Supplemental Data

The following materials are available in the online version of this article.

Figure S1. Quantile-quantile plots of F-test value for model comparisons.

Figure S2. Distribution of canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting under well-watered (C) and drought stress (D) conditions, respectively. The values were least square means calculated for all accessions across six environments.

Figure S3. Two-dimensional plots of significant correlations ($P<0.05$ level) among canopy and air air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting under well-watered (C) and drought stress (D) of perennial ryegrass.

Figure S4. Distribution of pairwise relative kinship estimates across 192 accessions of perennial ryegrass.

Figure S5. LD decay patterns of individual candidate genes of *LpCAT*, *LpChl Cu-ZnSOD, LpGPX, LpMnSOD, LpMDAR, LpDHAR, LpGR, LpFeSOD, LpCyt Cu-ZnSOD, LpMAPK, LpLEA3, LpPIP1,* and *LpTIP1* related to plant drought response.

Figure S6. Synonymous SNPs did not cause change amino acid sequence for putative LpFeSOD in perennial ryegrass.

Figure S7. Cleavage site prediction of putative *LpLEA3* gene in perennial ryegrass.

Figure S8. Phylogenetic tree of ~500 late embryogenesis abundant group 3 (LEA3) protein sequence from plants.

Figure S9. Relationship between chlorophyll fluorescence (*Fv/Fm*) and SNP markers in *LpCyt Cu-ZnSOD*.

Figure S10. The sequence similarity of Cyt Cu-ZnSOD between perennial ryegrass and *Arabidopsis*.

Table S1. Origin, status information and drought response traits of diverse perennial ryegrass accessions.

Table S2. Sampling date, soil moisture, and air temperatures in three locations before and after drought in year 2009 and 2010.

Table S3. Repeatability of phenotypic traits of perennial ryegrasses across six environments under the well-watered and drought conditions.

Table S4. Information of 109 simple sequence repeat (SSR) primers across seven linkage group of perennial ryegrass.

Table S5. Primers used for amplification of 14 candidate genes in perennial ryegrass.

Table S6. Analysis of variance (ANOVA) of phenotypic traits of perennial ryegrasses across six environments under the well-watered and drought conditions.

Table S7. Summary statistics for all accessions and individual groups (G1 to G5) within the panel of perennial ryegrass accessions identified by STRUCTURE analysis.

Table S8. Number of genotypes found in 12 candidate genes of *LpChl Cu-ZnSOD*, *LpGPX*, *LpMnSOD*, *LpMDAR*, *LpDHAR*, *LpGR*, *LpFeSOD*, *LpCyt Cu-ZnSOD*, *LpMAPK*, *LpLEA3*, *LpPIP1*, and *LpTIP1* across 192 accessions of perennial ryegrass.

Table S9. Number of associations among 14 genes and traits under drought conditions using simple linear model (S) and population structure (Q) controlled model in perennial ryegrass.

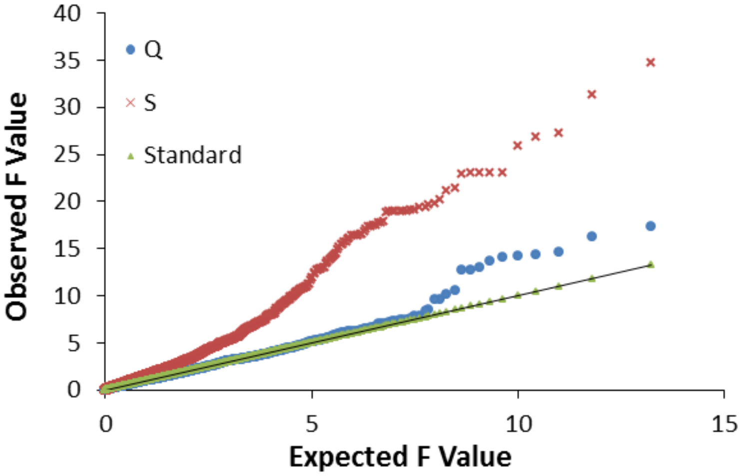


Figure S1. Quantile-quantile plots of F-test values for model comparisons. All traits were pooled together including canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting. The solid diagonal lines represent agreement between observed and expected F values. Data points represent ordered F value for each SNP-trait association analyzed with population structure (Q) and simple linear (S) model. These QQ plots of F-tests verified the adequate control of false positives for the Q model because the deviation of the observed F-statistics for 346 SNP markers from the expected value was much smaller than that of the simple linear model.

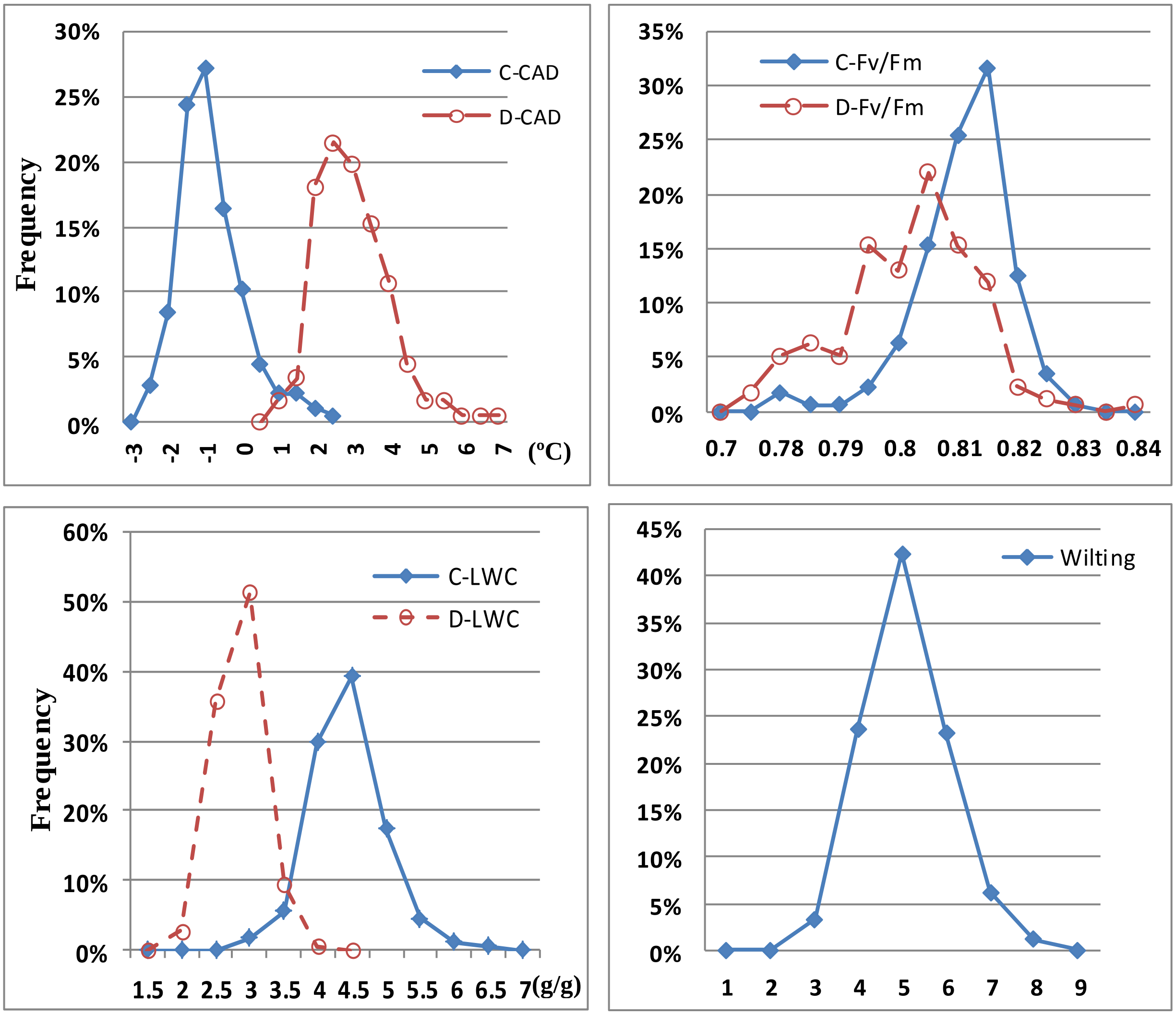
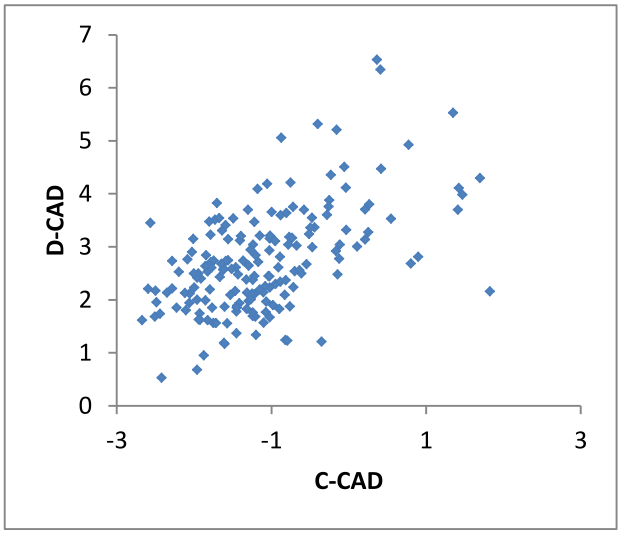
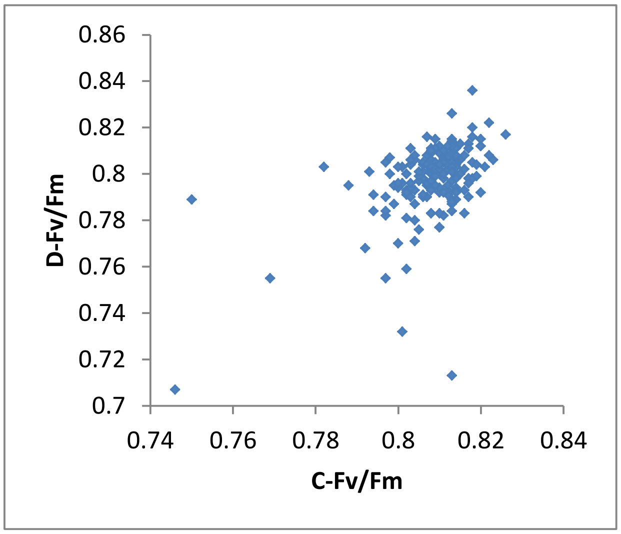
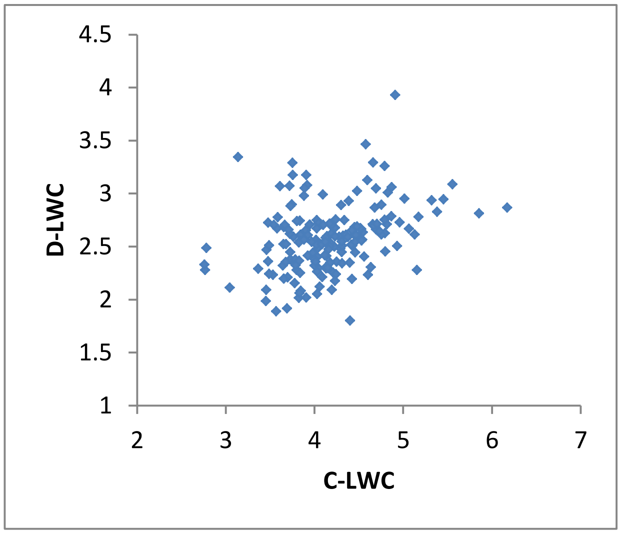
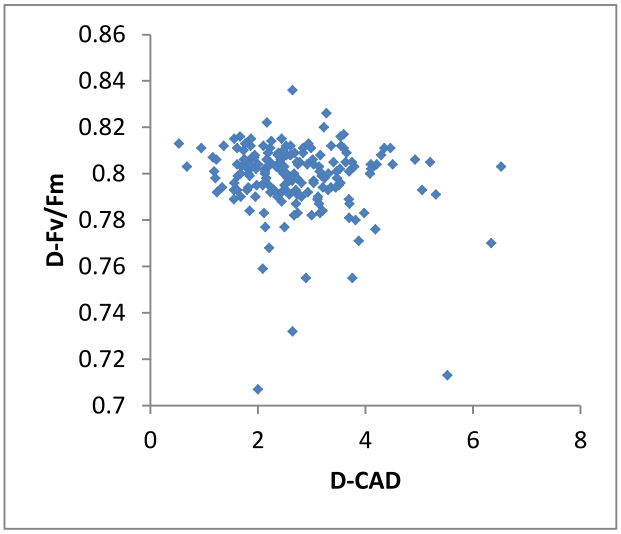


