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ORIGINAL PAPER

Genetic characterisation of seed yield and fertility traits in perennial ryegrass (*Lolium perenne* L.)

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Abstract Seed yield is a trait of major interest for the key grassland species *Lolium perenne* L. An F2 mapping population of perennial ryegrass (VrnA), recently characterised for vernalisation response, was assessed in a glasshouse for traits related to seed yield based on a lattice design with four replications over 2 years. The traits heading date, plant height, length of panicles, number of panicles per plant, seed yield per panicle, flag leaf length, flag leaf width and seed yield per plant revealed repeatabilities ranging from 41 to 76% and a considerable amount of genetic variation in the VrnA population. Path analysis partitioned the direct and indirect effects of seed yield components on seed yield per plant. Seed yield per panicle showed the highest effect on total seed yield. The adjusted mean values of each trait and a genetic linkage map consisting of 97 anonymous and 85 gene associated DNA markers were used for quantitative trait loci (QTL) analysis. Of particular interest were two QTL on linkage group (LG) 1 and LG 2, explaining 41 and 18%, respectively, of the observed phenotypic variation for the trait seed yield per panicle. Both QTL co-located with two major QTL for total seed yield per plant possibly representing the S and Z loci of the gametophytic self incompatibility (SI) system of perennial ryegrass. The diversity of SI alleles in mapping parents and the degree of heterozygosity at SI loci in the full sib progeny determines the interference of self incompatibility with seed production.

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Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grass species of temperate grassland worldwide. The major focus of ryegrass breeding has been on improvement of agronomic characters such as dry matter yield, disease resistance and forage quality (Humphreys et al. 2006). However, economic seed multiplication is crucial for novel grass cultivars to be competitive in the commercial market.

Although seed yield is a complex trait and affected by agricultural practices as well as the environment, traits related to seed production revealed considerable genetic variation (Elgersma 1990; Elgersma and van Wijk 1997), prerequisite for improvement by direct or indirect selection (Bugge 1987; Marshall and Wilkins 2003). However, progress in breeding for seed yield may negatively affect forage yield and quality (Bugge 1987; van Wijk 1980). For example, enhanced dry matter digestibility has been achieved partly by reducing the proportion of reproductive

tillers, leading to a reduction of seed yield (Wilkins 1995). In contrast, studies assessing the relationship between seed and dry matter production concluded that it should be possible to break this negative correlation, e.g., by combining a low number of tillers with high fertility, thereby producing an equivalent seed yield per plant with fewer panicles (Marshall and Wilkins 2003). Therefore, genetic components underlying traits related to seed production require detailed characterisation.

Seed yield can be divided into several components either reflecting seed yield potential (e.g., number of fertile tillers, panicle length, number of florets per panicle) or realisation of seed yield potential (e.g., panicle fertility, 1,000-seed weight) (Bean 1972). The relative contribution of these components to total seed yield per plant is still disputed. The number of fertile tillers is closely related to seed yield in grass species such as *Dactylis glomerata* L. (Chastain and Grabe 1989a), *Festuca arundinacea* Schreib. (Chastain and Grabe 1989b), *Phleum pratense* L. (Entz et al. 1994), *Agrostis stolonifera* L. (Cattani et al. 1997), and *Poa pratensis* L. (Chastain et al. 1997). In perennial ryegrass, the number of fertile tillers was the most important component affecting seed yield in a field experiment of 100 single spaced plants representing 6 perennial ryegrass varieties (Bugge 1987). In contrast, no association between seed yield and number of fertile tillers was found in stand densities used for commercial seed production (Chastain et al. 1995), which suggests compensation of tiller density in ryegrass swards.

Plants generally produce fewer mature seeds than the number of ovules available for fertilisation. The seed/ovule ratio, which is even lower for outcrossing species such as perennial ryegrass when compared to inbreeding species (Charlesworth 1989), indicate the importance of seed set for ryegrass seed production. In spaced genotypes of perennial ryegrass, considerable genetic variation for seed set has been found (Elgersma and Śnieżko 1988). Obvious explanations are that mature stigmata do not get sufficient viable pollen or that deposited pollen is not compatible, as perennial ryegrass exhibits a gametophytic self incompatibility system controlled by two loci S and Z (Cornish et al. 1979). On the other hand, pollen supply does not seem to be the only limiting factor, because seed abortion is reported in some plant species even when plenty of compatible pollen is available (Stephenson 1981; Sutherland and Delph 1984). Large flag leaves may decrease the rate of seed abortion, enabling assimilate reallocation via the stems to the inflorescence in the period of anthesis (Fang et al. 2004). This concept is well established in cereals and may also be of importance in forage grasses. However, seed abortion in ryegrass may be due to genetic defects associated with cross pollination (Marshall and Ludlam 1989) and is thus randomly distributed in the panicle (Elgersma and Śnieżko 1988).

Heading date, determined by a combination of photoperiod sensitivity and vernalisation requirements in *Lolium* spp., is an important seed yield component since late flowering cultivars of perennial ryegrass with a high forage yield performance often lack reproduction efficiency (Bugge 1987). To date, two genes controlling flowering time in perennial ryegrass were described. *LpVRN1* was identified based on DNA sequence homology to *TmVRN1* of *Triticum monococcum* and was mapped to a quantitative trait loci (QTL) for heading date on linkage group (LG) 4 in the mapping population VrnA (Andersen et al. 2006; Jensen et al. 2005). Furthermore, *LpCO* a *CO*-like, putative *Hd1*-orthologue from rice (*Oryza sativa*) mapped in close proximity to a QTL for vernalization response on LG 7 in the same mapping population (Andersen et al. 2006; Armstead et al. 2005; Martin et al. 2004). Thus, comprehensive syntenic relationship to major cereal grass species (Devos 2005) facilitate identification of seed yield candidate genes in perennial ryegrass. Genes with putative impact on seed yield components have been identified in monocots (e.g., genes affecting phytohormone biosynthesis and plant shape in maize; Doebley et al. 1995). Moreover, sequence homology to dicotyledonous model crop species *Arabidopsis thaliana* and *Glycine max* has been used to tag gene orthologous to *IAA1*, *RUB1* conjugating enzyme, *BRI1*, *SHOOT1* and *TB1* with putative functions in plant architecture, axillary tiller formation and hormone response in perennial ryegrass (Brazauskas and Pašakinskienė 2008).

