Validation of microsatellite markers for cytotype discrimination in the model grass *Brachypodium distachyon*

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Abstract: *Brachypodium distachyon* (L.) P. Beauv. ($2n = 2x = 10$) is a small annual grass species where the existence of three different cytotypes (10, 20, and 30 chromosomes) has long been regarded as a case of autopolyploid series with $x = 5$. However, it has been demonstrated that the cytotypes assumed to be polyploids represent two separate *Brachypodium* species recently named as *Brachypodium stacei* ($2n = 2x = 20$) and *Brachypodium hybridum* ($2n = 4x = 30$). The aim of this study was to find a PCR-based alternative approach that could replace standard cytotyping methods (i.e., chromosome counting and flow cytometry) to characterize each of the three *Brachypodium* species. We have analyzed with four microsatellite (SSR) markers 83 *B. distachyon*-type lines from varied locations in Spain, including the Balearic and Canary Islands. Within this set of lines, 64, 4, and 15 had 10, 20, and 30 chromosomes, respectively. The surveyed markers produced cytotype-specific SSR profiles. So, a single amplification product was generated in the diploid samples, with nonoverlapping allelic ranges between the $2n = 10$ and $2n = 20$ cytotypes, whereas two bands, one in the size range of each of the diploid cytotypes, were amplified in the $2n = 30$ lines. Furthermore, the remarkable size difference obtained with the SSR ALB165 allowed the identification of the *Brachypodium* species by simple agarose gel electrophoresis.

Key words: Brachypodium distachyon, *B. stacei*, *B. hybridum*, cytotype-specific markers, SSR analysis.

Résumé : Le *Brachypodium distachyon* (L.) P. Beauv. ($2n = 2x = 10$) est une petite graminée annuelle chez laquelle l'existence de trois cytotypes différents (à 10, 20 ou 30 chromosomes) a longtemps été considérée comme une série autopolyploïde avec $x = 5$. Cependant, il a été démontré que les cytotypes qui étaient présumés polyploïdes représentent en fait deux espèces distinctes du genre *Brachypodium* récemment nommées *Brachypodium stacei* ($2n = 2x = 20$) et *Brachypodium hybridum* ($2n = 4x = 30$). Le but de ce travail était de trouver une approche PCR permettant de remplacer les méthodes cytologiques (décomptes chromosomiques, cytométrie en flux) pour caractériser les trois espèces de *Brachypodium*. Les auteurs ont analysé 83 lignées de type *B. distachyon* provenant de différents sites en Espagne, incluant les Îles Baléares et Canaries, au moyen de quatre marqueurs microsatellites (SSR). Au sein de cette collection, 64, 4 et 15 lignées présentaient respectivement 10, 20 et 30 chromosomes. Les marqueurs examinés ont produit des profils spécifiques des cytotypes. Ainsi, un seul amplicon a été obtenu chez les échantillons diploïdes et les séries alléliques ne chevauchaient pas entre les cytotypes à $2n = 10$ et $2n = 20$, tandis que deux amplicons ont été obtenus chez les lignées à $2n = 30$, chacun de ces amplicons logeant à l'intérieur des séries alléliques observées chez les cytotypes diploïdes. De plus, la différence de taille remarquable observée entre les amplicons du SSR ALB165 permettait l'identification des espèces de *Brachypodium* sur un simple gel d'agarose.

Mots-clés : Brachypodium distachyon, *B. stacei*, *B. hybridum*, marqueurs spécifiques du cytotype, analyse SSR.

[Traduit par la Rédaction]

Introduction

The annual grass *Brachypodium distachyon* (L.) P. Beauv. (Pooideae) is increasingly becoming recognized as the most suitable model organism for temperate cereal crop research. Since the initial proposal of Draper et al. (2001), the development of analytical tools and genomic resources has been extraordinarily rapid and fruitful (Ozdemir et al. 2008; for recent updates see Mur et al. 2011; Vain 2011). Availability of a wide genetic diversity is also required to approach the molecular dissection of most agronomically relevant traits. Consequently, a number of studies have been focused on the characterization of *B. distachyon* natural accessions and derived lines (Filiz et al. 2009; Vogel et al. 2009; Mur et al. 2011; Catalán et al. 2012; Manzaneda et al. 2012). Germ-

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plasm resources, mainly originating from Turkey and Spain, are currently available to the scientific community. However, taking into account the ample spatial distribution of this wild grass (Garvin et al. 2008; Catalán et al. 2012), enlarging the *Brachypodium* gene pool remains as a feasible and valuable research objective.

