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RESEARCH ARTICLE

Genetic diversity of ribosomal loci (5S and 45S rDNA) and pSc119.2 repetitive DNA sequence among four species of *Aegilops* (*Poaceae*) from Algeria

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Abstract. In continuation of our previous research we carried out the karyological investigation of 53 populations of four *Aegilops* species (*A. geniculata*, *A. triuncialis*, *A. ventricosa*, and *A. neglecta*) sampled in different eco-geographical habitats in Algeria. The genetic variability of the chromosomal DNA loci of the same collection of *Aegilops* is highlighted by the Fluorescence In Situ Hybridization technique (FISH) using three probes: 5S rDNA, 45S rDNA, and repetitive DNA (pSc119.2). We found that the two rDNA loci (5S and 45S) hybridized with some chromosomes and showed a large genetic polymorphism within and between the four *Aegilops* species, while the repetitive DNA sequences (pSc119.2) hybridized with all chromosomes and differentiated the populations of the mountains with a humid bioclimate from the populations of the steppe regions with an arid bioclimate. However, the transposition of the physical maps of the studied loci (5S rDNA, 45S rDNA, and pSc119.2) with those of other collections revealed the existence of new loci in *Aegilops* from Algeria.

Keywords: *Aegilops*, Algeria, cytogenetic markers, eco-geography, genetic diversity, plant genetic resources

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Introduction

The genus *Aegilops* L., belonging to family *Poaceae*, tribe *Triticeae*, subtribe *Triticineae* Griseb., includes about 22 annual self-fertile species (Van Slageren, 1994' see also POWO, 2021: https://powo.science.kew.org/taxon/17369-1). It represents the main genetic reserve and a very important genetic resource for the breeding and improvement of cultivated wheat, *Triticum* L. spp. (Kilian et al., 2011). Therefore, an accurate genetic characterization of these species is desirable.

The genus *Aegilops* is represented in Algeria by several species, mainly by *A. geniculata* Roth, *A. neglecta* Req. ex Bertol., *A. ventricosa* Tausch, and *A. triuncialis* L. (Maire, 1955; Quezel, Santa, 1962). The populations of *A. geniculata* occur in very diverse habitats, from coastal hills, the Tell Atlas, and high steppe plains to the Saharan Atlas, while other species (*A. neglecta*, *A. triuncialis*, and *A. ventricosa*) are characterized by dispersed populations and have more limited distribution ranges (Bandou et al., 2009).

Cytogenetic studies of *Aegilops* species have been often used to clarify the systematic and phylogenetic position of certain taxa (Senyaninova-Korchagina, 1932; Chennaveeraiah, 1960; Kimber, Feldman, 1987; Van Slageren, 1994). As it comes out from these results, the diploid species of *Aegilops* represent seven genome types: C (*A. markgrafii* (Greuter) K.Hammer), M (*A. comosa* Sm.), N (*A. uniaristata* Steud.), D (*A. tauschii* Coss.), U (*A. umbellulata* Zhuk.), T (*A. mutica* Boiss.), and S (*A. speltoides* Tausch, *A. bicornis* (Forssk.) Jaub. & Spach, *A. longissima* Schweinf. & Muschl., *A. sharonensis* Eig, and *A. searsii* Feldman & Kislev). Indeed, the allopolyploid species possess different combinations of these genomes.

Previously, Badaeva et al. (1996, 2002, 2004, 2011) established the phylogenic relationships between polyploid species and their diploid parents by C-banding and cytomolecular techniques. They found that in the majority of polyploid *Aegilops* species, the genome is variously modified as compared to their ancestors. However, other molecular approaches have been developed, such as the study of reserved proteins (Fernandez-Calvin, Orellana, 1990; Rodriguez-Quijano et al., 2000; Sun et al., 2006) and DNA polymorphism (Zhang et al., 1996; Zaharieva et al., 2001; Sasanuma et al., 2004; Salina et al., 2006; Haider et al., 2008; Mahjoub et al., 2010; Giraldo et al., 2016) for better knowledge of speciation processes and genomic evolution in these species.

The main objective of the present research was to evaluate the genetic diversity of *Aegilops* populations sampled in a large range of ecological conditions in northern Algeria, using a cytogenetic approach based on analysis of the chromosomes structure by FISH. Moreover, the role of ecological factors in the differentiation and evolution of chromosomes and species is also discussed.

Materials and Methods

Plant materials

The collections used in these experiments are as follows: thirty-five populations of *A. geniculata*, eight of *A. neglecta*, six of *A. ventricosa*, and four of *A. triuncialis*, sampled from May 2012 to July 2015, according to an east-west rainfall gradient and north-south aridity gradient in Algeria (Fig. 1).

Each sampling site was characterized by the main ecological factors of the Mediterranean climate as the average annual rainfall (P), average temperatures of the hottest month in summer (M), average temperatures of the coldest month in winter (m), altitude (Alt), and the bioclimatic coefficient (Q2) (Table 1). At each sampling site, ten individuals were randomly sampled (each separated from another sample by at least one meter) for cytogenetic study (FISH).

Chromosome preparations

Chromosome preparations were made according to González-García et al. (2011), with some modifications. After seeds germination in Petri dishes at the room temperature, the roots of 1.0 to 1.5 cm were cut and pretreated by ice-cold water for 48 hrs. Fixation and conservation were made in a mixture alcohol-acetic acid (3:1) at 4 °C. Roots maceration was carried out in following mixture of enzymes: cytohelicase 1%, pectolyase 1%, and cellulase 1% (Sigma) at 37 °C for 105 min. After rinsing with water to remove excess of the enzyme mixture, the root tips were cut and mounted between the slide and cover slide in a drop of 45% acetic acid to increase the contrast of the chromosomes and cytoplasm. The squash was done with a match and the slides were subsequently frozen in liquid nitrogen to fix the chromosomes on slides. For the fluorescence observation of the chromosomes, the slides were washed successively by 1 × SSC, 2 × SSC, PBS, 4B solution and 4 Tween for 15 min.