The objectives of this study were to (1) assess the genetic variation of traits related to seed yield and fertility in the VrnA mapping population, (2) determine the relationship among seed yield components and their importance on seed yield per plant, (3) detect number and location of QTL controlling these traits, (4) explore specific genetic mechanisms and genes underlying these QTL, and (5) identify closely linked genetic markers for marker-assisted selection.

Materials and methods

Plant materials

The perennial ryegrass mapping population VrnA was derived from a cross between a plant of the Italian cultivar Veyo and a Danish ecotype Falster with contrasting vernalisation requirements and consisted of 184 F2 genotypes (Jensen et al. 2005). For this investigation, plants were clonally propagated, vernalised under short day and low temperature conditions in an unheated, unlit glasshouse over winter and grown in 13 cm diameter (1 l) pots.

Phenotypic characterisation

The traits heading date expressed as growing degree-days to heading (GDD), plant height (PH), length of panicles (LPa), number of panicles per plant (NPa), flag leaf length (FLL), flag leaf width (FLW), seed yield per panicle (SYPa) and seed yield per plant (SYP) were assessed in the glasshouse on single spaced plants based on a 12 × 8 lattice design with four clonal replications. GDD, PH, LPa and NPa were investigated in two consecutive years (2004 and 2005), whereas flag leaf dimensions (FLL and FLW) were assessed in 2005. Measurements of SYPa and SYP in 2005 focused on 40 F2 genotypes selected for maximal trait segregation. GDD was determined as the temperature sum from February 1 until heading using the formula $(T_{max} + T_{min}/2) - T_{base}$, where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature and T_{base} is the basal temperature set at 3°C, and defined as the temperature below which development of perennial ryegrass ceases (Jensen et al. 2005). FLL (cm) and FLW (mm) were evaluated at anthesis based on the average of the flag leaves of three different tillers representative for the respective plant. The traits PH (maximum length in cm from the base to the top of the plant), NPa and LPa were investigated at harvest. For LPa, the average of three panicles representative for each plant was determined (cm). The same panicles were separately harvested and threshed for the determination of SYPa. Their seeds were separated and cleaned by hand from glumes and weighed (g/panicle). The same cleaning procedure was applied to assess the trait SYP (g/plant).

Statistical analysis

Lattice analysis using the PLABSTAT software, version 2 P (Utz 2000) was performed in order to estimate quantitative genetic parameters. Repeatability was calculated by dividing the genotypic variance component σ_g^2 by the sum of σ_g^2 and the effective mean square of the error. The adjusted means of the 184 mapping individuals as well as standard error, least significant difference and the respective degrees of freedom of single lattice analysis were used for the ANOVA for each trait across both years. Heritability was calculated according to the formula $h^2 = \sigma_g^2 \Big/ \left(\sigma_g^2 + \sigma_{gy}^2/e + \sigma_{error}^2/\mathrm{er}\right)$ with σ_g^2, σ_{gy}^2 and σ_{error}^2 as variance components for the genotype, the genotype × year and the error. Heritability for SYPa and SYP is based on 20 selected genotypes with high and 20 genotypes with low seed yield. Since data for the traits FLL and FLW were only available from 2005, heritability is represented by repeatability.

The relationship among seed yield components and their relative importance on the target trait SYP were determined both by simple correlation and path coefficient analyses (Wright 1923) using R statistical software version 2.6.1 (R Development Core Team 2007). Direct and indirect path coefficients were estimated using regression analysis with SYP as the dependent variable. Path analysis was first performed using each seed yield character assessed in the same evaluation year (2004) as regressors and than compared to results obtained from a regression of SYP to the least square means (LSM) of single traits obtained from both years.

Genetic linkage mapping

The genetic linkage map of VrnA (Jensen et al. 2005) was complemented with genetic markers derived from *TmVRN2* of *Triticum monococcum* and *Hd1* of *Oryza sativa* (Andersen et al. 2006), from laccases and resistance gene analogs (Schejbel et al. 2008) and candidate genes putatively affecting seed yield components (*IAA1*, *RUB1* conjugating enzyme and *SHOOT1*) (Brazauskas and Pašakinskienė 2008). In order to increase the number of markers and map density for QTL analysis, 63 EST-derived SSR markers (Studer et al. 2008) were added, resulting in seven LGs consisting of 18–36 markers (mean of 26) and a total map length of 487 cM. In short, map construction was carried out according to Jensen et al. (2005) using the Haldane mapping function of the software JoinMap 3 (Van Ooijen and Voorrips 2001).

QTL analysis

QTL analysis was performed with MapQTL version 4.0 (Van Ooijen and Maliepaard 1996) using the multiple QTL model (MQM). Automatic cofactor selection (backward elimination, $P < 0.02$) was used for the detection of significantly associated markers as cofactors. LOD significance threshold levels were determined using 1,000 permutations. The two LOD support interval represents the distance between two map positions given in centiMorgan (cM) obtained at LOD scores 2 units lower than the maximum score (Van Ooijen and Maliepaard 1996).

To assess coincidence of QTL for seed yield components, the linkage map was divided into 5 cM intervals (bins). Assuming independence of QTL locations, presence or absence of a QTL within an interval was used to determine coincidence of QTL. Chi-square goodness of fit tests were applied to test for random or non-random coincidence of QTL.

