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NATURAL HYBRIDIZATION AMONG COCKSFOOT *(DACTYLIS GLOMERATA)* SUBSPECIES IN GALICIA (NORTH-WEST SPAIN)

R.LINDNER, M. LEMA, G. LINDNER AND A. GARCÍA.

Misión Biológica de Galicia (CSIC). Apartado 28. 36080 Pontevedra (Spain).
E-mail: csgporls@cesga.es

SUMMARY

Special cocksfoot (*Dactylis glomerata* L.) ecotypes from Galicia (Spain) were compared with *D. glomerata* subsp. h*ispanica* from Portugal, in order to determine if they belonged to that Mediterranean subspecies, which was not observed in Galicia to date (because of its Atlantic influence). Although multivariate analysis of quantitative traits separated subsp. *hispanica* from Galician cocksfoot, allelic frequencies of the diagnostic isozyme locus SOD1 (TO1) related subsp. *hispanica* to Mg1149, a natural population near Casaio (in the Southeast of Galicia, with Mediterranean climate). It is concluded that this population is a hybrid between subsp. *hispanica* and subsp. *izcoi* (an endemic subspecies of Galicia and north of Portugal), both tetraploids. The other populations from this zone present a genic flux from subsp. *hispanica.*

Key words: Plant genetic resources, taxonomy

INTRODUCTION

Dactylis glomerata, L. clasification is difficult because of it's numerous subspecies, ecotypes, geographical races and hybrids. In Galicia, the northwest part of the Iberian Peninsula, three main subspecies have been found to date: *D. glomerata* subsp. *glomerata* (a tetraploid cultivated worldwide), *D. glomerata* subsp. *izcoi* (Ortiz and Rodríguez-Oubiña, 1993) and *D.glomerata* subsp. *marina* (Borrill) Greuter (Lindner and García, 1997). Subspecies *izcoi* is endemic in Galicia and north of Portugal, and comprises two cytotypes: diploids (2n=14) and tetraploids (2n=28) (Borrill, 1978; Ortiz and Rodriguez-Oubiña, 1993). Subspecies *marina* (tetraploid) is a typical small bluish-green plant from the coast, with epidermal papillosity and smooth leaf margin (Borrill, 1961, 1978; Lindner, 1994).

D.glomerata subsp. *hispanica* (Roth) Nyman is a Mediterranean taxon not yet found in Galicia, although it was observed in León, near the Galician border (Acedo and Llamas, 1991). Like subspecies *glomerata*, *hispanica* type has a great phenotipic variability. After Borrill (1978): "subspecies *hispanica* has not pronounced habitat preferences, and in fact occupies a tremendous range of ecological situations in the summer- dry zone".

In the southeast of Galicia and also in the centre of the region, cocksfoot populations different from the rest of Galician ecotypes have been found by Ortiz and Rodríguez-Oubiña (personal communication). They are tetraploid, of small stature, quite woody, with narrow leaves and small panicles.

The objective of this study is to decide if these forms could belong to *D. glomerata* subspecies *hispanica*. To accomplish the objective of this study, quantitative traits (including inflorescence dimensions) are compared, and SOD1 allozymes (diagnostic of *Dactylis* subspecies) are determined

MATERIALS AND METHODS

Material

Ten populations of the species *Dactylis glomerata* have been studied. Six of them were collected in the southeast of Galicia, three in the centre of Galicia (Fig 1), and the last one (dh1230), used as a control, is a sample of *D. glomerata* subsp. *hispanica* collected in Evora (South Portugal) by the Institute of Grassland and Environmental Research (Aberystwyth, UK).

Three sites are in the same area of Ourense province, in the southeast of Galicia: Sobradelo (S) (415 m a.s.l.), Casaio (C) (550 m a.s.l.) and one kilometer further southeast (C1) (750 m a.s.l.), the nearest locality to the Galician border. The following populations come from this area: Mg1137 (Sobradelo) (S), Mg1144, Mg1145 and Mg1146 (Casaio) (C), Mg1149 and Mg1150 (C1). The fourth site is Barazon (B) (Coruña province), situated in the centre of Galicia (375 m a.s.l). Populations Mg1217, Mg1218 and Mg1223 were collected in this site.

