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SHORT COMMUNICATION

Genome size variation in *Chenopodium quinoa* (Chenopodiaceae)

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Abstract The extent and significance of intraspecific genome size variation were analysed in quinoa (*Chenopodium quinoa* Willd.), a pseudocereal important for human consumption in the Andean region of South America. Flow cytometry, with propidium iodide as the DNA stain, was used to estimate the genome size of 20 quinoa accessions from Ecuador, Peru, Bolivia, Argentina, Chile and the USA. Limited genome size variation was found among the analysed accessions. The differences between the accessions were statistically significant but the maximum inter-accession difference between the populations with the largest and the smallest genome reached only 5.9%. The largest genome was found in population C4 from Chile (mean 3.077 pg/2C) and the smallest in the Peruvian population P2 (mean 2.905 pg/2C). The variation was not correlated with collection site; however, the quinoa accessions analysed in this study belonged to three distinct geographical groups: northern highland, southern highland and lowland.

Keywords *Chenopodium quinoa* · Flow cytometry · Genome size · Intraspecific variation

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Introduction

Among angiosperms genome size variation is now approximately 2,400-fold, ranging from 0.065 pg/1C of DNA in *Genlisea margaretae* Hutch. to 152.23 pg/1C in *Paris japonica* Franch. (Greilhuber et al. 2006; Pellicer et al. 2010). Interest in the genome size has been fuelled by the fact that nuclear DNA content can affect various characteristics at the cellular, tissue and organism levels, and can have important ecological and evolutionary consequences (Knight et al. 2005). The DNA C-value for a species is the amount of nuclear DNA in the unreplicated haploid genome of a gamete, and it tends to be characteristic of a taxon and quite constant within a species (Swift 1950). However, since the beginning of the 1980s the number of studies showing significant intraspecific variation in genome size has continued to increase and the original view of species-specific constancy has been questioned (Michaelson et al. 1991; Reeves et al. 1998; Schmuths et al. 2004). Numerous studies have shown variation in C-values in many plant species ranging from a few to as high as 32% (for example, in *Arabidopsis thaliana* (L.) Heynh. and *Helianthus annuus* L. respectively) (Michaelson et al. 1991; Schmuths et al. 2004; Doležel and Bartos 2005). In several cases these findings have been rejected on reinvestigation using an improved technical approach (Greilhuber 2005). However, in recent years there has been an increase in the number of studies using meticulous methodologies showing intraspecific genome size variation (Lysak et al. 2000; Schmuths et al. 2004). Intraspecific genome size variation has particularly been found among geographically distant populations or among populations growing in contrasting macroclimatic conditions. Significant correlations have been found

between genome size and altitude, latitude, temperature, precipitation, seed mass, moisture and leaf anatomical traits; however, these trends may differ in different taxa (Knight et al. 2005).

Quinoa (*Chenopodium quinoa* Willd.) is one of the most important food crops in the Andean region of South America. Its use as a seed crop extends across a broad area including parts of Bolivia, Peru, Ecuador, Colombia, Argentina and Chile. Its grain has an excellent balance of carbohydrates, lipids and protein which provides an ideal balance of essential amino acids for human nutrition. The cultivated plant shows great morphological variability, and there is an enormous range of diversity including its tolerance to heat, cold and soil salinity, resistance to disease, growth period and other desirable characteristics (Popenoe et al. 1989). *C. quinoa* is an allotetraploid species ($2n = 4x = 36$) with a quite small genome. Previous studies have estimated the haploid genome size (1C value) at between 1.005 and 1.596 pg. Determination based on Feulgen photometry (1.330–1.596 pg) has suggested a bigger genome than flow cytometry (1.005–1.500 pg) (Bennett and Smith 1991; Stevens et al. 2006; Bhargava et al. 2007; Kolano et al. 2009). The aim of this study was to verify the presence of intraspecific variation within *C. quinoa* and to estimate its range.

Materials and methods

Experimental material

The *C. quinoa* material used in this investigation was obtained from several sources, namely 11 accessions from the Agricultural University La Molina (Lima, Peru), eight accessions from the USDA North Central Regional Plant Introduction Station of the US National Plant Germplasm System (Iowa, USA) and one accession from the international nursery collection at the Centro International de la Papa CIP-FAO (Puno, Peru) (Table 1). Plant material was cultivated under the same conditions in a greenhouse at the Silesian University (Katowice, Poland).