Fluorescence in situ hybridization (FISH)

FISH on chromosomal DNA was carried out using three DNA probes, 5S rDNA (pTa 794) and 45S rDNA (pTa 71) isolated from *Triticum aestivum*, and pSc119.2 (repetitive DNA sequence) isolated from *Secale cereale* L. (Bedbrook et al., 1980; Gerlach, Bedbrook, 1979; Gerlach, Dyer, 1980).

The three DNA probes were labeled by nick-translation: Biotin-16-dUTP for 5S rDNA and by Digoxigenin-11-dUTP for 45S rDNA and pSc119.2. Biotin and digoxigenin were detected by the antibody conjugated to the anti-streptavidin Cy3 (red) and antibody conjugated with anti-DIG fluorescein (green), respectively. The probing was made according to Kwiatek et al. (2013), using the two hybridization mixtures: the first – 160 µl (20 µl / slide) containing 5S rDNA and 45S rDNA probes, and the second – 160 µl (20 µl / slide) containing 5S rDNA and pSc119.2 probes. Chromosomal plaques with fluorescence signal were observed with an Olympus BX61 epifluorescence microscope (OLYMPUS corporation, Tokyo, JAPAN) equipped with a DP7 CCD camera. The captured images have been optimized for better contrast and brightness with Adobe Photoshop software (version 10.0).

Table 1. **Climatic and floristic characteristics of sampling sites of natural populations of *Aegilops* in Algeria**

Sites	Populations	Floristic characteristics	Alt (m)	M (C°)	m (C°)	P (mm)	Q_2	Bioclimat
Littoral								
El-Kala 1	**g29**	Lawn of *Poaceae*, wayside	30	27	2.8	1715	243.07	Humid
El-Kala 2	**g30**	Top of a hill with herbaceous plants	10	27	2.8	1715	243.07	Humid
Seraidi	**g24**	Lawn of *Poaceae* adjoining an olive grove (*Olea europaea*)	200	26.2	3.8	919	140.72	Sub-humid
Skikda	**g23**	Edge of way adjoining a grove of olive and lentisk (*Olea europaea* – *Pistacia lentiscus*)	200	34.33	4.9	704	85.02	Sub-humid
Ançor	**g2**	Lawn of *Poaceae*, roadside	150	25.5	7	457	75.9	Semi-arid
Ain-Tassa	**v1**	Herbaceous lawn	100	25	6.60	437	81.46	Sub-humid
Tell Atlas								
Zitouna	**g28**	Roadside adjoining a grove of olive and lentisk (*Olea europaea* – *Pistacia lentiscus*)	500	29	4.2	1773	245.21	Humid
Bougous	**g27**	Grass lawn at the edge of cork oak forest (*Quercus suber*)	250	29.6	4.8	1740	240.65	Humid
El-Afrine	**g26**	Lawn of *Poaceae* under *Eucalyptus camaldulensis*	300	29.5	4.7	1750	242.03	Humid
Souk-Ahras	**g25**	Degraded maquis of cork oak (*Quercus suber*)	500	24	6.3	729	78.4	Sub-humid
Constantine	**g22**	Roadside under *Eucalyptus camaldulensis*	600	32.8	2.8	515	58.88	Semi-arid
Mila	**g21**	Herbaceous field	450	30.7	2.5	603	73.34	Sub-humid
Sétif	**g20**	Roadside in herbaceous field	650	32.8	6	470	60.15	Semi-arid
Ourissia	**g19**	Lawn of *Poaceae* bordering a forest of *Pinus halepensis*	800	32	5.2	500	63.99	Semi-arid
Kherata	**g18**	Roadside in herbaceous field	600	32.3	4	1103	133.68	Sub-humid
Timzrite	**g17**	Edge of fig grove (*Ficus* sp.)	700	35.6	2.1	503	51.5	Semi-arid
Stita	**n1-g16**	Lawn of *Poaceae* adjoining an olive grove (*Olea europaea*)	300	28.9	6.3	1290	195.78	Humid
Ouaguenoune	**n2**	Roadside bordering a field of wheat (*Triticum durum*)	150	31.4	6.8	952	132.73	Humid
Taboukert	**g13-n3-t3**	Herbaceous lawns on the bank of the Oued Sebaou River	100	32.02	7.22	986	135.27	Humid
Timzguida	**g14-n4**	Roadside adjoining a grove of olive and lentisk (*Olea europaea* – *Pistacia lentiscus*)	300	30.7	3.9	1035	132.46	Humid
Tizi-Rached	**g15-n5-t4**	Lawn of *Poaceae* adjoining an olive grove (*Olea europea*)	400	30.0	4.7	1210	163.9	Humid
Iguenane	**n6**	Roadside bordering a holm oak forest (*Quercus ilex*)	600	32.0	2.7	1149	134.5	Humid
Ait-Meraou	**g12-n7**	Edge of fig grove (*Ficus* sp.)	650	31.6	2.9	1037	123.9	Humid
Benyenni	**g11-n8**	Grass lawn at the edge of a cork oak forest (*Quercus suber*)	850	32.6	2.8	1080	124.3	Humid
Tikjda 1	**g9**	Herbaceous lawn on flushed rocks	1200	30.2	2.1	1100	134.2	Humid
Tikjda 2	**g10-v4**	Edge of Atlas cedar formation (*Cedrus atlantica*)	1478	29	0.9	1190	145.3	Humid
Relizane	**t2**	Understory of a forest of *Pinus halepensis*	630	28.7	5.8	325	48.67	Semi-arid
Mascara	**g4**	Understory of a forest of *Pinus halepensis*	735	28.2	5.5	370	55.90	Semi-arid
Tessala 1	**v3**	Degraded maquis of cork oak (*Quercus suber*) on flushed rocks	850	32.5	2.9	678	80.02	Sub-humid
Tessala 2	**g3-v2**	Degraded maquis of holm oak (*Quercus ilex*) on flushed rocks	950	32.01	2.1	700	80.27	Sub-humid
Mansourah	**g1-t1**	Forest of *Pinus halepensis* and *Quercus ilex*	700	39.9	0.6	435	37.96	Sub-humid
Hamdania	**g5**	Lawn of *Poaceae* on the riverbank	450	31.6	3.3	755	91.50	Sub-humid
Benchicao	**g6**	Roadside in a lawn of *Poaceae*	1200	35	1.3	625	63.7	Semi-arid
Médea	**g7**	Formation of *Pinus halepensis*	600	32.2	2.7	800	93.3	Sub-humid
Berrouaghia	**g8**	Roadside adjoining a holm oak forest (*Quercus ilex*)	850	34.2	6	593	72.12	Semi-arid
Steppe highlands								
Mesrane	**g31**	Steppe with *Caroxylon vermiculatum* (*Salsola vermiculata*)	1050	28.4	5	309	45.29	Arid
Guelt-Estel	**g32**	Steppe with *Lygeum spartum*	900	28.9	5.8	285	43.32	Arid
Saharan Atlas								
Senalba	**g33**	Forest of *Pinus halepensis*	1350	33	–1	402	43.08	Semi-arid
Col des Caravanes	**g34-v5**	Clearing, with herbaceous lawn	1300	36	–1.5	352	34.99	Arid
Messaad	**g35**	Clearing, with herbaceous lawn	750	34.5	–2	342	36.09	Arid
Taadmit	**v6**	Clearing, with herbaceous lawn	850	36	–1.25	321	32.38	Arid