Brachypodium distachyon ($2n = 2x = 10$) has recently been taxonomically separated from two other *Brachypodium* species: the diploid *Brachypodium stacei* ($2n = 20$) and the allotetraploid *Brachypodium hybridum* ($2n = 30$) (Catalán et al. 2012). These three species had been earlier collected and catalogued under the same botanical name, *B. distachyon*, evidencing their great similarity for most external traits. The existence of distinct cytotypes, with 2*n* values of 10, 20, and 30 chromosomes (Robertson 1981), was assumed to be a case of an autopolyploid series with $x = 5$. Nonetheless, solid experimental evidences support that *B. hybridum* derives from *B. distachyon* and *B. stacei* (Hasterok et al. 2004, 2006; Idziak et al. 2011; Catalán et al. 2012). Under the framework established by Catalán and co-workers, all newly collected or yet uncharacterized *B. distachyon*-type accession must be unambiguously assigned to the right species to be valuable for further research. This is especially important to guarantee that any germplasm, genetic, or genomic tool eventually developed corresponds to the true model species, *B. distachyon* ($2n = 10$).

Catalán et al. (2012) have described significant phenotypic differences among the three cytotypes (i.e., species) for several quantitative characters. Nonetheless, the overlapping ranges reported for some of them, as well as the uncertainty about their actual level of intraspecific natural variation, make it risky to exclusively use morphometric traits for taxonomic assignment of any accession or line. Currently, chromosome counting and flow cytometry are among the best alternatives. The former provides reliable species identification by conducting direct cytotyping. However, flow cytometry sharply distinguishes the allotetraploid *B. hybridum* from the others (2C genome size values ranging from 1.3 pg (e.g., Draper et al. 2001) to 1.6 pg (Manzaneda et al. 2012)), but could not discriminate between *B. distachyon*, with 2C DNA content estimations from 0.42 pg (Draper et al. 2001) to 0.9 pg (Manzaneda et al. 2012), and *B. stacei* (2C DNA value of 0.56 pg in Catalán et al. 2012). Nevertheless, the main limitation of these two methods is that both are time-consuming and require expertise or equipment that are sometimes unavailable.

Our study aimed to validate the utility of a widely used PCR-based approach for species identification of annual *Brachypodium* specimens. For this, we have conducted SSR (single sequence repeats) analysis of *B. distachyon*, *B. stacei*, and *B. hybridum* lines whose cytotypes had been earlier confirmed by direct chromosome count.

Materials and methods

Plant material

The study has been carried out on the collection of *Brachypodium* lines held at UPM (Universidad Politécnica de Madrid, Spain). It is composed of 64 lines of *B. distachyon* ($2n = 10$), 2 lines of *B. stacei* ($2n = 20$), and 12 lines of *B. hybridum* ($2n = 30$) that derive from single seed descent of *B. distachyon*-type specimens collected by our group in about 70 locations across a wide area of the Iberian Peninsula (latitude: 36°44′N to 42°30′N; longitude: 0°09′E to 6°40′W) and the Balearic Islands (Menorca). Five lines from the germplasm bank of the National Centre of Plant Genetic Resources of Spain (CRF-INIA) have also been analyzed. These lines were established from individual plants collected in 1995 in the Canary Islands by a Spanish–Japanese consortium. Two of them correspond to the $2n = 20$ cytotype, while the remaining three lines correspond to the $2n = 30$ cytotype. Figure 1 shows the location of collection sites for all the Spanish lines examined; detailed information regarding their geographical origin is given as supplementary material (Table S1)[1]. The type specimens of *B. distachyon* (line Bd21; originating from Irak), *B. stacei* (line ABR114; from Formentera, Balearic Islands, Spain), and *B. hybridum* (line ABR113; from Lisbon, Portugal) have also been included in the study.

