



Water saving traits co-map with a major terminal drought tolerance quantitative trait locus in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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DOI: <http://dx.doi.org/10.1007/s11032-012-9720-0>

Supplement Material

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Water saving traits co-map with a major terminal drought tolerance quantitative trait loci in pearl millet (*Pennisetum glaucum* (L.) R. Br.).

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Supplementary Table 1. Average minimum and maximum temperature (Tmin, Tmax), humidity (RHmin, RHmax) and VPD of distinct experimental sets 14 days prior to assessment of physiological traits

Set Number	Tmin	RHmin	Tmax	RHmax	VPDmin	VPDmax
set 1	15.69	32.33	35.84	94.16	0.10	3.93
set 2	16.02	31.71	35.81	92.69	0.13	3.96
set 3	15.74	30.78	35.76	91.71	0.15	4.01
set 4	14.93	29.28	35.70	91.67	0.15	4.10
set 5	15.06	30.90	35.11	93.50	0.11	3.88

Supplementary Table 2. The variation within and between experimental RIL sets approximated by the set average, standard deviation (SD), minimum and maximum range of trait values and differences between parental genotypes across experimental sets. Below the trait values, the results of ANOVA showing the significance of variation across sets, genotypes and genotype x set interactions at the levels of significance; p<0.001 (***)^a; p<0.01 (**); p<0.05 (*); “ns” stands for non-significant variation and MS for mean square. The LSD of means is visualized as letters next to trait/parent average where different letters indicate significant difference of means and *vice versa*.

These traits are; leaf dry weight (LDW [g]), root dry weight (RDW [g]), shoot dry weight (ShDW[g]), stem dry weight (StDW[g]), biomass dry weight (BDW[g]), leaf area (LA [cm²]) specific leaf weight (SLW [g cm⁻²]), transpiration rate (Tr [g water cm⁻² leaf area h⁻¹]) and absolute transpiration (T [g]). The measurements between 7:30-10:30 a.m. are indicated with suffix M and 10:30 a.m.-2:30 p.m. with suffix A and particular set’s suffix indicates the date of measurement in December 2009.

trait-set	average	SD	Range	H77/833-2	PRLT2/89-33
BDW 23	7.47(c)	1.89	4.05-11.81	6.47	7.44
BDW 28	10.49(a)	1.77	6.09-14.03	9.5	10.86
BDW 31	9.00(b)	1.99	4.85-13.29	9.38	12.77
variation source	set	genotype	genotype x set	8.45±0.99(a)	10.36±1.56(a)
Significance	***	***	***		
LA 18	467.04(d)	112.61	206.75-766.25	285.69	-
LA 21	1072.49(b)	222.16	615.89-1604.31	995.45	1269.54
LA 23	898.30(c)	230.17	419.99-1402.59	547.46	965.94
LA 28	1198.35(a)	217.5	657.86-1749.9	1215.89	1303.97
LA 31	1052.60(b)	221.54	582.54-1649.22	916.85	1770.99
variation source	set	genotype	genotype x set	792.3±166.25(b)	1327.6±166.16(a)
Significance	***	***	***		
LDW 18	3.07(d)	0.59	1.68-4.34	2.19	-
LDW 21	4.00(c)	0.77	1.76-5.42	3.64	5.1
LDW 23	3.95(c)	0.98	2.24-7.06	3.2	3.6
LDW 28	5.10(a)	0.79	3.12-6.98	5.5	4.85
LDW 31	4.38(b)	0.89	2.51-6.76	3.61	5.86
variation source	set	genotype	genotype x set	3.63±0.54(a)	4.86±0.47(a)
Significance	***	***	***		
RDW 23	1.69(c)	0.65	0.58-3.22	1.97	1.93
RDW 28	2.92(a)	0.91	1.07-5.36	2.48	4.02
RDW 31	2.35(b)	0.72	1.2-4.5	4.36	4.25
variation source	set	genotype	genotype x set	2.93±0.73(a)	3.4±0.74(a)
MS	***	ns	***		
ShDW 18	4.34(d)	0.87	2.36-6.34	3.18	-
ShDW 21	5.78(c)	1.1	2.98-8.08	5.26	6.82
ShDW 23	5.75(c)	1.37	3.23-9.36	4.5	5.51
ShDW 28	7.51(a)	1.22	4.69-9.7	7.02	6.84
ShDW 31	6.65(b)	1.41	3.55-10.07	5.02	8.52
variation source	set	genotype	genotype x set	5±0.62(a)	6.9±0.62(a)
Significance	***	***	***		
SLW 18	0.0067(a)	0.001	0.0046-0.0109	0.0077	-
SLW 21	0.0038(d)	0.0009	0.0021-0.006	0.0037	0.004
SLW 23	0.0045(b)	0.0006	0.0032-0.0063	0.0058	0.0037
SLW 28	0.0043(bc)	0.0004	0.0035-0.0055	0.0045	0.0037
SLW 31	0.0042(c)	0.0005	0.0035-0.006	0.0039	0.0033
variation source	set	genotype	genotype x set	0.0051±0.0007(a)	0.0037±0.0001(a)
Significance	***	***	***		
StDW 18	1.24(d)	0.36	0.08-2.02	0.99	-
StDW 21	1.76(c)	0.49	0.59-2.96	1.62	1.72
StDW 23	1.78(c)	0.5	0.54-3.04	1.3	1.91
StDW 28	2.40(a)	0.57	0.88-3.78	1.52	1.99

