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Genetic Relationships Among Soluble Carbohydrates, Anthocyanins and Growth Characteristics in *Leymus* (Gramineae) Detected with Molecular Markers

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Abstract: Low-temperature soluble carbohydrate accumulations are commonly associated with anthocyanin coloration, attenuated growth and cold adaptation of cool-season grasses. The *vrn-1* gene has potent effects on vernalization requirement, growth, and soluble carbohydrate accumulations of the winter-annual Triticeae species. Two hundred and four unmapped AFLP markers and genome-specific DNA markers genetically linked to the *vrn-1* gene were used to detect QTL controlling soluble carbohydrate accumulations, anthocyanin coloration and growth characteristics in a segregating population derived from open pollinated *Leymus cinereus* × *L. triticoides* hybrids. These perennial Triticeae grasses are distinguished by adaptation and growth habit. As expected, positive trait correlations and pleiotropic gene effects were detected for soluble carbohydrate accumulations and anthocyanin coloration. Likewise, positive trait correlations and pleiotropic gene effects were detected for tillering, leaf development, leaf growth, regrowth and rhizome spread. However, soluble carbohydrate accumulations were not associated with attenuated growth. In fact, several DNA marker alleles, including one near *vrn-Xm1*, had positive effects on soluble leaf carbohydrate concentrations and low temperature growth. The corresponding DNA marker near *vrn-Ns1* had more specific effects on tillering. We speculate that *vrn-1* exerts quantitative effects on low-temperature soluble leaf carbohydrate accumulations and growth habit of the perennial *Leymus*. However, a number of other DNA markers displayed highly significant effects on soluble carbohydrate accumulations and various growth characteristics. Findings indicate that anthocyanin coloration may be a useful phenotypic marker for soluble carbohydrate accumulation. Although variation for soluble carbohydrates was not associated with attenuated growth in this population, this trait was under genetic control.

Key words: *Leymus*; molecular markers; genetic relationship

Species of genus *Leymus* are cool-season perennial grasses with circumpolar distribution. North American representatives of this genus include *Leymus triticoides* (creeping or beardless wildrye) and *L. cinereus* (basin wildrye). Aggressive rhizomes and adaptation to poorly drained alkaline sites, primarily within the western United States, characterize *L. triticoides*. *L. cinereus* is a tall, caespitose species widely distributed from British Columbia to Saskatchewan in the North (Canada) and California to Arizona and Colorado in the South (United States). *Leymus* is one of several perennial Triticeae genera that have been used for alien gene transfer to wheat[1]. Although most attention has been given to salinity tolerance and disease resistance, *Leymus* is also valued for tolerance to low temperature, freezing, and ice encasement[2]. Hybrids of *Leymus* and wheat may also provide a useful perennial grain crop in cold regions that are not suitable for conventional crops[3]. Although wild *Leymus* grasses do not have directly economic importance comparable to major food crops such as wheat, species of this genus are important for land reclamation across their native range. Beach wildrye is a circumpolar species complex, including North American *L. mollis* and Eurasian *L. arenarius*, uniquely suited for stabilization of erosion prone sites in high northern latitudes such as Alaska and Iceland, respectively[4,5].

Most cool-season grasses reversibly synthesize and store water-soluble fructan and sucrose during the fall and spring[6-8]. These soluble carbohydrates (CHO) can be rapidly metabolized to facilitate plant growth under fluctuating low temperatures or supply a profusion of fructose through periplasmic space as a rapid response to freezing[9]. Although fructan may not serve directly as cryoprotectant, fructan accumulation during fall may provide the primary carbon source for synthesis of sucrose during winter[7,8,10]. Sucrose accumulates during the coldest winter months[7,8] and is the most abundant cyroprotective solute in the cytosol[11].

The accumulation of nonstructural carbohydrates is thought to occur when growth is attenuated and photosynthate demand decreases[7]. The cold hardening of winter rye is dependent on irradiance and day length, which evidently control the partitioning of photoassimilation between growth and frost tolerance[12]. Genetic studies of segregating spring- and winter-annual wheat[13] and barley[14] indicate that fructan accumulations are closely associated with vernalization requirement, which also controls growth habit. However, genetic differences in photosynthe-

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sis[15-20], enzyme activity[21], or translocation between shoots and roots[22] may also contribute to variability in the accumulation of soluble CHOs. In some cases, the accumulation of soluble CHOs cannot be ascribed to anatomical or morphological differences[21].

