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Morphological variation and distribution of cytotypes in the diploid-tetraploid complex of the genus *Dactylis* L. (Poaceae) from Algeria

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Abstract. Morphological and cytological characters were analysed among thirty populations of the genus *Dactylis* L. from Algeria, to understand its infraspecific diversity. Principal Component Analysis using quantitative characters allowed discriminating the tetraploid populations into two subspecies, *marina* (Borrill) Greuter, localised on sea cliffs, and *hispanica* (Roth) Nyman, very widespread. Some individuals of these latest populations formed a distinct group, identified as subsp. *glomerata* Hayek. Three diploid taxa are described in the literature: subspecies *santai* Stebbins & Zohary, *castellata* Parker & Borrill and *mairei* Stebbins & Zohary that are considered as prevalent in Algeria, distributed in Tellian Atlas, in forest ecosystems within mesophytic habitats. Canonical Discriminant Analysis on natural populations and on experimental cultures showed two main groups: the first group corresponds to subspecies *mairei*, with a narrow distribution; the second one exhibits a wide morphological variability and belongs to *santai* type. Based on this study, a key to aid in identification of the subspecies is presented.

Key words: *Dactylis glomerata* L., cocksfoot, morphology, numerical taxonomy, polyploidy, cytotypes, Algeria.

Introduction

Cocksfoot (*Dactylis glomerata* L.) is one of the most important natural forage grasses that have a worldwide distribution. It has long been a subject of great interest for many taxonomists. *Dactylis glomerata* is a polyploid complex composed of fourteen diploid (2n = 2x = 14) and three tetraploid (2n = 4x = 28) taxa (Domin 1943, Stebbins and Zohary 1959, Borrill 1978). Hexaploid populations were also cited by Jones and Borrill (1961) in Cyrenaica.

Biosystematic and evolutionary relationships between diploids and tetraploids are a problem for taxonomists because they are morphologically indistinguishable. The Czech botanist, Domin (1943), already told "It is necessary to establish experimentally the basic types, define these stable forms morphologically, and study their economic properties"…. In fact, some cytotypes and/or taxa have more important agronomic potential than others such as subsp. *lusitanica* Stebbins & Zohary well known for its biomass production.

The considerable variability observed in *D. glomerata*, within and between populations, has stimulated many taxonomists and regional florists to describe morphotypes, ecotypes or geographical races as new species, subspecies, varieties or forms. The different taxa recognised are climatically distributed into subtropical, Mediterranean and temperate groups (Stebbins and Zohary 1959, Borrill 1978, Lumaret 1988). The diploids belonging to subsp. *aschersoniana* (Graebner) Thell., *himalayensis* Domin and *parthiana* Parker & Borrill characterise the temperate group; the subsp. *smithii* (Link) Stebbins & Zohary, the subtropical group and the 'Galician' type, the subspecies *lusitanica*, *reichenbachii* (Hausm.) Stebbins & Zohary, *woronowii* (Ovcz.) Stebbins & Zohary, *judaica* Stebbins & Zohary, *juncinella* (Bory) Stebbins & Zohary, *ibizensis* Stebbins & Zohary, *castellata* Parker & Borrill, *santai* Stebbins & Zohary and *mairei* Stebbins & Zohary correspond to a large Mediterranean group. The tetraploid subspecies *glomerata* exhibits a northern distribution and is linked to the temperate group while the other tetraploids, *hispanica* (Roth) Nyman and *marina* (Borrill) Greuter, are typically Mediterranean subspecies.

Numerous morphological and cytogenetic data highlighted the geographical distribution in some European and Mediterranean countries (Müntzing 1937; McCollum 1958; Borrill 1961a, b, c; Jones et al. 1961; Jones 1962; Borrill and Carroll 1969; Guignard 1980, 1987; Cenci 1982; Mizianty 1986, 1988, 1990; Acedo and Llamas 1991; Wetschnig 1983, 1984, 1991; Lindner and Garcia 1997). Diploids and tetraploids are frequently observed growing sympatrically (Borrill and Lindner 1971, Lumaret and Barrientos 1990, Lindner and Garcia 1997).

Genetic studies, carried out on enzyme polymorphism in the Mediterranean region from collections of the Welsh Plant Breeding Station, Aberystwyth (Lumaret 1981a, b, 1982), revealed a high level of heterozygosity in tetraploids at tetrasomic loci, as compared to diploids. In Italian populations, Speranza and Cristofolini (1986, 1987) reported little differentiation for both diploid and tetraploid *Dactylis*. Flavonoid biochemistry indicated that the two cytotypes showed different patterns (Ardouin et al. 1985, Fiasson et al. 1987). These authors found a polyphyletic origin for the Mediterranean diploid group, showing its complexity. Based on DNA chloroplast variation, the origin of polyploidy in this complex is known to result from autopolyploidy (Lumaret et al. 1989, Lumaret and Barrientos 1990). The use of chloroplast DNA RFLP's on endemic diploid and tetraploid *Dactylis* from Madeira and Canary Islands demonstrated a possible introgression from the Mediterranean into the subtropical material (Sahuquillo and Lumaret 1999). Moreover, RAPD markers indicate a high level of gene flow among tetraploid populations from Thrace, the European region of Turkey (Tuna et al. 2004).

In the Flora of Algeria, the botanists (Battandier and Trabut 1902, Maire 1955, Quezel and Santa 1962) described only one species, *Dactylis glomerata* L. s. l.. Since the works of Stebbins and Zohary (1959), Borrill (1961a, c) and Parker and Borrill (1968), three diploid subspecies (*santai*, *mairei*, *castellata*) and two tetraploid subspecies, (*marina* and *hispanica*) have been recognised. The tetraploids are widespread from the northern coast to the southern Saharian Atlas through the Steppe Highlands, whereas diploid taxa are quite rare and more restricted to gorges and forest habitats of the northern region. Subspecies *mairei* is confined to the gorges at Kherrata, Algeria (Stebbins and Zohary 1959, Borrill and Lindner 1971) and *santai* was localised in western Algeria and Spanish Morocco (Stebbins and Zohary 1959). Subspecies *castellata* was further cited and studied by Parker and Borrill (1968) and Borrill and Carroll (1969). In spite of several studies (Borrill 1978, Lumaret 1981b, Lumaret et al. 1989, Sahuquillo and Lumaret 1999, Wetschnig 1991) including samples of *D. glomerata* from Algeria, the data remain restricted to localities that mainly correspond to Stebbins'

Table 1. Ploidy level, climatic and floristic characteristics of collecting sites in Algeria Alt = altitude, P is the annual rainfall; M and m are the average of the maximum temperature of the hottest month and the average of the minimum of the coldest month, respectively