Ecological conditions of the collection sites

The nearest Sobradelo and Casaio weather station (Pumares, 365 m.a.s.l) records an annual mean temperature of 12.9ºC and a total rainfall of 595 mm. It has a quite long summer drought period , from mid May to the end of September (summer rainfall of 60 mm) (Fig.2 a), and could be considered as a Mediterranean climate. The vegetation

FIGURE 1

Localities of studied populations in Galicia (Spain). B=Barazón; C=Casaio; C1=one kilometer southeast from Casaio; S=Sobradelo; locality of D.g. subsp. hispanica described by Acedo and Llamas 1991 (not studied in this paper) P=Puente de Domingo Flórez.

Localidades de las poblaciones estudiadas en Galicia (España). B=Barazón; C=Casaio; C1=un kilómetro más hacia el sur de Casaio; S=Sobradelo; localidad de D.G. subsp. hispánica descrita por Acedo y Llamas 1991 (no estudiado en este artículo) P=Puente de Domingo Flórez.

is composed mainly of *Erica australis, Thymus mastichina, Origanum virens, Cystus ladaniferus, Rubus* spp. and *Lavandula pedunculata.* The soil had abundant slate, mixed in some places with limestone rocks.

The nearest Barazon metheorological station (Portodemouros, 255 m a.s.l.) records 1381 mm annual rainfall and shows also 12.9°C mean annual temperature. The mean temperature in winter and autumn is higher than in the southeast, but mean summer temperature is lower and the summer drought period is much shorter, from mid June to mid July (164 mm summer rainfall) (Fig.2 b). The climate of this zone has degraded from Oceanic towards Mediterranean, and it is considered as humid Mediterranean. The vegetation is composed of a small flora such as *Ulex nanus, Plantago serpentina, Erica cinerea* and *Calluna vulgaris.* The soil, on a cropping out of serpentinic ultrabasic rocks, is not deep.

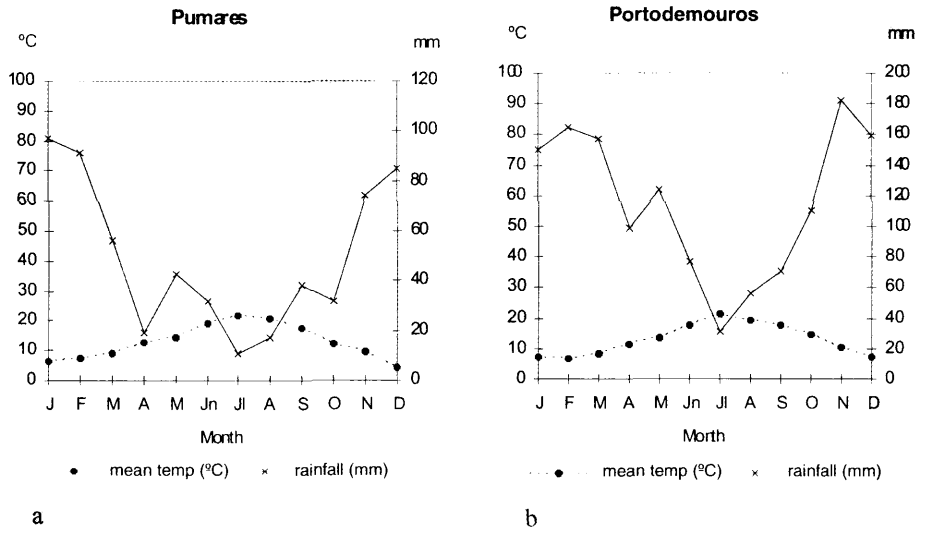

FIGURE 2

Gaussen diagrams of the two weather stations. The shadowed shows the drought period.

Diagramas de Gaussen de las dos estaciones meteorológicas. Las áreas sombreadas indican los períodos de sequía.

Methods

Twenty seedlings were transplanted into boxes and kept in an unheated greenhouse for two months. The plants were transplanted on December 16 to the field, with spacings 40 x 40 cm in two replicates of ten plants per population. The experimental field was situated at the Misión Biológica de Galicia (Pontevedra). No fertilization was applied to the soil.

