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Evaluation of Italian Ryegrass Populations Naturalized in the Flooding Pampa of Argentina. II. Phenotypic Variability among Populations Growing in Different Soils

Sara Isabel Alonso* • **Andrea Martina Clausen**

Unidad Integrada: Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata - Estación Experimental Agropecuaria Balcarce, INTA, CC 276 (7620) Balcarce, Buenos Aires, Argentina

Corresponding author: * **salonso@balcarce.inta.gov.ar**

ABSTRACT

Naturalized populations of Italian ryegrass (*Lolium multiflorum* Lam.) grow in different environments in Buenos Aires Province (Argentina). Juvenile plants of a cultivar and 20 populations collected in central-east areas of this Province were analysed in two environments with different soil types (Argiudol and Natracuol). At each site, 40 plants per plot were transplanted isolated, in a complete randomized blocks with three replicates. Morphological and agronomic traits were registered and ANOVA between environments were performed, with the replicates nested in the environments, and the genetic determination grade (GDG) was estimated. With the data registered during the trial as well as other previously obtained for the same populations, the affinity among populations was calculated by multivariate analysis. The genotype × environmental interaction resulted negative only for plant diameter and dry matter production as a result of the poor variation of the populations in the Natracuol soil type. Differences among populations and between environments in the majority of the characters were detected and the GDG was low to medium. The best performance was detected in Argiudol soil as a result of the limiting conditions of the Natracuol soil on the populations. Cluster analysis of the data in each environment showed differences in the affinity of the populations. The provenance of the most different populations was in the periphery of the area collected, while the ones distributed in the central area presented an affinity superior to 50%. Principal components analysis and the minimum spanning tree contributed to establish the affinity of the populations.

Keywords: affinity, dry matter, flowering, leaf characters, population, variability
Abbreviations: DM, dry matter; **DMAc**, dry matter accumulated; **EC**, electrical conductivity; **F-In**, flowering index; **F-50%**, 50% flowering date; **GDG**, genetic determination grade; **LL**, leaf length; **LW**, leaf width; **MST**, minimum spanning tree; **NDF**, neutral detergent fibre; **PCA**, principal components analysis; **Soil A**: Argiudol soil; **Soil N**: Natracuol soil

INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam.) is an excellent annual forage species adapted to humid temperate climates, with high capacity of re-sowing, forage production, palatability and quality (Beddows 1973; Arano and Ramuno 1978; Oliveira 2002; Ojuez *et al.* 2006). It originated in the Mediterranean basin, but has dispersed as a naturalized species in many countries of Europe, Asia and South and North America (Beddows 1973; Breese and Tyler 1986; Fujimoto 1992; Castaño 2005). In Argentina, it is widespread in the Pampean grasslands (Vervoorst 1967) and is actually found naturalized throughout the whole country, in environments with different degrees of human modifications (Castaño 2005; Alonso and Ispizúa 2008). It is cultivated in fields in the Pampean region for grazing, as winter forage or for silage (Castaño 2005; Ojuez *et al.* 2006; Ruiz *et al.* 2006; Borrajo *et al*. 2010; Ré *et al*. 2010), although it can appear as a weed in wheat and other winter cereals (Acciaresi *et al.* 2001). It grows spontaneously in roadsides and natural or semi-natural grasslands of the flooding Pampa of the Buenos Aires Province, associated to different vegetable communities (Vervoorst 1967; Omacini *et al*. 1995; Alonso and Ispizúa 2008).

The soils of the flooding Pampa are predominantly hydro-allomorphic and those with better edaphic conditions alternate with other with severe problems due to salinity or alkalinity (Vervoorst 1967; Musto and Maddaloni 2005). These soils present less favourable conditions for the growth of Italian ryegrass as it is a species moderately tolerant to salinity (Maas 1986); concentrations higher than 200 $mol.m^{-3}$ of NaCl (Marcar 1987) or electrical conductivities of 2 $dS.m^{-1}$ in the substrate (Alonso *et al*. 1999), can modify its germination behaviour as well as its initial development. Although some populations develop on these soils (Vervoorst 1967; Alonso and Ispizúa 2008), Italian ryegrass generally grows on the higher sites within the lowland areas, associated with more favourable conditions (fertile and humid soils, with an acid to alkaline pH and good drainage) (Beddows 1973; Castaño 2005; Oliveira 2002).

Italian ryegrass grows as a dominant species in favourable environments of the flooding Pampa of Argentina (Vervoorst 1967) and constitutes a key species for cattle feeding as a result of its high biomass production, nutritional quality and palatability (Castaño 2005; Ojuez *et al*. 2006; De Battista and Ré 2008, Rosso *et al.* 2010). With the objective of increase its presence in the grasslands, germination and tillering is promoted with different management practices. These practices include the burning of grasslands with a high density of dead material, intensive grazing, herbicide applications and fertilization and sowing of adapted varieties (Fernández Grecco 2000; Castaño 2005; Ojuez *et al*. 2006).

Italian ryegrass has been evaluated in different countries in a wide range of environments and variability has been detected for diverse morphological, ecological, physiological and agronomic characters (Edwards and Cooper 1963; Rhodes and Mee 1980; Bugge 1984; Fujimoto 1992; Oli-

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Table 1 Topographical level and habitat of collecting site, population means between soils for date of 50% flowering (F-50%), flowering index (F-Ind), leaf size and area, height and diameter of plant in soil A and N, of 21 populations (P) of Italian ryegrass from the Bs. As. Province.

