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Morphological Variation in Population of the Genus *Lolium* (Poaceae) in Iran

¹Mirjalili Seyyed Abbas and ²Bennett, J. Sarita

¹Agricultural Higher Education Center, Agricultural Research and Education Organization,
Ministry of Jihad-Agriculture, Tehran, Iran

²CRC for Plant-based Management of Dryland Salinity,
University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

Abstract: Forty-three accessions belonging to six species of the genus *Lolium* were analysed for morphological and phenological data following field trials. Principal Components Analysis (PCA) showed five distinct groups that separated most of the species except *L. loliaceum*, which grouped with the populations of *L. rigidum*. Cluster analysis, also, showed a close relationship between *L. rigidum* and *L. loliaceum*, linking them in a single group. These results confirm that these two species are not separable at the specific level. *L. temulentum* and *L. persicum*, as the inbreeding species, clearly separated from the cross-breeding species and were distinct from each other. According to the cluster analyses, *L. perenne* placed near the populations of *L. rigidum*. *L. multiflorum*, as a cross-breeding species, showed little similarity with the inbreeding species and was also very distinct.

Key words: Poaceae, *Lolium*, morphological variation, Iran

INTRODUCTION

The genus *Lolium* L. (Poaceae) consists of about eight recognised species (Loos, 1993a) all diploids, with a chromosome number of $2n = 14$ (Bennett, 2000). The genus is native to Europe, temperate Asia and North Africa (Loos, 1993a) but has been introduced in most temperate areas of the world (Bennett, 2000; Loos, 1993a). The two most economically important species are *Lolium perenne* L. and *L. multiflorum* Lam. which are both widely grown as forage grasses, especially in Europe, New Zealand, Australia and other temperate/mediterranean regions of the world (Bennett, 1997, 2000; Loos, 1993a). *L. perenne*, known as perennial ryegrass, is the only long-lived perennial in the genus. It is found growing further north than any of the other species. *L. multiflorum* (Italian ryegrass) is an annual or short-lived perennial. It has a similar distribution and cultivation to *L. perenne* but grows best in mediterranean climates. Both species are self-incompatible (Bennett, 2000; Terrell, 1968; Zwierzykowski and Naganowski, 1996). They will readily hybridise with each other, resulting in highly fertile F1 progeny although there may be some loss of fitness in the hybrids (Bennett, 1997, Mill, 1985). Bulinska-Radomska and Lester (1985) reported that along with *L. rigidum* the three species form one biological species (Bennett, 1997).

L. rigidum Gaud. is an annual species common in southern Europe and the mediterranean region. It is the third species of the allogamous group within the genus *Lolium*.

L. temulentum L. is only found growing as a weed among wheat fields and other cereals (Bennett, 1997). The species is a self-fertile annual (Bennett, 2000).

L. persicum Boiss and Hoh. is rare with few specimens collected in the wild (Bennett, 1997). The distribution of *L. persicum* is restricted to Southeast Asia (Bennett, 2000), although it has been widely found around Iran.

L. loliaceum Bory and Chaub. is considered by Terrell (1968) to be conspecific with *L. rigidum*. It has the same distribution and there are no clear boundaries between the characters scored. He therefore classified it as *L. rigidum* var. *rothbollioides* (Bennett, 1997). Although, Parsa (1950) in *Flore de l' Iran* didn't report the species of *L. loliaceum*, but this species is reported by Bor (1968, 1970) in *Flora of Iraq* and *Flora Iranica* as a separate species.

This study presents an analysis of quantitative morphological and phenological data observed in several populations of different *Lolium* species. Previous publications have shown that multivariate analysis of quantitative characters can be used to measure genetic

distances between populations within species (e.g., Souza and Sorrells, 1989; Loos, 1993a). Although the phenotype based on quantitative characters cannot be directly related to the genotype, it has a strong genotypic basis. Therefore, the quantitative, morphological and phenological characters used as a measure of genetic distance between species are those which are used in the determination keys for the species. The results will be compared with results obtained by other authors using various techniques.

