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Ecotypes of Italian ryegrass from Swiss permanent grassland outperform current recommended cultivars

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Abstract Italian ryegrass (*Lolium multiflorum* Lam. ssp. *italicum* Volkart ex Schinz et Keller) thrives well in permanent grassland in mild and moist regions of Switzerland. Ecotypes of Italian ryegrass from Swiss permanent meadows have often been used successfully as the base material of breeding programmes. However, their agronomic performance per se has never been studied in detail. Therefore, field trials were conducted to assess the potential of this semi-natural genetic resource for use in breeding. Twenty ecotype populations were collected in different geographical regions in 2003 and seed was increased. Plot trials with 20 ecotype populations were conducted 2005–2007 at three locations, supplemented by row trials at two additional locations, and including four currently recommended cultivars as controls. Average performance in terms of yield, vigour and resistance to snow mould of ecotypes was superior to the cultivars. Depending on the characteristic, between three and 16 of the 20 ecotypes performed significantly better than the cultivars mean. However, resistance against crown rust of all ecotypes was significantly poorer than that of the cultivars. Correlation and regression analysis showed that more eastern and more northern collecting sites harboured better performing ecotypes. However, the most consistent site related factor favouring performance of the ecotypes in terms of yield, vigour, snow mould and *Xanthomonas* resistance was a low abundance of species which are typical of *Arrhenatheretum* type meadows. Data from the plot trials were related to results obtained with spaced plants at a single site, either in a seedling nursery or in the greenhouse. Resistance against crown rust was extremely well predicted by spaced plant observations. *Xanthomonas* resistance observed after artificial inoculation in the greenhouse was also significantly, but less closely correlated with field observations. An early date of ear emergence as observed on spaced plants was associated with high annual yield in the plot trials. It is concluded that in intensively managed habitats, natural selection has resulted in ecotype populations of Italian ryegrass which are highly promising for use in breeding. Individual plant selection for resistance can be expected to efficiently reduce their high susceptibility to crown rust.

Keywords Diversity · Ecotypes · Grassland · *Lolium multiflorum* · Populations · Semi-natural · Resistance · Switzerland · Varieties · Yield

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Introduction

Italian ryegrass (*Lolium multiflorum* ssp. *italicum* Volkart ex Schinz et Keller) is an important species for forage production in temporary grassland. The species ranks second in gross grass seed consumption in Europe (Kaske 2008) and is mainly used for short rotation leys of one to two, occasionally 3 years of duration. Although Italian ryegrass is considered a short-lived perennial, it thrives well in permanent grassland in mild and moist regions of Central Europe. Stebler and Schröter (1892) concluded from literature studies that the species had been introduced as a forage plant into Swiss agriculture north of the Alps between 1811 and 1840, and had since "wildered out everywhere". Stebler and Volkart (1913) state that Italian ryegrass was introduced to Swiss agriculture in 1820 and to Great Britain in 1831. This corresponds with the date for introduction given for Great Britain by Borrill (1976). Stebler and Schröter (1892) stated that "nowadays, Italian ryegrass is a steady companion of intensive meadow culture of flatter Switzerland up to about 700–800 m". We can thus assume that naturalized Italian ryegrass populations have adapted to this region for at least 100 and up to nearly 200 years. Stebler and Volkart (1913) were the first to report on a more frequent occurrence of the species, and they described a particular permanent meadow near Zurich where it contributed 36% of total yield. Dietl and Lehmann (1975) observed that the proportion of Italian ryegrass in intensively managed meadows had increased markedly and proposed the name *Lolietum multiflori* for typical plant associations dominated by Italian ryegrass with yield proportions of 40–80%. Italian ryegrass also occurs frequently in yield proportions of 5–40% in rather intensively managed forms of *Arrhenatherion* meadows (*Dactylis-Heracleum* type, Dietl 1995) and in degenerated meadows for which Dietl (1995) proposed an association named *Poo trivialis-Ranunculetum repentis*. Thomet and Bassetti (1989) also reported on strong increase of Italian ryegrass in permanent meadows. Apparently, current agricultural practices with high amounts of fertilization and frequent cutting favour the development of Italian ryegrass populations in permanent grassland. The value of such naturalized populations for breeding has long been recognized (Nüesch 1959), and breeding based on Swiss ecotypes has resulted in the release of internationally successful varieties like 'Lipo' (Nüesch and Badoux 1972), 'Axis' and 'Oryx' (Boller et al. 2002). However, the agronomic potential of ecotype populations of Italian ryegrass as such has never been studied extensively. In his one site field study with 92 progenies of Italian ryegrass clones collected in permanent grassland of southern Germany and Switzerland and grown in small plots of 0.63 m^2, Krings (2001) found that only one progeny had a significantly higher annual fresh matter yield than the average of two standard cultivars, while 86 progenies had a significantly lower yield than the standards. A collection made in 1996 at 6 sites in Switzerland yielded ecotype populations with a fair yielding potential and promising resistance against bacterial wilt (Boller et al. 2005). However, also in this study, testing was restricted to a single site. Fostered by the National Plan of Action for the conservation and sustainable use of plant genetic resources, a more comprehensive collection of Italian ryegrass ecotype populations was carried out in 2003. Analysis of molecular markers showed that within a subset of 12 populations, there was little differentiation among the populations and their genetic structure was not influenced by site related factors (Peter-Schmid et al. 2008a). However, 19 ecotype populations from the same collection showed significant differences in some morpho-physiological characters like date of ear emergence, length, or number of culms (Peter-Schmid et al. 2008b). In this study, we report on agronomic performance of 20 populations, including those of the above mentioned molecular and morpho-physiological studies. Performance was tested in field plots at three locations and related to collection site factors to define criteria for targeted conservation and utilisation. Selected performance characteristics of the populations tested in plots were compared with characteristics of individual plants.

