Quinoa Cytogenetics

4

Bożena Kolano and Maja Orzechowska

Abstract

Chenopodium quinoa has rather a small genome (2.973 pg/2C DNA) and 36 mostly metacentric and submetacentric chromosomes. Like many other plants from the Chenopodiaceae family, it is a polysomatic species. Quinoa is an allotetraploid and its diploid parental taxa, which were suggested by GISH and molecular phylogenetic analyses, belong to the two different evolutionary lineages that are assigned as genome A and B. Two families of rRNA gene loci, 35S and 5S rDNA, were located on the different chromosome pairs for most of the analyzed *Chenopodium* species. In the quinoa karyotype, two pairs of 5S rDNA loci and only one pair of 35S rDNA loci were detected. The nrITS sequences were of a B-genome ancestry and the 35S rRNA gene locus was observed in the chromosome of the B subgenome. The chromosomal localization of other repetitive sequence, clone *12–13P* containing minisatellite repeats, was mainly restricted to pericentromeric regions of all quinoa chromosomes; however, the obtained hybridization signals showed variable intensity suggesting a difference in copy number of the repeat among the chromosomes of the karyotype. Recent analysis showed that sequences similar to the *12–13P* clone are also present in other diploid and polyploid species from *Chenopodium s.s.*. Hybridization signals of retrotransposons were enriched in pericentromeric regions of the quinoa chromosomes and were ordinarily excluded from the distal parts of the chromosomes. Two other repeats (*pTaq10* and *18–24 J*) showed also dispersed distribution in chromosomes of *C. quinoa* although they did not show homology to any known mobile elements. The amplification of the *pTaq10* repeat appears to be characteristic for chenopods with the A genome. However, hybridization signals of *pTaq10* were found on each *C. quinoa* chromosome. A second dispersed repetitive sequence, *18–24 J*, was present in most analyzed *Chenopodium s.s.* species. In quinoa genome hybridization signals of *18–24 J* repeat was mainly restricted to the chromosomes of B subgenome.

The genomes of eukaryotes are composed of multiple chromosomes. The chromosome is composed of a linear DNA molecule and associated proteins that pack the long DNA thread into a more denser and compact structure (Heslop-Harrison and Schwarzacher 2011). The number and structure of a chromosome complement (karyotype) is unique for each plant species

B. Kolano (✉) · M. Orzechowska
Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice, Katowice 40-007, Poland
e-mail: bozena.kolano@us.edu.pl

S. M. Schmöckel (ed.), *The Quinoa Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-65237-1_4

(Heslop-Harrison and Schwarzacher 2011). In plants, the nuclear DNA contains in addition to single- or low-copy coding sequences also different classes of repetitive DNAs, which can be found in hundreds or even thousands units in the genome (Heslop-Harrison and Schwarzacher 2011). Repetitive DNAs are largely responsible for the "C-value paradox" (the complexity of an organism is not associated with the size of its genome; Hawkins et al. 2008). To date, the significance of repetitive DNA, with respect to structure and function, is still poorly understood, although biological roles have been suggested for its specific families, for example, gene regulation, recombination, the maintenance of chromosome structure or a centromere function (Li et al. 2017).

Chromosomal diversification within genera has been a focal point of plant evolutionary studies (Schubert and Vu 2016). There are two prime reasons for this interest. First, a chromosomal change imparts a partial or complete barrier to an interspecific gene exchange. Second, chromosomal characteristics may provide clues to the relationships of species. Several mechanisms account for the high variability of number, length and shape of chromosomes, as well as in DNA content among eukaryotes (Schubert and Vu 2016). Polyploidy and dysploidy are considered to be the most important mechanisms responsible for high variation in chromosome number in plant (De Storme and Mason 2014). Polyploidy refers to the presence of more than two genomes and is a driving force in plant evolution that can induce various genetic and epigenetic alternation leading to reorganization of the polyploid nuclei (Leitch and Leitch 2008). It is now believed that a polyploidization event occurred in all angiosperms at least once in their history and some evolutionary lineages show evidence of several such events (Weiss-Schneeweiss et al. 2013; Jiao et al. 2011; Soltis and Soltis 2009). Polyploidy may induce a variety of chromosomal, genic and epigenetic changes that can take place quickly over a several generations and result in diploid-like chromosome pairing, segregation and gene function (Weiss-Schneeweiss et al. 2013; Soltis and Soltis 2009; Leitch and Leitch 2008). The shape and size of chromosomes can be modified by structural rearrangements (e.g., reciprocal translocations, pericentric inversions, etc.) or by sequence amplification/loss (Schubert and Vu 2016). Chromosome rearrangements can also change the chromosome numbers, a phenomenon called ascending or descending dysploidy (increase or decrease in chromosome numbers, respectively; Schubert and Vu 2016; De Storme and Mason 2014).

Studying the karyotype of selected species involves analyzing chromosome numbers, centromere position and the number and position of secondary constrictions and other chromosomal markers, e.g., banding patterns and the position of various DNA sequences that are uncovered by fluorescence *in situ* hybridization (FISH). Using FISH with single-copy (BAC-clones) and various repetitive DNA sequences (e.g., rDNA or satellite sequences), detailed physical chromosomal maps can be constructed that enable evolutionary patterns and processes to be determined (Kolano et al. 2013b; Hřibová et al. 2010; Lysak et al. 2006).

