roots were washed in cold distilled water twice for 5 minutes, followed by two washes in cold citrate buffer (0.01 M at pH 4.6) for 5 minutes. They were then macerated in a 2% cellulase solution (from *Aspergillus niger*, Sigma) and a 10% liquid pectinase solution (from *Aspergillus niger*, Sigma) dissolved in 40% glycerol in 0.01M citrate buffer at pH 4.6, at 37 °C for 1 hour. Then, they were washed four times with cold citrate buffer for 5 minutes (0.01 M at pH

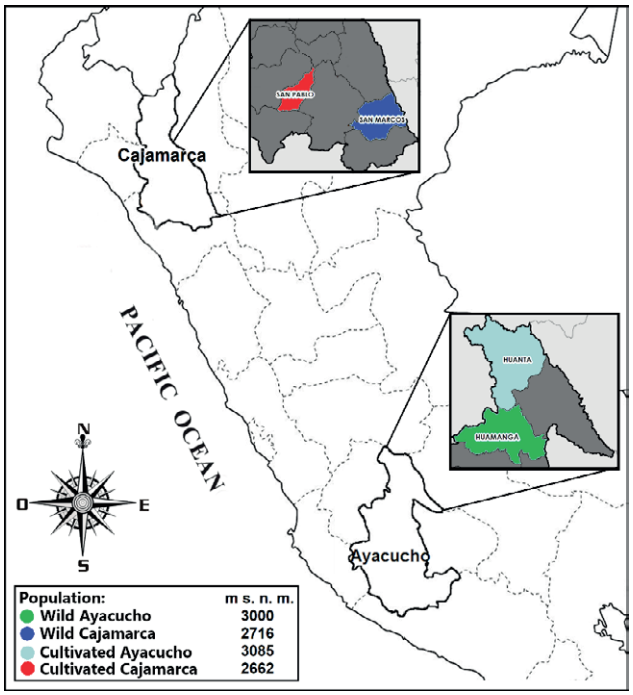

Figure 1. Map of Peru showing sampling locations for wild and cultivated populations of *Physalis peruviana* L.

4.6) and twice in ethanol 90° for 5 seconds. The apex of each root was then transferred to a slide, a drop of 45 percent acetic acid was placed on it, and the "squash" technique was performed. After that, the slide was frozen with dry ice to remove the coverslip slide, then air-dried and examined under a phase-contrast microscope to confirm the presence of cells with properly separated metaphase chromosomes. Finally, the selected slides were immersed in absolute ethanol at -20 °C until they were used.

2.2 Amplification of rDNA

Genomic DNA was extracted from seedlings grown from goldenberry seeds using the GF-1 Plant DNA extraction kit (Vivantis, Malaysia). To obtain the 5s rDNA probe, 5s rDNA amplification was performed using the primers pr5S14 (5'-GGCGAGTAGTACTAG-GATCCGTGAC-3') and pr5S15 (5'-GCTTAACTTCG-GAGTTCTGATGGGA-3') reported by Volkov et al. (2001). Because the 45s rDNA locus is too large to be completely amplified, it was decided to use the 18s rDNA gene as a probe. Since 18s rDNA is present within the gene structure of the 45s rDNA, mapping it would also show the location of the 45s rDNA locus. The following primers were used to achieve 18s rDNA amplification: primers F-566 (5'-CAGCAGCCGCGGTAATTCC-3') and R-1200 (5'-CCCGTGTTGAGTCAAATTAAGC-3') which were previously reported by Hadziavdic et al. (2014). The PCR reaction mixture for both cases had a final volume of 20 µl containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM dNTPs, 0.2 µM of each primer, 1 U of Taq polymerase (abm, Canada), and 30 ng of genomic DNA. The PCR program for 5s rDNA began with an initial denaturation at 95 °C for 9 minutes, followed by 45 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 45 seconds, and a final extension at 72 °C for 5 minutes. On the other hand, 18s rDNA involved an initial denaturation at 95 °C for 9 minutes, followed by 42 cycles of 95 °C for 30 seconds, 62 °C for 30 seconds and 72 °C for 60 seconds, and a final extension at 72 °C for 5 minutes. The amplicons were purified using the GeneJET PCR Purification kit (Thermo Scientific™) and quantified by spectrophotometry.

2.3 Labelling of DNA probes

The 5s rDNA amplicons were labeled with biotin-14-dATP for 90 minutes using the BioNick Labeling System commercial kit (Invitrogen, USA), and the 18s rDNA was labeled for 180 minutes with digoxigenin-11-dUTP using the DIG-Nick Translation Mix commercial kit (Roche, Switzerland).

2.4. FISH and signal detection

The FISH methodology used was slightly modified from that provided by Poggio et al. (2000). The slides with the chromosome preparation were treated with RNases and 4% (w/v) paraformaldehyde. The hybridization solution contained 15 µl of Formamide, 6 µl of 50% Dextran Sulfate, 3 µl of 20X SSC, 1 µl of Salmon DNA (10 mg/ml), 1µl of 10% SDS, and 50 ng of each 5s and 45s rDNA probe, with a final volume of 30 µl. This solution was applied to the chromosome preparations, and the hybridization program was as follows: 75 °C for 7 minutes, 55 °C for 6 minutes, 45 °C for 5 minutes, and 37 °C for 12 hours inside a hybridizer (Biobase, HS-500). After the hybridization, the following washes were performed to remove nonspecific binding: 2X SSC at 42 °C for 5 minutes, 20% formamide in 0.1X SSC at 42 °C for 10 minutes, 0.1X SSC at 42 °C for 5 minutes, 2X SSC for 5 minutes at 42 °C, and finally three washes with 0.2% (v/v) Tween 20 in 4X SSC for 5 minutes at room temperature. The 5s probe was detected with a Neutravidin-Oregon-Green 488 conjugate (Thermo Scientific™) while the 18s probe was detected with Anti-Digoxigenin-Rhodamine (Roche). The slides were mounted with Slow-

Fade™ Gold Antifade Mountant (Invitrogen™), which contained DAPI and an antifade solution.