Alt – altitude; **M** – average of the maximum temperatures of the hottest month; **m** – average of the minimum temperatures of the coldest month; **P** – average annual rainfall; **Q2** – Emberger's coefficient; **g** – *Aegilops geniculata*; **t** – *A. triuncialis*; **v** – *A. ventricosa*; **n** – *A. neglecta*.

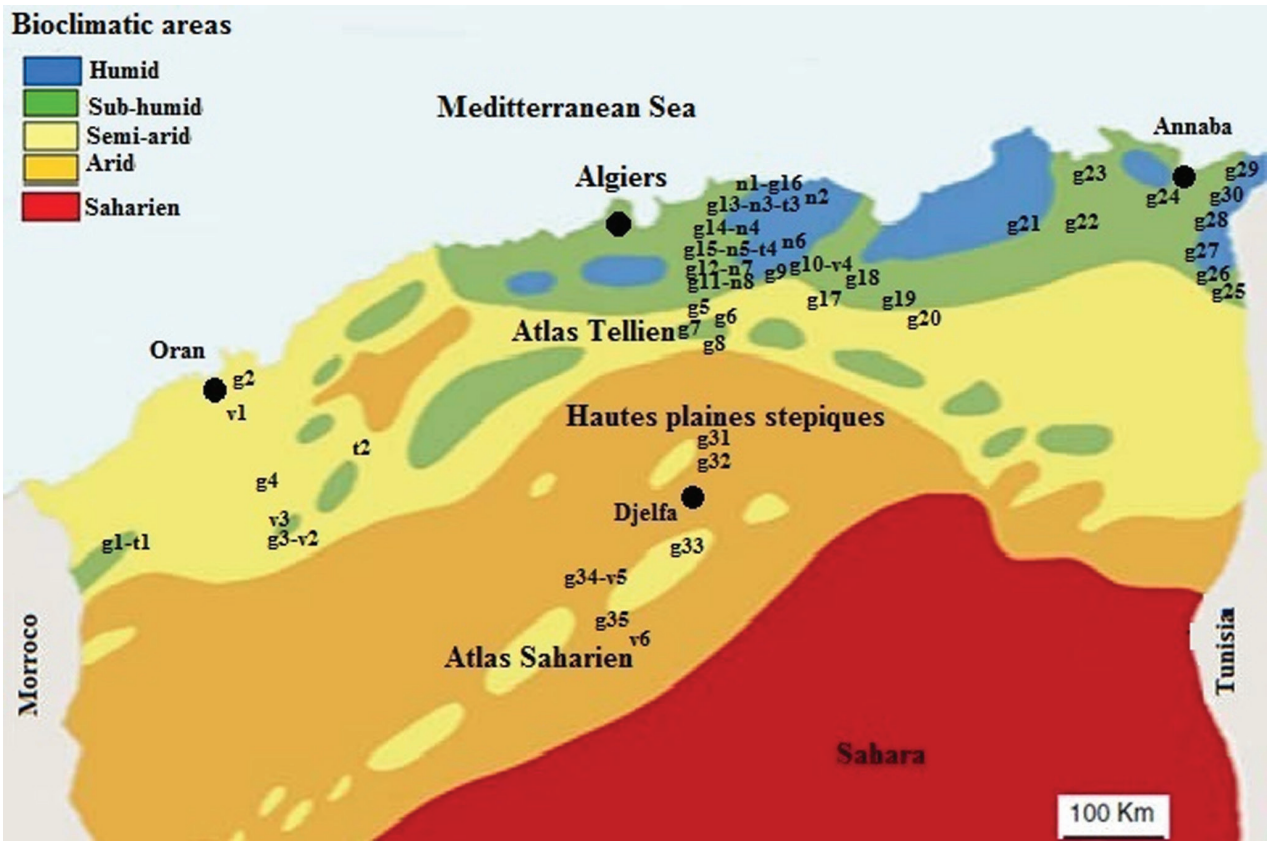

Fig. 1. Location of sampled populations of four species of *Aegilops* in Algeria (Aeg – *A. geniculata*, Aen – *A. neglecta*, Aev – *A. ventricosa*, and Aet – *A. triuncialis*). Bioclimatic limits are provided according to Stewart (1974)

Table 2. **The rates of 5S, 45S and pSc 119 in four *Aegilops* species from Algeria**

Species	Hybridization profile	5 S			45 S			pSc 119	
		▬	▬	▬	▬	▬	▬	⊙	○
A. geniculata	Aeg I	4	10	2	4	6	2	/	/
	Aeg II	4	4	0	4	8	0	/	/
	Aeg III	4	4	0	4	10	0	/	/
	PrH.1	/	/	/	/	/	/	11	8
	PrH.2	/	/	/	/	/	/	11	6
A. ventricosa	Aev I	2	4	0	2	0	0	/	/
	Aev II	2	4	0	2	4	4	/	/
	Aev III	2	6	0	2	0	0	/	/
	PrH.1	/	/	/	/	/	/	8	10
	PrH.2	/	/	/	/	/	/	10	14
A. triuncialis	Aet I	4	10	0	4	4	0	/	/
	Aet II	4	8	0	4	4	0	/	/
	PrH.1	/	/	/	/	/	/	21	16
A. neglecta	Aen I	4	6	2	4	4	2	/	/
	Aen II	4	6	2	4	4	0	/	/
	PrH.1	/	/	/	/	/	/	28	23

