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Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae)

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Abstract Quantitative trait loci (QTLs) controlling the morphological differences between pearl millet (*Pennisetum glaucum* ssp. *glaucum*) and its wild ancestor (*Pennisetum glaucum* ssp. *monodii*, form *mollissimum*) were investigated in a cultivated/wild F_2 population by means of RFLP markers. The most critical adaptive changes resulting from the domestication process involved the spikelet structure: non-shedding seeds with reduced bracts and bristles and long involucral pedicel. Major differences also concerned characters describing the plant architecture, phenology and spike sizes. Many morphological differences could be attributed to the effect of a small number of loci with relatively large effects. These loci are mainly concentrated on four linkage groups (2, 5, 6 and 7). The loss of shedding ability, due to the absence of a functional abscission layer, is controlled by a single locus on linkage group 6 (*al6*). Genetic control of the other spikelet traits involved factors with large effects which are located in the region of linkage group 6 close to *al6* and to an esterase gene, *Esterase-E*. Moreover, QTLs with large effects on plant and spike morphology traits such as plant height, number of spikes and weight of the spike were also mapped on linkage groups 6 and 7. This strong linkage of factors in the domestication syndrome may be involved in the maintenance of the phenotypic identity of wild and cultivated populations in sympatry. This result also brings new arguments in the understanding of the domestication process of this allogamous crop.

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Introduction

Pearl millet, *Pennisetum glaucum* (L.) R. Br. is a principal food cereal grown on about 27 million hectares of drought-prone soils in the semi-arid regions of Africa and the Indian subcontinent (ICRISAT 1996) with a grain yield averaging 500–600 kg/ha. It is also used as a forage in Australia, southern Africa, South America and the USA and ranks as the fifth cereal in the world in order of economical importance. Because pearl millet tolerates drought and low soil fertility and responds well to water and favourable soil conditions (Kumar and Andrews 1989), there is scope for increased production in the USA (Hanna et al. 1991) or in regions too arid for sorghum (Burton 1983).

Pearl millet is also of interest as a biological model for studying domestication and crop/wild complex evolution. The protogynic nature of its flowering promotes outcrossing. Moreover, the existence of sympatric situations between pearl millet and its wild ancestor in the sub-Sahelian zone of Africa allows gene flow to occur and provides the opportunity to study the dynamics of recombination between the two forms and the establishment of phenotypic divergence. The *Pennisetum glaucum* L. species, including both wild and cultivated forms, has a relatively small diploid genome ($2n=2x=14$) with a DNA content of 1C=2.36 pg. (Martel et al. 1997).

The term ”domestication” describes the selective process that yields genetic modifications of some original traits of the wild plants, thereby making them better adapted to the need of farmers (Hillman and Davies 1990). These traits, characteristic of a given crop, comprise the domestication syndrome (Harlan 1975).

Wild and cultivated pearl millet have a strikingly different plant architecture and spikelet structure (Fig. 1). The wild form is characterised by abundant tillering and

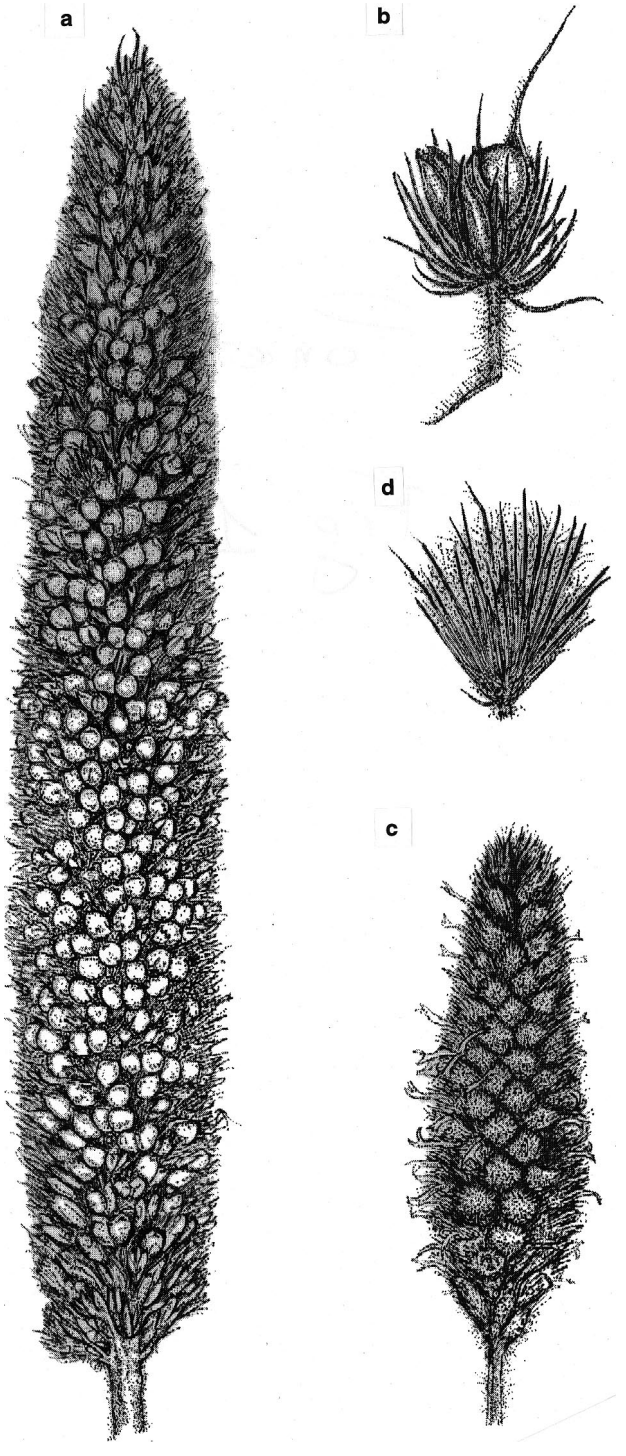


Fig. 1a–d Spike (**a**) and spikelet (**b**) from cultivated pearl millet *Pennisetum glaucum* subsp. *glaucum* and spike (**c**) and spikelet (**d**) from wild pearl millet, *Pennisetum glaucum* subsp. *monodii*. Domestication yields to increased spike size, non-shedding seeds with reduced bracts and bristles and long involucral pedicel

branching with short and thin spikes. Its spikelets have a short involucral pedicel length and the presence of an abscission layer that leads to shattering. The seeds of the wild form are small and enclosed by long bristles and bracts. The whole morphology tends to facilitate seed dispersal (scattering) and reproductive success of the wild plant in natural conditions. Conversely, the cultivated plants exhibit non-shedding seeds with reduced bracts and bristles that facilitate harvest and threshing. Branching and tillering are reduced, while the spike size is increased.