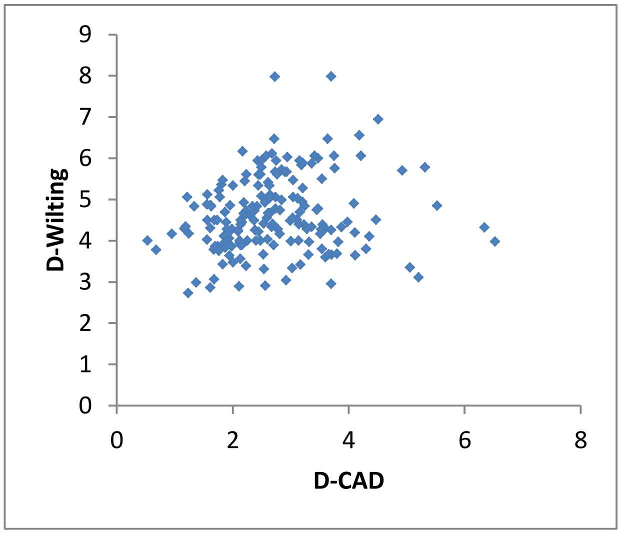
Figure S2. Distribution of canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting under well-watered (C) and drought stress (D) conditions, respectively. The values were least square means calculated for all accessions across six environments.

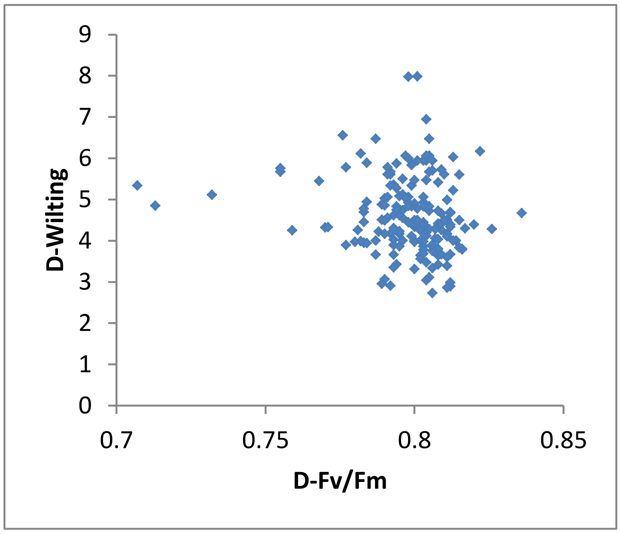

D-CAD
7
6
5
4
3
2
1
0
-3
-1
1
3
C-CAD


D-Fv/Fm
0.86
0.84
0.82
0.8
0.78
0.76
0.74
0.72
0.7
0.74
0.76
0.78
0.8
0.82
0.84
C-Fv/Fm


D-LWC
4.5
4
3.5
3
2.5
2
1.5
1
2
3
4
5
6
7
C-LWC


D-Fv/Fm
0.86
0.84
0.82
0.8
0.78
0.76
0.74
0.72
0.7
0
2
4
6
8
D-CAD


D-Wilting
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
D-CAD


D-Wilting
9
8
7
6
5
4
3
2
1
0
0.7
0.75
0.8
0.85
D-Fv/Fm

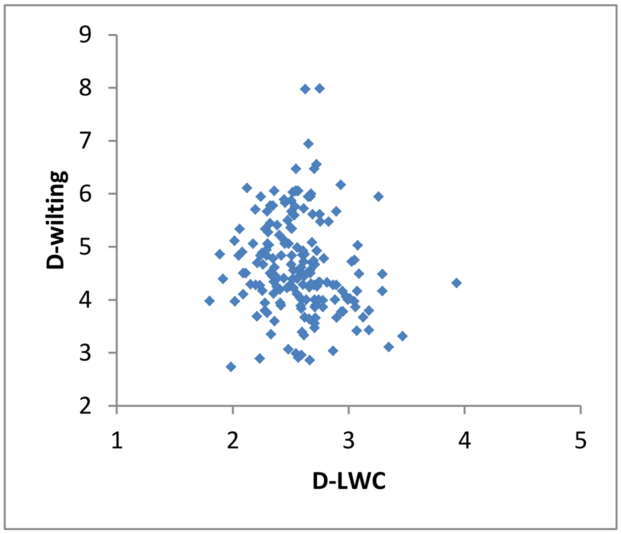


Figure S3. Two-dimensional plots of significant correlations (P<0.05 level) among canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting under well-watered (C) and drought stress (D) of perennial ryegrass.

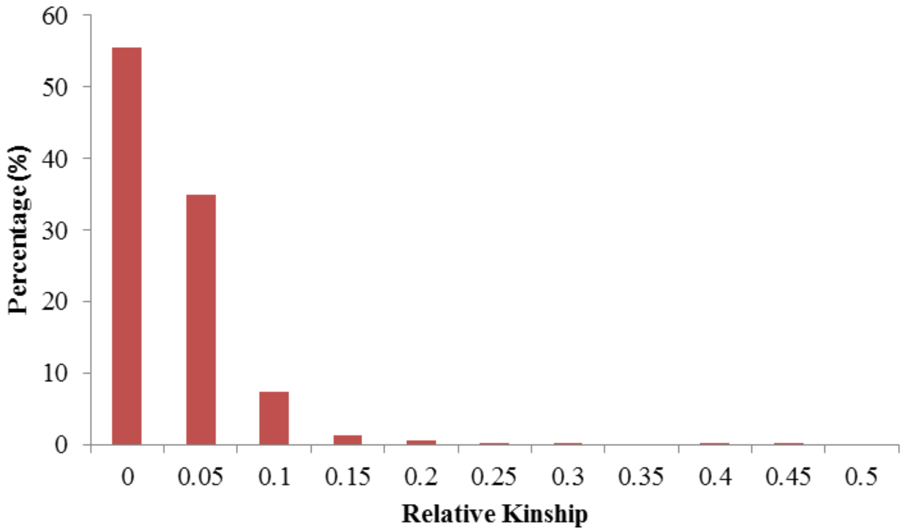
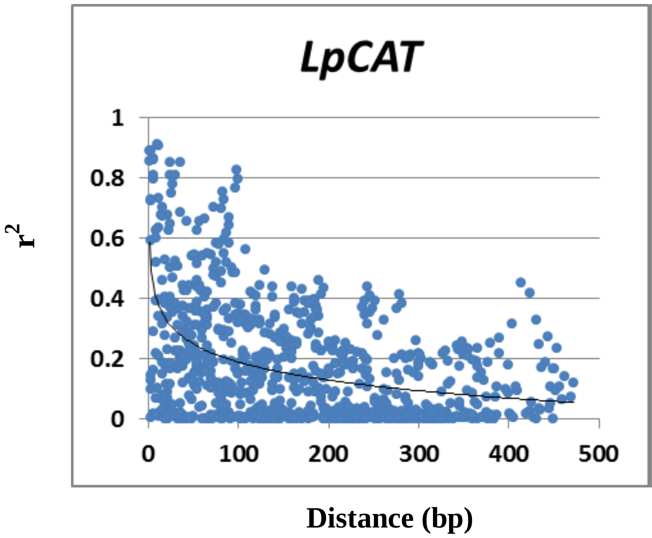
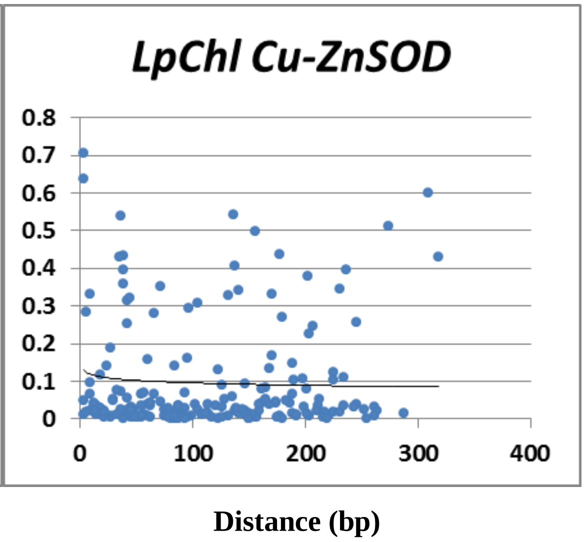
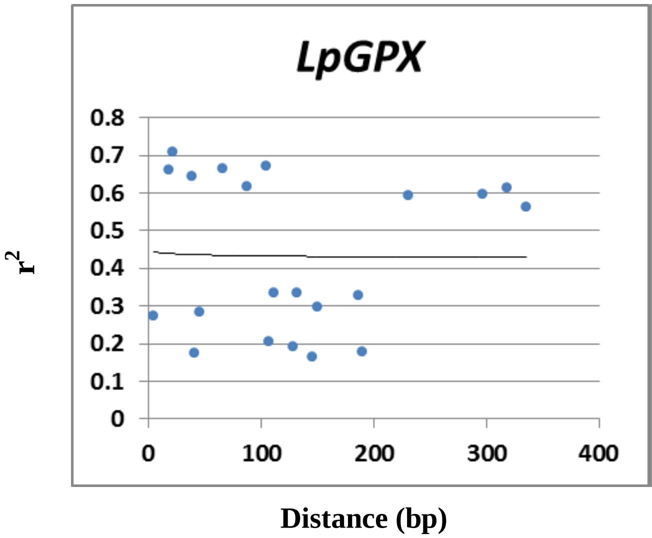
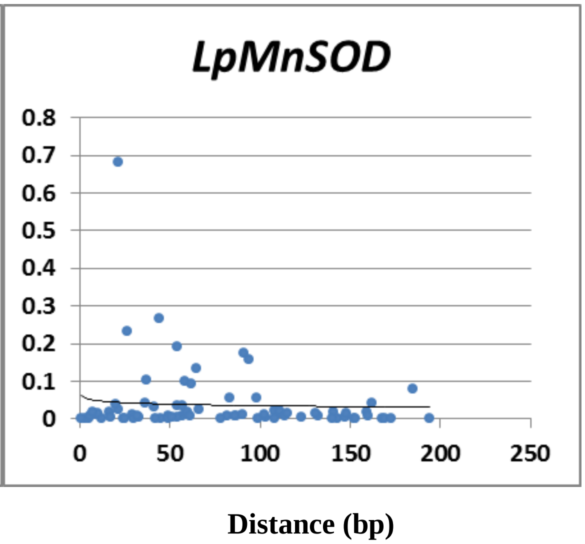


