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Short Communication

Genome size and repetitive sequences are driven by artificial selection on the length of the vegetative cycle in maize landraces from Northeastern Argentina

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Abstract

Variation in genome size and knob heterochromatin content was explored in relationship to altitudinal cline and length of the vegetative cycle in northern Argentina, USA and Mexico landraces. It was considering that the decrease in DNA and heterochromatin content could be an adaptation to a shorter growing season and the result of artificial selection by man. Guaraní landraces from Northeastern Argentina (NEA) show similar variation in genome size (3.81pg to 7.56pg) and knob heterochromatin content than maize growing across an altitudinal cline. The present analysis offers an overview of the genetic variability of NEA maize to explain why Guaraní landraces and those along an altitudinal cline share this similar variation. Karyotype and flow cytometry data were employed. The DNA content of Guaraní landraces which lacking B chromosomes, showed no significant relationship with knob heterochromatin, suggesting differences in the amount of interspersed DNA. A significant positive relationship was found between the length of the vegetative cycle and both number and percentage of knob heterochromatin. No significant correlation was found between genome size and vegetative cycle. All these results allow us to conclude that the variation in heterochromatin content among Guaraní maize is driven by the selection of farmers for flowering time.

Key words: genome size, knob heterochromatin, length of vegetative cycle, repetitive sequence variation, selective effect.

Resumo

La variación observada en el tamaño del genoma y el contenido de heterocromatina knob en relación con el cline altitudinal y la duración del ciclo vegetativo en razas de maíz nativas del norte de Argentina, Estados Unidos y México, permitió considerar que la disminución del contenido de ADN y heterocromatina se debería a una adaptación a temporadas cortas de crecimiento y al resultado de selección artificial por parte del hombre. Las razas Guaraníes nativas del noreste de Argentina (NEA), cultivadas a bajas altitudes y sin cromosomas B, muestran una variación similar en el tamaño del genoma (3.81 pg a 7.56 pg) y el contenido de heterocromatina knob a aquellos maíces que crecen a lo largo de un cline altitudinal. El presente análisis ofrece una visión general de la variabilidad genética del maíz del NEA y trata de responder por qué estas razas Guaraníes y los maíces que crecen en un cline altitudinal presentan la variación mencionada. Se emplearon datos de cariotipo y citometría de flujo. El contenido de ADN de las razas Guaraníes no mostró correlación significativa con la heterocromatina knob, lo que sugiere diferencias en el contenido de ADN repetitivo disperso. Se encontró una correlación positiva significativa entre la duración del ciclo vegetativo tanto con el número como con el porcentaje de heterocromatina knob. No se encontró una relación significativa entre el tamaño del genoma y el ciclo vegetativo. Estos resultados nos permiten concluir que la variación en el contenido de heterocromatina entre los maíces Guaraníes estaría impulsada por la selección del tiempo de floración realizada por los agricultores.

Palabras claves: tamaño del genoma, heterocromatina knob, duración del ciclo vegetativo, variabilidad de secuencias repetitivas, efecto selectivo.

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To the present, 51 morphological maize landraces have been described in northern Argentina (NA): 28 Northwestern (NWA) landraces distributed along an altitudinal cline, and 23 Northeastern (NEA) landraces cultivated without differences in altitude. In NEA, indigenous Guaraní communities from subtropical forests of Misiones Province cultivate up to 15 landraces (Cámara - Hernández *et al*. 2011). In NA landraces, genome size ranges between 4.4 pg and 6.9 pg (Tito *et al*. 1991; Poggio *et al*. 1998; Realini *et al*. 2016; Fourastié *et al*. 2017; Realini 2017). A similar genome size variation was reported for maize growing along altitudinal clines in USA and Mexico (Laurie & Bennett 1985; Rayburn *et al*. 1985; Rayburn & Auger 1990a, b; Díez *et al*. 2013; Bilinsky *et al*. 2017)