	Locality	Ploidy level	Alt. (m)	P (mm)	M°C	m°C	Bioclimate	Sites and floristic characteristics
1	Ténès 1	2x	150	513	30.9	8.6	SH	Pine forest of *P. halepensis*, *Callitris articulata, Pistacia lentiscus*
2	Ténès 2	4x	30	411	28.7	8.1	SA	Slope with *Chamaerops humilis*, *Ampelodesma mauritanicum*, *Lavandula denticulata*
3	Ténès 3	4x	10	411	28.7	8.1	SA	Maritime cliffs under wind-salt sprays with *Asteriscus maritimus*, *Lotus creticus*
4	Larhat	2x	270	634	28.3	7.1	SH	Pine forest of *P. halepensis*, *Callitris articulata*, *Arbutus unedo*, *Cistus monspeliensis*
5	Gouraya	4x	30	534	28.5	7.6	SH	Littoral prostrate shrubs of *Pinus halepensis*, *Quercus coccifera*
6	Mazafran	4x	10	690	37.7	5.9	SH	Edge of cultivated field
7	Ziama	4x	30	1181	30.2	8.3	H	Sublittoral maquis on sand dunes with *Quercus ilex*
8	Marsa 1	4x	20	568	28.9	7.6	SH	Top of maritime slope with *Thymelea hirsuta*, *Plantago coronopus*
9	Marsa 2	4x	10	568	28.9	7.6	SH	Bottom of maritime slope with *Asteriscus maritimum*, *Plantago coronopus*, *Lotus creticus*
10	Corso	4x	30	610	31.6	5.7	SH	Road border near cultivated field.
11	Figuier	4x	10	658	30.5	8.0	SH	Maritime rocks with *Plantago coronopus*, *Crithmum maritimum*

prospecting sites as underlined by Borrill (1978) ''Contemporary ideas about the evolution of *Dactylis* are based on the investigations of these collections''.

The aim of this paper is to analyse the phenotypic diversity of the *D. glomerata* complex in Algeria from a wider sampling, with particular focus on the weakly investigated diploid populations of this region. It is noteworthy that this region, including a large range of contrasted ecological conditions, is poorly explored and still lacks studies at the population level (Médail and Quezel 1997). For this purpose, morphological analysis was carried out by sampling natural populations, without *a priori* concerning their subspecific rank, under various bioclimatic conditions. Multivariate analyses based on a large number of quantitative characters were performed in order to highlight discontinuities between the diploid and the tetraploid Algerian cytotypes and to establish the importance and hierarchy of the characters with taxonomic significance. Validity of the morphological traits, traditionally employed for distinguishing of subspecific taxa and their geographic significance, is also examined. The taxonomic status of the subspecies remains of topic interest for this region where updating for the flora is greatly needed.

Materials and methods

Sampling. Thirty populations of *D. glomerata* were collected in very different bioclimatic and ecological conditions in North Algeria: coastal regions, hills, gorges and mountains of the Tellian Atlas (Table 1).

Table 1. (Continued)

	Locality	Ploidy level	Alt. (m)	P (mm)	M°C	m°C	Bioclimate	Sites and floristic characteristics
12	Lakhdaria	2x	650	814	33.8	6.3	SH	Gorges with *Olea europea*, *Ceratonia silica*
13	Tikjda 1	4x	1650	1190	29.0	0.9	H	Alpine grassland with *Festuca atlantica*, *Avena bromoides*
14	Tikjda 2	4x	1600	1005	30.5	1.1	H	Clearing forest with *Cedrus atlantica*
15	Kherrata 1, 2	2x, 4x	470	1103	30.0	6.0	H	On crest and in crevices of limestone cliffs in shady gorges
16	Babors 1	2x	1450	1668	32.0	1.6	H	Forest of *Quercus ilex*
17	Babors 2	2x	1500	1668	32.0	1.6	H	Clearing with *Quercus ilex*
18	Zaccar	4x	930	937	31.1	4.6	H	Forest of *Quercus ilex*, *Myrtus communis*, *Lonicera implexa*
19	Benchicao	2x	1250	584	35.0	1.3	SH	Mixed wood of *Quercus ilex*, *Quercus suber*
20	Berrouaghia	2x	980	570	34.2	0.6	SH	Clearing forest of *Pinus halepensis* and *Quercus ilex*
21	Senalba 1	4x	1360	392	33.4	−0.9	SA	Forest of *Pinus halepensis*, *Quercus ilex*, *Juniperus oxycedrus* north exposition.
22	Senalba 2	4x	1200	370	33.0	−0,8	SA	Reforests of *Pinus halepensis* in south exposition
23	Doui	2x	900	591	32.0	0.2	SH	Forest with *Quercus ilex*, *Pinus halepensis*, *Phillyrea media*, *Arbutus unedo*
24	Tessala	2x	750	514	34.5	1.3	SH	Wood with *Quercus ilex*, *Pinus halepensis*, *Phillyrea media*, *Arbutus unedo*
25	El Beldj	4x	45	580	32.4	6.2	SH	Sublittoral maquis with *Myrtus communis* and *Lonicera implexa*
26	Tipaza	2x	30	630	31.7	5.9	SH	Littoral forest of *P. halepensis*, with *Callitris articulata* and *Cistus monspeliensis*
27	Bouharoun	4x	50	610	32.8	6.4	SH	Road border near cultivated field.
28	Bouzareah	2x	324	740	30.9	5.2	H	Residual forest of *P. halepensis*, *Crataegus monogyna* and *lonicera implexa*.
29	Chréa	2x	1120	1005	30.5	1.1	H	Clearing forest with *Cedrus atlantica*
30	Chiffa	2x	450	735	28.5	3.1	SH	Forest with *P. halepensis*, *Pistacia lentiscus*, *Lavandula stoechas*

The sampling sites covered East-West and North-South transects of increasing aridity from the mesic coast, plains and mountains to steppic regions near Sahara. We have taken care to sample in the sites mentioned by Stebbins and Zohary (1959): ssp. *santai* was collected on the Mount of Tessala and on the top of Djebel Doui; ssp. *mairei* in the Gorges of Kherrata. These collecting sites correspond to localities investigated by Stebbins and the joint FAO/CSIRO in 1954. As cocksfoot is a rhizomatous perennial grass, individuals were sampled at least ten meters apart to avoid neighbouring effects. Individuals displaying a similar maturity stage were randomly collected. About 10–15 plants were collected per population. Dried individual plants were conserved in a herbarium for morphological analysis. Caryopses were collected in order to determine their ploidy level and for experimental cultures.