P	Topographic level	Habitat*	F- 50% (weeks)		F Ind (days)		Length (cm)	Width (cm)	Area (cm^2)	Height (cm)	Diameter (cm)	
			Not cut	With cut	Not cut	With cut					A	N
1	High	Embankment	1.00	1.1	1.7	3.7	15.9	0.66	5.5	15.9	60.0	32.9
2	Low	Grassland	2.3	3.3	10.2	14.4	14.9	0.62	4.6	14.9	47.6	34.8
3	High	Grassland	2.3	3.1	9.3	15.5	16.2	0.68	5.6	16.2	61.4	35.1
4	Middle	Grassland	3.0	3.5	13.5	18.4	15.9	0.64	5.2	15.9	59.9	29.3
5	High	Park area	3.0	3.5	15.0	19.3	15.9	0.66	5.3	15.9	51.8	31.5
6	Middle	Grassland	3..1	4.0	14.7	18.9	16.2	0.64	5.4	16.2	52.9	33.7
7	Low	Flooding road	1.3	2.1	4.2	10.1	16.8	0.65	5.6	16.8	58.3	31.2
8	Low	Flooding road	3.1	3.5	13.6	18.2	15.3	0.65	5.0	15.3	54.7	34.1
9	High	Hillside	2.3	3.0	11.6	15.1	15.8	0.68	5.6	15.8	56.2	34.6
10	Low	Grassland	2.3	3.0	11.1	16.4	15.9	0.65	5.3	15.9	53.9	33.8
11	Low	Grassland	1.7	2.3	6.8	11.3	18.1	0.70	6.4	18.1	62.1	39.9
12	Middle	Grassland	2.2	2.8	8.5	13.6	15.6	0.63	5.0	15.6	52.2	34.8
13	High	Grassland	3.2	3.5	14.6	19.2	17.5	0.68	6.0	17.5	61.0	38.2
14	Low	Grassland	3.00	4.0	15.5	20.6	17.1	0.67	5.9	17.1	64.3	31.3
15	Low	Grassland	3.2	4.0	15.7	20.8	15.4	0.63	5.0	15.4	55.1	31.8
16	High	Road side	2.2	3.3	9.8	17.0	17.6	0.68	6.2	17.6	60.0	39.2
17	Middle	Grassland	2.7	3.3	12.2	17.5	15.7	0.66	5.3	15.7	58.3	30.6
18	Middle	Roadside	3.0	3.3	13.7	17.3	15.5	0.61	4.9	15.5	48.3	29.3
19	Middle	Grassland	3.2	3.5	16.	19.3	15.5	0.62	4.9	15.5	56.8	35.5
20	Middle	Road	2.2	3.0	8.7	13.8	16.5	0.67	5.7	16.5	54.2	34.8
Cv#	High	Cultivated	2.0	2.8	7.2	13.8	18.3	0.76	7.2	18.3	61.7	35.9
LSD			*0.41*	*0.17*	*0.69*	*0.77*	*1.83*	*0.47*	*0.56*	*1.83*	*6.5*	*11.3*

Cv #= cultivar 'El Resero INTA'; LSD = least significant difference

Table 2 Type of soil based upon USDA Soil Classification, texture and chemical characteristics of the upper horizons at each site.

Soil	Type	pH	OM ($g.kg^{-1}$)	P ($mg.kg^{-1}$)	EC ($dS.m^{-1}$)	Na Int. %	CEC ($cmol.kg^{-1}$)	Texture
A	Argiudol	5.9	58.9	22.6	0.40	0.63	23.3	Loam
N	Natracuol	8.9	60.1	18.5	1.45	33.45	22.8	Clay-loam

OM = organic matter; P = phosphorus; EC = electrical conductivity; Na Int. = Na ion interchange; CEC = cationic exchange capacity.

veira 2002; Mittelmann *et al*. 2004). Comparative studies have been carried out in Argentina, between varieties and variability has been detected for different morpho-agronomic characters (De Batista *et al.* 2000; Di Nucci *et al.* 2000; Ruiz *et al*. 2006; Borrajo *et al*. 2010; Rosso *et al.* 2010).

The specific adaptations of the naturalized populations to diverse environmental conditions have permitted the detection of variability in flowering date, seed production, characters related to forage production, *Puccinia* spp. infection (Monteverde and De Battista 2008; Borrajo *et al*. 2010; Rosso *et al.* 2010), in entries conserved at the Germplasm Banks of the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. Alonso *et al.* (1999) observed differences within populations in germination, velocity and dormancy under different saline conditions when they compared 59 naturalized entries from the Province of Buenos Aires. The affinity among them varied according to the salinity and several populations presented a better behaviour in germination under saline conditions than the three cultivars used as checks. When isolated plants of 21 entries of the former 59 entries were cultivated in two soil types, differences in the establishment and growing of plants and juveniles were detected, as well as interaction genotype × environment for some characters (Alonso 2004). We did not measure germplasm variability in flowering, plant size, and production and forage quality. The evaluation of complex characters as production and quality, requires specific plot trials or conventional growing conditions with a dense cover (Bugge 1984; IPGRI 2000). Nevertheless, the evaluation based upon isolated plants would be valid if testers with known behaviour are included or trials that considerer an adequate control of the environmental variation (van de Wouw *et al.* 1999; IPGRI 2001).

The genetic variability present in the naturalized germplasm of the grasslands of the flooding Pampa could be employed in breeding projects to obtain locally adapted varieties or with a wide distribution (Borrajo *et al*. 2010; Rosso *et al*. 2010). Furthermore, the determination of population affinities will provide valuable information to decide upon future germplasm collecting and selection of progenitors to generate synthetic varieties (Franco and Hidalgo 2003). The evaluation of germplasm of Italian ryegrass naturalized in the Argentinean flooding Pampa were carried out with the objectives of: a) evaluate flowering date, leaf and plant size, forage quality and production per plant, b) estimate the better combination of resources to evaluated characters at adult stage, and c) determine the affinity among populations.

MATERIALS AND METHODS

The populations of Italian ryegrass evaluated for morphological and physiological characters al the juvenile stage (Alonso 2004) were employed in this experience. The 21 entries were cv. 'El Resero INTA' and 20 populations conserved at the Germplasm Bank of INTA, Estación Experimental Agropecuaria Balcarce. The cultivar was selected at Pergamino, located in the north of Buenos Aires Province, and the populations had been collected in different environments from the east to the middle-west of the Buenos Aires Province, Argentina, except for P1 collected in the south-west (**Table 1**, **Fig. 1**).

The evaluation of characters in plants at the adult stage were performed at Balcarce, Buenos Aires, Argentina (37° 45′ S - 58° 18′ W) in two sites separated by 4 km, each one with a representative soil of the humid Pampean region. The site A had a typical Argiudol soil and site N had a Natracuol one, the physic and chemical characteristics of each site are presented in **Table 2**. Sites were considered as different environments and entries as treatments arranged in a randomized complete block, with three replications per environment (Steel and Torrie 1988). In late autumn, 40 juvenile plants were transplanted in each experimental unit in two files of 20, separated by 50 cm between plants and files. The plants were watered only the day of planting, the weed control was made by hand and it was not necessary to applied supplementary