Table 1 Phenotypic characterisation of the seed yield and fertility traits (abbreviations and scale units in parenthesis) assessed in a glasshouse based on a lattice design with four replicates of the 184 F2 genotypes from the VrnA mapping population

Trait	Heading date (GDD[a])		Plant height (PH; cm)		Length of panicle (LPa; cm)		Number of panicles per plant (NPa)		Flag leaf length (FLL; cm)	Flag leaf width (FLW; mm)	Seed yield per panicle (SYPa; g)	Seed yield per plant (SYP; g)
Heritability	0.71		0.68		0.72		0.56				0.81[b]	0.85[b]
Year	2004	2005	2004	2005	2004	2005	2004	2005	2005	2005	2004	2004
Gen var[c]	8,993.0**(190)	46,908.0**(189)	45.1**(190)	101.1**(188)	4.0**(190)	9.3**(189)	229.0**(190)	870.0**(189)	7.8**(189)	0.3**(189)	1.6**(189)	1.5**(190)
Repeatability	0.65	0.76	0.44	0.49	0.50	0.63	0.41	0.53	0.56	0.46	0.65	0.63
Mean value	775.38	652.24	92.66	105.06	17.92	21.31	56.95	76.18	22.15	5.82	0.05	1.68
Maximum	1,031.00	1,031.75	110.56	131.25	23.97	29.16	101.07	182.00	31.26	7.23	0.20	6.54
Minimum	409.75	84.00	70.52	65.08	11.34	11.56	0[d]	0.51	13.80	4.50	0[d]	0[d]
LSD[e]	96.25	169.66	10.56	14.36	2.79	3.23	25.07	38.84	3.42	0.77	0.04	1.87

[a] GDD was determined as the sum of $(T_{max} + T_{min}/2) - T_{base}$ from February 1 until heading, where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature and T_{base} is the basal temperature set at 3°C, defined as the temperature below which development of perennial ryegrass does not progress

[b] Calculation based on 40 extreme values

[c] Genotypic variance components, degree of freedom is given in parenthesis

[d] Negative adjusted mean values were considered as 0

[e] Least significant difference at $P < 0.05$

** $P < 0.01$

Table 2 Product moment correlation coefficients for pair wise comparisons of seed yield components based on 184 VrnA F2 genotypes evaluated on single spaced plants in the glasshouse using four replicates per genotype

Trait	Year	Heading date (GDD)		Plant height (PH)		Length of panicle (LPa)		Number of panicles per plant (NPa)		Flag leaf length (FLL)	Flag leaf width (FLW)	Seed yield per panicle (SYPa)	Seed yield per plant (SYP)
		2004	2005	2004	2005	2004	2005	2004	2005	2005	2005	2004	2004
GDD	2004	–											
	2005	0.731**	–										
PH	2004	−0.404**	−0.324**	–									
	2005	−0.401**	−0.476**	0.556**	–								
LPa	2004	−0.186**	-0.122^{ns}	0.597**	0.234**	–							
	2005	−0.341**	−0.328**	0.478**	0.674**	0.600**	–						
NPa	2004	−0.309**	−0.276**	0.372**	0.350**	0.153*	0.290**	–					
	2005	-0.114^{ns}	−0.260**	0.064^{ns}	0.509**	0.017^{ns}	0.397**	0.471**	–				
FLL	2005	0.143*	0.084^{ns}	0.084^{ns}	0.341**	0.049^{ns}	0.389**	0.156*	0.317**	–			
FLW	2005	−0.311**	−0.247**	0.191**	0.329**	0.136^{ns}	0.399**	0.048^{ns}	0.127^{ns}	0.241**	–		
SYPa	2004	0.053^{ns}	0.123^{ns}	0.145*	0.01^{ns}	0.125^{ns}	0.042^{ns}	−0.144*	−0.252**	0.062^{ns}	0.026^{ns}	–	
SYP	2004	-0.139^{ns}	-0.051^{ns}	0.355**	0.244**	0.177*	0.294**	0.269**	0.019^{ns}	0.240**	0.155*	0.726**	–

^{ns} $P > 0.05$; * $P < 0.05$; ** $P < 0.01$

Table 3 Path analysis showing direct (diagonal, bold) and indirect effects of heading date (GDD), plant height (PH), number of panicles (NPa), length of panicles (LPa), flag leaf length (FLL), flag leaf width (FLW) and seed yield per panicle (SYPa) on seed yield per plant (SYP) in 184 VrnA F2 genotypes evaluated on single spaced plants in the glasshouse using four replicates per genotype

Traits	Heading date (GDD)		Plant height (PH)		Length of panicle (LPa)		Number of panicles per plant (NPa)		Flag leaf length (FLL)	Flag leaf width (FLW)	Seed yield per panicle (SYPa)	
	LSM[a] 04/05	AM[b] 04	LSM[a] 04/05	AM[b] 04	LSM[a] 04/05	AM[b] 04	LSM[a] 04/05	AM[b] 04	AM[b] 05	AM[b] 05	LSM[a] 04/05	AM[b] 04
GDD	**−0.06**	**−0.03**	−0.05	−0.06	−0.01	0.01	−0.06	−0.10	0.01	−0.01	0.08	0.04
PH	0.03	0.01	**0.10**	**0.14**	0.02	−0.04	0.09	0.12	0.03	0.01	0.06	0.11
LPa	0.02	0.01	0.06	0.09	**0.04**	**−0.06**	0.06	0.05	0.03	0.01	0.06	0.10
NPa	0.02	0.01	0.04	0.05	0.01	−0.01	**0.20**	**0.32**	0.03	0.00	−0.18	−0.11
FLL	−0.01		0.03		0.01		0.06		**0.10**	0.01	0.05	
FLW	0.02		0.03		0.01		0.02		0.02	**0.02**	0.02	
SYPa	−0.01	0.00	0.01	0.02	0.00	−0.01	−0.05	−0.05	0.01	0.00	**0.77**	**0.82**

[a] Least square mean; estimated using ANOVA for both evaluation years

[b] Adjusted means: estimated using lattice analysis of a replicated experiment in a single year

Results

Phenotypic characterisation

The assessed traits showed considerable variation within the VrnA mapping population. Lattice analyses revealed highly significant ($P < 0.01$) genotypic variance components for all traits (Table 1). Repeatabilities ranged from 0.41 for the trait number of panicles per plant (NPa) evaluated in 2004 to 0.76 for heading date (GDD) in 2005. Average seed yield per plant (SYP) was 1.68 g per plant with a maximum of 6.54 g per plant. Detailed description of the mean and maximum values as well as the least significant difference (LSD) is shown in Table 1.

Analysis of variance revealed significant ($P < 0.01$) variance components for genotypes as well as genotype × year interactions for all traits (data not shown). Trait heritabilities were moderate for NPa ($h^2 = 0.56$), high for plant height (PH), GDD, and length of panicles (LPa) ($h^2 = 0.68$–0.72) and highest for seed yield per panicle (SYPa) and SYP with h^2 estimates of 0.81 and 0.85, respectively.