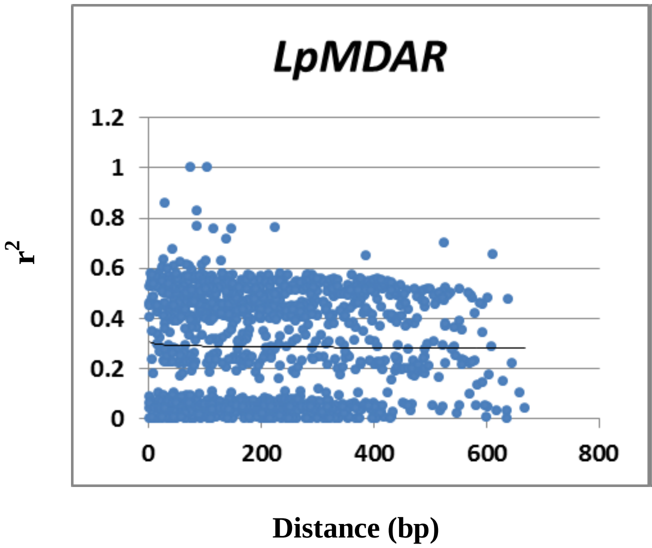
Figure S4. Distribution of pairwise relative kinship estimates across 192 accessions of perennial ryegrass. The peak around zero indicates no relationship. Percentages of relative kinship estimates were only shown from 0 to 0.5.

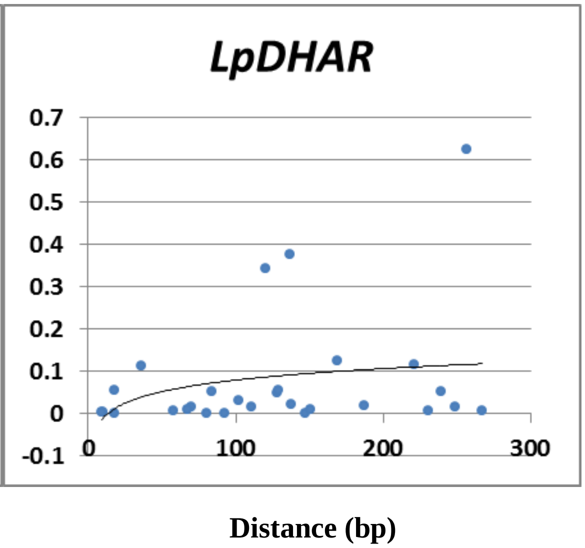

LpCAT
r2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
Distance (bp)

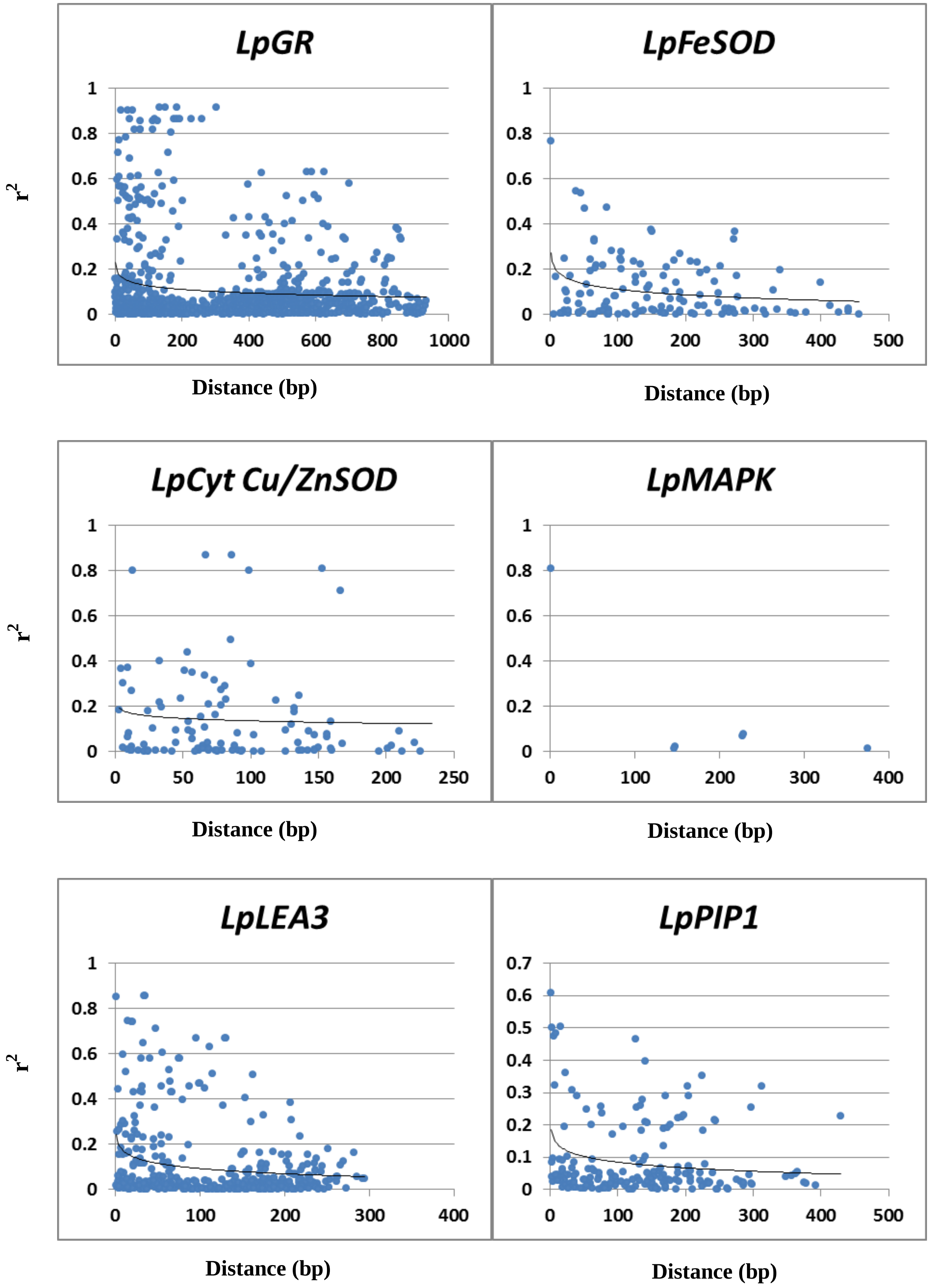

LpChl Cu-ZnSOD
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
Distance (bp)


LpGPX
r2
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
Distance (bp)


LpMnSOD
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
Distance (bp)


LpMDAR
r2
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
Distance (bp)


LpDHAR
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
100
200
300
Distance (bp)


LpGR
r^2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
Distance (bp)
LpFeSOD
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
Distance (bp)
LpCyt Cu/ZnSOD
r^2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
Distance (bp)
LpMAPK
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
Distance (bp)
LpLEA3
r^2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
Distance (bp)
LpPIP1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
Distance (bp)

