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Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., *Poaceae*) crosses

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Abstract Comparative mapping of Quantitative trait loci (QTLs) involved in domestication of adaptative syndrome traits of pearl millet was realized at the intra-specific level using two F_2 populations derived from domesticated (*Pennisetum glaucum* ssp. *glaucum*) × wild (*Pennisetum glaucum* ssp. *monodii*) crosses. The two domesticated parents analyzed differ in their geographical origins, agronomic characteristics and life cycles. In both populations, two regions of the genome were identified on linkage groups 6 and 7, that controlled most of the key morphological differences. The importance of these two linkage groups reveals their central role both in the developmental control of spikelet structure and in the domestication process of this crop. In contrast, QTLs involved in traits that are components of yield and measure differences in resource allocation (such as the shape of the spike, the number of spikes per plant and plant height) show a low level of correspondence among our two crosses. The results of the comparative mapping between cereals, although preliminary, reveal that genes involved in seed-shattering could correspond in maize, rice and sorghum. The evolutionary significance of our results, and especially the relationships between genome organization and cereal domestication, are discussed. The potential use of these results in pearl millet genetic-resources enhancement are presented.

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Introduction

Cereals have been domesticated for thousands of years through convergent selection for analogous agronomic characters, ensuring adaptation to the sowing/harvest cycle. The morphological differences between the domesticated forms and their wild ancestors, developed under human selection, represent the domestication syndrome (Hammer 1984), and mainly concern loss of seed dispersal and survival mechanisms in natural conditions (non-shattering, loss of seed ornamentations and dormancy), improved yield components (seed and spike sizes) and compact growth habit of the domesticated forms.

Although the grasses have a monophyletic origin over 70 million years ago (Clark et al. 1995) and display a huge variation in genome size (Arumuganathan and Earle 1991), there is an extensive conservation of gene repertoire and order across subfamilies. This has led to the suggestion that molecular resources and genetic knowledge acquired on small-sized genomes such as rice could benefit the entire family (Bennetzen and Freeling 1993, 1997; Moore et al. 1995; Devos and Gale 1997).

Whether synteny is conserved for genes that are submitted to strong selective pressures can still be questioned. For example, important and rapid genome reorganization has been reported for pathogen resistance (R) genes (Leister et al. 1998). Selection of traits simultaneously targeted can be facilitated by a tight linkage of the genes involved in these traits. Models simulating phenotypic evolution under disruptive selection and gene flow between adjacent domesticated and wild populations suggest that this could occur for genes involved in the domestication process (Le Thierry d'Hennequin et al. 1999). Cereal domestication is an appropriate model to tackle this question on the empirical side for two main reasons. First, the traits that have been the target of selec-

tion are often analogous between different species of the grass family. Second, at the intra-specific level, domestication can be considered, for some species at least, as a repeated evolutionary experiment of selection for truly homologous traits since it could have occurred independently at different times and locations in so-called 'non-centers of origin' (Harlan 1975). This is supposed to have been the case for pearl millet domestication, which took place in widely dispersed regions, from Sudan to the Atlantic coast of Africa (Pilate-André 1992).

Doebley and Stec (1993) and Dorweiler et al. (1993) showed that several of the morphological differences between maize and its wild ancestor, teosinte, were controlled by major genes and that few genomic regions were involved. In the case of pearl millet, the study of a domesticated × wild cross revealed that the domesticated spikelet structure is mainly under the control of major genes located on linkage groups 6 and 7 (Poncet et al. 2000). In order to investigate the correspondence of domestication traits across domesticated forms of pearl millet we analyzed another cross in which the domesticated parent differs in its geographical origin, agronomic characteristics and life cycle, from the domesticated parent in the previously studied population (Poncet et al. 2000).

Based on the study of two crosses involving different parents, this paper presents a comparison of the locations and effects of QTLs controlling the morphological differences between domesticated and wild pearl millet with a focus on the organization of linkage groups 6 and 7. We also present a preliminary analysis of the correspondence of QTLs affecting similar phenotypes of the domestication syndrome in pearl millet and other *Poaceae* taxa.

Materials and methods

The segregating F_2 population

The present experiment used a F_2 population derived from a domesticated × wild hybrid "Thiotandé × Wild" (T×W). The location and the effects of the QTLs detected in this cross are compared to those obtained in a previous analysis of an F_2 population derived