Chromosome counts. Chromosome counting was carried out on pollen mother cells collected from young panicles at anthesis and/or from root-tips of germinating caryopses. Root tips were pre-treated in α-bromonaphtalene–saturated water solution at room temperature for 2 hours. Following fixation in alcohol-acetic acid 3:1, v/v at 4°C, hydrolysis in 1N HCl for 8–12 min at 60°C was performed before the usual Feulgen staining. The same procedure for meiosis was applied on panicles after fixation in Carnoy's solution (alcohol-chloroform-acetic acid, 6:3:1, v/v) for 48h at room temperature. Preparations with a minimum of 30 chromosome squashes of good quality were analysed for each individual and at least five to ten individuals were scored for each population. Chromosome spreads were observed and photographed with a Leitz photomicroscope.

Morphological analysis. Morphological analyses were first conducted on 224 diploid and tetraploid individuals from representative locations of sampling from the field to appreciate the morphology under natural conditions. Second analyses were focused on diploid individuals. Experimental cultures were performed to evaluate phenotypic plasticity or stability of characters and were essentially used for comparative observations in the diploid populations. Individuals experimentally cultivated were originating from Kherrata, Lakhdaria, Ténès and Berrouaghia. Sixteen quantitative and one qualitative characters were selected on the basis of previously defined taxonomical criteria (Maire 1955, Stebbins and Zohary 1959) and morphological studies in *Dactylis* (Borrill 1978, Guignard 1980, Mizianty 1988, Horjales et al. 1997).

The frequency of the qualitative character "lobed or unlobed shape of the lemma", traditionally used in the identification of *Dactylis* subspecies was estimated among populations. This character, difficult to evaluate, was subdivided taking into account the apex shape, pubescence and ciliation of the keel. The quantitative characters (Table 2), five vegetative characters of culms (A, P, S, T) and leaves (C), seven characters of panicles (B, F, G, H, I, J K) and four characters of the spikelets (L, M, N, U) were measured on ten plants per population. Multivariate methods of analysis were carried out using the software package STATISTICA F, version 5.1 (StatSoft Inc.1984–1997).

Principal Component Analysis (PCA) and Discriminant Analysis (DA) were performed in sequence. PCA was used to detect phenetic groups and to estimate the contribution of each variable to the analysis. This analysis, concerned with continuous characters (Sneath and Sokal 1973), was based on correlation matrices of standardised quantitative variables. Discriminant Analysis (DA) was used to test differences among given groups and to point out the most discriminating variables. Two Discriminant Analyses were performed (i) on the 224 individuals of the diploid–tetraploid complex and (ii) on the 90 diploid individuals belonging to the three subspecies recognised in Algeria. Since DA requires that the individuals be assigned to groups, each plant was assigned to its corresponding putative taxon. Analyses were carried out by the automatic option of Statistica selecting the best variables to identify groups. Variables were chosen to enter or leave the discrimination model among groups based on the F test significance level. F statistics indicate the

Table 2. Morphological characters of *Dactylis glomerata* L. used in numerical analysis

Symbol	Character
	Culm **(cm)**
A	Length of the culm measured from the lowest node to the base of panicle
C	Length of the leave
P	Culm diameter.
S	Length of the first internode
T	Length of the second internode
	Panicle **(cm)**
B	Length of the panicle
F	Length of the first internode (panicle)
G	Length of the second internode (panicle)
H	Length of the third internode (panicle)
I	Second pedicel length
J	Second branch length (glomerulus + pedicel)
K	Number of glomeruli
	Spikelet **(mm)**
L	Length of the spikelet of the first glomerulus from the second branch
M	Length of the superior glume of this spikelet
N	Length of the lemma of the first flower of this spikelet
U	Awn size

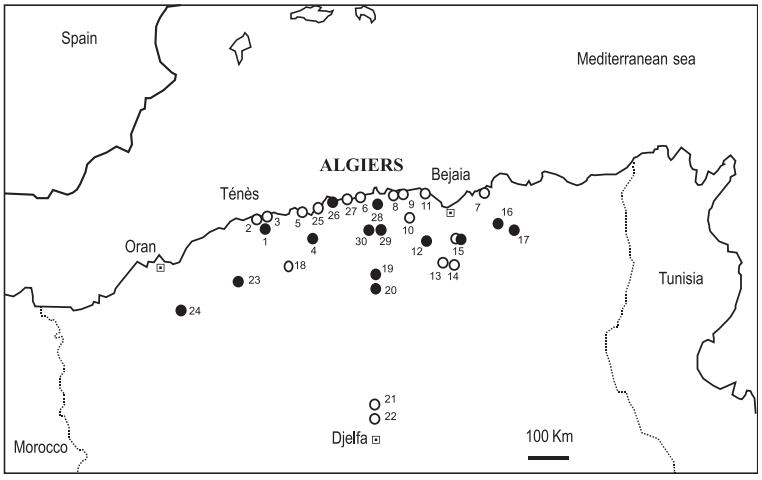

Fig. 1. Location of sampling sites of *Dactylis glomerata* L. in northern Algeria. Closed circles and open circles represent 2x and 4x populations respectively. See also Table 1

relative importance of the variables used in the analysis. A Canonical Discriminant Analysis (CDA) was performed to determine the successive discriminant functions, and individual discriminant scores were viewed and plotted.

Results

Cytology. Chromosome numbers of *Dactylis* were preferentially determined at first metaphase of meiosis. Mitotic chromosome preparations were also made in complement to meiosis. Between 150 and 300 diakineses / metaphases I were observed per population. All populations analysed by mitotic and/or meiotic chromosome counting had a consistent basic number of x = 7. Twelve populations were found to be diploid (2n = 2x = 14) and 18 populations tetraploid (2n = 4x = 28) (Fig. 1, Table 1). Only one mixed population containing both levels of ploidy was found, localised in the Gorges of Kherrata. Chromosome numbers from several new localities have been counted for the first time. The diploid cytotype is displayed in Fig. 2A. Bivalents were usually observed in the diploid populations. In the tetraploids, bivalents and tetravalents were encountered at diakinesis (Fig. 2B, C). Several abnormalities were observed in these 4x populations, like multivalents and chromosomes lagging. (Fig. 2C, D).