The accumulation of anthocyanin pigments is also associated with low-temperature exposure and changes in light intensity in monocots[17,23,24] and dicots[25]. Anthocyanins may modulate light intensity and photosynthesis during color accumulation (CA)[24]; however, anthocyanins per se do not impart freezing tolerance and their role in CA is unclear[17,25]. The anthocyanin synthesis pathway is well characterized and several early steps involve cold-inducible genes including phenylalanine ammonialyase and chalcone synthase[23,25]. Studies also suggest that anthocyanin synthesis and/or chalcone synthase gene expression are directly induced by sugars[26-28] and are associated with the accumulation of soluble CHO[29,30]. Therefore, anthocyanins are useful phenotypic markers for genetic and molecular studies of plant gene expression during cold stress and acclimation[23] and may facilitate selection of genotypes with higher concentrations of soluble CHO[29,30]. However, the genetic basis for this putative association of anthocyanin coloration and soluble carbohydrate accumulations has not been well characterized.

Genetic variation in vernalization requirement controls major differences in the growth, development (e.g. tillering, branch angle and reproductive flowering) and low-temperature soluble CHO accumulations between spring- and winter-annual forms of wheat, barley, and rye. The orthologous *vrn-1* genes on wheat 5A, 5D, 5B, rye 5R, and barley 5H chromosomes are the most potent genes reducing the vernalization requirements of spring-annual forms of these Triticeae species[13,14,31-33]. The perennial Triticeae grasses generally display vernalization requirements similar to winter-annual forms, which are fixed for the recessive *vrn-1* alleles. However, genetic variation for low-temperature soluble CHO accumulations has also been observed among winter-annual or perennial cool-season grasses[10,15,21,29,34]. Moreover, many closely related forms of the perennial Triticeae also display different growth habits related to tillering and branch angle (e.g. caespitose, stoloniferous, and rhizomatose growth habits). Genetic variation in growth habit and low-temperature soluble leaf CHO accumulations are important components of plant establishment, reproduction, cold-hardiness and general adaptation of the cool-season grasses.

The aim of this study was to use molecular genetic markers to elucidate genetic relationships between low-temperature soluble CHO accumulations, anthocyanin coloration, growth and development in a population of 132 second-generation progeny (replicated clones) of open pollinated *Leymus cinereus* × *L. triticoides* hybrids, with particular attention to the *vrn-1* gene. Both *L. cinereus* and *L. triticoides* are self-incompatible, allotetraploids (2n = 4x = 28). Hybrids of *L. cinereus* and *L. triticoides* generally form 14 bivalents at metaphase Ⅰ; and subsequent meiotic stages are correspondingly regular[35].

1 Materials and Methods

1.1 Plant materials

The *Leymus cinereus* × *L. triticoides* study population traces back to multiple crosses between the Acc:636 (*L. cinereus*) and Acc:641 (*L. triticoides*) accessions. Seeds for the Acc:636 plants, used as male parents, were received from the Agriculture Research Centre at Lethbridge, AB and presumably originated from Alberta or Saskatchewan. Seeds for the Acc:641 plants, used as female plants, were collected by Dr. Kay H. Asay near Jamieson, OR. The resulting hybrids were open pollinated thru two generations to produce an F_1OP_2 population The F_1OP_2 study population was grown from seed in cone containers and transplanted to the Utah State University Greenville Farm (North Logan, UT) in March, 1998. This population displayed wide variation for green and purple (anthocyanin) coloration during early spring growth. Tillers from each of 66 green and 66 purple F_1OP_2 selections were each cloned in two 6-cm wide cone containers, along with two clones from each of six Acc:641 plants and six Acc:636 plants taken from field plots of the original parent populations established in 1987. These plants were normalized in a greenhouse maintained between a daytime high of 25 ℃ and a nighttime low of 15 ℃ for approximately three months until all plants displayed a green, healthy appearance.

1.2 Low temperature soluble CHO accumulations, anthocyanin coloration, and growth

All leaves and tillers were counted and measured immediately before and after the growth chamber study. Clones were arranged in two randomized complete blocks (RCB design) and maintained under normal nutrient fertility conditions in growth chambers from Dec. 16, 1998 to Jan. 29, 1999. These growth chambers were maintained at 5 ℃ during the night and 10 ℃ during the day, with 11 h of light from a mixture of fluorescent and incandescent bulbs. Subsequent anthocyanin accumulation was rated on a scale of 0 to 9, where 9 indicates the highest observable levels of coloration.

Free and combined ketohexose was estimated by a ketose-specific modification of the anthrone method based on the method of Jermyn[36].

1.3 Rhizome development

Clones were transplanted to a cultivated (barren) field site near the Utah State University Greenville Farm (North Logan, UT) during the spring of 1999. These clones were established on one m centers in RCB design with two replications. The maximum length (l) and width (w) of spread for each clone was measured on April 21, 2000. Spread of each clone was determined by the location at which tillers break above ground level. The area (cm^2) of tiller spread, for each clone, was estimated using the formula for the area of an oval, $(\pi/4)lw$.