Table 1 List of morphological traits analyzed

Code	Units	Trait description
		Traits measured 1 month after sowing (juvenile stage)
NL		Number of leaves on the primary tiller (shoot)
NT		Number of basal tillers
LS	cm	Length of the sheath from base to the first leaf, on the primary tiller
DS	mm	Diameter of the sheath
LL	cm	Length of the 4th appearing leaf
WiL	mm	Width of the 4th appearing leaf
		Flowering time
Head	Days after sowing	Days to heading of the primary tiller
Hhead	cm	Plant height at first heading date
BFF	Days after sowing	Beginning of female flowering
PI	(BMF-BFF)/(DO)	Protogyny index. (with BFF: beginning of female flowering; BMF: beginning of male flowering on the primary tiller; DO: duration of the female flowering)
		Traits measured at maturity
NS		Total number of spikes/plant
NTM		Number of basal tillers
Hmax	cm	Plant height
HTP	cm	Height of the primary tiller
LoFL[a]	cm	Length of the flag leaf
WiFL[a]	cm	Width of the flag leaf
		Traits measured on the primary mature spike
WeS	g	Weight of the spike
LoS	cm	Length of the spike
WiS	mm	Width of the spike
LoPd[a]	cm	Length of the peduncle of the spike
WiPd[a]	cm	Width of the peduncle of the spike
DENS[a]	no./cm	Spikelet density: number of involucres attached on 1 cm length of rachis (measured at the middle of the spike)
WeG[a]	g	Weight of hundred seeds
PL	mm	Pedicel length of the floral involucre
AL	Presence or not	Functional abscission layer
Ct	scaling points 0-2	Seed coating (0 as domesticated phenotype: exposed seed)
GL	mm	Length of the upper floret lemma
BL[a]	mm	Length of the involucre bristles
LBL[a]	mm	Length of the longer bristle
FB[a]	Scaling points 0-3	Feathery bristles (0 as domesticated phenotype: bristles without ornamentation)
GS[a]	Scaling points 0-2	Shape of the caryopsis (0 as domesticated phenotype: globular seed)

[a]Traits only scored in the T×W F_2 population

from the cross "Souna × Mollissimum" (S×M) (Poncet et al. 2000). Souna is an early flowering landrace from Mali where sympatry with wild forms still occurs. Thiotandé is an S_2 inbred line selected from a Senegalese cultivar, which is cultivated during the off-season, i.e. in a situation where no gene flow between the wild forms and this domesticated line occurs. M and W were two individuals from two different populations of the wild subspecies, *P. glaucum* sp. *monodii*.

One hundred and twenty six F_2 plants from the S×M cross and 168 individuals from the T×W cross were analyzed.

Morphological observation

Characters known to discriminate between domesticated and wild phenotypes (Robert and Sarr 1992) were measured on the F_2 plants at different stages of development and maturity (Table 1). Most traits were scored in common with the S×M population for comparison. Some traits were scored only in the T×W F_2 population as a complement to the domestication syndrome description: length and width of the flag leaf (LoFL and WiFL), length and width of the peduncle of the spike (LoPd and WiPd), spikelet density (DENS), weight of 100 seeds (WeG), feathery/glabrous bristles (FB), and shape of the caryopsis (GS).

Genetic analysis

Analysis of polymorphism in the F_2 populations was performed as described by Sandmeier et al. (1981) for the following isozymes: α-esterase (EST), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), glutamate oxaloacetate transaminase (GOT) and isocitrate dehydrogenase (ICD). Only the α-*esterase E* (*Est-E*) locus was found to be polymorphic in our crosses.

The RFLP analysis was performed as described in Poncet et al. (2000). Sixty eight pearl millet genomic probes were tested on the T×W cross for polymorphism. In addition, 14 cDNA clones from barley (BCD), oat (CDO) and rice (RZ) (described by Causse et al. 1994, and Heun et al. 1991) were used as probes. Their selection was based on two criteria: (1) their map position in the orthologous chromosome regions affecting shedding ability in rice, sorghum and maize (Paterson et al. 1995a), and (2) the intensity of the hybridization signal when used as probes in Southern analysis of maize, sorghum and sugarcane DNA (Van Deynze et al. 1998), three genera belonging to the *Panicoideae* subfamily, of which pearl millet is a member. CDO795, RZ596, CDO457 and BCD1072 produced weak signals in pearl millet, whereas no signal was observed on Southern hybridization for CDO507, CDO204, CDO412 and RZ14. The RZ630, BCD450, CDO524, BCD926 and RZ614 probes, although producing fair signals, revealed single monomorphic bands in our cross. Therefore, the only probe that could be mapped was RZ404, which detected a single locus on pearl millet group 7.

Statistical analysis

The marker segregation was tested by a χ^2 test with three different hypotheses: (1) 3:1 or 1:2:1 genotypic ratios for dominant and co-dominant loci, respectively, (2) a 1:1 ratio for allelic segregation of co-dominant loci [when significant deviation from this ratio was observed for a locus, the distortion coefficient, d, was calculated as $d = \frac{1}{2} - f(B)$, with f(B) being the frequency of the wild allele], (3) a 1:1 ratio of heterozygotes against homozygotes (this ratio is not affected in presence of gametic or gametophytic selection in only one sex).

The linkage mapping and QTL detection were performed as described in Poncet et al. (2000). The data were analysed using the programme MAPMAKER (version 3) supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA. Linkage groups were obtained using two-point analysis with a LOD score of 4 and a maximum recombination fraction of 0.30. Three-point and multi-point analysis were then used to determine the relative order of markers in each individual group with a LOD threshold of 2.5.

Interval mapping of morphological trait loci was performed using the computer programme MAPMAKER/QTL version 1.1b (Lander et al. 1987). The LOD score threshold value to declare the presence of a putative QTL in a given genomic region was chosen as suitable for a map of the marker density and length observed (Lander and Botstein 1989). It was 2.3 and 2.0 for the S×M and T×W crosses, respectively. The percentages of variation explained by the QTLs for the traits, and the additive and dominance effects were estimated by MAPMAKER/QTL analysis. The multilocus model was used to estimate the percent of phenotypic variation accounted for by all significant QTLs. When one or more QTL(s) with large effects were observed, the "fix QTL" algorithm was used to eliminate their effects and to seek QTLs with smaller effects, which were otherwise likely to be hidden (e.g. Lin et al. 1995).