The slides were observed and photographed under an epifluorescence microscope (ZEISS Axio Scope.A1). For DAPI fluorophore, BP 340/30 excitation and BP 510/90 emission filters were used; for Oregon Green 488, BP 470/40 excitation and BP 540/50 emission filters were used; and for Rhodamine, BP 560/40 excitation and BP 630/75 emission filters were used. We specifically worked with cells in metaphase that contained 48 recognizable chromosomes to avoid variation in the results attributable to any somatic aneuploidy present in the meristematic tissue of the root of the species, a phenomenon already observed in prior studies (Carbajal et al. 2021; Franco et al. 2021). The following programs were utilized: GIMP 2.10.14 was used for picture overlaying and chromosomal individualization; IdeoKar1.2 (Mirzaghaderi and Marzangi 2015) for morphological measurements of chromosomes and the production of ideograms; and ImageJ for fluorescence quantification of probe signals. The latter was achieved in accordance with Fitzpatrick (2021).

2.5 Determination of nuclear DNA content

Each wild and cultivated population had eight plants evaluated. These were obtained after a three-month seed culture in pots. Flow cytometry was used to determine the nuclear genome size of each plant in duplicate using *Pisum sativum* cv. 'Ctirad' (2C = 9.09 pg DNA) as an internal reference standard. The nuclear suspension preparation protocol and nuclear DNA content estimation were performed as described by Doležel et al. (2007). Young leaves of the standard and goldenberry were utilized as plant material in the streamlined two-step process for nuclei suspension preparation. Furthermore, propidium iodide was employed as a fluorochrome. The nuclear DNA content was estimated using an Attune NxT flow cytometer (Thermo Fisher Scientific) and the following formula:

$$\text{2C value of the sample (pg de ADN)} = \frac{\text{Mean position of the 2C peak of the sample}}{\text{Mean position of the 2C peak of the reference standard}} \times 9.09\text{pg}$$

3. RESULTS

3.1 Number of 5s and 45s rDNA signals in wild and cultivated populations

FISH was used to identify the 5s and 45s rDNA probe signals in the metaphases of *Physalis peruviana* L. populations (Figure 2). For each population, 25 metaphases with 48 chromosomes were analyzed. After studying the metaphases of all the populations, a total of five combinations of the number of 5s and 45s rDNA sites were identified; three of these combinations were detected in wild populations (Figure 2A, B, E, F, I, J) and four combinations in cultivated populations (Figure 2C, D, E, G, H, K, L). There were no significant variations in the number of 5s and 45s rDNA sites across wild populations ($p=0.422$), nor between cultivated populations ($p=0.085$), however, there were significant differences across populations from the same region ($p<0.01$). Furthermore, it was discovered that the predominant rDNA combination in wild populations was six 5s rDNA sites and four 45s rDNA sites, whereas in cultivated populations it was six 5s rDNA sites and two 45s rDNA sites (Figure 3). In general, a higher frequency of six 5s rDNA sites is observed in all goldenberry populations, while for 45s rDNA a number of two sites predominates for cultivated populations and 4 sites for wild populations.

3.2 Characterization of chromosomes with rDNA loci

The 12 best metaphases with clear and distinct rDNA signals in relation to their chromosomal arms and fluorescent signals were selected from the 25 metaphases per population, considering at least one metaphase that showed each combination of the number of 5s and 45s rDNA sites from each wild and cultivated population. The morphological characterization, individualization, and pairing with their homologous chromosomes that displayed rDNA signals were performed in each of the selected metaphases (Figure 4, Figure S1). Based on the study of the chromosomes that showed rDNA signals in all populations, nine types of chromosomes were identified according to their morphological features, the position of the 5s or 45s rDNA locus, the fluorescence intensity of the rDNA probe, and their presence in the populations (Figure 5 and Table 1). The *N* chromosomes are those that are found in all populations: *N1* has a metacentric morphology and the 5s rDNA in the p arm; *N2* is submetacentric and has the 45s rDNA in the p arm, and *N3* is acrocentric and has the 5s rDNA in the q arm. The *S* chromosomes, which stand for wild, are found specifically in wild populations: *S1* has a submetacentric morphology and 45s rDNA in the q arm; *S2* is metacentric and has 45s rDNA in the q arm; *S3* is metacentric and has 5s rDNA in the p arm. It should be noted that, while *N1* and *S3* are described in the same way, the difference between them is determined by the length of the chromosome (Table 1). *C* chromosomes are those found solely in cultivated populations: *C1* has a submetacentric

