Supporting Information Figs S1–S3 and Tables S1–S5

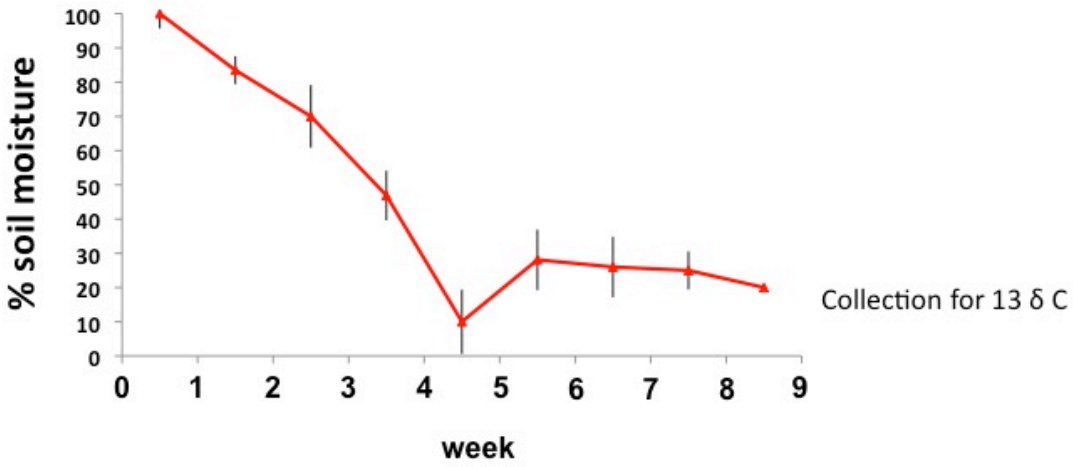

Fig. S1 Soil moisture variation during the drought tolerance experiment.

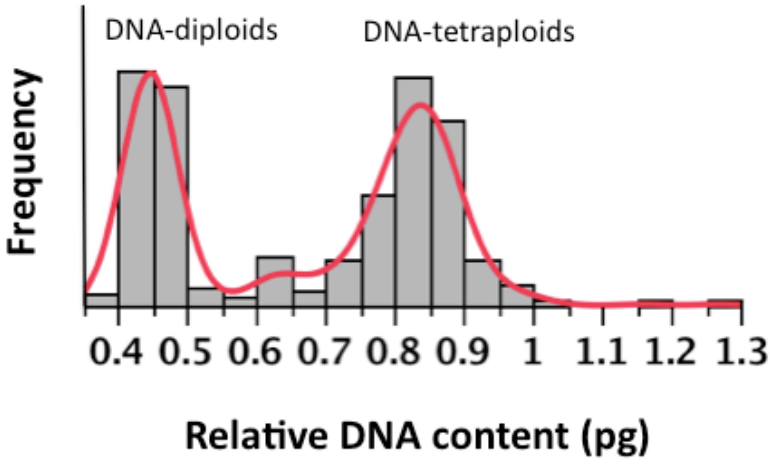

Fig. S2 Frequency distribution of relative DNA content (pg) obtained from the screening of 1274 individual plants from 637 *Brachypodium distachyon* accessions collected in 57 populations across the Iberian Peninsula.

