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ORIGINAL PAPER

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Construction of a comparative RFLP map of *Echinochloa crus-galli* toward QTL analysis of flooding tolerance

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Abstract To analyze quantitative trait loci (QTLs) affecting flooding tolerance and other physiological and morphological traits in *Echinochloa crus-galli*, a restriction fragment length polymorphism (RFLP) map was constructed using 55 plants of the F_2 population (*E. crus-galli* var. *praticola* × *E. crus-galli* var. *formosensis*). One hundred forty-one loci formed 41 linkage groups. The total map size was 1,468 cM and the average size of linkage groups was 35.8 cM. The average distance between markers was 14.7 cM and the range was 0–37.2 cM. Early comparisons to the genetic maps of other taxa suggest appreciable synteny with buffelgrass (*Pennisetum* spp.) and sorghum (*Sorghum* spp.). One hundred ninty-one F_2 plants were used to analyze QTLs of flooding tolerance, plant morphology, heading date, number of leaves, and plant height. For flooding tolerance, two QTLs were detected and one was mapped on linkage group 24. Other traits, including plant morphology, heading date, number of leaves, and plant height were highly correlated. Three genomic regions accounted for most of the mapped QTLs, each explaining 2–4 of the significant marker-trait associations. The high observed correlation between the traits appears to result from QTLs with a large contribution to the phenotypic variance at the same or nearby locations.

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Introduction

Comparative genetic mapping of closely related species such as wheat and barley or sorghum and maize has revealed conserved gene content and gene order among the species (for recent reviews, see Gale and Devos 1998; Paterson et al. 2000). These maps may allow model species to guide inquiry and provide insights into the genomes and evolution of other species (Paterson et al. 2000). RFLP markers, major genes and QTLs for important traits such as vernalization, flowering time, plant height, seed size and shattering all exhibit an orthologous relationship in barley, wheat, maize and rice (Lin et al. 1995; Paterson et al. 1995; Sarma et al. 1998; Bailey et al. 1999). However, analysis at the DNA sequence level demonstrates that microcolinearity of genes is complicated by small-scale rearrangements and deletions (Chen et al. 1997; Tikhonov et al. 1999). For this reason, Keller and Feuillet (2000) emphasized that a species as closely related as possible to the species of interest should be used for comparative genome analysis.

Echinochloa crus-galli (L.) Beauv. (barnyardgrass) is one of the world's worst weeds, with a worldwide distribution, and consisting of several varieties (Yabuno 1966; Holm et al. 1977). Of these, *E. crus-galli* var. *formosensis* Ohwi and *E. crus-galli* var. *praticola* Ohwi (hereafter, formosensis and praticola, respectively) exhibit physiological and morphological traits that are especially diverse. Formosensis is one of the paddy weeds found in flooded rice fields and exhibits an ability to germinate and grow under anaerobic conditions, whereas praticola inhabits roadsides and fields and requires oxygen for germination (Yamasue et al. 1990; Yamasue 2001; Fukao et al. 2003). These varieties are suitable materials for studying anaerobic germinability and flooding tolerance because they exhibit obvious differences in physiological and morphological traits and they can be crossed with each other to obtain fertile offspring.

Our previous data imply that anaerobic germinability and flooding tolerance in *E. crus-galli* are complex traits and are most likely regulated by several genes (Fukao et al. 2003). Until now, QTL analysis of flooding tolerance has been examined only in rice. Xu and Mackill (1996) reported that a major locus (*Sub1*) for flooding tolerance was located approximately 4 cM from the RFLP marker C1232 on rice chromosome 9. Sripongpangkul et al. (2000) identified several QTLs, including *Sub1*, that control plant elongation and submergence tolerance in rice. However, these studies base the criteria for the degree of flooding tolerance on plant elongation and leaf survival under submergence stress. They do not include anaerobic germinability and seedling growth under anaerobic conditions.

In this study, an RFLP linkage map of *E. crus-galli* for praticola and formosensis was constructed using buffelgrass (*Pennisetum ciliare* (L.) Link., syn. *Cenchrus ciliaris* L.) and other grass probes. In addition, based on anaerobic germinability and growth of seedlings under anoxia, QTLs for flooding tolerance were analyzed. This work includes QTL analysis of other common physiological and morphological traits that are different between formosensis and praticola.

Materials and methods

Genetic stocks

Both formosensis and praticola inbred lines were obtained by self-pollination through about ten generations. Formosensis seeds originated from a plant collected at a fallowed rice field in Uji City, Japan, and praticola was derived from a strain originally collected by Dr. T. Yabuno, Osaka Prefectural University. F_1 seeds of a praticola × formosensis cross were produced by conventional breeding methods. Ten F_1 plants were grown and selfed to collect F_2 seeds. Hybridity of the F_1 individuals was estimated based on intermediate heading date between formosensis and praticola, and hybridity of the F_2 plants was confirmed based on ADH zymograms (Fukao et al. 1998). One hundred ninety-one F_2 seedlings were transplanted to 7.5 l pots in a glasshouse (30°C day, 25°C night) at Texas A & M University, College Station, TX on 6 June 2000. At the time of transplanting, 5 g of osmocote (14-14-14) was provided to each pot. Of 191 F_2 plants, 55 were genotyped using the entire polymorphic RFLP markers for linkage mapping and all 191 were subjected to phenotype and QTL analysis using selected polymorphic markers.

