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Author: Bożena Kolano, Luz Gomez Pando, Jolanta Małuszyńska

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MOLECULAR CYTOGENETIC STUDIES
IN CHENOPODIUM QUINOA AND AMARANTHUS CAUDATUS

BOZENA KOLANO, LUZ GOMEZ PANDO1, JOLANTA MALUSZYNSKA
Department of Plant Anatomy and Cytology, Silesian University
Jagiellonska 28, 40-036 Katowice, Poland
e-mail: maluszyn@us.edu.pl
1University La Molina Lima, Peru
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ABSTRACT

Chenopodium quinoa Wild. and Amaranthus caudatus L., two plant species from South America, have small and numerous chromosomes. Looking for chromosome markers to distinguish pairs of homologous chromosomes double fluorescence staining, in situ hybridization with 45S rDNA and silver staining were applied. Fluorescent in situ hybridization with 45S rDNA has shown two sites of hybridization occurring on one pair of chromosomes in quinoa genome (lines PQ-1, PQ-3). The number of rDNA loci in Amaranthus caudatus L. genome depends on the accession. Kiwicha 3 line has one pair of chromosomes with signals and Kiwicha Molinera cultivar two pairs. All observed rDNA loci were active. After chromomycin/DAPI staining in all cases, except Kiwicha Molinera cultivar, the CMA+ positive bands co-localized with signals of in situ hybridization with rDNA. In Kiwicha Molinera the number of CMA+ bands was higher than the number of 45S rDNA signals after FISH.

KEY WORDS: Amaranthus caudatus, Chenopodium quinoa, chromosomes, cytogenetics, FISH, in situ hybridization, nucleolus, rDNA.

INTRODUCTION

Quinoa (Chenopodium quinoa Wild.) and kiwica (Amaranthus caudatus L.) were cultivated as important pre-Colombian „grains”. Both of these species are annual, self-pollinating C4 plants derived from South America. Until recently they have been cultivated only in the highlands of Argentina, Bolivia, Chile, Colombia, Ecuador and Peru. Today they are becoming more and more popular as crops in different regions. For this reason, they have started to attract scientific attention. Seeds of both species have a high nutritional value and a better amino acid balance than the proteins in most cereals. Their proteins are rich in the essential amino acids such as lysine, methionine, and cysteine, which make them complementary to both cereals and leguminous. They have a wide adaptability to different ecological niches. Several cultivars have been selected for their tolerance to heat and cold as well as for resistance to disease (Popcnoc et al. 1989).

Chenopodium quinoa Wild. (Chenopodiaceae) is a tetraploid species with the chromosome number 2n = 4x = 36 (Wang et al. 1993). There is little information about the genome and karyotype of quinoa. The small size and great number of quinoa chromosomes make cytogenetic analysis difficult.

Amaranthus caudatus L. (Amaranthaceae) is also a tetraploid species. The chromosome number for this species varies. Usually it was described as 2n = 4x = 32 and occasionally as 2n = 4x = 34 (Pal and Khosho 1973). It has been suggested that the gametic chromosome number n = 17 originated from the n = 16 through primary trisomy and the basic chromosome number is x = 8 (Pal et al. 1982). The chromosome number and karyotype of many varieties of these species should be established for better understanding of their origin and relationships, which occurred during evolution and plant breeding programs.

Today molecular cytogenetics offers excellent methods for analysis of plant genomes, even with small chromosomes (Osuji et al. 1997; Hasterok and Maluszynska 2000). One of the most useful methods is fluorescence in situ hybridization (FISH), which allows localisation of different genes and noncoding DNA sequences on chromosomes and in interphase nuclei. rDNA loci are widely used as chromosome markers for karyotyping and studying the evolutionary relationships within genera and families (Lee et al. 1999). In the presented study chromosomes of quinoa and kiwica were examined using in situ hybridization and differential chromosome staining. The number and localization of rDNA loci and their activity for the two species are presented.
MATERIAL AND METHODS

Plant material

Two lines of *Chenopodium quinoa*: PIQ-1 and PIQ-8 and *Amaranthus caudatus*: cultivar Kiwicha Molinere and line Kiwicah were used. Plants were grown in pots in the growth chamber. For cytogenetic analysis young leaves were collected and pretreated with 8-hydroxyquinoline (2 hours at room temp, 2 hours at 4°C) prior to fixation. The material was fixed in ethanol: glacial acetic acid (3:1) and stored at -20°C until use.

For chromosome preparation, the material was washed with 0.01 sodium citrate buffer (pH 4.8) for 15 min and digested with enzyme mixture: cellulase (4% v/v, Onozuka) and pectinase (20% v/v, Sigma) for 2 hours at 37°C. After digestion, the material was washed again with sodium citrate buffer for 30 min. Squash preparations were made in a drop of 60% acetic acid. After freezing the slide and removal of coverslip, preparations were dried overnight and stained with 2 μg/ml 4,6-diamidino-2-phenylindole (DAPI) to check the quality of the chromosome preparations.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization was applied according to the method described by Maluszynska and Heslop-Harrison (1991) with some minor modifications. 18S-25S rDNA isolated from *Arabidopsis thaliana* was used as a probe for in situ hybridization. The DNA probe was directly labelled with Cy3-11-dUTP by nick-translation (Amersham Life Sciences) according to the manufacturer’s instruction.