Results

Comparison of the level of polymorphism and the genetic map

The T×W map consists of 22 loci distributed among the seven linkage groups (LG) of the pearl millet map covering 176.9 cM (Table 2), which corresponds to 54.6% of the reference map (Liu et al. 1994). This is due to both a strong reduction in the recombination rate in our two crosses relative to the cross between cultivars used to build the reference map (Poncet et al. 2000), and to a partial map coverage. Linkage groups 6 and 7, which are the two smallest linkage groups of the reference map, are represented in the T×W map by segments with lengths of 25.5 cM and 22.9 cM, respectively. A low level of polymorphism is observed in this cross with 30.4% of the

Table 2 Genetic distances in the domesticated × wild (T×W) pearl millet cross (Haldane cM)

Group	Markers	Distances
LG1	*Xpsm515*	17.3
	Xpsm756	
LG2	*Xpsm592*	2.7
	Xpsm662	
LG3	*Xpsm174*	2.7
	Xpsm108	8.9
	Xpsm473	1
	Xpsm476	
LG4	*Xpsm409.1*	20
	Xpsm464	42.2
	Xpsm568	5.3
	Xpsm305	
LG5	*Xpsm616*	8.4
	Xpsm642	0.8
	Xpsm411	19.2
	Xpsm735	
LG6	*Xpsm696*	1.5
	Est-E	24
	Xpsm579	
LG7	*Xpsm618*	4
	Xpsm526	18.9
	Xrz404	

Table 3 Genotypic and allelic sample sizes for loci that show segregation distortion in the T×W population. AA, BB: domesticated and wild homozygotes, respectively; H: heterozygotes; d: distortion coefficient $d = \frac{1}{2} - f(B)$

Locus	LG	Genotypes AA:H:BB	*P* (1:2:1)	Alleles A:B	*P* (1:1)	d	Favored allele
515	1	22:75:59	0.0001***	119:193	0.000***	–0.1186	B
756	1	13:76:57	0.000***	102:190	0.0000***	–0.1507	B
592	2	58:78:24	0.0007***	194:126	0.0001***	0.1063	A
662	2	58:77:23	0.0004***	193:123	0.0001***	0.1108	A
696	6	27:75:57	0.0027**	129:189	0.0008***	–0.0943	B
Est-E	6	19:49:38	0.0245*	87:125	0.0091**	–0.0896	B
579	6	31:75:49	0.1141ns	137:173	0.0409*	–0.0581	B

*$P<0.05$. **$P<0.01$. ***$P<0.001$

Table 4 Biometrical parameters of individual QTLs and cumulated QTLs (*tot*) that affect the domestication syndrome traits specific to the T×W population. % Var: percentage of phenotypic variance explained, a: additive effect of the wild allele (when the positive effected is attributed to the wild allele, figures are in italics), d: dominance effect, d allele : the dominant allele is c, domesticated or +, wild allele

Variable	Group	Locus	LOD	% Var	a	d	d/a	d allele
LoFL	/							
WiFL	6	Est-E-579	2.4	8.0%	–0.25	0.22	–0.87	c
LoPd	/							
WiPd	6	Est-E-579	2.20	8.0%	–0.35	–0.06	0.17	+
	7	526-rz404	2.08	7.9%	–0.33	–0.13	0.37	+
	Tot		*4.48*	*16.3%*				
DENS	7	526-rz404	11.35	40.7%	–12.03	–9.90	0.82	+
WeG	2	592	3.2	9.1%	0.07	*0.06*	0.81	+
LBL	7	526-rz404	8.0	21.7%	2.39	*1.91*	0.80	+

RFLP probes and one out of six isozyme systems showing polymorphic patterns segregating within the F_2 progeny against 60.0% of the RFLP probes in the S×M F_2 population.

Comparison of the S×M and T×W maps on the basis of common sets of DNA probes revealed conservation of colinearity. The locus order in these two maps agreed with those of the published pearl millet map (Liu et al. 1994).

Segregation distortions

In the T×W population, highly distorted segregation has been observed for loci located on linkage groups 1, 2 and 6. No significant bias in the heterozygote:homozygote ratio was observed (data not shown) but the parental allele (A:B) ratios differ significantly from the expected 1:1 ratio (Table 3). Excess of the domesticated alleles was observed on linkage group 2, while segregation distortion on linkage groups 1 and 6 favored the wild alleles.

QTLs for characters only scored in the T×W population (Table 4)

Length and width of the flag leaf (LoFL and WiFL), as well as the length and diameter of the spike peduncle (LoPd and WiPd), were scored in the T×W population. A large flag leaf (54.2 cm × 3.5 cm on average) and a large peduncle (7 cm × 6 mm on average) are characteristics of domesticated pearl millet. A large flag leaf could be related to yield since it has been suggested that activity of the flag leaf may affect seed filling (Sarr, personal communication). The domesticated spike is, on average, composed of 56.1 involucres per cm of rachis (DENS). These involucres possess short bristles without the ornamentations (FB = 0) that give a feather-like aspect to the wild involucres (FB = 3). The seeds of domesticated forms are larger and more globular (GS = 0) than the wild grains (GS = 2).