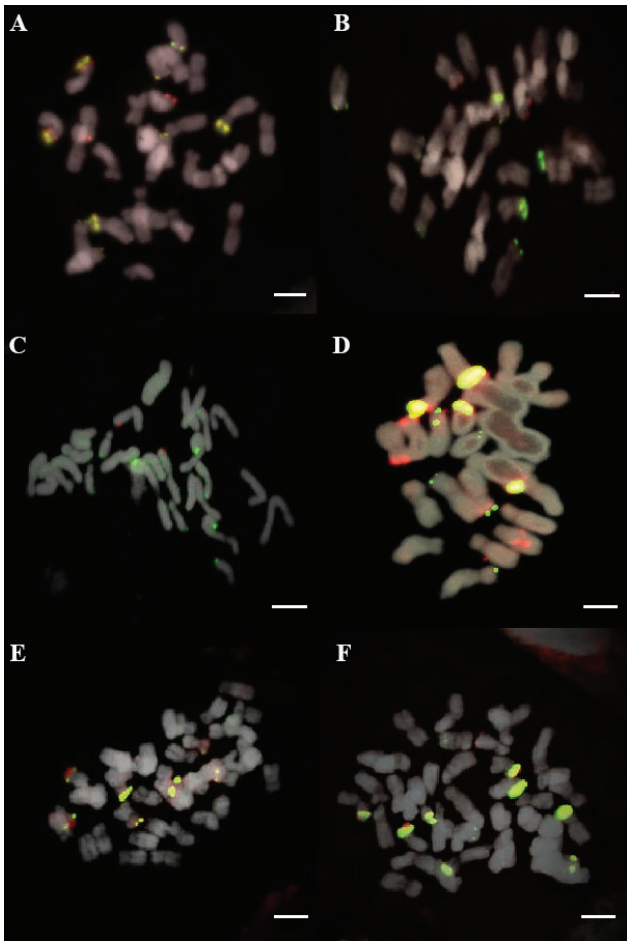

Fig. 2. FISH with rDNA 5S rDNA (Red) and 45S rDNA (Green) on mitotic chromosomes of *A. geniculata* (A: Aeg I; B, C: Aeg II; D: Aeg III) and *A. neglecta* (E: Aen I; F: Aen II). Scale bar: 10 µm

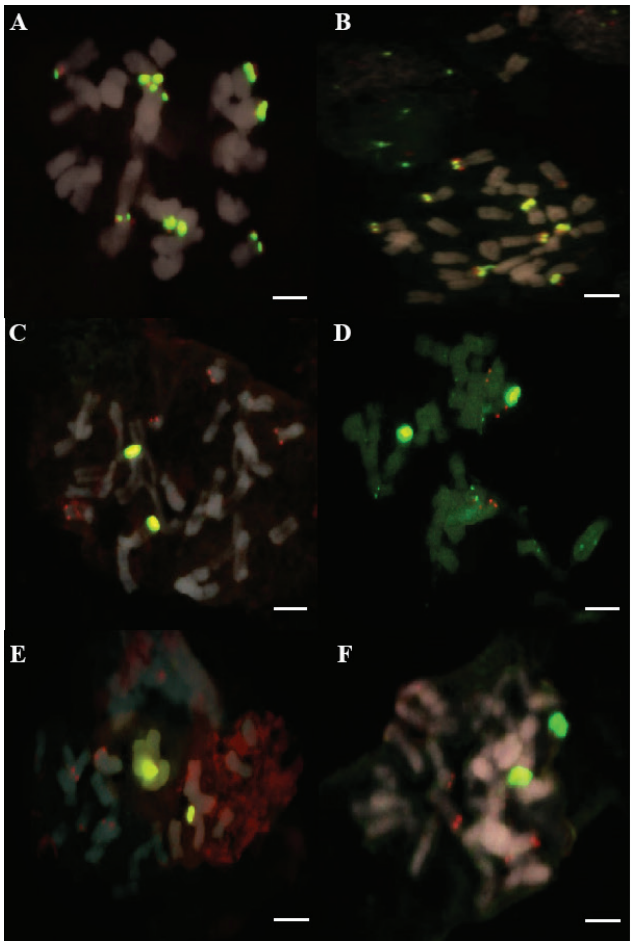

Fig. 3. FISH with 5S rDNA (Red) and 45S rDNA (Green) on mitotic chromosomes of *A. triuncialis* (A: Aet I, B: Aet II) and *A. ventricosa* (C: Aev I; D: Aev II; E, F: Aev III). Scale bar: 10 µm

Results

Genetic diversity of the rDNA loci (5S and 45S)

Analysis of the mitotic chromosomes, by FISH with two rDNA probes 5S and 45S (Table 2) revealed three hybridization patterns in the two species, *A. geniculata* (Aeg I, Aeg II, and Aeg III) and *A. ventricosa* (Aev I, Aev II, and Aev III), and two hybridization patterns in *A. triuncialis* (Aet I and Aet II) and *A. neglecta* (Aen I and Aen II) (Figs 2–4).

Aegilops geniculata

The **Aeg I** was found in the populations sampled in low altitudes with humid and subhumid bioclimates. It carries six chromosome pairs labeled as follows: the first pair, with two large 45S rDNA loci corresponding to active nucleolar organizing regions (NOR), two large 5S rDNA loci superimposed in the telomeric region of the short arm, two minor 5S rDNA loci and two minor 45S rDNA loci located in the subtelomeric region of the long arm; the second pair, with two large 45S rDNA loci (NOR) and two large 5S rDNA loci superimposed in the telomeric region of the short arm and two small 5S rDNA loci in the subtelomeric region of the same arm; the third pair, is labeled with two small 5S rDNA loci in the telomeric region of the short arm; finally, the fourth, the fifth and the sixth pairs, were labeled each in the same way by two small 5S rDNA loci and two small 45S rDNA loci on telomeric region of the short arm.