Quantitative traits

The following traits were evaluated:

-100-seed weight (g)

- seed length (average of five seeds, in mm)
- days to germination (from sowing to the appearance of the root)
- heading date of the 1st flower (days from the 15th April)
- anthesis (days from heading to appearance of the anthers)
- length, width and ligule length of the flag leaf (cm)

- length, width and ligule length of the 1st and 2nd leaves below the flag leaf (cm)
- number of flowering stems (culms)

Flower dimensions (on the first three flowering stems): (according to Lindner and García (1995) with some changes)

- culm length (cm)
- panicle length (cm)
- first branch length (cm)
- first pedicel length (cm)
- first internode length (cm)
- culm diameter (mm), one cm below the panicle

Allozymes

Zymograms of SOD1 (superoxide dismutase, E.C.1.15.1.1. = TO1) were obtained for each plant on polyacrylamide gels, following a modification of the methods of Vidal (1996) and Ramón (1995).

Statistical analysis

Analysis of variance (unbalanced ANOVA due to missing data) and correlation coeficients were calculated for each quantitative trait. Mahalanobis distances (principal component analysis followed by cluster analysis of the principal components with eigenvalues>1) were computed from the correlation matrix of mean quantitative traits with significant differences between populations. Nei's genetic distances between populations were obtained from allozyme allele frequencies (Rohlf, 1993).

RESULTS

Quantitative traits

Though, it was not possible to make statistical comparisons for the 100 seed weight, Table 1 displays the highest value for Mg1137 from Sobradelo; dh1230 (the *hispanica* check) and Mg1149 (from Casaio) also show high values.

All traits were significantly different at $P<0.001$ level except ligule length at $P<0.01$ between populations (Table 1). Mg1137 (the only population from Sobradelo) had the

longest seeds, while dh1230 had intermediate seed lengths. Most populations germinated quickly, but Mg1145 and Mg1149 (near Casaio) were the slowest. The smallest flower dimensions (lenghts of culm, panicle, basal branch, basal pedicel, first internode length, and culm diameter) belonged to dh1230 (*D.glomerata* subsp. *hispanica*) and Mg1217 (from Barazón). Mg1137 (from Sobradelo) had the biggest flower and general leaf dimensions. *D.glomerata* subsp. *hispanica* (dh1230) was the earliest heading, together with populations around Casaio (Mg1144 to Mg1150). The latest heading populations were Mg1217, Mg1218 and Mg1223 (from Barazón). However, population differences in anthesis date were less than in heading date: the earlier flowering populations took more days from heading to anthesis than the later flowering populations. Pearsons correlation coeficient between both traits was significantly negative (r = -0.698***). Correlation coefficients between the other traits were significantly positive.

TABLE 1

Means of quantitative traits separated by Waller-Duncan test.