Genome size variation in maize has been mainly attributed to differences in the amount of heterochromatin, principally due to the presence of heterochromatin knobs and of B chromosomes (Bs) (Laurie & Bennett 1985; Poggio *et al*. 1998; Fourastié *et al*. 2017). Other proposed cause includes differences in the amount of interspersed DNA (SanMiguel & Bennetzen 1998; Meyers *et al*. 2001; Bilinski *et al*. 2018). Although Bs are common in NWA landraces, showing large intra- and inter-population differences in number and frequency (Fourastié *et al*. 2017; Poggio *et al*. 1998; Rosato *et al*. 1998), they have not been previously detected in Guaraní landraces from NEA (Realini *et al*. 2018). In Argentinian landraces, variation in genome size and knob heterochromatin content was explored in relationship to altitudinal cline and length of the vegetative cycle (Fourastié *et al*. 2017; Realini *et al*. 2016; Realini 2017). The decrease in knob heterochromatin found at high altitudes was proposed to be related to the length of the growing season (Poggio *et al*. 1998) and to natural selection on flowering time across altitudinal clines (Bilinsky *et al*. 2017). Then, the following question arises: why do landraces living in sympatry in lowland restricted areas and those along an altitudinal cline share similar variation in genome size and heterochromatin content?

In this study, we investigated the parallel link of the vegetative cycle length with variation in intraspecific genome size and abundance of knob heterochromatin in Guaraní landraces from Northeastern Argentina (NEA). New genome size and heterochromatin data were estimates for NEA maize populations and were integrated with those previously reported for Guaraní maize populations (Realini *et al*. 2016, 2018; Realini 2017). The present jointly analysis offer a more comprehensive overview of the genetic variability source of the native maize of NEA region and allows us to develop a general discussion about the selective forces involved (Tab. 1).

The maize samples analyzed here were collected from Guaraní farms in Misiones Province, Argentina: VAV6843- Pororó Chico from Aldea Perutí, El Alcázar Depto. Libertador General San Martín; VAV6823- Overo, from Aldea Yuytu Pará, Ruíz de Montoya; VAV6604- Colorado from Pozo Azul, Depto. San Pedro; VAV2011/09- Pipoca Amarillo from Aldea Pozo Azul, Depto. Eldorado. The specimens were deposited at the seed bank of the Plant Genetic Resources Laboratory "N. I. Vavilov" in the Facultad de Agronomía, Universidad de Buenos Aires. DNA content was measured in three to five individuals from each ears and two to five ears per population, with three replicates per individual and data analysis was carried out according to Realini *et al*. (2016). The 2C DNA content was measured in three to five individuals of each corncob and two to five corncobs per population, with thee replicates per individual. The cell nucleus were stained with propidium iodide (PI). *Pisum sativum* cv. citrad (9.09 pg), used as internal standard, was kindly provided by Dr J. Doležel from the Institute of Experimental Botany, Sokolovska, Czech Republic (Doležel *et al*. 2007). For each individual, 100 mg offresh leave samples were co-chopped with 50 mg of *P. sativum* leaves in a Petri dish with 0.5 mL of buffer Otto I (citric acid 0.1 M and 0.5 % v/v of Tween 20), usinga stainless-steel razor blade. The sample was filtered through a nylon mesh (45 µm pore size) and then 0.5 mL of buffer Otto II (0.4 M Na_2HPO_4 $12H_2O$) supplementedwith PI (50 µg mL^{-1} of final concentration) and RNase (50 µg mL^{-1} of final concentration) were added. The samples were incubated in the dark for 40 min. Flow cytometry was performed at Instituto Nacional de Tecnología Agropecuaria (INTA-Catelar) with a CyFlow Ploidy Analyzer Cytometer (Partec). We adjusted the gain to 400 and the sample speed to 0.4 mL^{-1}. The samples were run until 5,000 nuclei were scored. The DNA content was estimated from gated fluorescence histograms of the PI area. Data analysis was performed using the software Flowing 2.5.0 (<http://www.flowingsoftware.com>). Genome size (2C DNA, in picograms) was determined comparing the peak of the sample to the peak of the standard according to Doležel

Table 1 – Percentages of heterochromatin, number of knobs, genome size and length of the vegetative cycle.