MATERIALS AND METHODS

Forty- three *Lolium* populations were obtained from several sources (Gene bank and Research Institutes) and collected around the country. Following Bor (1970) in *Flora Iranica*, these populations were classified into six *Lolium* species, most of the time agreeing with the species name under which the seed was supplied (Table 1). In Table 1, the used code, seed source, species

and abbreviations are tabulated for each population. Each species consisted of 8 to 14 plants sown in a plot. Each plot consisted of three rows of four plants, in which each plant was spaced 30 cm apart within and between rows. All populations were transplanted to the field on 9th April 2002. Seeds were germinated and vernalized, where required, by placing in petri-dishes in the 4°C refrigerator for one week. The field was situated at the Research Institute of Forests and Rangelands experimental farm at Tehran. The observed characters and the method of scoring the characters are given in Table 2.

The mean and standard deviation of each trait was estimated per species. The characters were standardized across populations and population averages were calculated for principal components analysis. Principal Components Analysis (PCA) was done using STSTATISTICA software, 99' Editon (1995). The populations were clustered using UPGMA and WPGMA methods and Percent Disagreement coefficient.

Table 1: Code, seed source, species and Abbreviations for *Lolium* populations included in study

No.	Code	Seed source	Origin	Species	Abbreviations
1	12000.9	Isfahan	Gene Bank	<i>L. perenne</i>	LPN1
2	1000.1	Tabriz University	Unknown	<i>L. perenne</i>	LPN2
3	309	RIFR	Gene Bank	<i>L. perenne</i>	LPN3
4	24.1764	RIFR	Gene Bank	<i>L. perenne</i>	LPN4
5	1000.3	Collected	Freidounkenar	<i>L. perenne</i>	LPN5
6	1000.8	Collected	Yazd	<i>L. perenne</i>	LPN6
7	1000.9	Collected	Amol- Babol	<i>L. perenne</i>	LPN7
8	1000.15	Imported	Denmark	<i>L. perenne</i>	LPN8
9	1000.16	Imported	Netherlands	<i>L. perenne</i>	LPN9
10	1000.21	Collected	Boushehr	<i>L. perenne</i>	LPN10
11	1000.26	Collected	Kelardasht	<i>L. perenne</i>	LPN11
12	12000.26	Isfahan	Gene Bank	<i>L. multiflorum</i>	LMU1
13	187	RIFR	Gene Bank	<i>L. multiflorum</i>	LMU2
14	23.1766	RIFR	Gene Bank	<i>L. multiflorum</i>	LMU3
15	1551.1253	RIFR	Gene Bank	<i>L. multiflorum</i>	LMU4
16	1557	RIFR	Gene Bank	<i>L. multiflorum</i>	LMU5
17	308.8268	RIFR	Gene Bank	<i>L. multiflorum</i>	LMU6
18	1000.4	Collected	Chalous	<i>L. multiflorum</i>	LMU7
19	1000.12	Collected	Rasht	<i>L. multiflorum</i>	LMU8
20	1000.17	Collected	Ramsar	<i>L. multiflorum</i>	LMU9
21	1000.25	Collected	Astaneh	<i>L. multiflorum</i>	LMU10
22	1000.28	Collected	Babol	<i>L. multiflorum</i>	LMU11
23	1000.5	Collected	Chalus	<i>L. temulentum</i>	LTE1
24	1000.19	Collected	Boushehr	<i>L. temulentum</i>	LTE2
25	1000.24	Collected	Babolsar	<i>L. temulentum</i>	LTE3
26	1000.27	Collected	Rostam abad	<i>L. temulentum</i>	LTE4
27	12000.4	Isfahan	Gene bank	<i>L. rigidum</i>	LRG1
28	2489	RIFR	Gene bank	<i>L. rigidum</i>	LRG2
29	2508	RIFR	Gene bank	<i>L. rigidum</i>	LRG3
30	1587	RIFR	Gene bank	<i>L. rigidum</i>	LRG4
31	200	RIFR	Gene bank	<i>L. rigidum</i>	LRG5
32	12000.52	RIFR	Gene bank	<i>L. rigidum</i>	LRG6
33	1000.2	Collected	Freidounkenar	<i>L. rigidum</i>	LRG7
34	1000.6	Collected	Chalous	<i>L. rigidum</i>	LRG8
35	1000.10	Collected	Babolsar	<i>L. rigidum</i>	LRG9
36	1000.11	Collected	Rasht	<i>L. rigidum</i>	LRG10
37	1000.20	Collected	Boushehr	<i>L. rigidum</i>	LRG11
38	1000.7	Collected	Chalous	<i>L. persicum</i>	LPS1
39	1000.14	Collected	Yazd	<i>L. persicum</i>	LPS2