StDW 31	2.24(b)	0.58	1.04-3.59	1.41	2.66
variation source	set	genotype	genotype x set	1.37±0.11(a)	2.07±0.2(a)
Significance	***	***	***		
TA 18	19.72(g)	3.3	8.03-28.28	16	-
TA 19	21.30(f)	3.85	8.78-31.1	15.2	-
TA 21	23.36(e)	3.88	10.8-32.78	21.97	26.03
TA 22	33.76(a)	5.53	10.98-45.15	33.8	36.88
TA 23	28.45(c)	6.09	14.18-40.18	20.8	31.27
TA 24	31.81(b)	6.83	16.22-48.15	24.9	36.02
TA 28	28.27(c)	4.61	19.1-39.77	30.4	28.15
TA 29	16.71(h)	2.4	11.52-21.88	19.3	18.58
TA 31	26.24(d)	4.49	18.4-37.85	21.35	36.55
variation source	set	genotype	genotype x set	22.64±2.06(b)	30.5±2.56(a)
Significance	***	***	ns		
TM 18	11.55(d)	2.11	4.9-18.17	9.43	-
TM 19	7.91(e)	1.35	3.7-10.97	6.2	-
TM 21	10.38(c)	1.68	5.57-14.17	9.7	11.17
TM 22	14.09(a)	2.44	7.33-19.33	14.73	16.57
TM 23	11.26(b)	2.7	5-18.9	10.5	13.8
TM 24	13.40(a)	2.89	7.2-20.23	11.43	14.87
TM 28	9.58(d)	1.26	5.93-12.07	10.9	9.8
TM 29	9.97(cd)	1.47	7.03-13.73	10.97	10.97
TM 31	10.11(cd)	1.69	6.83-14.47	8.2	13.5
variation source	set	genotype	genotype x set	10.23±0.78(b)	12.95±0.91(a)
Significance	***	***	**		
Tr A 18	0.0442(a)	0.0075	0.0323-0.0659	0.056	-
Tr A 19	0.0477(b)	0.0082	0.0356-0.0752	0.0532	-
Tr A 21	0.0227(f)	0.0036	0.0146-0.0311	0.0221	0.0205
Tr A 22	0.0331(d)	0.0061	0.0221-0.0485	0.034	0.029
Tr A 23	0.0328(e)	0.0043	0.0242-0.0423	0.038	0.0324
Tr A 24	0.0367(c)	0.0051	0.0245-0.0518	0.0455	0.0373
Tr A 28	0.0239(g)	0.0031	0.0176-0.0331	0.025	0.0216
Tr A 29	0.0141(g)	0.0018	0.0108-0.0188	0.0159	0.0142
Tr A 31	0.0258(f)	0.0034	0.0199-0.0357	0.0233	0.0206
variation source	set	genotype	genotype x set	0.035±0.0048(a)	0.025±0.003(b)
Significance	***	***	***		
Tr M 18	0.0259(a)	0.0052	0.017-0.0447	0.033	-
Tr M 19	0.0178(b)	0.0035	0.0117-0.0299	0.0217	-
Tr M 21	0.0101(g)	0.0019	0.0072-0.0152	0.0097	0.0088
Tr M 22	0.0138(d)	0.0031	0.0096-0.0221	0.0148	0.013
Tr M 23	0.0130(d)	0.0023	0.0078-0.0194	0.0192	0.0143
Tr M 24	0.0155(c)	0.0028	0.0079-0.0234	0.0209	0.0154
Tr M 28	0.0082(f)	0.0012	0.0057-0.0118	0.009	0.0075
Tr M 29	0.0084(h)	0.0011	0.0058-0.0111	0.009	0.0084
Tr M 31	0.0100(e)	0.0016	0.0069-0.0146	0.0089	0.0076
variation source	set	genotype	genotype x set	0.016±0.0027(a)	0.011±0.0013(b)
Significance	***	***	***		