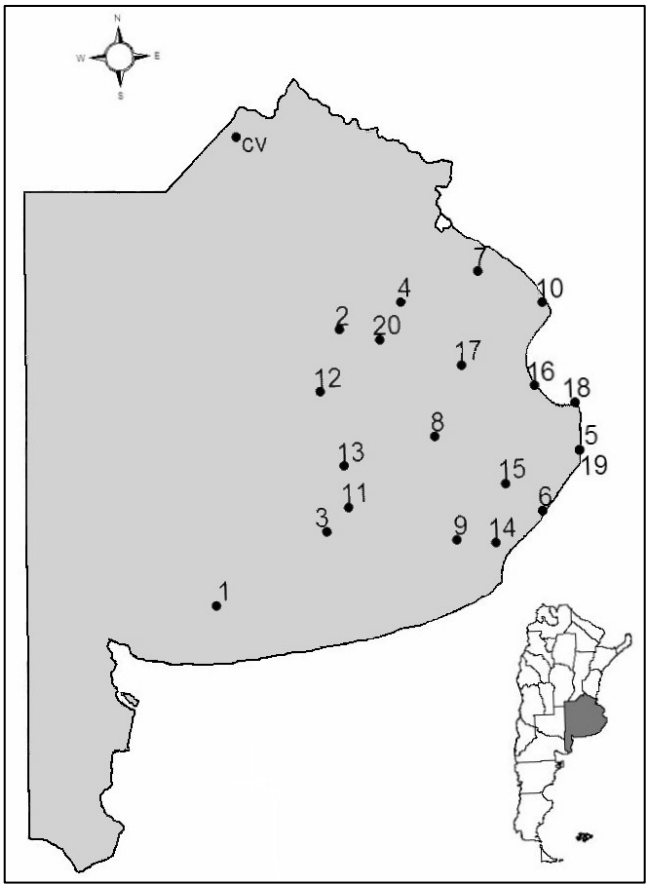

Fig. 1 Distribution of the collection sites of 20 Italian ryegrass populations naturalized in the Buenos Aires Province, Argentina, and site of selection of the cultivar 'El Resero INTA' (cv).

fertilization.

Two months after transplanted, leaf length and leaf width of the last emerged leaf of the main tiller were registered in 15 plants per experimental unit, and a month later, plant height and plant diameter were measured using the same plant number (van de Wouw *et al.* 1999). The leaf area per plant was calculated as LA = ½ (LL x LW). The forage production per plant was estimated thorough two forage cuts, the first (DM1) on 15 plant at the 12th week after the transplant, and the second cut (DM2) on week 16th on the first 8 plants used for the firs cut. The forage was oven-dried during three days to estimated dry matter per cut and accumulated production (DMAc = DM1 + DM2). Dry and milled forage was used to determine percentage of protein (Nelson and Sommers 1973), neutral detergent fibre (NDF) by Goering and van Soest (1970) and *in vitro* digestibility (Tilley and Terry 1963).

Thirty-four weeks after the sowing date, the number of flowering plants was registered once a week during 8 weeks, to estimate the date at which 50% flowering occurred. The velocity of flowering was estimated as the inverse of an index adapted from Alonso (2004) to estimate tillering velocity, being = ∑(Ni x Di) / NT, where Ni = number of flowering plants at day i, Di = number of days from the first day of week 35th till day i, NT = total number of flowering plants at final count, ∑ for i = 1 to n, n = number of days from first flowering registration date. Data were collected for all plants with and without a forage cut.

Analysis of variance was performed considering sites as different environments and blocks nested in each of them, according to the following mathematical model and with the assumption that environments, replications and populations were independent normal random factors (Steel and Torrie 1988): $Yijk = \mu + \varphi k + \beta j(k) + \delta i + (\varphi\delta)ki + \varepsilon ijk + \theta ijkl$, for Yijk = mean value of the ijkth plot; μ = general mean; φk = effect of kth environment; βj(k) = effect of the jth block nested in the kth environment; δi = effect of ith entry, (φδ)ki = interaction effect to the kth environment with the ith entry; εijk = common effect of the ijkth observation; θijkl corresponding to among plants error.

Using the variance components, the genetic determination grade were calculated as a way to obtain estimations of H^2 that involve populations, as GDG = δ^2 genotypic/ δ^2 phenotypic (Falconer and Mackay 1996). When significant interaction (soil × entry) was detected, an ANOVA within each soil type was performed. The comparisons of means were performed with the Duncan's multiple range test, considering an α= 0.05, and the genetic determination grade (GDG) were calculated according to Falconer and Mackay (1996), to obtain estimations of H^2 that involve populations. The most efficient allocation of environments, replications and plants to improve the GDG estimation to a value higher than 70%, was calculated according to Rasmusson and Glass (1967).

The affinity among population was established utilizing a clustering and ordination multivariate analysis on the standardized mean population values. The characters used were those measured in this experience, plus the characters registered on the same entries by Alonso *et al.* (1999) and Alonso (2004): a) seed: weight, length and awn length; b) germination behaviour of seed incubated at 0, 2 and 6 dS.m^{-1} of electrical conductivity (EC): vigor, standard germination, velocity, dormancy and mortality; c) seedlings: initial and final emergence, emergence index, d) juvenile plants: tillering index, growth habit, tiller number at 40, 55 and 75 days, leaf length, leaf width, leaf area, leaf number.tiller^{-1}, leaf area.plant^{-1}.

Three basic data matrix were made, Case 1 with a data set of 35 characters registered on original seeds and those under favourable environments (Argiudol soils or substrate with EC of 0 dS.m^{-1}); Case 2 with data of 40 characters measured on original seed and those under unfavourable environments (Natracuol soils or substrates with EC of 2 or 6 dS.m^{-1}), and Case 3 that included a data set constituted by the sum of characters of both situations, but original seed characters were included only once (72 characters). Cluster analysis was conducted on the Taxonomic distance matrix with the unweighted pair-group method based on arithmetic averages (UPGMA) with average linkage using NTSYS-pc version 2.1 (Rohlf 2001). The same programs was used to computed the cophenetic coefficients in order to test the goodness of fit of the clustering, as well as to performed a minimum spanning tree (MST) and a principal component analysis (PCA) using Case 3.

RESULTS AND DISCUSSION

Environmental conditions

The mean temperatures from planting to harvest (June to December) were coincident with the means of the last 30 years, but the rainfalls exceeded in 100 mm that value (Servicio Agrometeorológico, EEA Balcarce, INTA). For that reason, no heat or water stress was registered in the sites during the evaluation period. The edaphic conditions of the sites were different because the Natracuol (N) soil presented a poor drainage as a result of a high proportion of clay and reduced slope, and higher pH, electric conductivity and content of Na (**Table 2**).

The Natracuol soils are less suitable for the development of plants, nevertheless the growth of Italian ryegrass is possible when they have moderate to high salinity, as many naturalized ecotypes in the grasslands of the flooding Pampa develop in similar environments (Alonso and Ispizúa 2008). In evaluation trials of naturalized germplasm in different environments of Buenos Aires Province, many populations have germination, emergence and growing capacity, under high saline conditions in Natracuol soils. Nevertheless, the percentage of germination and germination velocity are less when compared to Argiudol soils, or under less saline or neutral conditions (Alonso *et al.* 1999; Alonso 2004).

Flowering

The 50% flowering date and the flowering index did not present significant interaction nor differences among environments for cut plants as well as for those not cut; for both types of plants and characters, differences between populations were detected (**Table 3**). When plants were cut, they flowered between 7 and 10 days after the non cut plants,

Table 3 Means squares (MS) for interaction (Int), soils (S) and populations (P), general mean (G-mean) and of each soil (A-mean, N-mean), range of means within the two soils and percentage of genetic determination grade (GDG) for characters of flowering (F) for plants with and without cut, leaf size, plant size and production and quality of the dry matter of 21 entries (E) of Italian ryegrass from the Bs. As. Province.