4.1 Genome Constitution of Polyploid *C. Quinoa* and Related Species

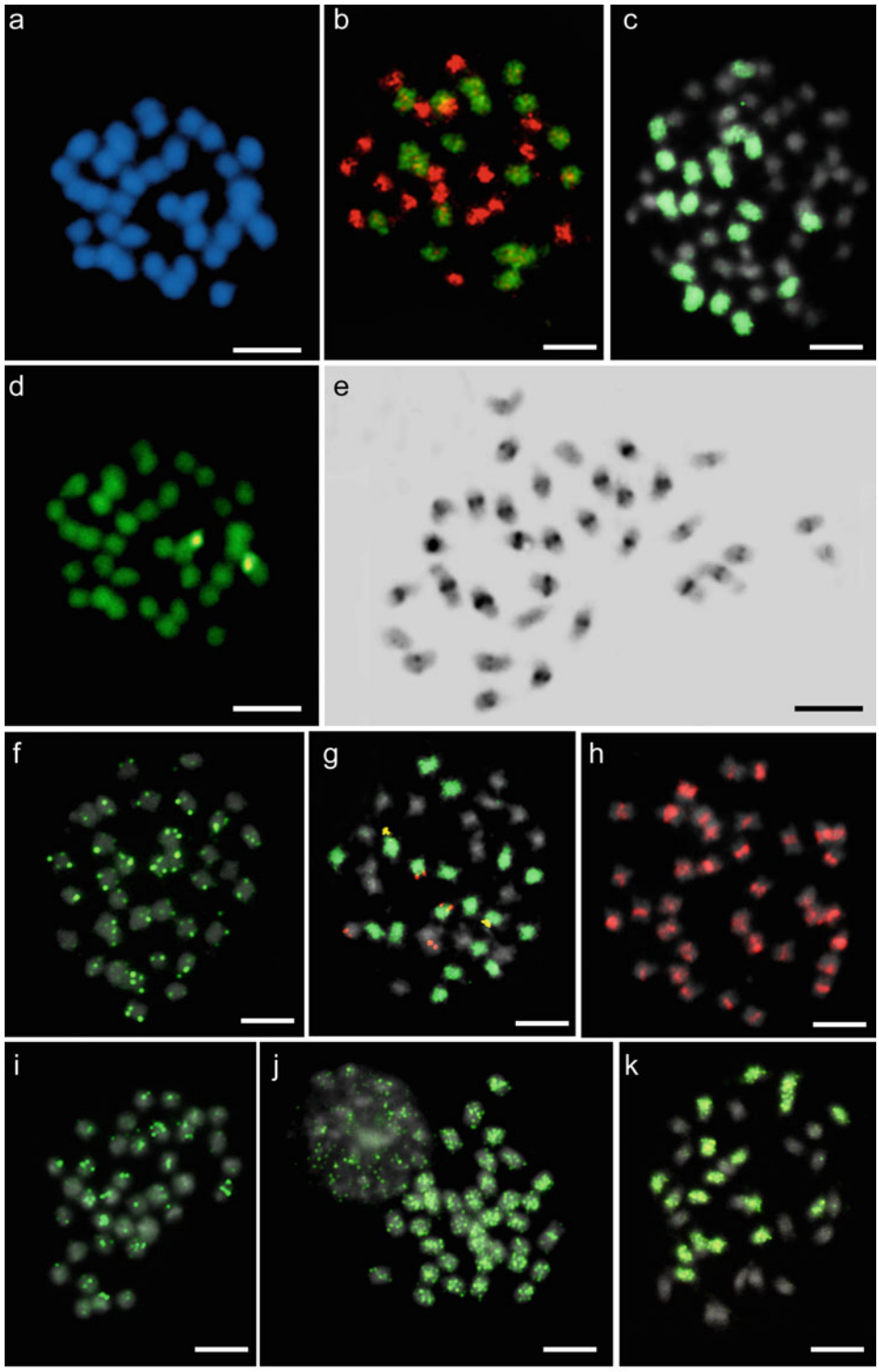
Quinoa belongs to *Chenopodium sensu stricto* (*Chenopodium s.s.*; Fuentes-Bazan et al. 2012). All species of this genus have the basic chromosome number $x = 9$. The diploid *Chenopodium s.s.* species ($2n = 2x = 18$) can be divided into three evolutionary lineages: (1) American diploids with the A genome (e.g., *C. hians*, *C. pallidicaule*); (2) the Old World species with the B genome (e.g., *C. ficifolium* and *C. suecicum*) and (3) *C. vulvaria*, which was recovered as a sister taxon to the rest of the *Chenopodium s.s.* diploids (Kolano et al. 2015; Walsh et al. 2015). In *Chenopodium s.s.*, as in most plant groups, polyploidization has a significant impact on speciation, and several polyploid species

representing three different polyploidy levels (tetraploid e.g., *C. berlandieri*; $2n = 4x = 36$; hexaploid e.g., *C. album*, 2n = 6x = 52 and decaploid e.g., *C. frutescens* $2n = 10x = 90$) have been described (Kolano et al. 2012b; Jellen et al. 2011; Lomonosova 2005). Polyploidy is generally classified into two types—allopolyploidy, which origin involves interspecific hybridization, and autopolyploidy consisting of more than two the same genomes. Whether autopolyploids or allopolyploids are more widespread among plants is a question that still remains unresolved (Weiss-Schneeweiss et al. 2013). Quinoa, which displays a disomic inheritance, is an allotetraploid, with $2n = 4x = 36$ chromosomes (Fig. 4.1a; Ward 2000). The allotetraploid nature of *C. quinoa* and the closely related *C. berlandieri* was supported by molecular phylogenetic studies that were based on different plastid and nuclear markers (nrITS, 5S rDNA NTS; *Flowering Locus T-Like* and *Salt Overly Sensitive 1*) and genomic *in situ* hybridization (GISH), which enable the parental subgenomes to be differentiated in the karyotype of a polyploid (Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015). A combination of molecular phylogenetic analyses and GISH showed that the two parental taxa of *C. quinoa* and *C. berlandieri* belong to two various genome pools. One of the parental species contributing the maternal genome was similar to extant American diploids (genome A, maternal genome), whereas, the second ancestral species was similar to contemporary species from Eurasia (B genome, paternal genome; Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015). The paternal species that contributed the B subgenome for *C. quinoa* resembled the present *C. ficifolium* or a closely related species (Fig. 4.1b), whereas the origin of the A-subgenome progenitor seems to be more vague (Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015). The ancestral species of the A subgenome of *C. quinoa* and *C. berlandieri* possible resembled extant *C. standleyanum*, *C. incanum* or *C. fremontii* based on molecular phylogenetic studies (Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015). In contrast, GISH suggested species similar to *C. watsonii* or *C. nevadense* for *C. quinoa* and *C. watsonii* for *C. berlandieri* as the A-genome progenitors (Fig. 4.1b; Kolano et al. 2016). B-genome diploid (*C. ficifolium* or related species) was also inferred to be one of the ancestral species of Eurasian hexaploids (*C. album, C. giganteum, C. pedunculare*; Fig. 4.1c; Krak et al. 2016; Walsh et al. 2015; Kolano et al. 2019).

4.2 Karyotype and Chromosome Banding

C. quinoa has 36 small and poorly differentiated chromosomes. Metacentric and submetacentric chromosomes whose lengths range between approximately 1–3 μm dominate in its karyotype (Palomino et al. 2008; Bhargava et al. 2006; Kolano et al. 2001). Palomino et al. (2008), basing on chromosome morphology, distinguished nine groups of chromosomes supporting the tetraploid origin of quinoa genome. The numerous small chromosomes of *C. quinoa* and other chenopods make cytogenetic studies that are based on procedures such as simple chromosome staining followed by karyotype analysis very challenging. Also, the scarcity of chromosome markers has made it difficult to match chromosomes in homologous pairs. Other techniques, such as chromosome banding, are useful tools for chromosome identification and they often permit conclusions about chromosomal evolution to be drawn (Guerra 2000). Various banding techniques allow to reveal a series of consistent landmarks along the length of metaphase chromosomes that allow identification of homologous chromosome pairs. Three different banding techniques are most often used in plant cytogenetics: C-banding, double-fluorescent banding (CMA_3/DAPI) and silver staining (Guerra 2000).