Supplementary Table 3 QTLs detected for transpiration rate (Tr) and absolute transpiration (T) by PLABQTL CIM analysis. In VPD columns the average VPD between 7:30-10:30 a.m. (with suffix M) and 10:30 a.m.-2:30 p.m. (with suffix A) is presented. QTLs are shown for particular replications (suffix of trait name indicates day of measurement in December 2009) and for replication based BLUPs means (indicated as BLUP suffix of the trait). The columns names further indicate the LG group and maximum confidence interval of QTLs encompassed in particular column. The QTLs detected are presented as; LG group, peak position (percentage of phenotypic variation explained by QTL; LOD). Red or blue colored font indicates that the positive effect comes from PRLT (tolerant) or H77 (sensitive) allele

Trait name	VPD	QTL	LG1 (126-134)	LG2 (0-14)	LG2 (100-122)	LG2 (234-244)	LG2 (250-278)	LG2 (276-318)	LG2 (310-324)	LG3 (18-30)	LG7 (78-84)	LG7 (96-114)
TrA 18	4.10	none										
TrA 19	3.19	none										
TrA 21	3.14					2, 240 (2; 3.6)						
TrA 22	4.05						2, 260 (6; 3.6)					
TrA 23	4.22											
TrA 24	4.50							2, 278 (6; 5.2)	2, 322 (13; 3.7)			
TrA 28	3.66	none										
TrA 29	2.04										7, 82 (7; 3.6)	
TrA 31	2.82				2, 112 (11; 2.7)							
TrA BLUP	3.53	none										
TrM 18	2.62	none										
TrM 19	0.89								2, 316 (1; 4.4)	3, 28 (1; 4.1)		
TrM 21	0.89			2, 2 (15; 2.9)			2, 276 (7; 2.7)					
TrM 22	1.78			2, 2 (14; 2.5)								
TrM 23	2.19			2, 2 (26; 5.0)					2, 322 (16; 6.3)			
TrM 24	2.53			2, 6 (16;3.8)			2, 262 (16; 4.2)					
TrM 28	1.05	none										
TrM 29	1.17										7, 102 (10; 2.7)	
TrM 31	0.98	none										
TrM BLUP	1.57					2, 260 (26; 7.8)	2, 314 (14; 3.6)					
TM18	2.62	none								3, 28 (13; 3.7)		
TM19	0.89											
TM21	0.89	none										
TM22	1.78	none										
TM23	2.19						2, 254 (9; 4.5)					
TM24	2.53						2, 254 (10; 4.0)			3, 22 (7; 3.8)		
TM28	1.05	none										
TM29	1.17	none										
TM31	0.98	none										
TM BLUP	1.57							2, 318 (8; 3.3)			7, 112 (5; 3.4)	
TA18	4.10	none										
TA19	3.19								3, 28 (10; 3.9)			
TA21	3.14		1, 130 (1; 6.1)								7, 112 (9 ; 6.1)	
TA22	4.05	none										
TA23	4.22	none										
TA24	4.50	none										
TA28	3.66	none										
TA29	2.04	none										
TA31	2.82	none										
TA BLUP	3.53			2,4 (18; 6.5)							7, 112 (5; 3.4)	

