BREEDING EARLY ITALIAN RYEGRASS (*Lolium multiflorum* LAM.) FOR ROTATION WITH MAIZE IN NORTHWEST SPAIN

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SUMMARY

Breeding procedures used for the development of an early Italian ryegrass variety from NorthWest Spain's landraces are described with the objective of a rotation with maize (a frequent Galician practice). Starting with an early *Westerwoldicum* landrace -whose annual nature and aftermath heading allows for rapid breeding-, two cycles of intrapopulation selection, one of individual selection and another of combined individual/half-sib family selection were applied. Although selected families were less productive than commercial cultivars, their winter vigour was better. Four families were independently selected in two climatically and edaphically different localities, showing a good adaptation to coastal areas of La Coruña and Pontevedra. The interest of a synthetic experimental cultivar obtained by crossing the best plants from these families is being evaluated.

Key words: Genetic resources, grass breeding, *Lolium multiflorum.*

INTRODUCTION

In France and in the North of Spain, farmers frequently rotate Italian ryegrass with forage maize, due to high production of high quality green forage and for conservation uses. This intensive double cropping system leads to the maximization of production from limited land resources, typical of Galicia's (Northwest Spain) property structure (Lloveras, 1987). An early-flowering Italian ryegrass allows the sowing of long-cycle maize after its harvest. However, there are no early commercial cultivars adapted to Atlantic conditions.

The present work continues the research started on seventeen Italian ryegrass landraces collected as seed in Northwest Spain (Galicia and Asturias) and characterized isoenzymatically and agronomically in three Galician localities, both as spaced plants and in miniplots, together with six cultivars (Bregu and Oliveira, 1994; Bregu, 1995; Lindner *et al.*, 1995, 1996). One Westerwoldicum (annual) type -an early landrace from Mos (Pontevedra, Galicia)- was selected for its good growth and productivity as the basis to breed a cultivar. The population was also characterized by high allozyme frequencies in alleles *Sod-1 b*, (p=0.53), *Got-3 a* (p=0.27) and *Pgi-2 c* (p=0.16). Since it showed enough phenotypic variability and it had presumably undergone little artificial selection, the objective of the present study was to apply intrapopulation selection to obtain a synthetic variety. Forage grass seeds are commercialized as synthetic varieties. They are a mixture of genotypes which provide population buffering against environmental changes and the farmer does not need to buy commercial hybrid seed each year (Allard, 1980).

MATERIALS AND METHODS

Two cycles of intrapopulation selection (one individual and another combined individual and half-sib family) were applied to the population in each of two Galician localities: Salcedo (Pontevedra), on a sandy loam soil with high organic matter and no fertilization, and Mabegondo (Coruña) on a fertilized clay loam soil. Average annual mean temperatures are 14.4°C and 12.8°C for Salcedo and Mabegondo, respectively. Corresponding average annual precipitations are 1586 mm and 975 mm (Carballeira *et al.*, 1983; CIFL, 1995). Since plants were annual, selection was made on the basis of a phenotypic index, allowing the crossing of selected individuals in the same year with aftermath heads.

Individual selection

Seeds were sown in soil trays in an unheated greenhouse in September 1994 and 1000 plants transplanted to the field (500 in each site) with a 60 cm x 60 cm spacing. The trial was divided into three blocks and plants were selected within each one. The following traits were measured:

- Winter growth (February 1995). 10 visual classes from 0- dead to 9-high.
- Heading date of third spike (1995)
- Early spring's disease symptoms (March 1995). Five visual classes from 1 (very susceptible) to 5 (healthy).
- Growth habit (March 1995). Five visual classes from 1 (prostrate) to 5 (erect).

- Early spring growth (4/4/95). Nine visual classes from 1- poor to 9-high.

All plants were cut and the best twenty-four plants were selected in each locality (4.8% selection pressure). The forty-eight plants were transplanted in Mabegondo within a rye border and aftermath heads were allowed to pollinate freely, obtaining mostly half-sib families.

Combined selection

From each of the 38 plants which survived transplantation, seeds were germinated in September 1995 in Petri dishes and plants were transplanted to soil trays in an unheated greenhouse and then four replicates of seven plant-rows at each site were transplanted to the field in October 1995, together with four annual cultivars (tetraploid 'Billion' and diploids experimental synthetic 'CIAM2', 'Vitesse' and 'Monasmo'). Within-row spacing was 25 cm; between rows was 80 cm.

Traits measured were:

- Winter growth (1/3/1996). Five visual classes from 1-poor to 5-high. Individual data in Mabegondo. Row data in Salcedo.
- Early spring's row disease symptoms (March 1996). Absence or presence.
- Row growth habit (March 1996): prostrate, intermediate or erect.
- Individual plant heading date of first spike (Salcedo, 1996).
- Row's early spring growth (2/4/96). Eight visual classes from 2-poor to 9-high.
- Row dry weight and % dry matter when all plants in the row headed (Salcedo). (Individual data were obtained dividing family data by number of surviving plants); row contents of crude protein (CP), acid detergent fibre (ADF), water soluble carbohydrates (WSC) and organic matter (%OM), were obtained by Near Infrared Reflectance Spectroscopy in three replicates.