Character	MS Int.	MS S	MS P	G-mean	A-mean	N-mean	Range s	GDG
F-50% with cut (weeks)	0.12 ns	0.008 ns	2.53**	3.1	3.13	3.11	1-4	93
F-50% without cut (weeks)	0.08 ns	1.55 ns	2.47**	2.5	2.38	2.6	1-4	95
F-Index with cut (days)	2.61 ns	0.53 ns	98.3**	15.9	15.89	16.03	1-22	96
F-Index without cut (days)	2.36 ns	73.9 ns	94**	11.2	10.4	11.9	1-20	97
Leaf length (cm)	6.3 ns	80**	>999**	16.2	19.6	12.9	9.6-25	43
Leaf width (cm)	3.2 ns	10**	>999**	6.6	0.47	0.64	4.9-9.1	61
Leaf area (cm^2)	740 ns	>999*	>999**	5.4	7.3	3.8	3.2-9.9	34
Plant height (cm)	26.7 ns	>999**	35*	12.6	12.6	9.4	6.8-17	50
Diameter (cm)	>999 **	493*	848 ns	45.3	56.7	33.9	25-68	42
MS 1º cut ($g.plant^{-1}$)	213 ns	>999 ns	432**	12.8	15.5	10.1	3.6-33	51
MS 2º cut ($g.plant^{-1}$)	305 *	>999**	463 ns	22.9	34.7	11.1	5-52	34
MS Acc. ($g.plant^{-1}$)	89 *	>999**	159**	35.7	50.2	21.2	11.73	46
Protein (%)	1.48 ns	33.1 ns	1.6 ns	21.1	20.6	21.6	17.1-26.6	5
NDF (%)	7.06 ns	180*	7.9 ns	42.8	40	45.8	30.6-52.7	6
Digestibility (%)	10.6 ns	>999*	15.9*	76.8	78	75.6	64.5-86	34

*, **, ns: differences significant at α= 0.05, α= 0.01 and no significant at α= 0.05, respectively.

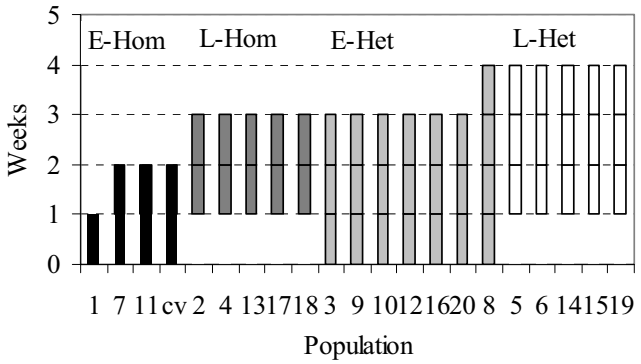

Fig. 2 Classification of Italian ryegrass populations as a function of earliness and homogeneity of flowering. E: early; L: late; Hom: homogeneous; Het: heterogeneous.

and the correlation between both types of cut management was highly significant for the date of F-50% (r = 0.96) as well as for flowering index (r = 0.98).

Some populations required only 1 week to reach F-50% from the moment the first spike appeared in the trial (population P1), while others needed more than 3 weeks (**Table 1**). The precocity estimated through F-50%, could be also established through F-In; both variables presented high correlation (r = 0.99) both on cut and not cut plants. The F-In determines the flowering uniformity, as at equal F-50%, a larger index means that the plants presented a dispersed flowering. Considering non cut plants, the populations were classified according to their uniformity and precocity (**Fig. 2**). The populations that flowered from the first week were considered early, and late the ones that flowered from the second week; homogeneous flowering were considered the ones that concentrated their flowering period in 1-2 weeks, and heterogeneous the ones that required 3 or more weeks to reach complete flowering of the population. The entry P8 was highly heterogeneous and P1 very early and uniform, more than the cultivar.

The forage cut only affected the date of flowering, and not the number plants that flowered. This behaviour is characteristic of Italian ryegrass as well as in other annual grasses, where the new tillers that persist after the cut as well as the new ones, are induced to flower, although the appearing of spikes are delayed (Gillet 1984). This author mentions that in Italian ryegrass the earliness between varieties is determined by the photoperiod and the earliest populations require less long days. On the other hand, the provenance of the early varieties are sites with late freezing dates and an early beginning of the dry season, while uniformity of flowering is frequent in cultivars and in annual species from areas with low rainfalls (Gillet 1984). These environmental conditions are characteristic of the site of origin of P1, and this explains why this population is the earliest and more homogeneous among the evaluated germplasm.

Leaf and plant size

The interaction population × site was not significant ($P > 0.05$) for length, width and foliar area and for plant height (**Table 3**). The behaviour of the populations was better in environment A, with a diminishing performance in environment N that varied among 21 and 48%, foliar area being the most affected character (**Table 1**). At both sites, the populations showed significant differences in the three characters of foliar size. As a result of a smaller foliar length in environment N, the diameter of the plants was reduced 40%, approximately, when compared to environment A. This variable presented a significant interaction as the populations only differed in this character in environment A (**Tables 1, 3**). In the environmental with constraints to growth, the populations did not express the variability existing for plant diameter, resulting in genotype-environment interaction.

The correlations among variables and leaf size, was positive and significant ($P \leq 0.01$) ranking between r = 0.9 and r = 0.97. The foliar area and its components, presented high correlation with plant height among environments and with the diameter only in site A (r = 0.8 to 0.84). The largest mean values between environments for leaf size was detected in the cultivar and in the populations 11, 13, 14 and 16, while the entries with smaller leaves and plants were observed in the populations 2, 12 and 8. The germplasm studied presented variability for length, width and foliar area of adult leaves, variation also detected for the same entries at the juvenile stage (Alonso 2004). In both cases, 'El Resero INTA' was one of the entries with the largest values for leaf length and width in environment A, nevertheless it did not differ among the values obtained for the wild populations, that possess equal capacity for production of long leaves. Another coincidence was the detection of the populations 2, 12 and 18 as the ones with short leaves and minor foliar area.

The averages for leaf length ranged from 14.9 to 18.3 cm, and for leaf width from 0.61 to 0.76 cm (**Table 1**). They only were slightly lower than those obtained by Rosso *et al*. (2010) for 43 populations of different countries grown in Pergamino, north of the Buenos Aires province.

Table 4 Mean for dry matter DM2 and DMAc for soil A and N, and mean between soils for dry matter DM1, percentages of protein, neutral detergent fibre (NDT) and *in vitro* digestibility (IVDDM) of 21 populations (P) of Italian ryegrass from Bs. As. Province.