Phenotype analysis

To estimate the degree of flooding tolerance, F_2 seeds were subjected to a germination test under anaerobic conditions. Two hundred F_2 seeds were placed on wet filter paper at 30°C in the light for 5 days in an anaerobic chamber (Forma Scientific, OH, USA) that was flushed continuously with a 90% nitrogen:10% hydrogen gas mixture. Oxygen concentration in the chamber was similar to that in soil under flooding conditions. In fact, germinating seeds of formosensis exhibited identical morphological traits to those germinating in soil of flooded rice fields (i.e., poor elongation of the radicle and pale yellow pigmentation in the coleoptile). Also, praticola seeds did not germinate in the chamber as well as they did in a flooded rice field.

After the germination test, shoot length was recorded. Both germinating and non-germinating seeds were incubated at 30°C in the light in an aerobic chamber for 5 days and 191 surviving seedlings were transplanted into pots and grown under the conditions described above. The morphological and physiological traits recorded for the F_2 plants (f, formosensis; p, praticola) included: plant morphology (f, upright; p, procumbent), heading date (f, neutral; p, short day), number of leaves on the main stem (f, more; p, less) and plant height (f, tall; p, short). Plant morphology was rated on a scale of 1–5 (1 being totally procumbent and 5 being totally upright). For heading date, the date of emergence of the inflorescence on the main stem was recorded and the number of days after transplanting counted. At that point, the number of leaves on the main stem was also counted. Plant height was measured when growth was stopped in the fall. Scores for all physiological and morphological traits were represented by numbers. All of the traits in formosensis exhibited larger values than those in praticola. Averages, standard deviations and phenotype correlation were analyzed using Microsoft Excel 2000.

RFLP analysis

Young leaves were harvested from both parents and each of the F_2 plants, frozen in liquid nitrogen and kept at −80°C until used. For DNA extraction, 3 g of frozen tissue were ground with dry ice in a coffee mill. DNA was extracted from the ground material using a DNeasy plant maxi kit (Qiagen). DNA digestion, blotting and probe preparation by PCR were carried out using published techniques (Chittenden et al. 1994). Approximately 25 ng of PCR-product was labeled with ^{32}P-dCTP using the Megaprime DNA labeling system (Amersham). Hybridization was performed in hybridization buffer containing 6× SSC, 5× Denhardts and 2.5% SDS (w/v) at 65°C overnight in a shaker. Blots were washed at 65°C in 2× SSC and 0.1% SDS for 30 min, 1× SSC and 0.1% SDS for 30 min, and 0.5× SSC and 0.1% SDS for 30 min, and autoradiographs produced using ImagePlus X-ray film (Diagnostic Imaging) with intensifying screens.

Cloning of enzyme genes

Total RNA was extracted from formosensis and praticola seeds (1 g fresh weight), imbibed under anaerobic conditions for 10 h, using Trizol (Invitrogen) and treated with PCR grade DNAse I (Invitrogen). cDNA was prepared from 1 μg of total RNA by the random primer method using a MasterAmp high fidelity RT-PCR kit (Epicentre). The primer sequences synthesized were based on GenBank cDNA sequences of other species such as maize, sorghum, wheat, rice and sugarcane and included: ADH, 5′GTYCTYCCTGTGTTCACTGG3′ and 5′GAAGGTBCCCTTSAGRGTCC3′; aldolase, 5′AGTACTACGMDGCYGGTGCC3′ and 5′WG-

Table 1 RFLP probes used for polymorphism survey for parents of the cross between *Echinochloa crus-galli* varieties

Name	Species	Origin	Attempted probes	Hybridized probes	Hybridization percentage	Polymorphic loci	Polymorphism percentage
Enzyme	*Echinochloa*	cDNA	5	5	100.0	5	100.0
pPAP	Buffelgrass	cDNA	355	306	86.2	95	31.0
pPAS	Buffelgrass	cDNA	286	241	84.3	49	20.3
BNL	Maize	Genomic	3	1	33.3	0	0.0
CSU	Maize	cDNA	132	88	66.7	12	13.6
RG	Rice	Genomic	13	5	38.5	0	0.0
RZ	Rice	cDNA	49	42	85.7	5	11.9
CDO	Oat	cDNA	9	6	66.7	2	33.3
pRC	Sorghum	cDNA	17	13	76.5	0	0.0
Total	–	–	869	707	81.4	168	23.6%

MCCARGCCTTGAGVGTGC3′; and PDC, 5′GCNGGNCC-NATHTTYAAYG3′ and 5′NGTRTCRTCYTTRTGNYC3′. PCR amplification was performed using the primers listed above with cDNA from formosensis (ADH and PDC) and praticola (aldolase) as templates following standard protocols (1st step, 5 min at 95°C; 2nd step, 45 cycles, 30 s- 60 s- 90 s each at 95°C, 40°C and 72°C; 3rd step, 15 min at 72°C) using *Taq* polymerase (Invitrogen). A partial *aldh2* fragment of *Echinochloa oryzicola* was a gift from Dr. Mikio Nakazono, the University of Tokyo, Japan. The enolase probe (*eno*) was isolated previously from *Echinochloa phyllopogon* by RT-PCR in our lab (Fox et al. 1995).