Genome size determination by flow cytometry

Fifteen plants per population were analysed; each plant was measured twice. All analyses were performed by the same operator when the plant was 4–5 weeks old; a maximum of five plants per accession were analysed in one working day. The youngest fully developed leaf sheath was used for the preparation of the suspension of intact nuclei. The leaves were chopped with a razor blade in a nucleus extraction

Table 1 Passport data on *Chenopodium quinoa* accessions used for genome size analysis

Sample ID/name	Geographical group	Accession no.	Country	Region	Source
A1	Southern highland	PI 587173	Argentina	Jujuy	USDA-NPGS
B1/Real	Southern highland	N/A	Bolivia	Oruro	CIP-FAO
B2/Chucapaca	Southern highland	Ames 13214	Bolivia	La Paz	USDA-NPGS
C1	Lowland	PI 614880	Chile	Los Logos	USDA-NPGS
C2/Pichilemu	Lowland	PI 634917	Chile	Pichilemu	USDA-NPGS
C3/Baer	Lowland	PI 634918	Chile	Cajon	USDA-NPGS
C4	Lowland	PI 614889	Chile	Bio–Bio	USDA-NPGS
E1	Northern highland	Ames 13228	Ecuador	Otavalo	USDA-NPGS
P1	Southern highland	PEQPC-1906	Peru	Arequipa	AULM
P2	Southern highland	PEQPC-1907	Peru	Arequipa	AULM
P3	Southern highland	PEQPA-1893	Peru	Arequipa	AULM
P4	Northern highland	PEQPC-0705	Peru	Cajamarca	AULM
P5	Northern highland	PEQPC-0706	Peru	Cajamarca	AULM
P6	Southern highland	PEQPA-0557	Peru	Cusco	AULM
P7	Southern highland	PEQPA-0361	Peru	Cusco	AULM
P8	Southern highland	PEQPC-0645	Peru	Cusco	AULM
P9	Southern highland	PEQPC-0768	Peru	Puno	AULM
P10	Southern highland	PEQPC-0820	Peru	Puno	AULM
P11	Southern highland	PEQPA-1887	Peru	Puno	AULM
U1	Lowland	Ames 13728	USA	New Mexico	USDA-NPGS

CIP-FAO Centro Internacional de la Papa, *USDA-NPGS-USDA* North Central Regional Plant Introduction Station of the US National Plant Germplasm System, *AULM* Agricultural University la Molina.

buffer (Doležel and Gohde 1995), filtered through a 30-µm nylon mesh and stained with propidium iodide using a Partec high-resolution DNA kit according to the manufacturer's instructions. Nuclei isolated from the leaves of *Lycopersicon esculentum* Mill. cv. Stupicke (2C DNA = 1.96 pg) (Doležel et al. 1994) were used as an internal standard. For each sample at least 10,000 nuclei were analysed. All samples were measured using a DAKO Galaxy flow cytometer equipped with an air-cooled argon ion laser. The nuclear genome size of *C. quinoa* was estimated according to the following formula (Doležel and Bartos 2005):

$$C.\ quinoa\ \text{2C nuclear DNA content} = \frac{C.\ quinoa\ G_1\ \text{peak mean}}{L.\ esculentum\ G_1\ \text{peak mean}} \times 1.96\,\text{pg}$$

Statistical analysis

The Chi-squared goodness-of-fit test was applied to check the normality of the distribution of each accession. As many of them demonstrated a non-normal distribution the Kruskal-Wallis test followed by a nonparametric post-hoc test were used to check the significance of differences between accessions. To test whether genome size variation was correlated with geographical group Spearman's rank correlation coefficient (r_s) was used to avoid problems of data non-normality.

Results and discussion

In the present study, nuclear genome size was analysed in 20 accessions of *Chenopodium quinoa*. All analysed accessions were tetraploids with the chromosome number $2n = 4x = 36$. Although *C. quinoa* is an polysomatic species (Kolano et al. 2009), the histograms of the nuclear DNA content in young leaves contained a single distinct peak corresponding to G1 nuclei (2C DNA content) and a minor peak representing nuclei in the G2 phase (4C DNA content). Analysis of the distributions showed that more than 90% of leaf cells were in the G0/G1 phase. No nuclei with DNA content higher than 4C were observed.

Replicate measurements within each accession were highly consistent. The standard deviation of genome size in the accessions ranged from 0.76% to 2.9% of the genome sizes. The mean 2C nuclear DNA content of individual accessions ranged from 2.905 pg to 3.077 pg. This corresponds to a 5.9% difference in genome size between the accessions, with P1 being the accession with the smallest genome and C4 being the accession with the largest genome. An overview of genome sizes in all the accessions investigated is given in Table 2. The mean *C. quinoa* genome size was 2C = 2.973 ± 0.043 pg.