Microsatellite (SSR) analyses

For the SSR analysis of the *Brachypodium* lines, DNA was extracted from leaf samples using the Ultra Clean Plant DNA Isolation kit (MOBIO), according to manufacturer's instructions. PCR was carried out in a 20 μL reaction volume containing 2 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 5 μmol/L of each primer, 100 ng of template DNA, and 1U of *Taq* DNA Polymerase (Biotools) using a MyCycler thermocycler (Bio-Rad). PCR cycling conditions were as follows: 2 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at annealing temperature (52–56 °C) of each primer pair, 1 min at 72 °C, and final extension of 10 min at 72 °C. The PCR products with different fluorescent labels were mixed with Hi Di formamide and Liz internal size standard, denatured at 95 °C for 5 min, and genotyped on a ABI PRISM 3100 DNA analyzer (Applied Biosystems, USA). Allele sizing for different DNA fragments was carried out by GeneMarker software (SoftGenetics, LLC, USA). In the case of ABL165 marker, amplification products were also analyzed in 2.5% Seakem LE agarose gels (Lonza) staining with GelRed (Biotium).

Results and discussion

An initial set of *B. distachyon*-type accessions was profiled with 15 microsatellites (SSRs) selected based on their genomic distribution and polymorphism level when data were available (Garvin et al. 2008; Vogel et al. 2009). Five of them (ALB165, ALB311, BdSSR207, BdSSR330, and R2-3-ABI) behaved as potential species-specific markers. BdSSR207, which maps very close to R2-3-ABI, was finally discarded as it failed to show a robust profile. The remaining four SSR markers were tested in a total of 64 *B. distachyon* ($2n = 2x = 10$), 4 *B. stacei* ($2n = 2x = 20$), and 15 *B. hybridum* ($2n = 4x = 30$) Spanish lines established at the UPM and in the reference lines of the three species, namely, Bd21, ABR114, and ABR113. See supplementary Table S2 for further description of the microsatellite marker loci used.

Invariably, amplification of ALB165, ALB311, BD330,

[1]Supplementary data are available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/g2012-039).

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Fig. 1. Location of collection sites of the *Brachypodium distachyon* (2*n* = 10), *B. stacei* (2*n* = 20), and *B. hybridum* (2*n* = 30) lines derived from populations represented in the Universidad Politécnica de Madrid (UPM) collection (Iberian Peninsula and Balearic Islands) and in the National Centre of Plant Genetic Resources of Spain (CRF-INIA) collection (Canary Islands). The province code of sampled populations is indicated by one or two letters (see Table S1 for details).