1.4 DNA markers

The DNA sequence of the CDO504 RFLP marker[37], near the *vrn-1* gene[13,33], was obtained from the GrainGenes database (http://ars-genome.cornell.edu). Primers designed from the original *Avena* CDO504 DNA clone, e.g. CDO504 F1 & R1 (Table 1), were used to amplify corresponding CDO504 genomic DNA sequences (hereafter aCDO504 sequences) from other cool-season grasses (results below). The resulting aCDO504 sequences were used to design gene specific primers, e.g. aCDO504 F2, R2, and NR (Table 1; results below). Amplification and segregation analysis of these aCDO504 sequences was performed using modifications[38] of the original method described by Saiki *et al*[39], with 60 ℃ annealing temperature for PCR.

The AFLP technique analysis was performed according to the methods of Vos *et al*[40], except that *Eco*RⅠ selective amplification primers were labeled with fluorescent 6-FAM (6-carboxy fluorescein) on the 5′ nucleotide. The AFLP adapter sequences, pre-amplification primer sequences, and selective amplification primer sequences are described in Table 1. The amplified fragments were fractionated and detected with an ABI373XL instrument (PE Applied Biosystems, Foster City, CA) using 34 cm well-to-read denaturing polyacrylamide gels and 1X TBE running buffer. Each sample lane included the GS500-ROX (PE Applied Biosystems) internal lane size standards (labeled with fluorescent rhodamine X) that range from 35 nucleotide base pairs (bp) to 500 bp. Fluorescent signals of the 6-FAM labeled AFLPs, between 35 bp and 500 bp, were identified by GeneScan 3.1 software (PE Applied Biosystems). The GeneScan sample (trace) files were subsequently analyzed for the presence and absence of AFLP products, in one bp intervals, using Genographer[41]. The AFLP fragments were scored as phenotypically dominant DNA markers with four possible genotypic classes (e.g. A = homozygous for *L. triticoides* allele and C = not homozygous for *L. triticoides* allele or B = homozygous for *L. cinereus* allele and D = not homozygous for *L. cinereus* allele).

1.5 Data analysis

The normality of data for each trait was analyzed by the Shapiro-Wilk statistic and calculated using the UNIVARIATE procedure of SAS version 6.12 (SAS Institute Inc., Cary, NC). Pearson correlation coefficients, and corresponding significant values, were calculated using the CORR procedure of SAS. QTL effects for normally distributed traits (e.g. soluble carbohydrates) were individually tested by analysis of variance, using the SAS GLM procedure, treating each marker as an independent class variable (fixed effect) and trait means (over two reps) for each of the 132 F_1OP_2 clones as dependent variables. Similarly, traits lacking normal distribution were tested by the nonparametric Kruskal-Wallis statistic for Wilcoxon rank sums, using the NPAR1WAY procedure of SAS.

Sequence divergence between aCDO504 marker sequences was calculated as p using PAUP version 4.0b4a (Sinauer Associates, Inc.). Dendograms that summarize phylogenetic relationships between genome specific aCDO504 marker sequences were developed using NTSYS-pc, version 2.0 (Exeter Software, Setauket, NY) using unweighted pair-group method, arithmetic average (UPGMA) cluster analysis. Bootstrap confidence levels (BCL) for the UPGMA cluster analysis of aCDO504 marker sequences were calculated using PAUP.

Table 1 Nucleotide sequence of AFLP adapters and PCR primers

Type	Primer code	Sequence
*Eco*RⅠ adapter		5′-CTCGTAGACTGCGTACC-3′
		3′-CATCTGACGCATGGTTAA-5′
*Mse*Ⅰ adapter		5′-GACGATGAGTCCTGAG-3′
		3′-TACTCAGGACTCAT-5′
*Eco*RⅠ + 1 primer	E01	5′-GACTGCGTACCAATTCA-3′
*Eco*RⅠ + 3 primers	E37	5′-GACTGCGTACCAATTCACG-3′
	E41	5′-GACTGCGTACCAATTCAGG-3′
*Mse*Ⅰ + 1 primer	M02	5′-GATGAGTCCTGAGTAAC-3′
*Mse*Ⅰ + 3 primers	M48	5′-GATGAGTCCTGAGTAACAC-3′
	M49	5′-GATGAGTCCTGAGTAACAG-3′
	M59	5′-GATGAGTCCTGAGTAACTA-3′
	M60	5′-GATGAGTCCTGAGTAACTC-3′
	M61	5′-GATGAGTCCTGAGTAACTG-3′
	M62	5′-GATGAGTCCTGAGTAACTT-3′
CDO504	F1	5′-AAGAAGATGGTGATGCCCAC-3′
	R1	5′-ATGAATGCAAAGCCACCAAT-3′
aCDO504	F2	5′-CGATGCTATGGCACAAGGT-3′
	R2	5′-CGGTGCTGGTGAAGAGAACT-3′
	NR	5′-GCCACAGTGTAGCACCTAAAC-3′