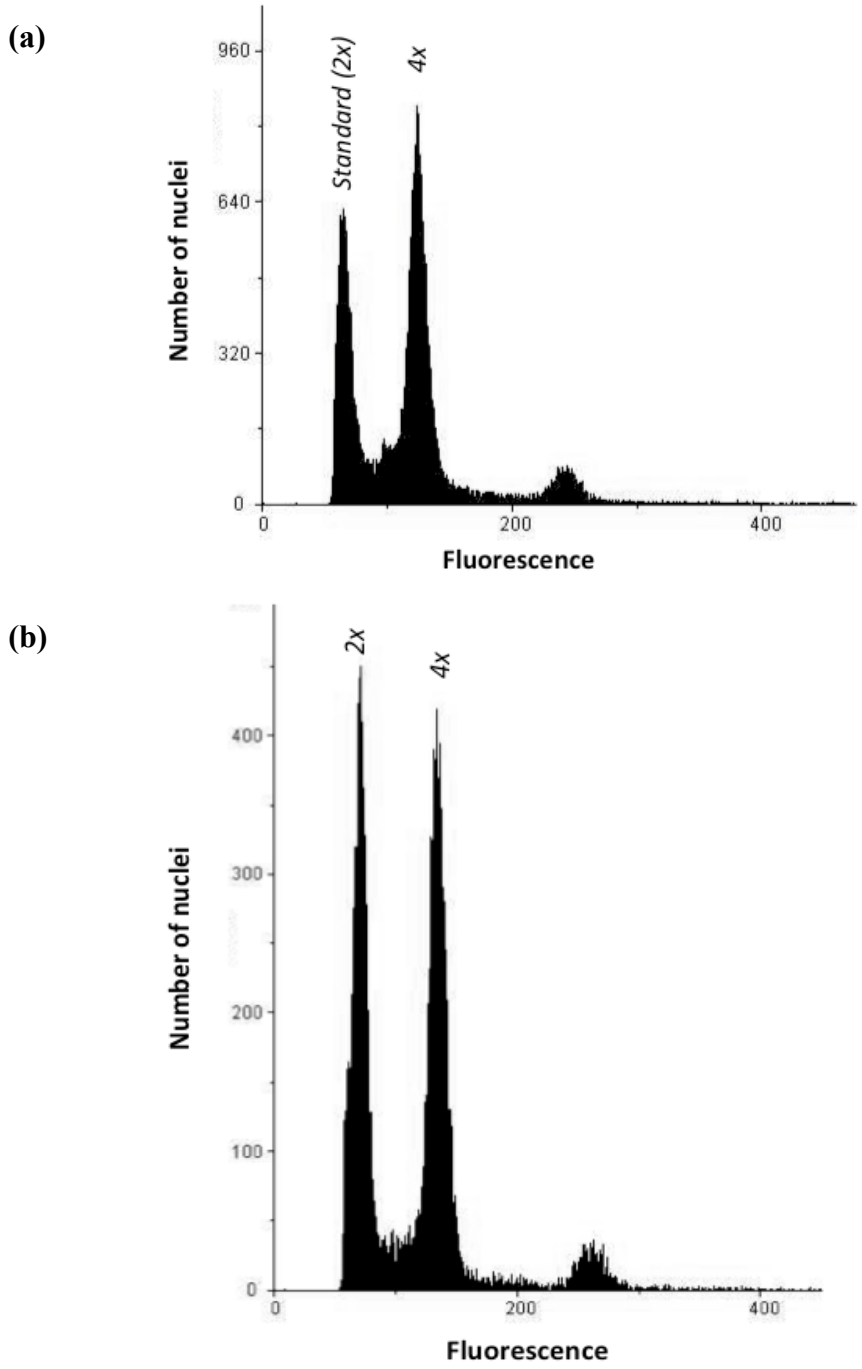

Fig. S3 Simultaneus flow cytometry analysis of DAPI-stained nuclei isolated from (a) the diploid *Brachypodium distachyon* Bd-21 inbred line, used as external standard in the study, and a DNA-tetraploid plant (Alplay_15 from population #45). (b) Simultaneus analysis of a DNA-diploid plant (Gua_15 from population #8) and a DNA-tetraploid plant (Alplay_15 from population #45).

Table S1 Localities and geographical coordinates of the 57 *Brachypodium distachyon* populations across the Iberian Peninsula used in this study