Prior to FISH, the slides were pretreated with 100 μl/ml RNase for 1 h at 37°C and dehydrated in 70% and 90% ethanol. The chromosomes and hybridization mixture were denatured separately. The chromosomes were denatured in 70% formamide for 5 minutes at 78°C on a hot plate (Hybaid Thermal Cycler PCR – in situ).

The hybridization mixture, consisting of 3 ng/µl of labelled probe 60% (v/v) formamide, 10% (w/v) dextran sulphate and 0.1 mg/ml salmon testes DNA in 2 x SSC (sodium sodium citrate), was denatured at 95°C for 10 minutes and immediately placed on ice for a few minutes. 15 μl of the hybridization mixture were added to each chromosome preparation and covered with a plastic coverslip. Hybridization was carried out at 37°C for about 72 hours. Stringent washing was performed at 40°C in 2 x SSC, 0.1 x SSC and 2 x SSC for 5 min each. Chromosomes were counter-stained with DAPI (2 mg/ml) and mounted in antifade medium CITTIFLUOR (Pelco).

Silver staining

Silver staining was performed following the method of Hizume (1980). Slides were treated with a borate buffer (pH 9.2) for 5 min and air-dried. 50 μl of freshly prepared 50% silver nitrate in distillate water was applied to each preparation. Slides were covered with nylon mesh and incubated in a humid chamber for 20 min at 42°C, washed in distillate water, and air-dried. Preparations were mounted in DPX medium. The number of nucleoli per nucleus was analysed in about 6000 cells for each type of plant.

Fluorescent banding

Fluorescent staining followed the method described by Schweizer (1976). Briefly, material was incubated with 0.5 mg/ml chromomycin A₃ (CMA3) and DAPI (2 mg/ml) for 60 min each with subsequent washing in distilled water. Preparations were mounted in a 1:1 mixture of glycerol and McIlvaine buffer with 2.5 mM MgCl₂. Slides were analysed after two weeks maturation at 37°C and then stored at + 4°C.

Chromosome analysis

Preparations were examined with an OLYMPUS PROVIS epifluorescent microscope using the proper filter set. Photos were taken on KODAK 400 or 100 ASA film and processed with an AnalySIS program (OLYMPUS). Measurements of chromosome length were done using the same AnalySIS programme for 6 metaphase plates, for each line.

RESULTS

The small and numerous chromosomes of quinoa and kiwicha do not facilitate cytogenetic analysis. Additionally, they are difficult to obtain from root tips grown in soil due to root cap resistance to enzyme digestion. Therefore, in the present study young leaves were used for chromosome preparation. The mitotic index in this tissue is relatively high and both metaphase and prometaphase chromosomes can be obtained. The chromosomes of all analysed species are relatively small and very poorly differentiated in their morphology. The centromer position cannot be easily determined. Chromosomes with secondary constriction (NOR-chromosomes) can only be recognised in quinoa chromosome complement.

Both analysed lines of quinoa have 36 small chromosomes (Figs 1a, 2a). Their length falls within the range of 1.0-3.3 μm. The chromosome number of the analysed kiwicha plants is different. Kiwicha 3 has 34 chromosomes (Fig. 3a) and Kiwicha Molinere possesses only 32 chromosomes (Fig. 4a). The size of chromosomes ranges from 0.9 to 2.4 μm.

Double fluorescence staining was applied to metaphase chromosomes and interphase nuclei of all analysed lines. Two CMA-positive (CMA⁺) bands were detected at the satellite chromosomes of both quinoa lines (Figs 1c, 2c). In interphase nuclei two separate signals were also seen (Figs 1f, 2f). Similarly, in Kiwicha 3 CMA⁺ bands were observed at the distal region of two chromosomes (Fig. 3e). In Kiwicha Molinere six chromosomes possess CMA⁺ bands. Four of them were major (Fig. 4e) and seen in all cells, but they differed in size. Two minor bands could be observed after prolonged (for six weeks) maturation of chromosome preparations stained with CMA. In the interphase nuclei the maximum number of CMA⁺ signals observed was equal to the number of bands in the chromosome complement of all analysed plants (Figs 3f, 4f).

After fluorescenc in situ hybridization with 45S rDNA, signals at the distal region of two chromosomes were observed in *Chenopodium quinoa* lines (Figs 1c, 2c). In the interphase nuclei, two separate sites of hybridization were seen (Figs 1d, 2d).

In the *Amaranthus caudatus* chromosomes of Kiwicha 3 line, two rDNA loci were observed, while in the Kiwicha Molinere cultivar, four signals of in situ hybridization were present in all metaphase plates and interphase nuclei (Figs 3c, d, 4c, d). The signals in the Kiwicha Molinere cells differ in their size, which may indicate that one pair of loci possesses more copies of rRNA genes than the other.