Materials and methods

Collection sites and plant material

Lolium multiflorum ecotype populations were collected in 2003 at a total of 20 sites in Switzerland. Elevation of the sites ranged from 430 to 980 m a.s.l. All sites were north of the Alps, seven were located in the region "Northern foothill of the Alps", and 13 in the Swiss plateau region. At least 60 tillers, each deriving from a separate plant, were randomly sampled at each collection site, and seed was harvested separately from each tiller. More details of the sampling procedure and complete site data are listed in Peter-Schmid et al. (2008b). Botanical composition was evaluated in spring of 2004 and 2006 at the sites of origin. Abundance of all species was assessed by the yield fraction estimation method (Dietl 1995) within an area of 5 × 5 m. The sites were assigned to the grassland types by summing up the abundance of all species per indicator group A to H proposed by Dietl (1995). The sum of abundances of groups A to C was considered the abundance of *Arrhenatheretum* species.

Seed increase

One hundred and twenty seedlings of each ecotype population were raised in the greenhouse, using a maximum of 5 seeds per collected tiller. Seedlings of each population were transplanted to a field at Zürich-Reckenholz in October 2003 in 20 rows of 6 plants, with a row-to-row distance of 50 cm, and 30 cm distance between plants within a row. Populations were separated from each other by a distance of 15 m. In between the seed increase plots, there was a barrier of rye which was well developed at the time of flowering in spring 2004. Spring growth of 2004 was used for seed harvest without pre-cutting. Sixty well developed plants were chosen for individual seed harvest, seed of the remaining plants was harvested as a bulk. Seed of all 20 populations was successfully increased (Table 1). With a single exception having only 44 seed setting plants, for each population, between 57 and 74 plants contributed to total seed yield. However, the coefficient of variation for seed yield per plant reached almost 70% on average. Moreover, the distribution of seed yield per plant was always skewed to the right, indicating that plants with very large seed yields occurred more frequently than would be expected from a normal distribution. Based on the Jarque-Bera test for normality, 13 of the 20 populations had non-normally distributed seed yield per plant, and in each case, positive values of skewness and kurtosis were responsible for the deviation from normality. Indeed, the largest plant contributed up to 10% to total seed yield. Therefore, in order to avoid a shift in gene frequencies within the populations in favour of such extreme plants, a partially balanced bulk was prepared from harvested seed by limiting the contribution of individual plants to the amount of seed harvested from the plant ranking 30iest within the population. Only a part of the bulk seed harvest of those plants not harvested individually was added to the bulk, namely, the part corresponding to the ratio of the seed yields of the 30th ranking to the best plant of the respective population. In this way, the expected contribution of the largest plant in that bulk harvest was limited to the same level as the contributions of individual plants to the balanced bulk. In the final partially balanced bulk, the largest plant contributed only 1.7–2.4% to total yield, with the single exception of the 'LIT' ecotype population having only 44 plants (Table 1).

Field trials

Plot trials (6 × 1.5 m plots) with 20 ecotype populations and four locally recommended diploid

Table 1 Seed yield characteristics of 20 ecotypes of Italian ryegrass increased in isolated plots using spaced plants

Characteristic	Values over 20 ecotypes			
	Mean	SD	Maximum	Minimum
Number of plants harvested in total	74	13.5	98	57[a]
Number of plants harvested individually	59	4.3	69	57[a]
Seed yield per plant (g) mean per ecotype, μ	7.53	2.12	11.82	4.42
Standard deviation of seed yield (g) per plant, σ	5.25	1.35	8.89	3.07
Coefficient of variation (%) for seed yield per plant	71	13	105	51
Skewness for seed yield per plant	1.10	0.60	2.46	0.45
(Kurtosis-3) for seed yield per plant	1.80[b]	2.50	8.55	−0.56
Prob. of deviation from normality (Jarque-Bera)	0.058	0.082	0.257	<0.0001
Seed yield (g) of largest plant per ecotype	24.23	6.51	38.15	13.28
Contribution (%) of largest plant to total seed yield	5.73	1.93	10.76	3.75

[a] Without ecotype 'LIT', having only 44 plants

[b] Mean of the absolute values

cultivars, 'Axis', 'Oryx', 'Abercomo' and 'Barlizzy' (Suter et al. 2002) were established with three replications in spring of 2005 on the experimental farms of Agroscope Reckenholz-Tänikon ART at Ellighausen (525 m a.s.l.) and Oensingen (452 m a.s.l.). A reduced set of 10 ecotype populations and two cultivars was investigated in a plot trial at Zürich-Reckenholz (440 m a.s.l.). All 24 entries were additionally sown in observation trials with 3 m rows 0.5 m apart at Watt near Regensdorf (465 m a.s.l.) and Gibswil (1,000 m a.s.l.). In all plot trials, fresh matter yield was determined with a Hege plot harvester for each of five cuts in 2006 (first full harvest year) and 2007 (second full harvest year). Dry matter content was determined for a sample from two replicates of each entry, location and cut to determine herbage dry matter (DM) yield. Disease and vigour scores were rated in both plot and row trials on a 1–9 scale (9 = best). Diseases scored included crown rust (*Puccinia coronata* Corda), snow mould (various fungi), leaf spots (various fungi, often dominated by *Drechslera* spp.), and bacterial wilt (*Xanthomonas translucens* pv. *graminis,* Vauterin et al. 1995). All data were analyzed using procedures GLM and CORR of SAS© software. Least square means of performance characteristics are reported.