C-banding has been used to stain constitutive heterochromatin in metaphase chromosomes (Tanaka and Taniguchi 1975). In plant species that have relatively large genomes and appreciable amounts of constitutive heterochromatin (e.g., *Triticum* or *Avena*), this technique produces rich banding patterns that often allows


a
b
c
d
e
f
g
h
i
j
k

◀ **Fig. 4.1** Somatic metaphase chromosomes of (**a**, **b**, **d**–**k**) *Chenopodium quinoa* and (**c**) *C. album*; **a** DAPI staining (from Kolano et al. 2001); **b** double GISH with gDNA isolated from the B-genome diploid *C. ficifolium* (green) and the A-genome diploid *C. watsonii* (red); **c** GISH with gDNA isolated from the B-genome diploid *C. ficifolium*; **d** differential fluorescent staining with chromomycin A3, CMA_3^+ bands indicate large GC-rich regions of chromatin (from Kolano et al. 2001); **e** C-bands indicate constitutive heterochromatin; **f** FISH with telomeric repeats; **g** GISH/FISH with gDNA isolated from *C. ficifolium* (green), 35S rDNA (yellow) and 5S rDNA (red); **h** FISH with the *12-13P* repeat; **i** FISH with the *rt* clone of Ty1-*copia* retrotransposons; **j** FISH with dispersed repetitive sequence *pTaq10*; **k** FISH with dispersed repetitive sequences *18–24 J*. Scale bar = 5 μm

identification of single chromosomes and can display morphological or karyotypic variations, such as large chromosomal rearrangements and aneuploidies (Jellen 2016; Gill and Kimber 1974). However, in the *C. quinoa* karyotype, as in many other plants with a small genome size, C-bands and constitutive heterochromatin are present only in the pericentromeric regions of chromosomes (Fig. 4.1e). Other types of heterochromatin bands could be demonstrated using a GC-specific fluorochrome—chromomycin A_3 (CMA_3). Double-fluorescent staining with CMA_3 and DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) allowed to distinguish GC-rich and AT-rich chromatin region (Schweizer 1876). Two CMA_3^+ bands were revealed in one pair of homologous chromosomes of quinoa (Fig. 4.1d). These CMA_3^+ bands co-localized with secondary constrictions and 35S rRNA gene loci (Kolano et al. 2001). A similar organization of GC-rich chromatin (colocalization with 35S rDNA locus) was also observed in other analyzed chenopods (*C. album, C. ficifolium, C. berlandieri*) and appears to be very often observed in many other angiosperms (e.g., *Amaranthus*; Kolano et al. 2001, 2013b; Guerra 2000; Maragheh et al. 2019). Silver staining, a marker of the transcriptional activity of 35S rRNA genes, revealed the presence of two distally located silver-positive bands in one pair of quinoa chromosomes. One or two nucleoli were observed in the interphase nuclei. However, most frequently, one nucleoli was present (Kolano et al. 2001). Thus, classical banding methods have not been very effective as chromosome markers and have allowed to identify only one pair of homologous chromosomes in *C. quinoa*. The elaboration of new chromosome markers using fluorescent *in situ* hybridization (FISH) with various DNA sequences was necessary for better understanding of chenopod karyotype structure and evolution.

4.3 Genome Size

Genome size (typically measured in picograms or as the total number of nucleotide base pairs in megabases) is one of the fundamental biological characters of all living organisms. The DNA 1C-value for a species is the amount of nuclear DNA in the unreplicated haploid genome of a gamete (Greilhuber et al. 2005). Genome size varies approximately 2,400-fold in angiosperms, ranging from 0.065 pg/1C of DNA in *Genlisea margaretae* to 152.23 pg/1C in *Paris japonica* (Pellicer et al. 2010; Greilhuber et al. 2006). *Chenopodium s.s*. consists of plants with small or very small genomes, which range between 0.47 pg/1C DNA (diploid *C. vulvaris*) and 3.22 pg/1C DNA (decaploid *C. frutescens*; Mandak et al. 2016; Kolano et al. 2015; Bhargava et al. 2007). The analysis of genome size in a phylogenetic background enabled to hypothesize about the direction and trends of genome size evolution during the diversification of the diploid species of *Chenopodium s.s.* The distribution of genome sizes in this genus reflects the phylogenetic grouping into three different evolutionary lineages. The Eurasian diploids (genome B) have 70% larger genomes than the diploids from the Americas (genome A). The smallest genome size among *Chenopodium s.s.* was estimated for *C. vulvaria* (Kolano et al. 2015).

There is very little data on the evolution of the genome sizes of polyploid chenopods. The mean *C. quinoa* genome size is 2C = 2.973 pg (Kolano et al. 2012a). Polyploidization can generate quick and often directional changes to the subgenomes

of allopolyploids that often result in genome downsizing (Leitch and Bennett 2004). This phenomenon, reported for many polyploid angiosperms, is usually due to the elimination of repetitive sequences or duplicated genes (Leitch and Bennett 2004). However, the genome size of the allotetraploid *C. quinoa* and other analyzed polyploid chenopods (*C. berlandier* and *C. album*) fit almost perfectly into the estimated additive values of the hypothetical parental species (Kolano et al. 2016; Mandák et al. 2012).

Although genome size is known to be quite constant feature of a species there are more and more studies showing significant intraspecific variation in this characteristic (e.g., *Tanacetum vulgare* or *Arabidopsis thaliana;* Schmuths et al. 2004; Keskitalo et al. 1998). An intraspecific genome size polymorphism also exists in *C. quinoa* (Kolano et al. 2012a). A statistically significant variation in genome size was found among the 20 accessions of *C. quinoa* collected in Peru, Bolivia, Ecuador, Argentina and Chile. The greatest intraspecific difference found between those accessions with the largest genome size and the smallest genome size was nearly 6%. The largest genome had the accession from Chile (1.539 pg/1C) and the smallest was found in the Peruvian accession (1.452 pg/1C). The intraspecific variation in genome size found in *C. quinoa* was rather small when compared to that observed in *Tanacetum vulgare* (27%; Keskitalo et al. 1998); however, it is consistent with other studies that investigated genome size polymorphisms in species that have small genomes, such as *A. thaliana* (Schmuths et al. 2004). The intraspecific polymorphism in genome size was reported to be correlated with various ecological conditions prevailing in specific areas (e.g., altitude or latitude and longitude; Knight et al. 2005). The cultivated populations of quinoa groups into two main clusters—the Andean highland and the coastal lowland from Chile (Christensen et al. 2007). No correlation was observed between the genome size and the geographical origin of the accessions (although the quinoa accessions analyzed in this study belonged to both the highland and lowland groups), possible due to the relatively large variation of genome size revealed among the highland quinoa populations collected on the Altiplano near Lake Titicaca in Bolivia and Peru (Kolano et al. 2012a).

