v. 3, n. 9, 2023

International Journal of Biological and Natural Sciences

DETERMINATION OF OPTIMAL CONDITIONS FOR CYTOGENETIC STUDIES IN QUINOA (*CHENOPODIUM SP.*)

Santina Rodrigues Santana
Universidade Federal de Rondônia – Fisheries Engineering Department
Presidente Médici, RO
https://lattes.cnpq.br/3559414297094574

Deisy Alexandra Rosero Alpala
``Universidad Nacional de Colombia``, Postgraduate Department
Palmira, Colombia
http://lattes.cnpq.br/2152492736392461

María Gladis Rosero
Colombian Agricultural Research Corporation - AGROSAVIA
Antioquia, Colombia

Abstract: Quinoa is a species of South American origin, of great importance for pre-Columbian peoples, it is currently recognized for its high nutritional value. The present study aimed to determine some tools of classical cytogenetics in Chenopodium species and establish the appropriate pretreatment for mitosis studies in two or three varieties of Chenopodium quinoa and Chenopodium album. The seeds from plants grown in the department of Nariño were subjected to three types of pretreatments (8-hydroxyquinoline -8AQ, 0.05% colchicine and 0.1% colchicine), a chemical digestion with HCl (1N) for 5, 10, 15 and 20 minutes at 60 ºC, and staining with 2% orcein. The results showed that the appropriate time for digestion with HCl was 15 minutes, for the treatments there were significant differences ($p<0.0026$), with an average chromatin observation of 32.3%; the pretreatment with higher percentages was presented for 8AQ with 57.5% on average, in contrast to the treatment with colchicine which was presented 27%; A similar response of the two Chenopodium species was found in phenological processes such as germination, and to the effect of the treatments, finding that 8-hydroxyquinoline (8AQ) is the most suitable pretreatment for visualization of chromatin and chromosomes. Ch. quinoa and Ch. album require a pretreatment for the observation of chromatin, both species respond in a similar way, with 8AQ being the best pretreatment for the two Chenopodium species, its use has been reported previously.
Keywords: 8-hydroxyquinoline; Colchicine; Chromatin; Chromosome

INTRODUCTION

Quinoa belongs to the Amaranthaceae family, and to the Chenopodium genus. The Chenopodium genus is the main one within the family with a wide global distribution, with about 250 species (FAO, 2011). It is a pseudo-cereal of great nutritional value whose nutritional qualities have been recognized throughout the world for being considered a complete food for nutrition (MUJICA and JACOBSEN, 2006). The high nutritional value and adaptability to different environmental conditions of this species makes it an alternative to contribute to the reduction of malnutrition and sustainable crop practices in marginal agricultural regions (PALOMINO et al., 2008).

Quinoa is a species native to South America, whose center of origin is in the Andes of Bolivia and Peru (FAO, 2011). It is one of the oldest crops in the Andean region, approximately 7000 years old, in whose domestication and conservation great cultures such as the Tiahuanacota and the Inca have participated (JACOBSEN, 2003). It is one of the domesticated species around Lake Titicaca, between Peru and Bolivia, this place is considered the center of origin of quinoa where the greatest diversity of this species is preserved in situ, as well as its wild relatives (BAZILE et al. al., 2013). The greatest diversity of wild relatives of quinoa is found in the Aynokas, ancestral systems of peasant organization as centers of conservation of genetic diversity in situ (MUJICA and JACOBSEN, 2006). In Colombia, the Chibchas indigenous peoples and others of the Cundiboyasense plateau cultivated and consumed quinoa, in addition to its uses in trade and fertility rituals for their crops. In the San Agustín-Huila area, products were exchanged with the Bogotá savanna and it is believed these communities helped with the dispersion of quinoa to the south of Colombia specifically to Nariño and from there to Ecuador (TAPIA, 2014).

The understanding of its origin is still complex, especially because many crossing options are involved in which two diploid species participated, so quinoa would be an

amphidiploid with disomic inheritance, being the closest wild relative of Chenopodium quinoa, Ch. hircinum and of wild Ch. nuttalliaeel from Ch. berlandieri, resulting in four complete sets of chromosomes, that is, an allotetraploid species 2n = 4x = 36, derived from two wild diploid species that were crossed in ancient times (JELLEN et al., 2014), Both Ch. quinoa and Ch. berlandieri are tetraploids with 2n = 4x = 36 and have symmetrical karyotypes with small, mostly metacentric and submetacentric chromosomes (KOLANO et al., 2016).