No QTLs were detected for the length of the flag leaf (LoFL) or the length of the peduncle (LoPd). A single QTL on linkage group 6 that influenced the width of the flag leaf (WiFL) explained only 8% of the phenotypic variation. Two QTLs affected the diameter of the peduncle (WiPd). They were located on linkage groups 6 and 7 and collectively accounted for 16.9% of the variation. Hundred-grain weight (WeG) was associated with a single QTL on LG2 accounting for 9.1% of the variation. For the density of involucres (DENS), one QTL of large effect (accounting for 40.7% of the variation) was identified on LG7. It was located in the same region as a QTL for the diameter of the peduncle (WiPd) and exhibited similar effects, suggesting that a single QTL may be involved in the control of the two traits. In fact, the larger the peduncle, the larger the rachis becomes and the more involucres it carries. The shape of the grain (GS) fitted the 3:1 segrega-

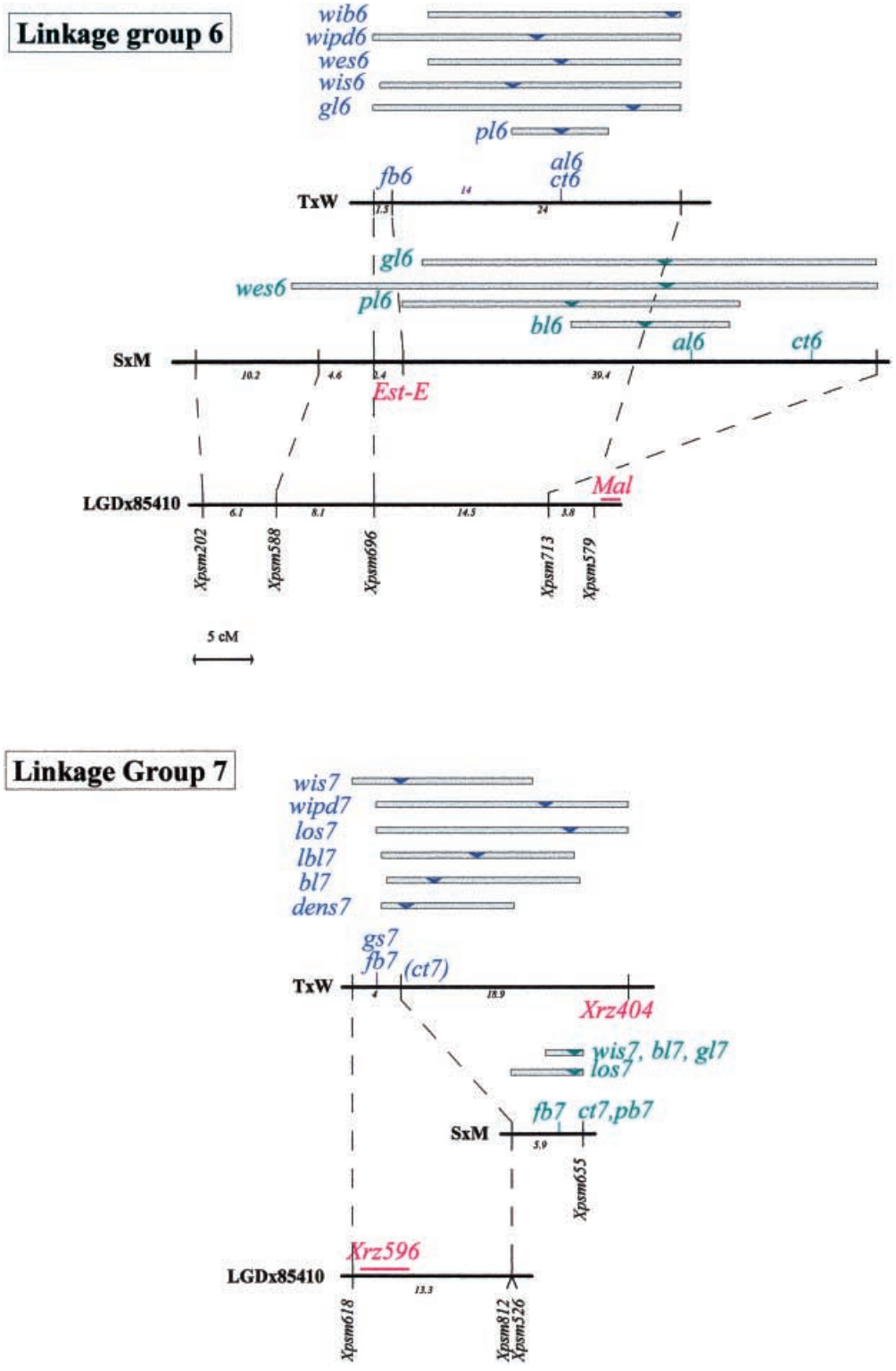

Fig. 1 Comparative genetic map for linkage groups 6 and 7 carrying QTLs of major effect for the differences in spike and spikelet architecture between wild and domesticated pearl millet. Figures on the left-hand side indicate linkage groups and the F_2 population in which QTLs were detected: S×M (Poncet et al. 2000) and T×W (present results), as well as the reference map (LGD × 85410 cross) (Liu et al. 1994). Haldane centiMorgans (cM) and loci are given at the bottom of the reference map. Markers that could not be placed unambiguously are indicated with *red horizontal lines*. QTL positions are indicated by the highest peaks (*dark triangles*) with their one-LOD support intervals (*grey boxes*). The genes or QTLs identified are named with a trait abbreviation followed by the linkage group number

tion ratio, which suggests that this trait is controlled by a single gene that is located on linkage group 7, with the wild alleles being dominant. Two unlinked genes control wild-like feathery bristles (FB), again with wild alleles being dominant. They have been located on linkage group 6 and linkage group 7 (segregation 9:7).