P	DM1 (g)	DM2 (g)		DMAc (g)		Protein (%)	NDF (%)	IVDDM (%)
		A	N	A	N			
1	18.8	47.5	13.7	72.2	26.5	20.9	44.0	75.9
2	11.7	34.1	11.8	46.3	22.9	20.6	43.0	76.8
3	14.3	36.0	13.5	53.5	24.4	21.2	43.1	77.3
4	14.3	37.3	6.7	56.9	15.91	21.6	41.8	75.2
5	9.6	29.1	10.0	40.3	17	21.6	42.9	75.4
6	12.1	26.2	8.6	39.6	19.2	21.0	43.1	76.8
7	12.7	35.5	9.2	51.7	18.4	20.8	40.7	78.8
8	11.8	32.4	7.9	46.4	17.5	21.9	42.9	77.9
9	11.6	35.1	10.9	48	20.9	21.1	42.3	74.5
10	12.7	27.9	14.9	42.1	26.1	21.3	41.0	77.2
11	14.6	38.6	10.7	55.6	22.8	20.7	43.4	78.1
12	11.7	28.2	9.7	40.7	20.4	20.8	42.3	77.8
13	14.2	46.0	12.8	62.4	24.5	20.8	43.7	76.0
14	13.7	36.6	11.0	54.6	20.1	21.1	44.6	76.0
15	11.8	31.3	9.3	45.4	18.9	21.2	42.7	75.3
16	15.7	37.1	13.2	55.3	26.3	20.5	44.7	76.1
17	13.6	36.6	10.0	54.4	19.3	21.5	45.2	73.5
18	9.0	25.8	10.4	36.6	24	21.5	42.0	77.6
19	11.8	32.5	14.0	45.9	20	21.4	42.7	77.2
20	10.0	36.8	12.1	48.8	20.8	21.6	43.6	78.9
Cv#	13.2	38.3	13.3	57.2	17.5	19.8	41.6	80.6
LSD	*3.51*	*9.1*	*5.67*	*13.2*	*8.8*	*1.70*	*4.7*	*1.19*

cv = cultivar 'El Resero INTA'; LSD = least significant difference

Dry matter production

The production of DM1 per plant differed among populations (**Table 3**). It did not differ between environments, although considering absolute values, the mean of DM1 at site N was 35% lower than in site A. The interaction genotype environment was significant for DM2 and DMAc per plant (P = 0.012 and p = 0.02, respectively). The populations did not differ in the environment N but they did in A, where the production of DM was higher (**Table 4**). For that reason, the correlation among the values obtained at both sites, resulted non significant (P > 0.01) both for DM2 (r = 0.53), and DMAc (r = 0.50). In the environment N the production of DM2 and DMAc was reduced by 58 and 68%, respectively.

As observed for diameter of the plant, the interaction genotype-environment in DM2 and DMAc was the result of variations in the magnitude of the responses of the populations as in all the entries, the smallest values were registered in the Natracuol soil. When comparing pastures of Italian ryegrass in three localities of the Pampean grassland, Freddi *et al.* (2001) observed that total forage production was larger in the locality with Argiudol soil type than in those with Hapludol soils. Borrajo *et al.* (2010) also detected genotype × environment for forage production, to analyze 71 cultivars of *L. multiflorum* in Corrientes Province (Argentina). In the Buenos Aires Province, phenotypic variation in DM production was detected among naturalized populations and/or varieties of Italian ryegrass by De Battista *et al.* (2000), and Monteverde and De Battista (2008).

When considering absolute values for DMAc, the entries with the highest production in A was 'El Resero INTA' and the wild populations P1, P4, P11, P13, P16, while in site N performed better P1, P3, P10, P13, P16 and P18 (**Table 4**). On the other hand, the populations with less absolute production of DMAc in site A were P5, P8 and P12 with less that 41 g.plant^{-1} and in site N, 'El Resero INTA' and P4, P5, P8. It is interesting to point out, that P5 and P8 present the smallest absolute values in DM at sites and P1, P13 and P16 the largest, while 'El Resero INTA' and P4 presented a different behaviour in each environment.

The net forage production of the grass pasture is directly related to the foliar area index, and this is directly related to number of tillers, number of leaves per tiller and leaf size (Lemaire and Chapman 1996). The relation among leaf size and DM production was low for environment N, but not for site A, as shown by the correlation among leaf area and production of DM1, DM2 and DMAc (r = 0.64, r = 0.74 and r = 0.73, respectively). Several entries with large leaves such as 'El Resero INTA', P11, P13 and P14 (**Table 1**), did not present high DMAc production (**Table 4**), while P18, with short leaves, did not show the lowest DM production. This could be explained as compensation between size and number of leaves because at the juvenile stage P11, P13 and P14 presented a less number a tillers/plant when compared to P18 (Alonso 2004).

Future yield evaluation of DM in the Italian ryegrass collection should be carried out in conventional trials, with a dense canopy, a traditional management and a number of replicates and environments that could reduce the experimental error. Furthermore, based upon the size of leaf and plant, and potential production of DM obtained in this work, it would be possible to diminish the number of entries to evaluate and make a more efficient use of the resources (van de Wouw *et al.* 1999).

Dry matter quality

The percentage of protein of the DM did not differ among populations, nor among environments, with an average of 21.6 ± 0.8%. This value was similar to those presented by Bugge (1984) for naturalized populations from South America, but high in relation to germplasm cultivated at Buenos Aires province (Acosta *et al*. 2008). The mean protein content among data registered in the South East of Buenos Aires Province for the cultivar 'Tama' (Cavallieri 1995), was also lower than those obtained in this trial. But when Freddi *et al.* (2001) analyzed this cultivar in three localities, they found that the protein values ranged between 18.9 and 27.8%.

Differences among environments were detected for the content of NDF and digestibility; for the latter character differences among populations also were detected (**Tables 3, 4**). In site N the percentage of NDF of the DM was 14% higher and the digestibility 3.1% less. The phenotypic variation over sites ranged among 73.5 and 80.6% for digestibility (**Table 4**). The entry with the highest value was 'El Resero INTA'; nevertheless it did not differ significantly

Table 5 Combinations of number of environments (ENV), replications (REP) and plants (PL) to reach estimation of degree of genetic determinations higher than 70%, for characters measured on Italian ryegrass.

Character	ENV	REP	PL
Flowering-50%	1	1	10
Flowering index	1	1	10
Leaf length	5	4	15
	5	5	10
	4	6	10
Leaf width	3	3	15
	3	4	8
	2	6	10
Height	4	4	15
	4	5	10
	3	7	15
Diameter	6	3	20
	6	4	11
	5	5	20
MS 1	4	4	10
	4	5	10
	3	6	15
MS 2	8	4	7
	8	3	20
	6	7	20
Protein	>10-	>10	15
NDF	>10	>10	15
Digestibility	7	5	15

from 11 wild populations, whose digestibility exceeded 76%. Of these, the ones with higher quality were P7, P10 and P18, if considering the lower absolute content of NDF (**Table 4**). The digestibility range of the evaluated entries in this trial where superior when compared to the ones registered by Bugge (1984) on European material, but was similar to the reports for the species by other researches (Tyler and Hayward 1982; Cavallieri 1995). This latter author also reported a content of NDF of 42.3% for cv. 'Tama', value coincident with the mean value of the entries in this experience.