Individual plant observations and disease screening

Morphological characterisation of individual plants of the same populations had been carried out in 2005 in a spaced plant field nursery at Zürich-Reckenholz. The procedure and results were described in detail by Peter-Schmid et al. (2008b). We used data obtained from this field nursery trial to assess the potential of observations on individual plants to predict related performance characteristics of the populations. Heading date was recorded as "days after first of May" for each individual, and average heading date for one population was calculated as the arithmetic mean of individual heading dates. Resistance to crown rust (*Puccinia coronata* Corda) was scored in the field nursery repeatedly, two times in autumn 2004 and once in autumn 2005, and the mean response of all individuals of a population was used for the calculation of the correlation with plot data presented here. Resistance to bacterial wilt, caused by *Xanthomonas translucens* pv. *graminis* (Vauterin et al. 1995), was screened in the greenhouse in 2008. The 104 seedlings per population were raised in boxes (30 × 40 cm) in groups of 26 (two rows of 13 plants with a row-to-row distance of 2.8 cm and a distance of 2 cm between plants within a row), replicated 4 times in a complete randomized block design. Twenty-six days after sowing, the plants were trimmed to 2 cm with scissors which had been immersed in a suspension of bacteria. Thereby, isolate RI-26 of *Xanthomonas translucens* pv. *graminis* was used. For details see Kölliker et al. (2006). Twenty-one days after inoculation, the plants were trimmed to 2 cm again. Twelve days after this second cut, the number of vigorous, weak and dead plants was counted and related to the number of plants inoculated. The percent proportion of vigorous plants was found to give the highest level of significance for differences among entries and was therefore preferred over disease severity scores for comparison with field data.

Results

Agronomic performance

The ecotype populations varied significantly in all performance characteristics investigated (Table 2). Two ecotypes produced significantly higher yields than the average of the cultivars in the first full harvest year (YTOTH1), and 15 additional ecotypes outyielded the cultivars but the difference was not statistically significant. Even 13 ecotypes outyielded the cultivars significantly in the second full harvest year (YTOTH2). The highest yielding ecotypes outyielded the highest yielding cultivar in both years, the difference being significant for 3 ecotypes in the second full harvest year. In both years, only one ecotype yielded significantly less total dry matter than the mean of the cultivars. First cut yield (YCT1) of the ecotypes was particularly high, with 12 ecotypes producing a significantly higher yield than the cultivars mean on average over both years, and none of the ecotypes yielded significantly less in the first cut than the cultivars. The yields of the summer cuts (YCT23) of three ecotypes were significantly below those of the cultivars and this was related to insufficient resistance to bacterial wilt (XANT). However, on average, resistance of the ecotypes against bacterial wilt was

Table 2 Performance characteristics of 20 ecotype populations of Italian ryegrass in plot and row trials, compared to recommended cultivars