The trait values between different evaluation years were positively correlated for the traits GDD (0.731, $P < 0.01$), PH (0.556, $P < 0.01$), NPa (0.471, $P < 0.01$) and LPa (0.600, $P < 0.01$; Table 2). Interestingly, GDD was negatively correlated to all other traits with two non-significant exceptions, namely flag leaf length (FLL) and SYPa. The target trait SYP showed no significant correlation to GDD,

Table 4 Detailed description of QTL for seed yield and fertility traits observed in the F2 mapping population VrnA

Trait	Year	Linkage group (LG)	Position	Two LOD support interval (cM)	LOD	% expl Vp	Closest marker (distance to the QTL maximum in cM)
Heading date (GDD)	2004	3	14.3	11.6–14.7	7.8	7.5	LpRGA5 (0.0)
		4	33.3	31.5–35.5	22.3	27.8	vrn-1 (0.0)
		6	23.5	22.7–25.6	3.3	3.5	B1A8 (0.0)
		7	40.3	38.5–40.8	12.9	13.7	vrn2_2 (0.0)
	2005	3	14.3	11.6–14.7	3.8	4.6	LpRGA5 (0.0)
		4	33.3	31.5–35.5	13.1	20.3	vrn-1 (0.0)
		7	40.3	38.5–40.8	16.5	25.9	vrn2_2 (0.0)
Plant height (PH)	2004	2	67.4	57.6–75.9	5.3	10.4	P15M47_159 (5.0)
		3	14.7	13.1–15.1	7.1	9.8	B1A2 (0)
		7	40.3	38.5–40.8	18.8	27.9	vrn2_2 (0.0)
	2005	1	31.9	28.0–33.6	3.0	5.3	M4213 (0.0)
		3	14.3	11.6–14.7	7.4	13.7	LpRGA5 (0.0)
		7	40.3	38.5–40.8	4.7	8.3	vrn2_2 (0.0)
Panicle length (LPa)	2004	2	35.6	30.1–36.9	4.0	7.8	SHOOT (0.0)
		3	28.1	25.0–29.0	6.9	10.4	G04_064 (0.0)
		6	21.8	20.0–22.7	5.6	8.1	G01_086 (0.0)
		7	38.5	37.1–39.0	10.0	15.3	P15M48_261 (0.0)
	2005	1	26.1	22.1–26.3	2.5	3.0	PR25 (0.0)
		2	11.0	5.0–16.7	3.5	4.5	LpRGA6 (0.0)
		2	35.6	35.1–38.9	7.8	14.4	SHOOT (0.0)
		3	6.2	2.8–7.2	3.2	5.6	LpRGA4 (0.0)
		4	19.7	15.0–20.0	4.5	5.4	G05_139 (0.0)
		5	39.0	36.7–40.4	5.5	10.5	P15M47_174 (0.0)
		7	40.3	38.5–40.8	9.6	13.1	vrn2_2 (0.0)
Number of panicles per plant (NPa)	2004	1	36.1	33.6–38.6	3.7	10.8	DLF27 (0.0)
		3	10.1	7.2–11.6	3.3	4.3	P15M49_188 (0.0)
		5	29.8	25.7–30.8	3.7	4.7	G05_044 (0.0)
		6	25.6	23.5–26.9	4.2	7.9	P16M47_241 (0.0)
		6	39.1	32.6–44.1	3.7	13.0	P16M47_150 (0.0)
		7	37.1	34.2–38.5	3.7	4.3	P15M48_159 (0.0)
	2005	–	–	–	–	–	–
Flag leaf length (FLL)	2005	2	43.5	42.0–43.7	7.6	11.1	G02_049 (0.0)
		4	0.0	0.0–3.3	9.4	13.4	G05_015 (0.0)
		4	33.3	31.5–35.5	8.4	11.8	vrn-1 (0.0)
		6	25.6	23.5–26.9	8.0	16.7	P16M47_241 (0.0)
Flag leaf width (FLW)	2005	1	36.1	34.3–38.6	2.8	3.7	DLF027 (0.0)
		4	33.3	31.5–35.5	3.7	6.5	vrn-1 (0.0)
		5	29.8	28.4–30.8	10.5	21.7	G05_044 (0.0)
		7	34.2	31.6–35.8	3.8	7.0	P15M48_152 (0.0)
Seed yield per panicle (SYPa)	2004	1	26.3	23.6–26.4	22.7	41.3	PRE (0.0)
		2	21.7	16.7–22.6	2.91	18.0	LpRGA7 (0.9)
Seed yield per plant (SYP)	2004	1	26.3	23.6–26.4	12.3	24.6	PRE (0.0)
		2	21.7	23.2–31.0	6.7	32.8	LpRGA7 (0.9)

Results are based on a multiple QTL model (MQM) mapping using MapQTL and a genetic linkage map based on 97 anonymous and 85 gene associated DNA markers

a low, but significant correlation with PH, NPa, LPa, FLL, and a close correlation with SYPa (0.73, $P < 0.01$). PH showed significant positive correlation coefficients with LPa, in particular within the same evaluation years (0.60, $P < 0.01$ in 2004 and 0.67, $P < 0.01$ in 2005, respectively). The dimension of the flag leaf, which was assessed in 2005, was moderately correlated to PH and LPa from 2005 (0.33–0.40, $P < 0.01$).

Path-coefficient analysis provided information about direct and indirect effects of the examined characteristics on SYP (Table 3). The highest direct positive effect on SYP was exhibited by SYPa (0.77–0.82). Likewise, the direct effects of NPa (0.20–0.32) and PH (0.10–0.14) were considerably small. A low negative effect on SYP was detected for GDD (−0.03 to −0.06). Indirect effects were generally negligible, except for the small positive effects conferred by SYPa via PH (0.06–0.11) and LPa (0.06–0.10).

QTL results

For GDD, two large QTL effects on LG 4 and 7 as well as a QTL on LG 3 were fully coincident in both 2004 and 2005 (Table 4). A minor QTL on LG 6 explained 3.5% of the phenotypic variation (Vp) in 2004 and was not identified in 2005. The markers derived from putative vernalisation gene orthologues (*VRN1* and *Hd1*) from *Triticum monococcum* and *Oryza sativa* co-localized with QTL on LG 4 and LG 7.