On an evolutionary scale, cereal domestication is a recent event, initiated some 5–10 thousand years ago. To some extent, this process is still happening as sympatric situations and hybridisation between cultivated populations and their wild progenitors are still occurring in the centre of origin of crops. Domestication is facilitated if the key traits of the domestication syndrome are governed by few major genes (Ladizinsky 1985). This is the case for seed or spikelet sheeding in maize (Dorweiler et al. 1993), millet (Darmency and Pernès 1986), barley and wheat (Hillman and Davies 1990) and sorghum (Paterson et al. 1995). Linkage of genes that are the target of the farmer's selection is also a parameter that could play a fundamental role in the success of domestication (Slatkin 1995).

It is important to establish the number of genetic factors involved in the domestication syndrome and their localisation on the chromosomes in order to understand the dynamics of introgression of genes from the wild forms into traditional landraces and the evolution of their phenotypic structure, especially for cereals with a preponderant outcrossing reproductive system. The pearl millet restriction fragment length polymorphism (RFLP)-based map developed by Liu et al. (1994) has been used to locate quantitative trait loci (QTLs) involved in the resistance to downy mildew (Jones et al. 1995). This reference map was employed to map QTLs involved in the domestication syndrome in pearl millet via the genetic analysis of a F_2 derived from a wild×cultivated cross.

Materials and methods

The F_2 segregating population

The F_2 population was derived from a cultivated×wild F_1 hybrid. The wild parent, *Pennisetum glaucum* ssp. *monodii*, form *mollissimum* (187-80(4)), referred to as "Molli" in the paper, is a S_4 line generated from a natural wild population collected near Gao in Mali. The cultivated parent, *P. glaucum* ssp. *glaucum* cv. 'Souna' [5338(1)], is an early-flowering landrace from Mali where sympatry with wild forms still occurs. The cultivated plant was used as the female parent. The F_2 population (364-87) was obtained from a single F_1 plant [272-86(1)]. The wild and cultivated parents were also self-pollinated to reconstitute the parental genotypes. All crosses were carried out at Gif sur Yvette and a F_2 population of 250 plants was sown. Plants were grown under a 16-h photoperiod for the first 6 weeks and a 12-h photoperiod during the subsequent 3 weeks to induce flowering with a day/night temperature of 28°/24°C. The plants were then planted randomly in the greenhouse with a spacing of 70 cm between plants.

Phenotypic observations

In total, 26 characters were measured on the F_2 plants at different stages of development and maturity (Table 1). Robert and Sarr

Table 1 List of morphological traits analysed

Code	Units	Trait description
Traits measured 1 months 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
TNL		Total number of leaves
Flowering time		
Head	Days after sowing	Days to head of the primary tiller
HHead	cm	Plant heigt at first heading date
PI	(BMF-BFF)/(DO)	Protogyny index. (BFF, beginning of female flowering; BMF beginning of male flowering on the primary tiller; DO, duration of the female flowering). Joly and Sarr 1985
Traits measured at maturity		
NNTP		Number of nodes on the primary tiller
NS		Total number of spikes/plant
NTM		Number of basal tillers
Hmax	cm	Plant height
HPT	cm	Height of the primary tiller
LLM	cm	Length of the 4th leaf of maturity
WiLM	mm	Width of the 4th leaf at maturity
Traits measured on the primary mature spike		
WeS	g	Weight of the spike
LoS	cm	Length of the spike
WiS	cm	Width of the spike
PL	mm	Pedicel length of the floral involucre measured from the abscission layer (or rachis) to the base of the involucre
BL	mm	Length of the involucre bristles
Ct	Scaling points (0 as cultivated phenotype)	Seed coating (exposed seeds as the cultivated phenotype or enclosed seeds as the wild phenotype)
AL	Presence (1) or not (0)	Functional abscission layer
PB	Presence (1) or not (0)	Existence of a longer bristle
GL	mm	Length of the upper floret lemma

(1992) had previously reported that spike and spikelet morphology and plant architecture highly discriminate these cultivated and wild phenotypes.

Molecular markers

DNA extraction

DNA was extracted from young leaves. Two or three grams of freshly harvested material was ground in liquid nitrogen and incubated in conical 50-ml tubes for 30 min at 60°C in 25 ml of 0.1 *M* TRIS-HCl, pH 8.0, 0.5 *M* NaCl, 50 m*M* EDTA, pH 8.0, 1.25% SDS; 3.8 g/l sodium bisulfite was added before use. Twenty-five milliliters of chloroform/isoamyl alcohol (24/1) was added to the extract, and the tubes were placed horizontally in ice and gently agitated on an orbital shaker for 15 min. After a 30-min centrifugation at 3500 rpm, the top layer was transferred to a new tube containing 13 ml of isopropanol. DNA was precipitated by rocking the tube gently, hooked out, washed in 70% ethanol, dried on a paper towel and resuspended in 1 ml TE (10 m*M* TRIS-HCl, 1 m*M* EDTA). After RNAse treatment (15 min at 37°C at a concentration of 50 µg/ml) DNA was precipitated with 500 µl of 7.5 *M* NH_4acetate and 2 ml of absolute ethanol, then hooked out, washed in 70% ethanol, dried and resuspended in 200 µl of TE.