The main reasons for the intraspecific and interspecies genome size polymorphism are polyploidy, the presence of B chromosomes and a different amplification of the repetitive DNA sequences (Hawkins et al. 2008). All of the analyzed *C. quinoa* accessions were tetraploid with $2n = 4x = 36$ chromosomes and there were no B chromosomes in the analyzed accessions. Thus, the main reason for intraspecific genome size polymorphism could be an amplification or reduction in the copy number of the repetitive DNA sequences in the genomes of the analyzed quinoa accessions (Greilhuber 2005).

4.4 Repetitive Sequence Organization and Evolution

Angiosperms vary in the proportion of DNA repetitive sequences in their genomes. Species with small genomes have a relatively small amount of repetitive DNAs, whereas in larger genomes, such as *Zea mays*, at least 85% of the genomes are repetitive sequences (Ibarra-Laclette et al. 2013 Schnable et al. 2009). Repetitive DNA elements can be divided into two major groups based on their genomic organization—dispersed and tandem repeats. Dispersed repetitive DNA elements are distributed throughout the whole chromosomes, intermingled with other sequences although some regions of chromosomes can show reduced or increased number of copies. The second group includes sequences that are organized in tandem repeating units in which the monomers are arranged adjacent to each other to form tandem arrays (Kubis et al. 1998).

4.4.1 Tandem Repetitive Sequences

Tandem repetitive sequences include rRNA genes, telomeric repeats and a very heterogenic group of satellite DNAs (Kubis et al. 1998). *C.*

quina, as many angiosperm species, has arabidopsis-type telomeric sequence. After probing with telomeric repeats, small but discrete signals were observed in all chromosome ends however the hybridization signals had various intensities, revealing the copy number variation among the chromosomes of the same complement (Fig. 4.1f).

rRNA genes are the most extensivly studied repetitive sequences in *Chenopodium s.s.* The 35S and 5S rDNA units consist of conserved genic regions encoding for 35S rRNA (18S–5.8S–25S rDNA) and 5S ribosomal RNA (5S rDNA) and fast evolving transcribed and non-transcribed spacer regions that are arranged as tandem arrays at one or more loci (Volkov et al. 2004). The coding sequences of rRNA genes as evolutionarily highly conserved regions are most often used as chromosome markers in cytogenetic analyses of non-model organisms (Weiss-Schneeweiss et al. 2013;). On the other hand, the non-coding sequences of rRNA genes evolve very quickly and they are often used in phylogenetic analyses (Álvarez and Wendel 2003). Combining a phylogenetic analysis with cytogenetic studies allows to improve the understanding of the evolution of 35S and 5S rDNA loci organization. Our studies indicated that the 35S rDNA and 5S rDNA sequences are organized in a low number of loci, mostly in the subterminal position of the chromosome arm in *Chenopodium* species (Kolano et al. 2015; Kolano et al. 2012b). An analysis of the chromosomal organization of rDNA loci in a phylogenetic context showed that the ancestral states for the diploid *Chenopodium s.s.* taxa were one 35S rDNA locus and one 5S rDNA locus per haploid chromosome set, both located in subterminal positions on different chromosomes. This pattern was also observed in several extant diploids from *Chenopodium s.s.* (e.g., *C. watsonii* or *C. nevadense*; Kolano et al. 2016; Kolano et al. 2015). The number of 5S rDNA loci increased from ancestral state (one locus) to two in the common ancestor of the B-genome diploids since most of them have two 5S rDNA loci (Kolano et al. 2015). Although all of the A-genome diploids had one locus of each rRNA gene, rearrangements of rDNA loci were found in some species. The pattern of 5S rDNA loci observed in two South American species, *C. pallidicaule* and *C. petiolare,* is likely the results of translocation from subterminal to interstitial position in chromosomes. The repositioning of the 35S rDNA locus to the chromosome with a subterminally located 5S rDNA appears to be most likely explanation of the chromosomal organization of rDNA loci revealed in *C. standleyanum* (Kolano et al. 2015, 2016).

The rDNA loci pattern in allopolyploid species are showed to evolve quickly and often the number of rDNA loci in an allopolyploid is not the sum of the loci of its ancestral species (Weiss-Schneeweiss et al. 2013). Polyploids often show genome rearrangements that involve the loss/acquisition of the 35S rDNA repeats or the silencing of rDNA sites as well as interlocus recombinations and complete or near-complete repeat replacements (Sochorová et al. 2017; Weiss-Schneeweiss et al. 2013; Kovarik et al. 2004). The direction of the conversion and homogenization of 35S rDNA can be incline toward the one of the ancestral taxa and may vary in the allopolyploids of the same parentage but independent origin (Sochorová et al. 2017; Weiss-Schneeweiss et al. 2013; Kovarik et al. 2004).

Neither *C. quinoa* nor closely related *C. berlandieri* showed an additive number of rRNA gene loci; but they both experienced a reduction in the number of 35S rDNA loci (Fig. 4.1g). *C. quinoa* has one locus of 35S rDNA in a subterminal position of chromosome (Maughan et al. 2006). Similarly, most analyzed *C. berlandieri* accessions also exhibited only one subterminally located locus of 35S rDNA (this species showed limited polymorphism in 35S rDNA loci number and some accession had two loci of 35S rDNA; Maughan et al. 2006). In both *C. quinoa* and *C. berlandieri*, only the 35S rDNA loci placed in the chromosomes of the B subgenome (paternal parent) were observed. The nrITS sequences were also of a B-genome ancestry (Fig. 4.1g; Kolano et al. 2016). The 35S rDNA loci from the maternal A subgenome was lost in these two allotetraploid (Kolano et al. 2016). A different

pattern of 35S rDNA loci evolution was exchibited by the Eurasian allohexaploids *C. album* and *C. giganteum*. The number of 35S rDNA loci in their genomes seems to be equal to the sum of the loci of their ancestral taxa. However, sequence analysis indicated that nrITS was in most analyzed accessions the subject of homogenization and that only one maternal type of nrITS existed in this hexaploid taxa (Krak et al. 2016; Kolano et al. 2019).