Population	Latitude	Longitude	Elevation	Annual rainfall (mm)	Annual temperature (°C)	R : T†	Type of habitat
(1) Campohermoso	42º 51' 17" N	5º 26' 5" W	1074	945	9	105	Roadside
(2) Cervera	42º 49' 32" N	4º 29' 27" W	1105	930	9	103.33	Roadside, oak forest margin
(3) Vª de Arriba	42º 47' 23" N	4º 47' 07" W	1112	1000	9	111.11	Roadside
(4) Roncal	42º 46' 50" N	0º 57' 48" W	594	1102	11	100.18	Roadside
(5) Yesa	42º 36' 35" N	0º 57' 53" W	562	873	9	97	Roadside
(6) Jaca	42º 33' 10" N	0º 40' 53" W	713	1062	9	118	Roadside
(7) Sobrado	42º 31' 54" N	6º 51' 7" W	464	1008	12	84	Roadside/ oak forest margin
(8) Guara	42º 24' 09" N	0º 22' 43" W	733	840	9	93.33	Roadside/Riverside
(9) Serrablo	42º 24' 07"N	0º 1' 48" W	1291	1423	9	158.11	Roadside
(10) Gerbé	42º 23' 18" N	0º 11' 10" E	585	843	13	64.85	Xerophytic meadow
(11) Bosia	42º 21' 22" N	0º 53' 1.9" E	842	969	10	96.9	Xerophytic meadow
(12) Rosas	42º 14' 45" N	3º 11' 1" E	27	591	14	42.21	Rocky cliff
(13) Aguilar de Ebro	41º 30' 50" N	0º 30' 30" W	177	349	15	23.27	Abandoned arable field
(14) Íscar	41º 22' 12" N	4º 32' 18" W	828	468	12	39	Xerophytic meadow
(15) La Fregeneda	40º 59' 39" N	6º 51' 50" W	508	546	15	36.4	Roadside, orchard margin
(16) Gallocanta	40º 58' 42" N	1º 27' 53" W	1053	479	12	39.92	Scrubland margin
(17) Aguatón	40º 39' 44" N	1º 15 ' 50" W	1084	463	11	42.09	Abandoned arable field
(18) El Bodón	40º 26' 23" N	6º 35' 28" W	768	573	13	44.08	Roadside
(19) Ademuz	40º 06' 54" N	1º 15' 50" W	780	466	14	33.29	Understory pine forest
(20) Monfrague	39º 49' 20" N	6º 02' 52" W	281	807	18	44.83	Understory oak forest
(21) Villatoya	39º 20' 32" N	1º 20' 47" W	383	397	16	24.81	Xerophytic meadow
(22) Casas Ibañez	39º 16' 09" N	1º 29' 22" W	721	409	13	31.46	Abandoned arable field
(23) Ruidera	38º 57' 42 N	2º 52' 17" W	765	433	14	30.93	Roadside, oak forest margin

(24) Los Pedroches	38º 36' 38" N	5º 9' 3" W	496	542	16	33.88	Roadside, pine forest margin
(25) La Cimbarra	38º 23' 28" N	3º 22' 13" W	723	509	14	36.36	Roadside, oak forest margin
(26) La Aliseda	38º 19' 48" N	3º 34' 44" W	699	552	15	36.80	Understory oak forest
(27) Nerpio	38º 11' 49" N	2º 15' 22" W	958	458	13	35.23	Roadside, xerophytic meadow
(28) Moratalla	38º 10' 38" N	1º 55' 39" W	919	543	14	38.79	Understory pine forest
(29) Cazorla	37º 52' 12" N	2º 56' 24 W	938	1385	12	115.42	Understory pine forest
(30) Espuña	37º 49' 14" N	1º 35' 59" W	673	335	17	19.71	Roadside, orchard margin
(31) Jodar	37º 48' 53" N	3º 19' 09" W	650	302	14	21.57	Xerophytic meadow
(32) Larva	37º 48' 38" N	3º 12' 53" W	575	500	14	35.71	Xerophytic meadow
(33) Tíscar	37º 46' 02" N	3º 1' 21" W	879	434	15	28.93	Understory pine forest
(34) Jaén	37º 45' 35" N	3º 47' 53" W	633	516	17	30.35	Roadside, orchard margin
(35) Hinojares	37º 43' 41" N	2º 58' 52" W	804	725	11	65.91	Xerophytic meadow
(36) Baza	37º 32" 38" N	2º 51' 30" W	1318	580	9	64.44	Understory pine forest
(37) Negratín	37º 32' 38" N	2º 56' 32" W	689	350	15	23.33	Xerophytic meadow
(38) Cubillas	37º 16' 38" N	3º 40' 11" W	650	453	15	30.2	Understory pine forest
(39) Lepe	37º 13' 44" N	7º 11' 55" W	30	564	18	31.33	Roadside
(40) Sorbas	37º 05' 79" N	2º 06' 35" W	330	342	18	19	Roadside, scrubland margin
(41) Tabernas	37º 02' 28" N	2º 24' 22" W	356	298	16	18.63	Scrubland margin, dry stream
(42) Sierra Nevada	36º 59' 43" N	3º 34' 18 W	1420	440	10	44	Roadside, xerophytic meadow
(43) Nigüelas	36º 59' 26" N	3º 30' 31 W	1220	440	10	44	Roadside, xerophytic meadow
(44) Antequera	36º 57' 49" N	4º 32' 44' W	1117	250	14	17.86	Roadside, xerophytic meadow
(45) Rodalquilar	36º 51'24" N	2º 00' 46" W	11	311	19	16.37	Roadside, scrubland margin
(46) Grazalema	36º 45' 21" N	5º 26' 30" W	1103	1773	16	110.81	Understory pine forest
(47) Cabo de Gata	36º 43' 51" N	2º 12' 4" W	77	311	19	16.37	Rocky cliff
(48) Ronda	36º 38' 32" N	5º 14' 42" W	808	1011	14	72.21	Roadside, xerophytic meadow
(49) Algatocín	36º 34' 54" N	5º 16' 37" W	814	931	16	58.19	Roadside, xerophytic meadow
(50) Botafuegos	36º 08' 44" N	5º 27' 54" W	8	968	17	56.94	Roadside, xerophytic meadow
(51) Algeciras	36º 08' 37" N	5º 30' 43" W	123	968	17	56.94	Roadside, xerophytic meadow
(52) Las Corzas	36º 04' 48" N	5º 31' 56" W	279	968	17	56.94	Roadside, xerophytic meadow
(53) Cala Arena	36º 03' 17" N	5º 29' 08" W	1	886	17	52.12	Roadside, xerophytic meadow
(54) Mogadouro	41º 25' 7" N	6º 49' 21" W	377	597	14	42.64	Roadside, oak forest margin