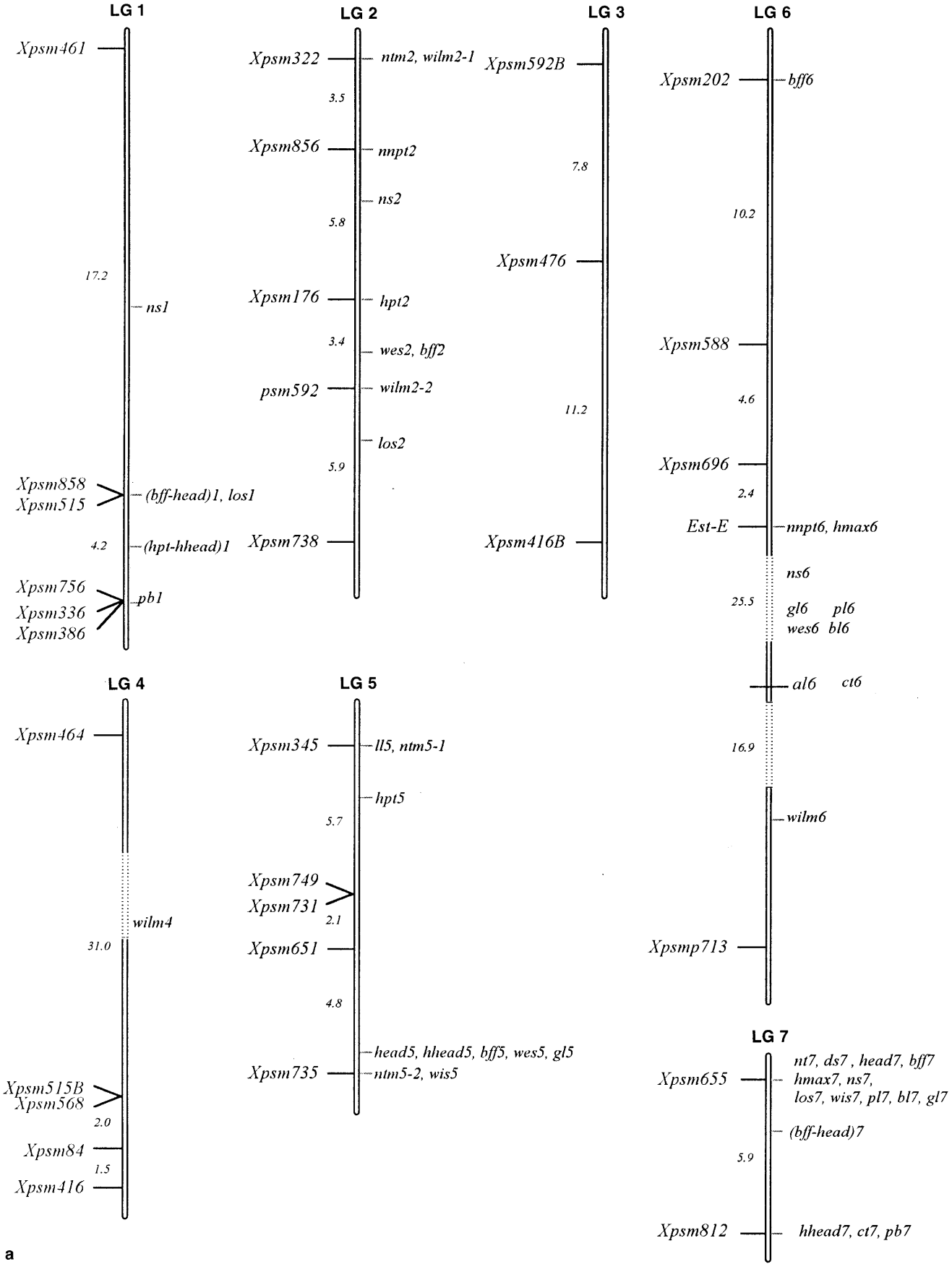
RFLP probes and Southern blot hybridisation

Ten micrograms of total DNA was digested at 37°C by *Eco*RV in the presence of 4 m*M* spermidine or 100 µg/ml casein (Dreyer and Schulte-Holthausen 1991). The DNA was fractionated on 0.8% agarose gels in TAE buffer (40 m*M* TRIS, 20 m*M* acetic acid, 1 m*M* EDTA) and alkaline-blotted onto Hybond N^+ nylon membranes (Amersham, UK) following the procedure described by Southern (1975).

Genomic clones were from a pearl millet *Pst*I library (Liu et al. 1994). Probes were labelled with [^{32}P] by random priming (Feinberg and Vogelstein 1983). After hybridisation, the membranes were washed twice in 2×SSC; 1% SDS for 15 min each at 65°C, followed by two washed in 0.2×SSC and 1% SDS for 15 min each at 65°C.

*Eco*RV is a restriction enzyme that has been shown to reveal high levels of polymorphism between the *Pennisetum* subspecies of the primary gene pool (Liu et al. 1992). Screeing for polymorphisms was carried out on 4 S_1 plants derived from each parent and 4 F_2 individuals.

A total of 32 RFLP markers were used in this study. They were selected to cover the pearl millet linkage groups. The RFLP-based map of pearl millet (Liu et al. 1994) comprises seven linkage groups (noted LG) and covers 303 cM with an average map distance between loci of about 2 cM. The reference pearl millet linkage map (Liu et al. 1994) was based on an inter-varietal cross: (LGD-1-B-10)-1×ICMP 85410, referred to below as LGD×85410.


LG 1
Xpsm461
17.2
ns1
Xpsm858
Xpsm515
(bff-head)1, los1
4.2
(hpt-hhead)1
Xpsm756
Xpsm336
Xpsm386
pb1
LG 2
Xpsm322
ntm2, wilm2-1
3.5
Xpsm856
nnpt2
ns2
5.8
Xpsm176
hpt2
3.4
wes2, bff2
psm592
wilm2-2
5.9
los2
Xpsm738
LG 3
Xpsm592B
7.8
Xpsm476
11.2
Xpsm416B
LG 6
Xpsm202
bff6
10.2
Xpsm588
4.6
Xpsm696
2.4
Est-E
nnpt6, hmax6
ns6
25.5
gl6 pl6
wes6 bl6
al6 ct6
16.9
wilm6
Xpsmp713
LG 4
Xpsm464
wilm4
31.0
Xpsm515B
Xpsm568
2.0
Xpsm84
1.5
Xpsm416
LG 5
Xpsm345
ll5, ntm5-1
hpt5
5.7
Xpsm749
Xpsm731
2.1
Xpsm651
4.8
head5, hhead5, bff5, wes5, gl5
Xpsm735
ntm5-2, wis5
LG 7
Xpsm655
nt7, ds7 , head7, bff7
hmax7, ns7,
los7, wis7, pl7, bl7, gl7
(bff-head)7
5.9
Xpsm812
hhead7, ct7, pb7

a

Fig. 2

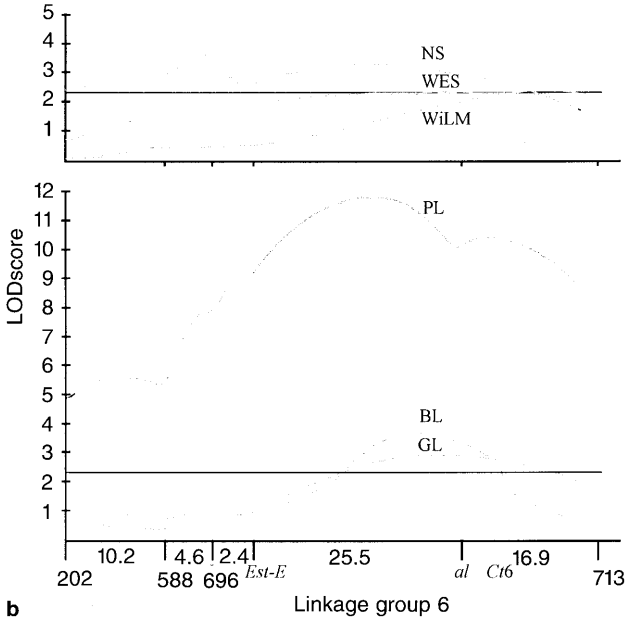


Fig. 2 a Locations of putative QTLs on pearl millet linkage map. CentiMorgan distances (Haldane distances) are indicated between markers on the *left* of the linkage groups. QTLs are designated by the (*trait linkage group number*) combinations to the *right* of the linkage groups (*LG*) (see also Table 5). **b** Likelihood map for QTLs detected on linkage group 6. The *X axis* represents the linkage group 6 with markers and genetic distances; the *Y axis* indicates the LOD scores (level of significance for the presence of a QTL is LOD 2.3)

The carboxylic esterase Est-E *gene (E.C.3.1.1.1)*

Analysis of the isozyme β-esterase E was performed as described by Sandmeier et al. (1981).