Media de los caracteres cuantitativos separados por el test de Waller-Duncan

Popul.	SW	SL	Ger	HD	Ant	NFS	FLL	FLW	Lig	LL1	LW1	Lig1	LL2	LW2	Lig2	CL	PL	BBL	BPL	BIL	CD
Mg1137	102.4	5.8	7.3	25.4	26.4	30.1	18.0	0.8	1.0	20.6	0.9	0.9	18.8	0.9	0.5	77.8	18.8	11.0	5.7	6.3	1.5
Mg1144	61.7	3.3	8.8	21.6	31.3	29.8	16.0	0.6	0.8	18.4	0.6	0.6	15.5	0.6	0.4	70.2	14.4	7.6	3.5	4.8	0.9
Mg1145	50.4	3.4	13.8	21.9	31.7	25.8	12.9	0.6	0.6	13.4	0.6	0.5	11.5	0.6	0.2	66.2	12.7	6.8	3.1	4.7	0.9
Mg1146	68.3	3.7	8.7	20.7	31.3	39.9	13.4	0.7	0.9	15.7	0.7	0.8	12.6	0.7	0.4	75.3	14.3	7.5	3.5	4.6	1.1
Mg1149	81.1	4.1	12.3	22.8	30.5	50.5	10.0	0.7	0.7	9.5	0.7	0.4	7.4	0.6	0.2	61.0	11.8	6.3	3.0	3.9	0.9
Mg1150	54.7	3.7	9.0	21.4	30.7	47.1	11.6	0.6	0.9	13.5	0.7	0.6	11.7	0.7	0.3	69.4	12.8	7.1	3.3	4.2	0.9
Mg1217	69.1	4.5	6.9	40.2	19.4	26.8	7.1	0.5	0.5	8.7	0.6	0.4	8.1	0.5	0.3	39.0	7.1	3.8	1.8	2.8	0.8
Mg1218	54.2	3.5	6.9	33.9	21.6	44.7	9.2	0.6	0.7	10.3	0.7	0.5	9.0	0.6	0.3	51.8	11.2	6.0	3.3	4.4	0.9
Mg1223	70.0	4.5	6.2	28.6	27.0	24.3	15.7	0.9	0.8	17.9	1.0	0.7	14.9	0.9	0.5	71.4	12.9	7.0	3.1	4.1	1.2
dh1230822	4.0	6.3	19.5	28.9	19.9	7.8	0.5	0.8	11.9	0.6	0.7	9.9	0.6		0.5	56.6	5.7	2.1	0.5	1.6	0.7
LSD	-	0.5	2.2	5.6	5.2	15.8	3.6	0.1	0.3	3.3	0.1	0.2	3.3	0.1	0.1	3.7	1.2	0.7	0.5	0.5	0.1
siglevel	-	***	***	***	***	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***	***

SW=100-seed weight, SL=seed length, Ger=days to germination; HD=heading date; Ant=anthesis; NFS= nº flowering stems; FLL, FLW and Lig=flag leaf length, flag leaf width and flag leaf ligule, respectively; LL1, LW1, Lig1=length, width and ligule length of the 1st. leaf below the flag leaf; LL2, LW2 and Lig2=length, width and ligule lenght of the 2nd leaf below the flag leaf; CL=culm length; PL=panicle length; BBL=basal branch length; BPL=basal pedicel length; BIL=basal internode length; CD=culm diameter.

The variation of leaf dimensions is analysed in Table 2. There was no significant interaction between populations and leaf order: the first leaf below the flag leaf was always the biggest (Gillet, 1984).

TABLE 2

Mean squares from the ANOVA of total leaf dimensions.

ANOVA de cuadrados medios del total de las dimensiones foliares

Source	d.f	Length M.S.	Width M.S.	Ligule length M. S.
Popul	9	413.6***	0.564***	0.575***
Plant (popul)	76	82.2***	0.042***	0.079***
Leaf	2	266.0***	0.166***	4.400***
Popul*leaf	18	415.9ns	0.023ns	0.047ns
Error	269	34.6	0.015	0.052
R^2		0.574	0.703	0.626
C.V. (%)		42.4	17.5	38.3

ns= not significant; *** $p \leq 0.001$

The dendrogram of Mahalanobis distances for all morphological evaluated traits, computed from the three first principal components, which explain 85.7% of the variance, shows four groups: Cluster I includes two populations from Barazón (B) (Mg1217 and Mg1218) characterized by late flower emergence and early anthesis; populations near Casaio (C): Mg1149, Mg1150, Mg1145, Mg1144 and Mg1146 are associated in cluster II is an intermediate group; cluster III includes one population from Barazon (B)(Mg1223) and the population from Sobradelo (S) (Mg1137), both populations with the biggest leaf dimensions; and the fourth group IV comprises dh1230 (subspecies *hispanica*) (Fig. 3).

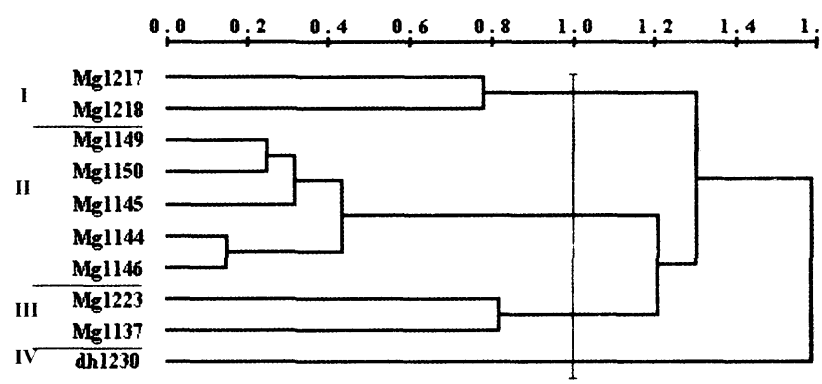

FIGURE 3

Dendrogram of Mahalanobis distances between cocksfoot populations in Galicia.