(55) Bemposta	41º 18' 3" N	6º 28' 41" W	437	597	14	42.64	Xerophytic meadow
(56) Faro	37º 0' 52" N	7º 58' 35" W	3	776	16	48.5	Roadside,
(57) Le Perthus	42º 28' 59" N	2º 50' 27" E	154	894	12	74.5	Roadside, oak forest margin

Annual average of precipitation (mm), average temperature (°C) and their ratio (R : T) (see the Materials and Methods section for details on the obtention of climatic parameters) of each population is shown. In addition, short descriptions of the habitat in which *B. distachyon* plants grow in each population are also given. Numbers in parentheses indicate the location of the population as shown in Fig. 1. †Rainfall to temperature ratio, this is the inverse of aridity since the lower its value the higher the aridity.

Table S2 Ploidy level variation among 637 *Brachypodium distachyon* accessions collected from 57 populations in the Iberian Peninsula

Population	Relative DNA content 1C-DNA (pg) ± 1SD	Chromosome number	Ploidy Level	Frequency of polyploidy (%)	*n*
(1) Campohermoso	0.43 ± 0.01	-	*2*x*	0	10
(2) Cervera	0.45 ± 0.01	2*n* = 2*x* = 10 (8)	2*x*	0	13
(3) Vª de Arriba	0.47 ± 0.02	-	*2*x*	0	12
(4) Roncal	0.46 ± 0.02	2*n* = 2*x* = 10 (8)	2*x*	0	14
(5) Yesa	0.42 ± 0.03	-	*2*x*	0	12
(6) Jaca	0.41 ± 0.003	-	*2*x*	0	18
(7) Sobrado	0.45 ± 0.014	-	*2*x*	0	10
(8) Guara	0.46 ± 0.03	-	*2*x*	0	12
(9) Serrablo	0.44 ± 0.01	-	*2*x*	0	11
(10) Gerbé	0.45 ± 0.016	-	*2*x*	0	11
(11) Bosia	0.44 ± 0.007	-	*2*x*	0	9
(12) Rosas	0.92 ± 0.068	-	*4*x*	100	8
(13) Aguilar de Ebro	0.87 ± 0.02	-	*4*x*	100	6
(14) Íscar	0.44 ± 0.007	2*n* = 2*x* = 10 (8)	2*x*	0	16
(15) La Fregeneda	0.9 ± 0.02	2*n* = 4*x* = 30 (7)	4*x*	100	9
(16) Gallocanta	0.45 ± 0.03	-	*2*x*	0	12
(17) Aguatón	0.45 ± 0.02	-	*2*x*	0	12
(18) El Bodón	0.85 ± 0.09	-	*4*x*	100	8
(19) Ademuz	0.45 ± 0.01	-	*2*x*	0	10
(20) Monfrague	0.83 ± 0.03	2*n* = 4*x* = 30 (12)	4*x*	100	15
(21) Villatoya	0.45 ± 0.03	-	*2*x*	0	11
(22) Casas Ibañez	0.46 ± 0.01	-	*2*x*	0	11