Map location and effects of domestication-QTLs segregating in both populations

A total of 18 QTLs were detected in the T×W F_2 population for traits measured in both populations (Table 5). For these traits, 36 QTLs were detected in the S×M

Table 5 Biometrical parameters of individual QTLs and cumulated QTLs (*tot*) that affect the domestication syndrome traits common to the S×M and T×W populations. % Var: percentage of phenotypic variance explained. a: additive effect of the wild allele (when the positive effect is attributed to the wild allele figures are in italic). d: dominance effect. d allele : the dominant allele is c domesticated or + wild allele. 5 (1) and 5 (2): two QTLs were detected on linkage group 5. QTLs in bold characters are those for which no accurate comparison can be done because they are detected in regions that are not well covered by markers in the other cross

Variable	S×M								T×W							
	Group	Locus	LOD	% Var	a	d	d/a	d allele	Group	Locus	LOD	% Var	a	d	d/a	d allele
NL	/								/							
NT	7	655	2.78	16.4%	*0.14*	0.7	5.09	+	1	515–756	2.48	9.6%	–0.32	–0.39	38.96	+
									3	108–473	2.08	6.1%	–0.19	–0.25	38.90	+
									Tot		*3.96*	*13.1%*				
LS	/								/							
DS	7	655	2.3	14.7%	*0.17*	1.2	6.99	+	/							
LL	5	345	2.8	14.7%	–2.61	0.18	–0.07	/	/							
WiL	/								/							
Hmax	7	655	2.73	16.1%	–16.42		24.48	–1.49	c	7	526–rz404		2.41	8.2%	–19.85	5.41
–0.27	c															
	6	*Est-E-713*			*19.3*	1.13	0.06	+	6	579			*14.87*	4.36	0.29	+
	Tot		*19.3*	*25.3%*					*Tot*		*4.16*	*12.5%*				
HPT	5	345–731	5.58	29.9%	–21.41	27.46	–1.28	c	/							
	2	176–592			–10.44	16.5	1.58	+								
	Tot		*7.66*	*38.6%*												
NS	2	856–176	2.96	15.2%	*8.85*	–0.63	–0.07		2	592–662	2.36	6.3%	*2.98*	0.02	0.01	+
	6	*Est-E 713*	4.33	37.0%	*13.79*	–6.31	–0.46	c	**4**	**409.1–464**	**2.42**	**28.6%**	**4.43**	**–6.76**	**–1.53**	**c**
	7	655	2.33	12.9%	*5.79*	7.67	1.32	+	*Tot*	*5.23*	*36.2%*					
	1	461–858			–1.27	10.89	–8.58	c								
	Tot		*10.89*	*67.8%*												
NTM	**2**	**332**	**2.68**	**13.7%**	**3.31**	**–1.24**	**–0.37**	/	/							
	5 (1)	345	4.31	21.4%	*4.54*	0.85	0.19	/								
	5 (2)	735	2.79	17.2%	*3.78*	–2.26	–0.60	c								
	Tot		*11.7*	*57.6%*												
HHead	5	651–735	6.65	34.7%	–24.75	26.13	–1.06	c	3	108–473	2.29	6.6%	*12.85*	6.57	0.51	+
	7	812	2.47	14.2%	–16.16	8.04	–0.50	c	7	526–rz404	2.36	8.1%	–14.91	2.28	–0.15	c
	Tot		*9.15*	*42.9%*					*Tot*		*4.84*	*14.5%*				
Head	5	651–735	10.75	59.0%	10.93	–10.23	–0.94	c	2	592–662	3.26	9.2%	–7.12	1.69	–0.24	c
	7	655	3.68	24.2%	–5.52	–7.05	1.28	+								
	Tot		*15.16*	*67%*												
BFF	5	651–735	12.78	64.0%	*10.24*	–11.1	–1.08	c	2	592–662	3.14	9.7%	–6.76	3.93	–0.58	c
	6	202	2.39	14.6%	*2.29*	–8.06	–3.52		5	616–642	2.3	8.4%	–6.09	2.3	–0.38	c
	6	*Est-E-713*	6.09	69.3%	*12.02*	–16.21	–3.53	c	*Tot*		*5.5*	*17.7%*				
	Tot		*14.41*	*69%*												
PI	/								/							
WeS	2	176–592	16.04	60.9%	–1.92	–0.56	0.29	+	6	*Est-E-579*	4.06	16.2%	–1.17	–0.83	0.73	+
	5	651–735	2.99	22.5%	–1.11	–0.82	0.73	+								
	6	*Est-E-713*			–0.60	0.10	–0.17	c								
	Tot		*24.52*	*77.1%*												