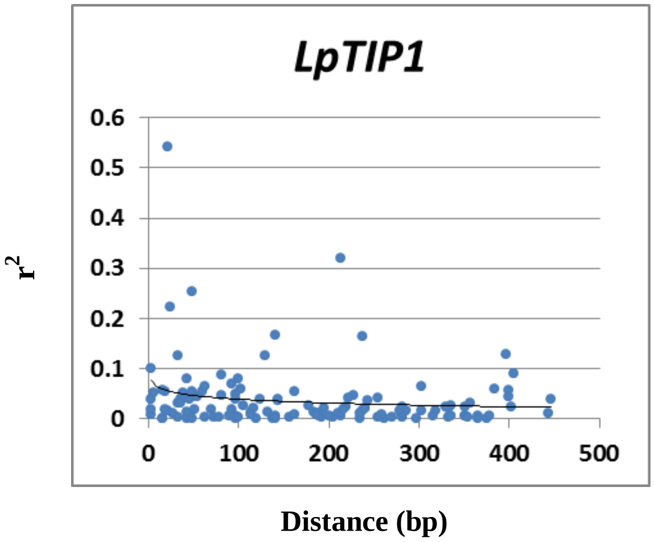


Figure S5. Linkage disequilibrium (LD) decay patterns of individual candidate genes of *LpCAT*, *LpChl Cu-ZnSOD*, *LpGPX*, *LpMnSOD*, *LpMDAR*, *LpDHAR*, *LpGR*, *LpFeSOD*, *LpCyt Cu-ZnSOD*, *LpMAPK*, *LpLEA3*, *LpPIP1*, and *LpTIP1* related to plant drought response.

```
GT1  575  KVVSYYGLTTPPYKTDALEPYMSKRVVELHWGKHQQDYVDGLNKQLDTSPFYGHTLEDMV  396
          KVVSYYALTTPPYKPEALEPYMSKRTVELHWGKHQQDYVDGLNKQLATSPFYGYTLEELV
GT2  575  KVVSYYALTTPPYKPEALEPYMSKRTVELHWGKHQQDYVDGLNKQLATSPFYGYTLEELV  396

GT1  395  KEAYNNGNPLPEYNNAAQVWNHHFFWESMQPGGGGSPEAGVLQQIEKDFGSFANFREEFM  216
          KEAYNNGNPLPEYNNAAQVWNHHFFWESMQPDGGGSPEGGVLRQIEKDFGSFFNFREEFV
GT2  395  KEAYNNGNPLPEYNNAAQVWNHHFFWESMQPDGGGSPEGGVLRQIEKDFGSFFNFREEFV  216

GT1  215  LSALSLLGSGWVWLVLKRNERKLSVVNTRNAINPLAFGDIPIISLDLWEHAYYLDYKDDR  36
          RSALSLLGSGWVWLVLKRNEKKLAVVRTRNAISPLAAGDIPIISLDLWEHAYYLDYKDDR
GT2  215  RSALSLLGSGWVWLVLKRNEKKLAVVRTRNAISPLAAGDIPIISLDLWEHAYYLDYKDDR  36

GT1  35   RAYVSN  18
          RAYVSN
GT2  35   RAYVSN  18
```

Figure S6. Synonymous SNPs did not cause change in amino acid sequence for putative LpFeSOD in perennial ryegrass. GT1, genotype 1 of perennial ryegrass LpFeSOD; GT2, genotype 2 of perennial ryegrass LpFeSOD; The corresponding SNP (T/C) in locus 411 resulted in nucleotide change from ACT to ACC, whereas both of these codons are coding amino acid residue threonine (T). Numbers on sequence comparison were nucleoside sequence reference position before they translated into amino acid.

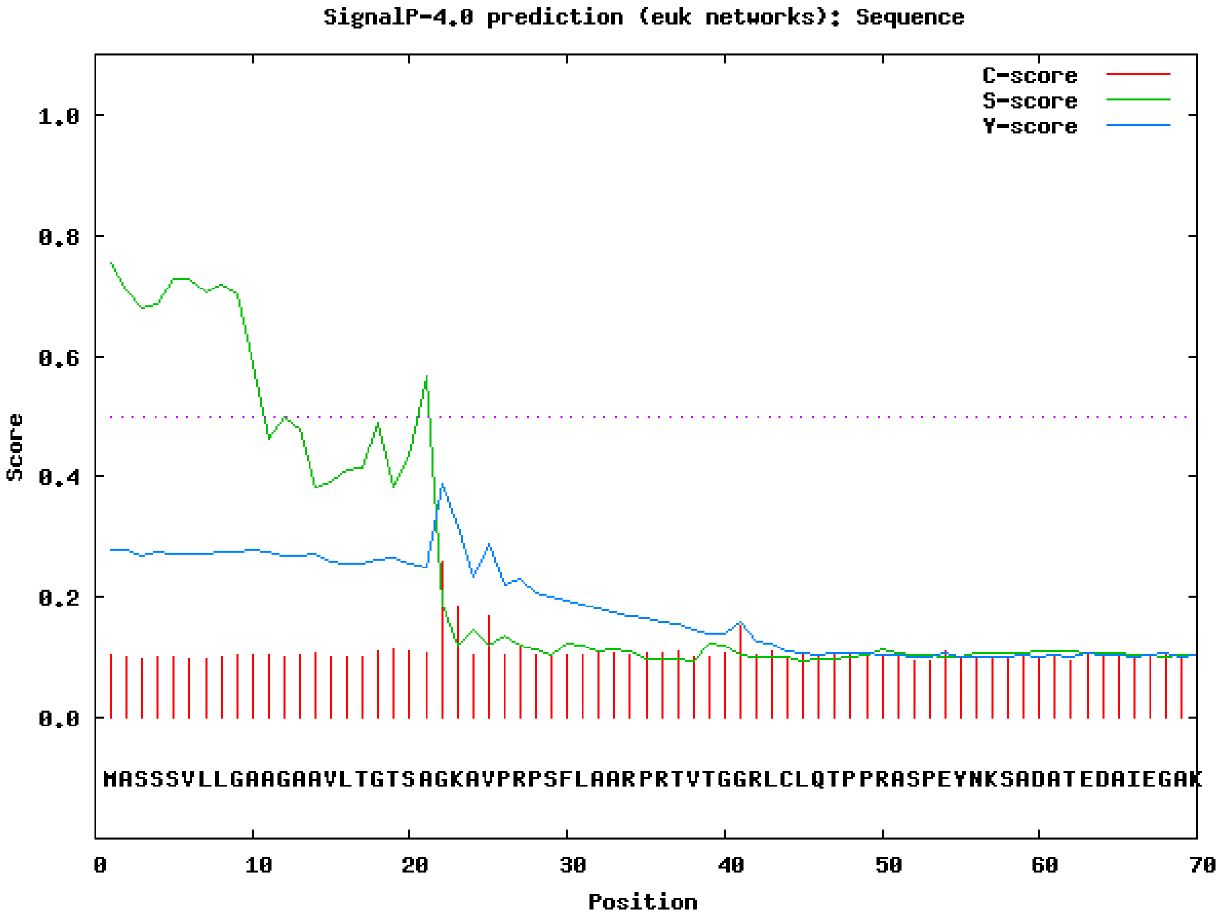


Figure S7. Cleavage site prediction of putative *LpLEA3* gene in perennial ryegrass.