Morphology

Qualitative character: the lemma shape. A considerable variation was revealed among the examined individuals. Six types of lemma (A, B, C, D, E, F) can be distinguished (Fig. 3) and their frequencies are given in Fig. 4. Type A is the most frequent with a very high rate

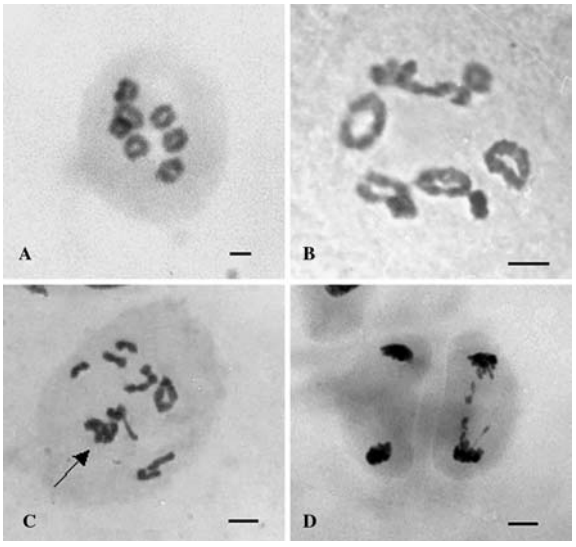

Fig. 2. A-D. Representative diploid and tetraploid meiotic configurations. **A** diakinesis with 7 ring II. **B** diakinesis with 4 ring II + 4 ring IV + 1 chain IV. **C** diakinesis with 3 rod II + 3 chain IV + 1 rod IV + 1 hexavalent (arrow). **D** Second telophase with chromosomes lagging. Scale bar = 5 μm

(> 75%) particularly in diploid (Kherrata 1, Berroughia, Benchicao, Babors 1, Babors 2) and some tetraploid (Kherrata 2, Tikjda 2) populations.

This type of lemma, lobed, strongly ciliate and pubescent, is characteristic of populations from mountains higher than 800 m. Type A is lacking in tetraploid samples collected on coastal cliffs (Marsa 1, Marsa 2, Gouraya and Ténès 3) and on sublitoral sand dunes (Ziama) under wind-salt spray. These latter populations are characterised by a lemma of type F, unlobed, smooth to scabrous, and sometimes by a lemma of type E. The presence of papillae in leaf epidermal cells (data not shown) from these samples is a diagnostic character of subspecies *marina*. The other lemma types (C, D and E) have an intermediate shape, being medium mucronate, slightly ciliate and pubescent. They are present in all populations with variable frequencies. Type B is present at a very low rate (< 10%) in all populations except those from the littoral zone. This character exhibits a tendency to vary according to the ecological conditions of the populations but it does not allow discrimination between diploid and tetraploid taxa.

Multivariate analyses

Diploid-tetraploid relationships. The first PCA analysis, performed on 224 individuals, explains for the first three axes 52.13 % of information (38.77 %, 8.36 % and 5.0 % respectively). Loadings of the 16 characters, eigenvalues and cumulative variance of the three first components are given in Table 3. PCA axis 1 is characterised by the following characters (presented in decreasing order of loadings): culm length (A), leaf length (C), length of the second internode of the culm (T), panicle length (B), length of the first internode of the culm (S), glomeruli number (K). PCA axis 2 is influenced by length of the superior glume (M), awn size (U), length of the second internode of the panicle (G); PCA axis 3, by lemma length (N) and culm diameter (P). The scatter plot (Fig. 5) of the two first axes shows that diploid individuals are located in the positive values of axis 1 with populations weakly separated, whereas the tetraploids are clustered into three main groups.

The first one, clearly distinguished in the negative part of axis 1 is attributed to the morphotype "*marina*". This group comprises most of the populations from the coastal rocky cliffs exposed to salt spray. (Marsa 2, Figuier, Ténès 3, Gouraya). It is characterised by a small plant with a mean length of the culm = 15.5 cm and a short panicle (1.5–3 cm). The lemma is lobed and glabrous and the leaves possess papillae in the epidermal cells.

The second group formed a cluster of points along the axis 2 essentially in the

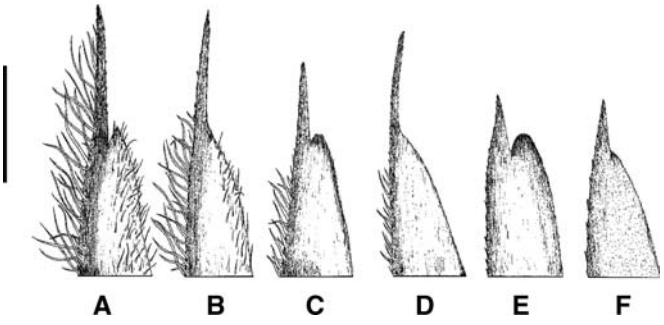

Fig. 3. The six types of lemma found in 2x–4x Algerian samples of *Dactylis*. **A** lemma lobed, strongly pubescent, ciliate on the keel, lengthy mucronate; **B** lemma unlobed, variably ciliate and pubescent, lengthy mucronate; **C** lemma lobed, sometimes ciliate and pubescent on margin, medium mucronate; **D** lemma unlobed, ciliate on keel, scabrous on margin, medium mucronate; **E** lemma lobed, keel scabrous to ciliate, shortly mucronate; **F** lemma unlobed, keel scabrous to ciliate, shortly mucronate. Scale bar = 1 mm

Ploidy	Populations	Types of lemma					
		A	B	C	D	E	F
2x	Benchicao	■	ρ				
4x	Tikjda 2	■	ρ				
2x	Kherrata 1	■	ρ				
4x	Kherrata 2	■	ρ	ρ			
2x	Babors 1	■	ρ	ρ			
2x	Babors 2	■	ρ	ρ			
2x	Berrouaghia	■	ρ	ρ			
2x	Ténès 1	■	ρ	■			
4x	Zaccar	■	ρ	■			
4x	Mazafran	■	ρ	■			
4x	Tikjda 1	■	ρ	■	ρ		
4x	Senalba 1	■	ρ	■	■		
2x	Tessala	■	ρ	■	■		
4x	Ténès 2	■	ρ	■	■	ρ	
2x	Lakhdaria	■	ρ	■	■	ρ	
4x	Senalba 2	■	ρ	■	ρ	■	
2x	Doui	ρ		■	■	ρ	
2x	Larhat	ρ		■	■	■	
4x	Gouraya			■	■	ρ	
4x	Ziama			■	■	■	
4x	Ténès 3			■	■	■	■
4x	Marsa 1			■	ρ	■	■
4x	Figuier			■	ρ	■	■
4x	Marsa 2				ρ	■	■

Fig. 4. Distribution of frequencies of the six lemma types in 2x–4x populations of *Dactylis*. Frequencies were calculated on 30 lemmas removed from ten individuals per population. ■ ≥ 75 % > ■ ≥ 50 % > ■ ≥ 25 % > ■ ≥ 10 % > ρ

negative part of the scatter plot, and it comprises populations collected in the humid bioclimate on Tellian Atlas (Tikjda 1, Zaccar) and in sublitoral habitats (Ziama, Marsa 1). This second group should correspond to the morphotype "*hispanica*". Two other populations from the semi-arid regions of Saharian Atlas (Senalba 1, Senalba 2) are clustered with this group. It is characterised by a spiciform panicle (less than 15.5 cm) with only 1 or 2 branches. They are the most common samples, found in various bioclimatic conditions, with higher mean culm and panicle length (31.5 cm and 3.5 cm, respectively). The lemma (3–4 mm) of the flower is sometimes lobed, weakly ciliate and pubescent on the keel.