Studies show that its genome is diploid of 967 Mbp, the chromosomes are very small, therefore establishing the karyotype or developing biomarker libraries is difficult in the traditional way (YANGQUANWEI et al., 2013). In the case of Ch. album, which is considered a relative because it shares a diploid ancestor and current crop weed, it is considered a complex species in which polyploidy occurs. Different cytotypes have been reported in Ch. album from its diploid form (2n=2x=18), tetraploid (2n=4x=36) and hexaploid (2n=6x=54) (ATUL BHARGAVA et al., 2006).

The Nariño variety of quinoa has been studied in productive terms (BERTERO et al., 2004) and at a genetic level in the development of a new set of polymorphic SSR markers (JARVIS et al., 2008), however, to date it has not been has carried out no cytogenetic study of the varieties grown in Nariño, necessary for quinoa improvement processes in this producing region.

The objective of the present study was to determine some tools of classical cytogenetics that contribute to the study of mitosis in two species of Chenopodium and to establish the appropriate pretreatment for studies of mitosis in three varieties of *Chenopodium quinoa* and *Chenopodium álbum*.

MATERIALS AND METHODS

The seeds were obtained from the conservation plots in the harvest stage, belonging to the ORII Tierra y Vida organization, located in the municipality of Cumbal at 0.5101 (Latitude) and -77.4607 (longitude) at an altitude of 3085 m.a.s.l. in the department of Nariño, Colombia (Figure 1).

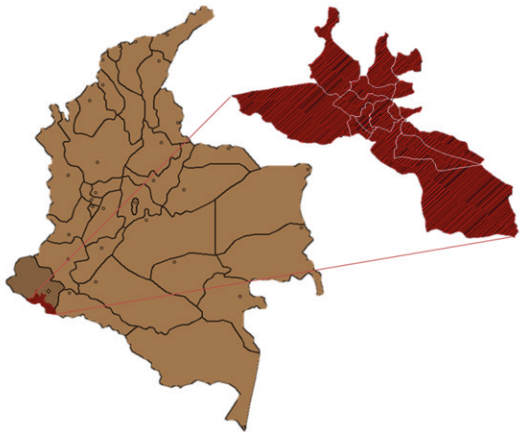


Figure 1. Location of the seed collection area, Municipality of Cumbal, Department of Nariño where the varieties of Ch. quinoa and Ch. album were collected.

Three varieties of Chenopodium quinoa were collected, two of which were improved varieties of Peruvian origin, Pasankalla and Rosada de Huancayo (Figure 2 A-B), a local variety (Figure 2 C), and a companion species Chenopodium album, known locally as alpaquinua (Figure 2D). The botanical seed of the materials was stored in plastic bags with their respective identification code and sent to the Cytogenetics Laboratory of `` Universidad Nacional de Colombia Campus Palmira``, for the respective study.

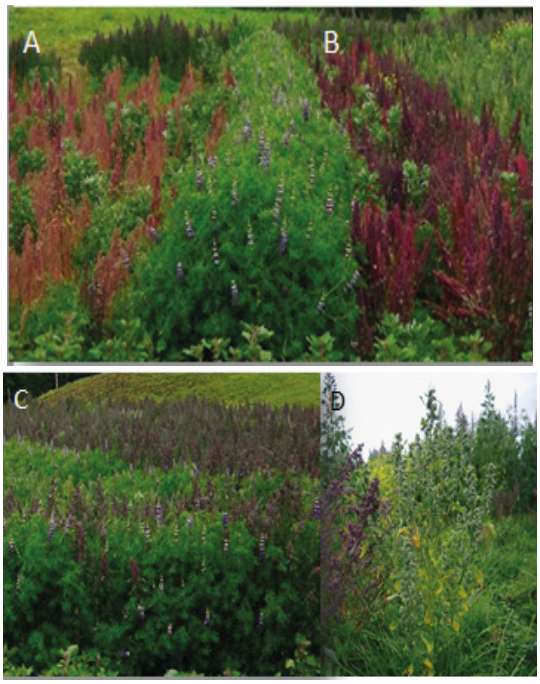


Figure 2. Quinoa cultivars and plants grown in the field under traditional production systems to obtain seeds. A-B) Ch. quinoa improved varieties (pasankalla quinoa and pink quinoa from huancayo), C) local variety (Tunkahuan), D) Ch. album (wild species - Alpaquinua).