Accession		Yields (dt/ha)[b]				Vigour (9=best, 1=poorest)[c]						Disease resistance (9=best)[d]			
Acronym	Nr	YTOTH1	YTOTH2	YCT1	YCT23	VIG1	VIG23	VGEND	VIG	VIGH2	VGALT	SNOM	RUST	XANT	LSPOT
EGG	1	109.1b	143.1a	39.0b	52.1b	7.22b	7.50a	7.11b	7.09b	7.19b	5.88b	6.28b	5.91c	6.84	6.75
LEN	2	105.6b	135.7b	37.6b	49.5b	6.94b	7.42a	7.72b	7.22a	7.24b	6.50b	6.64a	5.47c	7.34	6.41c
ROO	3	104.4	140.4a	37.3b	49.7b	7.26b	7.31a	7.63b	6.85b	7.75d	2.50	6.41b	6.33c	6.62	7.14
LIT	4	104.8	138.9b	37.7b	50.2b	7.26b	7.18a	6.97b	6.95b	7.09b	4.88b	6.35b	4.69d	7.47b	6.83
NIE	5	104.5	134.4b	36.8b	51.4b	7.00b	7.50a	7.39b	6.89b	7.18b	5.75b	6.05b	4.86d	7.40b	6.22c
EGL	6	104.8	140.2a	35.9	51.4b	6.34	6.64b	8.13b	6.38	7.57a	2.75	5.85b	5.13c	7.70b	7.04
WEI	7	105.8	136.6b	36.1	49.1	6.01	7.06a	7.38b	6.85b	7.45b	2.00	5.97b	5.75c	6.39	7.04
WER	8	104.2	130.5	37.9b	47.5	6.72b	7.42a	6.61	6.76b	6.80b	6.00b	6.32b	4.63d	7.03	6.66
OBE	9	101.9	130.2	36.5b	45.6	6.34	6.89b	6.38	6.28	6.87b	2.63	6.22b	6.60c	6.01c	7.21b
HÜ	10	105.1	131.3	35.7	49.6b	6.56	6.78b	6.11	6.51	6.41	5.00b	5.69	4.61d	7.22	6.53
BAZ	11	104.9	138.4b	37.4b	51.5b	6.94b	7.53a	7.39b	6.93b	7.22b	3.75	6.19b	5.17c	7.15	6.16c
GAC	12	103.5	135.0b	37.3b	47.1	6.33	7.11b	6.61	6.80b	7.13b	1.25c	5.91b	6.51c	6.09	7.12
GOM	13	106.2	126.5	36.8b	48.4	6.93b	6.64b	6.63	6.75	6.17	6.25b	6.03b	4.30d	7.32	5.90c
HÜT	14	100.7	137.1b	36.0	50.2b	6.06	6.72b	7.28b	6.40	7.10b	2.75	5.87b	6.12c	6.84	6.91
DOP	15	102.9	133.6b	38.2b	49.1	7.18b	7.31a	6.47	6.11	6.47	4.50	6.16b	5.28c	6.55	6.42c
TÜR	16	101.7	133.6b	36.2b	49.1b	6.06	6.28	7.00b	6.02	6.59b	3.63	5.69	6.33c	6.40	6.84
PFI[a]	17	96.6	119.0	33.6	44.1c	5.34c	6.10	5.55c	5.68c	5.77c	4.25	5.41	6.30c	5.93c	6.76
LAT	18	96.2	121.0	35.5	43.5c	5.59	6.48	6.13	5.68c	6.20	3.50	5.85b	5.13c	6.08	6.42
WOL	19	101.1	121.1	36.8	45.1	6.34	6.89b	5.55c	6.21	5.92	2.63	6.10b	4.92d	5.93c	6.17
REI	20	91.7c	107.0d	30.1	41.8d	5.83	5.78	5.22c	5.84c	5.10d	2.63	5.28	5.61c	5.84c	5.84d
Ecotypes mean		102.8	131.7	36.4	48.3	6.51	6.93	6.76	6.51	6.76	3.95	6.01	5.48	6.71	6.62

Table 2 continued

Accession		Yields (dt/ha)[b]				Vigour (9=best, 1=poorest)[c]						Disease resistance (9=best)[d]			
Acronym	Nr	YTOTH1	YTOTH2	YCT1	YCT23	VIG1	VIG23	VGEND	VIG	VIGH2	VGALT	SNOM	RUST	XANT	LSPOT
ABERCOMO		95.9	132.1	30.5c	50.6b	5.26c	5.81	7.47b	5.55	6.84	2.50	4.53c	6.54	5.24c	6.35c
AXIS		97.4	123.8	31.3	46.7	5.33c	5.50c	5.94	6.36	5.93c	1.38c	5.23	8.51b	7.72	7.22
BARLIZZY		105.6b	122.2	36.7b	45.2	7.01b	6.35	5.97	6.61b	6.10	4.88b	6.16b	5.54	7.16	6.97
ORYX		103.4	122.9	36.0	44.7	6.56	6.47	5.89	6.56	6.26	3.50	5.64	8.05b	6.59	6.91
Cultivars mean		100.6	125.3	33.6	46.8	6.04	6.03	6.32	6.27	6.28	3.06	5.39	7.16	6.68	6.86

Values of individual accessions followed by these letters are: a significantly superior to best cultivar; b: significantly superior to cultivars mean; c: significantly inferior to cultivars mean; d: significantly inferior to poorest cultivar. *T*-test, level of significance $P = 0.05$

[a] Ecotype 'PFI' was identified as being tetraploid

[b] Yields: YTOTH1, YTOTH2: total 1st, 2nd full harvest year; YCT1, YCT23: 1st cut, 2nd + 3rd cut; means 1st and 2nd full harvest year

[c] Vigour scores: VIG1, VIG23 1st cut, 2nd + 3rd cut, means 1st and 2nd full harvest year; VGEND at end of 2nd full harvest year; VIGH1, VIGH2 means 1st, 2nd full harvest year; VGALT at high altitude (1,000 m a.s.l.)

[d] Resistances: SNOM snow mould (different fungi); RUST crown rust (*Puccinia coronata*); XANT bacterial wilt (*Xanthomonas translucens* pv. *graminis*); LSPOT leaf spots caused by various fungal diseases

not inferior to the cultivars and 3 ecotypes had even significantly superior resistance. Vigour scores generally reflected the differences in yield except that the ecotypes were scored relatively best in summer (VIG23) where 9 ecotypes performed significantly better than the best cultivar. Ecotypes showed a remarkable resistance against snow mould (SNOM), with 16 out of the 20 ecotypes being significantly more resistant than the cultivars mean. Conversely, they were less resistant against leaf diseases than the cultivars. This was particularly striking for crown rust (RUST). Without exception, the ecotypes were significantly more susceptible than the cultivars mean, and 6 ecotypes were scored significantly poorer than the most susceptible cultivar.