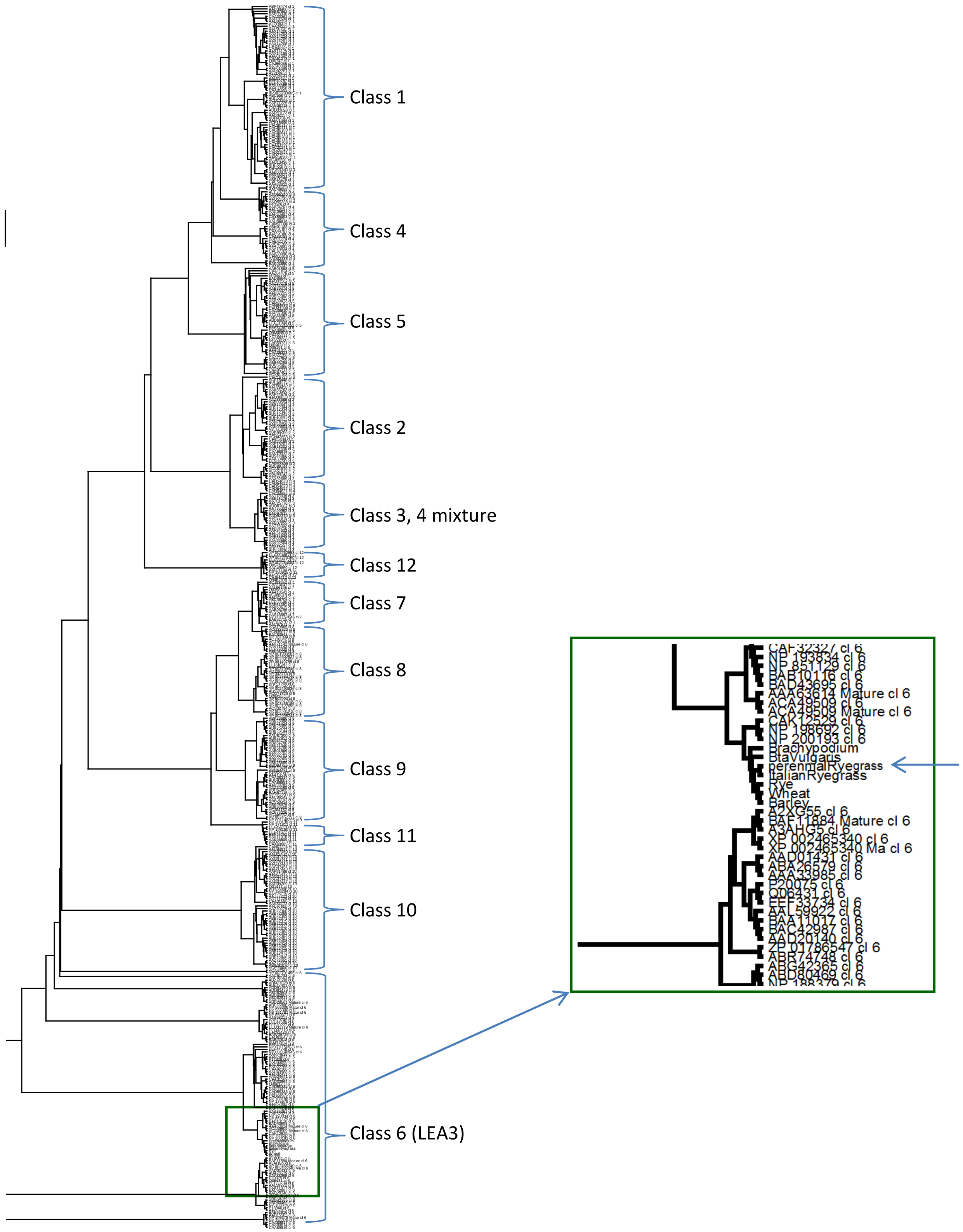

Class 1
Class 4
Class 5
Class 2
Class 3, 4 mixture
Class 12
Class 7
Class 8
Class 9
Class 11
Class 10
Class 6 (LEA3)
CAF32327 cl 6
NP 193834 cl 6
NP 851129 cl 6
BAB10116 cl 6
BAD43695 cl 6
AAA63614 Mature cl 6
ACA49509 cl 6
ACA49509 Mature cl 6
CAK12529 cl 6
NP 198692 cl 6
NP 200193 cl 6
Brachypodium
BtaVulgaris
perennialRyegrass
ItalianRyegrass
Rye
Wheat
Barley
A2XG55 cl 6
BAF11884 Mature cl 6
A3AHG5 cl 6
XP 002465340 cl 6
XP 002465340 Ma cl 6
AAD01431 cl 6
ABA26579 cl 6
AAA33985 cl 6
P20075 cl 6
Q06431 cl 6
EEF33734 cl 6
AAL59922 cl 6
BAA11017 cl 6
BAC42987 cl 6
AAD20140 cl 6
ZP 01786547 cl 6
ABR74748 cl 6
ABG42365 cl 6
ABD80469 cl 6
NP 188379 cl 6

Figure S8. Polygenetic tree of ~500 late embryogenesis abundant group 3 (LEA3) protein sequence from plants.

Most LEA proteins from varies species was obtained from LEAP database (LEAPdb) reported by Hunault and Jaspard (2010). Class 6 in this data base stood for LEA group 3 protein. Several defined LEA group 3 proteins were also included in this analysis to further confirm the results. These LEA3 proteins were obtained from NCBI, including the following species: wheat (*Triticum aestivum*, NCBI accession number: AAA16282.2), rye (*Secale cereal*, AAM09564.1), barley (*Hordeum vulgare*, ACJ68104.1), Italian ryegrass (*Lolium multiflorum*, BAJ07539.1) and *Brachypodium distachyon* (XP_003581642.1).

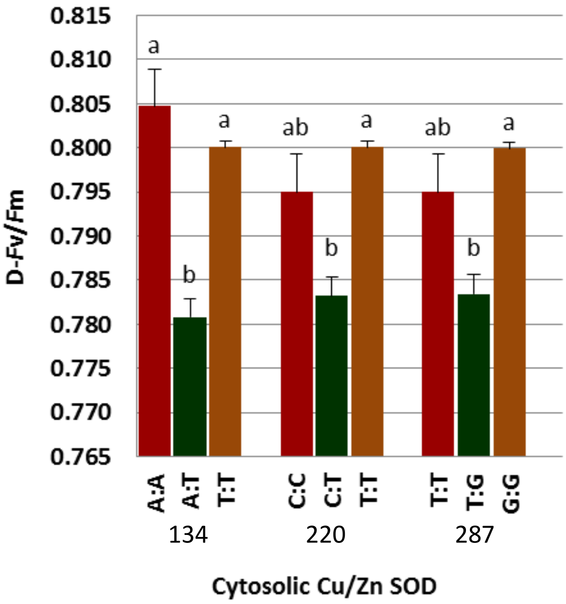


Figure S9. Relationship between chlorophyll fluorescence (*Fv/Fm*) and SNP markers in *LpCyt Cu-ZnSOD*. Locus 134, 220 and 287 were significantly associated SNP markers from *LpCyt Cu-ZnSOD* gene with chlorophyll fluorescence under drought stress (D-*F*v/*F*m). Means followed by the same letter within a marker position were not significantly different at $P < 0.05$. Bars indicate standard error.

```
PR     375  SSEGVKGTIFFTQEGDGPTTVTGSVSGLKAGLHGFHVHALGDTTNGCMSTGPHFNPAGHV  196
            SSEGV-GTIFFTQEGDG-TTV-G-VSGLK-GLHGFHVHALGDTTNGCMSTGPHFNP-G--
AT     10   SSEGVTGTIFFTQEGDGVTTVSGTVSGLKPGLHGFHVHALGDTTNGCMSTGPHFNPDGKT  69

PR     195  HGAPEDEIRHAGDLGNVTAGEDGVAPINFVDKHIPLTGPHSIIGRAVVVHGDADDLGKGG  16
            HGAPED--RHAGDLGN-T-G-DG-A-----D--IPLTGP-SI-GRAVVVH-D-DDLGKGG
AT     70   HGAPEDANRHAGDLGNITVGDDGTATFTITDCQIPLTGPNSIVGRAVVVHADPDDLGKGG  129

PR     15   HELS  4
            HELS
AT     130  HELS  133
```

Figure S10. The sequence of similarity of Cyt Cu-ZnSOD between perennial ryegrass (PR) and *Arabidopsis* (AT; ID: ABN50366.1). Score = 211 bits (538), Expect = 4e-73 Identities = 99/124 (80%), Positives = 105/124 (85%), Gaps = 0/124 (0%) Frame = -1

Table S1. Origin, status information and drought response traits of diverse perennial ryegrass accessions

ID[a]	Acc[b]	Origin	S[d]	Drought[e]				ID[a]	Acc[b]	Origin	S[d]	Drought[e]			
				CAD (°C)	Fv/Fm	LWC (g/g)	Wilting					CAD (°C)	Fv/Fm	LWC (g/g)	Wilting
1	187222	Belgium[c]	U	1.24	0.792	2.25	4.2	51	231587	Algeria	U	3.14	0.787	2.63	4.0
2	197270	Finland[c]	CD	1.67	0.816	2.94	3.8	52	231589	Algeria	U	4.36	0.811	2.09	4.1
3	200322	Denmark[c]	CD	1.68	0.790	2.48	3.1	53	231594	Algeria	U	3.60	0.811	2.36	3.6
4	202451	Argentina[c]	W	3.16	0.783	2.68	4.7	54	231598	Greece	U	2.48	0.800	2.99	4.0
5	204879	Turkey[c]	W	1.87	0.815	2.59	3.8	55	231604	Portugal	U	4.11	0.804	2.41	4.2
8	220178	Afghanistan[c]	W	5.53	0.713	2.60	4.8	56	231606	Portugal	U	4.11	0.802	2.70	3.6
9	229702	Iran[c]	W	6.34	0.770	3.93	4.3	57	231619	Iran	U	2.11	0.812	2.23	2.9
10	231569	Libya[c]	U	2.14	0.777	2.42	3.9	58	231620	Iran	U	2.54	0.800	3.46	3.3
11	231578	Algeria[c]	U	2.92	0.804	2.87	3.0	59	234779	Germany	U	1.94	0.807	2.98	4.1
12	231588	Algeria[c]	U	4.30	0.808	2.28	3.8	60	237184	Netherlands	CD	1.76	0.813	2.40	5.2
13	231595	Morocco[c]	U	3.70	0.789	2.59	3.0	61	237187	Netherlands	CD	1.17	0.807	2.61	4.3
14	231597	Greece[c]	U	1.74	0.806	2.78	3.9	62	238938	New Zealand	U	2.45	0.815	2.53	5.6
15	231605	Portugal[c]	U	4.93	0.806	2.19	5.7	65	251224	Yugoslavia	W	0.68	0.803	2.95	3.8
16	251141	Yugoslavia[c]	W	2.53	0.812	2.62	3.7	66	251667	Yugoslavia	U	1.56	0.793	3.01	4.0
17	265344	Ireland[c]	C	3.04	0.797	2.48	5.1	67	254898	Iraq	W	3.32	0.800	3.05	4.0
18	265351	Chile[c]	U	2.71	0.793	2.59	3.9	68	265336	Sweden	C	2.99	0.811	3.29	4.5
19	267059	Poland[c]	U	2.23	0.811	2.60	3.4	69	265340	France	C	1.80	0.793	2.50	5.4
20	275660	Australia[c]	CD	2.67	0.800	2.74	4.3	70	265341	France	C	3.11	0.789	2.59	4.5
21	287855	Spain[c]	U	3.70	0.781	2.49	4.3	71	265342	Ireland	C	2.56	0.798	2.72	4.9
22	298091	Hungary[c]	W	2.65	0.836	2.50	4.7	72	265349	Ireland	U	3.21	0.794	2.30	5.3
23	303011	UK[c]	C	2.19	0.809	2.71	4.7	73	266293	Netherlands	CD	1.56	0.815	2.32	4.5
24	303022	Netherlands[c]	CD	2.84	0.809	2.61	5.7	74	267058	Poland	U	3.41	0.805	2.56	6.1
25	303031	Netherlands[c]	CD	3.47	0.798	2.68	6.0	75	268333	Former USSR	W	3.55	0.812	1.92	4.4
26	632517	USA	U	2.56	0.792	2.57	2.9	76	272120	Poland	C	3.00	0.782	2.78	4.0
27	403889	Canada[c]	C	2.53	0.808	2.56	4.4	77	274637	Poland	U	1.97	0.802	2.66	4.2
28	632542	Hungary	C	2.14	0.801	2.67	4.5	78	Inspire	USA	C	2.65	0.732	2.02	5.1
29	418707	Romania[c]	W	1.98	0.795	3.06	3.9	79	277846	Yugoslavia	U	1.76	0.806	2.30	3.8
30	418714	Italy[c]	W	2.73	0.798	2.62	8.0	80	277848	Cyprus	U	1.85	0.784	2.41	3.9
31	418726	France[c]	W	3.70	0.801	2.75	8.0	81	278773	Canada	C	2.45	0.788	2.47	4.2
32	418727	France[c]	W	2.59	0.791	2.52	4.6	82	284823	Australia	U	2.25	0.814	2.71	4.0
33	423136	Spain[c]	W	4.09	0.800	2.08	4.9	83	284826	Australia	U	3.02	0.806	2.61	3.3
34	462339	New Zealand[c]	C	2.38	0.809	2.73	4.3	84	285101	Australia	C	2.37	0.808	3.02	4.7
35	182857	Czech Republic	U	1.21	0.798	2.18	5.1	87	290368	Hungary	U	3.12	0.790	3.08	5.0
36	189392	New Zealand	U	1.62	0.804	2.62	4.9	88	290373	Hungary	U	1.19	0.801	2.36	4.3
37	198070	Sweden	CD	2.30	0.793	2.36	4.6	89	303012	UK	C	2.61	0.808	2.38	5.4
38	204085	Cyprus	U	0.95	0.811	2.94	4.2	90	303020	Sweden	C	1.99	0.804	2.70	4.3
39	204880	Turkey	W	3.51	0.795	3.07	4.2	91	303021	Netherlands	CD	2.75	0.804	2.67	5.9
40	220528	Afghanistan	W	3.31	0.793	2.90	3.7	92	303026	France	C	1.83	0.794	3.18	3.4
41	220597	Afghanistan	W	5.21	0.805	3.35	3.1	93	303027	Denmark	C	2.78	0.805	2.87	4.3
43	225825	Denmark	U	2.93	0.792	2.89	5.7	94	303028	Denmark	C	2.23	0.805	2.61	4.7
45	231565	Libya	U	2.12	0.783	2.28	3.9	95	636643	Japan	CD	2.58	0.797	2.54	6.1
46	231566	Libya	U	4.47	0.811	2.11	4.5	96	303037	Sweden	C	1.83	0.800	2.83	5.5
47	231567	Libya	U	1.56	0.789	2.25	4.9	97	303044	Belgium	CD	2.94	0.813	2.52	6.0
49	231576	Algeria	U	2.43	0.806	3.26	5.9	98	634204	USA	C	2.64	0.799	2.06	5.3
50	231580	Algeria	U	1.69	0.803	2.71	3.9	99	306292	Bolivia	U	1.56	0.796	2.45	5.1