The seeds were suitable through cleaning and selection processes, subsequently the germination test was carried out on filter paper, placed in a humid chamber for 48 to 72 hours at room temperature in laboratory conditions, with values of 18 to 28 °C and relative humidity. 64% on average.

Root meristems of 1 to 2 cm in length were taken. The apical regions of the roots were subjected to three pretreatments with mitosis-inhibiting agents: A) 8-hydroxyquinoline - 8AQ (2mM), B) a solution of 0.05% Colchicine and C) 0.1% Colchicine. Additionally, a control without treatment was established. The samples were stored at 4ºC for 24 hours, they were washed three times for five minutes in distilled water. Subsequently, a Carnoy solution (methanol and glacial acetic acid in a 3:1 ratio) was used to fix chromosomes and they were stored at 4ºC. for 24 hours (ANDRADE et al., 2005). For chemical digestion or hydrolysis, acidic HCl solution (1N) was used in three times of 5, 10, 15 and 20 minutes at 60ºC (HUAMÁN, 1995).

Three root tips were taken per material, the roots were prepared in plates with 2% orcein stain, subsequently they were crushed according to the conventional McClintock crushing technique (ANDRADE et al., 2005).

Subsequently, a reading of ten regions per plate was carried out, observations of the cells in mitosis were carried out in a binocular microscope. From the observations, a matrix of zeros and ones was organized for the absence and presence of chromatin respectively, then the formula was applied to establish the percentage of observation per material.

$$\% \, chromatin = \frac{\sum Obs.chromatin * 100}{\text{Total of notes}}$$

An analysis of variance was performed for the pretreatments and quinoa materials evaluated and a 95% Duncan multiple range test was performed to evaluate the effect of the pretreatment using the statistical package SAS (Statistical Analysis System) and STATGRAPHICS centurion version XV.II.

RESULTS AND DISCUSSION

It was found that all quinoa materials presented favorable characteristics for obtaining root meristems, with 100% germination percentages under humid chamber conditions at room temperature, in a period of 48 to 72 hours (Figure 3). Despite the ease of obtaining the root tips, they are small which makes them difficult to handle and also requires a digestion process.

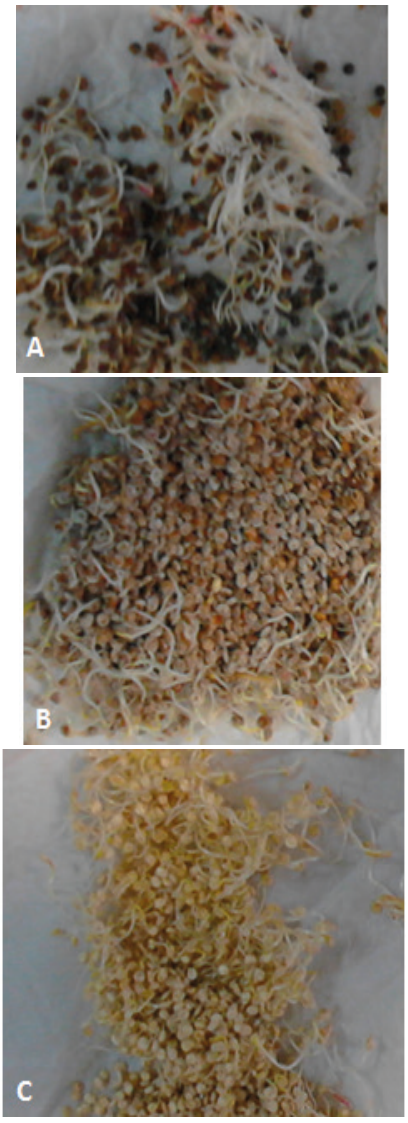


Figure 3: Root meristems of Chenopodium sp. obtained by pregermination after 48 hours for the observation of the mitosis phases. A) Chenopodium album (Alpaquinua), B) Chenopodium quinoa (Black Quinua - Pasankalla), C) Chenopodium quinoa (Quinua Tunkahuan).