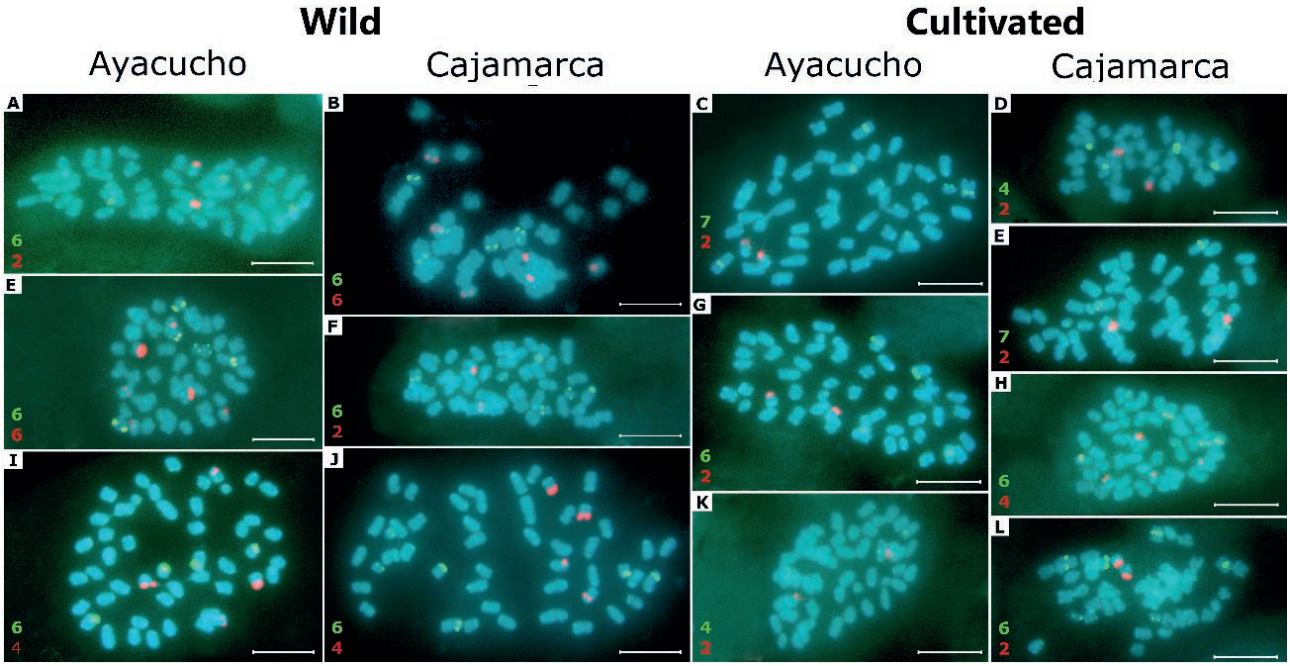

Figure 2 Fluorescent in situ hybridization signals of the 5s (green) and 45s (red) rDNA probes in *Physalis peruviana* L. metaphase chromosomes. The numbers at the bottom left of each image represent the number of 5s (green) and 45s (red) sites found in the metaphases. Combinations of the number of 5s and 45s rDNA sites identified in the population of wild Ayacucho are shown in A, E, and I; wild Cajamarca is shown in B, F, and J; cultivated Ayacucho is shown in C, G, and K; and Cultivated Cajamarca is shown in D, E, H, and L. The scale bar measures 10 μm.

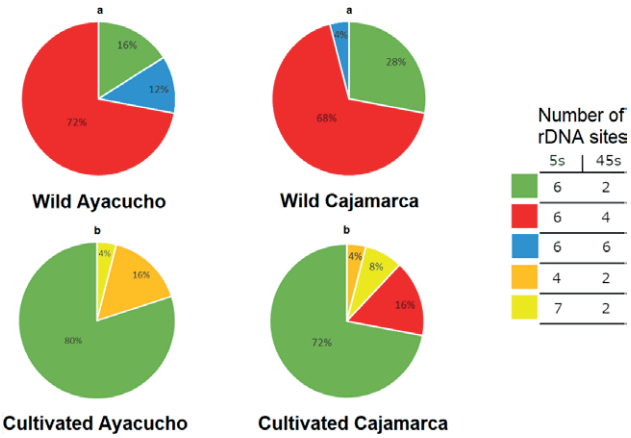

Figure 3 The frequency of 5s and 45s rDNA sites observed in the 25 metaphases of each goldenberry population. Different letters at the top of each frequency circle show significant differences (Fisher's Exact Test, $p<0.05$).

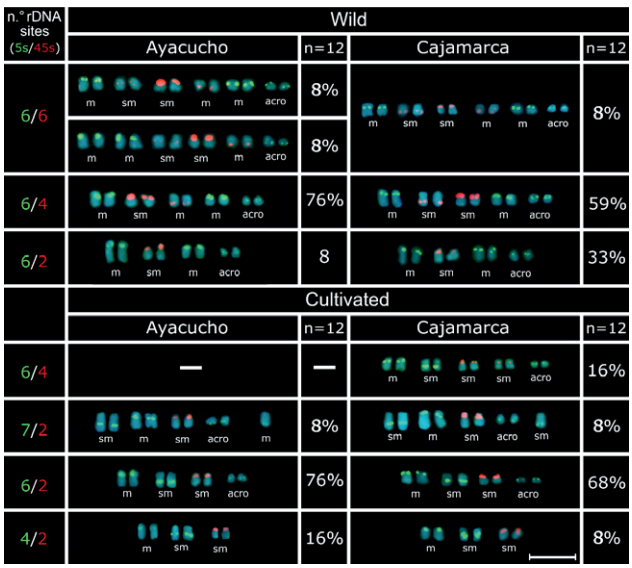

Figure 4 Chromosomes that showed hybridization of the 5s and 45s rDNA probes between the best 12 metaphases for each population, encompassing at least one metaphase for each of the combinations of the number of 5s and 45s rDNA sites reported for each population of goldenberry. m=metacentric, sm=submetacentric, acro=acrocentric.

morphology and has the 5s rDNA in the q arm, whereas *C2* has a metacentric/submetacentric morphology and has the 5s rDNA in the q arm, and a lower fluorescence intensity than *C1*. The morphology of the *C2* chromosome is attributable to the fact that only one *C2* chromo-

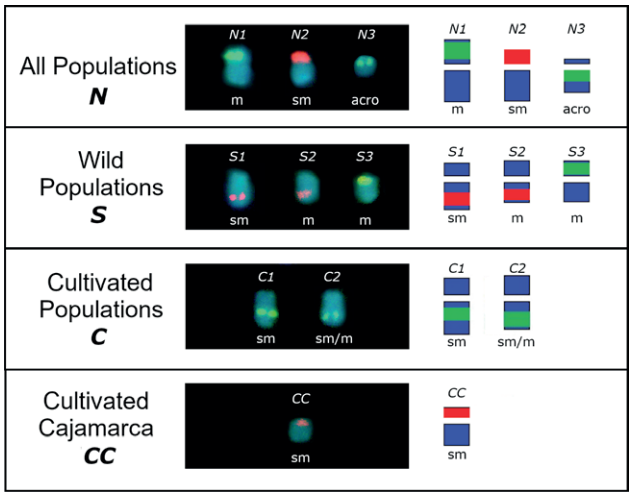

Figure 5 Characterization of the nine types of chromosomes that showed rDNA probe hybridization based on their existence in populations, morphology, size, presence of the 5s or 45s locus, position of the rDNA locus, and rDNA probe fluorescence intensity, m=metacentric, sm=submetacentric, and acro=acrocentric.

some was observed in each of the cultivated populations, resulting in a metacentric morphology in Ayacucho and a submetacentric morphology in Cajamarca (Figure 6). The *CC* chromosome, found only in the Cultivated Cajamarca population, has a submetacentric morphology and the 45s rDNA in the p arm, similar to *N2*, although it differs in terms of chromosome length and rDNA probe fluorescence intensity (Table 1).

The types of chromosomes that contain rDNA are conserved among wild populations. However, there is a difference in the observed frequency of chromosomes *S1*, which is higher in Cajamarca, and *S2*, which is higher in Ayacucho (Figure 6). Additionally, in Ayacucho, the 45s rDNA locus of *S1* chromosome is found in the centromeric position, whereas in Cajamarca, it is found in the interstitial position. The *CC* chromosome was found only in Cajamarca among the cultivated populations, while the other chromosomes were found in both groups with no variations in frequency or position of the rDNA (Figure 6).