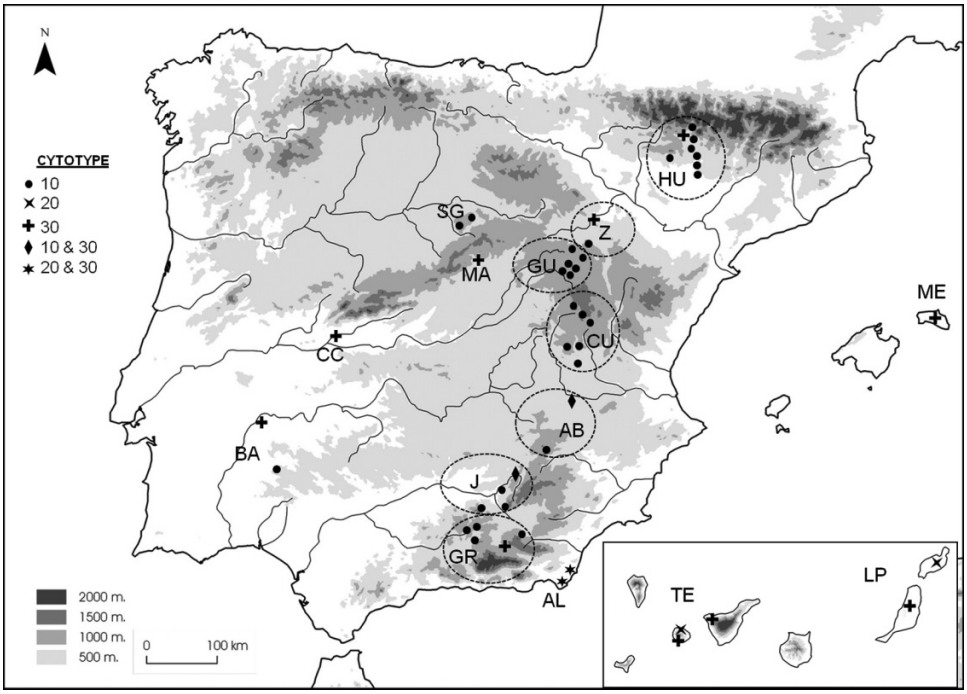


Table 1. Summary of the analysis of amplification profiles for ALB165, ALB311, BD330, and R2-3 SSR markers in the *Brachypodium distachyon* (2*n* = 10), *B. stacei* (2*n* = 20), and *B. hybridum* (2*n* = 30) lines examined.

			Marker amplification range							
			ALB165 (Bd1)		ALB311 (Bd5)		BD330 (Bd2)		R2-3 (Bd3)	
	2*n*	*N**	137–141	150–195	223–227	231	168	174–176	189	191–204
B. distachyon	10	65	–	+	+	–	–	+	–	+
B. stacei	20	5	+	–	–	+	+	–	+	–
B. hybridum	30	16	+	+	+	+	+	+	+	+

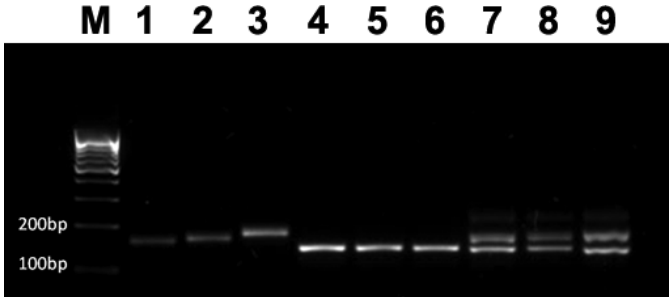
Note: +, amplification band in the range; –, no amplification band in the range. The chromosomal location of each marker in the *B. distachyon* genome is noted in brackets.

*Including 78 UPM lines, 5 lines derived from CRF-INIA accessions, and the reference lines for each of the three species.

and R2-3 in the diploid species has resulted in a single amplification band, with no overlap between the allelic variation found in 2*n* = 10 and 2*n* = 20 lines, while two fragments have been detected in the allotetraploid *B. hybridum* (2*n* = 30 lines), one of each being in the respective size range of the *B. distachyon* and *B. stacei* alleles (Table 1; see Table S3 for detailed results). Therefore, these four SSR markers offer a valid PCR-based alternative to direct chromosome counts or flow cytometry for reliable cytotyping and subsequent taxonomic assignment within this complex of *Brachypodium* species.

Furthermore, the remarkable size difference found for ALB165 alleles between 2*n* = 10 and 2*n* = 20 lines (150–195 bp vs. 137–139 bp, respectively) allows their discrimination in agarose gels: a unique band is observed in diploids, with distinct mobility for *B. distachyon* and *B. stacei*, whereas two bands are obtained for *B. hybridum* (Fig. 2). Thus this particular SSR marker provides a suitable tool for annual *Brachypodium* species identification. The analysis to

Fig. 2. Analysis of ALB165 SSR marker amplification in agarose gels. Lanes 1, 2, and 3 correspond to *Brachypodium distachyon* (2*n* = 10) samples and lanes 4, 5, and 6 correspond to *B. stacei* (2*n* = 20) samples. In both cases a single amplification band is visible. Lanes 7, 8, and 9 correspond to *B. hybridum* (2*n* = 30) samples. In this case, two distinct amplification fragments are clearly visible. The product sizes for these samples are given in Table S3. Lane M contains a 100-bp size marker.



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be conducted for cytotype assignation of a sample is not only easy and fast but also cheaper than other alternative methods, as fluorescent labeling and capillary electrophoresis of PCR amplification products is not required.

