ASSOCIATION MAPPING OF CANDIDATE GENES FOR DROUGHT TOLERANCE IN MEDITERRANEAN DURUM WHEAT LANDRACES

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Durum wheat is a Mediterranean crop that is typically grown under rainfed conditions. Therefore, the main yield constraint is the so called "terminal stress" (a combination of drought and high temperatures during grain filling). Yield and yield components are dramatically affected by drought, and breeding in such environments is difficult because of the low heritability of these traits. A genomics approach may help breeding under drought by finding marker/trait associations. In association mapping, genes/markers are related to traits using a diverse panel of genotypes/accessions. A candidate gene approach may take advantage of more fine, small scale research at the physiological and genetic level, which can be scaled up by the use of genomics.

A collection of 172 durum wheat landraces from 22 Mediterranean countries and 20 durum wheat cultivars was grown under irrigated and rainfed conditions in Lleida (Spain) during the 2006-07 growing season. Yield (T/ha at 12 % grain moisture), thousand kernel weight (g, TKW), and the number of spikes per m2 (NSM2) were measured at ripening. Yield was the combine grain weight, TKW was measured on a sub-sample of the harvested grain, and NSM2 was measured on a 1 m sample from a central row.

We prioritized 18 candidate genes reported to be associated with drought tolerance and designed primers spanning introns. Primer pairs were tested on Jennah Ketifa and Cham1 cultivars. Single