The correlation between protein and the other quality character of DM were not significant while the correlation between NDF and digestibility was negative and highly significant (r = -0.68; $P < 0.01$). 'El Resero INTA' presented high value for digestibility and low fibre content, consisting with the quality mentioned for this variety by Arano and Ramuno (1978). The nutritive value of the forage, measured through the digestibility and the fibre content of the dry matter, is influenced by numerous factors related to the plant, like maturity stage (Pujol Palol 1998). The P1 produced the largest amount of DM, but of low quality, probably as a result of its higher maturity at the cut date.

Heritability and distribution of resources

The GDG was high for the attributes related to flowering, resulting in values higher than 93% (**Table 1**), similar to those observed by Monteverde and De Battista (2008) for other cultivars and naturalized populations from the flooding Pampa. The selection of populations based upon precocity and/or uniformity at flowering, could be obtained based upon the results of this work; it would not be necessary to increase the replicates and/or environments to improve the estimations (**Table 5**).

The characters of leaf and plant size resulted in an intermediate GDG ranging from 42 to 61% (**Table 1**). Heritability estimations in the broad sense for leaf area components of similar magnitude, was reported for Italian ryegrass for Cooper and Edward (1961). On the other hand, in the same germplasm entries, but in the juvenile stage, the estimations of GDG carried out by Alonso (2004), were similar for leaf width but higher for leaf length (72%). The differences in GDG among both studies can be attributed to the increase of the phenotypic variance in relation to the genotypic one (Falconer and Mackay 1996), as a result of the environmental variation encountered when the plants were cultivated at two sites directly in the field, related with a trial in pots in one site.

The selection of promissory material on the basis of leaf size, plant height and diameter would be possible as variability was detected for those characters, although the heritability were low (GDG = 34 to 51%) and the genetic progress would be low (Falconer and Mackay 1996). As the correlation between length of adult and juvenile leaves is high (r = 0.75) and the GDG obtained in the juvenile stage was higher (72%), the selection for foliar length could be carried out on juvenile plants. Selection at this stage would be more efficient, less time and work consuming, avoiding the registration of information on adult leaves (Casler and Hovin 1985). In future experiences with Italian ryegrass, the evaluation of leaves and plant should be carried out with a more efficient distribution of resources. The possible combinations of environments, replicates and plants needed to reach estimations of GDG of 70%, are presented in **Table 5**.

The GDG for the characters of DM production were intermediate to low (**Table 3**), indicating that a low proportion of the phenotypic variability is a result of the genotype. These GDG values were lower than the ones reported by Monteverde and De Battista (2008) for biomass production, but they used a larger number of plants per experimental unit. Forage yield is a complex character, and its expression is generally influenced by the environment. Its evaluation requires trials in different environments, under traditional management, and plants growing under competitive conditions (IPGRI 2001). Nevertheless, the evaluation based upon isolated plants could be employed to establish the potential of the gemplasm *per se* or through the components of forage yield (Casler and Hovin 1985). In this case, it would be necessary to increase the number of environments, especially to estimate DM2 and DMAc (**Table 5**).

For protein and NDF the estimated GDG was very low and the value for digestibility resulted intermediate (**Table 3**); for this reason the selection of promissory materials for quality based upon these results, should not be pursued. To increase the precision of these evaluations, it would be necessary to increase the number of environments and the replicates (**Table 5**). Furthermore, as a result of the importance of the maturity stage and the relation steam-leaf on digestibility and the NDF, it would be convenient to standardize the material for precocity and homogeneity and evaluate in different trials (Pujol Palol 1998).

Affinity among populations

For cluster analysis, the cophenetic coefficient was 0.88, indicating a good fit of the similarity matrix and its representation on the phenogram obtained for Cases 1 and 2. In both cases, the major similarity was encountered at a taxonomic distance (TD) of 0.64, and the minor similarity at TD = 2.5 (values obtained from the similarity matrix, data not shown); these were observed between P1 and P12 for Case 1, and between P1 and 'El Resero INTA' for Case 2. Considering in the phenogram the 75% of the maximum TD, P1 was the only population that appeared isolated (**Fig. 3**), but at 50% similarity, three populations were detached in Case 1 and 5 in Case 2. At this similarity level, three clusters were formed, two minor ones formed by 2 to 3 entries and other with 12 populations (**Fig. 3**). When comparing both cases none of the clusters presented the same populations, although 8 of the 12 populations of the larger clusters were common (P5, P6, P10, P11, P14, P15, P17 and P20).

The location of some populations in the major cluster or in smaller groups depended upon the environment considered, for example for Case 1, P2-P12 formed one cluster, but for Case 2 they joined the largest group. The same occurred with part of group P8-P18 and the contrary with the entries P3, P11 and P13. According to Somajulu *et al*. (1970) the populations that presented a different location on the phenogram in relation to the environment, are con-

