Genetic variation in Spanish populations of the genus *Aegilops* revealed by RAPDs

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Abstract – Random amplified polymorphic DNA (RAPD) markers were used to analyse genetic variability and relationships in wild species of the genus *Aegilops*. Fifty natural populations, which included the species *Aegilops biuncialis* (UUMM), *Ae. neglecta* (UUMMNN), *Ae. ovata* (UUMM), *Ae. ventricosa* (DDNN) and *Ae. triuncialis* (UUCC) were selected. These populations are widely distributed in the Iberian peninsula and Balearic islands. Twenty primers were used to generate DNA amplification data. Genetic relationships were established by correspondence analysis. In the resulting dendrogram, *Ae. ventricosa* appears to be segregated from the other species, probably owing to the influence of the D genome. *Ae. biuncialis* and *Ae. ovata* are clearly separated suggesting that the superindex system should be used to differentiate the M genomes of both species. The separation of the M and N genomes was also confirmed, as was the proximity of N to U and C to M. In addition, correlation was found between RAPD markers and ecogeographical factors. (© Inra/Elsevier, Paris.)

Aegilops / RAPDs / genetic diversity / ecogeographical factors

Resumé – Variation génétique dans des populations espagnoles du genre *Aegilops* étudiées par RAPD. Les marqueurs RAPD (ADN polymorphe amplifié aléatoirement) ont été utilisés dans une étude des relations génétiques entre les espèces du genre *Aegilops*. On a sélectionné cinquante populations naturelles des espèces de *Aegilops biuncialis*, *Ae. neglecta*, *Ae. ovata*, *Ae. ventricosa*, et *Ae. triuncialis*. Ces populations ont une ample répartition sur la Péninsule Ibérique et les Îles Baléares. Vingt oligonucléotides ont été choisis pour obtenir les données de l'ADN amplifié. Les relations génétiques ont été estimées à l'aide des techniques d'analyse de correspondance. Dans le dendrogramme résultant *Ae. ventricosa* apparaît éloigné des autres espèces, peut être à cause de l'influence de génome D. La séparation nette de *Ae. biuncialis* et *Ae. ovata* permet de penser que le système de superindex devrait être utilisé pour différencier les génomes M de deux espèces. On confirme aussi la séparation de génomes M et N, ainsi que la proximité des génomes N et U et C et M. De plus des corrélations entre les marqueurs RAPD et des facteurs écogéographiques ont été observées. (© Inra/Elsevier, Paris.)

Aegilops / RAPDs / diversité génétique / facteurs écogéographiques

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1. Introduction

Cereal breeders refer to the species of the genus *Aegilops* as the wild relatives of wheat. They probably represent the main genetic reserve available for the improvement of the wheat cultivar [4, 14]. *Aegilops* species are mostly annual and autogamous. Polyploid forms exist, arising by evolutionary convergence of ancestral diploid species [27]. The distribution of the genus *Aegilops* extends from southwest and central Asia to the Mediterranean basin with Turkey as the centre of diversification. The genus has adapted in such a way that all the species are annual and survive the extreme Mediterranean summers as seeds. Spain is especially rich in wild populations of the polyploid species *Aegilops biuncialis* Vis. (UUMM), *Ae. triuncialis* L. (Uucc), *Ae. ovata* L. (UUMM) and *Ae. ventricosa* Tausch (DDNN), all of which are allotetraploids, and *Ae. neglecta* Req. ex Bert. (UUMM/UUMMNN) which is found naturally in both tetraploid and hexaploid forms [9, 14, 26]. The hexaploid variant (*Ae. neglecta* subsp. *recta* Zhuck and Hammer) was selected for the present study since the tetraploid is not found in the Iberian peninsula. *Aegilops ovata* and *Ae. triuncialis* are widely distributed in Spain except in the north and northwest, areas which are typified by cool, damp summers. *Aegilops neglecta*, *Ae. biuncialis* and *Ae. ventricosa* may be found in more restricted areas [20]. *Aegilops caudata* L (CC), *Ae. commosa* Sibth. and Sm (MM), *Ae. uniaristata* Vis. (NN), *Ae. umbellulata* Zhuck (UU) and *Ae. squarrosa* Coss. (DD) are the proposed diploid ancestors of the species mentioned above and were used as phylogenetic markers.