Sequence-tagged-site (STS) marker

A STS marker was generated from the single-copy *Pst*I clone, PSM713, by T.A. Money (personal communication) and identifies the locus *Xpsm713*.

Linkage analysis

Marker segregation was checked against the expected Mendelian 1:2:1 (for codominant markers) or 3:1 (for dominant markers) ratios using the chi-square statistic (Pearson's chi-square goodness-of-fit statistic, Snedecor and Cochran 1980). The data were analysed using the programme MAPMAKER (version 3) supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts. 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 multipoint analysis were then used to determine the relative order of markers in each individual group with a LOD threshold of 2.5.

The genetic control of the qualitative traits (AL, PB, Ct) and their co-segregation with marker loci located on the linkage map were tested with χ^2 tests. The probability threshold level for the rejection of the null hypothesis ($P<0.001$) was determined according to the sequential Bonferroni correction (see Rice 1989). Since the presence of a functional abscission layer was determined by a single gene, this gene was used in the mapping procedure as a marker.

Table 2 Genetic distances in the inter-subspecific ('Souna'× Molli) and inter-varietal (LGD×85410) crosses (Haldane cM)

	Marker interval	Souna×Molli	LGD×85410[a]
LG 1			
	461–858	17.2	25.4
	858–515	0	15.6
	515–756	4.2	19.4
	756–336	0	4.5
	336–386	0	12
LG 2			
	322–856	3.5	3.0
	856–176	5.8	6.3
	176–592	3.4	15
	592–738	5.9	11.5
LG 3			
	592B–476	7.8	/
	476–416	11.2	20.8
LG4			
	464–515B	31.0	37.0
	515B–568	0	/
	568–84	2.0	10
	84–416B	1.5	/
LG 5			
	345–731	5.7	9.5
	731–749	0	0
	749–651	2.1	/
	651–735	4.8	/
	(749–735)	6.9	6.8
LG 6			
	202–588	10.2	6.1
	588–696	4.6	8.1
	696-*Est-E*	2.4	/
	Est-E-al6	25.5	/
	al6–sts713	16.9	/
LG 7			
	655–812	5.9	/
Total length		171.6	

[a] Liu et al. 1994

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 of 2.3 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). The percentages of variation explained by the QTLs for the traits and the additive and dominance effects were estimated by MAPMAKER/QTL analysis. The additive (a) and dominance (d) effects at each significant QTL were calculated as (BB-AA)/2 and AB-(BB-AA)/2, respectively, where BB and AA were the phenotypic means for individuals homozygous for wild (Molli) alleles and cultivated (Souna) alleles, respectively, and AB was the phenotypic mean of the heterozygotes. The multilocus model was used to estimate the percentage 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 likely to be otherwise hidden (e.g. Lin et al. 1995). This algorithm adjusted the residual variance used for the detection procedure by subtracting the variance explained by the fixed QTL(s). However, in this case, the percentage of variation individually explained by the additionally detected QTLs could not be estimated.

The genes or QTLs identified are named with a trait abbreviation followed by the linkage group (LG) number.

Results

Establishment of the linkage map (Fig. 2a)

Polymorphism

Fifty-five low-copy-number genomic probes were tested on the parental and F_2 DNA digested with *Eco*RV: 60% revealed polymorphic patterns segregating within the F_2 progeny. Pearl millet has been shown to be highly polymorphic at the molecular level. In an analysis by Liu et al. (1992), low-copy probes hybridised to *Eco*RV-digested DNA for a range of cultivated genotypes revealed 69% of polymorphism.

Colinearity of the intra- and inter-subspecific maps

A comparison of the inter-varietal maps, constructed by Liu et al. (1994), with the inter-subspecific map (Fig. 2a) showed that with few exceptions, markers were located on the same linkage groups and in a similar order in both maps. Changes in linkage group assignment were observed for the markers detected with four clones, PSM515, PSM592, PSM655, PSM651. For example, the locus *Xpsm651* mapped to linkage group 1 in the inter-varietal map (Liu et al. 1994) and was located on linkage group 5 in our study (Fig. 2a). All four probes detected multicopy sequences, and it is likely that the loci segregating in our cross were different from the ones previously mapped. Two loci, *Xpsm53* and *Xpsm160*, could not be assigned to any linkage group. Five probes revealed a presence/absence type polymorphism, three with dominance of the cultivated allele (for the loci *Xpsm592B*, *Xpsm416B* and *Xpsm202*) and two with dominance of the wild allele (loci *Xpsm858* and *Xpsm515*). These are distributed on different linkage groups. No segregation distortion was noted except for the locus *Xpsm812*, located on linkage group 7. Bias was observed in favor of the wild allele.

Comparison of recombination distances

Our map covered 80% of the map based on the inter-varietal population, which has a total length of 303 cM (Liu et al. 1994). Several gaps remained, especially on linkage group 4 (Fig. 2a). Since these gaps are observed in both maps, they could not be attributed to the presence of chromosome regions common by descent in the two parents of our F_2. A comparison of the recombination rates between identical pairs of linked markers in the two maps (Table 2) showed an overall reduction of approximately 45% that was unevently distributed over the linkage groups. The recombination frequencies between the pairs *Xpsm756–Xpsm336* and *Xpsm336–Xpsm386*, located in the distal part of linkage group 1, were null. This may be the result of different rates of recombination in the inter-subspecific compared to the inter-varietal cross or could be due to an inversion that differentiates Molli and ‘Souna’. The probable presence of an inversion on linkage group 1 was also observed by Liu et al. (1996) in three-way crosses involving *P. glaucum* and *P. mollissimum*. A general reduction in genetic distances in inter-specific crosses was in agreement with observations made in other species (Causse et al. 1994; Bonierbale et al. 1988; Paterson et al. 1988) and is generally interpreted to be the consequence of genome divergence between the species involved in the cross. However, it is doubtful that this was the case in our pearl millet cross since genetic distances between cultivated and wild forms from the same geographic area have been shown to be very low (Pilate-André 1992).

Genetic analysis of domestication syndrome traits

Spikelet morphology (Fig. 1)

The most critical adaptive differences between wild and cultivated pearl millet involve spikelet structure (Table 3). In wild pearl millet, the presence of an abscission layer at the basis of the involucre allows fertile spikelets to be shed spontaneously at maturity. Abundant bristles and the presence of a long awn, all ornamented with backward-pointing barbs as on a fish-hook, enhance the dispersal abilities by adherence on mammalian manes or by wind transport. Once on the ground, these bristles and the arrow-shaped morphology of the sessile spikelets allow deeper penetration of the soil and thus contribute to the survival of the seed until its germination.