Dendrograma de las distancias de Mahalanobis entre poblaciones de dactilo en Galicia.

Allozymes

The most frequent SOD1 allele was 1.12 (Table 3). The following populations from Casaio showed high frequencies for 1.12 allele: Mg1144 (0.629), Mg1145 (0.875), Mg1146 (0.684) and Mg1150 (0.625). The next average frequent allele was 1.03. Mg1137 from Sobradelo showed the highest frequency of allele 0.88 (0.403) (Table 3). Populations were compared individually by χ^2 analyses of their allele frequencies, as an application of the method described by Mather (1951), (cited by Hayward and McAdam, 1977). Population dh1230 showed significant differences only with Mg1137 and Mg1145 at $P<0.05$ level (Table 4).

The dendrogram of Nei's genetic distances between populations from SOD1 allelic frequencies showed that there are two groups (Fig. 4). Population Mg1137 (Sobradelo) alone forms a cluster. The second cluster can be divided in two sub-groups (or sub-clusters). All populations from Casaio region and Barazón are included in a sub-cluster. The other sub-cluster is integrated by dh1230 (the *hispanica* check) and Mg1149 (near Casaio).

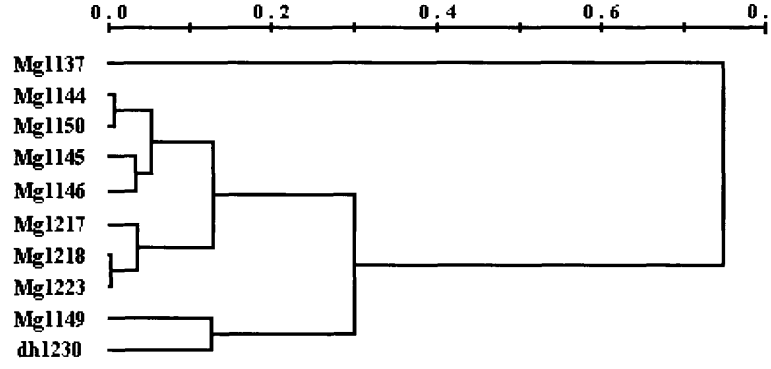

FIGURE 4

Dendrogram of Nei's genetic distances between cocksfoot populations in Galicia.

Dendrograma de las distancias génicas de Nei's entre poblaciones de dactilo en Galicia.

DISCUSSION

The only tetraploid subspecies with the SOD1 allele 1.12 are *hispanica* and the coastal *marina*; subsp. *izcoi* does not present it (Lumaret, 1988). In this study, the highest 1.12 frequencies were found in populations near Casaio with the exception of Mg1149 and dh1230 (Table 3). The highest frequencies of allele 1.00 were displayed by dh1230

TABLE 3

Population allelic frecuencies in SOD1.

Frecuencias alélicas de la poblaciones para SOD1.

Allel	Mg1137	Mg1144	Mg1145	Mg1146	Mg1149	Mg1150	Mg1217	Mg1218	Mg1223	dh1230
1.12	0.088	0.629	0.875	0.684	0.270	0.625	0.447	0.445	0.404	0.536
1.03	0.316	0.257	0.000	0.053	0.135	0.187	0.386	0.259	0.277	0.000
1.00	0.193	0.028	0.111	0.053	0.406	0.125	0.114	0.148	0.213	0.464
0.88 0.403	0.086	0.014	0.210	0.189	0.063	0.023	0.148	0.106	0.000	

TABLE 4

Significance of individual x^2 comparisons between populations for SOD1.