Maize landraces/ populations (VAV)	**Percentage of Knob heterochromatin (X ± SE) Range**	**Numbers of *knobs* (X ± SE) Range**	**DNA - 2C Content values (X pg ± SE) Range**	**Vegetative Cycle (days)**
Tupí Amarillo VAV6563	16.71% ± 1.26 13.90% - 20.02%	19.75 ± 1.25 17 - 23	6.26 ± 0.13 E 4.63pg - 7.56pg	82
Tupí Blanco VAV6851	--	--	6.07 ± 0.27 CE 5.79pg - 6.35pg	--
Tupí Blanco VAV6592	13.31% ± 0.47 12.15% - 14.38%	16.50 ± 1.04 14 - 19	6.05 ± 0.17 DE 4.88pg - 6.45pg	--
Pororó Grande VAV6826	--	--	6.04 ± 0.24 CE 5.89pg - 6.27pg	--
Pororó Grande VAV6827	--	--	5.98 ± 0.24 CE 4.88pg - 6.45pg	--
Pororó Chico* VAV6843	--	--	5.91 ± 0.14 CE 5.54pg - 6.12pg	--
Azul VAV6564	7.03% ± 1.16 5.35% - 11.51%	11.80 ± 1.98 8 - 18	5.84 ± 0.13 CE 5.08pg - 6.98pg	75
Overo VAV6559	7.19% ± 0.54 5.51% - 8.60%	11.50 ± 0.67 9 - 13	5.79 ± 0.14 CE 4.52pg - 7.37pg	76
Pororó Grande VAV6562	13.59% ± 0.59[2] 10.82% - 15.81%	17.00 ± 0.53[2] 15 – 20	5.79 ± 0.23 CE 5.68pg - 6.01pg	83
Pipoca Amarillo* 2011/09	12.69% ± 1.38[2] 9.33% - 16.66%	13.00 ± 1.18[2] 10 - 17	5.74 ± 0.23 CE 5.66pg - 5.77pg	--
Pipoca Colorado VAV6567	11.62% ± 0.76 9.04% - 13.46%	15.60 ± 0.68 14 - 17	5.70 ± 0.17 CE 5.02pg - 6.38pg	89
Colorado* VAV6604	--	--	5.70 ± 0.15 CE 5.48pg - 5.96pg	--
Pipoca Colorado VAV6607	12.47% ± 0.89 9.73% - 15.64%	17.83 ± 0.98 15 - 21	5.69 ± 0.15 CE 5.21pg - 6.08pg	--
Colorado VAV6573	8.56% ± 0.67 7.27% - 10.88%	12.20 ± 0.66 10 - 14	5.68 ± 0.16 CE 5.23pg - 5.97pg	64
Blanco Ancho VAV6560	7.34% ± 0.68[2] 5.21% - 10.66%	12.22 ± 0.95[2] 9 – 17	5.63 ± 0.13 CE 5.01pg - 6.10pg	77
Rosado VAV6565	8.34% ± 0.73 6.38% - 11.02%	13.33 ± 0.80 11 – 16	5.57 ± 0.17 CE 4.58pg - 6.19pg	76
Blanco Angosto VAV6574	6.37% ± 0.51[2] 5.06% - 8.51%	9.83 ± 0.40[2] 8 - 11	5.45 ± 0.14 CD[2] 5.29pg - 5.75pg	75
Overo* VAV6823	9.23 ± 0.65 8.13% - 10.92%	13.00 ± 0.91 11 - 15	5.41 ± 0.23 ACE 5.06pg - 5.69pg	--
Colorado VAV6837	8.43% ± 0.72 6.78% - 10.07%	13.00 ± 0.91 11 - 15	5.39 ± 0.16 BCD 4.93pg - 5.86pg	--
Amarillo Ancho VAV6569	7.78% ± 0.58 6.00% - 9.19%	12.50 ± 0.96 10 - 16	5.36 ± 0.12 CD 5.01pg - 5.80pg	80

Maize landraces/ populations (VAV)	Percentage of Knob heterochromatin (X ± SE) Range	Numbers of *knobs* (X ± SE) Range	DNA - 2C Content values (X pg ± SE) Range	Vegetative Cycle (days)
Pororó Chico VAV6575	12.12% ± 0.89 10.21% - 14.84%	17.20 ± 0.49 16 - 18	5.36 ± 0.13 BCD 4.67pg - 5.72pg	85
Amarillo Angosto VAV6556	7.07% ± 0.59[2] 5.79% - 10.32%	11.29 ± 1.06[2] 8 – 17	5.25 ± 0.13 AC 4.91pg - 5.62pg	66
Pipoca Amarillo VAV6568	12.24 ± 1.01[2] 9.91% - 15.80%	15.40 ± 0.93[2] 14 – 19	4.70 ± 0.13 AB 3.96pg - 5.92pg	85
Variegado VAV6557	7.27% ± 0.67 5.30% - 8.63%	11.80 ± 0.80 10 - 14	4.59 ± 0.13 A 3.81pg - 5.81pg	74