According to cytogenetic studies, some species require digestion processes with HCl (1N) for 15 minutes at 60º to improve visualization (HUAMÁN et al., 1995), some studies have found favorable results for the use of HCl (1N) for 11 min at 60°C (PALOMINO et al., 2008), others for their part report the use of HCl (5N) for 30 minutes (BHARGAVA et al., 2006). This study found that for Ch. quinoa and Ch. album the most appropriate time for digestion with HCl (1N) is 15 minutes at 60ºC according to the quality of plates obtained from times 5, 10, 15 and 20 minutes., in accordance with what was reported by Huamán (1995); Furthermore, it was found that at times greater than 15 minutes HCl can damage the cells (Figure 5-B).

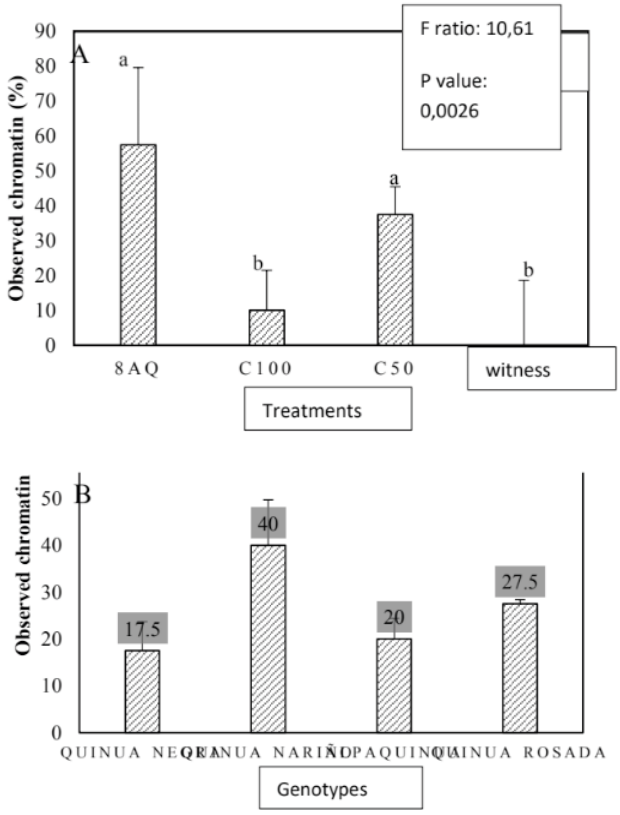
With the analysis of variance it was determined that the pretreatments present significant differences $p<0.0019$ (Table 1), which shows the effects of the pretreatments for the observation of chromatin in the cells.

Source of variation	Middle Square	F-Ratio	P-value
A: Pre-treatment	2741,67	10,61	0,0026
B: Genotype	408,333	1,58	0,2610
A(Pre-treatment) x B (Genotype)	2307,69231	6,6	0,0019
Residues	258,333		

Table 1: Analysis of variance of the pretreatments (8 hydroxyquinoline, Colchicine 0.05%, Colchicine 0.1%) in different genotypes of Ch. quinoa and Ch. album and their interactions.

With Duncan's multiple range test, differences were observed in two groups (Figure 4A) divided by their efficiency in observing chromatin. The effect of pretreatment with colchicine can be affected by the concentration with significant differences between colchicine 0.1% compared to 0.05% with a reduction in the observation percentage of 27.5%, where colchicine 0.1% allowed an observation of 10% that according to Duncan's multiple range test at 95% this treatment is grouped with the control with 0%, the above allows us to infer that some type of pretreatment is required because it is not possible to visualize cells with chromatin for the control (without treatment) and fixation with Carnoy is not enough.

In contrast to reducing colchicine to 0.05%, the percentage increases to 37.5% in efficacy and is grouped with 8-hydroxykineloin with greater efficiency with chromatin observations with 57.5% of the cells evaluated (Figure 4A).. The Chenopodium species evaluated require pretreatment, unlike other plant species in which an analysis of chromosomes due to the accumulation of cells in metaphase is possible with direct preparations (without pretreatment) (ANDRADE et al., 2005) similar to others. species of the Amaranthaceae family where colchicine is widely used for karyotype studies (BONASORA et al., 2013).