2 Results and Discussion

2.1 Overall trait means and correlations

The *L. triticoides* and *L. cinereus* parental populations displayed relatively large differences for all traits, whereas the segregating F_1OP_2 population generally showed intermediate trait means and greater variation compared to the parental populations (Table 2). Measurements of the same trait on the clonally replicated plants were significantly correlated (see diagonal of Table 3) and reflected at least some genetic control for these

Table 2 Trait means (SD) for the *L. triticoides*, *L. cinereus*, and segregating F_1OP_2 populations

Trait	*L. triticoides*	*L. cinereous*	F_1OP_2
	$n = 6$	$n = 6$	$n = 132$
New tillers[a]	13.6 (2.0)	0.4 (1.3)	2.7 (2.7)
New leaves[a]	22.9 (3.6)	1.8 (2.9)	10.1 (6.2)
Leaf growth[a] (cm)	125 (63)	16 (20)	96 (60)
Percent soluble CHO[a]	27.0 (4.2)	8.6 (2.6)	25.4 (8.4)
Color[a]	1.5 (0.5)	0.4 (0.2)	2.4 (1.6)
Regrowth[b] (mg dry wt.)	153 (56)	58 (34)	232 (229)
Rhizome development[c] (cm^2)	3 477 (2 375)	96 (43)	317 (244)

a, accumulation after 45 d controlled 10 ℃ light (11 h) and 5 ℃ dark (13 h) diurnal cycle (potted clones); b, regrowth after clipping (potted clones); c, rhizome spread after one year of field establishment.

variable traits. Genetic control of anthocyanin accumulations was also evident in the correlation between anthocyanins coloration during the growth chamber evaluations and anthocyanin coloration previously observed in the field during the early spring growing season of 1998 ($r = 0.55^{***}$). However, for some traits (e.g. number of new tillers), low correlations for measurements of the same trait between replications also reflect a substantial amount of stochastic variation, measurement error, or environmental effect (Table 2).

As expected tiller proliferation and rhizome development was much greater in the *L. triticoides* species (Table 2). However, a relatively low correlation between these traits (e.g. tiller proliferation and rhizome development) was observed in the segregating F_1OP_2 population (Table 3). *L. triticoides* also displayed higher growth trait means, e.g. number of new leaves, leaf growth (measured as total length of all leaves), and regrowth. Total leaf elongation was highly dependent on the number of new leaves, which in turn was highly dependent on the number of new tillers in the F_1OP_2 population (Table 3). However, the overall trait correlation between the number of new tillers and total leaf elongation was relatively less (Table 3), suggesting that the number of new leaves and leaf elongation per se contributed independently to total leaf elongation. Interestingly, the biomass of regrowth after clipping, a useful forage trait, was highly dependent on total leaf growth (cm) before clipping (Table 3).

Surprisingly, soluble CHO accumulations were higher in the *L. triticoides* species, which generally showed stronger growth characteristics under low temperatures (Table 2). Anthocyanin coloration was also higher in *L. triticoides* (Table 2) and showed significant correlation with soluble carbohydrate accumulations. However, soluble CHO accumulations were not correlated with most of the other growth attributes observed in *L. triticoides* except leaf growth (Table 3).

2.2 *vrn-Xm1* and *vrn-Ns1* DNA markers

The CDO504 F1 and R1 primers (Table 1), designed from the original Avena CDO504 RFLP marker (DNA clone), were used to amplify corresponding sequences from *Agropyron* and *Leymus*. The 740 bp *Agropyron* and 742 bp *Leymus* aCDO504 sequences both included 326 bp of apparent exon sequences that were highly homologous to the original CDO504 clone. However, the combination of CDO504 F1 and R1 primers failed to amplify aCDO504 sequences from other Triticeae species, including diploid *Psathyrostachys* which carries the Ns genome of *Leymus* (i.e. *Psathyrostachys* represents one of the diploid ancestors of *Leymus*). The second set of primers, aCDO504 F2 and R2 (Table 1), was designed from sequences conserved between the *Agropyron* and *Leymus* aCDO504 sequences and the original CDO504 clone. These primers (aCDO504 F2 and R2) were used to amplify corresponding aCDO504 sequences from *Poa*, *Psathyrostachys*, *Haynaldia*, *Hordeum*, and the other diploid ancestor of *Leymus* (Fig.1).