Unlike 35S rDNA, the interlocus homogenization of 5S rDNA has not been yet described for any polyploid systems that were studied (Weiss-Schneeweiss et al. 2013; Weiss-Schneeweiss et al. 2012). However, the number and position of 5S rDNA loci may be changed in the karyotype of a polyploid relative to its putative ancestral taxa (Weiss-Schneeweiss et al. 2012; Clarkson et al. 2005). The number of 5S rDNA loci in analyzed American allotetraploids (*C. quinoa* and *C. berlandieri*) and Eurasian allohexaploids (*C. album* and *C. giganteum*) seems to be additive as compared with their ancestor species and the 5S rDNA NTS sequences have not undergone homogenization (Kolano et al. 2016, 2019). In the *C. quinoa* karyotype, the chromosomes of B subgenome bear the subterminal locus of 5S rDNA like in the B-genome diploids. The second 5S rDNA locus in the A subgenome was observed in an interstitial position in place of subterminal position observed in its hypothetical ancestor species (Fig. 4.1g). The observed incongruence can suggest two scenarios: (i) different species (related to *C. watsonii* or *C. nevadense)* which possessed an interstitial 5S rDNA locus could have been the ancestral taxon or (ii) the localization of 5S rDNA was changed in chromosomes of A subgenome of *C. quinoa* after polyploidization. In *C. berlandieri* karyotype the localization of the 5S rDNA loci was unchanged compared to its putative diploid parents (Kolano et al. 2016).

A clone *12–13P* containing minisatellite repeats, was localized in pericentromeric regions of all quinoa chromosomes using FISH. The obtained hybridization signals showed variable intensity, thus suggesting a differences in copy number of the repeats among the chromosomes of the karyotype (Fig. 4.1h; Kolano et al. 2011). The minisatelite repeats from *12–13P* showed a high sequence similarity to the *Beta corolliflora* minisatellite pBC1447 (Gao et al. 2000). Minisatellites are short, usually 10 to 100 bp, tandemly repetitive sequences, which are predominantly localized in the subterminal or pericentromeric regions of chromosomes (Shcherban 2015; Gao et al. 2000). Recent analysis showed that sequences similar to the *12–13P* clone were present in the genomes of most diploid and polyploid species that belong to all *Chenopodium s.s.* lineages, while they were absent in the remaining lineages of *Chenopodium sensu lato* (*Chenopodiastrum, Oxybasis, Blitum, Dysphania, Lipandra*). This suggests that the *12–13P* sequence may be specific to *Chenopodium s. s.* (Orzechowska et al. 2018). In all analyzed species hybridization signals of *12–13P* were present in pericentromeric regions of all chromosomes (Orzechowska et al. 2018).

4.4.2 Disperse Repetitive Sequences

Disperse repetitive sequences primarily include transposable elements (DNA transposons and retrotransposons; Bennetzen and Wang 2014). Retrotransposons that multiple their copy number when transpose to new genome locations are particularly abundant in plant genomes and it has been clear for some time that these transposable elements have the major impact on plant genome size variation (Lisch 2013). To date, the chromosomal organization of the conserved domain of the reverse transcriptase (*rt*) of the LTR retrotransposons (both Ty1-*copia* and Ty3-*gypsy*) has been analyzed in the *C. quinoa* genome (Kolano et al. 2013a). The number of copies of the particular LTR retroelement families can differ remarkably from a few copies to thousands of copies in plant genomes (Du et al. 2010). *C. quinoa* has a relatively small genome and among isolated retrotransposon sequences only one family of Ty1-*copy* retrotransposons was highly

amplified. All of the other analyzed *rt* clones were present in the quinoa genome in a low number of copies (Kolano et al. 2013a).

Previous surveys of LTR retrotransposons divided the Ty1-*copia* elements isolated from plant genomes into four major evolutionary lineages (*Tork, Sire, Oryco* and *Retrofit*). Among Ty3-*gypsy* retrotransposons six major evolutionary lineages were distinguished (*Del, Reina, Galadriel, CRM, Athila* and *Tat*; Llorens et al. 2009). To date, four Ty1-*copia* lineages were found in the quinoa genome but among isolated clones dominate elements from *Tork* and *Retrofit* lineages. Most of the elements from the Ty3-*gypsy* superfamily that have been identified belong to *Del* lineage (Kolano et al. 2013a). The chromosomal organization of retrotransposons varies and depends on the retrotransposon families and the host species. The retrotransposons in the *C. quinoa* chromosomes showed an uneven distribution (Fig. 4.1i). Relatively strong hybridization signals were observed on approximately half of the chromosomes, whereas the rest of the chromosomes had only a very weak or no signal. The hybridization signals of retrotransposons were mainly observed in the pericentromeric regions of the chromosomes and were usually not detectable in the distal parts of the chromosomes. In the interphase nuclei, the hybridization signals were mainly observed in the heterochromatic regions (Kolano et al. 2013a).

Following the insertion of a retrotransposons into a genome, nucleotide substitutions accumulate in the elements and they undergo fragmentation over time. These sequences have a dispersed organization in a genome, similar to the transposable elements; however, their nucleotide sequences show a very low or no similarity to the transposable elements (Menzel et al. 2008; Kubis et al. 1998). Two sequences with disperse organization and without homology to known retrotransposons were described in the *C. quinoa* genome. One of these, *pTaq10*, was spread throughout the 36 chromosomes (Fig. 4.1j). Discrete hybridization signals (although weak) were shown in the pericentromeric, interstitial or terminal localizations on the chromosome arms (Kolano et al. 2008a). Hybridization signals of the *pTaq10* clone were present in the interphase nuclei, particularly in the heterochromatic regions. Although such localization of *pTaq10* may suggested that these repeats were present in genomes of both hypothetical ancestors of *C. quinoa*, however our recent studies suggested that *pTaq10* is present only in species with the A genome and that it is not present in the B-genome diploids (Orzechowska et al. unpublished). FISH analyses indicated that the hybridization signals for 5S rDNA and *pTaq10* partly overlapped, thus suggesting partial co-localization of these two repetitive sequences in some chromosome regions. Fiber-FISH, allowing mapping with higher resolution, showed that in *C. quinoa* chromosomes most of the 5S rDNA arrays were free of *pTaq10* repeats, except for a part of the interstitial 5S rDNA locus where *pTaq10* and 5S rDNA repeats co-localized (Kolano et al. 2008a). This interstitial 5S rDNA locus is placed very close or even partly overlaps with the pericentromeric heterochromatin, which implies that at least part of the locus is transcriptionally inactive (Kolano et al. 2008a).