Table 1: Continued

No.	Code	Seed source	Origin	Species	Abbreviations
40	1000.18	Collected	Ramsar	<i>L. persicum</i>	LPS3
41	1000.23	Collected	Hamadan	<i>L. persicum</i>	LPS4
42	1000.13	Collected	Rasht	<i>L. loliaceum</i>	LLO1
43	1000.22	Collected	Rasht	<i>L. loliaceum</i>	LLO2

RIFR: Research Institute of Forests and Rangelands at Iran

Table 2: Characteristics measured on *Lolium* populations, with abbreviation and description

No.	Character	Abbreviation	Description (Character States)
1	Date of ear emergence	DEE	Days after the 20th of April
2	Plant height at ear emergence	NHE	Height at ear emergence (cm)
3	Habit	HAB	Habit of plant from erect to prostrate (erect =1 to prostrate = 5)
4	Leaf colour	LCO	Leaf colour from light green to dark green (light green =1 to dark green =5)
5	Anthocyanin colouring	ANT	Anthocyanins in culms, leaf or node(only node =1, node and culms = 2, node, culms and leaf =3, about all of parts = 4 all of part with dark red = 5)
6	Rooting	ROT	Power of rooting in lower nodes. (scale: 1 = very weak to powerful = 5)
7	Tillering	TIL	Number of tillers per plant
8	No. of spikes	NOS	Number of spikes per plant
9	Plant width	PLW	Width of plant (average of two direction)
10	Flag leaf length	LFL	Length of the flag leaf (cm)
11	Flag leaf width	LFW	Width of the flag leaf (mm)
12	Plant height at maturity	HAE	Height 30 days after ear emergence (cm)
13	Internode plus Ear length	LNE	Length of the upper internode and ear (cm)
14	Internode length	LIN	Length of the upper internode (cm)
15	Ear length	ELN	Ear length (cm)
16	Rachis length	RAL	Rachis length (cm)
17	No. of spikelets	NSS	Number of spikelets per spike
18	Spikelet length	LSA	Spikelet Length, awns not included (mm)
19	No. of florets	NFL	Number of florets per spikelet
20	Percentage awned floret	PAF	Percentage awned florets
21	Length longest awn	LLA	Length longest awn (mm)
22	Length of glume	LGL	Length of glume (mm)
23	Spikelet glume length	SGL	Spikelet length (18)/length of glume (22)
24	Plant density	PLD	Plant density (scale: 1 = solitary to 5 = caespitose)

RESULTS

In Table 4, the estimated means and standard deviations for all characters of each species are given. These Figures are based on a minimum of two populations per species.

Principal components analysis (PCA) was found to explain the variation between specimens in the first three principal components. The first three components with the percentage variation and the characters with the greatest loadings are given in Table 3.