The "Steptoe" (AF236869) and "Morex" (AF236887) aCDO504 sequences displayed a single nucleotide polymorphism (SNP) at nucleotide 329 of these GenBank accessions, which destroys a highly conserved *Bst*UⅠ recognition site present in the other Triticeae aCDO504 sequences. Segregation analysis of this restriction site difference, in the "Steptoe"×"Morex" doubled haploid mapping population[42], confirmed that these "Steptoe" and "Morex" aCDO504 sequences are allelic to the CDO504 RFLP locus. Therefore, similar aCDO504 markers sequences from the perennial Triticeae (including those of *Leymus*) are most likely orthologous to the aCDO504 *vrn-H1* marker allele of *Hordeum* and the original CDO504 RFLP marker from *Avena* (Fig.1).

The *Leymus* sequence amplified by the CDO504 F and R primers (Table 1) is relatively unique compared to corresponding aCDO504 sequences of *Agropyron*, *Haynaldia*, *Hordeum*, or *Psathyrostachys* (Fig.1). Because *Psathyrostachys* is one of the known ancestors of allotetraploid *Leymus*, these amplification products (e.g. aCDO504.X) evidently correspond to the "unknown" *Leymus-Xm* genome[43]. The aCDO504.X sequences from the Acc.636 *L. cinereus* accession contained a SNP, at nucleotide 235 (see AF230517), which destroys a *Dpn*Ⅱ recognition site conserved in the corresponding intron sequences of other Triticeae aCDO504 sequences. Segregation analysis of the codominant aCDO504.X. *Dpn*Ⅱ restriction fragments were used as a genome specific DNA marker for the *vrn-Xm1* locus in the F_1OP_2 population

Table 3 Correlations of measurements overall correlations among rep means of different traits (lower left) in the segregating *L. cinereus* × *L. triticoides* F_1OP_2 population

	New tillers[a]	New leaves[a]	Leaf growth[a] (cm)	Percent soluble CHO[a]	Color[a] (0 – 9)	Regrowth[b] (mg)	Rhizome spread[c] (cm^2)
New tillers[a]	1						
New leaves[a]	0.73***	1					
Leaf growth[a]	0.37***	0.76***	1				
Soluble CHO[a]	NS	NS	0.23**	1			
Color[a]	NS	NS	0.23**	0.39***	1		
Regrowth[b]	0.20*	0.43***	0.70***	NS	NS	1	
Rhizome spread[c]	0.27**	0.31***	0.31***	NS	NS	0.25**	1

Pearson correlation coefficients: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS < not significant.
a, accumulation after 45 d controlled 10 ℃ light (11 h) and 5 ℃ dark (13 h) diurnal cycle (potted clones); b, regrowth after clipping (potted clones); c, rhizome spread after one year of field establishment.

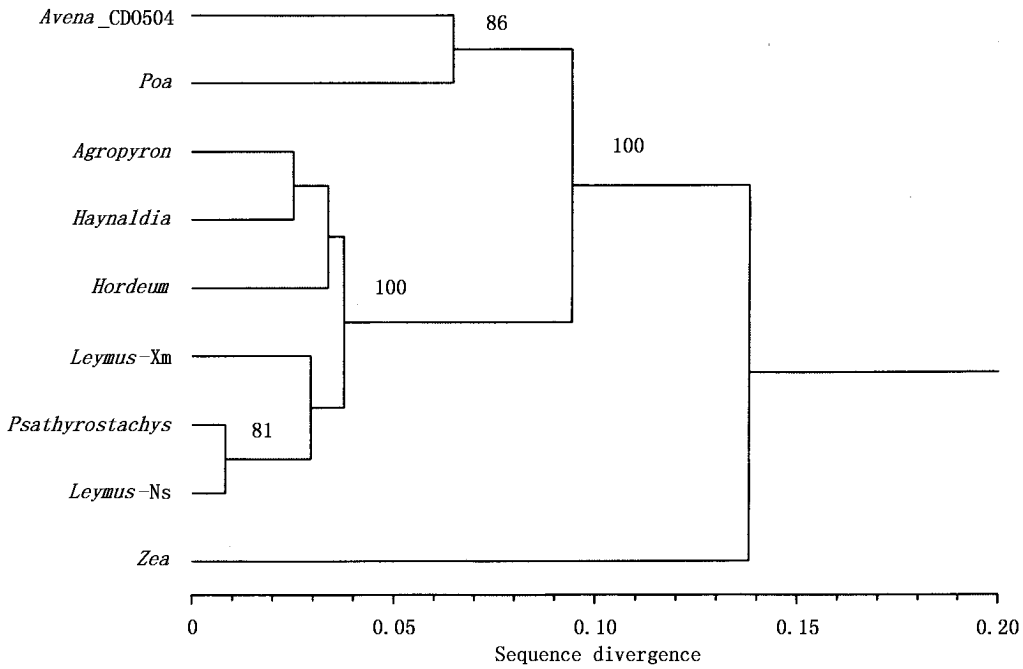

Fig.1. UPGMA analysis of the putative *vrn-1* marker sequences, including the original CDO504 clone (RFLP probe) from *Avena*, a homologous EST from *Zea* (AA979945), and the aCDO504 exon sequences from *Poa* (AF249677), *Psathyrostachys* (AF241509), *Leymus* Ns (AF241511), *Agropyron* (AF241512), *Leymus* Xm (AF230517), *Haynaldia* (AF241510), and *Hordeum* (AF236869). Allelism of the *Hordeum* (AF236869) sequence and CDO504 RFLP locus, near the *Sh*2 (*vrn-H1*) locus on barley chromosome 7(5H), was confirmed by STS-PCR analysis. Bootstrap confidence levels are indicated for clades present in the 50% major-rule consensus tree.