Linkage analysis

DNA from each parent was surveyed with 869 probes derived from *Echinochloa*, buffelgrass, maize, rice, oat and sorghum (Table 1). Of 869 probes, 148 detected 168 polymorphic loci between praticola and formosensis. DNA from 55 F_2 individuals was analyzed with the 148 polymorphic probes. Due to the allo-hexaploid nature of *E. crus-galli*, the probes could detect at least three homologous loci. Each polymorphic fragment per probe was scored by the methods used for allohexaploid oat (O'Donoughue et al. 1995). Linkage groups were constructed using MAPMAKER/EXP version 3.0 and the Kosambi mapping function. Both initial linkage associations and local ordering of markers were based on a significant threshold logarithm of odds (LOD) of 3.0 and a centimorgan distance threshold of 37.25.

QTL analysis

All 191 F_2 individuals were analyzed using polymorphic RFLP loci. To identify putative QTLs and estimate biometrical parameters, two analytical approaches were used, single marker regression and interval mapping, by Qgene (Nelson 1997) and Map Manager QTX (Manly et al. 2001). LOD thresholds of 2.5 and 2.0 were used to detect significant and possible QTLs, respectively (Lander and Botstein 1989; Jiang et al. 2000). Biometric parameters were calculated as described (Paterson et al. 1991). The additive effect was estimated assuming that the number of formosensis alleles influence phenotype in an additive manner. The possible modes of gene action for each QTL were determined based on testing for additive, dominance and recessive models as described in Paterson et al. (1991).

Results

RFLP map of *E. crus-galli*

One hundred sixty-eight loci were analyzed on 55 F_2 plants of praticola × formosensis. Of all the loci, 98 (58.3%) were dominant and 70 (41.7%) were codominant. The segregation ratios of all the loci of the F_2 generation were subjected to a χ^2 test to investigate if they fit to the expected 3:1 (dominant loci) or 1:2:1 (codominant loci) ratios. Nineteen loci (11.3%) mapping to fourteen unlinked genomic regions exhibited segregation distortion ($P<0.05$) (Table 2). One hundred and forty-one loci (83.9%) were linked on 41 linkage groups (Fig. 1) and 27 loci (16.1%) were unlinked. The total map size was 1468 cM and the average size of linkage groups was 35.8 cM. The average distance between markers was 14.7 cM and the range is 0–37.2 cM.

Table 2 RFLP probes that revealed segregation distortion in the F_2 generation. Segregation distortion was judged by a χ^2 test at the 5% level. Buffelgrass *pPAP* and *pPAS* probes are labeled *P* and *S*, respectively. Loci with names followed by a or b correspond to RFLP probes that detected polymorphism at more than one locus on the map

Linkage group	Loci	Observed value	Expected value	*P* value
1	*P10E03*	22:23:10	1:2:1	0.035
1	*S7F02*	32:22	3:1	0.008
2	*P3C08*	24:24:7	1:2:1	0.003
2	*P8C09*	22:24:9	1:2:1	0.030
2	*S4H05*	21:28:6	1:2:1	0.017
3	*S6B12*	12:37:6	1:2:1	0.020
5	*P9H07*	17:34:4	1:2:1	0.010
11	*P1D03*a	34:21	3:1	0.024
13	*S7B04*	30:21	3:1	0.008
22	*P3D08*	33:21	3:1	0.018
24	*P9E02*	33:20	3:1	0.032
34	*P9F07*	1:20:34	1:2:1	0.000
34	*CDO580*a	6:15:33	1:2:1	0.000
39	*P5F11*	4:31:18	1:2:1	0.012
39	*P10H05*	3:32:19	1:2:1	0.004
40	*P9F06*	34:21	3:1	0.024
Unlinked	*P4H03*a	48:6	3:1	0.015
Unlinked	*P4H03*b	33:20	3:1	0.032
Unlinked	*P8H04*	33:21	3:1	0.018