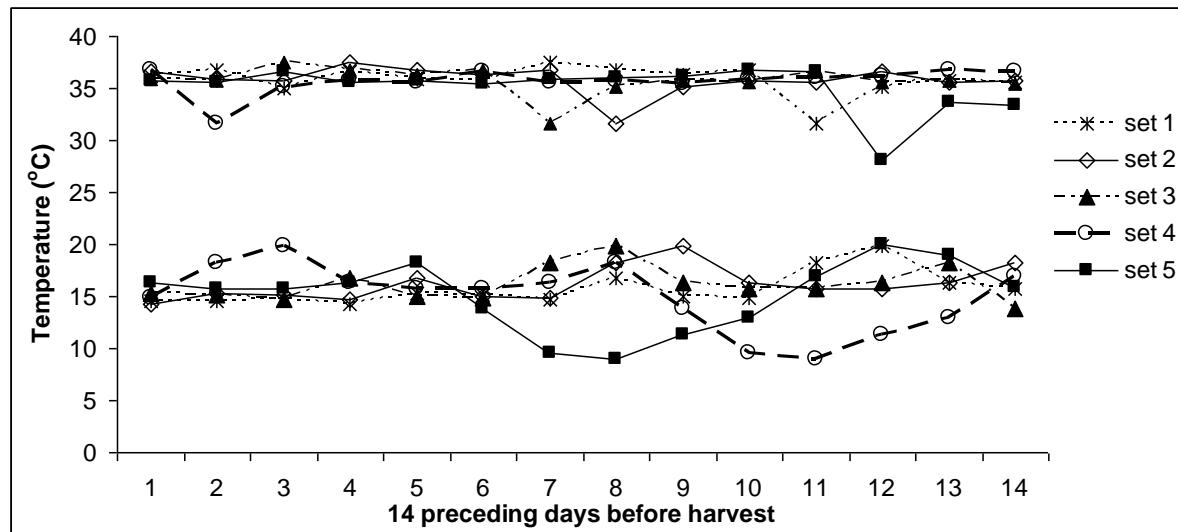
Supplementary Table 4 QTLs detected for Leaf dry weight (LDW), root dry weight (RDW), shoot dry weight (ShDW), stem dry weight (StDW), biomass dry weight (BDW), leaf area (LA) specific leaf weight (SLW) and principal components (PCA 1, 2, 3) by PLABQTL CIM analysis. QTLs are shown for particular replications (suffix of trait name indicates day of measurement in December 2009) and for replication based BLUPs (indicated as BLUP suffix of the trait). The columns names further indicate the LG group and maximum confidence interval of QTLs encompassed in particular column. The QTLs detected are presented as; LG group, peak position (percentage of variation explained by QTL; LOD). Red or blue colored font indicates that the positive effect comes from PRLT (tolerant) or H77 (sensitive) allele.

Supplementary Table 5. Correlation analysis of the best unbiased linear predicted (BLUP) means of traits from suppl. Table 2. Numbers represent correlation coefficient with p<0.001 (***) ; p<0.01 (**) ; p<0.05 (*) and “ns” stands for non-significant relation.