Over several years, the present authors have explored the Iberian peninsula and collected wild populations of *Aegilops* with the aim of introducing native material into plant breeding programmes, mainly those involving wheat and *Triticale*. Special care must be taken to preserve the original structure of the natural populations. The collection method of Hawkes [10] increases the genetic value of the material harvested. Indeed, if the genetic individuality of a plant is maintained,

genes of interest may be detected in individual plants, and their allelic frequencies calculated for each population. This is an important consideration in the selection of a plant breeding strategy.

Studies of genetic variability in natural populations are extremely useful during the process of introducing wild material into plant breeding programmes. Besides, although in the past many useful cytological and biochemical studies have examined the genetic relationships within the genus [2, 12, 18, 19], the information available is far from complete and further molecular data are required if valid taxonomic conclusions are to be drawn. The aim of the present work was to study the interpopulational variability in different *Aegilops* species and their interspecific genomic relationships.

RAPD markers are extensively used in the evaluation of variability and genetic relationships despite some limitations mainly concerning the lack of reproducibility in different laboratories. This screening method, based on DNA sequence polymorphisms in primer binding regions, is easy to perform, fast, inexpensive and efficient [3, 5, 7, 17, 22, 23, 25, 28, 29].

2. Materials and methods

2.1. Plant material

Fifty populations of five *Aegilops* species growing in Spain and samples of five diploid species regarded as the ancestral donors of these tetraploid forms were used. Ten populations per species of *Ae. biuncialis*, *Ae. triuncialis*, *Ae. ovata*, *Ae. ventricosa* and *Ae. neglecta* were selected for the study. This plant material belongs to the living collection of wild *Triticeae* collected from natural environments, maintained at the I.N.I.A. plant breeding unit (La Canaleja, Madrid, Spain). Specimens were collected according to the method of Hawkes [10]. These populations are representatives of the total geographical distribution of the five species in Spain [20]. The species found in Spain, populations and their geographical origin are shown in figure 1. Single populations of *Ae. caudata*, *Ae. commosa*, *Ae. uniaristata*, *Ae. umbellulata* and *Ae. squarrosa* provided by the Royal Botanical Gardens of London (UK) were also included in the study.

Figure 1. Geographical distribution of the Spanish *Aegilops* species and populations used in the study: *Ae. ventricosa* (V), *Ae. ovata* (O), *Ae. triuncialis* (T), *Ae. biuncialis* (B) and *Ae. neglecta* (N).

2.2. Genome extraction and PCR procedures

Genomic DNA was extracted from young, green leaves of 20–30 individual specimens of each population, according to the method of Dellaporta et al. [6]. PCR amplification was performed in a 25 μ L reaction volume containing 7.5 ng primer, 12.5 ng DNA, 2.5 μ L reaction buffer 10 $\times$, 100 mM dNTPs and 1.25 units of Taq DNA polymerase (Bioprobe). Eighty 10-mer random primers used were provided by M, R, S and T kits (Operon technologies). A further ten primers were synthesised. Amplifications were made in a PTC-100 MJ Research Inc. thermocycler as follows: 41 cycles of 1 min at 94 $^{\circ}$ C, 1 min at 36 $^{\circ}$ C, 2 min at 72 $^{\circ}$ C, and 5 min

at 72 $^{\circ}$ C [28]. The amplification products were separated by electrophoresis in 1.8 % agarose gel using TAE buffer and visualised by ethidium bromide staining. Boeringer Mannheim marker VI (174–2 176) was used as the molecular marker.