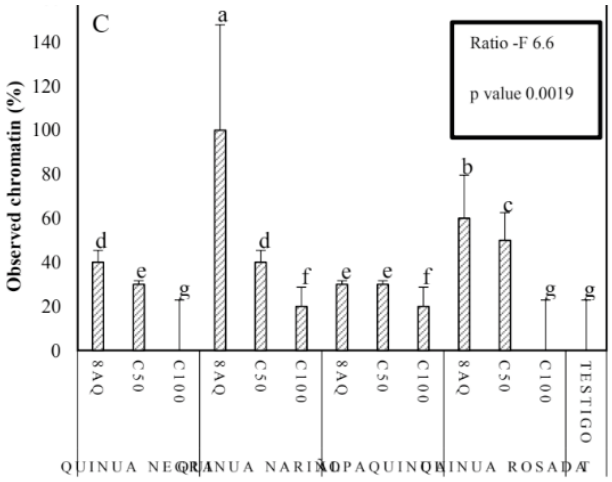


Figure 4: Percentage of chromatin observed (average of 30 cells) by pretreatment and genotype. A) effect of pretreatment (8 hydroxyquinoline, Colchicine 50gr., Colchicine 100gr.) on the observation of chromatin (* shows the significant differences between groups), B) Differences in percentage terms of each genotype of Ch. quinoa and Ch. album, C) Interaction of genotypes and pretreatment (letters show differences and groupings) Duncan test at 95% confidence.

Regarding the behavior of the Ch. quinoa genotypes for the Nariño varieties and the Rosada de Huancayo variety presented higher percentages of chromatin observation of 40 and 27.5% respectively, the Pasankalla quinoa variety and Ch. album (Alpaquinua) presented percentages between 17 and 20% (Figure 4B). The genotypes do not present statistically significant differences, which implies that the effective procedures for Chenopodium quinoa are similar for any variety and can be used in Ch. album.

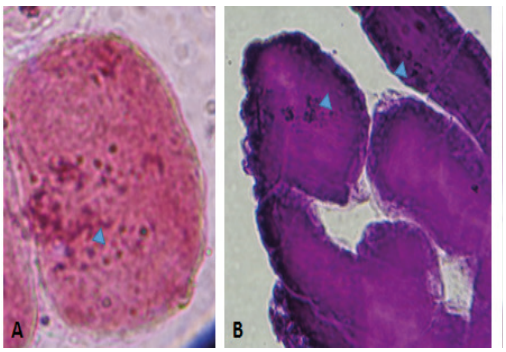
However, regarding interactions, the pretreatments evaluated provided responses with significant differences for the response variable (chromatin observation) for the treatments with colchicine and 8-hydroxyquinoline, which were grouped into 7 categories. The multiple range test for the presence of chromatin in 4 Chenopodium

materials showed significant variations between treatments with p<0.0026 with a normal distribution for each material, with a coefficient of variation of 5.7% and a standard deviation of 1. 8; the average chromatin observation was 32.3% (low).

For 8-hydroxyquinoline (8AQ), a higher response was observed for local quinoa materials with 100% observation of chromatin concentrations (category a), and in the remaining materials 44.3% was observed, already in previous cytogenetic studies in Chenopodium has been used 8AQ due to the ease of chromatin observation (KOLANO, 2001).

For colchicine 0.05% it had a homogeneous behavior in all the materials observed with an average percentage of 37.5%; while colchicine 0.1% obtained a response for local quinoa and Alpaquinua with an average of 20%, and 0% (category g) in the other materials (Figure 4C). Although colchicine is commonly used in cytogenetic studies of species of the Amaranthaceae family (BONASORA et al., 2013), it was the treatment that obtained the lowest percentages of chromatin observation.

Of the three pretreatments carried out only in the 8AQ treatment, chromosomes without a clear morphology were observed (Figure 5), other studies recommends the use of 8-hydroxyquinoline for cytogenetic analysis, using primary root tips pre-treated with 2 mM 8-hydroxyquinoline for 4 hours at room temperature (WANNER et al., 2000).