The **Aeg II** distinguishes the populations sampled in the mountains and plains at medium altitude under subhumid and semi-arid bioclimates. It comprises six chromosome pairs labeled as follows: the first pair, with two large 45S rDNA (NOR) and two large 5S rDNA superimposed in the telomeric region of the short arm of

chromosome 1 (in chromosome 2 these loci are deleted), and two minor 45S rDNA in the secondary construction; the second pair, with two large 45S rDNA (NOR) and 5S rDNA superimposed in the telomeric region of the short arm and two small 5S rDNA and two small 45S rDNA in the subtelomeric region of the same arm; the third pair, labeled with two small 5S rDNA in the telomeric region of the short arm; the fourth, fifth and sixth pairs, were similarly labeled each by two small 45S rDNA loci in the telomeric region of the short arm.

The **Aeg III** characterizes the populations of mountains at high altitudes with humid bioclimate. It consists of the following six chromosome pairs: the first pair, with two large 45S rDNA (NOR) and two large 5S rDNA in the telomeric region of the short arm; the second pair, with two large 45S rDNA (NOR) and two large 5S rDNA superimposed in telomeric region of the short arm as well as two small 5S rDNA and two small 45S rDNA in the subtelomeric region of the same arm; the third, fourth and sixth pairs were labeled each by two small 45S rDNA in the telomeric region of the short arm, while the fifth pair was labeled by two small 5S rDNA and two small 45S rDNA on telomeric region of the short arm.

Aegilops ventricosa

The **Aev I** characterizes the populations sampled in the low altitudes with semi-arid bioclimate. These populations carried three chromosome pairs labeled as follows: the first pair with two small 5S rDNA loci in the subtelomeric region of the long arm; the second pair with two large 45S rDNA loci (NOR) and two large 5S rDNA loci superposed in the telomeric region of the short arm; the third pair labeled with two small 5S rDNA loci in the telomeric region of the short arm.

The **Aev II** was found in populations of the Tell Atlas Mountains at high altitudes under humid bioclimate. It contains seven chromosome pairs: the first pair, with two small 5S rDNA in the subtelomeric region of the long arm; the second pair, with two minor 45S rDNA in the telomeric region of the short arm; the third pair labeled by two small 45S rDNA on telomeric region of the short arm; the fourth pair, with two small 45S rDNA in interstitial site on the long arm; the fifth pair labeled by two large 45S rDNA (NOR) and two large 5S rDNA superimposed in telomeric region of the short arm; the sixth pair, with two small 5S rDNA in the telomeric region of the short arm; the seventh pair labeled with two 45S rDNA minor loci in the subtelomeric region of the long arm.

The **Aev III** distinguishes populations of the Saharan Atlas Mountains at high altitudes under arid bioclimate. It comprises three chromosome pairs labeled in the same way as **Aev I** and is differentiated by two small 5S rDNA loci in the subtelomeric region of the short arm of the second chromosomal pair.

Aegilops triuncialis

The **Aet I** was found in populations of steppe highlands with semi-arid bioclimate. It contains four chromosome pairs labeled as follows: the first and second pairs labeled by two large 45S rDNA loci (NOR) and two large 5S rDNA loci superposed in the telomeric region of the short arm and two small 5S rDNA loci in the subtelomeric region of the same arm; the third pair labeled with two small 5S rDNA loci and two small 45S rDNA loci in the telomeric region of the short arm; the fourth pair, with two small 5S rDNA loci and two small 45S rDNA loci in the telomeric region of the short arm and two small 5S rDNA loci in the subtelomeric region of the same arm.

The **Aet II** characterizes populations sampled in low altitudes with humid bioclimate. It is constituted by four chromosome pairs labeled in the same way as **Aet I**, but is differentiated by the deletion of two small 5S rDNA loci of the first chromosomal pair.

Aegilops neglecta

The **Aen I** characterizes the populations sampled in mountains at high altitude under humid bioclimate. It contains six chromosome pairs labeled as follows: the first pair, with two large 45S rDNA loci (NOR) and two large 5S rDNA loci on telomeric region of the short arm; the second pair with two large 45S rDNA loci (NOR), two large 5S rDNA loci and two small 5S rDNA loci in telomeric region of the short arm; the third pair, with two small 45S rDNA loci in the telomeric region and two small 5s rDNA loci in subtelomeric region of the short arm; the fourth pair labeled in the same manner as the third pair, with the reverse localization of these two loci; the fifth pair, with two minor 5S rDNA loci on the long arm; the sixth pair with two minor loci of 45S rDNA on the long arm.

The **Aen II** was found in the populations sampled at low altitudes under sub-humid bioclimate. It contains five chromosome pairs labeled in the same way as **Aen I**, but is differentiated by the deletion of the two 45S rDNA loci in the sixth chromosomal pair.