Table S1. Continued

ID[a]	Acc[b]	Origin	S[d]	Drought[e]				ID[a]	Acc[b]	Origin	S[d]	Drought[e]			
				CAD (°C)	*Fv/Fm*	LWC (g/g)	Wilting					CAD (ºC)	*Fv/Fm*	LWC (g/g)	Wilting
100	311075	Romania	U	2.64	0.797	2.31	5.0	147	577254	Luxembourg	W	3.45	0.794	3.05	4.8
101	317452	Afghanistan	W	3.36	0.812	2.75	4.3	148	577260	UK	W	3.75	0.805	2.54	6.1
103	321397	Czech Republic	U	0.53	0.813	2.59	4.0	149	577265	UK	W	2.68	0.782	2.12	6.1
104	321681	France	C	1.87	0.812	2.21	4.7	150	577268	UK	W	2.09	0.795	2.52	4.2
105	340104	Turkey	U	2.16	0.798	2.34	4.4	151	577269	Norway	W	4.19	0.776	2.72	6.6
106	340108	Turkey	U	2.69	0.809	2.44	4.4	152	577273	Turkey	W	2.01	0.707	2.51	5.3
107	371952	Bulgaria	U	2.12	0.796	2.74	4.0	153	Manhattan4	USA	C	1.94	0.808	2.66	3.6
108	376878	New Zealand	C	3.04	0.796	2.67	4.6	154	578760	USA	C	4.21	0.804	2.36	6.1
109	384478	Poland	C	3.04	0.804	2.76	5.5	155	578763	USA	C	3.20	0.784	2.29	4.9
110	634205	USA	C	2.50	0.777	2.32	5.8	156	636539	Italy	W	3.24	0.798	2.42	4.8
111	403847	Canada	C	1.61	0.811	2.66	2.9	158	595046	UK	W	5.32	0.791	2.35	5.8
112	403851	Canada	C	2.48	0.810	2.75	5.6	159	598434	Italy	W	2.17	0.822	2.93	6.2
113	SilverDollar	USA	C	2.90	0.755	2.30	5.7	160	598437	Belgium	W	3.21	0.784	2.44	5.9
114	403868	Canada	C	1.85	0.799	2.56	4.1	161	598441	Switzerland	W	3.71	0.787	2.71	3.7
115	403869	Canada	C	2.50	0.795	2.68	5.1	162	598443	Switzerland	W	3.14	0.803	2.55	4.4
116	403886	Canada	C	3.19	0.799	2.45	5.8	163	598447	UK	W	2.84	0.811	2.56	5.0
117	403899	Canada	C	1.61	0.793	2.68	4.3	164	598452	UK	W	3.64	0.805	2.70	6.5
118	403907	Canada	C	2.49	0.803	2.65	5.9	165	598453	Romania	W	3.15	0.801	2.24	5.9
120	418706	Italy	W	2.13	0.800	2.52	4.4	166	598515	Turkey	W	3.54	0.816	3.17	3.8
121	418708	Romania	W	2.74	0.783	2.35	4.8	167	598517	Turkey	W	2.13	0.802	2.70	3.6
122	418712	Italy	W	2.00	0.804	2.70	3.5	168	598518	Turkey	W	1.37	0.812	2.55	3.0
123	418721	Belgium	W	2.21	0.795	2.59	4.6	169	598839	Morocco	W	5.06	0.793	2.33	3.3
124	418722	Luxembourg	W	1.89	0.803	2.61	4.4	171	598877	Morocco	W	3.60	0.817	2.15	4.3
125	418723	Luxembourg	W	3.36	0.794	2.50	5.9	172	598892	Morocco	W	3.17	0.808	3.07	3.4
126	418736	Switzerland	W	2.60	0.808	2.58	4.0	173	598911	Tunisia	W	3.28	0.826	2.20	4.3
127	418741	France	W	2.16	0.806	2.72	3.9	174	598928	Italy	W	4.52	0.803	1.83	6.0
128	420124	Japan	C	3.88	0.771	2.81	4.3	175	Divine	USA	C	2.10	0.759	2.38	4.3
129	420126	Japan	U	2.73	0.805	2.51	5.7	176	Catalina	USA	C	2.61	0.812	2.26	4.7
130	634278	USA	W	2.44	0.792	2.28	5.3	177	600783	USA	C	2.74	0.791	2.30	5.1
131	420128	Japan	C	2.40	0.802	2.88	4.0	178	600878	USA	C	1.78	0.803	2.45	5.1
132	BrightStar	USA	C	2.21	0.768	2.32	5.4	179	610802	Norway	W	2.77	0.791	2.69	5.6
133	440474	Former USSR	W	3.23	0.820	2.38	4.4	180	610802	UK	W	3.54	0.796	2.47	5.5
134	462335	New Zealand	CD	2.81	0.790	3.29	4.2	182	610925	Tunisia	W	1.23	0.806	1.98	2.7
135	462336	New Zealand	CD	1.90	0.807	2.89	4.3	183	610926	Tunisia	W	6.53	0.803	1.80	4.0
136	462337	New Zealand	CD	2.71	0.787	2.54	6.5	184	610928	Italy	W	3.65	0.809	3.13	3.7
137	502412	Russia	W	2.42	0.805	2.05	4.8	185	610950	Tunisia	W	3.53	0.802	2.23	4.3
138	504539	Greece	W	1.95	0.790	1.89	4.9	186	610965	Italy	W	3.98	0.783	2.37	4.5
139	505840	Former USSR	CD	3.47	0.801	2.79	4.8	187	611036	Russia	W	1.68	0.800	2.09	4.5
140	505841	Former USSR	CD	2.19	0.803	2.61	4.9	188	619003	Norway	W	1.85	0.804	2.35	4.1
141	505842	Former USSR	CD	1.63	0.799	2.24	4.8	189	619012	UK	W	4.51	0.804	2.65	6.9
142	505843	Former USSR	CD	2.24	0.792	2.52	5.6	190	619474	Romania	CD	2.37	0.790	3.09	4.5
143	516605	Morocco	W	3.80	0.803	2.21	3.7	191	619554	UK	W	3.76	0.755	2.54	5.8
145	547390	Iran	W	2.81	0.796	2.70	4.7	192	628717	Bulgaria	W	3.82	0.780	2.02	4.0
146	577251	Yugoslavia	CD	2.34	0.803	2.29	4.8	LSD[f]				1.36	0.023	0.60	0.97

Table S1 continued

[a] ID numbers used in this study to represent accessions (discontinuous numbers).
[b] Acc: Accession.
[c] Core collection.
[d] Improvement status obtained from USDA germplasm bank. U: uncertain; W: wild; C: cultivar; CD: cultivated.
[e] Data were lsmeans across six environments under drought stress. CAD: canopy and air temperature difference; LWC: leaf water content; *Fv/Fm*: chlorophyll fluorescence.
[f] Least significant difference.

Table S2. Sampling date, soil moisture, and air temperatures in three locations before and after drought in year 2009 and 2010.

Year	Location	Sampling date		Soil Moisture (%)		Temperature [Mean, (min-max)] °C	
		C	D	C	D	C	D
2009	Wanatah	05/30/09	07/07/09	13.9	9.2	17.1 (10.6-23.9)	19.7 (14.7-24.4)
2009	W. Lafayette	06/09/09	07/03/09	17.7	18.7	21.4 (16.2-26.3)	23.3 (18.0-28.6)
2009	Vincennes	06/12/09	06/30/09	12.2	8.0	23.7 (19.1-27.9)	27.4 (21.5-33.0)
2010	Wanatah	05/28/10	07/07/10	14.5	11.3	22.1 (16.2-28.3)	22.4 (15.9-28.6)
2010	W. Lafayette	05/14/10	07/03/10	20.4	17.9	15.6 (10.5-20.6)	22.0 (15.2-27.9)
2010	Vincennes	05/23/10	07/05/10	11.4	7.6	23.7 (17.9-29.1)	23.8 (17.5-29.9)

Temperature: control was averaged from 1 week (3 days before sampling, sampling day, and 3 days after sampling); Drought was averaged from irrigation shutdown to sampling date.