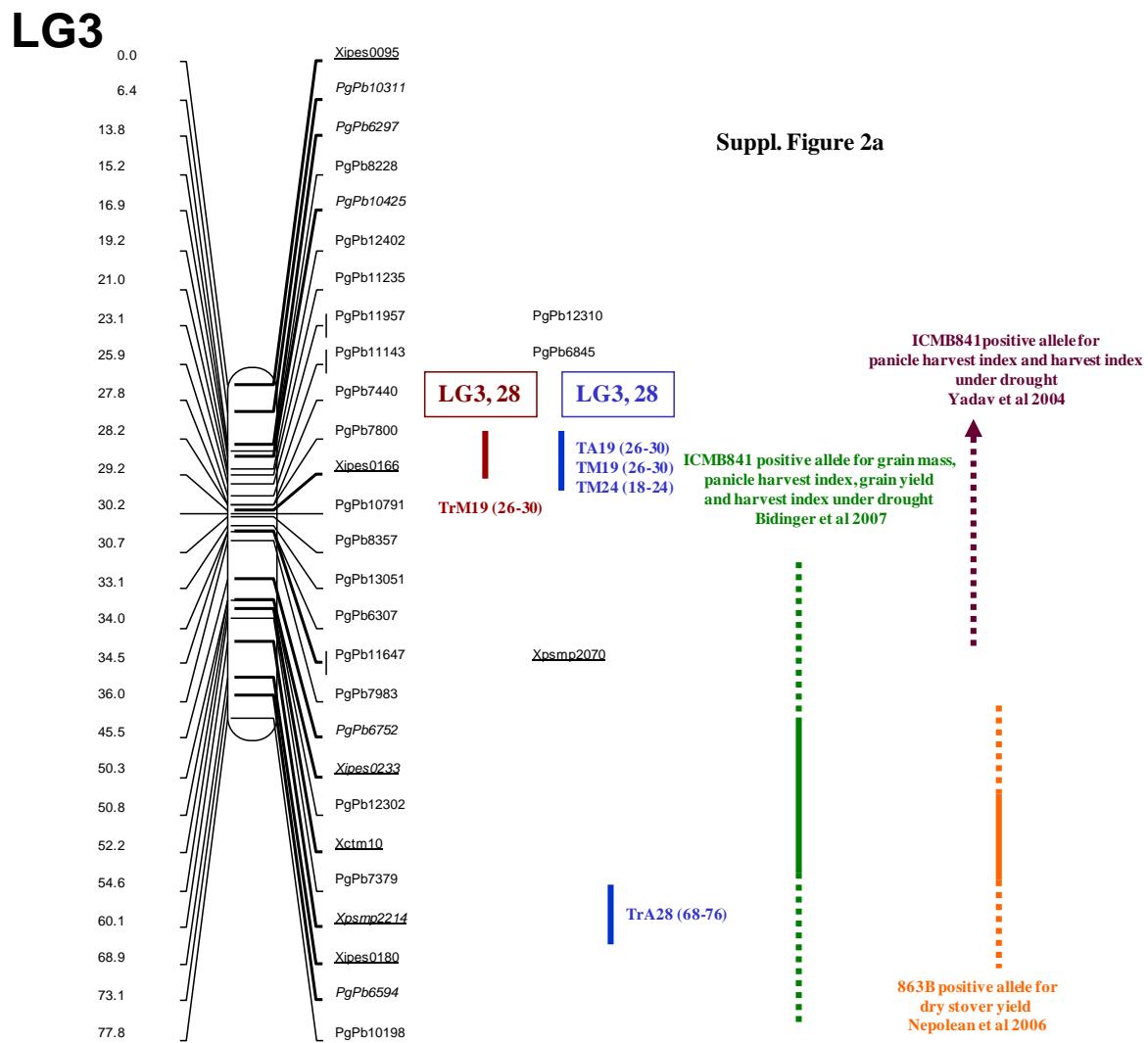
Supplementary Table 6 Numerical output of principal component analysis (PCA) based on BLUP means of investigated traits, i.e. transpiration rate (Tr), absolute transpiration (T), leaf dry weight (LDW), root dry weight (RDW), shoot dry weight (ShDW), stem dry weight (StDW), biomass dry weight (BDW), leaf area (LA) specific leaf weight (SLW). Traits with suffix M were measured during 7:30-10:30 a.m. and traits with suffix A were measured during 10:30 a.m.-2:30 p.m. In the top part of the table the standard deviation (SD), proportion of phenotypic variance (PV) and cumulative PV for three major principal components (PC1, 2, 3) is shown. Bottom part of the table shows the values of traits loadings in particular PC

Statistics	PC 1	PC 2	PC 3
SD	2.565	1.481	0.813
PV	0.598	0.199	0.060
Cumulative PV	0.598	0.797	0.858
Loadings:			
Trait name	PC 1	PC 2	PC 3
TrM	-0.249	-0.479	negligible
TrA	-0.233	-0.455	negligible
LDW	0.351	-0.132	negligible
StDW	0.334	negligible	negligible
ShDW	0.366	-0.112	negligible
LA	0.362	0.153	negligible
SLW	-0.169	-0.518	negligible
RDW	0.254	-0.276	-0.632
BDW	0.347	-0.171	-0.386
TA	0.319	-0.161	0.37
TM	0.261	-0.329	0.544