3.3 Size of the nuclear genome

All flow cytometric analyses showed high-resolution histograms with a coefficient of variation (CV) of the G0/G1 peaks between 1.63% and 2.85% (mean 2.04%). Representative histograms are shown with the peaks corresponding to the G0/G1 nuclei of *Physalis peruviana* L. and the internal standard *Pisum sati-*

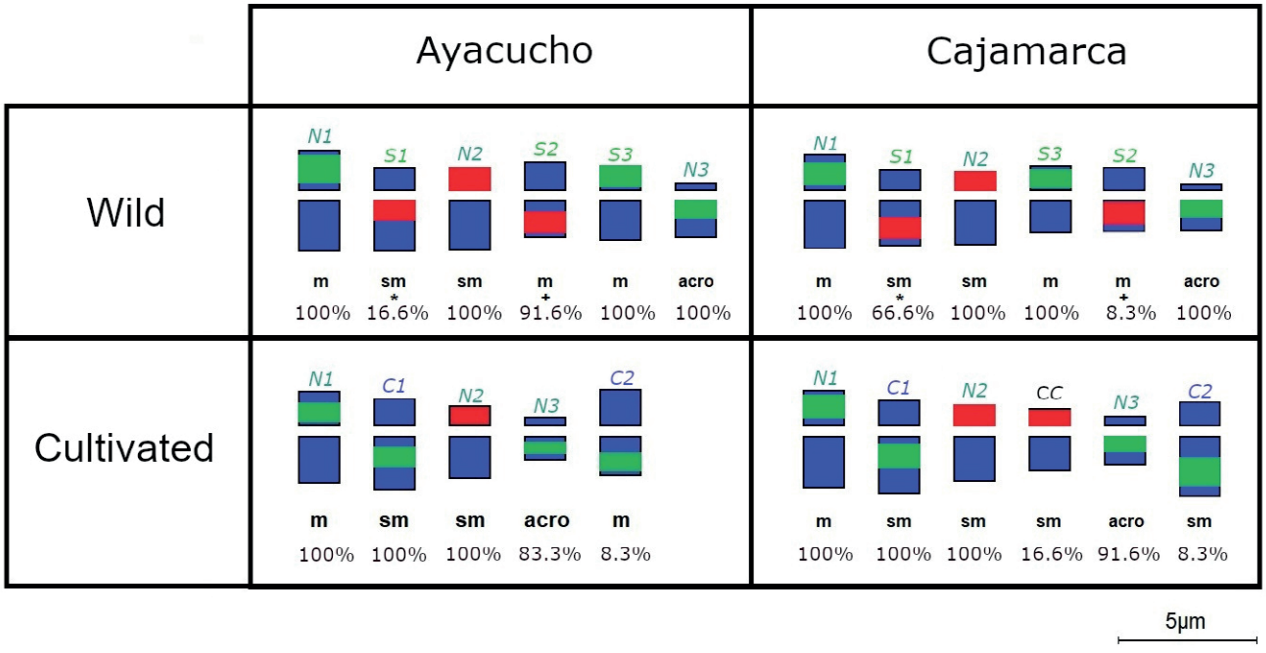

Figure 6 The average ideogram of the types of chromosomes that included rDNA. The proportion observed in the total of the metaphases analyzed by each population is indicated beneath each chromosome. Equal symbols above the percentages indicate a significant difference between the observed frequencies of both populations (Fisher's Exact Test, $p<0.05$). m=metacentric, sm=submetacentric, and acro=acrocentric.

Table 1. Morphometric and fluorescence data of the nine types of chromosomes found in *Physalis peruviana* L. populations.

rDNA-bearing chromosome	n	Chromosome Length	Arm ratio	Log (DNA probe fluorescence intensity)	CM	Position of rDNA locus
N1	48	3.120 ± 0.416	1.363 ± 0.161	3.943 ± 0.265	m	5s -> p
N2	48	2.436 ± 0.332	2.191 ± 0.265	3.909 ± 0.404	sm	45s -> p
N3	45	1.499 ± 0.230	3.919 ± 0.641	3.078 ± 0.307	acro	5s -> q
S1	10	2.516 ± 0.341	2.189 ± 0.240	3.383 ± 0.318	sm	45s -> q
S2	12	2.417 ± 0.216	1.471 ± 0.112	3.371 ± 0.164	m	45s -> q
S3	24	2.241 ± 0.287	1.284 ± 0.167	3.995 ± 0.319	m	5s -> p
C1	24	3.002 ± 0.461	2.220 ± 0.292	3.930 ± 0.240	sm	5s -> q
C2	1	2.908 ± 0.106	1.951 ± 1.238	3.180 ± 0.001	m/sm	5s -> q
CC	2	1.922 ± 0.047	2.027 ± 0.366	3.212 ± 0.062	sm	45s -> p

The values are expressed as mean ± standard deviation. The "n" sample values are per chromosomal pair. CM = chromosome morphology; m = metacentric; sm = submetacentric; acro= acrocentric.