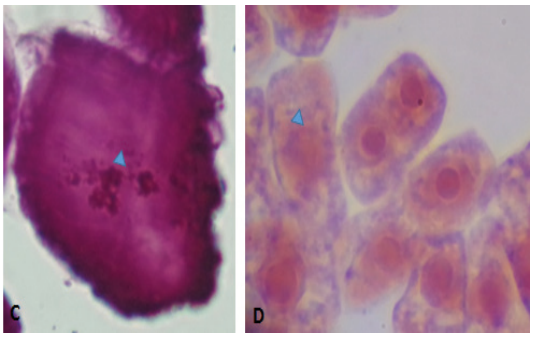


Figure 5. Chenopodium cells treated with 8AQ. A) material 8AQ-2, Ch. quinoa var. Tunkahuan –Nariño, cells with condensed chromosomes without clear definition; B-C) Material 8aQ-3, Ch. album with condensed chromosomes; D) C50-3 material, Ch. album, cells with visible nuclei.

Although the 8AQ pretreatment allows the observation of chromosomes, the flaws in the methodology in terms of digestion and staining do not allow a clear observation and identification to be made. Since Chenopodium species are known to have symmetric karyotypes with small meta- or sub-metacentric chromosomes (BHARGAVA et al., 2006; PALOMINO et al., 2008). Which makes it difficult to observe and identify chromosomes (JELLEN et al., 2014). Staining with 2% acetic orcein in pre-treated cells pigments the entire cell, this prevents the visualization of chromatin (Figure 5 –D). Therefore, research recommends that the material must be transferred to Feulgen's reagent for 25 minutes (PALOMINO et al., 2008).

Since genetic research on quinoa began systematically in the late 1970s, until now the FISH technique is the most used for cytogenetic studies with the 18-24J repetitive sequence that has allowed us to clarify the origin of quinoa (ZURITA -SILVA et al., 2014). However, this species continues to be of interest at a cytogenetic level given that the cytogenetic characterization of Chenopodium karyotypes has been limited (JELLEN et al.,

2014), research on this species continues in order to clarify the tetrapliode origin and identify the diploid origins. through phylogenetic analyzes (KOLANO et al., 2016). Thus, the tools of classical cytogenetics allow low-cost cytogenetic studies to be carried out in Ch. quinoa and Ch. album for evaluations of local varieties, in order to begin the characterization of karyotypes of local genotypes and identify their ploidy levels in future studies.

CONCLUSIONS

The tools of classical cytogenetics are useful for the cytogenetic study of the Chenopodium species evaluated. The similar response of the two species in phenological processes such as germination, and the effect of the treatments, made it easy to determine that 8-hydroxyquinoline (8AQ) is the most appropriate pretreatment for visualization of chromatin and chromosomes with a digestion of HCl for 15 minutes. However, the digestion processes with HCl at different concentrations must be evaluated and other types of stains must be tested.

THANKS

To the technical team of the cytogenetics laboratory of `` Universidad Nacional de Colombia Campus Palmira``, Indigenous Organization for Land and Life Research and to the Professor: Creuci María Caetano.

REFERENCES

ANDRADE, E., ET AL. **Manual de guías de laboratorio**. Universidad Nacional de Colombia-Sede Bogotá, 2005, p219.

BAZILE, D.; BERTERO, D.; NIETO, C. Estado del arte de la quinua en el mundo en 2013. Disponível em: https://www.fao.org/3/I4042S/i4042s.pdf> Acesso em: 09 set. 2023.

BERTERO, H.; DE LA VEGA, A.; CORREA, G.; JACOBSEN, S. Y.; MUJICA, A. Genotype and genotype-by-environment interaction effects for grain yield and grain size of quinoa (*Chenopodium quinoa* Willd.) as revealed by pattern analysis of international multi-environment trials. **Field Crops Research**, v. 89, n. 2–3, p. 299–318, 2004. http://doi.org/10.1016/j.fcr.2004.02.006

BHARGAVA, A.; SHUKLA, S. Y.; OHRI, D. *Chenopodium quinoa*—An Indian perspective. Industrial **Crops and Products**, v. 23, n.1, p. 73–87, 2006. http://doi.org/10.1016/j.indcrop.2005.04.002

BHARGAVA, A.; SHUKLA, S. Y.; OHRI, D. Karyotypic studies on some cultivated and wild species of Chenopodium (Chenopodiaceae). **Genetic Resources and Crop Evolution,** v. 53, n. 7, p. 1309–1320, 2006. http://doi.org/10.1007/s10722-005-3879-8

BONASORA, M. G.; POGGIO, L. Y.; GREIZERSTEIN, E. J. Cytogenetics Cytogenetic studies in four cultivated Amaranthus (Amaranthaceae) species. **Comparative Cytogenetics**, v. 7, n. 1, p. 53–61, 2013. http://doi.org/10.3897/CompCytogen.v7i1.4276

FAO. 2011. *La quinua : Cultivo milenario para contribuir a la seguridad alimentaria mundial*. (Organización de las Naciones para la Alimentación y la Agricultura -FAO, Ed.) (Wilfredo R). Regional de las americas: FAO. Retrieved from http://www.fao.org/docrep/017/aq287s/aq287s.pdf

HUAMÁN, Z. **Tecnicas Citologicas para determinar el numero cromosomico y la fertilidad de las papas**. Centro Internacional de La Papa (CIP), 1995.