Statistical and genetic analysis

Since Bartlett's test showed that the localities had heterogeneous error variances, their analyses were made independently.

For individual heading date, the following model of analysis of variance was used:

$$X_{ij} = \mu + hs_i + bl_j + (hs*bl)_{ij} + e_{ij}$$

where X_{ij} is the phenotypic value of half-sib family i on block j, μ is the grand mean, hs_i is the effect of half-sib family i, bl_j is effect of block j, $(hs*bl)_{ij}$ is the interaction effect between half-sib family i and block j and e_{ij} is the residual error (within row phenotypic variation).

Row measurements were analysed using the model:

$$X_{ij} = \mu + hs_i + bl_j + e_{ij}.$$

For variables showing significant family effects,

we estimated σ^2hs = variance between half-sib families, σ^2hs*bl = variance of the familyxblock interaction, and σ^2e = residual variance.

For heading date we estimated the narrow sense heritability as

$$h^2 = 4\sigma^2hs / (\sigma^2hs + \sigma^2hs*bl + \sigma^2e).$$

For half sib families obtained by open pollination, σ^2hs estimates 1/4th of the additive genetic variance (Falconer, 1981).

For row measurements the narrow sense heritabilities based on half-sib family means were calculated as $h^2 = \sigma^2hs / (\sigma^2hs + \sigma^2e/b)$ where b is the number of blocks.

For both estimates, confidence intervals were obtained according to Burdick and Graybill (1992).

RESULTS AND DISCUSSION

Mass selection

Most seedlings emerged four days after sowing. Table 1 shows other trait means in the initial population and selected parents. The latter showed significantly higher winter and spring growths, and a more erect habit.

TABLE 1

Means of initial population and selected parents.

Medias de las poblaciones inicial y seleccionada.

Population	WG	SDT	GH	ESG	HD
Global	5.9	4.1	3.6	4.0	29/3/95
Selected †	8.3***	4.2	4.0***	7.0***	28/3/95

WG:winter growth; SDT:early spring disease tolerance;
GH: growth habit; ESG:early spring growth; HD:heading date.
† asterisks represent t-test significance.

Combined selection

Since error mean squares of growth scores in each locality were not homogeneous after Bartlett's test (data not shown), separate analyses of variance (unbalanced due to missing data) were carried out.

TABLE 2

Mean squares from family analyses of variance (ignoring cultivars) and other parameters in Salcedo.

Cuadrados medios de análisis de varianza familiares y otros parámetros de caracteres de campo en Salcedo.

Source of V.	d.f.	WG	ESG	DW
Family (F)	37	0.6NS	3.2**	206.3*
Block (B)	3	2.4**	7.9**	1407.3***
Error	10	80.6	1.6	132.8
R^2		0.34	0.46	0.45
CV(%)		39.0	20.8	41.7
h^2#				0.36[0,0.57]
Family mean		2.3	5.8	27.6
Selected F mean		2.3	6.5	28.8
Cultivars mean +		1.8	4.1***	73.8***

WG:winter growth; ESG:early spring growth;
DW:dry weight at heading (g).
narrow sense heritability [95% confidence interval].
+ asterisks represent significant differences from family means.

Commercial cultivars were more productive (Table 2), later heading (Table 3) and more tolerant to diseases (data not shown) than the families. They also had less ADF and more WSC (Table 4), but selected families showed higher winter growth (Tables 2 and 5), earlier spring growth and higher crude protein in Salcedo. There were no significant differences between families for percentage dry matter (data not shown). Although not shown in the Tables, prostrate plants presented significantly higher winter growth (mean=5.4) than intermediates (mean=4.3) and erects (mean=3.9) (Waller/Duncan F=15.5***). Families derived from plants selected in Salcedo flowered significantly later than those from Mabegondo (two days on average) (data not shown).

TABLE 3

Mean squares (M.S.) from family analysis of variance and other parameters for heading date in Salcedo.

Cuadrados medios (M.S.) del análisis de varianza familiar y otros parámetros para fecha de espigado en Salcedo.

Source of V.	d.f.	M.S.
Family (F)	37	415.9***
S vs M†	1	999.5***
Block (B)	3	34.1NS
F x B	108	79.2***
Error	875	38.8
R^2		0.50
CV(%)		25.7
h^2#		0.85[0.48,1]
Family mean		30/3/96
Selected F mean		27/3/96
Cultivars mean+		10/5/96***

† mother plants selected in Salcedo versus Mabegondo
# narrow sense heritability[95% confidence interval].
+ asterisks give significant differences from family mean.