Ref. SE = standard error. Data of vegetative cycle were taken from Melchiorre *et al*. 2006. Genome size data were taken for Realini *et al*. 2016. Data of number and percentages of heterochromatin knob were taken for Realini *et al*. 2018. ∗ = New genome size estimates. 2 = Heterochromatin data incorporated into a new integrative analysis. Data Populations with different letters have significantly different means of genome size, Tuckey's HSD pairwise comparisons ($P < 0.05$).

et al. (2007). All samples with a coefficient of variance ≤ 5 were included in the present study. Conversion from picograms to megabase pairs was done according to Doležel *et al*. (2003).

All statistical analyses were performed using the programs Infostat, FCA, National University of Córdoba (Di Rienzo *et al*. 2012) and R (R Development Core Team 2012). Statistical significance was set at $P < 0.05$.

The variation in genome size of the Argentinian Guaraní maize populations was analyzed using analysis of variance (ANOVA), followed by the Tukey's HSD test for pairwise comparisons. The DNA content (2C values) varied from 3.81pg to 7.56pg, and there were significant differences among populations ($Pv < 0.0001$, $F_{23.501} = 7.25$, $n = 578$, Tab. 1). In addition, intra-population variation in DNA content was evaluated through the intraclass correlation coefficient at the ear level ($ICC_{ear} = 0.252$) and at the individual-within-ear level ($ICC_{individual/ear} = 0.818$). The individual and ear random effects accounted for about 82% of the total residual variance, while the ear random effect alone explained only about 25% of the variance. The inter-population variation was similar to those obtained in previous studies involving lines and landraces from Argentina, Mesoamerica and USA (Laurie & Bennett 1985; Rayburn & Auger 1990a, b; Tito *et al*. 1991; Poggio *et al*. 1998; Díez *et al*. 2013; Bilinsky *et al* 2017; Fourastié *et al*. 2017).

In regard to knobs repetitive DNA, the Guaraní maize populations showed variation in number (from 8 to 23), and percentage of knob heterochromatin (from 5.30 to 20.02% of total chromosome length) (Fig. 1) (Realini 2017; Realini *et al*. 2018). Moreover, variation in the number of knobs and percentage of heterochromatin has been reported at the intra-population level (Tab. 1) (Realini *et al*. 2016, 2018; Realini 2017). Analysis of karyotype data showed that the correlation between the number of knobs and percentage of knob heterochromatin (Tab. 1), using the Spearman coefficient (SC), was positively significant ($Pv < 0.0001$, $SC = 0.85$; $n = 105$; $Y = -2.05 + 0.86\ X$, $R = 0.72$, $Pv < 0.0001$). It is important to point out that individuals of the same population with the same number of knobs, not always exhibited similar percentages of heterochromatin. This suggests that the percentage of heterochromatin depend the number of knob and the numbers of copies of the satellite DNA repeats that conform them (Fig. 1). Although, thus allowed us to suggest that the variation in the number of copies of repetitive scattered DNA sequences are important sources of content variation (SanMiguel & Bennetzen 1998; Meyers *et al*. 2001; Tenaillon *et al*. 2011, 2016; Chia *et al*. 2012; Bilinsky *et al*. 2017).

The DNA content of the Guaraní populations showed no relationship either with the number of knobs ($Pv = 0.2106$, $SC = 0.30$, $df = 18$) or with the percentage of heterochromatin ($Pv = 0.1110$, $SC = 0.38$, $df = 18$). In Figure 2 are represented the relations between the abundance of repeat DNA from knob heterochromatin, genome size and length of the vegetative cycle in Guaraní landraces, interestingly not always the populations

with a major percentage of heterochromatin content has the highest values of genome size. In contrast, a recent study carried out on NWA maize showed that the percentage of knob heterochromatin in a set of A chromosomes (A-HC) and DNA amount were positively correlated (Fourastié *et al.* 2017). Thus suggesting that the balance between knob heterochromatin and genome size is different in Guaraní maize than NWA landraces, this could explain for the presence of B chromosomes in NWA landraces. In the present work, the joint analysis of published and new data reveals positive significant relationships between the length of the vegetative cycle and both heterochromatin percentage (Pv = 0.0068, SC = 0.69, df = 13) and knob number (Pv = 0.0007, SC = 0.80, df = 13) (Fig. 2). A clear relationship between genome size and vegetative cycle length was not found in Guaraní landraces (P = 0.66779, SC = 0.13, df = 13) (Fig. 2). In fact, the populations VAV6563 and VAV6568, have 6.26pg and 4.70pg as average values of genome size and they possess longer vegetative cycle, 82 and 84 days, respectively. Despite the differences in DNA content, both populations have a higher percentage of heterochromatin (16.71% and 12.24%, respectively, Fig. 2).