The allopolyploid origin of *B. hybridum* is currently out of debate mainly based upon the strong cytogenetic evidences reported by Hasterok and co-workers (Hasterok et al. 2004, 2006; Idziak et al. 2011). It has also been confirmed by the analysis of plastid and nuclear gene sequences (Catalán et al. 2012) and prolamin storage protein profiles (Hammami et al. 2011) on collections of *B. distachyon* (s.l.) including samples representative of the three cytotypes. Results in Table 1 provide additional support that *B. hybridum* derives from *B. distachyon* and the recently described *B. stacei*. The finding that 4 out of 15 SSR markers behave additively in the allotetraploid genotypes suggests that roughly 25% of the diploid genomes of *B. distachyon* and *B. stacei* remain intact after the polyploidization process. It is worthy of noting that each of the species-specific SSR markers is located on a different *B. distachyon* chromosome.

As microsatellite markers are co-dominant, the observation of a single amplification fragment in all diploid lines and two in the allotetraploids indicates full homocigosity for the surveyed loci in the genotypes examined. This agrees with the expected results from the self-breeding lifestyle of the annual *Brachypodium* species that in some cases (e.g., *B. distachyon*) is greatly imposed by cleistogamous flowers (Khan and Stace 1999). However, it cannot be totally excluded that cross pollination may have sporadically succeeded in these autogamous plants. In fact, heterozygotes have been reported at very low frequencies in *Brachypodium* collections examined by SSR markers (e.g., Bakker et al. 2009; Vogel et al. 2009). Nonetheless, the *B. distachyon*-type specimens genotyped as heterozygotes by Bakker et al. (2009) were $2n = 30$ (*B. hybridum*) plants, where two SSR amplification products could in fact reflect homozygous allotetraploid genotypes. The species-specific SSR markers described in this study can serve to confirm or discard the eventual existence of heterozygotes in *Brachypodium* samples. So, the nonoverlapping amplification fragment ranges in the diploid species genomes (Table 1) provides a tool to discern whether two bands generated in a given individual correspond to a heterozygous diploid genotype, of either *B. distachyon* or *B. stacei*, or to a homozygous genotype of *B. hybridum*. The whole set of markers should be checked in those samples where the amplification profile for one of them resulted inconclusive as heterozygosity for four unlinked SSR loci is highly unlikely to occur.

ALB165 shows the highest level of intra-cytotypic (i.e., intraspecific) allelic diversity among the four species-specific SSR markers (see detailed data as supplementary material in Table S3). Fourteen distinct ALB165 alleles have been detected in our collection of 64 Spanish lines of *B. distachyon* ($2n = 10$), whereas the number of alleles scored at the remaining loci was as few as two or three. The level of diversity assessed with ALB165 marker in our collection (PIC = 0.8672; Table S2) was greater than that found in 187 samples of *B. distachyon* collected in Turkey (PIC = 0.5586; Vogel et al. 2009). It is worthy of noting that the ALB165 allele found in the *B. distachyon* reference line Bd21 is the commonest in both collections as it is present in 119 out of 187 Turkish lines and 20 out of 64 Spanish lines (Table S3). Although the number of samples for the other two *Brachypodium* species under study is restricted, it is remarkable that ALB165 is the only polymorphic marker in the *B. stacei* lines examined as well as the only marker for which a new fragment size, not detected in any of the diploids, has been found in the allotetraploid *B. hybridum* lines.