Table 5 (continued)

Variable	S×M								T×W						
	Group	Locus	LOD	% Var	a	d	d/a	d allele	Group	Locus	LOD	% Var	a	d	d/a	d allele
LoS	1	858–515	2.45	13.2%	*1.27*	–0.11	–0.09		7	526–rz404	2.35	8.6%	–1.71	–0.63	0.37	+
	2	592–738	7.37	35.9%	–2.13	–0.74	0.35	+								
	7	655			*0.42*	1.18	2.81	+								
	Tot		*12.31*	*55.7%*												
WiS	5	735	2.62	15.4%	–0.34	–0.10	0.31	/	6	*Est-E-579*	4.17	14.4%	–2.03	–1.00	0.49	+
	7	655	15.58	62.0%	*0.54*	0.21	0.39	/	7	526–rz404	2.162	6.4%	*1.325*	0.356	0.27	+
	Tot		*22.2*	*75.8%*					*Tot*		*6.79*	*21.4%*				
PL	6	*Est-E-713*	12.93	61.9%	–1.6	–0.89	0.56	+	6	*Est-E-579*	18.0	66.6%	–2.49	–2.06	0.83	+
	7	655			*0.66*	0.16	0.25	+								
	Tot		*17.28*	*71.7%*												
BL	6	*Est-E-713*	3.55	54.7%	–0.68	4.14	–6.09	c	7	526–rz404	9.4	29.2%	*1.46*	0.46	0.32	+
	7	655	8.9	43.3%	2.08	1.51	0.73	+								
	Tot		*10.02*	*51.1%*												
GL	5	651–735	3.24	17.9%	–0.44	–0.02	0.05	/	6	*Est-E-579*	2.3	7.6%	*0.24*	0.24	1.02	+
	6	*Est-E-713*	2.87	16.4%	*0.23*	0.39	1.70	+								
	7	655	10.72	49.9%	*0.52*	0.37	0.71	+								
	Tot		*15.86*	*63.4%*												

cross. However, the T×W and S×M mapping analyses differ by their map coverage levels. In the T×W cross, a maximum of two QTLs were detected for each trait and the proportion of the phenotypic variance explained ranged from 6.1% to 66.6%. In general, fewer QTLs with large effects were detected in the T×W F_2 compared to the S×M population.

In the T×W cross, effects of the QTLs generally fit the a priori expectation that the domesticated alleles should be associated with the domesticated-like phenotypes, and the wild alleles associated with the wild-like phenotypes. There were, however, several exceptions. The width of the spike (WiS) and the plant height at heading date (HHead) were both affected by two QTLs, one of which received the positive-effect allele from the domesticated parent while in the other QTL, the positive-effect allele was contributed by the wild parent.

Plant architecture and flowering

Results of QTL mapping in the S×M and T×W populations revealed different suites of QTLs for traits associated with plant architecture and flowering characteristics (Table 5). We considered a QTL for a trait in the T×W population to be putatively the same as a QTL for that trait in the S×M population if there was overlap in their one-LOD support intervals.

For the number of spikes (NS), a QTL was identified on LG2 in both populations but the intervals were not overlapping. Neither were any of the other QTLs affecting this trait common to the two populations (Table 5). For the number of basal tillers (NTM), three QTLs explaining altogether 57.6% of the phenotypic variation have been detected in the S×M population against none in the T×W population. However, the QTL detected on LG2 for NTM and the QTL detected on LG4 for NS were located in regions that were not fully covered by markers in the other cross, so that an accurate comparison for these two positions is not possible.

Similar results have been observed for the diameter of the sheath (DS), the length of the sheath (LL), and the height of the primary tiller (HTP). Likely explanations for the lack of QTLs in the T×W cross for these traits are the presence of QTLs with minor effects that are below the detection threshold and/or the incomplete genome coverage in the T×W population. For plant height at maturity (Hmax), both populations carried two QTLs at similar positions on LG6 and LG7 with the QTL of largest effect on LG7 (Fig. 1). For plant height at heading date (HHead), two QTLs were detected in each cross. Linkage group 7 harbored a QTL in the two populations, while the other QTLs mapped on LG5 and LG3 in S×M and T×W, respectively. A QTL on linkage group 5 was detected for the start-date of female flowering (BFF) in both populations. However, these are not the same loci since they are located at opposite distal ends of the linkage group. The distribution of other QTLs for BFF is also different. Hash et al. (1995) identified one QTL of

large effect (accounting for 35% of the variation) for flowering time on LG6 that could correspond to one of the QTLs for the beginning of female flowering (BFF) detected on linkage group 6 in the S×M cross. Heading date (Head) showed a different distribution of QTLs in both populations (Table 5).

Spike morphology

For traits describing the spike morphology, QTLs are found on linkage groups 6 and 7 in both populations. A QTL for weight of the spike (WeS) was identified on linkage group 6 in both populations, and for width and length of the spike (WiS and LoS) both populations possess QTLs on linkage group 7. However, in the S×M population, QTLs of major effect on LoS and WeS were also detected on linkage group 2.