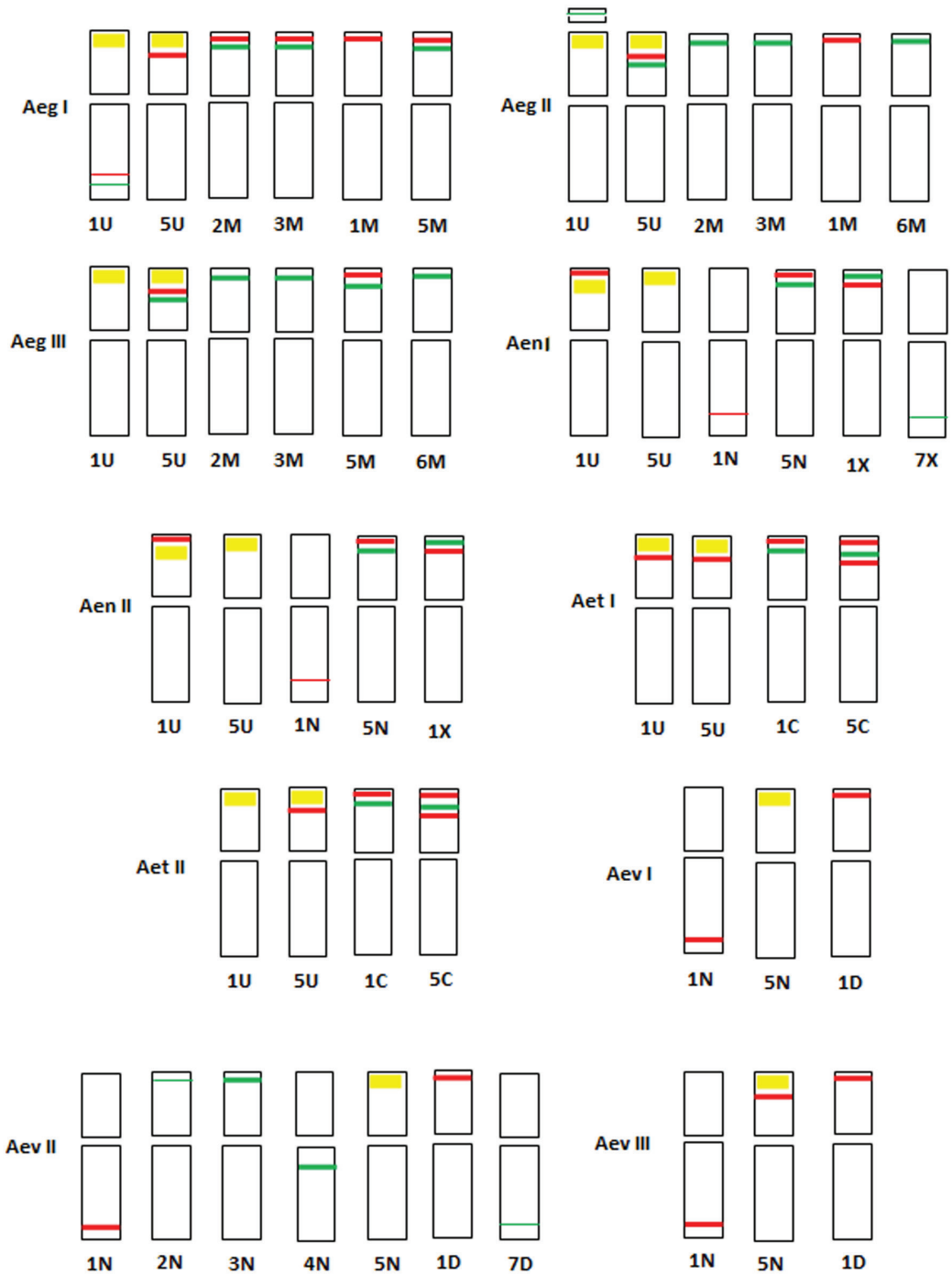

Fig. 4. Physical mapping (idiograms) showing distribution of 5S rDNA (red rectangle), 45S rDNA (green rectangle) and collocation of 5S rDNA and 45S rDNA (yellow rectangle) on mitotic chromosomes of four species of *Aegilops* from Algeria

Genetic variability of the repetitive DNA sequence (pSc119.2)

FISH with the pSc119.2 probe (Fig. 5, 6, Table 2) revealed two types of loci (large and small) distributed differently within the four studied species: two hybridization profile (**PrH1** and **PrH2**) in each of the species, *A. geniculata* and *A. ventricosa*, while *A. neglecta* and *A. triuncialis* each show only one hybridization profile (**PrH1**).

Aegilops geniculata

The **PrH1** was found in the populations sampled in low altitudes with humid and sub-humid bioclimates. It consists of nine chromosome pairs labeled with pSc119.2 probes as follows: 4 chromosome pairs (two small loci in the subtelomeric region of the short arm), 4 chromosome pairs (two large loci in the subtelomeric region of the short arm), and 1 chromosome pair (three large loci in the subtelomeric region of the short arm).

The **PrH2** characterizes the populations of mountains at high altitudes with humid and arid bioclimate. It consists of seven chromosome pairs labeled with pSc119.2 probes as follows: 2 chromosome pairs (two small loci in the subtelomeric region of the short arm), 3 chromosome pairs (two large loci in the subtelomeric region of the short arm), 1 chromosome pair (three large loci in the subtelomeric region of the short arm), and 1 chromosome pair (two large loci in the subtelomeric region of the short arm and two small loci in the subtelomeric region of the long arm).

Aegilops ventricosa

The **PrH1** distinguishes populations sampled in plains and mountains at medium altitude under sub-humid and semi-arid bioclimates. It has seven chromosomal pairs labeled by the pSc119.2 probe as follows: 1 chromosome pair (two small loci in the subtelomeric region of the long arm), 1 chromosome pair (two small loci in the subtelomeric region of the short arm), 1 chromosome pair (two large loci in the subtelomeric region of the long arm), 2 chromosome pairs (two large loci in the subtelomeric region of the short arm), 1 chromosome pair (two large loci in the subtelomeric region of the short arm and two small loci in the subtelomeric region of the long arm), and 1 chromosome pair (four small loci in the subtelomeric region of the long arm).

The **PrH2** characterizes populations of Tell and Saharan atlas at high altitudes under humid and arid bioclimate, respectively. It is distinguished by ten chromosomal pairs marked with the pSc119.2 probe as follows: 1 chromosome pair (two small loci in the subtelomeric region of the long arm), 3 chromosome pairs (two small loci in the subtelomeric region of the short arm), 1 chromosome pair (two large loci in the subtelomeric region of the long arm), 3 chromosome pairs (two large loci in the subtelomeric region of the short arm), 1 chromosome pair (two large loci in the subtelomeric region of the short arm and two small loci in the subtelomeric region of the long arm), and 1 chromosome pair (four small loci in the subtelomeric region of the long arm).