2.3. Statistical analyses

RAPD data were scored in a global matrix. Amplified bands of identical size were considered to be the same marker and the presence (1) or absence (0) of bands were considered as character states. The data matrix was analysed with the NTSYS computer package (version 1.50) [21]. Correspondence analysis [17] and

Table 1. Primers used and numbers of RAPD markers obtained in the ten taxonomic units of the genus *Aegilops* investigated. Also represented is the number of polymorphic bands per species.

Primers	Sequence 5'.....>3'	Total number	Size (bp)		Number of polymorphic bands and percentage									
			min	max	Ae ven	Ae ova	Ae tri	Ae biu	Ae neg	Ae cau	Ae com	Ae squ	Ae uni	Ae umb
OPM-08	TCTGTTCCCC	5	390	550	1(20)	0	0	3(60)	0	0	0	0	1(20)	1(20)
OPR-01	TGCGGTCCT	10	380	950	2(20)	3(30)	2(20)	3(30)	1(10)	1(10)	1(10)	1(10)	1(10)	1(10)
OPR-09	TGAGCACGAG	7	400	1,240	3(42.9)	2(28.6)	2(28.6)	5(71.4)	4(57.1)	0	2(28.6)	2(28.6)	1(14.3)	1(14.3)
OPR-14	CAGGATTCCC	7	320	650	1(14.3)	1(14.3)	2(28.6)	4(57.1)	4(57.1)	3(42.9)	2(28.6)	0	1(14.3)	1(14.3)
OPS-03	CAGAGGTCCC	10	220	900	2(20)	3(30)	3(30)	2(20)	2(20)	1(10)	2(20)	1(10)	2(20)	3(30)
OPS-04	CACCCCTTG	15	350	1,230	3(20)	5(33.3)	6(40)	9(60)	6(40)	3(20)	3(20)	3(20)	3(20)	3(20)
OPS-05	TTTGGGGCT	10	420	850	1(10)	5(50)	2(20)	3(30)	1(10)	1(10)	2(20)	2(20)	2(20)	2(20)
OPS-09	TCCTGGTCCC	10	250	650	1(10)	1(10)	1(10)	4(40)	3(30)	2(20)	3(30)	3(30)	1(10)	3(30)
OPS-10	ACCGTCCAG	7	300	1,000	3(42.9)	1(14.3)	2(28.6)	3(42.9)	0	2(28.6)	2(28.6)	3(42.9)	1(14.3)	1(14.3)
OPS-14	AAAGGGTCC	5	350	900	0	0	0	1(20)	0	1(20)	0	2(40)	0	0
OPS-19	GAGTCACCAG	4	520	800	1(25)	1(25)	2(50)	0	0	1(25)	1(25)	0	1(25)	1(25)
OPS-20	TCTGGACGCT	14	220	900	3(21.4)	2(14.3)	2(14.3)	4(28.6)	3(21.4)	1(7.1)	3(21.4)	3(21.4)	4(28.6)	1(7.1)
OPT-01	GGGCCACTCA	6	350	1,000	1(16.7)	1(16.7)	2(33.3)	0	0	1(16.7)	2(33.3)	2(33.3)	2(33.3)	2(33.3)
OPT-02	GGAGAGACTC	6	200	1,000	0	2(33.3)	2(33.3)	1(16.7)	2(33.3)	1(16.7)	2(33.3)	1(16.7)	1(16.7)	1(16.7)
OPT-03	TCCACTCCTG	6	200	950	3(50)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	2(833.3)	1(16.7)	1(16.7)
OPT-05	GGGTTGGCA	2	390	450	0	0	1(50)	0	0	0	0	0	0	0
OPT-08	AACGGCGACA	3	250	480	2(66.7)	2(66.7)	1(33.3)	1(33.3)	2(866.7)	0	0	0	1(33.3)	1(33.3)
OPT-13	AGGACTGCCA	5	300	1,000	2(40)	3(60)	1(20)	2(40)	3(60)	1(20)	1(20)	1(20)	1(20)	1(20)
OPT-19	GTCCGTATAG	7	320	420	1(14.3)	4(57.1)	2(28.6)	2(28.6)	0	0	0	0	0	0
OPT-20	CACCAATCCT	7	300	1,000	2(28.6)	0	1(14.3)	2(28.6)	1(14.3)	1(14.3)	3(42.9)	1(14.3)	1(14.3)	1(14.3)