The presence of an abscission layer, AL, is controlled by a single dominant gene (3:1 segregation ratio), *al6*, on linkage group 6 (Table 4). Seed coating (Ct) fits a 9:7 segregation, which suggests that this trait is controlled by two genes with the wild alleles being dominant. One of the two genes, *ct6*, was linked to the *al6* locus on LG6, and the other *ct7*, was located on LG7. Seed coating describes to which extent the glumes enclose the seed, and this trait depends in part on the length of the glumes (GL). Three unlinked genetic factors were identified for GL. A major QTL, *gl7*, which explained 49.9% of the phenotypic variance, mapped to the same region as *ct7* on group 7, and two other QTLs with minor effects, *gl5* and *gl6*, were located on LG5 and LG6, respectively (Table 5). The wild parental alleles of QTLs *gl6* and *gl7* increased glume length with over-dominant and dominant effects, respectively. The positive effect allele for *gl5* is attributed to the cultivated parent without any dominance effect. Two unlinked genes controlled the presence (wild type) or absence (cultivated type) of a long awn (PB). They are located on linkage group 1 (*pb1*) and linkage group 7 (*pb7*), with wild alleles being dominant for both genes (segregation 9:7). For the length of the bristles (BL), a large part of the phenotypic variance is explained by two major QTLs, which mapped on LG6 (*bl6*) and LG7 (*bl7*). The length of the pedicel (PL) was controlled by a major QTL, *pl6*, which is responsible for about 60% of the phenotypic variation. The peak

Table 3 Mean phenotype values, (standard deviations) for parents, F_1 hybrids and F_2 population

Trait	Cultivated parent (5)[a]	Wild parent (3)[a]	Mid-parent[b]	F_1 (9)[a]	F_2 (128)[a]
NL	6 (1.22)	5.33 (1.53)	5.66	6.22 (0.83)	6.78 (1.1)
NT	0.2 (0.44)	0.33 (0.58)	0.26	0.55 (0.73)	0.74 (0.89
LS	5.88 (1.64)	2.63 (1.15)	4.25	5.32 (2.1)	6.27 (2.03)
DS	4.7 (1.82)	2.50 (1.32)	3.60	4.11 (2.16)	4.75 (1.69)
LL	17.4 (5.08)	7.67 (3.55)	12.53	16.44 (6.68)	16.87 (5.11)
WiL	0.96 (0.43)	0.47 (0.23)	0.71	0.80 (0.24)	0.90 (0.25)
TNL	0.4 (0.89)	0.33 (0.58)	0.36	0.89 (1.36)	1.35 (2.09)
Hmax	174.2 (23.38)	110.67 (48.23)	142.43	217.29 (39.06)	204.79 (40.74)
HPT	186.2 (10.99)	92.33 (43.15)	139.26	201.11 (43.82)	190.35 (37.15)
KNTP	10.2 (0.45)	7 (1.73)	8.60	8.89 (0.93)	9.6 (1.28)
NTM	1.8 (1.3)	22.33 (2.52)	12.06	6.11 (4.81)	7.98 (6.59)
LLM	47.8 (9.98)	37.33 (5.51)	42.56	44.22 (7.61)	45.63 (7.92)
WiLM	3.48 (0.94)	1.47 (0.55)	2.47	2.5 (0.48)	2.81 (0.57)
Head	93.6 (4.39)	82.33 (6.43)	87.96	82.22 (5.78)	84.73 (10.33)
HHead	149 (11.11)	64 (36.66)	106.50	154.11 (31.92)	147.65 (37.49)
PI	2.97 (1.48)	5.11 (2.46)	4.040	2.48 (0.89)	3.16 (1.70)
NS	5 (1.22)	87.33 (68.16)	46.16	28 (11.20)	28.67 (15.89)
WeS	15.3 (4.8)	0.77 (0.45)	8.03	3.97 (1.57)	3.31 (1.89)
LoS	14.6 (1.85)	7.13 (1.48)	10.86	14.34 (1.92)	12.97 (2.6)
WiS	2.64 (0.15)	1.73 (0.31)	2.18	2.06 (0.24)	2.2 (0.56)
PL	5.92 (0.62)	0 (0)	2.96	1.57 (0.51)	2.2 (1.69)
BL	4.96 (0.21)	8.33 (0.76)	6.64	8.17 (0.77)	7.31 (2.68)
GL	3.52 (0.34)	4.27 (0.64)	3.89	5.18 (0.45)	4.81 (0.64)

[a] Sample size
[b] (Mean cultivated+mean wild)/2

Table 4 Genetic determinism of qualitative traits and their association with genetic markers

Variable	Segregation ratio	Group	Marker	χ^2	*P*
AL	3:1	6	713	20.45	0.000***
Ct	9:7	6	*al6*	29.96	0.000***
		7	812	29.80	0.000***
PB	9:7	1	756	20.50	0.000***
		7	812	23.56	0.000***

*** $P<0.001$

of the LOD score for *pl6* was located between *al6* and *Est-E* on linkage group 6. The wild allele of *pl6* decreased the length of the pedicel. Once *pl6* was fixed in the MAPMAKER algorithm, a minor QTL, *pl7*, was detected on LG7 which exhibited a positive additive effect of the wild allele. The wild alleles of both QTLs were dominant. When the effects of both *pl7* and *pl6* were cumulated they explained 71.7% of the phenotypic variation.

Thus, genetic control of the domestication syndrome at the spikelet level involved factors with large effects that were located on linkage group 6 (Fig. 2b) close to the *Est-E* and *al6* loci and/or on linkage group 7. The evolution from wild to cultivated spikelet morphology occurred mostly through recessive mutations.

Spike morphology

The average spike of the cultivated parent had a length of 14.6 cm, a width of 2.64 cm and a weight of 15.3 g, whereas the wild spike had, on average, a length of 7.13 cm, a width of 1.73 cm and a weight of 0.77 g (Table 3). Distributions for the length of the spike (LoS) (Fig. 3) and the width of the spike (WiS) revealed transgressive individuals among the F_2 progenies.

The weight of the spike (WeS) was controlled by at least 3 QTLs: *wes2* (LG2), *wes5* (LG5) and *wes6* (LG6) that collectively accounted for 77.1% of the phenotypic variation. The major QTL, *wes2*, alone explained more than 60.9%. For all QTLs, the cultivated alleles increased weight, as predicted by the parental means. The length of the spike (LoS) was mainly determined by 3 QTLs. The major QTL, *los2*, (35.9% of the phenotypic variance) is located on linkage group 2. The additive effect of the cultivated allele increased the length. In contrast, the positive effect of the 2 minor QTLs located on LG1 (*los1*) and LG7 (*los7*) was attributed to the wild alleles. Thus, when positive-effect alleles of the 3 QTLs are cumulated in the same genotype, transgressive phenotypes with longer spikes than the cultivated parent could be obtained. This can occur quite easily as these QTLs are located on different chromosomes. One of the QTLs for both weight and length of the spike, *wes2* and *los2*, coincided on LG2. For these 2 QTLs, the magnitude and direction of allele effects were similar. They may represent a single QTL with pleiotropic effect. This QTL could be responsible for the high positive correlation coefficient observed between the two characters (0.65). Two QTLs were detected for the width of the spike (WiS). The effect of *wis7* (LG7) was the largest and explained 62% of the phenotypic variance. The additive effect of the cultivated allele of *wis7* reduced the width by 0.54 cm. A minor QTL on LG5 was also de-