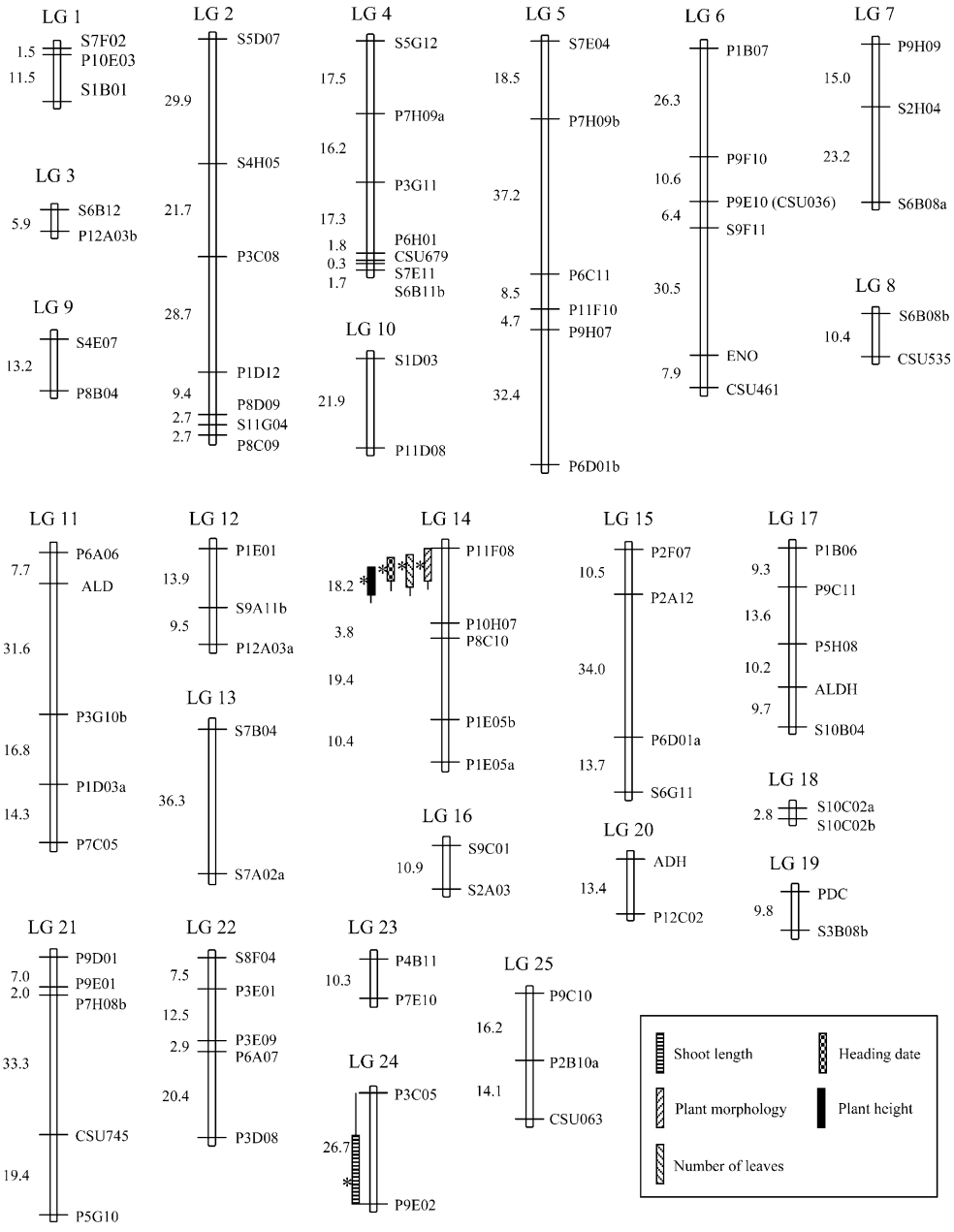

Fig. 1 An RFLP linkage map of *Echinochloa crus-galli* based on the cross, praticola × formosensis, and QTLs affecting physiological and morphological traits. Buffelgrass probes *pPAP* and *pPAS* are labeled *P* and *S*, respectively. Loci with names followed by '*a*' or '*b*' correspond to RFLP probes which detected polymorphism at more than one locus on the map. Loci in *parentheses* indicate that there is no interval (0 cM) between two loci. Maximum likelihood locations (*) and 1-LOD (*box*) and 2-LOD (*line*) likelihood intervals for each QTL are to the *left* of appropriate linkage groups. When the 2-LOD likelihood interval was less than 2 cM, only the maximum likelihood location was shown by an *asterisk*

Across a diversity of probe sources evaluated, cDNA clones from other taxa hybridized to *Echinochloa* DNA in 66.7–86.2% of cases and genomic clones in 33.3–38.5% of cases, reflecting the generally higher level of conservation of cDNAs. RFLP probe polymorphism percentages between formosensis and praticola are shown in Table 1. All enzyme probes from *E. crus-galli* exhibited polymorphism between the parents. Both buffelgrass (*pPAP* and *pPAS*) and oat cDNA probes (*CDO*) detected polymorphism at relatively high levels (31.0%, 20.3% and 33.3%, respectively), although the total number of oat probes used was small. Maize and sorghum cDNA probes exhibited lower hybridization percentages than buffelgrass probes and the polymorphism percentages were low (range 0–13.6%). The hybridization percentage of rice cDNA probes was similar to that of buffelgrass probes, but the polymorphism percentage was only 11.9%.

Loci of five enzymes associated with anaerobic metabolism were mapped on the *E. crus-galli* genome (Fig. 1). Since *E. crus-galli* is allohexaploid and has three genome sets (AABBCC) per cell, it is believed that each enzyme should have a locus on each genome for a total of three loci per whole genome. Southern blot analysis revealed that all enzyme probes hybridized at several locations (data not shown). However, only one polymorphic band between formosensis and praticola was found for each enzyme probe and the other bands proved monomorphic between parents. A locus for each enzyme was located on the *E. crus-galli* genome using an RFLP linkage map.