Finally, a third group, smaller, is restricted to the positive part of axis 1. It is constituted

Table 3. Principal component analysis of 224 individuals from 23 populations of *Dactylis glomerata* L.: loading of 16 characters on the first tree axis, eigenvalue and cumulative variance

Characters	PC1	PC2	PC3
A	**0.924**	−0.024	0.008
B	**0.845**	0.060	−0.075
C	**0.873**	0.074	−0.023
F	−0.032	0.476	−0.108
G	0.040	**0.547**	−0.032
H	−0.054	0.371	0.482
I	−0.091	0.297	0.456
J	0.073	0.161	−0.038
K	**0.760**	−0.003	−0.296
L	−0.081	0.059	0.469
M	0.000	**0.600**	0.051
N	0.038	0.194	**0.699**
P	−0.169	−0.286	**0.684**
S	**0.801**	−0.094	−0.004
T	**0.847**	−0.110	0.028
U	0.187	−0.598	−0.041
Eigenvalue	4.459	1.743	1.337
Cumulative variance (%)	38.77	47.13	52.13

by a few individuals from two populations sampled on the edge of cultivated fields in the irrigated coastal plain (Mazafran, Corso). These individuals can be attributed to the '*glomerata*' type distinctly separated from the other ones reflecting differences in overall size. This morphotype has the tallest plants in the genus with a plant size over 80 cm, a panicle more than 15.5 cm, pyramidal and open, with several lateral branches.

The Discriminant Analysis was carried out using the same set of 224 individuals and variables, with the three putative tetraploid subspecies and the diploid ones. The results (Table 4A) show that individuals are classified with 78.37 % success based on 13 quantitative characters selected in the model. Two culm characters (A and S), and two panicle ones (K and B) are the best characters for distinguishing the different subspecies. The Canonical Discriminant Analysis (Fig. 6) revealed that the diploids and the tetraploids are clustered together. They form two major groups, the first one corresponding to the main 4x taxa *marina* and *hispanica* overlapping the diploid taxa on one side, the second group corresponding to individuals attributed to the *glomerata* type. The Discriminant Analysis shows that individuals from this latter subspecies can be classified with 100% success based on quantitative variables (Table 4A).

Because of the weak differentiation between the diploid natural populations, further analyses were conducted on both field samples and experimental cultures.

Diploid taxa. A new Discriminant Analysis was performed using 90 individuals from the field and the same number of variables (16). The three groups to be discriminated were designed according to their putative subspecific taxonomic rank (Stebbins and Zohary 1959, Parker and Borrill 1968, Borrill 1978, Lumaret 1988): subsp. *santai* (group 1), subsp. *castellata* (group 2) and subsp. *mairei* (group 3). The results (Table 4B) show that individuals are classified with 80.0 % success based on 11 (S, T, A, N, L, F, C, B, U, J, K) quantitative characters selected in the model. The scatter plot of CDA (Fig. 7) shows a cluster centred on the origin with numerous individuals overlapping between group 1 and 2. It is clear that the third best-individualised group, comprising populations of Kherrata and Babors from East Algeria corresponds to subsp. *mairei*. The other populations from Central and West Algeria represent subsp. *castellata* and subsp. *santai*, respectively.

A Principal Component Analysis (Fig. 8) was performed on the values of the quantitative characters measured on the 40 diploid individuals that survived after three years of cultivation in experimental garden (Table 5). Principal components 1, 2 and 3 explain 80.54% of total information. All the individuals of Kherrata and part of those of Lakhdaria, are in the negative part of axis 1. They are characterised by a slender habit, with frail panicle (2.5–5 cm). All the individuals from Ténès, Berrouaghia and some of those of Lakhdaria, with a stiff panicle (5–6.5 cm), are in the positive part of this axis. The DA,

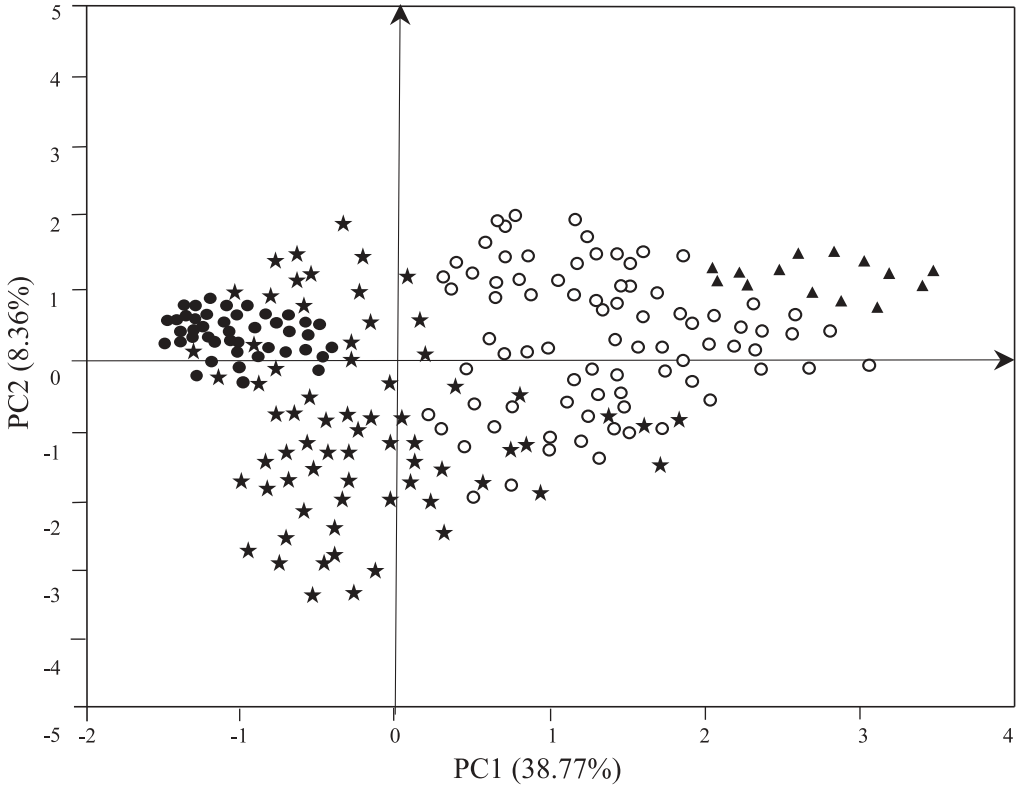

Fig. 5. Principal component analysis of 224 individuals from 23 populations of *Dactylis glomerata* L. belonging to tetraploid subspecies *marina* ● hispanica ★, *glomerata* ▲; and diploid subspecies ○

performed on the same material after allocation of the group 1 to the subsp. *mairei* and of the group 2 to the subsp. *santai*, shows that these groups 1 and 2 are 100% successfully classified.