Table 5 Biometrical parameters of individual QTLs and cumulated QTLs (*tot*) affecting the domestication syndrome traits

Variable	QTL	Group	Locus	LOD	% var[a]	a[b]	d[b]	d/a	d allele[b]
NL		/							
NT	*nt7*	7	655	2.78	16.5%	*0.14*	0.7	5.09	+
LS		/							
DS	*ds7*	7	655	2.3	14.7%	*0.17*	1.20	6.99	+
LL	*115*	5	345	2.8	14.7%	–2.61	0.18	–0.07	/
WiL		/							
Hmax	*hmax7*	7	655	2.73	16.1%	–16.42	24.48	–1.49	c
	hmax6	6	*Est-E-al6*			*19.30*	1.13	0.06	
		tot		*19.3*	*25.3%*				
HPT	*hpt5*	5	345–731	5.58	29.9%	–21.41	27.46	–1.28	c
	hpt2	2	176–592			–10.44	16.50	1.58	+
		tot		*7.66*	*38.6%*				
HPT-HHead	*(hpt-hhead)1*	1	515–756	2.68	15.7%	4.61	–11.57	–2.50	c
NNPT	*nnpt2*	2	856–176	3.5	17.6%	–0.29	0.86	–2.97	c
	nnpt6	6	*Est-E-al6*	2.7	13.9%	*0.29*	–0.82	–2.82	c
		tot		*6.0*	*28.5%*				
NS	*ns2*	2	856–176	2.96	15.2%	*8.85*	–0.63	–0.07	
	ns6	6	*Est-E-al6*	4.33	37.0%	*13.79*	–6.31	–0.46	c
	ns7	7	655	2.33	12.9%	*5.79*	7.67	1.32	+
	ns1	1	461–858			–1.27	10.89	–8.58	c
		tot		*10.89*	*67.8%*				
NTM	*ntm2*	2	322	2.68	13.7%	*3.31*	–1.24	–0.37	/
	ntm5–1	5 (1)	345	4.31	21.4%	*4.54*	0.85	0.19	/
	ntm5–2	5 (2)	735	2.79	17.2%	*3.78*	–2.26	–0.60	c
		tot		*11.7*	*57.6%*				
LLM		/							
WiLM	*wilm2–1*	2 (1)	322	5.72	27.6%	–0.42	–0.041	0.10	/
	wilm2–2	2 (2)	592	4.8	24.3%	–0.41	–0.15	0.37	+
	wilm4	4	464–515B	3.36	30.9%	–0.36	–0.35	0.99	+
	wilm6	6	*al6*–713	2.34	20.8%	–0.27	0.35	–1.28	c
		tot		*10.53*	*54.9%*				
HHead	*hhead5*	5	651–735	6.65	34.7%	–24.75	26.13	–1.06	c
	hhead7	7	812	2.47	14.2%	–16.16	8.04	–0.50	c
		tot		*9.15*	*42.9%*				
Head	*head5*	5	651–735	10.75	59.0%	*10.93*	–10.23	–0.94	c
	head7	7	655	3.68	24.2%	–5.52	–7.05	1.28	+
		tot		*15.16*	*67%*				
BFF	*bff2*	2	176–592			–3.20	–3.04	0.95	+
	bff5	5	651–735	12.78	64.0%	*10.24*	–11.10	–1.08	c
	bff6	6	202	2.4	14.6%	*2.28*	–8.07	–3.53	c
	bff7	7	655			–2.62	–0.57	0.22	/
		tot		*19.2*	*73.5%*				
BFF-Head	*(bff-head)1*	1	858–515	5.04	26.2%	*1.88*	–0.12	–0.06	/
	(bff-head)7	7	655–812	9.4	43.4%	*1.96*	1.38	0.70	+
		tot		*13.67*	*58.1%*				
BMF-BFF		/							
FFF-BFF		/							
PI		/							
WeS	*wes2*	2	176–592	16.04	60.9%	–1.92	–0.56	0.29	+
	wes5	5	651–735	2.99	22.5%	–1.11	–0.82	0.73	+
	wes6	6	696-*Est-E*			–0.60	0.10	–0.17	c
		tot		*24.52*	*77.1%*				
LoS	*los1*	1	858–515	2.45	13.2%	*1.27*	–0.11	–0.09	
	los2	2	592–738	7.37	35.9%	–2.13	–0.74	0.35	+
	los7	7	655			*0.42*	1.18	2.81	+
		tot		*12.31*	*55.7%*				
WiS	*wis5*	5	735	2.62	15.4%	–0.34	–0.10	0.31	/
	wis7	7	655	15.58	62.0%	*0.54*	0.21	0.39	/
		tot		*22.20*	*75.8%*				

Table 5 continued

Variable	QTL	Group	Locus	LOD	% var[a]	a[b]	d[b]	d/a	d allele[b]
PL	*pl6*	6	*Est-E-al6*	12.93	61.9%	–1.60	–0.89	0.56	+
	pl7	7	655			*0.66*	0.16	0.25	+
		tot		*17.28*	*71.7%*				
BL	*bl6*	6	*Est-E-al6*	3.55	54.7%	–0.68	4.14	–6.09	c
	bl7	7	655	8.90	43.3%	*2.08*	1.51	0.73	+
		tot		*10.02*	*51.1%*				
GL	*gl5*	5	651–735	3.24	17.9%	–0.44	–0.02	0.05	/
	gl6	6	*Est-E-al6*	2.87	16.4%	*0.23*	0.39	1.70	+
	gl7	7	655	10.72	49.9%	*0.52*	0.37	0.71	+
		tot		*18.48*	*69.5%*				

[a] % Var, Percentage of phenotypic variance explained
[b] a, Additive effect of the wild allelic (when the positive effect is attributed to the wild allele; figures in italics); d, dominance effect; d allele, the dominant allele is cultivated (c) or wild allele (+)

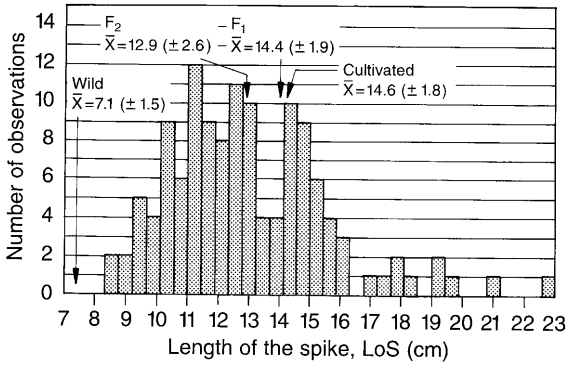


Fig. 3 Frequency distribution for spike length in the F_2 population. The means and standard deviations (in brackets) are given for parents, F_1 and F_2 populations

tected where the wild allele exerted a positive effect (a=–0.34 cm).