Spikelet morphology

The two F_2 populations possessed similar suites of QTLs for traits describing the spikelet architecture. In both populations, a QTL with large effect on the pedicel length (PL) was found on linkage group 6 and a QTL for the bristle length (BL) was found on linkage group 7. A QTL controlling the length of the awn (LBL) in the T×W population was located in the same region of linkage group 7 as a gene found for the presence/absence of this awn in the S×M population. Moreover, the gene controlling the generation of an abscission layer (AL) in the wild parent has a similar location on linkage group 6 in both populations. The positions of the genes affecting the seed coating (Ct) on linkage groups 6 and 7 are also similar in both crosses.

Finally, six out of seven QTLs detected for spike and spikelet characteristics in the T×W cross could correspond to QTLs detected in the S×M cross.

Comparison with other *Poaceae*

To assess the correspondence of the mapped QTLs with those identified in other *Poaceae*, QTL locations were determined in reference to the rice genetic map (Causse et al. 1994) based on nearby markers inferred from comparative maps of rice, maize, sorghum and pearl millet (Whitkus et al. 1992; Ahn and Tanksley 1993; Ahn et al. 1993; Wilson et al. 1999; Devos et al. 2000).

Correspondence of QTLs located on pearl millet linkage groups 6 and 7 across cereals

Pearl millet linkage group 6. The region of linkage group 6 that is mainly involved in the shattering and spikelet structure in pearl millet is associated with the Est-E-Mal interval (Esterase locus E-malate dehydrogenase locus, Devos et al. 2000; data herein) which corresponds to the ESTI-2-Mal I interval of rice chromosome 1 (Causse et al. 1994). This rice interval harbors a QTL for shattering (Xiong et al. 1999; Cai and Morishima 2000) and might also correspond to regions of maize chromosomes 3 and 8 that affect seed dispersal ability in the Zea mays race Reventador ¥ Z. mays ssp. Parviglumis cross (Paterson et al. 1995a).

Moreover, Xiong et al. (1999) observed a QTL for panicle length (pl) and QTLs for spikelet density (sd) and number of spikelets per panicle (sp) at opposite locations on rice chromosome 1. The distance between the two QTLs corresponds to a small region of less than 5 cM on pearl millet LG6 (Devos et al. 2000).This pearl millet region carries QTLs for weight of the spike (WeS) and width of the spike (WiS). Moreover, the corresponding regions in maize (on chromosomes 3 and chromosome 8) also harbor QTLs involved in the ear morphology, especially the number of cupules per rank (CUPR) (Doebley and Stec 1993).

Pearl millet linkage group 7. Paterson et al. (1995a) reported the presence of a QTL affecting seed dispersal at corresponding locations in rice (chromosome 9) and sorghum (linkage group C). The sorghum linkage group-C locus that affects shattering accounted for virtually 100% of the phenotypic variation (Paterson et al. 1995b). This QTL is associated with the marker interval *rz404–rz596*. The probes *rz596* and *rz404* both mapped at the bottom of pearl millet linkage group 7 (Devos et al. 2000, data therein). This pearl millet linkage group carries many QTLs influencing the spikelet architecture (Fig. 1). Although no gene for shattering has been mapped on this linkage group in the T×W or S×M crosses, the analysis of other crosses (Poncet et al. 1998) revealed a digenic control of shattering with the presence of a major gene potentially located on LG7 (Poncet et al. 2000).

A rice QTL for plant height (ph) is located on chromosome 9 between *rz698* and *rz206* (Xiong et al. 1999) which correspond to a region included in the interval *Xpsm618–Xpsm812* of the pearl millet linkage group 7 (Devos et al. 2000). Regardless of the pearl millet domesticated × wild cross, QTLs for height (Hhead, Hmax) are detected on this region that could correspond to the rice QTL. Similarly, another plant height QTL detected on rice chromosome 7 corresponds to pearl millet QTLs located closely to the *Xpsm812* locus. Two rice QTLs (Xiong et al. 1999) involved in the number of spikelets per panicle (sp) and panicle length (pl) are also located in regions that correspond to the pearl millet regions close to the *Xpsm812* locus and *Xpsm618* locus, respectively (Devos et al. 2000). These regions of linkage group 7 carry QTLs for length of the spike (LoS) and density of spikelet (DENS).

Discussion

Segregation distortions

Deviations from the expected Mendelian segregation ratios have been observed for loci on linkage groups 1, 2 and 6 (Table 3). The distortions in linkage group 6 were most severe at the top of the group and decreased progressively further down the linkage group. These distortions were always benefiting the wild alleles. This suggests that the effect is caused by a single gene or gene cluster located at the top of linkage group 6. The coverage of linkage groups 1 and 2 was too partial to estimate a putative position for a single locus. Thus, a minimum of three genetic factors contributed to the observed segregation distortion. This result is in accordance with the quantitative inheritance trend already observed by Sarr et al. (1988). The deviation in allelic segregation, but not in the heterozygotes:homozygotes ratio, suggests that selection took place on the haploid phase and in only one sex. Indeed, previous studies on pearl millet reported the occurrence of male gametophyte selection when pollen from different cultivars (Sarr et al. 1988) or pollen from wild and domesticated plants (Robert et al. 1991) are in competition on the same stigma.