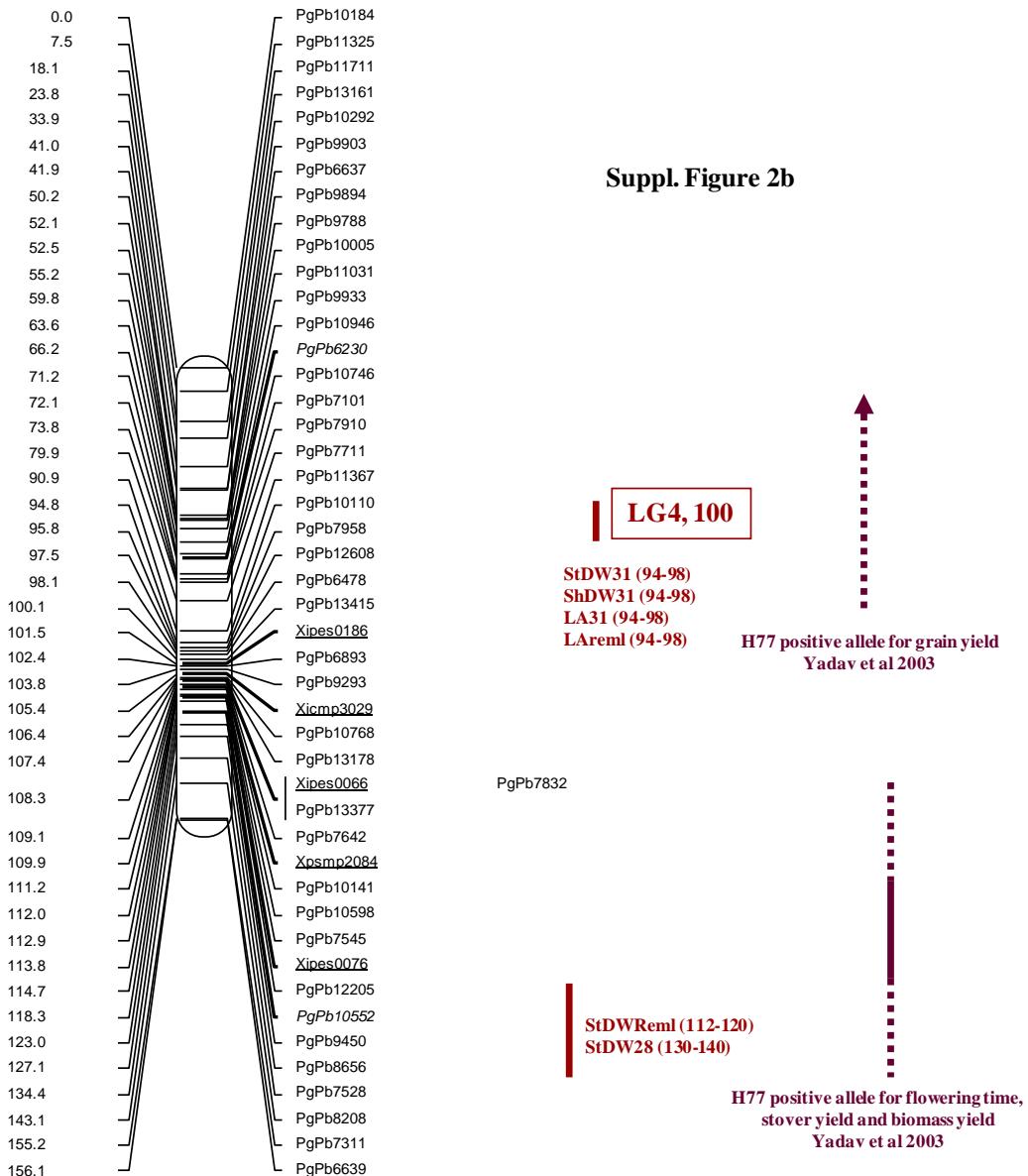
Supplementary Fig. 1. Maximum and minimum temperature to which the different experimental sets were exposed during 14 days prior to their assessment for Tr and related traits.