Discussion

Dactylis is a widely distributed genus. In Algeria, since the field collecting in the early nineteen fifties by Stebbins, few new localities have been prospected by other authors. So, we have performed a wide sampling in ecologically contrasting sites of North Algeria, from the northern mesic coastal hills and mountains of the Tellian Atlas to the steppic regions near Sahara.

The morphological diversity of various diploid and tetraploid populations of *D. glomerata* from Algeria was analysed on the basis of a large set of traits including the diagnostic characters that have been previously used to distinguish different subspecies.

Our study reveals that the lemma shape, usually considered as a diagnostic character (Battandier and Trabut 1902, Domin 1943, Maire 1955, Stebbins and Zohary 1959, Borrill 1978, Lumaret 1981b, Guignard 1985) has not a significant value, especially in the diploid populations. The lobed or unlobed lemma criteria were primarily established to distinguish the *hispanica* type from the *glomerata* type. Nevertheless, our results show the important polymorphism of this proposed diagnostic character in subsp. *hispanica* and in all the other subspecies.

Our morphological data confirm the difficulties in distinguishing the different ploidy levels previously highlighted in the literature (Borrill 1978, Lumaret 1988, Lindner and Garcia 1997). The multivariate analyses based on quantitative characters show consistent

Table 4. A-B. Results of discriminant analysis (DA), best variables, classification and discriminant functions of grouping. **A** in the diploid-tetraploid *Dactylis glomerata* complex. **B** in diploids subspecies *mairei*, *santai* and *castellata*. See also Table 2 for the abbreviation of characters.

A

Best variables	Wilks Lambda	F-to-remove	p
A	0.214044	36.60757	0.000000
S	0.170569	15.22481	0.000000
K	0.182146	20.91885	0.000000
B	0.156181	8.14845	0.000370
F	0.156004	8.06158	0.000042
N	0.159309	9.68696	0.000005

Lambda Wilks: 0.13961 F appro. (39.610) = 14.788 p < 0.0000

	Diploid subspecies P = 0.39640	Tetraploid subspecies			Total Correct
		marina	*hispanica*	*glomerata*	
		p = 0.22523	p = 0.31532	p = 0.06306	
Diploids	75	3	10	0	85.22
marina	3	45	2	0	90.00
hispanica	15	15	40	0	57.14
glomerata	0	0	0	14	100.00
Percentage	93	63	52	14	78.37

B

Best variables	Wilks Lambda	F-to-remove	p
S	0.338808	6.11442	0.003431
T	0.397363	13.82496	0.000007
A	0.318453	3.43406	0.037274
N	0.318213	3.40243	0.038373
L	0.326431	4.48467	0.014377
F	0.318668	3.46231	0.036320
C	0.330607	5.03455	0.008813
B	0.312150	2.60402	0.080481
U	0.302904	1.38650	0.256118
J	0.301348	1.18164	0.312277
K	0.300333	1.04801	0.355581

Lambda Wilks: 0.29237 F appro. (22.154) = 5.9458 p < 0.0000

	santai	*castellata*	*mairei*	Total Correct
	p = 0.44444	p = 0.33333	p = 0.22222	
santai	34	5	1	85.00
castellata	4	25	1	83.33
mairei	2	5	13	65.00
Percentage	40	35	15	80.00

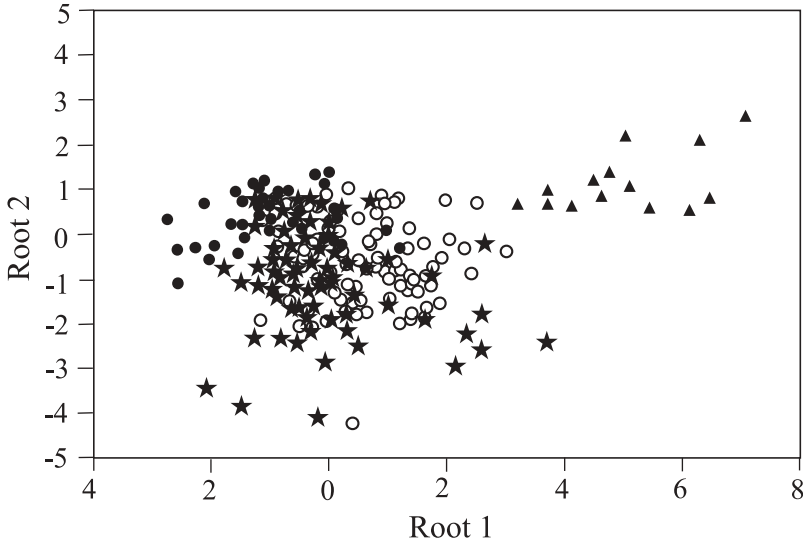

Fig. 6. Canonical discriminant analysis of 224 diploid–tetraploid individuals of *Dactylis glomerata* L. Discrimination based into four groups. Group 1: diploid subspecies ○; Group 2, 3 and 4 subspecies tetraploids *marina* ●, *hispanica* ★ and *glomerata* ▲ respectively

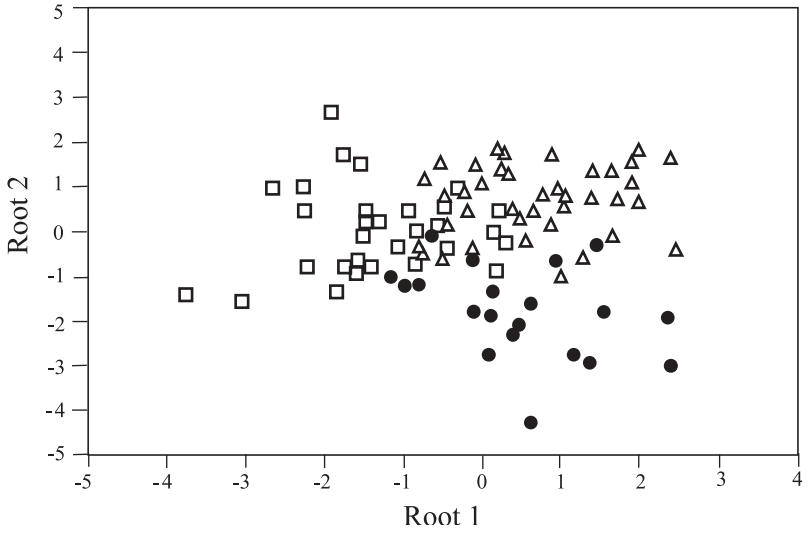

Fig. 7. Canonical discriminant analysis of three diploid subspecies of *Dactylis glomerata*. Group 1: subsp. *santai* △; Group 2: subsp. *castellata* □; Group 3: subsp. *mairei* ●

variability in both diploid and tetraploid populations. In PCA, which was used to visualise the differences or the similarity, some subspecies were poorly or not separated. PCA axes 1 and 2 (Fig. 5) show together a continuum of variability, nevertheless some tetraploid taxa have been identified by six characters of culm and panicle (A, C, T, S, B, K). In the Discriminant Analysis (Table 4A), six principal characters (A, S, K, B, F, N), two of culms and two of panicles are the same as those pointed out by the PCA. In this DA, 78.37% of the samples were classified correctly, showing the diagnostic values of