The domestication process

The weight of the seed, a major component of the yield, is a key trait of the domestication syndrome. In the T×W population, 100-grain weight (WeG) was associated with a single QTL on LG2 accounting for 9.1% of the variation. The low contribution of this QTL is quite unexpected since grain size has been submitted to strong selection during pearl millet domestication and is generally highly heritable. Even though part of the residual variation can be due to environmental factors, it is highly plausible that several QTLs with minor effects were not detected in our experiment. If this were the case, increase in grain size during the domestication of the Thiotandé ancestors would have been very gradual. This should be apparent from archeological records which, up to now, are unfortunately very scarce. It is also plausible that major QTLs were segregating in regions that are not well-covered by markers in this cross and were therefore not detected. Hash et al. (1995) detected three QTLs of large effect for 1,000-grain mass, one on LG2 and two on LG4. A correspondence with our QTL for 100-grain weight (WeG) on linkage group 2 remains to be confirmed. Interestingly, no QTL for flag length and width (LoFL and WiFL), shape of the seed (GS), or weight of spike (WeS) was identified which corresponded to the QTL for the weight of the grain (WeG) on linkage group 2.

In both the S×M and the T×W population we identified two regions of the genome, on linkage groups 6 and 7, that control most of the key differences between wild and domesticated phenotypes. Both regions contribute mainly to the phenotypic divergence between domesticated pearl millet and its wild relatives.

The concentration of effects in these two regions of the genome may partially be explained by linkage and partially by pleiotropy. Seed coating, lengths of the bristles and bracts, length of the awn as well as feathery bristles are components of the ornamentation of the seed and their development may be regulated by the expression of common genes. The importance of these two linkage groups across other cultivars has also been shown by Poncet et al. (1998), revealing their central role both in the developmental control of the spikelet structure and in the domestication process of this crop.

Similar architectural differences between wild and pearl millet spikelets are found across cultivars and environments. They were probably selected unconsciously, early in the evolution of the domesticates. Indeed, archaeological records from Mauritania have shown that domesticated-like phenotypes for spikelet structure were already present at 3000 BP (Amblard and Pernès 1989).

The hypothesis of a single domestication event at the origin of both cultivars, Souna and Thiotandé, can be considered, taking into account the similar genetic organization underlying the spikelet architecture. Under this assumption, the differences observed between the two crosses for some plant and spike traits would be the consequence of subsequent selections, exercised after the first stages of the domestication process or gene flow from local wild populations. However, if the number of genes in which mutations could potentially lead to a domesticated phenotype is small, the number of different genetic organizations that could be obtained through different domestication events would also be limited.

The analysis of several cultivars from diverse origins (Joly 1984; Poncet et al. 1998) revealed that modification of key traits [non-shedding ability (Al), nude seeds (Ct), and long pedicel (PL)] can occur through independent mutations in at least two different genes located on two linkage groups. Different combinations of these genes were observed in the cultivars. This observation suggests several, and independent, domestications of this crop.

The results of comparative mapping between cereals, although preliminary, reveal that genes accounting for a major part of the variation in traits of the spikelet architecture, especially seed shattering, correspond in maize, rice and sorghum. Thus, convergent evolution of the domestication syndrome in these crops might involve the action of orthologous genes. This is again an argument in favor of the hypothesis that the evolution of the domesticated phenotype, at least for the spikelet architecture and shattering, has occurred under the constraint of a small number of genes that can lead to a domesticated phenotype.

The linkage of the genes that are responsible for the alternative wild/domesticated types would therefore be prior to the domestication. Moreover, correspondence of QTLs on duplicated chromosome segments within the maize genome has been suggested elsewhere (Paterson et al. 1995a). For example, pairs of loci affecting shattering of maize in the maize inflorescence fall on corre-

sponding duplicated segments (Doebley and Stec 1993), whose duplication preceded domestication by millions of years through alloploidization.

In contrast, traits which are components of yield and measure differences in resource allocation (such as shape of the spike, number of spikes per plant and plant height) show considerable variation among cultivars (Portères 1976). Our results demonstrate that correspondence of the QTLs involved in these traits is low among our two cultivars. This supports the model according to which these traits have evolved independently through the selection of different combinations of genes. However, to identify more precisely the level of synteny between grasses in regions harboring domestication genes, an improved resolution of the existing genetic maps and the development of physical maps in these genomic regions are needed.

Implications for the use of genetic resources

Knowledge of mechanisms controlling genetic recombination *sensu lato* and of the genetic basis of phenotypic differences between wild and domesticated forms is a pre-requisite for the exploitation of genetic resources from the wild and traditional landraces to broaden the genetic basis of modern varieties. The existence of favorable wild alleles that reinforce the domesticated phenotype and enhance yield components was observed in both F_2 populations (WiS, LoS and WeG). This opens promising possibilities for pearl millet improvement through the introgression of genes from wild phenotypes. Moreover, our results show that the two wild genotypes could be sources of different useful genes. This suggests that combining wild genotypes from different origins in the same breeding scheme could be an efficient strategy to cumulate useful genes. In addition, the strong linkage of the genetic factors involved in the pearl millet domestication syndrome could facilitate massive introgression of wild genes into cultivars while maintaining a domesticated phenotype.

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