The 2C values given here agree with some previously published values obtained with flow cytometry (Palomino et al. 2008; Kolano et al. 2009) and they are only slightly

Table 2 Nuclear DNA content in various accessions of *Chenopodium quinoa*

Accession	Geographical group	DNA content (pg/2C)			Significance[a]
		Mean (pg)	SD	Range	
P2	Southern highland	2.905	0.076	2.784–3.020	a
P1	Southern highland	2.909	0.069	2.791–3.022	a
P11	Southern highland	2.931	0.071	2.804–3.017	a,b
P6	Southern highland	2.934	0.058	2.811–3.001	a,b
P3	Southern highland	2.945	0.085	2.826–3.099	a,b,c
P4	Northern highland	2.949	0.069	2.858–3.094	a,b,c
B1	Southern highland	2.950	0.063	2.840–3.042	a,b,c
P8	Southern highland	2.955	0.033	2.881–3.022	a,b,c
C1	Lowland	2.962	0.025	2.897–3.022	a,b,c
E1	Northern highland	2.966	0.070	2.830–3.085	a,b,c
A1	Southern highland	2.977	0.076	2.848–3.092	a,b,c
P7	Southern highland	2.979	0.052	2.897–3.069	a,b,c
P10	Southern highland	2.981	0.059	2.836–3.063	b,c,d
C2	Lowland	2.990	0.080	2.872–3.101	b,c,d
U1	Lowland	2.992	0.066	2.888–3.106	b,c,d
B2	Southern highland	2.995	0.023	2.940–3.032	b,c,d
C3	Lowland	3.013	0.082	2.878–3.151	c,d,e
P5	Northern highland	3.013	0.041	2.940–3.088	c,d,e
P9	Southern highland	3.046	0.031	2.985–3.108	d,e
C4	Lowland	3.077	0.037	2.997–3.154	e

[a] Accessions with different letters have significantly different means ($P < 0.05$).

lower than those estimated using Feulgen cytophotometry (Bhargava et al. 2007). The data presented here differ greatly from the results reported by Stevens et al. (2006) (2C = 2.01 pg), although they also analysed quinoa 'Real' as we did (B1). 'Real' is an ecotype consisting of multiple genotypes with similar seed characteristics from the Potosi Department of Bolivia (Ganadillas 1968) and it is possible that there are some variations in genome size within this ecotype. However, differences between our results and those reported earlier might be also due to the use of different reference standards, sample preparation, staining protocols and flow cytometers (Doležel et al. 2007).

The genome sizes of most *C. quinoa* accessions were not normally distributed. Therefore, the nonparametric Kruskal-Wallis test was used to evaluate the differences in genome size among the analysed accessions. Significant differences ($P < 0.05$) in genome size were found between *C. quinoa* accessions. Statistical analysis using a multiple comparison test revealed significant differences between the four accessions with the largest genomes (B3, P5, P9, C4) and the four accessions with the smallest genomes (P2, P1, P11, P6) (Table 2). The remaining populations had very similar genome sizes and clustered into overlapping groups.

Intraspecific variation of up to 43% has been found in *Prospero autumnalis* L., which corresponds with the karyological heterogeneity of the species (Ebert et al. 1996). Similarly, variation of up to 27% has been found among Finnish genotypes of *Tanacetum vulgare* L. (Keskitalo et al. 1998). Considering these findings, the intraspecific variation revealed in *C. quinoa* (5.9%) should be regarded as limited; however, it is in agreement with the findings of a number of recent studies which showed genome size variation in species with rather small genomes; for example, *A. thaliana* (Schmuths et al. 2004) and *Sesleria albicans* Kit. ex Schult. (Lysak et al. 2000).

The differences in nuclear DNA content among quinoa accessions do not seem to be caused by aneuploidy because the chromosome numbers in all the analysed *C. quinoa* accessions were identical. The main reason for a such variation may be an increase or decrease in the copy number of repetitive DNA sequences, especially retrotransposons, which is known to be a frequent source of genome size variation in angiosperms (Bennetzen et al. 2005). Several studies have shown a correlation between genome size variation and different ecological conditions that predominate in particular areas (e.g. altitude or latitude/longitude). However, the results are not straightforward and the relationship between intraspecific variation and environment is still not clear (Knight et al. 2005). For example, a negative correlation has been observed between genome size and latitude in *A. thaliana* (Schmuths et al. 2004), whereas no such correlation was found in *S. albicans* (Lysak et al. 2000).

Quinoa is cultivated in distinct geographical and ecological areas that range from sea level in Chile (36°S) to over 4,000 m a.s.l. in the Andes near the equator. Wilson (1988) indicated, based on morphological data combined with data from six isozyme loci, that the accessions of quinoa cluster into two main groups: Andean highland and coastal lowland. This division was later supported by the work of Christensen et al. (2007) who additionally suggested that the highland group should be divided into northern highland (northern Peru/Ecuador) and southern highland (southern Peru/Bolivia/northern Argentina) subgroups. Most of the quinoa accessions analysed in this study belonged to the southern highland group (12 accessions), but the northern highland group (3 accessions) and lowland group (5 accessions) were also included. Accession U1, collected in the USA, according to Christensen et al. (2007), appears to originate in the coastal Chilean region and was included in the lowland group. The lowland accessions tended to have larger genomes than most of the highland accessions. However, Spearman's test for correlation showed no correlation between genome size and the geographical origin of the accessions. This may have been due to the relatively high diversity of genome size observed among the southern highland quinoa accessions. There was 4.85% difference in genome size among southern highland accessions, with P2 being the accession with the smallest genome and P10 being the accession with the largest genome, and the Kruskal-Wallis test showed that the differences were statistically significant.