A similar consistency among evaluation years was observed for QTL affecting PH on LG 3 and 7, explaining 9.8 and 27.9% Vp in 2004, and 13.7 and 8.3% Vp in 2005, respectively. Although the QTL on LG 3 is identified by two different markers in 2004 and 2005, their positions are only 0.4 cM apart, indicating that the QTL is identical. Additional minor QTL in each evaluation year were found on LG 2 in 2004 explaining 10.4% Vp and on LG 1 in 2005 explaining 5.3% Vp.

For LPa, four QTL were detected in 2004 and seven in 2005, two of which mapped on the same LG within a 2 cM interval in both years. One was located at the candidate gene-derived marker SHOOT on LG 2 explaining 7.8 and 14.4% Vp in 2004 and 2005, respectively. The second QTL for LPa on LG 7 explained 13.1–15.3% Vp and differed 1.8 cM in its position between the 2 years (Fig. 1).

QTL for NPa explaining 4.3–13% Vp were detected on LG 1, 3, 5, 6 and 7 in 2004, but not in 2005. For FLL, QTL of moderate effects (11.1–16.7% explained Vp) were obtained on LG 2, 4 and 6, whereas a QTL explaining a large effect of 21.7% Vp was found for flag leaf width closely linked to the SSR marker G05_044.

Significant QTL for SYPa and SYP were observed on LG 1 and LG 2, co-locating for both traits at position 26.3 cM on LG 1 and 21.7 cM on LG 2. For SYPa, a high

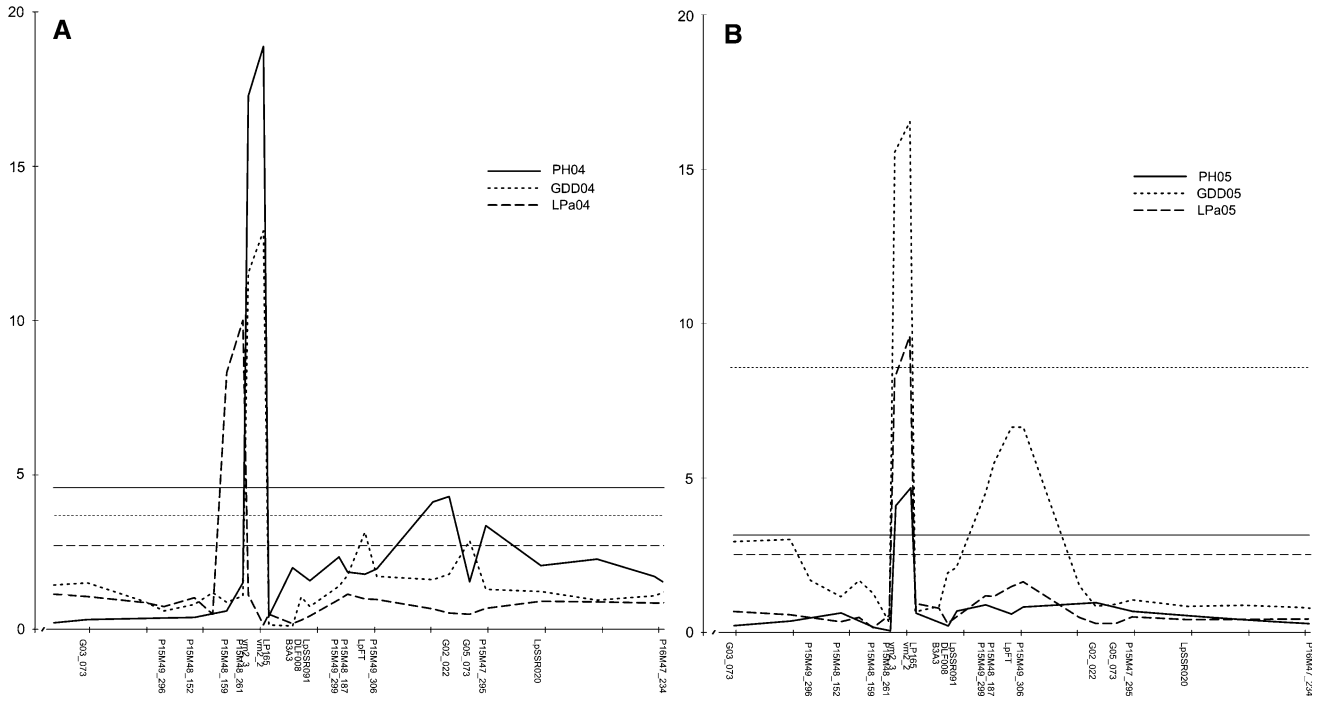

Fig. 1 Genetic linkage map and LOD profiles of multiple QTL model mapping on linkage group (LG) 7 for heading date (GDD; *dotted line*), plant height (PH; *solid line*) and panicle length (LPa; *dashed line*) assessed on 184 F2 genotypes of the VrnA mapping population in 2004 (**a**) and 2005 (**b**). The *horizontal lines* indicate the LG specific significance threshold of the corresponding trait

proportion (41.3%) of observed Vp was explained by the QTL on LG 1, revealing the highest LOD value at the map position of the SSR marker PRE. The QTL on LG 2 explained 18.0% of Vp and was closely linked to the CAPS marker LpRGA7 with a distance of 0.9 cM. Similar results were found for SYP QTL with 24.6 and 32.8% explained Vp for both LGs.

To illustrate the relationship of QTL for GDD, PH and LPa on LG 7 (Fig 1), the mean values of the marker classes identifying the QTL maximum were compared (Table 5). Plants belonging to genotype class hh at the marker vrn2_2 were early flowering and showed high values of PH and LPa. Similarly, the kk class, whose alleles were derived from the late flowering Danish ecotype Falster, was associated with reduced PH. The mean values of the three marker classes hh, hk and kk were significantly different ($P < 0.01$) for all traits. An exception was the PH assessment in 2005, in which hh and hk could not be distinguished at $P = 0.05$. Similar results were found for the marker P15M48_261, explaining the highest Vp for LPa in 2004 (Table 5).