Another dispersed repetitive sequence with no homology to any known retroelements is clone *18–24 J,* which is highly amplified in only half of the quinoa chromosome complements (18 chromosomes; B subgenome; Fig. 4.1k). Similar results were obtained in the related species *C. berlandieri* and the Eurasian hexaploid *C. album* (Kolano et al. 2011). Such a localization could suggest that *18–24 J* is specific to one parental genome of these species. However, a recent study on the *18–24 J* repeat, which comprised a putative ancestral species of *C. quinoa*, revealed the presence of the *18–24 J* repeat in the B-genome diploids as well as in most of the A-genome diploids. Moreover, this repeat was also present in genomes of species which are more distantly related to quinoa (*C. vulvaria* and *Lipandra polysperma*; Orzechowska et al. 2018). The pattern of the *18–24 J* chromosomal localization in *C. quinoa* and the related allotetraploid *C. berlandieri* might be explained in two ways. First, the level of the *18–24 J* repeat amplification in the A-genome ancestral species may have been very low, as has been observed in the case

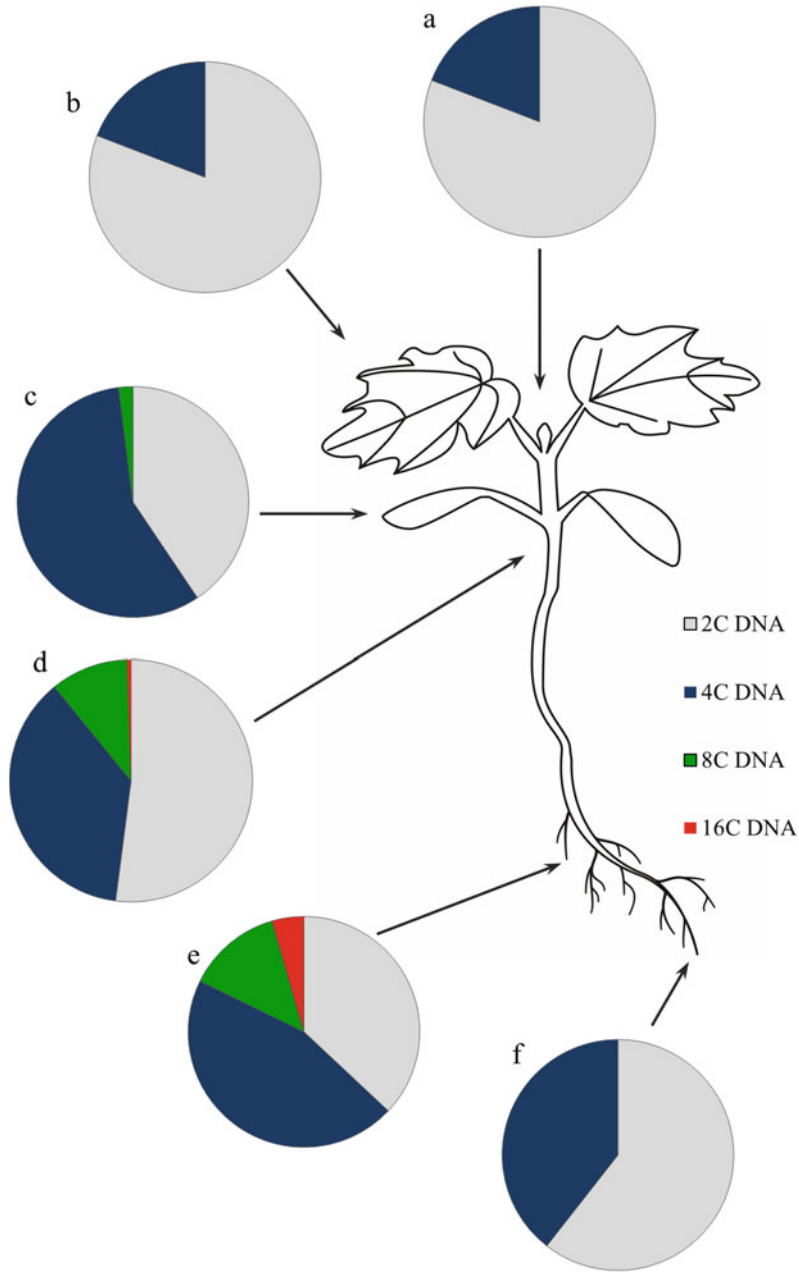


Fig. 4.2 Comparison of the endopolyploidy patterns in quinoa seedlings; **a** shoot apex; **b** young leaf; **c** cotyledons; **d** hypocotyl; **e** differentiated part of a root; **f** root apex (based on data published in Kolano et al. 2009)

of some putative ancestral species of the A subgenome (e.g., *C. watsonii;* Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015; Orzechowska et al. 2018). On the other hand, *18–24 J* repeats may have been eliminated from the A subgenome during the evolution of the allopolyploid genomes, because another putative ancestral species, *C. nevadense*, has shown a high amplification of *18–24 J* (Kolano et al. 2016; Storchova et al. 2015; Walsh et al. 2015; Orzechowska et al. 2018). A preferential elimination of various repetitive sequences (retroelements, rDNA) was reported for a number of allopolyploids, e.g., tetraploid *Nicotiana tabacum* or *Melampodium* species (Weiss-Schneeweiss et al. 2012; Kovarik et al. 2004).

4.5 *Chenopodium quinoa* Is a Polysomatic Plant

Polysomaty concern the plant organ/tissue which consists of cells which differ in ploidy levels (endopolyploid cells; Traas et al. 1998). Endopolyploidy is a term that described the outcome of the multiplication of nuclear DNA without cell division and plays an important role in determining cell fate and in interactions with symbiotic and pathogenic organisms (De Veylder et al. 2011). In single plant, different organs can vary in patterns of endopolyploidization. Some species revealed endopolyploid cells in most organs, whereas in other taxa, endopolyploidyzation is very limited and present only in few organs. Moreover, endopolyploidy patterns are usually correlated with plant development (Maluszynska et al. 2012).

C. quinoa, similar to many other species from Chenopodiaceae (e.g., *C. album, Spinacia oleracea*, *Beta vulgaris*), is a polysomatic plant (Kolano et al. 2008b, 2009; Barow and Meister 2003). Endopolyploid nuclei appear very early in quinoa plant development, already in matured embryo radicle (Kolano et al. 2009). During successive seedling development, the pattern of endopolyploidization differed between organs and changed over time (Fig. 4.2). The highest level of endopolyploidization was present in the hypocotyls and primary roots of quinoa seedlings whereas in the leaves and the shoot apex the endopolyploid cells were absent (Fig. 4.2; Kolano et al. 2009). The lack of endopolyploidization at the shoot apex, reported for many angiosperms, could be one of the mechanisms that protect the genetic stability of the germ line (Maluszynska et al. 2012; Kudo and Kimura 2001). The high degree of endopolyploidyzation in hypocotyls and primary roots possible is connected with large amount of vascular tissue in these organs (Kolano et al. 2009). The increase of endopolyploidy observed in root and hypocotyl during germination and seedling development seems to be correlated with their quick elongation and with vascular tissue development (Alarcón and Salguero 2017; Kolano et al. 2009).