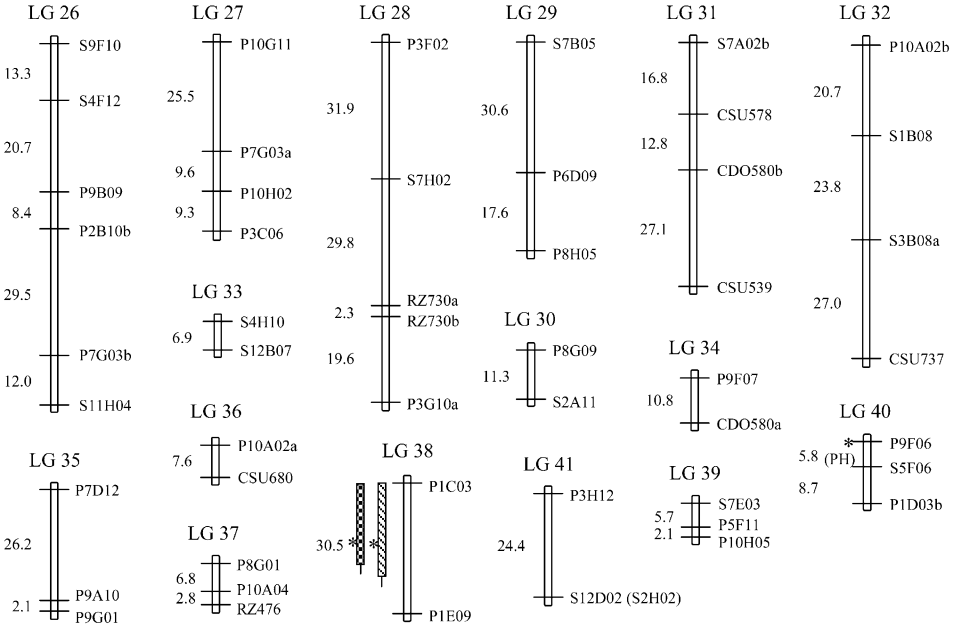

Fig. 1 (continued)

Table 3 Comparison of map location of markers in *Echinochloa*, buffelgrass and sorghum

Echinochloa LG	Buffelgrass Linkage group			Sorghum	
	Male	Female	Syntenic marker	Linkage group	Syntenic marker
4	–	13a	P7H09a, P3G11	J	P7H09a, CSU679
	–	13b	P7H09a, P3G11,	–	–
	–	–	P6H01	–	–
5	17a	–	P11F10	J	P7H09b, P11F10
	–	17a	P6D01b	–	–
	–	17b	P7H09b	–	–
11	–	4b	P3G10b, P1D03a	–	–
	–	5a	P6A06, P3G10b,	–	–
	–	–	P1D03	–	–
14	–	26	P11F08, P10H07	F	P10H07, P1E05a
	–	–	–	–	P1E05b
15	–	17a	P6D01a	–	–
	17b	–	P2F07	–	–
22	24	–	P6A07	–	–
	–	24	P3E09	–	–
23	–	8a	P4B11	–	–
	–	8b	P7E10	–	–
25	–	–	–	C	P9C10, CSU063
27	–	–	–	G	P10G11, P7G03a
35	5a	–	P7D12	A	P7D12, P9G01
	–	5a	P9A10	–	–
	5b	–	P9A10	–	–
37	–	–	–	F	P8G01, P10A04
	–	–	–	–	RZ476
39	1a	–	P5F11, P10H05	–	–
40	–	4a	P9F06, P1D03b	–	–
	–	4b	P9F06, P1D03b	–	–

Comparison among the *Echinochloa*, buffelgrass and sorghum genomes

In view of their close relationship, the *Echinochloa* map was compared with an RFLP map of the buffelgrass (*Pennisetum ciliare*) genome (Jessup et al. 2002, 2003) and the sorghum genome (Bowers et al. 2003). While this level of comparative data does not provide for a detailed alignment of the three genomes, early patterns can be discerned (Table 3). Locus *pPAP6D01* on *Echinochloa* LG 5 and LG 15 was located on female buffelgrasss LG 17a. Also, Locus *pPAP1D03* on *Echinochloa* LG 11 and