Discussion

Consistency of QTL for seed yield components

Seed yield is a complex trait affected by many genes and environmental interactions. Therefore, QTL mapping, a key tool for complex trait dissection (Holland 2007), often suffers from low percentages of explained Vp resulting in non-consistent QTL detection across environments and years. Nevertheless, the present study revealed significant consistencies of QTL results across years, e.g., for the traits heading date (GDD) and plant height (PH), between traits affecting similar morphological traits such as PH and panicle length (LPa) as well as between the highly correlated traits seed yield per panicle (SYPa) and seed yield per plant (SYP). Heading date, for example, is a highly heritable trait ($h^2 = 0.71$) as generally reported in earlier studies (Fang et al. 2004; Yamada et al. 2004) and revealed considerable genetic variation in the VrnA mapping population, which was originally developed to study vernalisation response in ryegrass (Jensen et al. 2005). As a consequence, QTL assigned to CAPS markers derived from *TmVRN1* and *TmVRN2* homologues on LG 4 and 7 explained large proportions (up to 27.8%) of observed Vp and co-located for both years. In addition they mapped to the similar chromosome regions as QTL found in previous heading date studies in VrnA (Jensen et al. 2005).

Similarly, a chromosome region affecting the developmental pattern of large, vigorous plants was detected for the traits PH and LPa around the *TmVRN2* derived marker vrn2_2 on LG7. This QTL mapped within a 1.8 cM interval for both traits in each year (explaining 7–28% Vp) and reflected the high phenotypic correlation found between these traits, illustrating that tall plants often exhibit longer panicles. Moreover, the QTL for PH and LPa found in 2005 on LG 1 mapped to chromosome regions known to contain QTL for PH and LPa in perennial ryegrass (Yamada et al. 2004) and QTL for PH in annual ryegrass (Inoue et al. 2004).

The number of fertile tillers per plant is one of the traits largely affected by agricultural practices and environment due to the ability of grasses to compensate for tiller density in field swards (Bahmani et al. 2003; Garay et al. 1999). Thus, several studies reported a highly significant genotype × environment interaction (Ergon et al. 2006; Fang et al. 2004) or significant replicate effects (Yamada et al. 2004). Even when assessed on single spaced plants under constant glasshouse conditions, NPa revealed the lowest heritability ($h^2 = 0.56$) of all plant characters investigated. As a

Table 5 Mean trait values of the marker classes identifying QTL for heading date (GDD), plant height (PH) and panicle length (LPa) on linkage group (LG) 7 estimated on 184 F2 genotypes of the VrnA perennial ryegrass mapping population

Trait	Year	Position (cM)	Marker (segregation type)	Mean value of the marker class			SD[b]
Heading date (GDD; [a])	2004	40.3	vrn2_2 (hh, hk/kh, kk)	700.12[1]	756.90[2]	838.26[3]	116.69
	2005	40.3	vrn2_2 (hh, hk/kh, kk)	462.72[1]	650.99[2]	785.59[3]	221.54
Plant height (PH; cm)	2004	40.3	vrn2_2 (hh, hk/kh, kk)	98.69[1]	94.43[2]	85.29[3]	7.94
	2005	40.3	vrn2_2 (hh, hk/kh, kk)	108.02[1]	107.48[1]	98.22[3]	11.37
Panicle length (LPa; cm)	2004	38.5	P15M48_261 (h-, kk)	17.51[1]	19.21[2]		2.26
	2005	40.3	vrn2_2 (hh, hk/kh, kk)	22.76[1]	21.68[2]	19.20[3]	3.12

[a] GDD was determined as the sum of $(T_{max} + T_{min}/2) - T_{base}$ from February 1 until heading, where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature and T_{base} is the basal temperature set at 3°C, defined as the temperature below which development of perennial ryegrass does not progress

[b] Standard deviation

[1,2,3] Trait values with variable numbers are significantly ($P < 0.05$) different

consequence, none of the QTL on LG 1, 3, 5, 6 and 7 detected in 2004 could be reproduced in the following year.

Relationship among seed yield components and their effect on total seed yield per plant

Phenotypic correlation and path coefficients allowed the determination of direct and indirect effects of seed yield components on SYP. Heading date reflects flower initiation and is associated with pollen availability during stigma maturity, both affecting seed set efficiency. However, in the present study heading date had no significant effect on SYPa and SYP. The stable environmental conditions in the glasshouse as well as the experimental design with four completely randomised replications balanced the effect of early and late flowering genotypes. Moreover, GDD was negatively correlated to PH (-0.48 to -0.32, $P < 0.01$) and to a lesser extent to LPa (-0.34 to -0.19, $P < 0.01$). Similar observations that prostrate growth habit is associated with late flowering has been previously described (Shah et al. 1990; Yamada et al. 2004). Indeed, alleles of the markers vrn2_2 and P15M48_261 identifying the QTL for GDD, PH, and LPa on LG7, which were associated with late flowering of the Falster ecotype, displayed inverse effects on PH and LPa. This indicates pleiotropic effects of one gene or close linkage of a few major genes in these regions, inversely affecting both heading date and growth characteristics. A consistent QTL for LPa on LG 2 was associated with the position of the gene orthologue to *SHOOT1* from *Glycine max*, describing an interesting genome region for the identification of allelic sequences associated with length growth. An impact of PH on SYP has previously been reported in *Festuca pratensis* Huds. (Fang et al. 2004). However, path analysis of the present ryegrass data revealed negligible indirect and only small direct positive effects of PH (0.10–0.14) and LPa (-0.06 to 0.04) on SYP.

Fang et al. (2004) were among the first demonstrating a significant relationship between flag leaf length and seed yield in forage grasses by means of path coefficient analysis. In annual cereals, the transportation of assimilates from the flag leaf to the reproductive plant tissues during grain filling is well established (Blake et al. 2007; Lopes et al. 2006). Such competitive resource allocation between plant organs might even be more pronounced in perennial grass species because seed development has to compete for assimilates with perennial vegetative organs serving as sinks. However, path analysis revealed low direct effects of FLL (0.10) and FLW (0.02) on SYP, suggesting that the competition for assimilates is not of major importance for seed production in VrnA. An explanation might be optimal fertilisation applied during the experiment minimizing the competition for nutrients, or that the panicle itself is more important for resource allocation. Indeed, seed abortion in perennial ryegrass was found at all floret positions, i.e., did not increase from the basal to the distal florets (Elgersma and Śnieżko 1988) and is rather due to lack of fertilization than to competition for nutrients and assimilates (Marshall and Ludlam 1989).