(results below).

Comparison of the aCDO504 sequences of the various Triticeae species was used to design the *Psathyrostachys* specific aCDO504 NR primer. The combination of CDO504 F1 and aCDO504 NR primers amplified the aCDO504.N sequence (AF241511) from the Acc #641 *L. triticoides* accession, which is almost identical to the aCDO504 sequence of *Psathyrostachys* (Fig.1). Therefore, this sequence evidently corresponds to the *Leymus-Ns* genome[43]. However, the CDO504 F1 and aCDO504 NR primer pair does not amplify DNA from the *L. cinereus* Acc #636 accession. Segregation analysis of this *L. triticoides* aCDO504-Ns sequence was used as a genome specific DNA marker for the *vrn-Ns1* locus in the F_1OP_2 population (results below). All aCDO504 sequences (see Fig.1) were submitted to GenBank for immediate release.

2.3 AFLP markers

Seven AFLP primer combinations (E37M60, E37M62, E41M48, E41M49, E41M59, E41M60, and E41M61) yielded 204 polymorphisms that effectively distinguish the two parental sample populations of *L. triticoides* and *L. cinereus* (i.e. dominant allele fixed in one accession and absent in the other accession). Although these markers are suitable for QTL detection, recombination frequencies and linkage map order could not be determined using the F_1OP_2 population. A total of 175 other AFLPs were also variable within the parental accessions, however were not considered suitable for QTL detection.

2.4 QTL detection

Of the 204 AFLPs that effectively distinguish the *L. triticoides* and *L. cinereus* parental accessions, 21 markers had highly significant effects ($P < 0.01$) on at least one trait (Table 4). In addition to these 21 putative QTL markers, the aCDO504.X and aCDO504.N genome specific markers both showed highly significant effects on at least one trait (Table 4). Although significant correlations between AFLP markers were detected (Fig.2), interpretation of these coefficients is not well understood. As expected, the highest marker correlations were between *cis*-dominant AFLPs. However, meaningful associations between *trans*-dominant markers, such as the allelic aCDO504.X.Lt and aCDO504.X.Lc fragments, were also detected (Fig. 2). Therefore, DNA markers showing highly significant effects were arranged in ten putative QTL groups (Table 4), each comprised of markers having highly significant correlations coefficients (e.g. $r > 0.2$), in the same order shown on Figure 2. The effects of these QTLs are largely congruent with differences between the parental species (Table 2) and the overall trait correlations in the segregating F_1OP_2 population.

The *L. triticoides* marker alleles for QTLs 2, 3, 4, 5, and 7 (Table 4) had positive effects on tillering, which might be expected from a highly rhizomatous species. However, only two QTLs (Table 4) had significant effects on rhizome spread, one of which (e.g. QTL 10) did not affect tillering. The *L. triticoides* marker allele for QTL 2 had positive (pleiotropic) effects on tillering, leaf number, leaf length, soluble carbohydrate accumulation, regrowth, and rhizome spread (Table 4). Relatively strong tillering effects of QTL 3 were associated