Relationship of performance characteristics with site factors

Table 3 lists those collection site factors which were correlated significantly with at least one performance characteristic. Longitude, latitude, Ca content, and abundance of *Arrhenateretum* species showed a consistent relationship with at least four performance characteristics, which was always in the same direction: Soils with a low Calcium content, located east and north, and covered by a vegetation with a low abundance of *Arrhenateretum* species harboured the best performing ecotypes. Altitude of the origin of the ecotypes was positively correlated with performance in altitude, whereas a high pH was associated with low performance in altitude. Response to rust was least related with site factors, with only Mg content of the soil showing a small but significant positive correlation with resistance.

Relationship between performance of populations and individual plant data

Observations on individual plants were useful to predict related performance characteristics of the populations. The two characters measured with the greatest precision in both situations were crown rust resistance and date of ear emergence, and these two showed the highest positive correlation between individual plant and population plot data (Table 4). A very strong negative correlation was found between date of ear emergence measured on individual plants and first cut yield of the plots, indicating that the

Table 3 Significant ($P < 0.05$) Pearson correlation coefficients (r) and probability (P) for $r \neq 0$ between selected performance characteristics (see Table 2 for explanation) and collection site data for 19[a] ecotypes of Italian ryegrass from Swiss permanent grassland

Collection site data	Yields (dt/ha)				Vigour (9 = best, 1 = poorest)						Disease resistance (9 = best)					
	YTOTH1		YTOTH2		VIGH1		VIGH2		VGALT		SNOM		RUST		XANTH	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Geographic data																
Longitude	**0.67**	0.002	**0.54**	0.016	**0.74**	<0.001	**0.48**	0.035	0.42	0.072	0.38	0.114	−0.04	0.883	**0.54**	0.017
Latitude	**0.58**	0.008	**0.64**	0.003	**0.52**	0.022	**0.65**	0.003	−0.11	0.667	0.31	0.188	0.33	0.164	0.28	0.239
Altitude	0.12	0.626	−0.18	0.456	−0.09	0.728	−0.41	0.078	**0.50**	0.031	0.03	0.910	−0.21	0.385	−0.08	0.742
Soil data																
pH	**−0.49**	0.034	−0.08	0.734	−0.30	0.213	0.07	0.772	**−0.49**	0.035	−0.32	0.188	0.43	0.064	−0.182	0.456
Ca content	**−0.87**	<0.001	**−0.66**	0.002	**−0.66**	0.002	**−0.55**	0.015	−0.26	0.278	**−0.56**	0.014	0.06	0.822	−0.41	0.081
Mg content	−0.24	0.323	0.04	0.882	−0.09	0.701	0.06	0.815	−0.14	0.577	−0.07	0.768	**0.48**	0.039	−0.144	0.557
Vegetation data																
Abundance of *Arrhenateretum* species	**−0.59**	0.007	**−0.61**	0.005	**−0.49**	0.034	**−0.49**	0.035	−0.44	0.058	**−0.60**	0.006	0.38	0.112	**−0.61**	0.006

Significant ($P < 0.05$) correlations are indicated in bold type

[a] Ecotype 'PFI' was excluded from correlation analysis because it had been identified as being tetraploid

Table 4 Correlation between related performance characteristics assessed on individual plants and plots of 20 ecotype populations and four cultivars

Characteristic of individual plants[a]	Values over 20 ecotypes and 4 cultivars				
	Characteristic of plots[b]	*F*-value[d] for differences among ecotypes/cultivars		Pearson correlation statistics ($n = 24$)	
		Individuals	Plots	r	Prob ($r > 0$)
Crown rust resistance	Crown rust resistance	18.97	48.86	0.914	<0.0001
Xanthomonas resistance[c]	*Xanthomonas* resistance	4.43	4.09	0.499	0.013
Resistance to winter damage	Snow mould resistance	1.74 (0.02)	6.41	−0.016	0.941
Natural height in spring	Yield 1st cut	5.84	5.12	0.416	0.043
Date of ear emergence	Date of ear emergence	9.80	15.41	0.866	<0.0001
Date of ear emergence	Yield 1st cut	9.80	5.12	−0.710	<0.0001
Date of ear emergence	Total yield 1st full harvest year	9.80	3.16	−0.517	0.010
Number of culms	Yield 1st cut	5.00	5.12	0.181	0.397
Number of inflorescences in aftermath	Yield 2nd + 3rd cut	6.50	7.90	0.542	0.006

[a] Data from Peter-Schmid et al. 2008b, except *Xanthomonas* resistance

[b] Data from Table 2; date of ear emergence measured in row trials

[c] Data from greenhouse test

[d] From analysis of variance; all values highly significant ($P < 0.0001$) except where p is indicated in brackets

earlier the ears emerged the higher was the yield. This effect also resulted in a significant association of early ear emergence with high annual dry matter yield. Similarly, a strong tendency to form inflorescences in aftermath was related to a higher yield of the aftermath cuts 2 and 3. A loose but still significant correlation was found between natural height of individual plants in spring and population yield of the first cut, and for *Xanthomonas* resistance assessed on either individual plants in the greenhouse or on populations in field plots. Analysis of the latter correlation revealed that some of the cultivars and the tetraploid ecotype accession ‘PFI’ were mainly responsible for the lack of a strong correlation. If all cultivars and the tetraploid ecotype accession were omitted, the correlation between greenhouse and field resistance of the remaining, diploid ecotypes against *Xanthomonas* rose from 0.50 to 0.65.