Aegilops triuncialis

In this species, one hybridization profile (**PrH1**) was found. It includes eleven chromosome pairs labeled with the pSc119.2 probe as follows: 1 chromosome pair (2 small loci in the subtelometric region of the short arm), 3 chromosome pairs (2 large loci in the subtelometric region of the short arm), 1 chromosome pair (3 large loci in the subtelometric region of the short arm), 2 chromosome pairs (2 small loci in the subtelometric region of the short arm and 2 small loci in the subtelometric region of the long arm), 2 chromosome pairs (2 large loci in the subtelometric region of the short arm and 2 small loci in the subtelometric region of the long arm), 1 chromosome pair (2 large loci in the subtelometric region of the short arm and 2 large loci in the subtelometric region of the long arm), and 1 chromosome pair (2 large loci in the subtelometric region of the short arm, 2 large loci in the subtelometric region of the long arm and 2 small loci in the middle position of the long arm).

Aegilops neglecta

This species showed one hybridization profile (**PrH1**). It encompasses seventeen chromosome pairs labeled with the pSc119.2 in the telometric region (short and long arm), except the following four chromosome pairs: 1 chromosome pair (with 2 interstitial loci and 2 loci in the middle part of the long arm), 1 chromosome pair (with 2 loci in the subtelomeric region on the long arm), 1 chromosome pair (with 2 loci in the middle region and 2 subtelomeric loci of the long arm), and 1 chromosome pair (with 2 loci in the middle part of the long arm).

Discussion

The FISH profiling and physical maps (idiograms) enabled to identify and characterize the families of genes that encode the ribosomal DNAs (5S and 45S rDNA loci) and repetitive DNA sequence (pSc119.2) in the

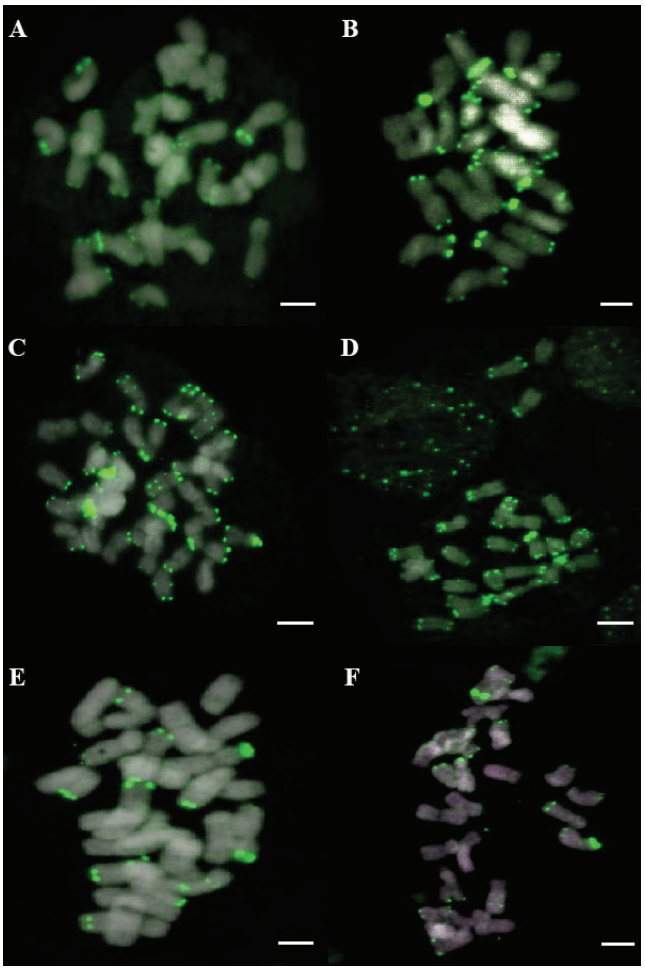

Fig. 5. FISH on mitotic chromosomes with repetitive DNA pSc119.2 (green circle): *A. geniculata* (A–B), *A. neglecta* (C), *A. triuncialis* (D) and *A. ventricosa* (E–F). Scale bar: 10 µm

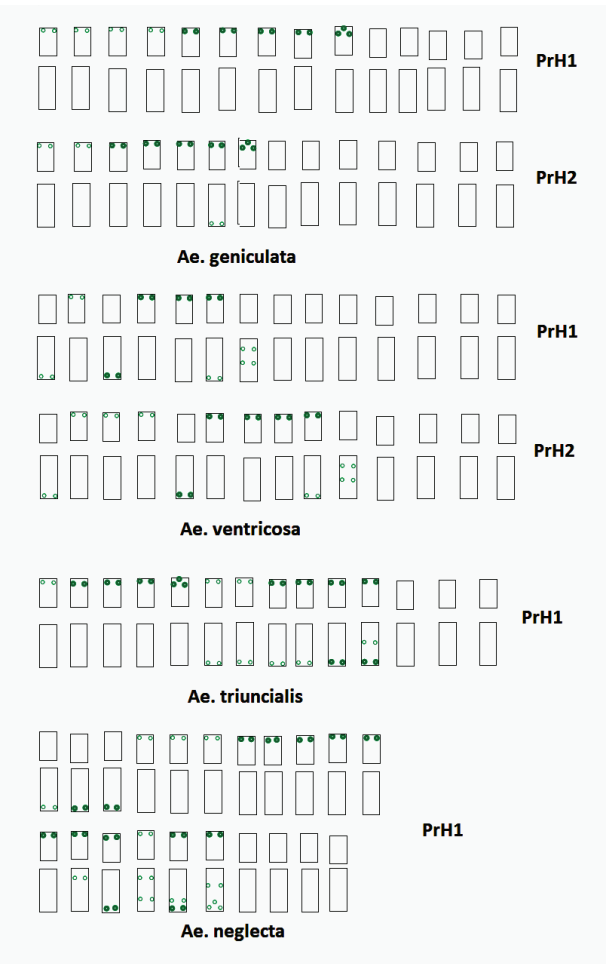

Fig. 6. Physical mapping (idiograms) showing distribution of repetitive DNA pSc119.2 (wide circle: large locus / slim circle: small locus) in four species of *Aegilops*

populations of the four species of *Aegilops* sampled in different eco-geographical areas in Algeria.