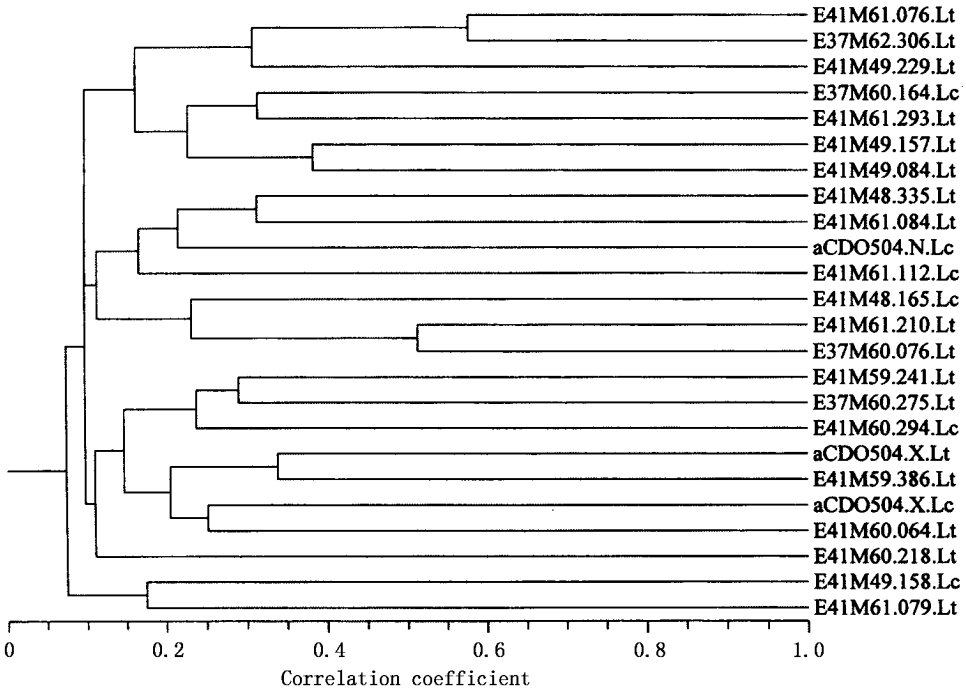

Fig.2. UPGMA analysis of phi coefficients between AFLP markers, as a measure of genetic association (e.g. linkage) between markers in the F_1OP_2 population. Coefficients greater than 0.18, 0.22, and 0.28 are significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 4 DNA markers showing highly significant ($P < 0.01$) effects for at least one trait in the segregating *L. cinereus* × *L. triticoides* F_1OP_2 population. Effects measured as the *L. cinereus* genotypic class mean subtracted from *L. triticoides* genotypic class mean.

Marker	Marker allele	QTL	New tillers[a]	New leaves[a]	Leaf growth[a] (cm)	Percent soluble CHO[a]	Color[a] (0 – 9)	Regrowth[b] (mg)	Rhizome spread[c] (cm^2)
E41M61.076	Lt	1	NS	– 2.5*	– 41**	– 3.4*	– 0.8*	– 190***	NS
E37M62.306	Lt	1	NS	– 2.8**	– 51***	NS	NS	– 243***	NS
E41M49.229	Lt	1	NS	NS	– 26**	NS	NS	– 125*	NS
E37M60.164	Lc	21.3*	4.6**	52**	NS	NS	122*	126*	
E41M61.293	Lt	21.2*	3.6**	33**	4.4**	NS	NS	NS	
E41M49.157	Lt	2	1.3**	3.1**	NS	NS	NS	124**	95*
E41M49.084	Lt	2	1.4***	1.9*	NS	NS	NS	NS	106**
E41M48.335	Lt	3	NS	3.1**	NS	NS	NS	NS	NS
E41M61.084	Lt	3	1.3*	4.1***	28*	NS	NS	NS	NS
aCDO504.N	Lt	3	1.7***	3.2**	NS	NS	NS	NS	NS
E41M61.112	Lc	4	2.6**	5.3***	NS	NS	NS	NS	NS
E41M48.165	Lc	5	1.7*	5.0**	NS	NS	NS	NS	NS
E41M61.210	Lt	5	2.1***	2.7*	NS	NS	NS	NS	NS
E37M60.076	Lt	5	2.0***	3.2*	NS	NS	NS	NS	NS
E41M59.241	Lt	6	NS	NS	– 34**	NS	NS	– 114**	NS
E37M60.275	Lt	6	NS	– 2.9**	NS	NS	NS	NS	NS
E41M60.294	Lc	6	NS	NS	NS	NS	0.8**	NS	NS
E41M59.386	Lt	7	1.2**	2.4*	NS	NS	NS	NS	NS
aCDO504.X	Lt/Lc	7	1.3*	5.4**	49*	5.8*	NS	NS	NS
E41M60.064	Lt	7	NS	2.2*	25*	NS	NS	107**	NS
E41M60.218	Lt	8	NS	NS	29**	NS	NS	100*	NS
E41M49.158	Lc	9	NS	NS	35**	NS	NS	70*	NS
E41M61.079	Lt	10	NS	NS	NS	NS	NS	NS	124**

Effects: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS < not significant.
a, accumulation after 45 d controlled at 10 ℃ in light (11 h) and at 5 ℃ in dark (13 h) diurnal cycle (potted clones); b, regrowth after clipping (potted clones); c, rhizome spread after one year of field establishment.

with aCDO504.N and two other *cis* dominant linked *L. triticoides* marker alleles (Table 4). Although the latter QTL is probably associated with the *vrn-Ns1* region of chromosome 5 NS, this does not necessarily mean the *vrn-Ns1* gene is controlling this QTL effect. However, the pleiotropic effects of QTL 7, associated with the codominant aCDO504.X marker, are somewhat more analogous to that of the *vrn-1* gene. The latter QTL displayed pleiotropic, recessive effects on tillering, leaf number, total leaf length, soluble carbohydrates, and regrowth. Soluble carbohydrate accumulations, and prostrate growth habit, are also associated with the recessive *vrn-1* genes of winter wheat, winter barley, and winter rye. Therefore, we speculate that effects of QTL 7 (Table 4) may be associated with the *vrn-Xm1* gene. However, two other QTLs (Table 4) also had significant effects on soluble carbohydrate accumulations.