References

Alarcón MV, Salguero J (2017) Transition zone cells reach G2 phase before initiating elongation in maize root apex. Biol Open 6:909–913

Álvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. Mol Phylogenet Evol 29:417–434

Barow M, Meister A (2003) Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. Plant Cell Environ 26:571–584

Bennetzen JL, Wang H (2014) The contributions of transposable elements to the structure, function, and evolution of plant genomes. Annu Rev Plant Biol 65:505–530

Bhargava A, Shukla S, Ohri D (2006) Karyotypic studies on some cultivated and wild species of *Chenopodium* (Chenopodiaceae). Genet Resour Crop Evol 53:1309–1320

Bhargava A, Shukla S, Ohri D (2007) Genome size variation in some cultivated and wild species of *Chenopodium* (Chenopodiaceae). Caryologia 60:245–250

Christensen SA, Pratt DB, Pratt C, Nelson PT, Stevens MR, Jellen EN et al (2007) Assessment of genetic diversity in the USDA and CIP-FAO international nursery collections of quinoa (*Chenopodium quinoa* Willd.) using microsatellite markers. Plant Genet Resour 5:82–95

Clarkson JJ, Lim KY, Kovarik A, Chase MW, Knapp S et al (2005) Long-term genome diploidization in allopolyploid *Nicotiana* section *Repandae* (Solanaceae). New Phytol 168:241–252

De Storme N, Mason A (2014) Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant Biol 1:10–33

De Veylder L, Larkin JC, Schnittger A (2011) Molecular control and function of endoreplication in development and physiology. Trends Plant Sci 16:624–634

Du J, Tian Z, Hans CS, Laten HM, Cannon SB et al (2010) Evolutionary conservation, diversity and specificity of LTR-retrotransposons in flowering plants: insights from genome-wide analysis and multi-specific comparison. Plant J 63:584–598

Fuentes-Bazan S, Uotila P, Borsch T (2012) A novel phylogeny-based generic classification for *Chenopodium sensu lato*, and a tribal rearrangement of Chenopodioideae (Chenopodiaceae). Willdenowia 42 (1):5–24

Gao D, Schmidt T, Jung C (2000) Molecular characterization and chromosomal distribution of species-specific repetitive DNA sequences from *Beta corolliflora*, a wild relative of sugar beet. Genome 43:1073–1080

Gill BS, Kimber G (1974) Giemsa C-banding and the evolution of wheat. Proc Natl Acad Sci USA 71:4086–4090

Greilhuber J (2005) Intraspecific variation in genome size in angiosperms: identifying its Existence. Ann Bot 95:91–98

Greilhuber J, Borsch T, Müller K, Worberg A, Porembski S, Barthlott W (2006) Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. Plant Biol 8:770–777

Greilhuber J, Doležel J, Lysák MA, Bennett MD (2005) The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-Value' to describe duclear DNA contents. Ann Bot 95:255–260

Guerra M (2000) Patterns of heterochromatin distribution in plant chromosomes. Genet Mol Biol. 23:1029–1041

Hawkins JS, Grover CE, Wendel JF (2008) Repeated big bangs and the expanding universe: directionality in plant genome size evolution. Plant Sci 174:557–562

Heslop-Harrison JS, Schwarzacher T (2011) Organisation of the plant genome in chromosomes. Plant J 66:18 33

Hřibová E, Neumann P, Matsumoto T, Roux N, Macas J et al (2010) Repetitive part of the banana (*Musa acuminata*) genome investigated by low-depth 454 sequencing. BMC Plant Biol 10:204–204

Ibarra-Laclette E, Lyons E, Hernandez-Guzman G, Perez-Torres CA, Carretero-Paulet L et al (2013) Architecture and evolution of a minute plant genome. Nature 498:94–98

Jellen EN (2016) C-banding of plant chromosomes. In: Kianian SF, Kianian PMA (eds) Plant Cytogenetics: methods and protocols. Springer, New York, pp 1–5

Jellen EN, Kolano B, Sederberg MC, Bonifacio A, Maughan PJ (2011) *Chenopodium*. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin Heidelberg, pp 34–62

Jiao YN, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L et al (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97–U113

Keskitalo M, Lindén A, Valkonen JPT (1998) Genetic and morphological diversity of Finnish tansy (*Tanacetum vulgare* L., Asteraceae). Theor Appl Genet 96:1141–1150

Knight CA, Molinari NA, Petrov DA (2005) The large genome constraint hypothesis: evolution, ecology and phenotype. Ann Bot 95:177–190

Kolano B, Bednara E, Weiss-Schneeweiss H (2013a) Isolation and characterization of reverse transcriptase fragments of LTR retrotransposons from the genome of *Chenopodium quinoa* (Amaranthaceae). Plant Cell Rep 32:1575–1588

Kolano B, Gardunia BW, Michalska M, Bonifacio A, Fairbanks D., Maughan PJ, Coleman CE, Stevens MR, Jellen EN, Maluszynska J (2011) Chromosomal localization of two novel repetitive sequences isolated from the *Chenopodium quinoa* Willd. genome. Genome 54(9):710–717

Kolano B, McCann J, Orzechowska M, Siwinska D, Temsch E et al (2016) Molecular and cytogenetic evidence for an allotetraploid origin of *Chenopodium quinoa* and *C. berlandieri* (Amaranthaceae). Mol Phylogenet Evol 100:109–123

Kolano B, McCann J, Oskedra M, Chrapek M, Rojek M, Nobis A, Weiss-Schneeweiss H (2019) Parental origin and genome evolution of several Eurasian hexaploid species of Chenopodium (Chenopodiaceae). Phytotaxa 392:163–185

Kolano B, Pando LG, Maluszynska J (2001) Molecular cytogenetic studies in *Chenopodium quinoa* and *Amaranthus caudatus*. Acta Soc Bot Pol 70:85–90

Kolano B, Plucienniczak A, Kwasniewski M, Maluszynska J (2008a) Chromosomal localization of a novel repetitive sequence in the *Chenopodium quinoa* genome. J Appl Genet 49:313–320

Kolano B, Saracka K, Broda-Cnota A, Maluszynska J (2013b) Localization of ribosomal DNA and CMA3/DAPI heterochromatin in cultivated and wild *Amaranthus* species. Sci Hortic 164:249–255

Kolano B, Siwinska D, Gomez Pando L, Szymanowska-Pulka J, Maluszynska J (2012a) Genome size variation in *Chenopodium quinoa* (Chenopodiaceae). Plant Syst Evol 298:251–255

Kolano B, Siwinska D, Maluszynska J (2008b) Comparative cytogenetic analysis of diploid and hexaploid *Chenopodium album*. Acta Soc Bot Pol 77:293–298

Kolano B, Siwinska D, Maluszynska J (2009) Endopolyploidy patterns during development of *Chenopodium quinoa*. Acta Biol Cracov Bot 51:85–92