Silver staining, an indicator of the transcriptional activity of rDNA, revealed the presence of two distally located silver-positive bands in one pair of quinoa chromosomes of both lines.
(Figs 1g, 2g). In the interphase nuclei one (Figs 1h, 2h) or two nucleoli were observed. However, most frequently one nucleolus was present (Fig. 5).

In *Amaranthus caudatus* in the investigated line and cultivar all loci of rRNA genes were transcriptionally active. Two Ag-positive bands and one or two nucleoli in the interphase nuclei were observed in the Kiwicha 3 cells (Figs 3g, h). The Kiwicha Molinera cultivar had four Ag-NORs and one to four nucleoli in the interphase nuclei (Figs 4g, h). Similarly as after in situ hybridization Ag-NORs were different in size, two small and two larger.

### DISCUSSION

**Chromosome numbers**

Both lines of *Chenopodium quinoa* examined in the present work have chromosome number $2n = 36$ and according to Wang et al. (1993) they are tetraploids. This was also reported $2n = 32$ for *C. quinoa* (Kawatani and Ohno 1950). Among other *Chenopodium* species different ploidy levels with the basic chromosome number $x = 9$ have also been described, e.g. diploid ($2n = 18$) for *C. glaucum* and *C. album*, tetraploid ($2n =$...
Fig. 3. Mitotic chromosomes and interphase nuclei of *Amaranthus caudatus*, Kiwicha 3, LW-95 B.

Fig. 4. Mitotic chromosomes and interphase nuclei of *Amaranthus caudatus*, Kiwicha Molinere LM-95-B
a, b – DAPI staining; c, d – in situ hybridization with 45S rDNA (red) and DAPI counterstaining; e, f – CMA₃ staining; g, h – silver staining.
ly older. The reduction of loci number in polyploid species has been described for several species (Maluszynska and Heslop-Harrison 1993; Leitch et al. 1998).

All rDNA loci of C. quinoa and A. caudatus detected by in situ hybridization are transcriptionally active. In other Amaranthus species (A. cruentus, A. hypochondriacus and A. mantegazzianus) only one pair of NOR chromosomes was described (Greizerstein and Poggio 1994). The number of nucleoli in the interphase nuclei observed in this study indicates a tendency toward fusion in cells of young leaves. One or two nucleoli per nucleus in A. caudatus cells were reported in a previous work (Greizerstein and Poggio 1994). The lower number of nucleoli than number of NOR-chromosomes was observed in many plant genera such as Hypochaeris (Cerbo et al. 1998), Brassica (Hasterok and Maluszynska 2000) and Arabidopsis (Weiss and Maluszynska 1998).

In the investigated plants the 45S rDNA sites co-localise with CMA+ bands. In Kiwicha Molinera the number of CMA+ bands was higher than the number of 45S rDNA sites after FISH. In this genotype CMA+ bands differed in the strength of fluorescence, which may imply that the blocks of GC-rich heterochromatin are of a different size, similarly as the signal after FISH with 45S rDNA and silver staining. Only the two strongest pairs of CMA+ coincide with rDNA sites. This is a common feature among a wide variety of plants. The coincidence of CMA-band and rDNA loci was described for Vicia faba (Huizume 1992), Clivia (Ran et al. 1999) and Brassica species (Hasterok and Maluszynska 2000).

The knowledge of the genome structure of Chenopodium and Amaranthus species is still limited. But recently developed genetic studies should bring new information. Genetic polymorphism has been documented using allozyme analysis (Wilson 1988) and DNA markers (Raus et al. 1999).

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CYTOGENETYKA MOLEKULARNA

CHENOPODIUM QUINOA I AMARANTHUS CAUDATUS

STRESZCZENIE

Genomy dwóch południowoamerykańskich gatunków, Chenopodium quinoa Wild. i Amaranthus caudatus L., charakteryzują się dużą liczbą niewielkich i morfologicznie mało zróżnicowanych chromosomów. Dla opracowania karyotypu tych gatunków niezbędne jest znalezienie cytogenetycznych markerów pozwalających odróżnić pary chromosomów homologicznych. W tym celu zastosowano barwienie fluorescencyjne (CMA/DAPI) i hybrydyzację in situ z 45S rDNA oraz srebrzenie dla określenia jąderkowej aktywności transkrypcyjnej. Po fluorescencyjnej hybrydyzacji in situ z 45S rDNA w genomie C. quinoa (linie PQ-1, PQ-8) obserwowano dwa sygnały występujące w jednej parze chromosomów. Liczba loci rDNA w genomie A. caudatus była różna. W linii Kiwica 3 obserwowano dwa sygnały a u odmiany Kiwica Molinera cztery. Wszystkie obserwowane loci rDNA są aktywnie transkrypcyjnie. We wszystkich przypadkach, za wyjątkiem odmiany Kiwica Molinera, pozycyjne prążki CMA pokrywały się z sygnałami hybrydyzacji w situ. U Kiwica Molinera liczba pozytywnych prążków CMA była większa niż liczba sygnałów po hybrydyzacji z 45S rDNA.

SŁOWA KLUCZOWE: Amaranthus caudatus, Chenopodium quinoa, chromosomy, cytogenetyka, FISH, hybrydyzacja in situ, jąderko, rDNA.