Path analysis clearly showed that SYPa was the most important component trait affecting total seed yield with a direct effect on SYP of 0.77–0.82. Similarly, the product moment correlation of phenotypic data between SYP and SYPa (0.79, $P < 0.01$) was larger than between other traits under investigation. Similar results were found in the closely related grass species *F. pratensis* Huds., where path coefficient analysis identified panicle fertility to be a major trait contributing to SYP (Fang et al. 2004). Moreover, Peng et al. (1999) concluded for rice that a seed yield increase is associated with tillers revealing high fertility and seed set abilities.

Impact of self incompatibility on seed yield

The low number and large genetic effects of QTL detected for SYP was unexpected, since this trait is known to be highly complex. Co-location of the two major QTL for both SYPa and SYP on LG 1 and LG 2 suggested the presence of a common genetic mechanism affecting these traits. The markers most closely linked to the respective QTL were highly distorted ($P < 0.0001$) in VrnA. Significant segregation distortion is indicative for self incompatibility (SI) loci, and has been used to map SI genes in rye (Fuong et al. 1993). In ryegrass like in rye, SI is mediated by a gametophytic system mainly based on two loci (Cornish et al. 1979). In a F2 population design based on full sib progenies of an initial cross between completely unrelated grandparents (as used to generate VrnA), nine out of 16 combinations among F1 genotypes lead to distorted F2 progenies (Sh1, ESM), affecting linkage tests, the estimation of genetic distances and the order of markers on the same LG (Zhu et al. 2007).

Selected F1 genotypes not sharing common S and Z alleles reveal an average of 75% compatible pollen-stigma combinations among F2 progeny (Sh2, ESM). Assuming one common S or Z allele in F1, the average of compatible pollen–stigma combinations is reduced to 62% for F2 individuals heterozygous at the loci with the common allele (75% for homozygous individuals) (Sh3, ESM). This discrepancy between hetero- and homozygous genotypes increases with the number of common SI alleles and can reach up to 44% in case the F1 mapping parents differ in only one SI allele (Sh4, ESM). Indeed, the QTL for SYPa and SYP in VrnA were reflecting the degree of heterozygosity at SI loci, as heterozygous F2 individuals exhibit the maximum number of different SI alleles and, therefore,

showed a significantly ($P < 0.01$, data not shown) reduced seed set. As a consequence, the major QTL for SYPa and SYP co-locate with S and Z loci in ryegrass, which map to LG 1 and LG 2, respectively (Thorogood et al. 2002).

In contrast to the hypothesis that already some compatible pollen in the pollen cloud will be sufficient to achieve full seed yield, our results indicate that substantial amounts of incompatible pollen in the cloud significantly reduced SYPa and SYP. However, it is noteworthy that the genetic impact of the pollen cloud composition on seed yield was quantitative, overlaid by environmental and biological mechanisms leading to the generally high seed abortion rate of obligate outbreeding species.

Breeding for high seed yielding forage grasses

For commercial breeding and seed production purposes, the genetic characterisation of SI alleles of future cultivars by means of functional markers for S and Z loci (Andersen and Lübberstedt 2003) is of major interest in order to avoid SI-mediated seed yield losses. This is of particular importance for second cycle breeding or synthetics based on few components strongly selected for traits that are linked to S and Z, thereby narrowing the diversity of SI alleles.

Generally, the present study substantiate that seed set is of major importance for total seed yield. It should thus be possible to breed for efficient realisation of seed yield potential rather than to increase the size of the reproductive system with possible negative effects on forage performance. Molecular markers associated with traits controlling seed yield and fertility may substantially contribute to genetically improve seed yield of future ryegrass cultivars.

References

Andersen JR, Lübberstedt T (2003) Functional markers in plants. Trends Plant Sci 8:554–560

Andersen JR, Jensen LB, Asp T, Lübberstedt T (2006) Vernalization response in perennial ryegrass (*Lolium perenne* L.) involves orthologues of diploid wheat (*Triticum monococcum*) *VRN1* and Rice (*Oryza sativa*) *Hd1*. Plant Mol Biol 60:481–494

Armstead IP, Skøt L, Turner LB, Skøt K, Donnison IS, Humphreys MO, King IP (2005) Identification of perennial ryegrass (*Lolium perenne* (L.)) and meadow fescue (*Festuca pratensis* (Huds.)) candidate orthologous sequences to the rice *Hd1*(*Se1*) and barley *HvCO1 CONSTANS*-like genes through comparative mapping and microsynteny. New Phytol 167:239–247

Bahmani I, Thom ER, Matthew C, Hooper RJ, Lemaire G (2003) Tiller dynamics of perennial ryegrass cultivars derived from different New Zealand ecotypes: effects of cultivar, season, nitrogen fertiliser, and irrigation. Aust J Agric Res 54:803–817

Bean EW (1972) Clonal evaluation for increased seed production in two species of forage grasses, *Festuca arundinacea* Schreb. and *Phleum pratense* L. Euphytica 21:377–383

Blake NK, Lanning SP, Martin JM, Sherman JD, Talbert LE (2007) Relationship of flag leaf characteristics to economically important traits in two spring wheat crosses. Crop Sci 47:491–496

Brazauskas G, Pašakinskienė I (2008) SNP genotyping for axillary tiller formation in perennial ryegrass. In: Studer B, Lübberstedt T (eds) Proceedings of XXVIIth EUCARPIA symposium on improvement of fodder crops and amenity grasses, Copenhagen, KU LIFE (in press)

Bugge G (1987) Selection for seed yield in *Lolium perenne* L. Plant Breed 98:149–155

Cattani DJ, Gjuric R, Feindel DE, Miller PR, Smith SR (1997) Management requirements for dryland creeping bentgrass seed production. In: Proceedings of the 18th international grassland congress, Saskatoon, pp 51–52

Charlesworth D (1989) Why do plants produce so many more ovules than seeds? Nature 338:21–22

Chastain TG, Grabe DF (1989a) Spring establishment of orchardgrass seed crops with cereal companion crops. Crop Sci 29:466–471

Chastain TG, Grabe DF (1989b) Spring establishment of turf-type tall fescue seed crops with cereal companion crops. Agron J 81:488–493

Chastain TG, Young WC, Garbacik CJ, Quebbeman BM (1995) Post-harvest residue management for perennial ryegrass seed crops. Agronomy Abstracts. American Society of Agronomy, Madison