Comparaciones significativas de x^2 para SOD1 entre poblaciones.

Popul	Mg1144	Mg1145	Mg1146	Mg1149	Mg1150	Mg1217	Mg1218	Mg1223	dh1230
Mg1137	9.19*	15.58**	13.72**	3.52 ns	8.72*	7.06 ns	5.48 ns	4.89 ns	8.41*
Mg1144		3.27ns	1.32 ns	5.35 ns	0.25 ns	1.19 ns	1.11 ns	1.72 ns	5.11 ns
Mg1145			2.58 ns	12.27**	2.22 ns	9.07*	8.37*	9.06*	10.29*
Mg1146				6.80 ns	0.99 ns	5.11 ns	8.46*	4.27 ns	5.97 ns
Mg1149					22.64**	4.16 ns	2.51 ns	1.71 ns	2.81 ns
Mg1150						1.35 ns	1.20 ns	1.27 ns	2.74 ns
Mg1217							0.80 ns	0.68 ns	1.28 ns
Mg1218								0.20 ns	4.86 ns
Mg1223									3.91 ns

ns = not significant; * p≤0.05; ** p≤0.01; ***p≤0.001

(0.464) and Mg1149 (0.406). Therefore, genetic distances between populations clustered dh1230 with Mg1149 (near Casaio) (Figure 4). This suggests that this population is a hybrid between subsp. *hispanica* and Galician cocksfoot. On the other hand, allele 1.03 was frequent in Mg1217 (from Barazón), and even Mg1149 had this allele. This allele appears in subspecies *izcoi* and *marina*, but not in subsp. *hispanica* nor in subsp. *glomerata* (Lumaret, 1988; Lindner *et al.*, 1999).

With respect to quantitative traits, dh1230 and populations near Casaio were early heading with a long interval between heading date and anthesis (Table 1; Figure 3). This

suggest that flower organogenesis at heading was less advanced in the early populations than in the late ones.

Subspecific hybridization within *Dactylis glomerata* is frequent in Galicia (Lindner and García, 1997; Lindner *et al.*, 1999). Following Mousset (1995) in transition areas between temperate and mediterranean climates, several intermediate subsp. *hispanica* and subsp. *glomerata* types have been observed. In addition, Mizianty (1991) discovered a hybrid between subspecies *glomerata* x subspecies *hispanica* in Poland. Also Mizianty (1996), mentions the possibility that some Bulgarian populations are hybrids of subsp. *glomerata* and subsp. *hispánica.* Since Acedo and Llamas (1991) described *D. glomerata* subsp. *hispanica* near the border of Galicia, Castilla and León (Puente de Domingo Flórez, Fig.1), it is probable that subsp. *hispanica* hybridized with subsp. *izcoi* in the southeast of Galicia, and that hybrids persist in zones of Mediterranean climate, with a large dry summer period, like Casaio (Fig.2a). The evidence presented in this study suggests several populations are hybrids between the two subspecies mentioned above

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HIBRIDACIÓN NATURAL ENTRE LAS SUBESPECIES DE DACTYLIS GLOMERATA, L. EN GALICIA (NOROESTE DE ESPAÑA).

RESUMEN

Se han comparado ecotipos de *Dactylis glomerata L.* de Galicia (España) con *D. glomerata* subsp. *hispanica*, con objeto de determinar si pertenecen a ésta subespecie mediterránea, la cual no se ha observado hasta ahora en Galicia (debido a su influencia atlántica). Aunque los análisis multivariantes de los caracteres cuantitativos separaban la subsp. *hispanica* de los dactilos gallegos, las frecuencias alélicas isoenzimáticas del locus de diagnóstico SOD1 (TO1)relacionaron la subsp. *hispanica* con Mg1149, una población natural próxima a Casaio (en el sureste de Galicia, de clima mediterráneo). Se llegó a la conclusión de que dicha población es un híbrido entre la subsp. *hispanica* y la

subsp. *izcoi* (una subespecie endémica de Galicia y el Norte de Portugal), ambas tetraploides. Las restantes poblaciones de ésta zona presentan un flujo génico de la subsp. *hispanica*.

Palabras clave: Recursos fitogenéticos, taxonomía.