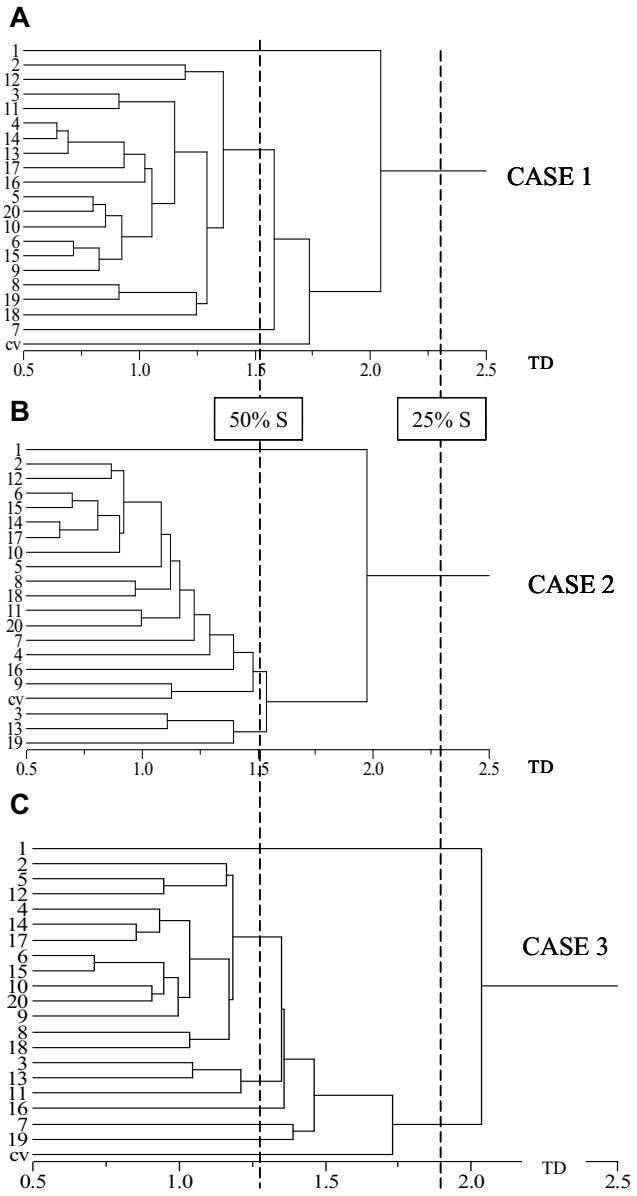

Fig. 3 Phenograms for 21 Italian ryegrass populations developed under (**A**) favourable environment (Case 1), (**B**) unfavourable environment (Case 2) and (**C**) both conditions (Case 3). TD = taxonomic distance; S = similarity; cv = cultivar 'El Resero INTA'.

sidered to be less divergent while the very different ones did not modify their position. For the combined matrix Case 3, the adjustment of the data to the phenogram was good (cophenetic coefficient = 0.81). The minimum similarity was established at TD = 0.7 and the maximum at TD = 2.4, between 'El Resero INTA' and P1 (values obtained from the similarity matrix, data not shown). The similarity of P1 with the other populations was less that 25% and among P7, P16, P19 and 'El Resero INTA' with the remaining ones, varied between 25 and 50% (**Fig. 4**). At 50% of TD two clusters were formed, C1 with 13 populations, among them the 8 commons in the major clusters, and C2 with the populations P3, P11 and P13, all included in one major cluster of Case 1 (**Fig. 3**).

Independently of the environment where the plants were grown, 8 populations whose provenance were the central area, always grouped with a TD less than 50%, and the entries from the periphery, P1, P7, P16 'El Resero INTA' and in some cases P19, always appeared isolated at that similarity level (**Fig. 1**). The less affinity of the peripheral populations could be a result of their adaptation to environments with different climatic and habitat conditions, when compared to the site comprising the central group. The specific effect of the habitat was clearly observed analyzing the minor affinities among P19 and P5, both from the same site, but from different micro-environment (**Table 1**).

The largest differences with the group from the central area were evident with P1 and 'El Resero INTA'. The first was the most distant population, which was collected in sandy and neutral soil, in the SW of the Province of Buenos Aires, in a sub-humid region (Musto and Maddaloni 2005). Furthermore, 'El Resero INTA' was selected to be cultivated in fertile soils in the northwest of the Province (Arano and Ramuno 1978). On the other hand, the isolated population belonged to the central area, but from a particular habitat; P7 was collected in a flooded road north of the studied area, while P16 was found growing on a calcareous soil, with a gross texture, located on a coastal belt of Samborombón bay (Alonso and Ispizúa 2008).

The small clusters generally included populations from the periphery of the central area, with the exception of P8 that in Case 1 resulted similar to the coastal population P18, sharing with it the same environment (roadsides frequently flooded). In this case, similar environments and not the spatial proximity, may have determined the morphological similarity in coincidence with observations in *Fragaria* (Jensen and Handcock 1982). The opposite was valid for P5 and P19. Nevertheless the affinity between P8 and P18 did not turn up when they were developed on unfavourable conditions or when both environments were considered, as both joined to the central group. The same occurred with P2-P12 formed by entries from NO of the central area (**Fig. 1**). Under unfavourable conditions for germination and growing of the plants, the populations of both clusters presented minor values of plant and leaf sizes than the other populations, explaining why they did not cluster with the central group. On the other hand, the majority of the central populations did not express their potential under non favourable conditions and could not be differentiated from them.

The cluster P3-P13 collected SW of that area, presented affinities with the central group under favourable conditions, but this relation was less than 50% under unfavourable environmental conditions of alkaline soils. Although all the populations performed less under unfavourable conditions, the entries P3 and P13 were more affected, as was observed by Alonso (2004) at their juvenile stage, and presented a different behaviour compared to the central group. Atlin and Frey (1989) found that the response of populations of oats was a function of their genetic background and their behaviour under favourable conditions was controlled by different genes than the ones acting under stress conditions. It is likely that in Italian ryegrass different genes could be acting under unfavourable environments.

The grouping obtained in Case 3 was similar to Case 1, because the populations could express their potential to a higher degree than under an unfavourable environment. Nevertheless, these conditions permitted a clearly detection of the divergence among populations, as they showed a differential adaptation to this environment. In relation to the genetic adaptation, Bradshaw (1965) mentions that the majority of the species are formed by populations genetically adapted whose properties are determined by the most severe environmental conditions.

The degree of adjustment of the PCA to the plot was high (Cophenetic coefficient = 0.96). The analysis indicated that 100% of the variability was explained by the first 20 principal components (PC), but only the first 4 contributed with more than 10%, and explained 61% of the variation. The location of the entries coincided with the observed in the clusters C1 and C2, presented in **Fig. 4** by groups G1 and G2, respectively. The most distant entries in the cluster were located far from the general mean, especially P1, P16 and 'El Resero INTA'. The entry P7 did not present a separation from the groups G1 and G2 considering the first three PC, but separated considering PC4 (**Figs. 4A-C**), and the same was observed for P19. In G2, the three first PC showed entry P13 closer to P11 than P3, but not in the space limited by PC1 and PC4 (**Fig. 4C**). The imposition of