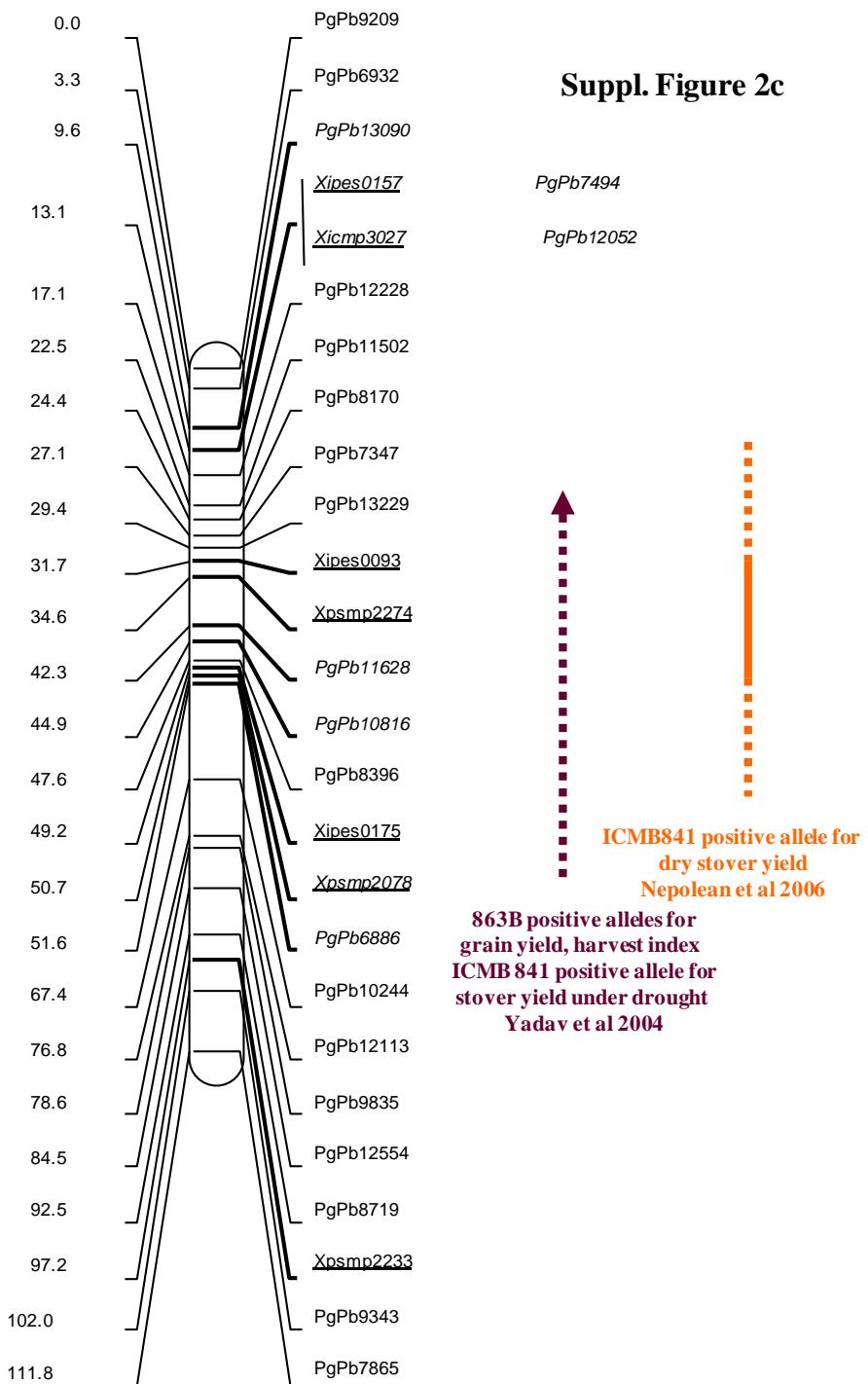
Supplementary Fig.2. Visualization of approximate QTLs positions within the linkage groups of minor importance (LG; chromosomes 3, 4, 5, and 6, letter a to d) consisting of SSR and DArT markers and their positions in cM.



LG4



LG5



LG6

Suppl. Figure 2d