Discussion

High performance of ecotypes

This comprehensive study reveals a surprisingly high agronomic potential of Italian ryegrass ecotypes from Swiss permanent grassland. We converted the results presented in Table 2 into a single final score according to the current regulation for the admission of cultivars to the Swiss national catalogue of cultivars (Anonymous 1998). The same score is considered when updating the list of recommended cultivars. Of the 20 ecotype populations, 13 obtained a final score more than 0.2 units better than the mean of the 4 cultivars, 0.2 units being the threshold which is applied for the decision about admission for recommendation (Anonymous 1999). The 6 best ecotypes were judged more than 0.5 units better than the cultivar mean. In the most recent official variety trials for Switzerland carried out 2002–2004 (Suter et al. 2005), only two out of 24 new cultivars tested reached the threshold level of an 0.2 unit improvement over the mean of all recommended cultivars, and none outperformed them by more than 0.5 units. The ranking of the four cultivars in the study presented here was very similar to the ranking in the official trial (Suter et al. 2005), where they occupied the first three and the 6th position of the 11 current, recommended cultivars. Therefore, more than half of the ecotype populations would have had a good chance of being admitted to the list of recommended cultivars, had they been applied for testing and provided that they were homogeneous enough to pass DUS. The 5 best ecotypes (EGG, LEN, ROO, LIT, NIE) originated from a relatively small region in east central Switzerland (Fig. 1). Their sites of origin were close to the foothills of the Alps. Conspicuously, the centre of this region is close to the origin of the ecotype populations which gave rise to the cultivar 'Oryx' (Boller et al. 2002), namely, south of the upper end of lake Zurich. Cham (canton of Zug) where Thomet and Bassetti (1989) found an extremely high yielding performance of a permanent meadow with high proportions of Italian ryegrass lies very near the collecting site Root which ranked third among the best performing ecotypes. Apparently, this region harbours highly performing ecotype populations of Italian ryegrass and this may be an important reason why permanent meadows with high proportions of Italian ryegrass are particularly frequent in that region since many years (Dietl and Lehmann, 1975).

While the variability among ecotype populations of forage grasses in terms of molecular or morpho-physiological characters has often been studied, e.g. for *Lolium perenne* by Bolaric et al. (2005), Balfourier and Charmet (1991), and Hayward et al. (1982), and for *Lolium multiflorum* by Peter-Schmid et al. (2008a, 2008b), few comparable data of the actual agronomic performance of ecotype populations can be found in literature. The results presented here confirm the promising agronomic performance of four out of six other Swiss ecotype populations tested in a previous, less comprehensive study at a single location (Boller et al. 2005). However, the excellent yielding performance of many ecotype populations is in sharp contrast to the study of Krings (2001) with progenies of ecotypes of Italian ryegrass from Bavaria and Switzerland. He concluded that most ecotypes performed clearly poorer than cultivars. One reason for this discrepancy might be that in the study of Krings, isolation among seed increasing plots was insufficient, thus masking possibly larger differences in performance of the ecotypes. Another possibility is that he compared only fresh matter yields and one of the two standard cultivars used was tetraploid. Tetraploids have lower dry matter content than diploids, thus the performance of the diploid ecotypes may have been underestimated. Finally, even though the majority of the ecotypes performed poorly, some exhibited a high yielding potential and one was significantly superior to the standard cultivars. Posselt and Willner (2007) studied German ecotypes of perennial ryegrass, a close relative of Italian ryegrass. In their great majority, they were also judged inferior to current cultivars in terms of yielding ability. However, one out of 23 ecotype populations clearly outyielded the mean of the corresponding cultivars with comparable earliness. The particular example of this outstanding ecotype population shows that potentially, ecotypes with high yielding ability can be found in permanent grassland.

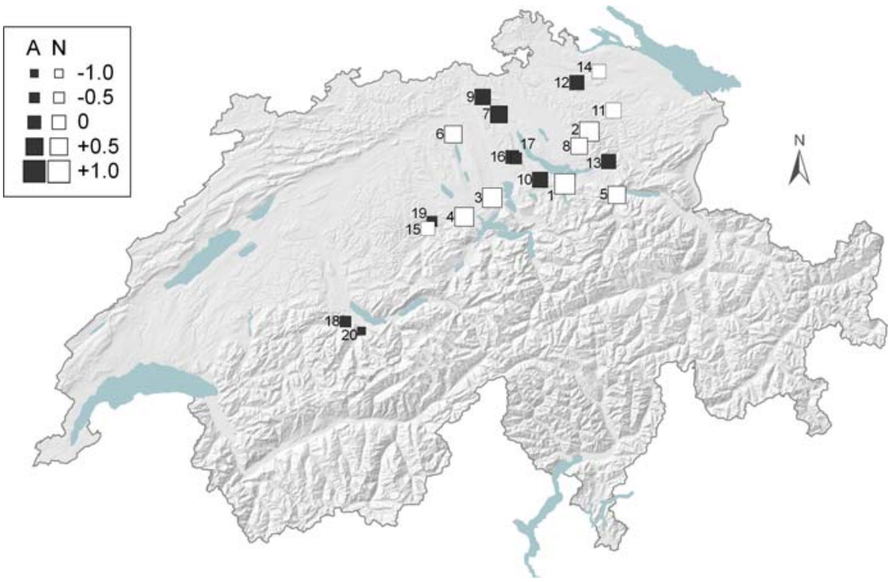

Fig. 1 Vegetation class at their origin and performance of 20 ecotype populations of Italian ryegrass. Black squares: *Arrhenatherion* (A) vegetation; white squares: Non-*Arrhenatherion* (N) vegetation. The size of the squares is proportional to their final performance score in terms of difference to the mean of recommended cultivars (see examples in graph). Numbers correspond with accession numbers in Table 2 and indicate the final performance ranking. Reproduced by permission of swisstopo (BA081816)