Chastain TG, Kiemnec GL, Cook GH, Garbacik CJ, Quebbeman BM, Crowe FJ (1997) Residue management strategies for Kentucky bluegrass seed production. Crop Sci 37:1836–1840

Cornish MA, Hayward MD, Lawrence MJ (1979) Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. Heredity 43:95–106

Devos KM (2005) Updating the 'crop circle'. Curr Opin Plant Biol 8:155–162

Doebley J, Stec A, Gustus C (1995) *teosinte branched1* and the origin of maize: Evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Elgersma A (1990) Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). Plant Breed 105:117–125

Elgersma A, Śnieżko R (1988) Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). Euphytica 39:59–68

Elgersma A, van Wijk A (1997) Breeding for higher seed yield in grasses and forage legumes. In: Fairey DT, Hampton JG (eds) Forage seed production. 1 Temperate species. CAB International, Oxon, pp 243–272

Entz MH, Smith SR, Cattani DJ, Storgaard AK (1994) Influence of post-harvest residue management and cultivar on tiller dynamics and seed yield in timothy. Can J Plant Sci 74:507–513

Ergon Å, Fang C, Jørgensen Ø, Aamlid TS, Rognli OA (2006) Quantitative trait loci controlling vernalisation requirement, heading time and number of panicles in meadow fescue (*Festuca pratensis* Huds.). Theor Appl Genet 112:232–242

Fang C, Aamlid TS, Jørgensen Ø, Rognli OA (2004) Phenotypic and genotypic variation in seed production traits within a full-sib family of meadow fescue. Plant Breed 123:241–246

Fuong FT, Voylokov AV, Smirnov VG (1993) Genetic studies of self-fertility in rye (*Secale cereale* L.). 2. The search for isozyme marker genes linked to self-incompatibility loci. Theor Appl Genet 87:619–623

Garay AH, Matthew C, Hodgson J (1999) Tiller size/density compensation in perennial ryegrass miniature swards subject to differing defoliation heights and a proposed productivity index. Grass Forage Sci 54:347–356

Holland JB (2007) Genetic architecture of complex traits in plants. Curr Opin Plant Biol 10:156–161

Humphreys MW, Yadav RS, Cairns AJ, Turner LB, Humphreys J, Skøt L (2006) A changing climate for grassland research. New Phytol 169:9–26

Inoue M, Gao ZS, Cai HW (2004) QTL analysis of lodging resistance and related traits in Italian ryegrass (*Lolium multiflorum* Lam.). Theor Appl Genet 109:1576–1585

Jensen LB, Andersen JR, Frei U, Xing Y, Taylor C, Holm PB, Lübberstedt T (2005) QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat *VRN1*. Theor Appl Genet 110:527–536

Lopes MS, Cortadellas N, Kichey T, Dubois F, Habash D, Araus J (2006) Wheat nitrogen metabolism during grain filling: comparative role of glumes and the flag leaf. Planta 225:165–181

Marshall C, Ludlam D (1989) The pattern of abortion of developing seeds in *Lolium perenne* L. Ann Bot 63:19–28

Marshall AH, Wilkins PW (2003) Improved seed yield in perennial ryegrass (*Lolium perenne* L.) from two generations of phenotypic selection. Euphytica 133:233–241

Martin J, Storgaard M, Andersen CH, Nielsen KK (2004) Photoperiodic regulation of flowering in perennial ryegrass involving a *CONSTANS*-like homolog. Plant Mol Biol 56:159–169

Peng S, Cassman KG, Virmani SS, Sheehy J, Khush GS (1999) Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci 39:1552–1559

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org

Schejbel B, Jensen LB, Asp T, Xing Y, Lübberstedt T (2008) Mapping of QTL for resistance to powdery mildew and resistance gene analogues in perennial ryegrass (*Lolium perenne* L.). Plant Breed (in press)

Shah SG, Pearson CJ, Read JW (1990) Variability in habit, flowering and seed production within the Kangaroo Valley cultivar of *Lolium perenne* when grown in a range of environments. Aust J Agric Res 41:901–909

Stephenson AG (1981) Flower and fruit abortion: Proximate causes and ultimate functions. Annu Rev Ecol Syst 12:253–279

Studer B, Asp T, Frei U, Hentrup S, Meally H, Guillard A, Barth S, Muylle H, Roldán-Ruiz I, Barre P, Koning-Boucoiran C, Uenk-Stunnenberg G, Dolstra O, Skøt L, Skøt K, Turner L, Humphreys M, Kölliker R, Roulund N, Nielsen KK, Lübberstedt T (2008) Expressed sequence tag-derived microsatellite markers of perennial ryegrass (*Lolium perenne* L.). Mol Breed 21:533–548

Sutherland S, Delph LF (1984) On the importance of male fitness in plants: Patterns of fruit-set. Ecology 65:1093–1104

Thorogood D, Kaiser WJ, Jones JG, Armstead I (2002) Self-incompatibility in ryegrass 12. Genotyping and mapping the S and Z loci of *Lolium perenne L.* Heredity 88:385–390

Utz HF (2000) PLABSTAT: a computer program for statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart

Van Ooijen JW, Maliepaard C (1996) MapQTL™ version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Van Ooijen JW, Voorrips RE (2001) JoinMap® 3.0. Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

van Wijk A (1980) Breeding for improved herbage and seed yield in *Setaria sphaecelata* (Schumach). In: Hubbard SA (ed) Agricultural Research Report 900, Wageningen

Wilkins PW (1995) Independence of dry matter yield and leaf yield among perennial ryegrass varieties differing in seasonal yield distribution. Grass Forage Sci 50:155–161

Wright S (1923) Correlation and causation. J Agric Res 20:557–587

Yamada T, Jones ES, Cogan NOI, Vecchies AC, Nomura T, Hisano H, Shimamoto Y, Smith KF, Hayward MD, Forster JW (2004) QTL Analysis of morphological, developmental, and winter hardiness-associated traits in perennial ryegrass. Crop Sci 44:925–935

Zhu CS, Wang CM, Zhang YM (2007) Modeling segregation distortion for viability selection I. Reconstruction of linkage maps with distorted markers. Theor Appl Genet 114:295–305