Most of the remaining QTLs (e.g. 1, 6, 8, and 9) were similar in that these markers had pleiotropic effects on total leaf growth (length) and regrowth. Unlike leaf growth effects of QTLs 2, 3, and 7 these QTLs (e.g. 1, 6, 8, and 9) evidently enhanced leaf elongation rather than the total number of new leaves. The positive alleles of QTLs 1, 6, 8, and 9 were divided among *L. cinereus* and *L. triticoides* (Table 4). Interestingly the most prominent leaf growth and regrowth effects were associated with the *L. cinereus* allele of E37M63.306 (QTL 1). The latter QTL also had pleiotropic effects on soluble carbohydrate accumulation and anthocyanin coloration. Therefore, the effects of QTLs 1 and 6 may be associated with the tall stature and larger leaves of caespitose *L. cinereus*.

3 Conclusions

Phenotypic variation for low-temperature soluble carbohydrate accumulations, anthocyanin coloration, and growth in winter-hardy grasses, such as *L. cinereus* and *L. triticoides*, may be attributed (in part) to the segregation of multiple QTLs with different effects. As expected, positive trait correlations and pleiotropic gene effects were detected for soluble carbohydrate accumulations and anthocyanin coloration. However, soluble carbohydrate accumulations were not associated with attenuated growth. In fact, several DNA marker alleles, including one near *vrn-Xm1*, had positive effects on soluble leaf carbohydrate concentrations and low temperature growth. We speculate that *vrn-1* exerts quantitative effects on low-temperature soluble leaf carbohydrate accumulations and growth of the perennial *Leymus* wild ryes. However, a number of other DNA markers displayed highly significant effects on soluble carbohydrate accumulations. Not surprisingly, the highly rhizomatous *L. triticoides* parental species contributed positive QTL alleles for tillering and rhizome development. However, tillering and rhizome development was weakly correlated in the segregating F_1OP_2 population and only one QTL had pleiotropic effects on tillering and rhizome development. Several other QTLs affected tillering or rhizome development, but not both.

Efforts are underway to develop a *Leymus* RFLP map to identify the chromosomal loci associated with low-temperature soluble carbohydrate accumulations and other important characteristics such as tillering, regrowth after clipping, and rhizome development. Although anthocyanin coloration may be a useful phenotypic marker for soluble carbohydrate accumulations, molecular markers may provide more effective selection pressure for genetic manipulation of this trait. Moreover, identification of the genes controlling these traits may enable genetic manipulations impossible through conventional plant breeding.

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用分子标记研究禾本科赖草属植物可溶性碳水化合物、色素积累和生长特性之间的遗传相关性

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摘要： 在低温条件下，冷季草的可溶性碳水化合物通常与紫色素的积累、生长的缓慢性及对寒冷的适应性具有相关性。*Vrn-1* 基因对一年生冬性禾本科物种的春化、生长和可溶性碳水化合物的积累有重要作用。本研究以 *Leymus cinereus* × *L. triticoides* 的杂种 F_1 开放授粉获得的 F_2 群体为材料，用 204 个未定位的 AFLP 分子标记和几个基因组特定的与 *vrn-1* 相连锁的 DNA 标记检测了控制可溶性碳水化合物的积累、紫色素的积累和生长特性等几个数量性状的 QTL。根据生长特性和适应性可将 *Leymus cinereus* 和 *L. triticoides* 区分开来。研究表明可溶性碳水化合物与紫色素的积累呈正相关，而且有关基因对这两种性状具有多效性。与之相类似，分蘖、叶发育、叶生长、草被剪后的再生长和地下茎的蔓延性这些性状之间也呈正相关，控制这些性状的基因具有多效性。但是可溶性碳水化合物的积累与生长的缓慢性无相关性。有几个分子标记包括与 *vrn-Xm1* 邻近的一个分子标记对叶可溶性碳水化合物的浓度和低温生长具有正效应。而与 *vrn-Ns1* 邻近的一个 DNA 标记对分蘖具有更加特别的效应。我们推测 *vrn-1* 对多年生赖草低温下叶可溶性碳水化合物的积累及生长习性具有数量效应。发现几个 DNA 标记对可溶性碳水化合物的积累及多个生长特性有较强的作用。研究结果暗示在赖草中紫色素的积累可作为可溶性碳水化合物积累的形态标记。

关键词： 赖草属；分子标记；遗传相关性

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