Table II. Number of RAPD products generated (diagonal) and shared (remaining data) by 20 primers in ten *Aegilops* species. The number of specific markers found in each taxonomic unit is indicated in brackets.

	Ae ven	Ae ova	Ae tri	Ae biu	Ae neg	Ae cau	Ae com	Ae squ	Ae uni	Ae umb
<i>Ae. ventricosa</i>	47(9)	26(0)	23(0)	26(0)	26(1)	20(0)	21(1)	28(7)	23(0)	22(0)
<i>Ae. ovata</i>		51(7)	34(1)	38(3)	32(1)	22(0)	21(1)	16(0)	29(0)	33(2)
<i>Ae. triuncialis</i>			51	36	31	27	29	21	30	32
<i>Ae. biuncialis</i>				69(14)	40(2)	25(0)	32(1)	23(0)	35(0)	35(0)
<i>Ae. neglecta</i>					54(8)	21(1)	26(0)	20(0)	29(0)	30(0)
<i>Ae. caudata</i>						35(4)	27(2)	12(1)	25(0)	23(0)
<i>Ae. comosum</i>							49(6)	22(0)	28(0)	28(1)
<i>Ae. squarrosa</i>								42(10)	23(0)	21(0)
<i>Ae. uniaristata</i>									39(0)	33(0)
<i>Ae. umbellulata</i>										42(3)

Figure 2. Intra- and interspecific polymorphisms revealed by amplification with primer R1 in six populations of *Ae. ovata* and ten *Ae. triuncialis*.

the unweighted pair group method (UPGMA) [24] were used to construct a dendrogram. Correlations between RAPD markers and ecogeographical variables were determined by the Spearman rank order correlation coefficient, using SPSS software (SPSS Inc., 1989–1995, version 6.1.3).

3. Results and discussion

3.1. Interpopulational and interspecific analyses

Of the 90 arbitrary 10-mer primers tested, 20 produced adequate DNA amplification patterns in each species. Bias based polymorphisms were carefully avoided [3]. RAPD amplifications were

performed in triplicate and only reproducible bands were scored (figure 2). A total of 146 RAPD products were recorded of which 131 (89.7 %) detected polymorphisms. Fifty-six showed variability at the interpopulational level, and 75 could discriminate only at the species level. The number of amplified bands per primers ranged from 15 (S-4) to 2 (T-5). Fragment size ranged from 200 to 1 240 bp (table I). *Ae. biuncialis* showed the highest degree of variation with 43.7 % polymorphic bands between populations. *Ae. neglecta* showed the least variation (7.5 %). The remaining values were 21.6 % (*Ae. ovata*), 21.6 % (*Ae. triuncialis*) and 10.5 % (*Ae. ventricosa*). Only nine bands were specific to single populations (6.2 %) while 68 (46.6 %) were present only in single species as described in table II.

The dendrogram generated (figure 3) was of sufficient resolution to clearly separate all the species and most populations. However, several populations of *Ae. neglecta*, *Ae. ventricosa* and *Ae. triuncialis* were not resolved. The low level of intraspecific variation detected in *Ae. ventricosa* and *Ae. neglecta* (table III) is consistent with the tendency of these species to colonise similar ecological habitats in different geographical locations of the Iberian peninsula [20].

Ae. ventricosa was associated with *Ae. squarrosa* (DD) and was clearly segregated from the other species. This is probably due to the influence

Figure 3. Dendrogram of the *Aegilops* species and populations used generated by NTSYS and UPGMA. The Pearson correlation coefficient was used to estimate genetic distances.