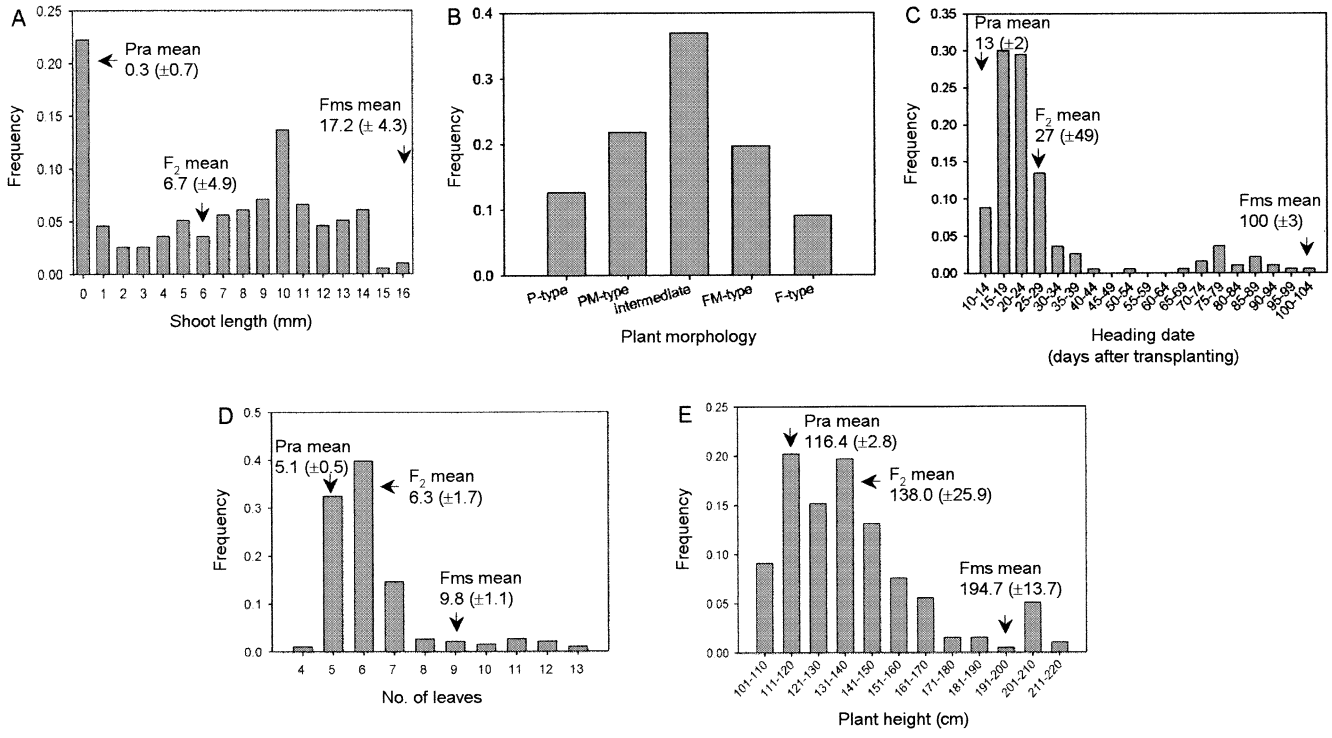

Fig. 2A–E Frequency distribution for physiological and morphological traits in the F_2 progeny. **A** Shoot length, **B** plant morphology, **C** heading date, **D** number of leaves, **E** plant height. Shoot length was recorded 5 days after incubation in an anaerobic chamber. Plant morphology was classified as 5 different types: *P-type*, most procumbent; *PM-type*, partially procumbent; *intermediate*, intermediate between P- and F-type; *FM-type*, partially upright; *F-type*, most upright. Heading date was recorded when emergence of inflorescence was observed on the main stem. The number of leaves on the main stem was counted at heading date. Plant height was the length of a whole plant from soil surface to the top of the panicle and measured when growth was stopped in the fall. Mean values for praticola (*Pra*), formosensis (*Fms*) and the F_2 progeny are indicated

Table 4 Correlations between physiological and morphological traits in the F_2 population of two *E. crus-galli* varieties

	Shoot length	Plant morphology	Heading date	No. of leaves	Plant height
Shoot length	x	–	–	–	–
Plant morphology	0.01	x	–	–	–
Heading date	0.01	0.55**	x	–	–
No. of leaves	0.08	0.47**	0.88**	x	–
Plant height	0.04	0.39**	0.77**	0.75**	x

** Indicates significant correlation at the 1% level

LG 40 corresponded to a locus on female buffelgrass 4b. Locus *pPAP7H09* on *Echinochloa* LG 4 and LG 5 was located on sorghum LG J. The dupulicated loci and synteny to the same buffelgrass and sorghum linkage groups suggests that *Echinochloa* LG 5 and LG 15, LG 11 and LG 40, and LG 4 and LG 5 may be part of a single linkage group. Loci *pPAP8G01*, *pPAP10A04* and *RZ476* on *Echinochloa* LG 37 corresponded to loci that are linked on sorghum LG F, but they do not overlap with the association between LG 14 and sorghum LG F.

Statistics of physiological and morphological traits

Average values and standard deviations for physiological and morphological traits of the parents and F_2 progeny are shown in Fig. 2. Shoot length (SL), heading date (HD), number of leaves on the main stem (NL), and plant height (PH) in the F_2 population exhibited intermediate values between those of the parents, but they were skewed toward the lower values. The frequency distribution for plant morphology (PM) was normal. Correlations between physiological and morphological traits in the F_2 population are shown in Table 4. Positive correlations were observed between heading date, the number of leaves, and plant height (r=0.75–0.88). Plant morphology showed lower, but still positive and significant correlations with the above three traits (r=0.39–0.55). No traits were correlated with shoot length (diagnostic of flooding tolerance).