(23) Ruidera	0.85 ± 0.03	-	*4*x*	100	8
(24) Los Pedroches	0.88 ± 0.04	-	*4*x*	100	9
(25) La Cimbarra	0.82 ± 0.005	-	*4*x*	100	7
(26) La Aliseda	0.77 ± 0.037	-	*4*x*	100	18
(27) Nerpio	0.82 ± 0.03	-	*4*x*	100	5
(28) Moratalla	0.44 ± 0.018 0.85 ± 0.037	- /2*n* = 4*x* = 30 (1)	*2*x*, 4*x*	68.5	19
(29) Cazorla	0.8 ± 0.023	2*n* = 4*x* = 30 (2)	4*x*	100	13
(30) Espuña	0.47 ± 0.01 0.82 ± 0.06	2*n* = 2*x* = 10 (1) / 2*n* = 4*x* = 30 (2)	2*x*, 4*x*	61.11	18
(31) Jodar	0.79 ± 0.06	-	*4*x*	100	17
(32) Larva	0.42 ± 0.008 0.8 ± 0.031	-/ 2*n* = 4*x* = 30 (3)	*2*x,* 4*x*	94.74	19
(33) Tíscar	0.43 ± 0.016 0.83 ± 0.00	2*n* = 2*x* = 10 (1) / 2*n* = 4*x* = 30 (1)	2*x*, 4*x*	25	5
(34) Jaén	0.83 ± 0.026	2*n* = 4*x* = 30 (3)	4*x*	100	7
(35) Hinojares	0.45 ± 0.037 0.85 ± 0.04	-	*2*x*, *4*x*	89.47	19
(36) Baza	0.83 ± 0.017	-	*4*x*	100	9
(37) Negratín	0.88 ± 0.01	-	*4*x*	100	8
(38) Cubillas	0.63 ± 0.019	2*n* = 4*x* = 30 (7)	4*x*	100	10
(39) Lepe	0.87 ± 0.01	-	*4*x*	100	5
(40) Sorbas	0.44 ± 0.001 0.81 ± 0.04	2*n* = 2*x* = 10 (2) / 2*n* = 4*x* = 30 (5)	2*x*, 4*x*	77.78	9
(41) Tabernas	0.44 ± 0.017 0.86 ± 0.014	2*n* = 2*x* =10 (1) / 2*n* = 4*x* = 30 (1)	2*x*, 4*x*	33.33	6
(42) Sierra Nevada	0.44 ± 0.004 0.82 ± 0.02	-	*2*x*, *4*x*	63.63	11
(43) Nigüelas	0.45 ± 0.018	2n = 2*x* =10 (4)	2*x*	0	11
(44) Antequera	0.87 ± 0.019	-	*4*x*	100	5
(45) Rodalquilar	0.42 ± 0.038	-	*2*x*, *4*x*	89.47	19

	0.84 ± 0.09				
(46) Grazalema	0.44 ± 0.02	-	*2*x*	0	12
(47) Cabo de Gata	0.43 ± 0.005	-	*2*x*, *4*x*	25	8
	0.84 ± 0.019				
(48) Ronda	0.85 ± 0.04	2*n* = 4*x* = 30 (2)	4*x*	100	5
(49) Algatocín	0.86 ± 0.04	2*n* = 4*x* = 30 (1)	4*x*	100	10
(50) Botafuegos	0.42 ± 0.03	2*n* = 2*x* =10 (1)	2*x*	14.28	7
	0.84 ± 0.01	2*n* = 4*x* = 30 (3)			
(51) Algeciras	0.82 ± 0.027	-	*4*x*	100	9
(52) Las Corzas	0.78 ± 0.09	2*n* = 4*x* = 30 (8)	4*x*	100	12
(53) Cala Arena	0.46 ± 0.03	2*n* = 2*x* =10 (3)	2*x*, 4*x*	14.28	14
	0.88 ± 0.07	2*n* = 4*x* = 30 (1)			
(54) Mogadouro	0.86 ± 0.06	-	*4*x*	100	20
(55) Bemposta	0.86 ± 0.035	2*n* = 4*x* = 30 (10)	4*x*	100	12
(56) Faro	0.85 ± 0.05	2*n* = 4*x* = 30 (2)	4*x*	100	9
(57) Le Perthus	0.81 ± 0.05	-	*4*x*	100	12

Average relative DNA content is obtained using DAPI flow cytometry and using the diploid (2*n* = 2*x* = 10) *B.distachyon* 'Bd21' inbred line as external standard. Chromosome counts were performed on root tip meristematics cells to study the linearity between DNA content variations and chromosome number. When the relationship between chromosome number and DNA content was not verified by chromosome counts (denoted by an asterisk in the table) DNA-ploidy level is considered. In parentheses, the total number of accessions whose chromosome counting was performed within the population.