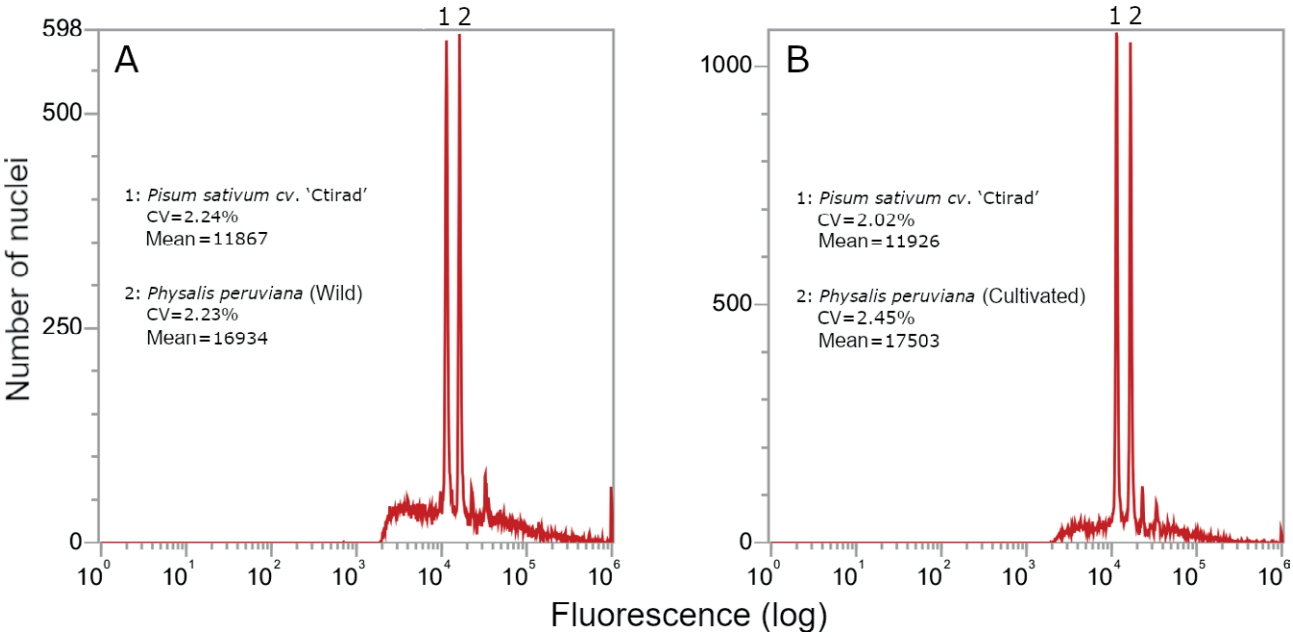

Figure 7 Fluorescence histograms of the nuclei of goldenberry and *Pisum sativum cv*. Ctirad populations (2C = 9.06 pg as internal reference standard) were analyzed simultaneously. Each peak denoted by the numbers 1 or 2 corresponds to the populations of nuclei in the G0/G1 phase of each species. Each histogram includes the percentage of the coefficient of variation (CV) and average absolute fluorescence for each of these peaks.

vum cv. Ctirad of the wild and cultivated populations in Figure 7. The nuclear DNA estimation content based on the amount of the 2C internal standard (9.09pg of DNA) displayed a substantial variation between both groups (Table 2).

4. DISCUSSION

The determination of the number of rDNA sites and their distribution on chromosomes by FISH has proven to be a reliable molecular cytogenetic marker when establishing genetic relationships of plant species that contain a significant number of small and homogenous chromosomes (Su et al. 2020). The current study

Table 2. Flow cytometry was used to quantify the nuclear DNA content and genome size of wild and cultivated populations of *Physalis peruviana* L. using *Pisum sativum cv*. Ctirad as the internal reference standard.

Population	n(R)	Average Values		CV (%)
		Nuclear DNA content 2C ± DE (pg)	Genome size 1C ± DE (x 10^9 pb)**	
Wild	8(2)	12.955 ± 0.086*	6.335 ± 0.041*	2.124±0.360
Cultivated		13.262 ± 0.087*	6.485 ± 0.042*	1.960±0.216

The n represents the sample number, while the R represents the number of repetitions per sample, CV: The coefficient of variation of G0/G1 peaks in flow cytometry histograms.
* Indicates a significant difference between values of the same column $p < 0.05$.
** 1pg DNA equal to 0.978×10^9 bp, according to Doležel et al. (2007).

found a substantial difference in the number of 5s and 45s rDNA sites between wild and cultivated populations of goldenberry (Figure 3). This is the first mapping of the 45s rDNA in *Physalis peruviana* L., where a number of two, four, and six sites have been reported for all the populations, prevailing a number of four sites in wild populations and two sites in cultivated populations. This intraspecific variation at the species level, as well as its variability among the populations analyzed, in terms of the number of 45s rDNA sites, can be explained by its nature as a fragile location within the plant genome (Huang et al. 2012). This trait increases the likelihood of chromosomal breakage in the rDNA region, resulting in a chromosomal rearrangement. Furthermore, because of its repetitive nature, it is an unstable genomic region that promotes homologous recombination (Rosato et al. 2016), resulting in increased variability in the amount of 45S rDNA sites within the genome. This variability has also been observed in other Solanaceae species, such as *Solanum* spp., which presents between 2 and 4 sites (Pendinen et al. 2008; Rego et al. 2009; Moyetta et al. 2017); *Capsicum* spp., which presents between 2, 4, 6, 8 up to 16, 24, 28, 36 sites (Youn-Kyu et al. 1999; Kwon and Kim 2009; Romero-da et al. 2017); *Nicotiana* spp. likewise shows considerable variety, reporting between 2, 4, 6, and, to a lesser extent, 8 and 10 sites (Lim et al. 2000; Nakamura et al. 2001; Kitamura et al. 2001; Matyasek et al. 2003; Kovarik et al. 2004). All of this suggests that the number of 45s rDNA sites of 2, 4, and 6 reported in the populations studied in this research is within the predicted range for the Solanaceae family. The variation between wild and cultivated populations was influenced by the unstable character of the 45s rDNA region and was favored by the selective breeding process throughout the domestication of the species (Doebley et al. 2006).

In comparison to the number of 45s rDNA sites, the number of 5s rDNA sites has been found to remain reasonably consistent throughout most angiosperm and gymnosperm taxa (Garcia et al. 2017). This is consistent with the findings of the current study, which found a predominance of six 5s rDNA sites in all goldenberry populations, with a small fraction of four and seven sites in the cultivated populations (Figure 3). Similarly, Siles et al. (2021) discovered a prevalence of six 5s rDNA sites in goldenberry cultivated populations. However, other Solanaceae species have a lower number of 5s rDNA sites, such as *Solanum spp.*, which has a prevalence of two 5s rDNA sites and, to a lesser extent, four sites (Pendinen et al. 2008; Rego et al. 2009; Aguilera et al. 2016; Romero-da et al. 2017), *Capsicum spp.*, which typically presents two sites (Youn-Kyu et al. 1999; Park et al. 2000; Kwon and Kim 2009; Aguilera et al. 2016; Romero-da et al. 2017); *Nicotiana spp.*, which generally alternates between two and four sites (Nakamura et al. 2001; Kitamura et al. 2001; Fulneček et al. 2002; Matyasek et al. 2003; Kovarik et al. 2004), and *Cestrum spp.*, which has two 5s rDNA sites (Fregonezi et al. 2006; Fernandes et al. 2009; Urdampilleta et al. 2014). These data indicate that goldenberry populations have more 5s rDNA sites than other species in the family. However, not enough studies have been conducted on this clade or the *Physalis* genus to be conclusive. Furthermore, the presence of a considerable number of 5s rDNA sites may be due to ribosomal gene copy number amplification during crossing over or transposition events (Kapitonov and Jurka 2003; Su et al. 2020). In accordance to prior research (Garcia et al. 2017), the majority of angiosperm species have more 45s rDNA sites than 5s rDNA sites; this is also shown with molecular cytogenetic results in the Solanaceae family to a higher extent (Vitales et al. 2017). This contrasts with the findings in the goldenberry populations analyzed in this study, which revealed six 5s rDNA sites and two/four 45s rDNA sites (Figure 3). Therefore, these results will be helpful as precedents for the molecular cytogenetic characterization of the genus *Physalis* and the cytotaxonomic understanding of the Solanaceae family.