Flowering

Wild plants tend to head and flower earlier than cultivated ones. Heading of the primary tiller from a wild individual takes on average 82 days from sowing compared to 94 days for a cultivated one (Table 3). Pearl millet spikes exhibit a pronounced tendency to protogyny. This promotes out-crossing although selfing can occur. The protogyny deviation indicates the number of days elapsed from the beginning of female flowering (BFF) to the beginning of male flowering (BMF) on the primary tiller. The protogyny index, PI, evaluates the potential incidence of self pollen on receptive stigmas of the same spike. When PI>1, all the stigmas of the inflorescence will have emerged before anthesis and self-pollination is excluded. When PI≤1, self-pollen as well as out-pollen can cause fertilisation. For both parents, the protogyny index value was high, 3 for the cultivated parent and 5.1 for the wild parent. The larger value for the wild parent could be associated with a higher disposition of Molli towards out-crossing. However, fertilisation between tillers within the same wild plant is still possible due to the high number of tillers on each wild plant and their overlap in flowering.

No QTL was detected that could be associated with the variation in the duration of the female flowering (DO), the protogyny deviation (BMF-BFF) or the protogyny index (PI), although strong heritability of PI had previously been estimated in cultivated populations (Sarr 1987).

The dates of heading and female and male flowering are highly correlated (r>0.95). These dates were all associated with a major QTL on LG5 that was responsible for a minimum of 50% of the phenotypic variation observed for each trait. The cultivated alleles at this QTL delayed heading and flowering in a dominant manner. Linkage groups 6 and 7 also carried QTLs with lesser effects on these characters. Variation in the delay between heading (Head) and beginning of stigma emergence (BFF) was observed in the F_2 progenies. Two QTLs on LG7 and LG1 collectively accounted for 58% of the phenotypic variation.

Plant architecture

Differences between the wild and cultivated phenotypes were observed at the juvenile stage, especially for sheath (LS) and leaf (LL) length (Table 3) and, to a lesser extent, for the number of leaves (NL, TNL), the width of the leaf (WiL) and the number of basal tillers (NT).

The few QTLs detected (Table 5) for the juvenile traits, *nt7*, *dg7* and *lof5*, explained only a low proportion of the phenotypic variation. The QTLs *nt7* and *dg7* were mapped in the same interval on linkage group 7. In both cases, the wild allele exhibited a positive additive effect and over-dominance in their favour. The effects of the wild allele of *dg7* were therefore contrary to those predicted by the parental means (Table 3).

At maturity, the maximum height (Hmax) of the Molli parent was, on average, 1.1 meters, and a total of 87 fertile tillers (NS) gave a bushy plant habit (Table 3). In contrast, the ‘Souna’ parent carried an average of 5

spikes and reached a maximum height of 1.74 meters. A maximum height (Hmax) greater than the height of the primary tiller (HPT) was observed when the development of axillary tillers exceeded the primary tiller. This was associated with a reduced apical dominance and is characteristic of the wild phenotype. The leaves of the cultivated plants were broader (3.48 cm compared to 1.74 cm) and longer (47.8 cm compared to 37.33 cm).

The maximum height of the plant (Hmax) was influenced by a QTL on LG7 that was responsible for 16.1% of the phenotypic variation. The additive effect of the cultivated allele of *hmax7* increased the height by about 16 cm. Once *hmax7* was fixed by MAPMAKER, a minor QTL was detected on LG6 which exhibited a positive additive effect of the wild allele. When the effects of both *hmax7* and *hmax6* were cumulated they explained 25.3% of the phenotypic variation.

Four unlinked regions contributing to variation in the total number of fertile tillers (NS) were identified: *ns1, ns2, ns6* and *ns7*, explaining 67.8% of the phenotypic variation. As expected, the wild alleles of the three main QTLs increased the number of fertile tillers.

The QTLs detected for the number of basal tillers (NTM) collectively accounted for 57.6% of the phenotypic variation and were located on LG2 and LG5. They were distinct from the QTLs for the total number of tillers (NS). However, *ns2* and *ns6* were closely linked to 2 QTLs controlling the number of nodes on the primary tiller (NNPT). *Ns7* occupied a similar position as *nt7*, a QTL for the number of basal tillers at the juvenile stage. The QTLs *ns7* and *nt7* may correspond to the same QTL, whereas *ns1*, *ns2* and *ns6* could be involved in axillary tillering. Induction of basal and axillary tillering therefore seemed to involve different genetic mechanisms.

Discussion

Genomic similarities

The level of polymorphism revealed in our cross was similar to that observed in another predominantly cross-pollinating species, maize (Helentjaris et al. 1985) and between two subspecies of barley, *Hordeum vulgare* subs. *vulgare* and *H. vulgare* subs. *spontaneum* (Saghai Maroof et al. 1995). Higher levels of polymorphism in inter-specific or inter-subspecific combinations compared to inter-varietal crosses have generally been observed in cereals (barley, Sherman et al. 1995; rice, Causse et al. 1994; sorghum, Chittenden et al. 1994; wheat, Gill et al. 1991). However, the levels of polymorphism we observed did not differ significantly from the average amount detected between varieties of pearl millet (Liu et al. 1992). This may reflect the close genetic relatedness of the two subspecies, which has previously been shown at the cytogenetic level (Martel et al. 1996). Moreover, Pilate-André (1992) showed in an isozyme diversity analysis that genetic distances between populations of wild and cultivated pearl millet in Africa depend more on their geographic distances than on their taxonomic status (wild vs. cultivated). This can be explained by gene flow between the two forms as witnessed by the presence of intermediate phenotypes often reported in pearl millet fields in the Sahel (Marchais 1994).

Genetic basis and chromosome organisation of the domestication syndrome

Although pearl millet has a genome size (2C=4.7 pg) similar to that of maize (Arumuganathan and Earle 1991; Martel et al. 1997), its genetic map covers only 303 cM (Liu et al. 1994), whereas the maize map is 1723 cM long (Ahn and Tanksley 1993). This may reflect a low level of recombination within the pearl millet genome. The presence of outlying points (e.g. on group 4) supports the idea that recombination could be more localised at the end of the chromosomes and that the pearl millet maps may, as yet, be incomplete (Devos et al. 1995). Our linkage map comprises seven linkage groups which could correspond to the seven pearl millet chromosomes.

The domestication syndrome factors appear to be concentrated mainly on four linkage groups: LG2, LG5, LG6 and LG7. No QTLs were detected on LG3, and LG4 carried only *wilm4*. Linkage groups 2 and 5 greatly affected growth habit, phenology and spike morphology. Linkage groups 6 and 7 carried almost all the factors involved in spikelet architecture and shedding but also characters describing the plant and spike morphologies and flowering. Thus, although LG6 and LG7 represented the smallest linkage groups of the pearl millet genome in terms of genetic distances, they appeared to play a central role in determining the domestication syndrome. Close linkage of individual genes or/and the expression of pleiotropic factors could explain this clustered distribution.