According to the results obtained by Badaeva et al. (2002, 2004), the chromosomal pairs identified by 5S and 45 S rDNA, correspond approximately to: 1U, 5U, 1M, 2M, 3M, 5M and 6M in *A. geniculata*, 1U, 5U , 1C, 5C in *A. triuncialis*, 1N, 2N, 3N, 4N, 5N, 1D and 7D in *A.ventricosa* and 1U, 5U, 7U, 1X, 7X, 1N and 5N in *A. neglecta*. Indeed, the chromosomes we identified differ from those identified by these authors, who reported changes in chromosome structure between individuals of the same species sampled in different geographical sites.

In other polyploid species of the genus *Aegilops*, such as *A. cylindrica* Host (Linc et al., 2012) and *A. crassa* (Badaeva et al., 1998), the rDNA sequences that code for these same RNAs genes do not vary between populations. At the same time, populations of the diploid species *A. speltoides* Tauch, considered to be a common ancestor of several species of the *Sitopsis* section, show a high variability in their cytogenetic structure revealed by a great polymorphism of the 5S rDNA and 45S rDNA sequences in relation to environmental conditions and changes in their ecological habitats (Belyayev, Raskina, 2013).

Some species of the family *Poaceae*, such as *Festuca pratensis* Huds. (now accepted as *Lolium pratense* (Huds.) Darbysh. or *Schedonorus pratensis* (Huds.) P.Beauv.), *Lolium perenne* L., *Hordeum vulgare* L. (Książczyk et al., 2010; Leitch et al., 1992), and some species of the family *Rosaceae*, such as species the genera *Sanguisorba* L., *Agrimonia* L., *Rosa* L., and *Rubus* L. (Mishima et al., 2002), show several patterns

of hybridizations of the 5S rDNA and 45S rDNA loci expressed by the duplication or deletion of the copies of these genes in some chromosomal pairs.

The repetitive DNA sequence (pSc119.2) revealed intraspecific differentiation of four species sampled at high altitudes, at low altitudes, and in the arid area by doubling and/or loss of some loci. This result agrees with those found by karyological analysis which showed that chromosome size varies significantly between the samples of these populations (Baik et al., 2017). Hence, these changes in the size and morphology of chromosomes have beneficial consequences on the evolutionary process and adaptation of species to eco-geographical conditions (Gorenflot, Raicu, 1980; Levin, 2002).

Abdolmalaki et al. (2019) and Parisod and Badaeva (2020) using FISH with repetitive DNA probes (GAA, pSc119.2, pTa535-1, pAs1-1) found a large intraspecific polymorphism of the hybridization patterns between different genotypes in each *Aegilops* species and a restructuring of the genome, which is associated with the processes of adaptation to the ecological condition and evolution of species and their chromosome structure.

Earlier, Salina et al. (2006) studied the evolution of DNA sequences (Spelt 1 and Spelt 52) repeated in tandem, in the species of *Triticum* and *Aegilops*, donors of the B genome, by FISH. Their data showed a large polymorphism in the number and patterns of hybridization of these sequences within species of each genus and determined phylogenetic relationships among species of the two genera. It has been proved that FISH is a good approach to characterize the genomes of interspecific hybrids between cultivated and related wild species, which has allowed understanding the role of cytogenetic markers in the function, structure and evolution of chromosomes and in plant breeding for the selection of suitable lines and populations (Cifuentes, Benavente, 2009; Kwiatek et al., 2013).

Meanwhile, the large-scale intraspecific and interspecific variability of the DNA loci and repetitive DNA sequence (pSc119.2) found in different cytotypes of the four species of *Aegilops* sampled in northern Algeria may be explained by the loss of genetic information by mutations, such as fissions and deletions, which have beneficial effects on the evolutionary process and adaptation (Gorenflot, Raicu, 1980; Soltis D., Soltis P., 1999). On the other hand, the structural diversity of chromosomes is often reflected in the phenotypic variability of several neopolyploid species (Ramsey, Schemske, 2002). This fact can favor adaptive evolutionary changes (Otto, Whitton, 2000) and would make it possible to adapt to eco-geographical conditions such as mountains at high altitudes and arid lands where some populations are sampled, thus promoting colonization of new ecological habitats (Levin, 2002).

Conclusion

Our results endorse that Algerian populations of *Aegilops* represent a polyploid complex with impressive genetic diversity and new landmarks of genes encoding rRNAs. Moreover, the north Algerian part has specific features and its transitional position between two contrasting areas (the desert to the south and the Mediterranean Sea to the north) offers the opportunity to study evolutionary trends of species and natural populations. We conclude that the environmental factors and geographical location might have an effect on the genetic structure and evolution of chromosomes. Furthermore, the cytogenetic markers are valuable tools in the identification and characterization of populations for assessing and enhancing plant genetic resources.

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Реферат. Продовжуючи наші попередні дослідження, ми провели каріологічне вивчення 53 популяцій чотирьох видів роду *Aegilops* (*A. geniculata*, *A. triuncialis*, *A. ventricosa* і *A. neglecta*), відібраних із різних за екологічними та географічними особливостями оселищ в Алжирі. Генетичну мінливість локусів хромосомної ДНК кожного зразка видів *Aegilops* виявляли методом флуоресценцентної гібридизації *in situ* з використанням трьох зондів: 5S рДНК, 45S рДНК і повторюваної ДНК (pSc119.2). Нами було встановлено, що два локуси рДНК (5S і 45S) гібридизувалися з деякими хромосомами і показали значний генетичний поліморфізм як у межах видів, так і між чотирма видами *Aegilops*, тоді як повторювані послідовності ДНК (pSc119.2) гібридизувалися з усіма хромосомами і відрізнялися в популяціях з гірських територій з вологим кліматом від популяцій зі степових районів із посушливим кліматом. Транспозиція фізичних карт досліджуваних локусів (5S рДНК, 45S рДНК і pSc119.2) з картами інших колекцій виявила існування нових локусів у представників роду *Aegilops* з Алжиру.

Ключові слова: *Aegilops*, Алжир, генетичне різноманіття, генетичні ресурси рослин, екологічна географія, цитогенетичні маркери