The nine types of chromosomes that contain rDNA (Figure 5) allowed cytogenetic distinction of wild and cultivated populations of goldenberries (Figure 6). These discrepancies are indistinguishable when only the chromosomal number (2n=48) is taken in consideration. Therefore, FISH significantly reduces the number of chromosomes that must be studied to determine cytoge-

netic differences, emphasizing its relevance in the study of plant species with multiple chromosomes (Su et al. 2020). The existence of different chromosomes in wild (*S1*, *S2*, and *S3*) and cultivated (*C1*, *C2*, and *CC*) populations could be attributed to multiple breeding events that happened throughout the domestication of the species, leading to chromosomal rearrangements (Bashir et al. 2018; Su et al. 2020). There is no variation in the presence of rDNA-containing chromosomes between the two wild populations, implying that there is no structure between these populations. However, discrepancies in the frequency of chromosomes *S1* and *S2* may imply a slight genetic distinction between the two wild populations as a result of their geographical separation (Figure 1). The presence of the *CC* chromosome in Cajamarca could be explained by the same trait found in cultivated populations (Figure 6). The 5s rDNA locus is present in three different chromosomal sites: telomeric (*N1* and *S3*), interstitial (*C1* and *C2*), and centromeric (*N3*) (Figure 5). The dispersed distribution of 5s rDNA has already been observed in numerous mappings of plant chromosomes (Vitales et al. 2017), which could be linked to the activity of mobile genetic elements such as transposons, which have been detected in association with ribosomal gene sequences (Kapitonov and Jurka 2003; Raskina et al. 2004). The 45s rDNA, on the other hand, has a more restricted position, with telomeric (*N2* and *CC*) and interstitial (*S1* and *S2*) sites reported (Figure 6). This more restricted variability is typical of angiosperms, and it has also been revealed that the 45s rDNA ancestral location is terminal (Garcia et al. 2017). The 45s rDNA locus is known to be involved in the creation of nucleoli during the cell cycle, especially if it is positioned in the satellite sections of the chromosomes since it correlates with the existence of nucleolar organizing areas (Long and Dawid 1980; Lopez et al. 2021). If the species has a large number of 45S sites, however, not all of them will be functional (Grabiele et al. 2018; Báez et al. 2020). In the current investigation, no satellites correlated with an active 45s rDNA locus were found in the analyzed chromosomes, however, the *N2* chromosome is likely to contain a functional locus because it is conserved in all goldenberry populations and is the only type of chromosome that has a 45s rDNA locus in the Ayacucho cultivated population. Garcia et al. (2017) propose that if a karyotype has a single 5s or 45s rDNA locus, that locus must be functional.

The fluorescence intensity of the rDNA probes that are highlighted in the differentiation of chromosome types *C1* and *C2*, as well as between *CC* and *N2*, could be related to the number of rDNA tandem repeats that they have between the loci, implying that low fluorescence would indicate a comparatively low number of repeats (Prado et al. 1996). Furthermore, events such as amplification, deletion, and unequal crossing over are known to change the number of repeats and result in variations in fluorescence signals (Su et al. 2020).

Differences in the amount of nuclear DNA between wild and cultivated populations of goldenberries may be due to the domestication process of the species, since artificial selection during domestication affects the evolution of its genome (Díez et al. 2013). The results reported in this study (Table 2) for the wild (12,955 pg) and cultivated (13,262 pg) populations are higher than those reported by Liberato et al. (2014) in Colombian accessions (between 5.77 pg and 8.12 pg). The values of the cultivated populations in this study are remarkably close to those published by Trevisani et al. (2018) in Colombian (13.29 pg), Brazilian (13.32 pg), and Peruvian populations (13 pg). It is important to note that all of these studies report a 2n=48 chromosome number. This could imply that they are related to the same population of goldenberries, however, more research into the molecular cytogenetics of these groups is needed to prove it. According to the plant genome size database (Pellicer and Leitch 2020), estimations of the amount of 2C DNA for the Solanaceae family are 5.68 ± 5.27 pg. This would place the *Physalis peruviana* L. populations studied here in the same family as the Solanaceae species with large genome sizes. Expansion of transposons, particularly retrotransposons with lengthy terminal repeats, has been identified as one of the main causes of genome size disparities in plants (Michael 2014), in addition to genome doubling events during speciation (Wendel et al. 2016). Based on the amount of nuclear DNA content in the goldenberry, all of our findings would be indicative of potential biodiversity and should be considered for future genomic investigations.

This is the first study in Peru to use molecular cytogenetics and genome size to compare wild and cultivated goldenberry populations. In conclusion, the molecular cytogenetic technique revealed a genetic variation in the number of 45s rDNA sites between wild and cultivated populations, as well as different types of chromosomes containing rDNA. This genetic difference was also detected when the amount of nuclear DNA in the populations was compared.

Finally, the findings show no difference between populations from different places, both for cultivated and wild, suggesting that they probably originated from the same population. This study also underlines the utility of these methodologies for analyzing and characterizing the genetic variability of *Physalis peruviana* L. populations, and it will be valuable for future genetic, genom-

ic, and phylogenetic research on this species, as well as the design of genetic improvement programs.

GEOLOCATION INFORMATION

The cultivated populations of goldenberry were collected from the province of Huanta (Department of Ayacucho) (GPS: 13°01'50.9"S, 74°10' 14.3"W) and the province of San Pablo (Department of Cajamarca) (GPS: 7°05'43.9"S, 78°49'13.9"W), the wild populations from the province of Huamanga (Department of Ayacucho) (GPS: 13°03'53.5"S, 74°09'13.1"W) and San Marcos (Department of Cajamarca) (GPS: 7°20'47.3"S, 78°06'0.3"W). The research procedure was developed in the Faculty of Biological Sciences of the UNMSM, Lima (GPS: 12°03'35.1"S, 77°04'55.6"W).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Ing. Gean Carlo Ciprian Salcedo of the Program for Research and Social Projections in Cereals and Native Grains of the Universidad Nacional Agraria La Molina in measuring the genome size using flow cytometry. The authors would like to express their gratitude to the AgroAndino company in Cajamarca and the Andrea family in Ayacucho for providing us with goldenberry samples. Finally, we would like to express our gratitude to the UNMSM Vice Rectorate for Research and Postgraduate Studies for providing study financing.