band amplicons were cloned, sequenced and aligned with the original EST/gene sequence. Primer pairs aligning with the EST/gene sequence and spanning an intron were used for the assessment of polymorphism in the landrace collection. However, only 16 showed enough quality to be used. Additional 33 unlinked or weakly linked SSR were used to determine the genetic structure of the population, which was used in a mixed model to assess the associations of the candidate genes with yield and yield components.

Soil Moisture
The pattern of soil moisture at three depths during the growing season showed the differences in water soil content between irrigated and rainfed treatments, especially between jointing and anthesis (solid bar on x-axis).
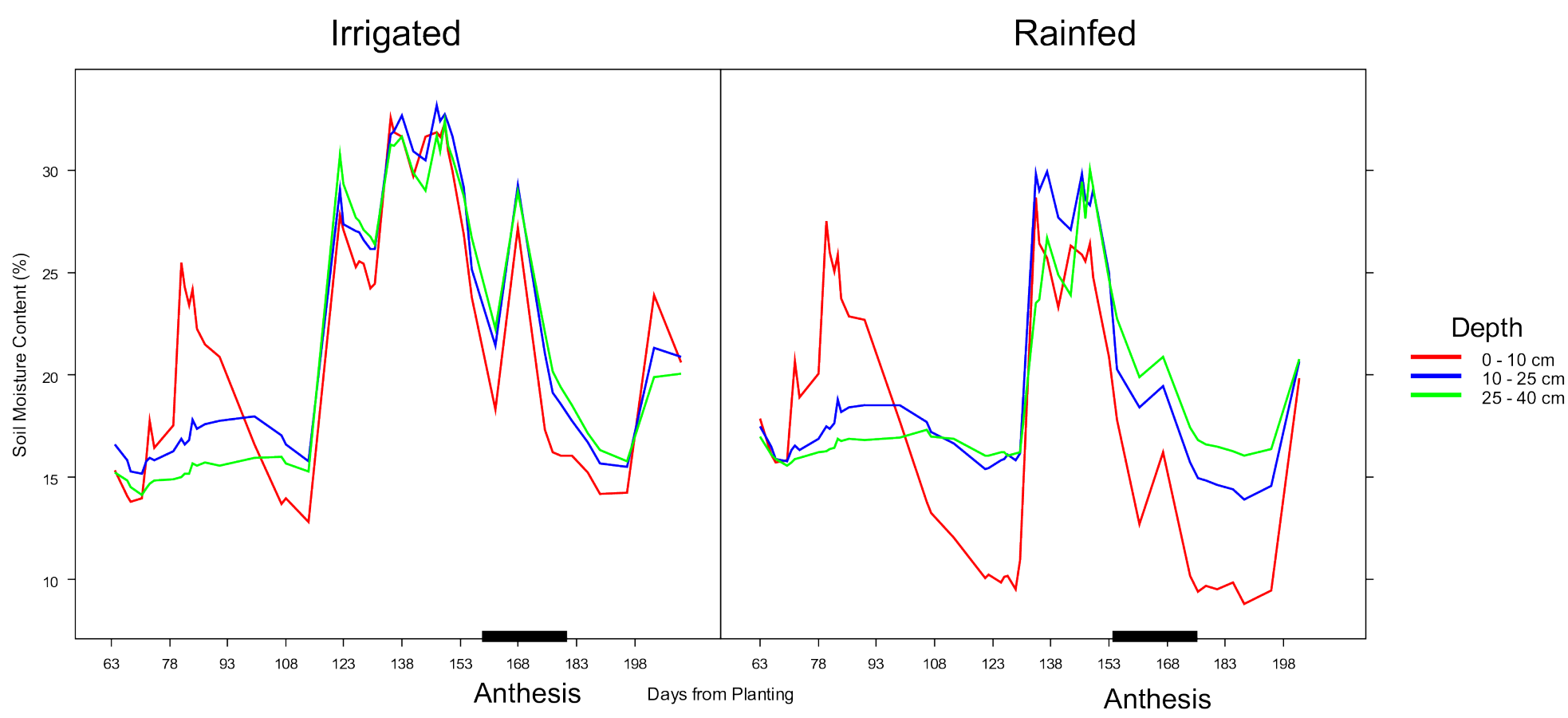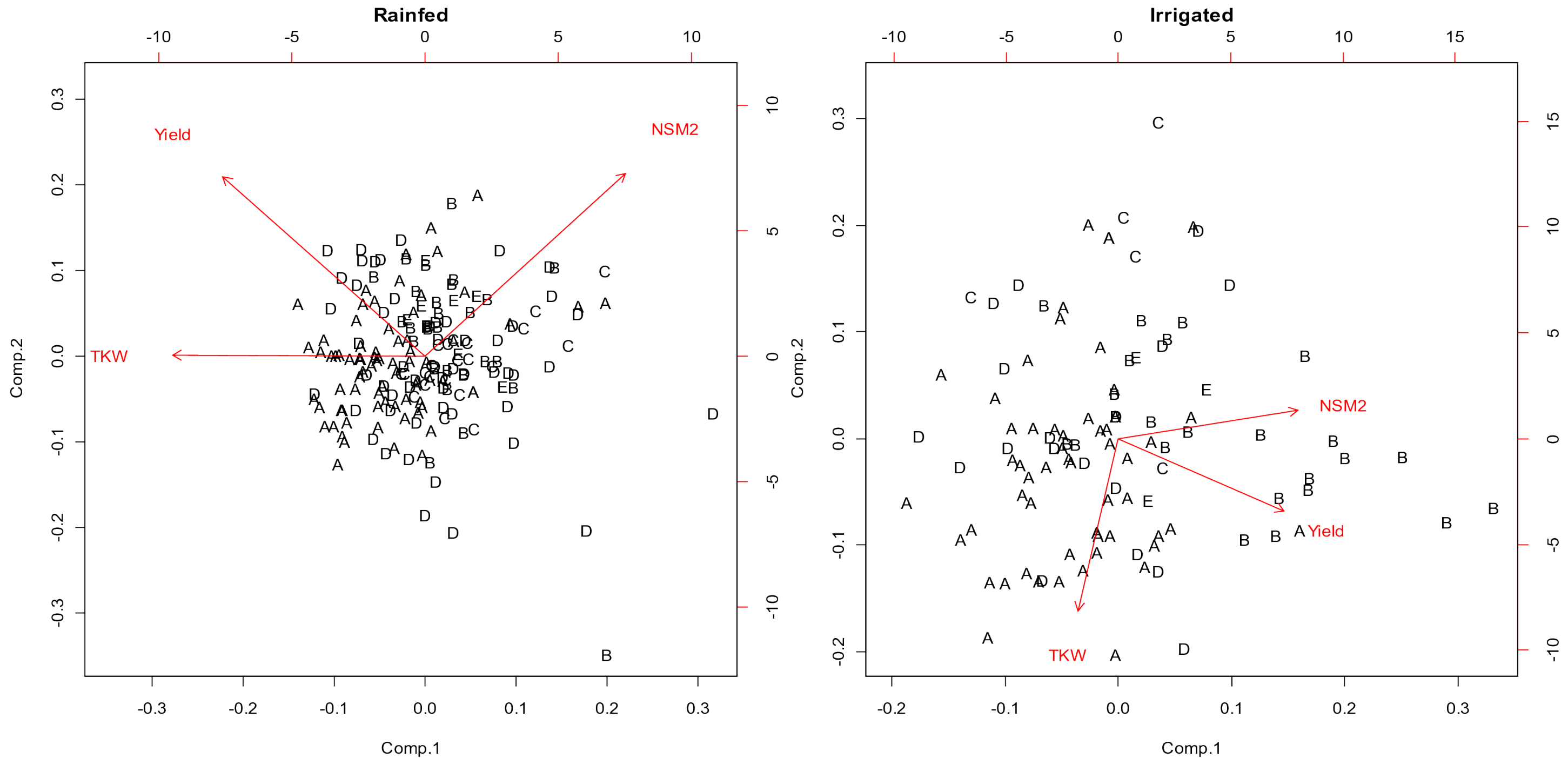