Importance of proper seed multiplication

One asset of the Italian ryegrass ecotype populations presented here is that before starting the evaluation, much care was taken to produce a seed lot which well represents the population of origin. The populations were collected as seed, and the seed was multiplied in isolations allowing random pollination among the individuals. Both at the original sampling and during seed increase, adequate numbers of individuals were considered, and care was taken to balance the contribution of seed by the individuals. The latter prerequisite has been discussed extensively within the European forage genetic resources community (Sackville Hamilton et al. 1997; Boller et al. 2008). The results presented here confirm that balancing the seed of individual plant harvests is necessary if only a limited number of plants are used for seed increase. Typically, the largest plant yielded 3.33 times the mean seed yield (Table 1). Consequently, if the aim is to limit the contribution of an individual plant to 3.33%, as is the case when making a balanced bulk of 30 individuals as suggested by Sackville Hamilton et al. (1997), at least 100 ($3.33/3.33 \times 100$) plants have to be harvested as a bulk. To cover the most extreme case where the largest plant yielded 5.97 times the mean, 179 ($5.97/3.33 \times 100$) plants would be necessary. The current recommendation is to use at least 100 plants for bulk harvest. A large variation for seed yield among individual plants has also been found in the seed increase experiments with perennial ryegrass reported by Hinton-Jones et al. (2007). One slight drawback of the seed increase setup used for our study was the relatively short distance of 15 m between plots, whereas 30 m are the preferred distance (Boller et al. 2008). However, a tall and dense barrier crop of rye was used which largely exceeded the ryegrass plants in height at the time of flowering. Such a barrier has been shown to effectively reduce pollen contamination to a level of 3.7% with a distance of 15 m between plots when these consisted of just 49 individual plants (Marum et al. 2007). Therefore, considering that our seed increase plots consisted of 120 plants and that genetic contamination of the progeny is only half the level of pollen contamination, we can assume that the seed increased was at least 98.5% true-to-type of the parent material. The much broader genetic base of our ecotype populations and the fact that they were multiplied in proper isolation may have favoured differentiation among the populations, thus making it more likely to detect superior ecotype populations than in the study of Krings (2001).

Disease resistance needs improvement

The high crown rust susceptibility of the ecotypes (see Table 2) would in most cases prohibit their direct use in agriculture and is a severe problem for their use in breeding. However, the close correlation between crown rust resistance scored in a single spaced plant nursery and crown rust resistance observed in rows or plots grown at several locations suggests that phenotypic selection of disease free plants is promising. This is supported by the strong correlations of crown rust disease scores of a set of Italian ryegrass cultivars among sites of the EUCARPIA multisite rust evaluation trial (Boller et al. 2003; Schubiger et al. 2006). Studer et al. (2007) also found crown rust resistance of individual genotypes used for a QTL mapping study to be detected consistently over a number of environments. The Italian ryegrass populations described here varied significantly for crown rust resistance, providing opportunities for selection. However, strict recurrent selection for rust resistance within a gene pool may lead to a loss in productivity (Boller et al. 2005). Therefore, as an alternative strategy, the most promising ecotypes in terms of yield and vigour may be crossed with cultivars with high crown rust resistance, followed by recurrent selection in the gene pool broadened in this way.

Resistance of many of the ecotype populations against *Xanthomonas* was surprisingly high (Table 2), and this was confirmed in a greenhouse test, which showed a fair correlation with field data. The coefficient of correlation of 0.65 between greenhouse and field data for the group of diploid ecotype populations is comparable to that of 0.71 found by Schmidt and Nüesch (1980) for a set of Italian ryegrass cultivars. A good correlation of greenhouse and field data on *Xanthomonas* resistance of Italian ryegrass was also found by Studer et al. (2006), based on field observations of a mapping population in an individual plant nursery. In that study, resistance against *Xanthomonas* was found to be controlled to a large extent by a single major gene. This would suggest rapid progress from phenotypic selection since the ecotype populations showed large, significant differences in *Xanthomonas* resistance. However, field resistance of the cultivar 'Oryx', which had been obtained from recurrent, greenhouse screening based selection for *Xanthomonas* resistance within a broad based ecotype population (Boller et al. 2002), appeared to be less pronounced than would be expected from its response in the greenhouse. It appears that on the one hand, screening for *Xanthomonas* resistance by artificial inoculation in the greenhouse may not sufficiently cover resistance factors becoming effective in a field situation. On the other hand, the high level of *Xanthomonas* resistance of the best performing ecotypes suggests that natural selection had been quite effective.