Table 5 Biometrical parameters of individual QTL affecting physiological and morphological traits. Modes of gene action are represented by *A* additive, *D* dominant, *R* recessive. The LOD score and percent phenotypic variance explained (% variance) for each QTL were estimated from single-marker models with unconstrained gene action. The additive effect, dominance deviation, and possible models of gene action for each QTL were calculated as described (Paterson et al. 1991). Modes of gene action that could not be deemed unlikely by 1-LOD or more are listed in order of decreasing likelihood. Gene action mode D indicates that the formosensis allele is dominant

Locus	LOD	% variance	Additive effect	Dominance deviation	Mode
Shoot length		5.3[a]			
Unlinked *P4H03a***	2.7	6.8	2.61	4.83	DAR
LG24; *P3C05-P9E02**	2.2	5.1	1.72	1.22	DR
Plant morphology		9.7[a]			
Unlinked *P4H03a***	4.2	9.9	0.76	1.05	RA
LG14; *P11F08-P8C10***	4.2	9.7	0.59	1.18	ADR
Unlinked *P9H10**	2.3	5.2	–0.46	-0.87	DAR
Heading date		30.7[a]			
Unlinked *P4H03a***	3.6	9.2	12.03	19.49	ARD
LG14; *P11F08-P8C10***	11.9	25.9	15.94	31.86	ADR
LG38; *P1C03- P1E09**	2.4	6.4	5.69	4.90	DA
Number of leaves		31.5[a]			
Unlinked *P4H03a***	2.8	7.4	0.94	1.66	ADR
LG14; *P11F08-P8C10***	11.5	25.1	1.38	2.76	ADR
LG38; *P1C03- P1E09***	2.8	7.3	0.57	0.43	AD
Plant height		35.4[a]			
LG14; *P11F08-P8C10***	15.7	31.6	24.18	48.33	ADR
LG40; *P9F06***	2.5	5.2	9.16	5.81	RAD
Unlinked *P2A09**	2.0	4.9	–7.34	-6.64	RA

** and * indicate that QTLs were detected with LOD $\geq$2.5 and 2.0$\leq$ LOD <2.5, respectively
[a] Overall phenotypic variance explained by a regression model that contains all QTLs for the trait

QTL analysis

Marker-trait associations and biometrical parameters of individual QTL affecting physiological and morphological traits are shown in Fig. 1 and Table 5, respectively. First, single marker regression was carried out for all RFLP markers to identify those flanking QTLs affecting their traits. Upon analysis, a total of 14 markers were detected by selecting markers in which the LOD score was greater than 2.5 (significant QTL) or 2.0 (possible QTL).

One QTL for shoot length was detected as an unlinked locus with LOD of 2.7, and one possible QTL was suggested on LG 24 with LOD 2.2. Formosensis alleles (+ alleles) increase shoot length under anoxia at the two loci, consistent with the phenotype expected based on the difference between the parents.

Two QTLs for plant morphology were found (unlinked *pPAP4H03a* and QTL on LG14) with LOD $\geq$2.5 and one more possible QTL was suggested (unlinked *pPAP9H10*) with LOD 2.3. Formosensis alleles increased the tendency to upright morphology at two loci (unlinked *pPAP4H03a* and QTL on LG14) and reduced it (toward procumbence) at unlinked *pPAP9H10*.

Two QTLs for heading date were detected (unlinked *pPAP4H03a* and QTL on LG14) with LOD $\geq$2.5 and one more possible QTL was suggested on LG38 with LOD 2.4. Of the three loci, a QTL on LG14 exhibited high phenotypic variance (25.9%). Formosensis alleles increase heading date at the three loci, consistent with the phenotype expected based on the difference between parents. When combined in the same statistical model, the three QTLs together explained 30.7% of phenotypic variance in heading date.

Three QTLs for number of leaves were found (unlinked *pPAP4H03a*, QTL on LG14 and QTL on LG38) with LOD $\geq$2.5. Of the three loci, a QTL on LG14 accounted for a large part of the phenotypic variance (25.1%). Formosensis alleles increase the number of leaves at the three loci, consistent with the phenotype expected based on the difference between parents. When combined in the same statistical model, the three QTLs together explained 31.5% of phenotypic variance in number of leaves.

Two QTLs for plant height were detected on LG14 and LG40 with LOD $\geq$2.5 and one more possible QTL was suggested (unlinked *pPAP2A09*) with LOD 2.0. A QTL on LG14 contributed a large part of the phenotypic variance (31.6%). Formosensis alleles increased plant height at two QTLs on LG 14 and LG40 and decreased it at the unlinked locus *pPAP2A09*. Only the latter QTL deviated from the effect expected based on the difference between parents. When combined in the same statistical model, the three QTLs together explained 35.4% of phenotypic variance in plant height.

A QTL or QTLs at an unlinked locus *pPAP4H03a* affected shoot length, plant morphology, heading date and number of leaves and formosensis alleles increased these traits at the locus. The most likely gene action of the locus is dominant for shoot length, recessive for plant morphology, and additive for heading date and number of leaves. A QTL on LG14 was detected for plant morphology, heading date, number of leaves and plant height with high phenotypic variance (9.7–31.6%). Formosensis alleles increased all the traits at this locus. The possible gene action of the locus was the same for the four traits. A QTL on LG38 was found for heading date and number of leaves. Formosensis alleles increased these traits at the locus. The most likely gene action of the locus was dominance for heading date and additivity for number of leaves.