Heading date heritability was high. Therefore, this trait can be easily adjusted by selection. There were no significant differences between families for ADF (Table 4), which is a trait negatively correlated with in vitro digestibility of dry matter (Oliveira and Castro, 1994). Heritabilities of the rest of quality traits were relatively high. Plant dry weight of headed plants was not significantly correlated with PB, ADF or WSC (data not shown). This is not a normal result (Oliveira and Castro, 1994), and is probably due to the late cutting date. Pearson's correlations of %OM with CP (r=-0.78***), with WSC (r=0.63***), with HD (r=0.54***) and with ADF (r=-0.23*) were significant. Partial and phenotypic correlations between %OM and HD were still significant (r>0.48***). This suggests that, apart from the decrease of CP and WSC, and the increase in ADF with reproductive maturity, biomass samples had also less content of mineral elements, due to translocation to the spikes.

TABLE 4

Mean squares from family analyses of variance and other parameters for quality traits in Salcedo.

Cuadrados medios de análisis de varianza familiares para caracteres de calidad en Salcedo.

Source of V.	d.f.	CP	ADF	WSC	%OM
Family (F)	37	3.0**	6.3NS	48.1**	0.9***
Block (B)	2	4.9*	4.8NS	47.2NS	0.1NS
Error	73	1.3	4.2	20.6	0.3
R^2		0.56	0.44	0.56	0.58
CV(%)		11.3	7.5	14.7	0.6
h^2†		0.57		0.57	0.63
[ ] #		0.27-0.71		0.27-0.72	0.38-0.76
F mean		10.1	27.3	30.8	91.3
Selected F mean		10.4	27.3	30.7	91.1
Cultivars mean+		9.1**	23.9***	40.2***	92.2***

CP:crude protein-NIRS; ADF:acid-detergent fibre-NIRS.
WSC:water-soluble carbohydrates-NIRS.
%OM:% organic matter in dry matter.
† narrow sense heritability.
# 95% confidence interval of h^2.
+ asterisks represent significant differences from family means.

Family 31 was the earliest heading (25/3/96, on average) but the most prostrate (as prostrate as the experimental 'CIAM2'). Families 30 and 3 did not show disease symptoms in any replicate, like 'Vitesse'. Family 38 was the latest heading, too late for our purposes, showing the lowest growth on the 2/4/96 but being the most productive, while having the least ADF.

The same four families were selected independently in both localities: 27, 28, 30 and 31. These families showed, then, wide adaptation. Families 27 and 31 had high CP and low WSC, while 28 and 30 had the opposite.

The 50% best plants in each of the four selected families in each locality (total~100) were planted together for aftermath cross-pollination as before. To evaluate the possible cultivar, Syn-1 seed obtained was sown at the beginning of October 1996 in both localities with three replicates at 20 kg/ha in plots of 5m x 1.3m (as it is current practice in variety evaluation trials for Northern Spain), together with commercial cultivars, intermediate and late experimental cultivars. Dry weight of frequent cuts will be assessed, together with fungal disease infection scored in rows. In the establishment cut at Mabegondo, the Syn-1 was more productive than the cultivars. In Salcedo, that cut was not weighed due to an abundance of weeds, probably because of the high temperatures in Pontevedra coast. In that location, sowing rate must be 25 kg/ha, in order to avoid the occurrence of weeds.

TABLE 5

Mean squares from the analyses of variance of combined selection growth scores in Mabegondo.

Cuadrados medios de análisis de varianza de valoraciones visuales de crecimiento en Mabegondo.

Source of V.	d.f.	WG	Source of V.	d.f.	ESG
Family (F)	37	5.8***	Family	37	1.3***
Block (B)	3	13.2***	Block	3	1.1***
F x B	111	2.9***	Error	100	0.5
Error	894	1.0			
R^2		0.48			0.50
CV(%)		34.7			25.7
Family mean		2.8			4.7
Selected F mean		3.9			6.0
Cultivars mean		3.2			6.1

WG: Winter Growth; ESG: Early Spring Growth.

CONCLUSIONS

Although selected families were less productive than commercial cultivars, their winter growth was better. The creation of a synthetic variety has been initiated by intercrossing the best plants from these families. Net gain in this synthetic variety should exceed that accomplished by selection at any single locality and result in improved stability of this material.

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MEJORA DE RAIGRAS ITALIANO PRECOZ PARA ROTACION CON MAIZ EN EL NOROESTE ESPAÑOL

RESUMEN

Se describen los procedimientos usados para obtener una variedad de raigrás italiano precoz a partir de razas locales del noroeste español, para su rotación con maíz (una práctica frecuente en Galicia). Comenzando con una población precoz tipo *Westerwoldicum* -cuya naturaleza anual y reespigado permite una mejora rápida-, se aplicaron dos ciclos de selección intrapoblacional, uno de selección individual y otro de selección combinada (individual/familiar de medios hermanos). Aunque las familias seleccionadas fueron menos productivas que los cultivares comerciales, su crecimiento invernal fue mejor. Cuatro familias fueron seleccionadas independientemente en dos localidades de distinto clima y suelo, sugiriendo una buena adaptación a la zona costera de La Coruña y Pontevedra. El interés de una variedad sintética experimental obtenida por el cruzamiento de las mejores plantas dentro de dichas familias se está evaluando actualmente.

Palabras clave: *Lolium multiflorum*, recursos fitogenéticos, mejora de gramíneas pratenses.