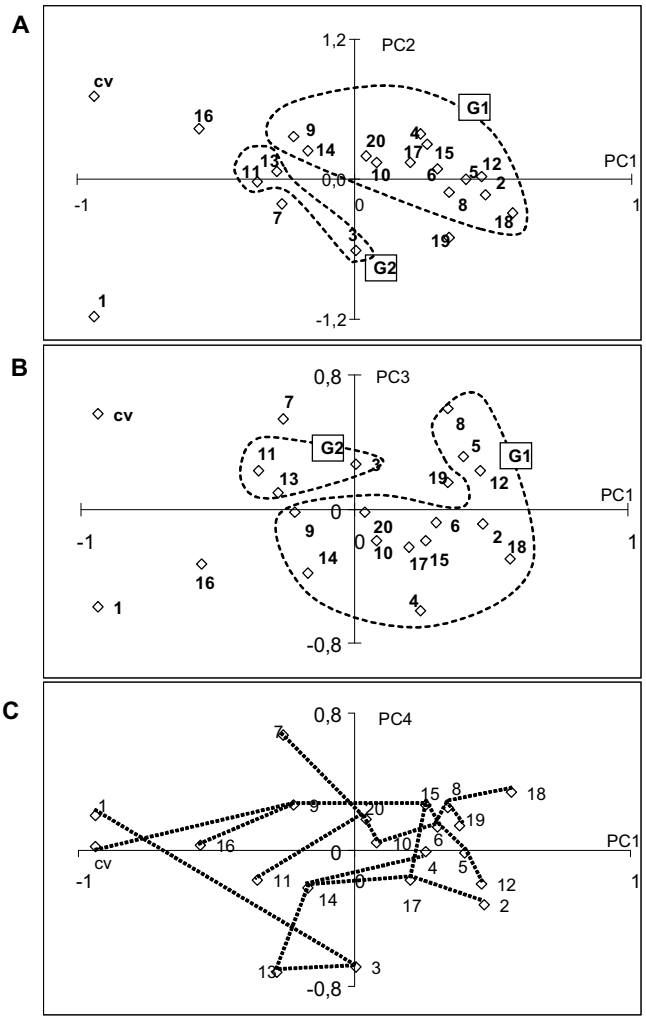

Fig. 4 Distribution of 21 Italian ryegrass populations determinate by (**A**) PC1 and PC2, (**B**) PC1 and PC3, (**C**) PC1 and PC4, and minimum spanning tree. G = groups; cv = cultivar 'El Resero INTA'.

Table 6 Characters with eigenvectors higher than 0.45 in any of the four principal component axes from PCA of 21 Italian ryegrass populations x 72 characters (A = argiudol soil; N = Natracuol soil; CE = electric conductivity).

Stage	Characters	PC1	PC2	PC3	PC4
Seed	Length				0.47
	Weight				0.47
	Vigor CE 0		0.89		
	Germination CE 0		0.82		
	Dormancy CE 0		-0.67		
	Germin.Index CE 0		-0.78		
	Vigor CE2		0.89		
	Germination CE 2		0.83		
	Dormancy CE 2		-0.77		
	Germin.Index CE 2		-0.49		
	Vigor CE 6		0.83		
	Germination CE 6		0.77		
	Dormancy CE 6		-0.65		
Emergence	Index-A			0.52	0.61
	Index-N		-0.64		-0.55
	Initial-N		0.66		
Juvenile	Tillers at 40 days-A			-0.74	
	Tillers at 55 days-A			-0.86	
	Tillers at 75 days-A			-0.75	
	Tillers at 40 days-N			-0.47	
	Tillers at 55 days-N			-0.53	
	Tillers at 75 days-N			-0.59	
	Tillering Index-A		-0.54		
	Habit-A	-0.50			-0.49
	Nº Leaves.plant^{-1}-A			-0.82	
	Leaf length-A	0.72			
	Leaf width-A	0.70			
	Leaf area-A	0.76			
	Leaf length-N		0.56		
	Leaf width-N		0.56		
	Leaf area-N		0.58		
	Plant area-A	0.63			
	Plant area-N		0.69		
Adult	Leaf length-A	0.74			
	Leaf width-A	0.86			
	Leaf area-A	0.71			
	Leaf length-N			0.51	
	Leaf width-N	0.47		0.49	-0.45
	Leaf area-N			0.53	-0.45
	Height-A	0.76			
	Diameter-A	0.77			
Dry matter	Cut 1-A	0.76			
	Cut 2-A	0.74			
	Accumulated-A	0.79			
	Protein-A	-0.58			
	Digestibility-N			0.59	
	Fiber-N				-0.76
Flowering	50%-A	-0.62			
	50%-N	-0.64			
	Index-A	-0.63			
	Index-N	-0.60			

the minimum spanning tree (MST) on the PCA established that the proximity of P11 was not as a result of its affinity with P3 and P13, but as a result of local distortions. The same is applied to P2 and P12, that appeared related through the cluster and the PCA, but not by the MST. On the other hand, this technique affirmed the relation among P19 with populations P8-P18 as observed on the phenogram of Case 1.

In the definition of PC1, the variables with more weight were related to leaf and plant size, production of dry matter in environment A, and flowering in both environments (**Table 6**). In PC2 the largest weight was represented by the characters related to germination capacity in the three substrates, emergence and tillering indexes, and juvenile foliar size in Natracuol soil. The attributes with largest weight in PC3 were number of tillers in both environments and number of leaves per plant in Argiudol soil. In the PC4 the major weight was presented by seed size, emergence of seedlings, habit, leaf size and fibre content in Natracuol soil.

The entries 'El Resero INTA', P1, P7, P16 and part of group G2 were characterized by its earliness and homogeneity, good development of leaves and plants under favourable conditions, which determined high dry matter production under these conditions. On the other hand P3, P19 and the majority of the group G1 presented less development, near the mean or less in some populations. The most extreme entries, P1 and 'El Resero INTA', can be differentiated mainly because 'El Resero INTA' presented the best germination capacity in the three saline conditions, but less number of tillers in the juvenile stage. These characters are common in forage species varieties, as they can be selected by leaf size, character inversely related to number of tillers (Edwards and Cooper 1963; Breese and Tyler 1986). The population P16 revealed similar germination capacity than 'El Resero INTA'. However, heterogeneous flowering, minor leaf and plant size and number of tillers that resulted in a high number of leaves per plant. The compensation among leaf size and number of tillers and leaves resulted in a good dry matter production (Lemaire and Chapman 1996). Population P7 was located near G1, although the results of the MST show its divergence (**Fig. 4C**); it was caused by its larger seed size, rapid emergence in Argiudol soils, but slow in Natracuol ones, as well as minor leaf size and high content of fibre of the DM produced in this soil type. Within G1, the populations P2, P5, P8, P12 and P18 were located in the right quadrants, indicating a reduced leaf and plant size and low DM. Of these, P2 and P18 presented a high

number of tillers, although they did not compensate the reduced leaf size to reach a good DM production.

CONCLUSIONS

The naturalized populations of Italian ryegrass in the central-east region of the Province of Buenos Aires, presented phenotypic variability for characters related to leaf and plant size, dry matter production and quality, registered on isolated plants. The unfavourable conditions of site N restricted growth of the plants in general, but some populations were more affected, revealing divergences not detected under favourable environments. Phenotypic variability also was detected for germination capacity under different saline conditions (Alonso *et al.* 1999), the establishment of seedlings and morpho-agronomic characters in juvenile plants (Alonso 2004). Based upon all these characters the affinity of populations was established as well as different degrees of divergence among entries.

The variability detected can be employed in plant breeding with the objective of obtain varieties for the Pampean grasslands for soils with or without limiting conditions to plant grow. Future collections of Italian ryegrass with the objective of increasing the variability in the germplasm banks of the region, should concentrate on areas with micro-environmental differences not included in former collecting initiatives.

The influence of the environments on the expression of many of the characters of agronomic importance was high, so for this reason in future evaluations of Italian ryegrass we recommend increase the number of environments and repetitions, as a way to reduce the environmental variation and detect specific variants.

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