The generally high correspondence among traits other than shoot length is consistent with their relative high phenotypic correlation. The low correlation of shoot length (diagnostic of flooding tolerance) to the other traits tends to suggest that additional QTLs specific to shoot length may remain to be discovered in regions of the genome not yet mapped.

Discussion

An RFLP linkage map of *E. crus-galli* was constructed using heterologous grass probes. Because the number of chromosomes of *E. crus-galli* is 54 ($2n=6x=54$), the complete linkage map should consist of 27 linkage groups. However, the linkage map obtained here is partial, consisting of 41 linkage groups, and 27 loci out of 168 (16.1%) were not linked. Additional loci will be needed to link the map into the expected 27 groups.

The effectiveness of heterologous probes at hybridizing to *Echinochloa* DNA generally reflected the taxonomic relationships among the respective grasses. Buffelgrass probes were generally more useful than maize, rice and sorghum cDNA probes (Table 1), consistent with the fact that *E. crus-galli* and buffelgrass belong to the same tribe, *Paniceae*, but maize, rice and sorghum are not taxonomically close to *E. crus-galli* (Watson and Dallwitz 1992). Hypomethylated genomic DNA probes from maize and rice were also used in the parent surveys, but the hybridization percentage was low (approximately 35%) and no polymorphic bands were observed between formosensis and praticola. This is consistent with the well-established finding that hypomethylated genomic DNA is less well-conserved than protein-encoding DNA sequences (cDNAs).

Several QTLs affecting physiological and morphological traits were identified in *E. crus-galli*. For shoot length, one significant QTL and one possible QTL were detected. Xu and Mackill (1996) reported that a major QTL for submergence tolerance (*Sub1*) is found on chromosome 9 in rice and exhibits 59% phenotypic variation for submergence tolerance. Other groups also found a QTL in the same or closely linked region of *Sub1* in rice (Nandi et al. 1997; Kamolsukyunyong et al. 2001). In *Echinochloa*, both QTLs explained relatively small portions of phenotypic variance for the trait, in contrast to rice *Sub1*. To investigate if one of the two QTLs is closely linked to *Sub1*, several rice chromosome 9 probes were applied to *Echinochloa*, but none of the probes detected polymorphisms. Rice *Sub1* was detected based on seedling elongation and leaf emergence under submergence stress (Xu and Mackill 1996; Nandi et al. 1997; Kamolsukyunyong et al. 2001). On the other hand, QTLs found in *E. crus-galli* were identified based on shoot elongation from seed under anoxia and the age of plant materials (stage). In fact, *Sub1* contributed to restricted growth under submergence, whereas QTLs found in this study exhibited an effect of rapid growth of the coleoptile under anoxic conditions. The opposite phenotypic effects suggest that QTLs affecting flooding tolerance in *E. crus-galli* may not be identical to those affecting flooding tolerance in rice.

The findings of the QTL mapping generally supported our evaluations of phenotypic correlations among the measured traits. Plant morphology, heading date, number of leaves, and plant height were highly correlated with one another (all significant at the 1% level; Table 4). Three chromosomal locations accounted for most of the variance that we were able to map for these traits (Fig. 1). A QTL on LG 14 exhibited the largest portion of phenotypic variance for HD, NL and PH, and second largest portion for PM. A QTL linked to the marker *pPAP4H03a* affected four traits (SL, PM, HD and NL), but no correlation between SL and the other traits was observed (Table 4) because the phenotypic variance was not high compared to other QTLs affecting these traits. Finally, a QTL on LG 38 affected both HD and NL, but explained relatively small portions of phenotypic variance. The finding of a different mode of gene action for PM than for the other traits at the LG14 locus suggests (but does not necessarily prove) that this QTL region represents the actions of at least two different genes. However, in the other two regions, it remains premature to assert whether these correlations represent multiple ('pleiotropic') actions of individual genes, or independent actions of closely-linked genes.

The relatively close taxonomic relationship between *E. crus-galli* and buffelgrass was generally consistent with early comparative data, albeit based on a small number of probes that detected polymorphism in both taxa. Ten linkage groups of *E. crus-galli* were homologous to buffelgrass linkage groups. In comparison to the more distantly-related genome of sorghum, some early hints of colinearity are observed, but there appears to be generally more rearrangement than with *Pennisetum*. Of QTL-containing regions, *E. crus-galli* LG14 (containing QTLs for PM, HD, NL and PH) is homologous to female buffelgrass LG26 and sorghum LG F. Although plant height and flowering time have been studied extensively in sorghum, no QTLs for these traits appear to be present in the corresponding genomic region. Better delineation of the corresponding arrangements of *E. crus-galli* and sorghum, together with ongoing QTL mapping in buffelgrass, may help to shed more light on whether common traits are under corresponding genetic control in these taxa.

Further surveying using more heterologous probes to achieve complete coverage of the *E. crus-galli* genome is in progress. A complete map of the common harmful weed may provide new insights into weed management, as well as useful new material for studies of polyploid evolution. Another valuable future experiment would be to further analyze flooding tolerance, a complex trait that may be better resolved with the replication that could be done by using F_3 or more advanced progenies rather than an F_2 population.

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