C and D represent control and drought stress, respectively.

Wanatah: Wanatah, IN, 41° 26' N and 86° 54' W

W. Lafayette: West Lafayette, IN, 40° 25' N and 86° 54' W

Vincennes: Vincennes, IN, 38° 40' N and 87° 31'W

Table S3. Repeatability of canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv/Fm*), leaf water content (LWC), and leaf wilting of perennial ryegrasses across 6 environments under both the well-watered (C) and drought conditions (D).

Treatment	Trait	h^2
C	CAD	0.64
	Fv/Fm	0.70
	LWC	0.51
D	CAD	0.63
	Fv/Fm	0.72
	LWC	0.64
	Wilting	0.86

Table S4. Information of 109 simple sequence repeat (SSR) primers across seven linkage group of perennial ryegrass.

SSR	**Forward primer**	**Reverse primer**	**Linkage group**	**SSR motif**	**Number of allele** [a]
LpSSR006	CAATGGAGTCCCAACAG	TACCTGGGCAAATCTTG	4	(CT)23	25
LpSSR011	AAATGTTCATCGTATCG	CAGGTCCCTGCTTAC	4	(CA)15(CA)9	35
LpSSR017	TGAGCACCATGAAGGAG	GGTTGTCCGCAGGTATT	7	(GA)26(GA)2(GA)2	31
LpSSR020	GGGGAAATACAGTTCTGC	GATGCTCCTGCCTACTTTA	5	(GA)5(GA)2(GA)16	27
LpSSR021	AACAAGTCAATGGACAGATT	TTTGTTTTCCCTTTTGG	2	(GA)21	28
LpSSR023	ATGCACGGGTTTTATTCATT	CGCGAGGCTTAAGGTGT	4	(GT)4(GT)19(GA)23	46
LpSSR027	CACCACCTTCTCCAAC	AACAAGCACTTAGGAACA	1	(CT)17	34
LpSSR057	TAGCCTCCAGAAACAAAGTC	CATAGCAGTACAGCCAGTCA	1	(GA)21	35
LpSSR058	CGATGAACTCAAGGGGGATT	GCACCGGTCTAGGGACAGAA	6	(GA)14	34
LpSSR059	GATCGGATCGGTACAGGAGA	GAAGCGCACCTTCTGTTTCT	5	(CT)20	33
LpSSR066	GCCAGTGCCCATTCCGATAA	CCCCACTCCAACCAAAGCAA	7	(TG)24	42
LpSSR076	CCCATACTTCGAGGCATAAA	AAATTCCCCCATCAGAGAAC	2	(CA)28	27
LpSSR082	CTAAACTAAATGTTCATCGT	CCTGCTTACTCCTGTT	4	(CA)25	32
LpSSR085	GCCAGATCCCTTGTAGAAG	GCACCATTTAAAACCAAAGA	1	(CA)47	26
LpSSR091	CACTCTCGGTCTCGCCTTAT	TTCGCATGCATACAACACAT	7	(TG)25	23
LpSSR100	AATACTTGAGTTGGCATTTC	CGGCTCACTGAACATTC	3	(TG)8(TG)2	7
LpSSR112	GACCCCGAGACAGCCTA	ACGCATATGGTCTTCAGAA	2	(CA)20	18
DLF008	CCGTTGCTTGATACTTGGAC	GAACGAGCATTCTTCCTTTCT	7	(ACT)7	13
DLF013	GTAGTCCAGCGGAGGTCAAT	ATAGCAAACGTTGGCACACAT	3	(GTT)6(GCT)3	3
DLF020	ATGACGACGAGGAGGAAT	ATAGCGACGAGAGAAAGGTAA	7	(CGA)5	27
DLF025	CGGCGAGAAGCTAACAGA	TCACGGAGAGGGCAAGT	4	(CT)10	13
DLF027	CGCTTTGTCAACTCATACC	CAAACCCGTTCTTCTACATT	1	(TA)11	6
rye012	GGTCTAATTGTCGTCCTTTC	GAGTGATTTGGAGGTGAGAA	4	(CA)23	29
rye014	CTGCTCTGTGTTTGTGTGAC	GCCTTTCATCGTTACTGTCT	6	(CA)26	18
uni001	AGCCACACTTTACCTAATGCTG	CCCGCAAAACTTACAATTAAA	3	(AC)17	21
B1A2	GTGCAGCAGTTTGAATTGGA	AGCATCGGGAGCTATGAATG	3	(GA)14	38
B1A8	GACTTTCAGGCATCGGTCAT	CCCAGCTCCATTCTTAATGC	6	(TG)7	22
B1A10	GCGACAGGAGTGAACACTGA	TAAGGCGTAAGGCAGCAGTG	3	(CA)12	23
B1B3	AGGTGTCCTGTTGCTTTGGA	TTTACCCCCAGGGATCAAAT	3	(TG)7	15
B1B6	GGAGCTGCATCTTTCTTGCT	GCAAACCCAGACACCCATTA	1	(CTT)4(GT)2	12
B1C8	TTCTGGCCATGTTGATTTGC	GTCTACGGGTTGGAGCAGTG	7	(CA)8(CT)6	7
B1C9	GAGCCGATGCACAGGTTACT	AAAGGAAGCCGGCTAATCAC	3	(CT)8	28
B2F1	CCAACCATATGCAACGATGA	TCCATTTGTTCTTGGGGAGA	5	(CA)5	7
B2G6a	CCAACTAGACAAAGGGGATTG	GGAGAGCACCATTCATCCAT	1	(TGA)8	24
B3A1	CTTGTCGTCCTTGTTGGGAG	ATATTCTGGATCGTGGCGTT	2	(AC)5(AG)5	12
B3A3	GGGTGAAGTGCTCTTTGTGA	ATGGTGAAGGCCTGAAACTG	7	(GT)8	6
B3B7	AGGCGACCAATACGTCTGTC	ATCTCTGATGGCTTTGTGGC	1	(TG)9	8
B3B8	TGTCATGTCCGCTGTCTACG	GAGAGTGGGCGATCATCTTC	3	(TG)10	15
B3C5	TGTCATGTTCAGAAAGTGCG	TGTCCACATAAATGCACCTCA	7	(GT)8	13
B3C10	CTACAACTCCGTGCTGCTGA	TGCATGGTTTCTCAAATGCT	7	(CTC)4	10
B3C11	ATTCACCTCGCTCGAAAATG	AACACCAAGCTAGCCACCAC	7	(CATG)3(TG)9	7
B3D2	ATACGAGCGAATTGCCTCTC	TCTCCCATCGCTTATGTTCC	1	(AT)4	6
B3D4	AAACCCATACCGACATACCG	GGCGTCTGTGAGAGTGAGTG	6	(CG)4	8
B3D12	GGGCATCACTGAGAAGAGGA	TACAAAGGAAGTCGGGCATC	2	(TC)7	10
B3E6	CTGTAACAACAGCCGCTGAG	GTCTCGAGCACAGGAGTTCA	3	(AG)4(GT)7	5

Table S4. Continued

SSR	**Forward primer**	**Reverse primer**	**Linkage**	**SSR motif**	**Number of allele** [a]
B4C4	TGCATGCACCCTTGTAGC	GGAGACTTTGTGTGTGCAGC	7	(GT)4(GT)3(TC)3	15
B4D7	CGGGAGCTCTCTCCTTCT	TCCAGAACCTTCTCGAGGTC	1	(CT)10	27
B4D9	GACGTCATACCTGCGTGCTA	GCGAATCAAAGAAGCATGTG	4	(TC)10(TC)4	16
B5E1	AAACATCAACGGAAGGATGC	TGATATGCATTGTGATGGAGG	5	(CA)3(CA)5	9
B5G4	TGGAGTTGTGGACCTTTTCC	AGATGCTGGTTGGTTTCCAG	7	(CA)8	6
LPSSRH01A02	AAAGACCGCATACGAAGT	AACCAAAGCCTCAAGACA	5	(CA)27	27
LPSSRH01E10	CGCAGCTTAATTTAGTC	GCTTTGAGTATGTAAAGTT	4	(CA)6	7
LPSSRH01H06	ATTGACTGGCTTCCGTGTT	CGCGATTGCAGATTCTTG	4	(CA)9	16
LPSSRH02C11	TGGAATAACGATGAAAAF	CATCACGAATTAACAAGAG	3	(CA)4(CA)4	16
LP165	CCATCACCTCCACTAT	AGCTCGCAGTCTGTTG	7	(CT)14	21
LP20a	ACCGCTGTGCTAAATCTG	ATGCGCTGCTGTCTGCCCT	4	(GA)16(A)5(GA)	23
LP204a	GAGCTTCTCTCGATCCT	AGTGGATGTGACTACA	4	(CT)20	14
LP8a	TGACTTCTCTCGATCCT	ATGTGACTACAAAACCA	4	(CT)17	10
M10138	TAGAGGATCAGTTGCATC	TAGTTCCGAGTTAGCTGA	3	(CA)13	31
M144	CAGAAGGAGGTCGTCGA	CTGAAACCTAGGCTATCTGAG	4	(CT)16	22
M15185	GGTCTGGTAGACATGCCTAC	TACCAGCACAGGCAGGTTC	2	(GA)5(GA)17	34
M16B	TGCTGTGGCTCTTGTGAC	AGCCGAGGCTCAGCTCGA	1	(GA)28	23
M4213	CACCTCCCGCTGCATGGCATGT	TACAACGACATGTCAAGGT	1	(GT)9	10
PR3	GTATAGTACCCATTCCGT	GCCGCCCTGCCATGCTG	2	(CA)22	26
PR8	AGGGTTCGTCTGCATTC	GCCGTCGCACCCCTG	1	(GT)22	16
PR14	CCTTTTCGCCTTCGTA	CACCAACATTGCCGAGTG	4	(GT)12	14
PR24	TGCTGTGATGCTGAATG	GTATAGTACCCATTCCGTTGTC	2	(GT)16	15
PR25	AGGGTTCGTCTGCATTC	CCTGCATACATTCATCCA	1	(GT)15	17
PR37	TCTGCATTCGTTGTCTCACTG	GAGCCGTCGCACCCCTG	1	(GT)18	17
PR39	CATTCATCCACGTTAGAC	CTTCCACGACTGCTTC	1	(CA)17	20
PRE	CATTCATCCACGTTAGAC	GTTAGGTTCGTCTGCAT	1	(CA)12	25
PRG	GCCGAGTGTCATCAAGGT	CTTTTTCGCCTTCGTA	4	(CA)13	11
LP194	GCGTAAGAGAGAGGGCGAT	ACGTATGTCCAACAGGT	Unknown	(GA)10GG(GA)G	20
M844	TAGCTTTCTATGCAAAGCT	CACTTCACTTTTCTTGCA	Unknown	(GT)8	15
PR10	CTTCTAATCCCTCGCCT	TGCCGAGTGTCATCAAGGT	Unknown	(GT)2GG(GT)11	16
rv0913	GGCTTACAGCGAAGAAGACATT	CAGTTGAGCTCATTGGAGACAC	1	(CT)9	19
rv0659	TCTCTCCAAGTTCCTCTTCTGC	TCTGCTTCCATCAAGAAAGGTT	1	(CT)19(CA)11	33
rv0244	CAGATTGCAACTCACGAGGA	ATTTTCCCGTCTGTATTGCG	1	(CT)21	22
rv0959	GTAAACTGGGTTGATGGGATGT	CATTGTACGATTTATGCTTCTCCA	2	(TAA)9	24
rv0706	TTCATGTTAGCCCTCCTGTTTT	AATCAAGCCATCTCGTCCTCTA	2	(CT)18	28
rv1133	CGGTTCTGAATTTTCTGCTTCT	AAAGCTGAGAACCGAATCCATA	3	(CG08	6
14ga1	GTTTGCATTCCTTGTGCT	TCCTTGGGTGTGTCTACG	3	(CT)25	30
25ca1	ATCGAGTGCACATTTCGT	TGTGATCGATGGTGCATA	3	(GT)16	19
rv0941	ACTTGCATAGCGTACAAGCAAA	AAAAACTGTACACTGCGCTCAC	4	(CT)9	15
rv0454	AGATGTGAGCTGTGTGCCTT	CGATATATCAGCCTCACGCA	4	(CT)19	28
rv0992	CAATGCCATCACCAACACTACT	TTTGTAGCATGGAGAGATTACCA	4	(TAA)11	21
rv1112	TTTGTTAGCCCGTCCTTACCTA	AACATGTGGAATGGAATGGAAT	5	(CT)11	17
rv0757	AGATGATGATGCAGTGGACAAG	CACCACTTCAATCACGCAGTAT	5	(CT)27	23
rv0985-1	CTTATTATTGCAAGCGATGGTG	GTATCGTCCCTCAGTCCTTTTG	6	(CT)18	15
rv0641	TGCATAACTTCACTGCAGCATA	AGAAACTCGGTAGAAGGACCTC	6	(CT)10	27