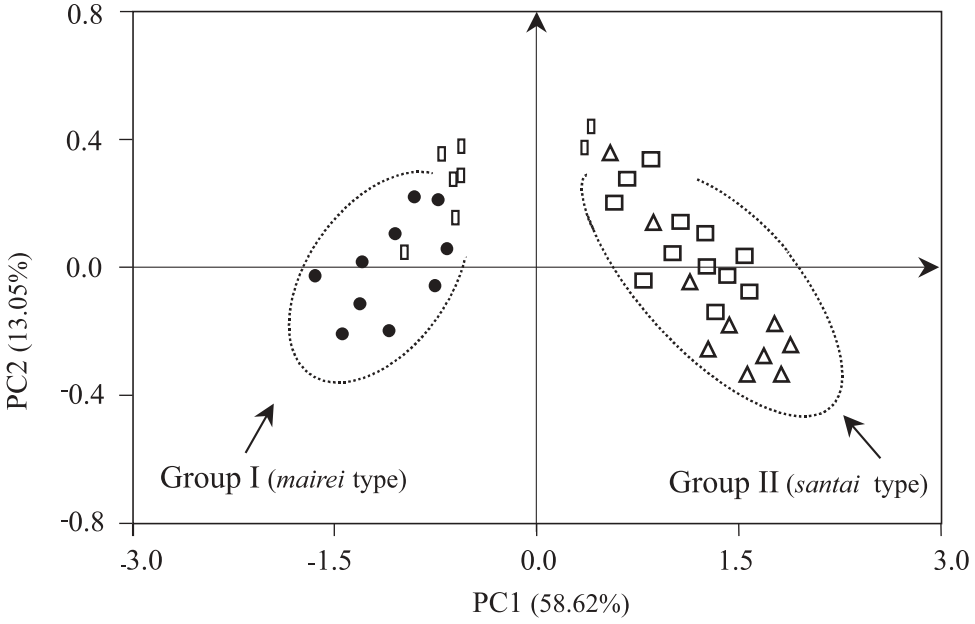

Fig. 8. Principal component analysis of 40 diploid individuals cultivated in experimental garden. ● *mairei* type, population from gorges of Kherrata; △ *santai* type, population from Ténès; samples previously belonging to *castellata* from gorges of Lakhdaria (▯) and those from Berrouaghia (□)

Table 5. Descriptive statistics of twelve morphological characters for the main two taxa of Algerian diploid *Dactylis*. Mean and SD in mm

Characters	Group I (subsp. *mairei*)		Group II (subsp. *santai*)	
	Field	Culture	Field	Culture
A	478.7 ± 33.0	290.9 ± 48.4	520.4 ± 65.1	415.8 ± 107.9
C	50.4 ± 18.5	26.6 ± 9.8	55.7 ± 22.8	46.3 ± 17.5
S	130.9 ± 11.2	89.5 ± 26.6	140.3 ± 17.0	101.8 ± 23.1
T	61.7 ± 10.7	38.0 ± 15.5	79.8 ± 13.7	69.9 ± 19.4
B	50.1 ± 16.6	24.3 ± 8.3	50.0 ± 17.5	62.5 ± 24.4
F	18.5 ± 9.6	4.3 ± 1.2	15.5 ± 8.5	23.8 ± 13.6
G	7.6 ± 3.8	2.8 ± 1.2	7.2 ± 3.7	9.1 ± 5.0
H	3.6 ± 1.0	2.2 ± 1.0	3.9 ± 1.1	3.9 ± 2.30
J	11.4 ± 3.0	8.4 ± 1.6	12.3 ± 4.3	13.0 ± 4.20
L	4.08 ± 0.36	4.98 ± 0.64	4.25 ± 0.36	5.37 ± 0.87
M	3.15 ± 0.37	3.5 ± 0.66	3.24 ± 0.34	3.66 ± 0.45
N	3.27 ± 0.21	4.43 ± 0.54	3.3 ± 0.20	4.24 ± 0.71

these characters. In fact, both vegetative and reproductive characters delineate three main phenotypic groups within the tetraploid populations. The samples easily identified were well separated along the first axis of PCA (Fig. 5). The subsp. *marina* is in the negative part and the subsp. *glomerata* in the positive one, with subsp. *hispanica* distributed between them. Until now subsp. *glomerata* was considered as absent in North Africa (Stebbins and Zohary 1959, Borrill 1978, Lumaret 1988). These samples are probably derived from

hybridisation between the native *hispanica* and some commercial cultivated varieties of *D. glomerata* subsp. *glomerata*.

Diploid entities are less distinguishable from each other and cannot be kept as three separated subspecies. They are morphologically similar to the tetraploid populations as clearly shown in CDA (Fig. 6). This agrees with the presumption of autopolyploidy in this complex. Stebbins (1950) termed *Dactylis glomerata* as an 'intervarietal autopolyploid'.

Nevertheless, the different analyses, allowed us to consider the existence of two taxa within the diploid populations. In fact, Fig. 8 shows clearly that the individuals of Berrouaghia and Lakhdaria, previously identified to subsp. *castellata,* are scattered after cultivation with Ténès and Kherrata respectively, because they share the morphological characters of each subspecies. The subsp. *mairei* with a typical morph (including some individuals previously identified as "*castellata*") is restricted to particular ecological niches as gorges and crevices of limestone cliffs, generally at low altitude. The subsp. *santai*, more polymorphous and associated with different habitats, includes most of the individuals attributed to the putative subsp. *castellata*. This latter subspecies was named by Parker and Borrill (1968) after examination of some samples collected by Stebbins, in Oued Chiffa on the railway embankment and in Le Kef (Tunisia). But it seems that no diagnosis was published concerning this new subspecies. Our results suggest that there is no substantial difference between subsp. *santai* and *castellata*. These two subspecies form a morphological homogeneous entity. They were initially differently named in regard to their geographical origin.