A high level of genetic diversity among the southern highland group of quinoa accessions when compared with the northern highland and lowland accessions has been indicated previously (Wilson 1988; Christensen et al. 2007; del Castillo et al. 2007). These authors suggested that the Peruvian and Bolivian southern highlands represent the centre of diversity for quinoa. A recent study based on RAPD markers has indicated that the high variation among the *C. quinoa* population in southern Peru and Bolivia might be due to population isolation, which is not simply linked to distance, but more probably to climatic and orographic barriers. Additionally, this population structure is also reinforced by the limited exchange of seeds among farmers (del Castillo et al. 2007).

In conclusion, only a limited amount of intraspecific genome size variation was found in *C. quinoa* and the variation was not correlated with geographical origin.

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References

Bennett MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R S Lond B Biol Sci 334:309–345

Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot 95:127–132. doi: 10.1093/aob/mci008

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, Coleman CE, Fairbanks DJ, Bonifacio A, Maughan PJ (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

del Castillo C, Winkel T, Mahy G, Bizoux JP (2007) Genetic structure of quinoa (*Chenopodium quinoa* Willd) from the Bolivian altiplano as revealed by RAPD markers. Genet Resour Crop Evol 54:897–905. doi: 10.1007/s10722-006-9151-z

Doležel J, Bartos J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot 95:99–110

Doležel J, Gohde W (1995) Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high resolution flow cytometry. Cytometry 19:103–106

Doležel J, Doleželova M, Novak FJ (1994) Flow cytometric estimation of nuclear-DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). Biol Plant 36:351–357

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:2233–2244. doi:10.1038/nprot.2007.310

Ebert I, Greilhuber J, Speta F (1996) Chromosome banding and genome size differentiation in *Prospero* (Hyacinthaceae): diploids. Plant Syst Evol 203:143–177

Ganadillas H (1968) Razas de quinua. Instituto Boliviano de Cultivos Andinos. División de Investigaciones Agrícolas, Ministerio de Agricultura, La Paz

Greilhuber J (2005) Intraspecific variation in genome size in angiosperms: identifying its existence. Ann Bot 95:91–98. doi: 10.1093/Aob/Mci004

Greilhuber J, Borsch T, Muller K, Worberg A, Porembski S, Barthlott W (2006) Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. Plant Biol 8:770–777. doi: 10.1055/s-2006-924101

Keskitalo M, Linden 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. doi:10.1093/Aob/Rnci011

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

Lysak MA, Rostkova AR, Dixon JM, Rossi G, Doležel J (2000) Limited genome size variation in *Sesleria albicans*. Ann Bot 86:399–403

Michaelson MJ, Price HJ, Johnston JS, Ellison JR (1991) Variation of nuclear DNA content in *Helianthus annuus* (Asteraceae). Am J Bot 78:1238–1243

Palomino G, Hernandez LT, Torres ED (2008) Nuclear genome size and chromosome analysis in *Chenopodium quinoa* and *C. berlandieri* subsp *nuttalliae*. Euphytica 164:221–230. doi: 10.1007/s10681-008-9711-8

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

Popenoe H, King S, Kalinowski L (1989) Lost crops of the Incas. In: Ruskin FR (ed) Little-known plants of the Andes with promise for worldwide cultivation. National Academy Press, Washington, pp 139–161

Reeves G, Francis D, Davies MS, Rogers HJ, Hodkinson TR (1998) Genome size is negatively correlated with altitude in natural populations of *Dactylis glomerata*. Ann Bot 82:99–105

Schmuths H, Meister A, Horres R, Bachmann K (2004) Genome size variation among accessions of *Arabidopsis thaliana*. Ann Bot 93:317–321. doi: 10.1093/aob/mch037mch037

Stevens MR, Coleman CE, Parkinson SE, Maughan PJ, Zhang HB, Balzotti MR, Kooyman DL, Arumuganathan K, Bonifacio A, Fairbanks DJ, Jellen EN, Stevens JJ (2006) Construction of a quinoa (*Chenopodium quinoa* Willd.) BAC library and its use in identifying genes encoding seed storage proteins. Theor Appl Genet 112:1593–1600. doi: 10.1007/s00122-006-0266-6

Swift H (1950) The constancy of desoxyribose nucleic acid in plant nuclei. Proc Natl Acad Sci USA 36:643–654

Wilson HD (1988) Quinoa biosystematics I: domesticated populations. Econ Bot 42:478–494