FUNDING

The Vice Rectorate for Research and Postgraduate Studies at the National University of San Marcos supported this research under the Research Projects Program for Research Groups (N°. RR-03202-R-18, project code B18100191) and the Program for the Promotion of Undergraduate Thesis. (N°. RR-4692-19, project code B19100214).

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(1): 117-125.

Aliyeva-Schnorr L, Ma L, Houben A. 2015. A fast air-dry dropping chromosome preparation method suitable for FISH in plants. Journal of Visualized Experiments. 106: e53470.

Azeez S, Faluyi JO. 2019. Karyotypic studies of four *Physalis* species from Nigeria. Acta Botánica Hungarica. 61(1-2): 5-9.

Báez M, Souza G, Guerra M. 2020. Does the chromosomal position of 35S rDNA sites influence their transcription? A survey on *Nothoscordum* species (Amaryllidaceae). Genetics and molecular biology. 43(1): e20180194.

Bashir T, Chandra Mishra R, Hasan MM, Mohanta TK, Bae H. 2018. Effect of hybridization on somatic mutations and genomic rearrangements in plants. International journal of molecular sciences. 19(12): 3758.

Carbajal Y, Bonilla H, Siles-Vallejos M, López A. 2021. Comparative cytogenetics of *Physalis peruviana* in three cultivated populations from Cajamarca, Peru. Revista peruana de biología. 28(2):e20462.

Ciprian-Salcedo GC, Jimenez J, Zolla G. 2020. Flow cytometry applications in plant breeding. Revista Peruana De Biología. 27(1): 079-084.

Díez CM, Gaut BS, Meca E, Scheinvar E, Montes-Hernandez S, Eguiarte LE, Tenaillon MI. 2013. Genome size variation in wild and cultivated maize along altitudinal gradients. The new phytologist. 199(1): 264–276.

Doebley JF, Gaut BS, Smith BD. 2006. The molecular genetics of crop domestication. Cell. 127(7): 1309–1321.

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols. 2(9): 2233–2244

Fernandes T, de Almeida Rego L do NA, Nardy M, Yuyama PM, Vanzela ALL. 2009. Karyotype differentiation of four *Cestrum* species (Solanaceae) revealed by fluorescent chromosome banding and FISH. Genetics and molecular biology. 32(2): 320–327.

Fitzpatrick M. 2021. The Open Lab Book. Release 1.0. Readthedocs. p. 50-53;[accessed 2022 Apr 24]. https://buildmedia.readthedocs.org/media/pdf/theolb/latest/theolb.pdf

Franco CV, Liberato Guio SA, Sanchez Betancourt EP, García Arias FL, Nuñez Zarantes VM. 2021. Cytogenetic and cytological analysis of Colombian cape gooseberry genetic material for breeding purposes. Caryologia. 74(3): 21-30.

Fregonezi JN, Fernandes T, Torezan JMD, Vieira AOS, Vanzela ALL. 2006. Karyotype differentiation of four *Cestrum* species (Solanaceae) based on the physical mapping of repetitive DNA. Genetics and molecular biology. 29(1): 97–104.

Fulneček J, Lim KY, Leitch AR, Kovařík A, Matyášek R. 2002. Evolution and structure of 5S rDNA loci in allotetraploid *Nicotiana tabacum* and its putative parental species. Heredity. 88(1): 19-25.

Garcia S, Kovařík A, Leitch AR, Garnatje T. 2017. Cytogenetic features of rRNA genes across land plants: analysis of the Plant rDNA database. The Plant journal: for cell and molecular biology. 89(5): 1020–1030.

Grabiele M, Debat HJ, Scaldaferro MA, Aguilera PM, Moscone EA, Seijo JG, Ducasse DA. 2018. Highly GC-rich heterochromatin in chili peppers (*Capsicum* -Solanaceae): A cytogenetic and molecular characterization. Scientia Horticulturae. 238: 391–399.

Hadziavdic K, Lekang K, Lanzen A, Jonassen I, Thompson EM, Troedsson C. 2014. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PLoS One. 9(2):e87624.

Herrera JC. 2007. La citogenética molecular y su aplicación en el estudio de los genomas vegetales. Agronomía colombiana. 25(1): 26–35.

Huang M, Li H, Zhang L, Gao F, Wang P, Hu Y, Yan S, Zhao L, Zhang Q, Tan J, Liu X, He S, Li L. 2012. Plant 45S rDNA clusters are fragile sites and their instability is associated with epigenetic alterations. PLoS One. 7(4): e35139.

Jedrzejczyk I, Rewers M. 2020. Identification and genetic diversity analysis of edible and medicinal Malva species using flow cytometry and ISSR molecular markers. Agronomy. 10(5): 650.

Kapitonov VV, Jurka J. 2003. A novel class of SINE elements derived from 5S rRNA. Molecular biology and evolution. 20(5): 694–702.

Kitamura S, Inoue M, Shikazono N, Tanaka A. 2001. Relationships among *Nicotiana* species revealed by the 5S rDNA spacer sequence and fluorescence in situ hybridization. Theoretical and applied genetics. 103(5): 678–686.

Kovarik A, Matyasek R, Lim KY, Skalická K, Koukalová B, Knapp S, Chase M, Leitch AR. 2004. Concerted evolution of 18-5.8-26S rDNA repeats in Nicotiana allotetraploids. Biological journal of the Linnean Society. Linnean Society. 82(4): 615–625.

Kwon J-K, Kim B-D. 2009. Localization of 5S and 25S rRNA genes on somatic and meiotic chromosomes in *Capsicum* species of chili pepper. Molecules and Cells. 27(2): 205–209.

Liberato S, Sánchez-Betancourt E, Argüelles JH, González C, Núñez VM, Barrero LS. 2014. Cytogenetics of *Physalis peruviana* L. and *Physalis floridana* Rydb. genotypes with differential response to Fusarium oxysporum. Corpoica Ciencia & Tecnología Agropecuaria. 15(1): 51-61.

Lim KY, Matyásek R, Lichtenstein CP, Leitch AR. 2000. Molecular cytogenetic analyses and phylogenetic studies in the *Nicotiana* section Tomentosae. Chromosoma. 109(4): 245–258.

Long EO, Dawid IB. 1980. Repeated genes in eukaryotes. Annual review of biochemistry. 49(1): 727–764.