Early heading related with high annual yield

Date of ear emergence was negatively correlated with dry matter yield, particularly in the first cut (Table 4). This is in contrast to the general trend of higher yields with later heading observed e.g. in perennial ryegrass (*Lolium perenne* L.), as reflected by often higher scores of late heading varieties for first cut and annual yield in official variety lists. However, the higher yielding potential of late heading varieties is only expressed if the first cut is taken adequately late (Gilliland et al. 1995). In our trials with Italian ryegrass, all accessions were cut at the same date, irrespective of their earliness. This regime apparently favoured high yield of early heading populations. Heading time of ecotypes of different grass species has been reported to be either positively (Peter-Schmid et al. 2008b for meadow fescue) or negatively (Balfourier and Charmet 1991) correlated with altitude of their origin. However, this was not the case for the ecotypes of Italian ryegrass investigated here (Peter-Schmid et al. 2008b). Therefore, altitude of origin was not involved in the observed relationship between earliness and dry matter yield. More likely, management intensity at the sites of origin played a role. Peter-Schmid et al. (2008b) observed that ecotype populations from intensively managed meadows headed earlier than those from less intensively managed sites. Intensive management may at the same time have favoured natural selection of highly productive and competitive plant types.

Vegetation class of collection sites points to performance

Of the site factors considered to assess the relationship between performance data and environmental data at the origin of the ecotypes, the negative influence of the abundance of *Arrhenateretum* species on performance was particularly striking (Table 3). This points to a possible conflict between maintenance of species-rich grassland of high nature conservation value and maintenance of grassland in the interest of conserving valuable genetic resources for food and agriculture. To analyze this further, we classified vegetation of all sites according to the proposal of Dietl (1995). Considering 2 years of observation (2004 and 2006), 10 sites resulted to be classified as an *Arrhenaterion* association: 4 as a *Lolio perennis-Arrhenatheretum* (OBE, WEI, GAC, REI), and 6 as a *Dactylis-Heracleum* type (HÜ, GOM, TUE, PFI, LAT, WOL). These associations, although managed rather intensively, still contain sufficient typical *Arrhenateretum* species to revert to more species-rich grassland types (Dietl 1995). The remaining 10 sites were classified as non-*Arrhenatherion*: 5 as *Lolietum multiflori* (ROO, LIT, NIE, EGL, HÜT), 2 as transition forms between *Lolietum multiflori* and *Trifolio repentis-Alopecuretum* (DOP, BAZ), 2 as degenerated vegetation approaching *Poo trivialis-Ranunculetum repentis* (LEN, WER), and 1 (EGG) as *Polygonum-Dactylis* type of *Trisetetion* (Dietl and Jorquera 2003). All these non-*Arrhenatherion* types of vegetation are considered critical in terms of nature conservation as they are unlikely to revert to species-rich grassland even when management intensity is reduced (Dietl 1995). The distribution of these sites, along with overall performance of the Italian ryegrass ecotype populations collected there, is represented in Fig. 1. Clearly, non-*Arrhenatherion* sites harboured the better performing ecotype populations, having an average performance score of +0.51 (0.11–0.84), compared to −0.06 (−0.96–0.47) for *Arrhenatherion* sites. Both *Arrhenatherion* and non-*Arrhenatherion* sites were geographically well distributed, and their distributions overlapped to a large extent. Therefore, the apparent association between vegetation class and performance of ecotypes was not biased by a regional differentiation of vegetation. A similar conflict between conserving *Arrhenateretum* type grassland as a contribution to biodiversity at the species and ecosystem level, and conserving valuable plant genetic resources for food and agriculture was identified by Peter-Schmid et al. (2008a). They reported on a reduced occurrence of rare alleles in *Festuca pratensis* ecotype populations from extensively managed habitats. Moreover, the fact that many of the best ecotype populations originated from *Lolietum multiflori* or even *Poo-trivialis-Ranunculetum repentis* vegetations shows that current political efforts focussing on the conservation of extensively managed grassland for enhancing biodiversity are not *a priori* suitable to conserve highly performing, valuable genetic resources of grassland species in situ. This is because the most valuable sites in terms of conserving Italian ryegrass genetic resources like *Lolietum multiflori* tend to have a low conservation value with regard to plant and animal species diversity. Conserving such valuable populations of Italian ryegrass in situ would require a specific management of the respective sites. They need sufficient nitrogen fertilization, and they should not be utilized too frequently. This was shown by Thomet and Bassetti (1989) who observed a marked decline of Italian ryegrass proportions when passing from a five cut to a seven cut regime, especially when only a moderate amount of fertilizer nitrogen was applied. Moreover, it is advisable to allow Italian ryegrass to reseed naturally by delaying summer cuts occasionally. Bassetti (1989) showed that cutting intervals of 6 weeks in summer permit large amounts of Italian ryegrass seed to become viable, as opposed to 4 weeks where very little viable seed was formed. When natural reseeding is allowed, there is an opportunity for genetic recombination within the population, favouring further adaptation.

Conclusion

Ecotypes of Italian ryegrass from Swiss permanent grassland exhibit very promising agronomic performance characteristics, justifying their further use to aliment breeding programmes. Populations from east central Switzerland growing on soils with a low calcium content and in habitats with vegetation classified as non-*Arrhenatherion,* particularly those classified as *Lolietum multiflori* appear to be the most valuable, even when such habitats degenerate to *Poo-trivialis-Ranunculetum repentis*. This observation points to the necessity of complementing current conservation efforts with new target sites when forages as plant genetic resources for food and agricultures are considered. High susceptibility to crown rust was the major drawback of the ecotypes. Efficient ways to introgress powerful rust resistance genes will greatly help exploiting their high performance potential.

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