Correlation between traits
The best linear unbiased predictor of each accession in the two treatments (Rainfed, Irrigated) was calculated. In the rainfed environment, grain weight was positively correlated with grain yield and negatively correlated with the number of spikes per m2 (NSM2), while NSM2 was not correlated with yield. Yield, grain weight and NSM2 were related to each other in the irrigated environment, but the relationship between grain weight and yield or NSM2 were weaker than in the rainfed environment.
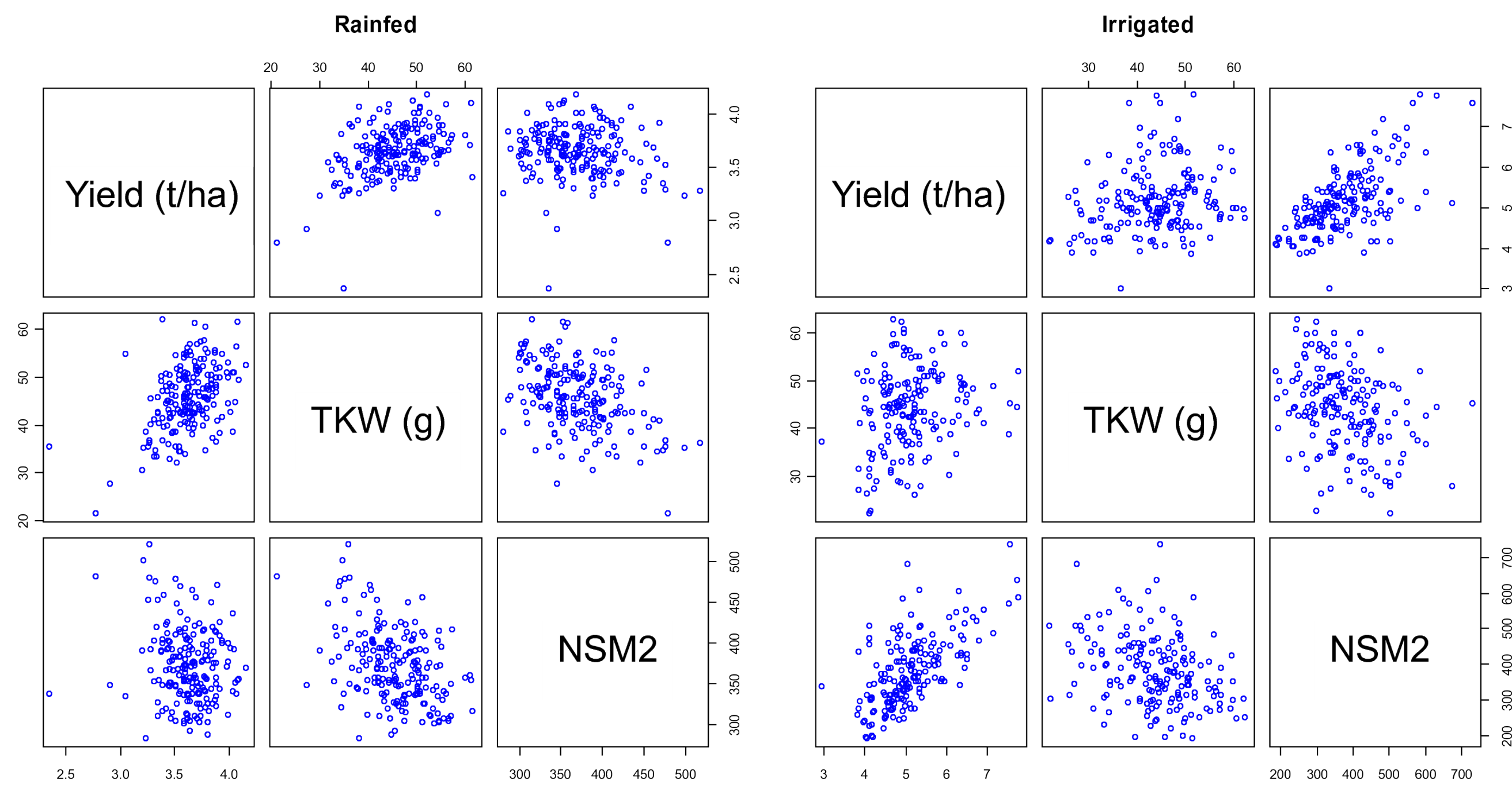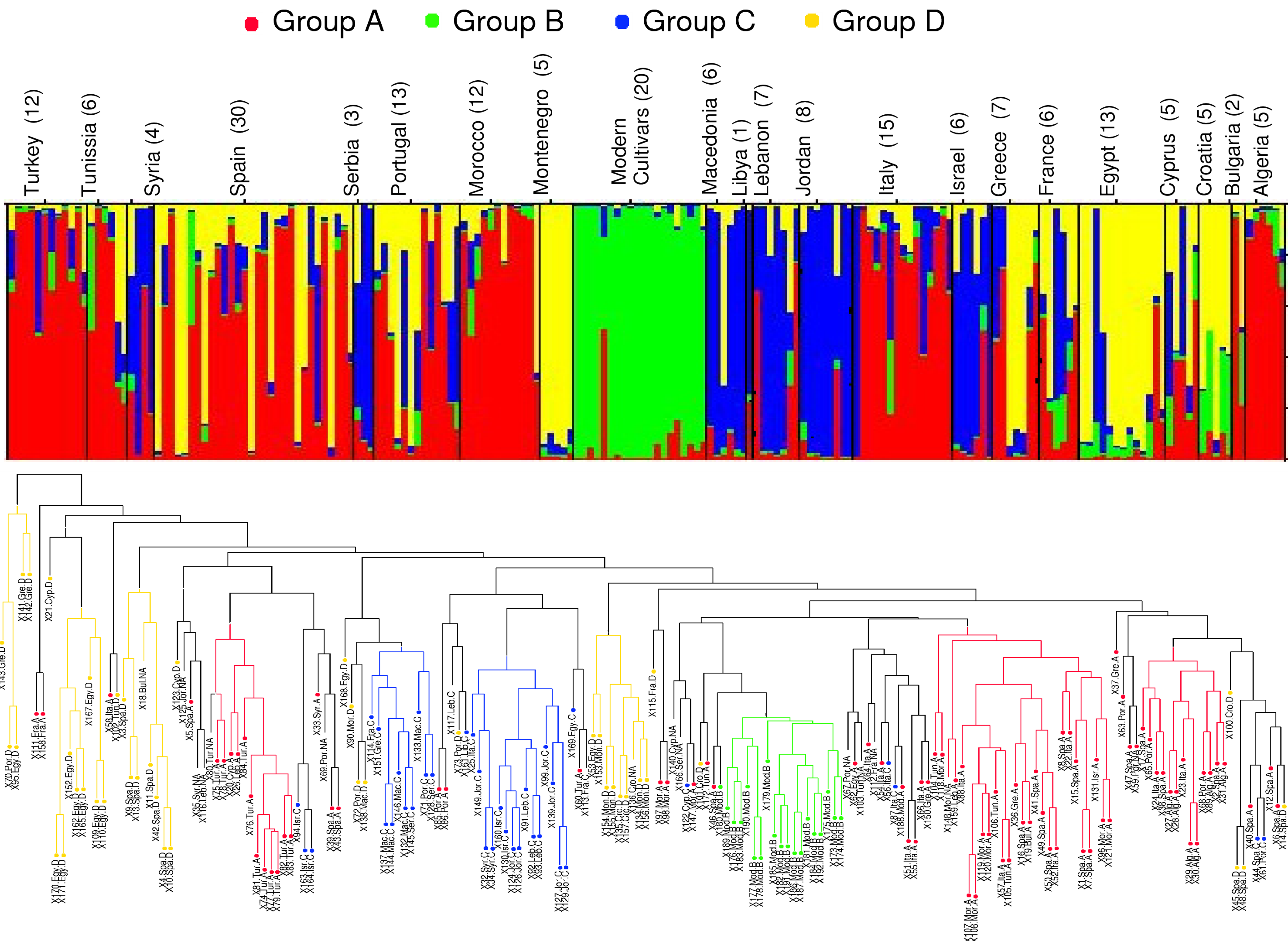

Principal Component Analysis
Principal component analysis (PCA) was applied on the correlation matrix between yield, thousand kernel weight (TKW) and the number of spikes per m2 in each environment. The correlation between traits, related to the angle between the eigen vectors, confirmed the results from the correlation analysis. Each point in the biplot represents an accession and they are coded as the subpopulation they belong to according to SSR data. The accessions from group B (modern cultivars) tend to cluster apart from the landraces when the potential of the environment is increased by irrigation. This cluster is in the direction of higher yield and NSM2, therefore modern cultivars take more advantage of better environments than landraces.

Genetic Structure
The profiles of 33 unlinked or fairly linked SSR loci revealed 4 subpopulations and cluster analysis showed that the accessions tended to group according to their geographical origin.