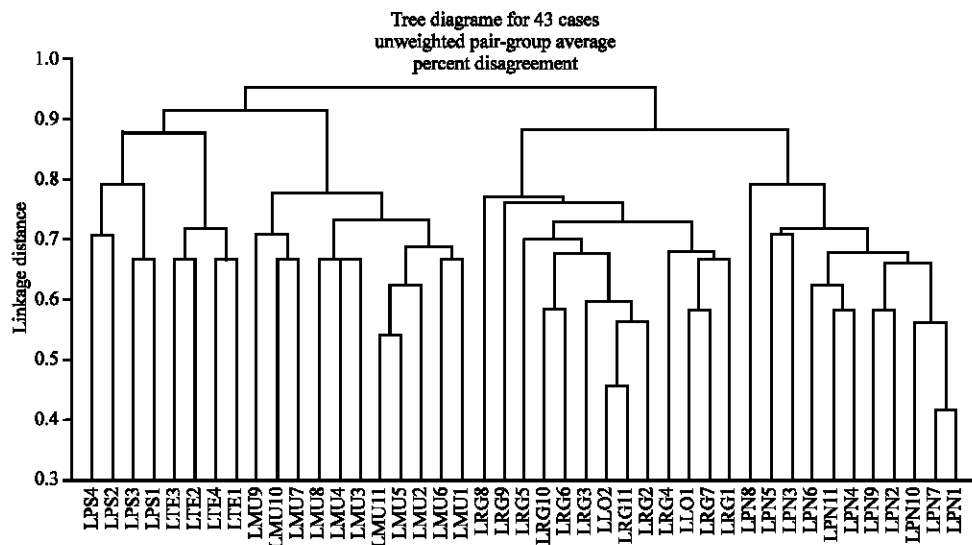
According to Table 3, cumulative percent in the first three components exceeded on 80% variation. In the first component, PLW (plant wide), PLD (plant density) and RAL (rachis length) have greatest loadings by over 52% of total variation. In the second, the characters SGL (spikelet glume length), LCO (leaf colour) and NFL (number of florets per spikelet) consist about 16% of variation.

The clustering of the populations showed five distinct group, which doesn't agree with the number of

Table 3: Principal components and factor loadings with corresponding variation

Principal components	1	2	3
Eigenvalues	12.492	3.844	3.065
Total variation (%)	52.050	16.020	12.771
Cumulative eigenvalues	12.492	16.336	19.402
Cumulative (%)	52.050	68.070	80.841
Factor loadings	PLW : -0.907 PLD : -0.899 RAL : 0.857	SGL : 0.903 LCO: 0.751 NFL: -0.612	ANT : -0.714 LLA: 0.711 NHE: -0.508

species entered in the analysis (Fig. 1-4). Despite of using two different methods (UPGMA and WPGMA) for clustering, the results were nearly the same (Fig. 1 and 2). In these Figures, *L. temulentum* and *L. persicum* placed at the left side of dendrograms and *L. perenne* and *L. rigidum* as the out breeding species at the right. As shown in Fig. 4, the populations formed five groups. Within this the cross- and inbreeding species are clearly separated, with *L. temulentum* and *L. persicum* populations forming two groups in the upper right-hand corner of the plot. *L. loliaceum* populations with the *L. rigidum* populations formed a single group. Table 3



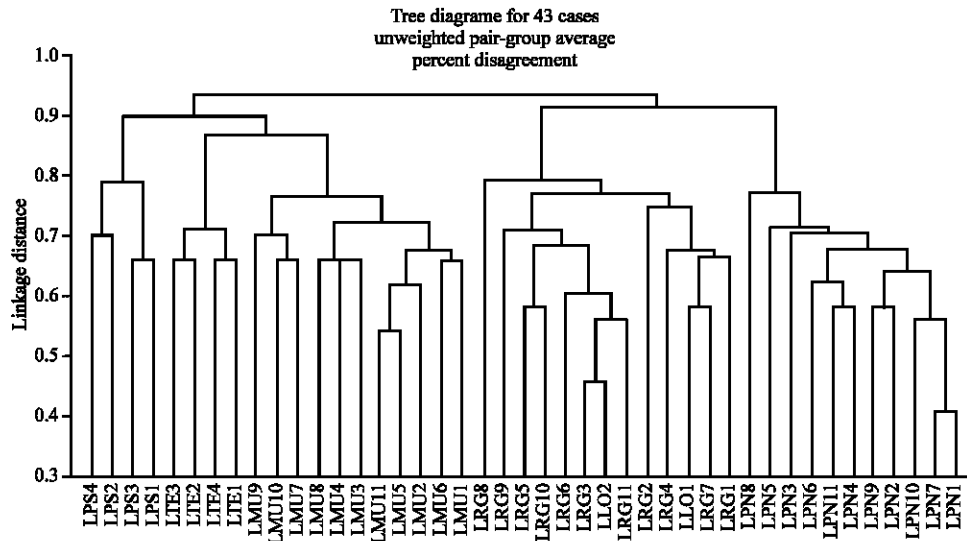


Fig. 2: Diagram of 43 *Lolium* populations on the first two principal components based on WPGMA method and percent disagreement coefficient