Moreover, our results show clearly that the diploids are much more abundant in Algeria than supposed in previous studies (Stebbins and Zohary 1959, Borrill 1978, Lumaret 1988). In fact, besides populations mentioned in this study, several diploid populations were found in the central region near Algiers (Tipaza, Bouzareah and Monts de Chréa). The diploid populations are generally distributed in altitude ranging from 150 to 1500 meters on Tellian Atlas in protected or open habitats like oak or pine woods in humid and subhumid bioclimates. In contrast, diploids are rare in other regions such as Italy (Speranza and Cristofolini 1986, 1987), they are lacking in France (Lumaret 1988, Guignard and Huon 1983) and in Thrace (Tuna et al. 2004). This particular pattern of diploid distribution can be interpreted as resulting from postglacial processes in which polyploids have excluded their diploid relatives in specific ecological niches (Hamrick and Godt 1997, Thompson 1999). This would explain the abundance of diploids that would have found refuge in southern Mediterranean areas, mainly in North Africa. These patterns of habitat and geographical differentiation of the diploid-tetraploid areas have been observed in *Dactylis* in other Mediterranean regions (Thompson and Lumaret 1992, Soltis et al. 2003) and in several autopolyploids like *Heuchera micrantha* (Ness et al. 1989), *Vaccinium oxycoccos* (Mahy et al. 2000), and *Chamerion angustifolium* (Husband and Sabara 2003). *Dactylis* in Algeria may be considered as a mature polyploid complex (*sensu* Stebbins 1971) because diploids and tetraploids are in parapatric and sometimes, in sympatric situation, like in the Kherrata region. According to Borrill and Lindner (1971), the Kherrata gorges are a relict habitat, and subsp. *mairei* can be considered as an isolated fragment of a previously widespread diploid type, restricted to its present habitat by the progressive deterioration of the North African climate during the last 10,000 years. In addition to the diploids, the gorges contain tetraploids which are slightly more vigorous than the diploids. A similar distribution of polyploidy in Algeria has been also observed on annual polyploid complexes from Poaceae like *Bromus* L. (Aïnouche et al. 1995) and *Hordeum* L. (Amirouche and Misset 2003).

In conclusion, this study on cytological and morphological variability within the species *D. glomerata* L., points out the wide distribution

of the tetraploid populations and the importance of the diploids in this region. The existence of many wild diploid populations of *Dactylis* in Algeria allows considering this region as a reservoir of cocksfoot that is critical in conservation programs of this forage grass. Moreover, our analyses have highlighted morphological variables that have better taxonomic value, particularly for the diploid taxa. In view of these results, we propose a key in complement to the treatment of this taxon by Quezel and Santa (1962) in their Algerian Flora. Nevertheless, further molecular analyses associated to these first investigations could provide a complementary picture of the distribution patterns of this polyploid complex in Algeria.

KEY

1a. Height of plant over 80 cm, panicle more than 15.5 cm, pyramidal and open with several lateral branches. Usually found on edge of cultivated field in humid area. Chromosome number 2n = 4x = 28

..... ● subspecies ***glomerata*** Hayek.

1b. Height of plant ≤ 80 cm, culms mean length with 31.5 cm, panicle laterally compressed, less than 15.5 cm, lemmas lobed and unlobed, weakly ciliate and pubescent on the keel.

..... **2**

2a. Plant small, length of the culms: 10–15 cm, panicle short, 1.5- 3 cm, lemmas lobed, glabrous, papillae on leaf, localised on maritime cliffs and rocks under wind-salted sprays. Chromosome number 2n = 4x = 28

..... ● subspecies ***marina*** (Borrill) Greuter, 1983

2b. Plant of intermediate length, culms length comprises between 30.5–50.5 cm, panicle length 3–15 cm, lemma lobed or weakly lobed, pubescent.

..... **3**

3a. Length of the second internode of culm 4–7 cm, Panicle intermediate length 3–6.5 cm, typically spiciform with only 1or 2 branches (sometimes 3), length of the lemma 3–4 mm, widely spread in different bioclimatic conditions. Chromosome number 2n = 4x = 28

..... ● subspecies ***hispanica*** (Roth) Nyman, 1882

3b. Characteristics similar concerning the length of the second internode of culm and panicle but lemma length < 4 mm and chromosome number 2n = 2x = 14

.... **4**

4a. Length of culm 30–50 cm, panicle frail, 2.5–5 cm, plant slender, localised on gorges and in crevices of limestone cliffs. Chromosome number 2n = 2x = 14

..... ● subspecies ***mairei*** Stebbins & Zohary (1959), Univ. Cal. Pub. Bot. p.15

4b. Length of culm ≥ 50 cm, panicle stiff, 5–6.5 cm, widely distributed in much degraded areas. Chromosome number 2n = 2x = 14

..... ● subspecies ***santai*** Stebbins & Zohary (1959), Univ. Cal. Pub. Bot. p.14 (included *castellata*)

★★★

TAXONOMIC TREATMENT

Dactylis glomerata L. Sp., p. 71, 1753.

Dactylis glomerata subspecies ***glomerata*** Hayek.

– *D. glomerata* subsp. *euglomerata* Jansen & Walchner. Netherlands. Kruidk. Archief. 47:174 1912.

• *Dactylis glomerata* subspecies ***marina*** (Borrill) Greuter, 1983.

– *D. marina* Borrill, 1961.

– *D. glomerata* var. *marina* (Borrill) Speranza & Cristof., 1986.

– *D. glomerata* subsp. *hispanica* (Roth); f. *hackelii* Asch. & Gr., 2, p.380. (1900) provar.

– *D. glomerata* subsp. *hackelii* (Asckers. & Graebn.) Cif. & Giacomm.

– *D. hispanica* var. *maritima* Hack., Gram. Port., p. 23,1880.

– *D. glomerata* var. *maritima* (Hack.) Richt., Pl. Eur. 1, p. 81, (1890); non Hallier, 1863.

• *Dactylis glomerata* subspecies ***hispanica*** (Roth) Nyman, 1882

– var. *hispanica* (Roth) Koch, Syn. ed.1, p. 808, 1837.
– *D. glomerata* var. *sibthorpii* Hack., Oest. Bot. Zeitschr. 28, p. 192 ,1878.
– *D. hispanica* Roth, Cat. Bot. 1, p. 8, 1797.
- *Dactylis glomerata* subspecies ***mairei*** Stebbins & Zohary, 1959. Univ. Cal. Pub. Bot. p. 15
- *Dactylis glomerata* subspecies ***santai*** Stebbins & Zohary, 1959. Univ. Cal. Pub. Bot. p.14
- *Dactylis glomerata* subspecies ***castellata*** Parker & Borrill, 1968; Borrill & Carroll, 1969.

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