JACOBSEN, S. E. The Worldwide Potential for Quinoa (Chenopodium quinoa Willd.). **Food reviews international**. v. 19, n. 1, p. 167–177. 2003. http://doi.org/10.1081/FRI-120018883

JARVIS, D. E.; KOPP, O. R.; JELLEN, E. N.; MALLORY, M. A.; PATTEE, J.; BONIFACIO, A. Y.; MAUGHAN, P. J. Simple sequence repeat marker development and genetic mapping in quinoa (*Chenopodium quinoa* Willd.). **Journal of Genetics**. v. 87, n. 1, p. 39–51, 2008. http://doi.org/10.1007/s12041-008-0006-6

JELLEN, E.; MAUGHAN, P.; FUENTES, F. Y.; KOLANO, B. 2014. Botany, Phylogeny and Evolution. In BAZILE. D.; BERTERO, NIETO, D. Y. C. (Eds.), "Estado del arte de la quinua en el mundo en 2013" (FAO / CIRA, p. 724). Santiago de Chile y Montpellier. Disponível em: <http://www.fao.org/3/a-i4042s.pdf>. Acesso em: 16 mar. 2016.

KOLANO, B.; MCCANN, J.; ORZECHOWSKA, M.; SIWINSKA, D.; TEMSCH, E. Y.; WEISS-SCHNEEWEISS, H. 2016**.** Molecular and cytogenetic evidence for an allotetraploid origin of *Chenopodium quinoa* and *Ch. berlandieri* (Amaranthaceae). **Molecular Phylogenetics and Evolution,** v.100, p. 109–123, 2016. http://doi.org/10.1016/j.ympev.2016.04.009

MUJICA, A. Y.; JACOBSEN, S. E. 2006. La quinua (Chenopodium quinoa Willd.) y sus parientes silvestres. **Botánica Económica de Los Andes Centrales.** v, 1, p. 449–457. Disponível em: <http://quinua.pe/wp-content/uploads/2013/03/La-quinua-y-sus-parientes.pdf>. Acesso em: 18 ago. 2018.

PALOMINO, G.; TREJO HERNÁNDEZ, L. Y.; DE LA CRUZ TORRES, E. 2008**.** Nuclear genome size and chromosome analysis in *Chenopodium quinoa* and *Ch. berlandieri* subsp. nuttalliae. **Euphytica**, v. 164, n. 1, p. 221–230. http://doi.org/10.1007/s10681-008-9711-8

TAPIA, M. El largo camino de la Quinoa: ¿quiénes escribieron su historia? In: BAZILE, D.; BERTERO, D.; NIETO, C. (eds). Estado del arte de la quinua en el mundo en 2013. FAO (Santiago de Chile) y CIRAD, (Montpellier, Francia). p3-10, 2014.

WANNER, G., Et Al. A New Chromosome Model. **Journal of Structural Biology**, v. 132, n. 2, p. 147–161, 2000. http://doi.org/10.1006/jsbi.2000.4310

YANGQUANWEI, Z.; NEETHIRAJAN, S. Y.; KARUNAKARAN, C. Cytogenetic analysis of quinoa chromosomes using nanoscale imaging and spectroscopy techniques. **Nanoscale Research Letters**, v. *8, n.*1, p. 1. http://doi.org/10.1186/1556-276X-8-46, 2013.

ZURITA-SILVA, A.; FUENTES, F.; ZAMORA, P.; JACOBSEN, S. E. Y.; SCHWEMBER, A. R. Breeding quinoa (*Chenopodium quinoa* Willd.): Potential and perspectives. **Molecular Breeding**, v. 34, n. 1, p. 13–30, 2014. http://doi.org/10.1007/s11032-014-0023-5