Lopez FB, Fort A, Tadini L, Probst AV, McHale M, Friel J, Ryder P, Pontvianne FDR, Pesaresi P, Sulpice R, et al. 2021. Gene dosage compensation of rRNA transcript levels in *Arabidopsis thaliana* lines with reduced ribosomal gene copy number. The plant cell. 33(4): 1135–1150.

Matyasek R, Lim KY, Kovarik A, Leitch AR. 2003. Ribosomal DNA evolution and gene conversion in *Nicotiana rustica*. Heredity. 91(3): 268–275.

Michael TP. 2014. Plant genome size variation: bloating and purging DNA. Briefings in functional genomics. 13(4): 308–317.

Mirzaghaderi G, Marzangi K. 2015. IdeoKar: an ideogram constructing and karyotype analyzing software. Caryologia. 68(1)31-35.

Moyetta NR, Urdampilleta JD, Chiarini FE, Bernardello GL. 2017. Heterochromatin and rDNA patterns in *Solanum* species of the Morelloid and Dulcamaroid clades (Solanaceae). Plant biosystems. 151(3): 539–547.

Nakamura R, Kitamura S, Inoue M, Ohmido N, Fukui K. 2001. Karyotype analysis of *Nicotiana kawakamii* Y. Ohashi using DAPI banding and rDNA FISH. Theoretical and applied genetics. 102: 810–814.

Park YK, Park KC, Park CH, Kim NS. 2000. Chromosomal localization and sequence variation of 5S rRNA gene in five *Capsicum* species. Molecules and Cells. 10(1): 18–24.

Pellicer J, Hidalgo O, Dodsworth S, Leitch IJ. 2018. Genome size diversity and its impact on the evolution of land plants. Genes (Basel). 9(2): 88.

Pellicer J, Leitch IJ. 2020. The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist. 226(2): 301-305.

Pendinen G, Gavrilenko T, Jiang J, Spooner DM. 2008. Allopolyploid speciation of the Mexican tetraploid potato species *Solanum stoloniferum* and *S. hjertingii* revealed by genomic in situ hybridization. Genome. 51(9): 714-20.

Poggio L, Confalonieri V, Comas C, Gonzalez G, Naranjo CA. 2000. Evolutionary relationships in the genus *Zea*: analysis of repetitive sequences used as cytological FISH and GISH markers. Genetics and Molecular Biology. 23(4):1021–1027.

Prado EA, Faivre-Rampant P, Schneider C, Darmency MA. 1996. Detection of a variable number of ribosomal DNA loci by fluorescent in situ hybridization in *Populus* species. Genome. 39(5): 1020–1026.

Raskina O, Belyayev A, Nevo E. 2004. Activity of the *En/Spm*-like transposons in meiosis as a base for chromosome repatterning in a small, isolated, peripheral population of *Aegilops speltoides* Tausch. Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology. 12(2): 153–161.

Rego LN, da Silva CRM, Torezan JM, Gaeta ML, Vanzela AL. 2009. Cytotaxonomical study in Brazilian species of *Solanum, Lycianthes* and *Vassobia* (Solanaceae). Plant systematics and evolution. 279: 93–102.

Rodríguez NC, Bueno ML. 2006. Study of the cytogenetic diversity of *Physalis peruviana* L. (Solanaceae). Acta Biológica Colombiana, Bogotá. 11(2): 75-85.

Romero-da MV, Urdampilleta JD, Forni ER, Moscone EA. 2017. Cytogenetic markers for the characterization of *Capsicum annuum* L. cultivars. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology. 151(1): 84-91.

Rosato M, Kovařík A, Garilleti R, Rosselló JA. 2016. Conserved organisation of 45S rDNA sites and rDNA gene copy number among major clades of early land plants. PloS one. 11(9): e0162544.

Salim D, Gerton JL. 2019. Ribosomal DNA instability and genome adaptability. Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology. 27(1–2): 73–87.

Sánchez EP. 2014. Ploidy Level of Cape Gooseberry Plants from Anther Cultivation. [master's thesis]. Bogotá: Universidad Nacional de Colombia.

Sierra y Selva Exportadora. 2021. Analysis of the Goldenberry Market from 2015 to 2020. Peru: Unidad de Inteligencia Comercial; 9-18.

Siles M, Bracamonte O, García M, López A. 2021. The first report on the identification of the 5s rDNA locus in *Physalis peruviana* L. chromosomes. Scientia Agropecuaria. 12(2): 149-153.

Singh N, Singh S, Maurya P, Arya M, Khan F, Dwivedi DH, Saraf SA. 2019. An updated review on *Physalis peruviana* fruit: Cultivational, nutraceutical and pharmaceutical aspects. Indian Journal of Natural Products and Resources. 10(2): 97-110.

Su D, Chen L, Sun J, Zhang L, Gao R, Li Q, Han Y, Li Z. 2020. Comparative chromosomal localization of 45S and 5S rDNA sites in 76 purple-fleshed sweet potato cultivars. Plants. 9(7): 865.

Trevisani N, Melo, de Melo RC, Oliveira PM, Colli MP, Meirelles JL, Frederico A. 2018. Ploidy and DNA content of cape gooseberry populations grown in southern Brazil. Caryologia. 71(4): 414–419.

Urdampilleta J, Chiarini F, Stiefkens L, Bernardello G. 2014. Chromosomal differentiation of Tribe Cestreae (Solanaceae) by analyses of 18-5.8-26S and 5S rDNA distribution. Plant Systematics and Evolution. 301(5): 1325–1334.

Vitales D, D'Ambrosio U, Gálvez F, Kovařík A, Garcia S. 2017. Third release of the plant rDNA database with updated content and information on telomere composition and sequenced plant genomes. Plant Systematics and Evolution. 303(8): 1115–1121.

Volkov RA, Zanke C, Panchuk II, Hemleben V. 2001. Molecular evolution of 5S rDNA of *Solanum* species (sect. *Petota*): application for molecular phylogeny and breeding. Theoretical and Applied Genetics. 103: 1273–1282.

Wendel JF, Jackson SA, Meyers BC, Wing RA. 2016. Evolution of plant genome architecture. Genome biology. 17(1): 37.

Youn-Kyu P, Kim B-D, Kim B-S, Armstrong KC, Kim N-S. 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(4): 149–157.

ONLINE SUPPLEMENTARY MATERIAL

Figure S1. Chromosomes from the 12 best metaphases among the populations of goldenberries studied.

https://drive.google.com/file/d/1FzgeeRgtUxS2GkD588N8m3cSwzF8Ss1_/view?usp=sharing