Association of Candidate Genes with Yield and Yield Components
The primer pairs designed successfully amplified introns. However, some of them showed more than one band. Usually, those bands (or patterns) could be clearly differentiated and, hence, scored as independent loci. All genes were scored as codominant markers, but BJ269350_246 and CJ655209_280 that were dominant and each band was used as an independent locus in the association analysis. The intron 12 of sucrose synthase I (SSI_Int-12) was associated with yield and grain weight in the rainfed, but not in the irrigated environment. The activity of sucrose synthase in the grain is increased by water stress during grain filling (Yang et al., 2001). The Cu/Zn superoxide dismutase located in chromosome 7BS (SOD_1.2) strongly influenced TKW and NSM2 in the rainfed environment, and was associated with NSM2 under irrigation. Additionally, the MnSOD (SOD_3.1 and SOD_3.2) were associated with yield only under rainfed conditions. MnSOD are drought inducible and decrease after rehydration, whereas Cu/ZnSOD are not induced by drought stress but increase after rehydration (Wu et al., 1999)

Gene		Rainfed			Irrigated		
		Yield	TKW	NSM2	Yield	TKW	NSM2
BE585626_450	A	0.39	0.20	0.74	1.00	0.37	0.64
BE585626_450	B	1.74	0.35	0.91	3.01	1.94	1.33
BE585626_450	C	0.80	0.23	1.59	0.96	0.52	0.87
BJ269350_246	A1	1.78	3.96	3.23	0.58	0.75	3.32
BJ269350_246	A2	2.58	1.15	0.58	1.00	0.16	2.33
BJ269350_246	B	0.02	1.50	3.08	0.74	0.29	1.13
BJ278757_130		1.11	0.33	1.79	0.11	0.27	1.40
CA728461_179	A	0.20	2.27	4.34	3.17	0.04	3.42
CA728461_179	B	3.46	2.08	1.84	1.47	1.36	1.10
CJ655209_280	A1	0.37	0.94	0.58	0.91	0.98	2.28
CJ655209_280	A2	0.01	4.52	0.60	8.80	0.50	5.61
CJ655209_280	B1	0.29	0.66	0.48	0.89	0.91	0.74
CJ655209_280	B2	0.55	1.81	0.89	1.11	0.51	0.43
CJ655209_280	B3	0.01	1.66	1.06	0.74	0.10	0.10
CJ655209_280	B4	0.04	2.07	0.11	0.47	0.73	1.49
CJ655209_280	B5	0.12	2.44	0.20	2.29	0.52	2.62
CJ655209_280	B6	0.26	0.08	1.62	0.86	1.43	0.18
CK162986_280		5.01	4.08	2.45	1.00	0.81	5.31
CV772381_305		0.67	0.64	0.42	0.28	0.33	0.58
gstA1_Int-2	A	1.33	5.93	3.51	1.86	3.62	5.09
gstA1_Int-2	B	2.24	1.05	2.29	1.54	0.12	2.37
gstA1_Int-2	C	0.22	1.58	3.87	1.46	0.40	3.58
P5CDH_Int-10		1.56	1.58	1.75	2.57	0.04	1.74
P5CDH_Int-3	A	0.92	3.60	0.39	1.39	2.33	4.39
P5CDH_Int-3	B	0.41	5.39	5.66	4.86	0.60	2.37
SOD1.1_Int-1		0.15	1.42	0.66	0.47	1.53	0.97
SOD1.2_Int-2		1.88	7.62	6.91	1.93	1.63	3.41
SOD3.1_Int-2	A	0.71	1.88	2.11	0.89	0.56	1.62
SOD3.1_Int-2	B	3.51	0.01	1.97	1.78	2.99	0.02
SOD3.2_Int-2		4.07	2.20	0.57	1.32	0.77	1.90
SPS7		1.29	1.52	1.23	1.84	3.12	2.74
SSI_Int-11		0.46	1.71	0.47	0.88	0.25	0.62
SSI_Int-12	A	4.46	1.26	4.30	0.24	0.34	0.70
SSI_Int-12	B	3.21	3.03	1.22	2.37	1.54	2.01
SSI_Int-12	C	4.03	0.92	2.33	0.21	0.60	0.74
WIS1.SSR		1.87	1.64	0.94	0.05	0.11	0.24

References
Wu, Guohai, et al. "Isolation, chromosomal localization, and differential expression of mitochondrial manganese superoxide dismutase and chloroplastic copper/zinc superoxide dismutase genes in wheat." Plant Physiology (Rockville) 120.2 (1999): 513-20.
Yang, J., et al. "Water Deficit-Induced Senescence and Its Relationship to the Remobilization of Pre-Stored Carbon in Wheat during Grain Filling." Agron J 93.1 (2001): 196-206.