Kolano B, Siwinska D, McCann J, Weiss-Schneeweiss H (2015) The evolution of genome size and rDNA in diploid species of *Chenopodium s.l.* (Amaranthaceae). Bot J Linn Soc 179:218–235

Kolano B, Tomczak H, Molewska R, Jellen EN, Maluszynska J (2012b) Distribution of 5S and 35S rRNA gene sites in 34 *Chenopodium* species (Amaranthaceae). Bot J Linn Soc 170:220–231

Kovarik A, Matyasek R, Lim KY, Skalicka K, Koukalova B et al (2004) Concerted evolution of 18–5.8–26S rDNA repeats in *Nicotiana allotetraploids.* Biol J Linn Soc 82:615–625

Krak K, Vit P, Belyayev A, Douda J, Hreusova L et al (2016) Allopolyploid origin of *Chenopodium album s. str.* (Chenopodiaceae): a molecular and cytogenetic insight. Plos One 11

Kubis S, Schmidt T, Heslop-Harrison JS (1998) Repetitive DNA elements as a major component of plant genomes. Ann Bot 82:45–55

Kudo N, Kimura Y (2001) Patterns of endopolyploidy during seedling development in cabbage (*Brassica oleracea* L.). Ann Bot 87:275–281

Leitch AR, Leitch IJ (2008) Genomic plasticity and the diversity of polyploid plants. Science 320:481–483

Leitch IJ, Bennett MD (2004) Genome downsizing in polyploid plants. Biol J Linn Soc 82:651–663

Li SF, Su T, Cheng GQ, Wang BX, Li X et al (2017) Chromosome evolution in connection with repetitive sequences and epigenetics in plants. Genes 8:290

Lisch D (2013) How important are transposons for plant evolution? Nat Rev Genet 14:49–61

Llorens C, Muñoz-Pomer A, Bernad L, Botella H, Moya A (2009) Network dynamics of eukaryotic

LTR retroelements beyond phylogenetic trees. Biol Direct 4:41

Lomonosova MN (2005) Chromosome numbers of Chenopodiaceae species from Russia and Kazakhstan. Botanicheskii Zhurnal 90:1132–1134

Lysak M, Berr A, Pecinka A, Schmidt R, McBreen et al (2006) Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related *Brassicaceae species*. Proc Natl Acad Sci USA 103:5224–5229

Maluszynska J, Kolano B, Sas Nowosielska H (2012) Endopolyploidy in plants. In: Leitch IJ, Greilhuber J, Dolezel J, Wendel J (eds) Plant Genome Diversity. Springer, Wien, pp 99–119

Mandak B, Krak K, Vit P, Pavlikova Z, Lomonosova MN et al (2016) How genome size variation is linked with evolution within *Chenopodium sensu lato*. Perspect Plant Ecol 23:18–32

Mandák B, Trávníček P, Paštová L, Kořínková D (2012) Is hybridization involved in the evolution of the *Chenopodium album* aggregate? An analysis based on chromosome counts and genome size estimation. Flora 207:530–540

Maragheh FP, Janus D, Senderowicz M, Haliloglu K, Kolano B (2019) Karyotype analysis of eight cultivated *Allium* species. J Appl Genet 60:1–11

Maughan P, Kolano BA, Maluszynska J, Coles ND, Bonifacio A et al (2006) Molecular and cytological characterization of ribosomal RNA genes in *Chenopodium quinoa* and *Chenopodium berlandieri*. Genome 49:825–839

Menzel G, Dechyeva D, Wenke T, Holtgräwe D, Weisshaar B et al (2008) Diversity of a complex centromeric satellite and molecular characterization of dispersed sequence families in sugar beet (*Beta vulgaris*). Ann Bot 102:521–530

Orzechowska M, Majka M, Weiss-Schneeweiss H, Kovařík A, Borowska-Zuchowska N, Kolano B (2018) Organization and evolution of two repetitive sequences, 18-24 J and 12-13P, in the genome of Chenopodium (Amaranthaceae). Genome 61:643–652

Palomino G, Hernández LT, de la Cruz Torres E (2008) Nuclear genome size and chromosome analysis in *Chenopodium quinoa* and *C. berlandieri* subsp. *nuttalliae*. Euphytica 164:221

Pellicer J, Fay MF, Leitch IJ (2010) The largest eukaryotic genome of them all? Bot J Linn Soc 164:10–15

Schnable PS, Ware D, Fulton RS, Stein JC, Wei FS et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Schmuths H, Meister A, Horres R, Bachmann K (2004) Genome size variation among accessions of *Arabidopsis thaliana*. Ann Bot 93:317–321

Schubert I, Vu GTH (2016) Genome stability and evolution: attempting a holistic view. Trends Plant Sci 21:749–757

Schweizer D (1876) Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma 58:307–324

Shcherban AB (2015) Repetitive DNA sequences in plant genomes. Russ J Genet 5:159–167

Sochorová J, Coriton O, Kuderová A, Lunerová J, Chèvre AM et al (2017) Gene conversion events and variable degree of homogenization of rDNA loci in cultivars of *Brassica napus*. Ann Bot 119:13–26

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol 60:561–588

Storchova H, Drabesova J, Chab D, Kolar J, Jellen EN (2015) The introns in FLOWERING LOCUS T-LIKE (FTL) genes are useful markers for tracking paternity in tetraploid *Chenopodium quinoa* Willd. Genet Resour Crop Evol 62:913–925

Tanaka R, Taniguchi K (1975) A banding method for plant chromosomes. Jpn J Genet 50:163–167

Traas J, Hülskamp M, Gendreau E, Höfte H (1998) Endoreduplication and development: rule without dividing? Curr Opin Plant Biol 1:498–503

Volkov R, Medina F, Zentgraf U, Hemleben V (2004) Organization and molecular evolution of rDNA nucleolar dominance and nucleolus structure. In: Esser K, Luttge U, Beyschlag W, Murata J (eds) Progress in botany. Springer, Berlin Heidelberg New York, pp 106–146

Walsh BM, Adhikary D, Maughan PJ, Emshwiller E, Jellen EN (2015) *Chenopodium* polyploidy inferences from Salt Overly Sensitive 1 (SOS1) Data. Am J Bot 102:533–543

Ward SM (2000) Allotetraploid segregation for single-gene morphological characters in quinoa (*Chenopodium quinoa* Willd.). Euphytica 116:11–16

Weiss-Schneeweiss H, Blöch C, Turner B, Villaseñor JL, Stuessy TF et al (2012) The promiscuous and the chaste: frequent allopolyploid speciation and its genomic consequences in american daisies (*Melampodium* sect. *Melampodium*; Asteraceae). Evolution 66:211–228

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenet Genome Res 140:137–150