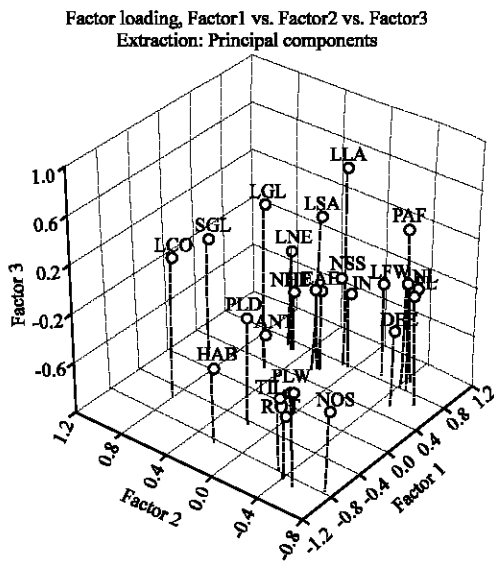


Fig. 3: Factor loadings in the analysis of 43 *Lolium* populations on the first three principal components

close similarity between *L. rigidum* and *L. perenne* using herbarium specimens and Loos (1993a) found that little differentiation was apparent between the three cross-breeding species, although they were distinct.

To date, it appears that *L. perenne*, *L. multiflorum* and *L. rigidum* are three separate species, but that their close association and possible recent evolutionary divergence (Terrell, 1968) has resulted in varying degrees of similarity between them. The magnitude of

differentiation, as assessed, will also differ according to the experimental techniques used.

Based on compatibility and hybrid-fertility data (Jenkin, 1954a, b, c), Terrell (1968) recognized *L. temulentum*, *L. remotum* and *L. persicum* as one group. *L. perenne* and *L. multiflorum* form another group. While *L. rigidum* is a polymorphic complex made up of several elements (Charmet and Balfourier, 1994).

L. temulentum and *L. persicum*, as two obligate in-breeders, have clearly diverged from the three cross-breeding species (Bennett, 1997; Jenkin, 1954b; Stammers *et al.*, 1995) with crosses between *L. temulentum* and the cross-breeding species giving poor results (Naylor, 1960; Thorogood and Hayward, 1992). They therefore provide a good control for the comparison of the three outbreeding species.

L. temulentum and *L. persicum* are clearly separated from the cross-breeding species (Fig. 1, 2 and 4). Both in principal components analysis and cluster analysis, these two species are joined together. The DNA amount of both inbreeding species is clearly much larger (about 40%) than that of the cross-breeding species (Hutchinson *et al.*, 1979). The results from this study agree with the literature (Loos, 1993a) that both species are clearly distinct from the rest of the genus.

The results from this trial indicate that the cross-breeders are easily separated from the in breeding species. As shown in Fig. 1, 2 and 4, two of the inbreeding species (*L. temulentum* and *L. persicum*) are clearly distinct from the cross-breeding species and place in the left of diagrams. These species, also, showed two distinct groups on the scatter plot.

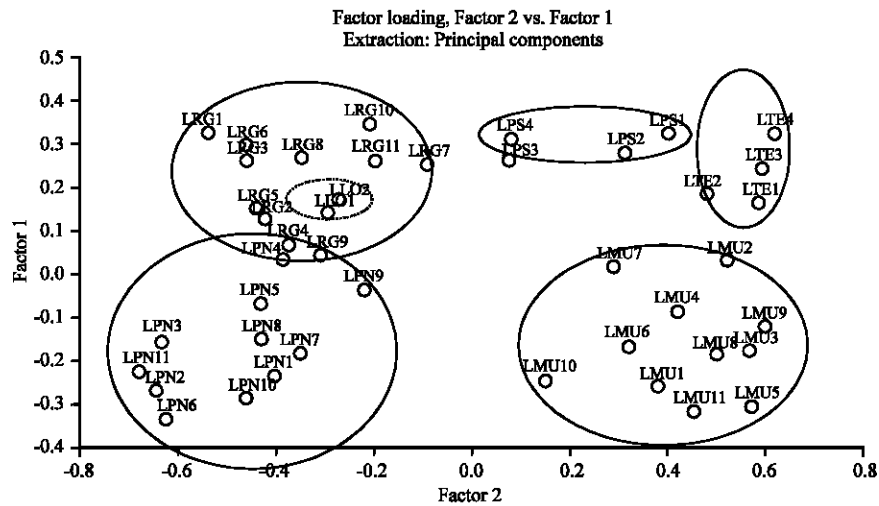


Fig. 4: Scatter plot to show cluster analysis of 43 populations of the genus *Lilium*

According to the clustering analysis, *L. multiflorum* is closest cross-breeding species to the in breeders. In previous studies such as Loos (1993a) and Bennett (1997), this species placed at the near of cross-breeders.