Table III. Mean, coefficients of variation and variance for each *Aegilops* species according to similarity indices between populations.

	Mean	Variance	Coef. of Variation
<i>Ae. ventricosa</i>	0.875206	0.010062	11.46 %
<i>Ae. ovata</i>	0.717279	0.023189	21.23 %
<i>Ae. triuncialis</i>	0.742287	0.031991	24.10 %
<i>Ae. biuncialis</i>	0.506344	0.076132	54.49 %
<i>Ae. neglecta</i>	0.900663	0.013363	12.83 %

of the D genome. This genome has undergone less change than other diploid genomes during evolu-

tion and therefore appears to be less modified and is well separated within the *Triticeae* [1, 4]. *Ae. biuncialis*, *Ae. ovata* and *Ae. triuncialis*, the tetraploid species which share the U genome, cluster together owing probably to the influence of this genome. However, *Ae. neglecta*, another species of the U group, although shown to be related to these species, was relatively distant. Given that *Ae. neglecta* is hexaploid, the tree seems to reflect differences at the ploidy level. All the diploid species, except *Ae. squarrosa*, were closely associated with *Ae. triuncialis*. The U and C genomes of *Ae. triuncialis* have undergone almost no evolutionary change with respect to the same genomes in other

Table IV. Significant correlations ($P = 0.01$) between ecogeographical variables and RAPD markers. The sign of the correlation (+/-) is indicated after the species containing the marker (V = *Ae. ventricosa*, T = *Ae. triuncialis*, O = *Ae. ovata*, B = *Ae. biuncialis*, N = *Ae. neglecta*). The markers are indicated with the primer used followed by a code number. The ecogeographical variables evaluated were: soil, Tanu (mean annual temperature), Tmin (mean minimum temperature of the coldest month), Panu (mean annual rainfall), Pver (summer rainfall), Alt (altitude), Lat (latitude), Lon (longitude), TI (thermal index = Tanu + Tmin + Tmax (mean maximum temperature of the coldest month), MI (Mediterranean factor index = evapo-transpiration potential in June, July and August/mean rainfall for the same period), FFD (frost-free day index = number of days per year with temperatures above 0 °C).

	Soil	Tanu	Tmin	Panu	Pver	Alt	Lat	Lon	TI	MI	FFD
T20-1	---	---	---	---	---	---	---	T ⁺ B ⁺ N ⁺	---	---	---
T20-2	---	V ⁻	---	---	---	---	---	---	---	---	---
S14-1	---	---	---	---	---	B ⁺	---	---	---	---	---
S09-1	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
S09-2	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
S09-3	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
S09-4	---	---	---	---	---	---	---	---	---	O ⁺ B ⁺ N ⁺	---
T08-1	---	---	---	---	---	V-O-N ⁻	---	---	---	---	---
T13-1	---	---	---	N ⁺	---	---	---	---	---	---	---
R09-1	---	O ⁺ B ⁺ N ⁺	---	---	---	---	---	---	---	---	---
R09-2	---	---	---	---	---	---	B ⁺	---	---	---	---
S20-1	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
S20-2	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
S20-3	---	---	---	---	N ⁺	---	---	---	---	---	---
T02-1	---	---	---	O ⁺ N ⁺	---	O-N ⁻	---	---	---	---	---
R01-1	---	---	---	---	---	B ⁺	---	---	---	---	---
R01-2	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
R01-3	---	---	---	N ⁺	---	---	---	---	---	---	---
R01-4	---	---	---	---	---	---	---	V-O ⁻	---	---	---
R14-1	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
R14-2	---	---	---	B ⁺ N ⁺	---	---	---	---	---	---	---
R14-3	---	---	---	N ⁺	---	---	---	---	---	---	---
T19-1	---	---	---	---	---	---	---	---	O ⁺ T ⁺ B ⁺	---	---
S04-1	---	---	---	V-O-T-B ⁻	---	---	---	---	---	---	---
S04-2	---	---	---	N ⁺	---	---	---	---	---	---	---
S04-3	---	---	---	---	---	B ⁺	---	---	---	---	---
S04-4	---	---	---	---	---	---	N ⁺	---	---	---	---
M08-1	---	---	---	---	---	B ⁺	---	---	---	---	---
M08-2	---	---	---	N ⁺	---	---	---	---	---	---	---