The genetic analysis of cultivars from diverse origins (Joly 1984) revealed that each of the three traits, non-shedding ability (AL), nude seeds (Ct) and long pedicel (PL), can occur through independent mutations in one or two different genes according to the cultivar. All these genes were mapped in two linkage groups defined as segments I and II (Poncet et al. 1998). The genetic distances between the genes appeared to be highly conserved in the different crosses suggesting that the genes tagged in those various cultivars are homologous. In our cross, non-shedding ability is controlled by a single gene, *al6*, on linkage group 6, close to *pl6*, the major QTL involved in the length of the pedicel. Seed coating is controlled by two genes, *ct6* and *ct7*, on linkage groups 6 and 7, respectively. Linkage groups 6 and 7 could correspond to those previously described as segment I and II.

The strong linkage of the genetic factors involved in the domestication syndrome may facilitate the maintenance of the phenotypic identity of wild and cultivated populations in sympatric situations, especially in allo-

gamous species such as pearl millet. This has also been observed in other species. Several of the morphological differences between maize and its wild ancestor, teosinter, have been shown to be controlled by major genes and only in few genomic regions (Doebley and Stec 1991, 1993; Dorweiler et al. 1993). Gene orders have been shown to be highly conserved throughout the evolution of grasses (Devos and Gale 1997). Loss of shattering appears to be controlled by homologous loci in maize, sorghum and rice (Paterson et al. 1995). In pearl millet, mutations in either of the two genes previously identified can lead to the loss of shedding ability. Moreover, seed dispersal in cereals is ensured by very different mechanisms depending on the species: for example, in wild pearl millet seed-shedding is due to the presence of a functional abscission layer at the pedicel basis, whereas in wild wheats the fragile rachis disarticulates at maturity (Sharma and Waines 1980). A comparative study of the main characters of the domestication syndrome remains to be done especially between allogamous and autogamous crops. It has been suggested by Pernès (1983) that linkage of genes controlling the differences between wild and cultivated forms could be more crucial for allogamous crops, whereas autogamy by itself reduces gene flow and favours multilocus associations even among unlinked loci through linkage disequilibrium. Hence, the effect of the reproductive system on the evolution of the genome organisation has to be investigated in depth.

No QTLs were detected for some of the juvenile plant descriptors (NL, LS, WiL), for the protogyny index (PI), although this trait had previously been shown to have a high heritability in cultivated populations (Sarr 1987), and for the length of the leaf at maturity (LLM). Only a relatively small number of QTLs were detected for traits such as height (Hmax). This could be explained by the fact that these traits were determined by many genes with minor effects that did not reach the threshold using our mapping conditions and/or that the phenotypic variance of these characters has a major environmental component.

A sizeable proportion of the phenotypic variation observed for most quantitative traits can be explained by genetic factors. However, the percentage of phenotypic variation explained by each QTL should be considered with caution. Indeed, Beavis (1994) has shown through theoretical simulation that the percentage of phenotypic variance explained by QTLs is generally overestimated unless the size of the mapping population is very large. Nevertheless, the presence of QTLs that explained a large percentage of the variation with a large LOD score strongly suggests the effect of single genes. This was the case for traits determined by *wes2*, *wis7*, *pl6*, *Head5* and *bff*5. In maize, the *teosinte glume architecture 1* (*tga1*) and the *teosinte branched* 1 (*tb1*) were identified as major contributors to the maize/teosinte evolution (Doebley et al. 1995; Dorweiler and Doebley 1997). Maize and teosinte alleles of *tb1* differed in their level of expression (Doebley et al. 1997) and the pleiotropic effects of *tga1* suggested that it was regulatory in nature (Dorweiler and Doebley 1997). This supported the argument that major changes determining adaptation to disturbed habitats such as cultivated environments has proceeded by an alteration in gene regulation and could therefore involve the action of transcription factors.

Nature of the polygenes, candidate genes

Dwarf phenotypes can be considered as a consequence of mutations which occur on genes involved in the height expression (Lin et al. 1995). Dwarf mutants of pearl millet have been studied (Kadam et al. 1940; Burton and Forstan 1966; Appa Rao et al. 1986) and four single genes have been reported up to date: *d1* and *d2* (Burton and Forstan 1966) and *d3* and *d4* (Appa Rao et al. 1986), with the possible presence of modifying factors. None of these genes has been mapped. *d2* may have a pleiotropic effect since *d2* near-isogenic lines have longer and narrower panicles, wider leaves and smaller seeds than their tall counterparts (Rai and Hanna 1990). The *d2* effects on the phenotype could correspond to the effects observed at the *hmax7* locus. Indeed, this QTL had an effect on the height of the plant. Moreover, *hmax7* was mapped within overlapping (1-LOD) confidence intervals of QTLs involved in the width and length of the spike (WiS and LoS) and a QTL associated with glume development (GL). The map location of *d2* should show whether *hmax7* could putatively be *d2*.

In maize, mutations in the *teosinte branched* 1 (*tb1*) led to the outgrowth of basal lateral branches as tillers, and upper lateral branches as shoots, replacing the ear. Interactions with other loci also contributed to the overall architecture with pleiotropic effects (Doebley et al. 1995; Doebley et al. 1997). Comparative maps of maize and pearl millet genomes should provide a basis for examining the potential nature of *tb1* as a candidate gene underlying pearl millet plant architecture.

Transgressions and genetic resources

For most major QTLs, the directions of the effects of the parental alleles were as predicted by the parental phenotypic means. However, for some traits, Hmax, LoS, WiS, BL and GL, positive-effect alleles were contributed by both parents. When an individual received positive-effect alleles from both parents for a particular trait, it out-performed them (e.g. Fig. 3). Transgressive phenotypes are all the more easily obtained when a trait is controlled by unlinked QTLs (e.g. LoS).

The existence of wild alleles that reinforce the cultivated phenotype in the F_2 background (WiS, LoS, Hmax...) raised questions on the mechanism allowing their maintenance in wild populations while they are expected to decrease the adaptive value of the wild plant in natural conditions. This paradox could be explained by epistasis of the wild genetic background on the effect of the QTL leading to its silencing. This hypothesis is still

to be tested. However, such results open promising possibilities to enhance pearl millet landraces by using genetic resources from the wild. Indeed, despite their overall inferior appearance in terms of agronomic value, the closest wild relatives of crops can be sources of novel genes not only to enhance stress tolerance but also to substantially increase yield. Such favorable genes have already been detected for fruit weight in tomato (Fulton et al. 1997) and for the number of grains per panicle in rice (Xiao et al. 1996). Introgression of *Oryza rufipogon* wild alleles at 2 specific loci into a top-performing rice variety was associated with yield increases of 18% and 17%. Our results suggest that improvement of yield components in pearl millet, such as length of the spike (LoS), could be obtained by introgression of wild alleles.

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