L. loliaceum, appears more closely related to the cross-breeding groups. Charmet and Balfourier (1994) reported that *L. loliaceum* has about 20% cross pollination and classified it as an in-breeder. The close relationship between *L. loliaceum* and *L. rigidum* (Fig. 1 and 2) is due to great similarity between them. According to Terrell (1968) and Mill (1985), these species are two varieties of *L. rigidum*, var. *rotbollioides* having an indurated rachis, with spikelets sunken into the rachis and shorter spikes.

Thomas (1981) suggested that *L. loliaceum* should be considered as intermediate between the cross-breeding and inbreeding group, based on the intermediate DNA content of this species and the great similarity of the C-banding pattern of this species with that of the cross-breeding species. The results from the present study agree with Thomas' (1981) view that *L. loliaceum* is a species distinct from the other inbreeders, but that its position is not intermediate but closest to *L. rigidum*.

L. rigidum and *L. loliaceum* have the most similarity between species. Terrell (1968) and Mill (1985) separated *L. rigidum* into two varieties: var. *rigidum* and var. *rotbollioides*. They were separated on the basis of var. *rotbollioides* having an indurated rachis, with spikelets sunken into the rachis and shorter spikes. Humphries (1980) separates them into two subspecies on the basis of the characters listed above, but refers to them as; subsp. *rigidum* and subsp. *lepturoides*. Principal components analysis separate two species of *L. rigidum* and

L. loliaceum into two clusters with some overlap between them. Cluster analysis, also, confirms this separation, but similarity level is very high. Thus separation of this two species, according to high similarity might be due to grouping those as one species with two variety or subspecies. Cluster analysis showed a greater similarities between these two species and *L. perenne* than to others. The reason for this can be explained in part by the large variation found in nearly all of the characters of *L. rigidum* and *L. loliaceum* and by high similarity of them to *L. perenne* other than the spikelet being sunken into the rachis in *L. loliaceum*, the majority of the other character appear to be within the range of character variation of *L. loliaceum*. In fact the only characters that show no overlap between *L. rigidum* and *L. loliaceum* is the degree in which the spikelet is sunk into rachis and the width of the spikelet and between *L. rigidum* and *L. perenne* is perenniality and leaf veneration. The results of this study confirm that these two species are not separable at the specific level. *L. perenne* separated clearly from the other species in the cluster analysis (Fig. 1 and 2) but grouped with *L. rigidum* populations. *L. perenne* and *L. multiflorum*, also, separated clearly in both principal component analysis and clustering.

Naylor (1960) and Bulinska-Radomska and Lester (1985) concluded that *L. perenne*, *L. multiflorum* and *L. rigidum* should be re-classified as subspecies of a single species, despite Jenkin (1954a-c) showing that F1 crosses between the three species show a reduction in percentage germination and fertility at flowering.

Bulinska-Radomska and Lester (1985) found that on the basis of protein similarities *L. multiflorum* and *L. rigidum* are more closely related than any other

combination of cross-breeding species. The karyomorphology of the three species is also very similar, although they differ in DNA-amount (Loos, 1993 a).

CONCLUSIONS

It appears that the genus *Lolium* can be divided into two groups: An inbreeding group, containing *L. persicum* and *L. temulentum* and an out-crossing group containing the remaining species. This second group contains all three cross-breeding species (*L. perenne*, *L. multiflorum* and *L. rigidum*); these species are closely related but seem to be distinct, although introgression between them is possible and some characters are similar in all three species. The one remaining inbreeding species (*L. loliaceum*) does not form a distinct group and is not separable at specific level from *L. rigidum*, forming a single group.

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