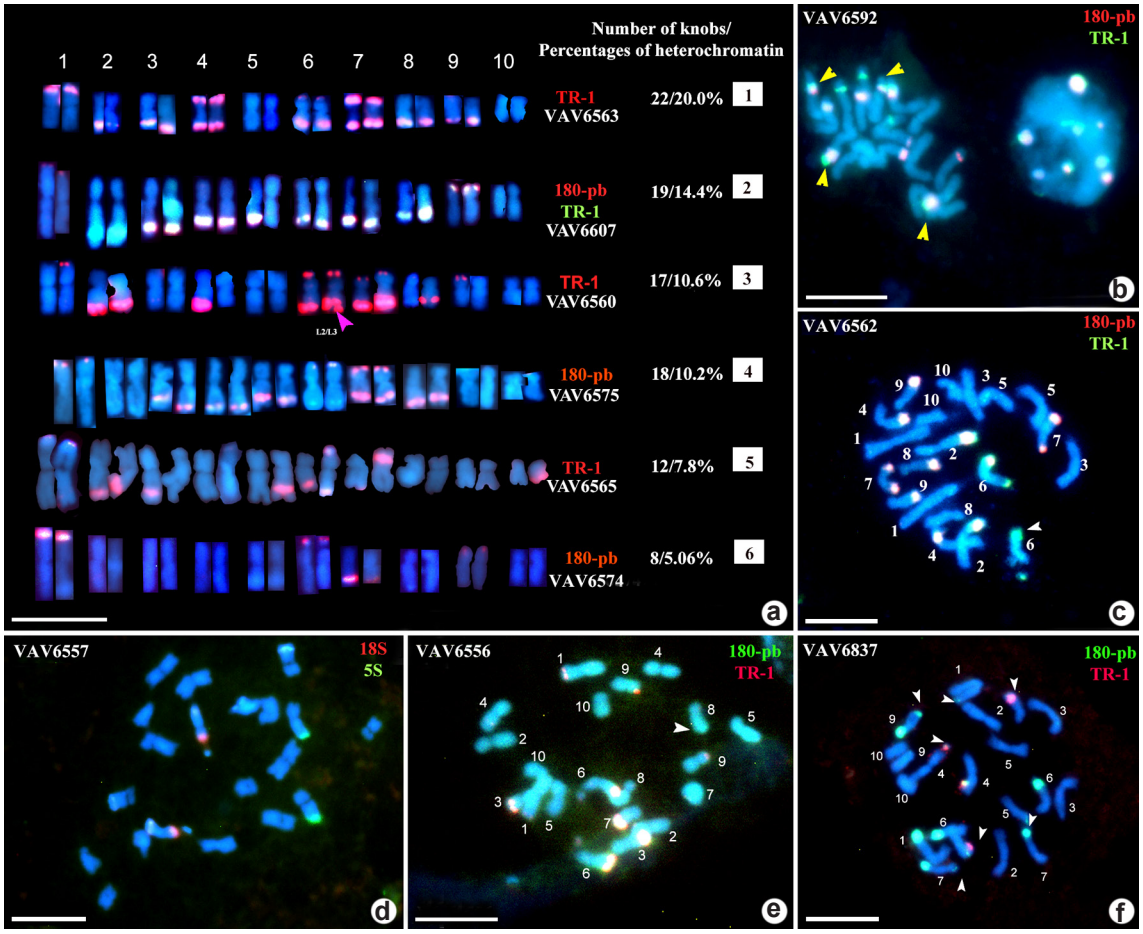

Figure 1 – a-f. Comparative analysis of knob chromosomal distribution, estimated by FISH – a. Cariograms DAPI/FISH in six Guaraní maize landraces (1 = V6563, Tupí Amarillo; 2 = VAV6607, Pipoca Colorado; 3 = VAV6560, Blanco Ancho; 4 = VAV6575, Pororó Chico; 5 = VAV6565, Rosado; 6 = VAV6574, Blanco Angosto). b-f. Metaphase chromosomes by FISH with knobs, 18S and 5S rDNA probes – b. VAV6592, Tupí Blanco; c. VAV6562, Pororó Grande; d. VAV6565, Rosado; e. VAV6556, Amarillo Chico; f. VAV6837, Colorado. The probes were labeled with digoxigenin and biotin, and revealed with antidigoxigenin-FITC (green) and Cy3 (red), respectively. Ref. The violet arrowhead indicated the 6L2 and 6L3 knob positions. The white arrowhead indicates knobs hybridized only with single knob sequence. The numbers indicate the chromosomal pairs. Scale bars = 10µm.

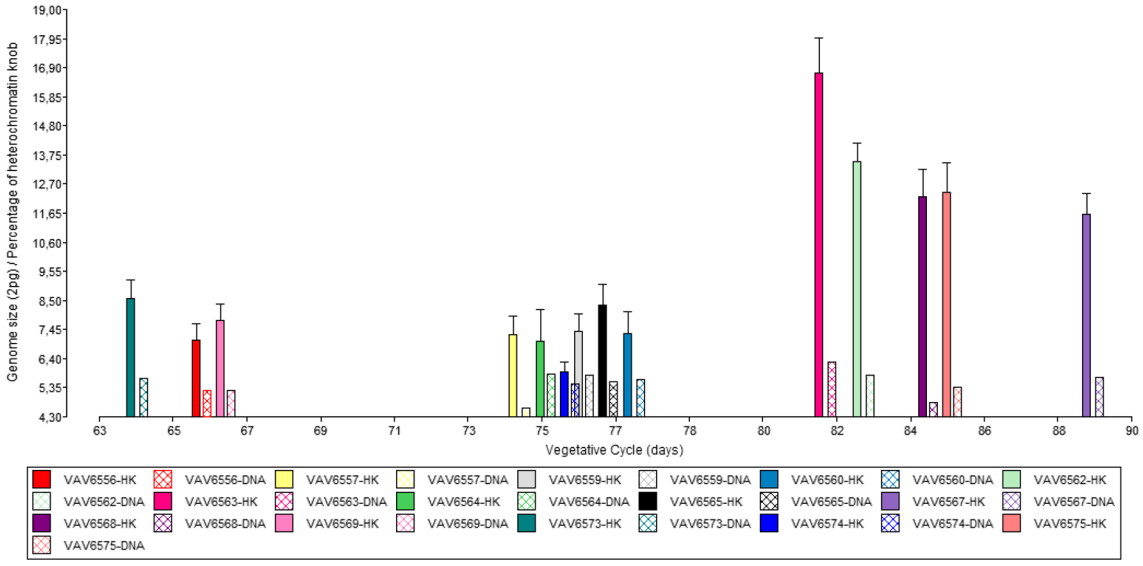

Figure 2 – Correlations between abundance of repeat DNA from knob heterochromatin, genome size and length of vegetative cycle in Guaraní landraces. Ref. Solid filled bars show the average percentages of the heterochromatin knob. Bars with crossed lines indicate the means values of genome size. Whiskers represent standard errors.

The data of the present work support that vegetative cycle is more related with heterochromatin than with total DNA content. This could be explained taken into account that the knob heterochromatin is the last component in completing DNA replication since increased DNA packaging leads to a longer synthesis, resulting in a longer cell cycle that may impact on the rate of cell division and plant development (Pryor *et al*. 1980; Buckler *et al*. 1999; Greilhuber & Leitch 2013). In fact, several studies on maize growing at high altitudes have determined that large amounts of heterochromatin are not favored under harsher climates and shorter growing seasons (Reeves *et al*. 1998; Buckler *et al*. 1999; Poggio *et al*. 1998; Rayburn *et al*. 1994; Bilinsky *et al*. 2017; Fourastié *et al*. 2017).

The analysis of the data obtained led us to propose that the populations of NEA analyzed here, which grew in similar altitudinal, climatic and / or ecological conditions, are isolated by temporary pre-zigotic barriers through differences in the duration of the vegetative cycle (*ie*, the flowering time). This isolation is developed artificially by the farmers with the purpose of maintaining the morphological characteristics of each race, avoiding the presence of hybrids with undesirable intermediate morphological and agronomic characteristics. The selection in the flowering time implies an indirect artificial selection for differences in heterochromatin content.

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