Table S3 Location and geographical provenance of the 96 genotypes used in the assessment of the variation in water use efficiency (carbon isotope composition and discrimination, $\delta^{13}C$ and $\Delta^{13}C$ values respectively) and flowering time (the number of days from germination until the opening of the first spike) among 24 natural *Brachypodium distachyon* populations across the Iberian Peninsula

Population	Ploidy level	Mean $\delta^{13}C$ (‰)	Mean $\Delta^{13}C$ (‰)	Mean flowering time (number of days)
(1) Campohermoso	2*x*	−31.28	24.21	67.8
(2) Cervera	2*x*	−31.15	23.89	61
(4) Roncal	2*x*	−30.65	23.37	136.4
(7) Sobrado	2*x*	−30.69	23.41	47.7
(11) Bosia	2*x*	−30.74	23.46	99
(12) Rosas	4*x*	−30.07	22.75	38
(13) Aguilar de Ebro	4*x*	−30.94	23.67	52
(14) Íscar	2*x*	−30.01	22.69	93.4
(18) El Bodón	4*x*	−30.71	23.43	45.7
(20) Monfrague	4*x*	−29.95	22.63	46.4
(21) Villatoya	2*x*	−30.7	23.42	107
(23) Ruidera	4*x*	−31.05	23.79	53.8
(24) Los Pedroches	4*x*	−29.87	22.54	104.7
(28) Moratalla*	2*x*	−30.73	23.45	51.8
(28) Moratalla*	4*x*	−31.6	24.37	44.5
(29) Cazorla	4*x*	−30.92	23.65	34.4
(30) Espuña*	2*x*	−32.51	25.33	32.3
(30) Espuña*	4*x*	−30.64	23.36	45.3
(35) Hinojares*	2*x*	−31.89	24.68	50

(35) Hinojares*	4*x*	−30.5	23.21	37.6
(41) Tabernas*	2*x*	−32.25	25.06	35.8
(41) Tabernas*	4*x*	**	**	36.5
(42) Sierra Nevada	2*x*	−33.0	25.85	35.8
(45) Rodalquilar	4*x*	−31.45	24.21	36.3
(46) Grazalema	2*x*	−31.27	24.02	48.6
(53) Cala Arena*	2*x*	−30.64	23.36	135.2
(53) Cala Arena*	4*x*	−32.4	25.22	146
(54) Mogadouro	4*x*	−29.53	22.19	-†
(56) Faro	4*x*	−29.92	22.60	49

At each population 4 genotypes were selected and drought tolerance traits characterized under water-limited conditions. Numbers in parentheses indicate the location of the population as shown in Fig. 1 and Table S1. Lower values of $\Delta^{13}C$ indicate higher water use efficiency (WUE). *, both diploid and polyploid accessions are present in the sampling population; **, only diploids were screened for isotopic ratio WUE; †, all plants dead without flowering.

Table S4 Spearman's correlation coefficients among the geographical and climate parameters considered in this study

Parameter	Latitude	Longitude	Elevation	Precipitation	R : T[†]
Latitude	1	**0.37**	0.18	**0.27**	**0.38**
Longitude		1	0.14	−0.18	−0.04
Elevation			1	0.06	**0.29**
Precipitation				1	**0.93**
R : T[†]					1

Significant coefficients ($P > 0.05$) are shown in bold. [†]Rainfall to temperature ratio, this is the inverse of aridity since the lower its value the higher the aridity.

Table S5 Principal components analysis conducted on two climatic and three geographical parameters of 57 *Brachypodium distachyon* populations across the Iberian Peninsula

Parameters	Correlation to principal components (PC)	
	PC1	PC2
Latitude	0.421	**0.7**
Longitude	−0.105	**0.885**
Elevation	0.379	0.243
Rainfall	**0.935**	−0.125
Rainfall/temperature[†]	**0.965**	0.189

Values highlighted in bold type are those parameters with correlations above 0.7 with each principal component. PC1 depicts an inverse to an aridity gradient and precipitation. PC2 depicts geographical variation. [†]Rainfall to temperature ratio, this is inverse to aridity since the lower its value the higher the aridity. Thus, PC1 is inversely related to aridity.