Triticeae polyploids [16]. In this particular case, the clustering pattern shown by the tree is, once again, consistent with previous evolutionary information. Although *Ae. ovata* and *Ae. biuncialis* cluster together according to their genomic constitution (both are UUMM), both species are clearly separated in the dendrogram. This suggests that despite the latest genome nomenclature for the *Aegilops* group [15, 26], superindex should be used

to differentiate the M genomes of both species. This has been proposed in the past by Kihara [11].

A recent study based on molecular markers [1] has demonstrated the phylogenetic proximity of the N and U genomes of *Ae. uniaristata* (NN) and *Ae. umbellulata* (UU). This finding was confirmed in the present study in which these species appear as the most closely related diploids. *Ae. caudata* (CC) and *Ae. commosa* (MM) share similar morphologi-

cal features despite their different genomes. The proximity observed between the C and M genomes is consistent with this phenotypical similarity [14]. The difference between the genomes M and N of *Ae. comosa* and *Ae. uniaristata* is also supported by the present findings. In the past, N was regarded as a modification of M [11] although recent data suggest that it is a different genome [1, 13].

Population 434 of *Ae. biuncialis* was dissociated from the other *Ae. biuncialis* populations and clustered with the *Ae. neglecta* group. *Ae. biuncialis* and *Ae. neglecta* share the U and M genomes. Although mainly self-fertilising, some degree of allogamous behaviour has been described and artificial hybrids have been obtained between species [16]. The natural formation of hybrids by polyploids which share genomes and habitats has been described as a common process [8]. A similar process might explain the segregation shown by population 434.

3.2. Correlation between RAPD variability and environmental factors

Dawson et al. [4] reported some of the RAPD diversity detected in the *Triticeae* species to be associated with ecogeographical factors. In the present study, some significant correlations ($P = 0.01$) were found between 29 amplified bands and eight bioclimatic and ecogeographical variables (table IV). This suggests adaptative variation at certain loci. Variables associated with rainfall were those most frequently correlated. Fifteen markers were correlated with mean annual rainfall (Panu), one with summer rainfall, six with altitude (one of which showed simultaneous correlation with Panu), two with longitude, two with latitude, and the remaining four with thermal variables. *Ae. neglecta* is found in the western half of the Iberian peninsula which has higher rainfall. Fifteen bands corresponding to this species were positively correlated with annual rainfall, which reflects the ecogeographical conditions in which the populations are found. Further, the markers S04-1 and S04-2 generated by the S04 primer were negatively and positively correlated with Panu, respectively. S04-1

(about 900 bp) appeared in all the species but *Ae. neglecta*, while S04-2 (about 850 bp) was only present in *Ae. neglecta*. This suggests that both bands correspond to allelic forms of a gene involved in the adaptation to water conditions. At a significance level of $P = 0.05$ (data not shown), the correlations scored showed 17 involving *Ae. ventricosa* bands which simultaneously and negatively affected the mean annual temperature, the mean maximum temperature of the coldest month and the Mediterranean index, all of which is consistent with the distribution pattern of this species. *Ae. ventricosa* mainly colonises the colder highlands of the Iberian peninsula which suggests that these bands are possible molecular markers of cold adaptation. The negative correlation with the Mediterranean index is consistent with the distribution of the species restricted to the mountains of the Mediterranean region. Future work on *Aegilops* within the current plant breeding programme will include the isolation and cloning of the markers of most interest.

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