Table S4. Continued

SSR	**Forward primer**	**Reverse primer**	**Linkage**	**SSR motif**	**Number of allele** [a]
rv1266	AAGAGGAAACAAATGGCAACAT	AGCTTCCTTACATGCTCTGTAT	6	(CAG)10	11
v0005	GGCAACTAGGAGAAAAATGAGAA	GATTGTAGTAGTGGGTAATCCT	7	(TAGA)22	21
rv0663	ATTAGTTCAAACACCCGCAAGT	CGTCTGCACCTCTACAGCATAG	7	(CT)15	9
rv1284	TTTGACTATTTCTCGCACTTCG	GAATTCCGCACATATCCAAACT	7	(TA)9	8
LPSSRH01A07	TGGAGGGCTCGTGGAGAAGT	CGGTTCCCACGCCTTGC	3	(GT)9	9
LPSSRK01A11	CGGCCACCCTTGATAGAG	TCGTCAAGGATCCGGAGA	4	(CA)21	15
LM15	TCATTGCCCTGACTACCAGG	GCATCGCCTTTCTCAGAGTG	unknown	(GT)5	7
LpACT13H2	TAACATTGATGCATGGGTTTC	ACTATTTAACTAGGATCCAAC	6	(CT)35	43
LpACT14A4	CATGCTTGGACACTGTTAGCC	CTCTGTGCATATTCACGAGGG	5	(CT)21	34
LpACT14C9	AATGATGGCACGGAGCAATCG	CTGTAATTCCAGGTCACTACC	6	(CT)22	26
LpACT15H3	GACATCCATGCAAAATGTAAG	TCGTCACTTGCAAATATGAAC	2	(CT)33	44
LpACT43C6	AGCGGGAAAGATACAGCGAAG	GAGCAAATTGGTGATCGCTAC	7	(CT)29	17
LpACT44A7	CACGTAGAAGCCACACTTTAC	GTCACATTCCATTCACTTCCG	3	(CT)60	42
LpACT44B9	TATGCAGCTTATGGGATCTTG	CGCATGCTGCACAATATCTGG	3	(CT)38	23
LpHCA16B2	TGACAGTGTAGGCTAGTGATG	GAGTACAACATAGCAGATACC	4	(CA)16	16
LpHCA17C6	AACAGCATCATGGATGCTAGG	ACGGTATGGGTATGCTGATCC	7	(CA)20	25
LpHCA17C11	ACCGGGACAAAGGGCTAGTAC	CGCTCGATCGATCTGATCGTG	2	(CA)23	28
LpHCA18F11	TTCACATGGCATGCACAAACC	AGGTATGACGTGCCGACATGC	7	(CA)15	27
LpHCA20F6	GGGTCATCAGAGATCTTGCTC	TGCAGCTGGATGGATCAGCTC	4	(CA)5	12
Total					2180

[a] Identified in this study.

Table S5. Primers used for amplification of 14 candidate genes in perennial ryegrass.

Putative gene	Protein name	Forward primer	Reverse primer
LpCAT	Catalase	TGTTCDCCTTCCTCTTYGACGAT	GTGGTGGTTGTTGTGGTATGC
LpChl Cu-ZnSOD	Chloroplast copper/zinc superoxide dismutase	TCGYGGACGCYACTAAGAAG	CAACAACACCACATGCCAGTCT
LpGPX	Glutathione peroxidase	GGTAACGATGTGAGTTTGAGCC	GTCCCARCAGAGCTTGAATRTC
LpMnSOD	Manganese superoxide dismutase	ATGGCBCTCCGCACGYTG	CCGTTGAACTTGATGGCGCTCT
LpAPX	Ascorbate peroxidase	GCYTCATCGCCGAGAAGAAC	CGTCCTCATCCGCAGCATAT
LpMDAR	Monodehydroascorbate reductase	AGCTCTGCATCATCTCCGAA	ACTGCCAGGACAATGTGAAGAC
LpDHAR	Dehydroascorbate reductase	CTCGGCGACTGYCCATTCWC	TTTGGTGCCCATCCAGCAAT
LpGR	Glutathione reductase	CCAAGGTYGCGATCTGCGAG	GCAACAGCAATGCCCTGCATAA
LpFeSOD	Iron superoxide dismutase	GCBCTCACTACTCCKCCGTAT	TGCGTAGAGTGACAGTATCCCA
LpCyt Cu-ZnSOD	Cytosolic copper/zinc superoxide dismutase	TTRCTAGCAGTGAGGGTGTCAA	ATGATTCCGCAAGCAACTCGG
LpMAPK	Mitogen activated protein kinase	TATATCCGCCAACTTCCCCG	TCAATCATCACCTGCCCACC
LpLEA3	Late embryogenesis-abundant, group 3	ATGGCTTCTTCTTCCGTGCTGCT	TACTCTTTGGCCGCGGCTTT
LpPIP1	Plasma membrane Intrinsic Protein, type 1	ACCTTCCTCTTCCTCTACGTGACCAT	AGGTGTGCTCCCTGTTGTAGATGA
LpTIP1	Tonoplast Intrinsic Protein, type 1	TCATCTCCACGCTCATCTTCGTCT	CGACCCAGTACACCCACTGGTAT

Table S6. Analysis of Variance (ANOVA) of canopy and air temperature difference (CAD), chlorophyll fluorescence (*Fv*/*Fm*), leaf water content (LWC), and leaf wilting of perennial ryegrasses across 6 environments.

Source	df	CAD	*Fv*/*Fm*	LWC	Wilting
Treatment (Treat)	1	24.19**[a]	1.85	1739**	N/A
Accession (Acc)	178	3.49**	6.00**	2.38**	8.16**
Treat×Acc	178	1.18	1.81**	1.34*	N/A

[a] Numbers used here were F statistics.

*$P < 0.05$; **$P < 0.01$.

df, degree of freedom; N/A, not applicable.

Table S7. Summary statistics for all accessions and individual groups (G1 to G5) within the panel of perennial ryegrass accessions identified by STRUCTURE analysis.

Statistics	All	G1	G2	G3	G4	G5
Sample size	192	121	21	25	13	12
Number of alleles per locus	20	15.4	9.2	10.4	8.2	7.1
Major allele frequency	0.34	0.38	0.40	0.37	0.36	0.40
Gene diversity	0.79	0.74	0.72	0.76	0.75	0.71
Heterozygosity	0.50	0.53	0.47	0.42	0.50	0.44
PIC [a]	0.76	0.71	0.69	0.73	0.73	0.68

[a] Polymorphism information content

Table S8. Number of genotypes found in 12 candidate genes of *LpChl Cu-ZnSOD, LpGPX, LpMnSOD, LpMDAR, LpDHAR, LpGR, LpFeSOD, LpCyt Cu-ZnSOD, LpMAPK, LpLEA3, LpPIP1, and LpTIP1* across 192 accessions of perennial ryegrass.

Genotype	**Number**	**Frequency (%)**
A	8396	17.5
T	7358	15.3
C	9935	20.7
G	12077	25.1
C/T	2511	5.2
C/A	914	1.9
C/G	1498	3.1
A/G	3781	7.9
A/T	609	1.3
T/G	972	2.0
Total	48051	100

Table S9. Number of associations among 14 genes and traits under drought conditions using simple linear model (S) and population structure (Q) controlled model in perennial ryegrass.

	Simple model	Q Model	False positive rate under S model
Significant associations	20	6	70%