The set of *B. hybridum* lines examined, including the reference line ABR113, covers a wide area of the Iberian Peninsula as well as the Canary and Balearic Islands (Fig. 1), thus representing very distinct eco-climatic conditions. It is then likely that, despite small sized, it encompasses much of the genotypic variability of this species in the western Mediterranean. Our study suggests that *B. hybridum* shows a much lesser allelic variation than *B. distachyon* for marker ALB165. Thirteen out of the 16 *hybridum* lines genotyped are monomorphic in the amplification range shared with *B. distachyon* (PIC = 0.3281). Furthermore, 11 of the allotetraploid samples have identical ALB165 profiles (see Table S3). It is noticeable that, as stated earlier for *B. distachyon*, the commonest ALB165 profile in the collection of *B. hybridum* lines described here is the same as that detected in the reference-type of this species (line ABR113). For the remaining species-specific SSR markers, the amplification product within the *B. staceii* size range was invariably the unique allele present in the $2n = 20$ lines, while certain variation was obtained for fragments in the size range of *B. distachyon*. However, the commonest allele in the $2n = 30$ lines was not usually the most frequent in the $2n = 10$ lines. The disagreement is striking for ALB311, as the 223-bp fragment, amplified in the majority of the *B. hybridum* lines, is present in a unique one of the 65 *B. distachyon* lines studied. To confirm whether these preliminary findings reflect the evolutionary history of the western Mediterranean accessions of the allotetraploid *B. hybridum*, enlarging the pool of molecularly characterized *Brachypodium* germplasm resources is required, especially concerning the rarest species *B. stacei*. Such a goal will surely be addressed soon as this species triangle is indeed attracting a great interest for polyploid origin and genome evolution studies (Bakker et al. 2009; Catalán et al. 2012; Manzaneda et al. 2012).

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TABLE S1.

Geographical details of the Spanish locations represented in the UPM and CRF-INIA Brachypodium collections. Each population is coded by one or two letters (province code) plus a number (location within a province). For populations from the CRF-INIA collection, the catalogue number (NC) is also indicated.

Collection	Poulation code	Province	Location	2n*	Latitude	Longitude	Alt (m)
UPM	AB1	Albacete	Riopar	10	38º 27' 40'' N	2º 27' 55'' W	1117
	AB3	Albacete	La Gineta	10+30	39º 10' 33'' N	1º 57' 57'' W	658
	AL1	Almería	Cala Chica	20+30	36º 43' 57'' N	2º 07' 59'' W	60
	AL5	Almería	San José	20+30	36º 46' 00'' N	2º 06' 23'' W	10
	BA3	Badajoz	Badajoz	30	38º 53’ 16’’ N	6º 54’ 47’’ W	195
	BA6	Badajoz	Fregenal de la Sierra	10	38º 10’ 10’’ N	6º 39’ 43’’ W	608
	CC3	Cáceres	Almaraz	30	39º 48’ 16’’ N	5º 41’ 11’’ W	277
	CU1	Cuenca	Gabaldón	10	39º 38' 10'' N	1º 56' 16'' W	924
	CU2	Cuenca	Pass of Tordiga	10	39º 55' 06'' N	2º 03' 38'' W	1193
	CU3	Cuenca	Pass of Rocho	10	39º 55' 48'' N	1º 56' 05'' W	1077
	CU4	Cuenca	Huerta del Marquesado	10	40º 10' 14'' N	1º 40' 22'' W	1295
	CU5	Cuenca	Mountain range of Cuenca	10	40º 12' 18'' N	1º 41' 31'' W	1525
	CU6	Cuenca	Source of Cuervo river	10	40º 25' 42'' N	1º 53' 54'' W	1429
	GR1	Granada	Pass of Onitar	10	37º 27' 04'' N	3º 36' 57'' W	935
	GR2	Granada	Pass of Zegri	10	37º 25' 22'' N	3º 35' 52'' W	1079
	GR3	Granada	Iznalloz	10	37º 24' 02'' N	3º 28' 35'' W	925
	GR5	Granada	Gor	30	37º 22' 00'' N	2º 57' 54'' W	1340
	GR6	Granada	National Park 'Sierra de Baza'	10	37º 26' 22'' N	2º 53' 25'' W	1194
	GU1	Guadalajara	Prados Redondos	10	40º 48' 44'' N	1º 48' 30'' W	1138
	GU2	Guadalajara	Molina de Aragón	10	40º 51' 18'' N	1º 52' 14'' W	1120
	GU3	Guadalajara	Embid	10	40º 58' 14'' N	1º 42' 54'' W	1084
	GU4	Guadalajara	Cerro Pelado	10	40º 48' 44'' N	1º 55' 18'' W	1177
	GU5	Guadalajara	Valsalobre	10	40º 49' 06'' N	1º 54' 24'' W	1122
	GU6	Guadalajara	Cubillejo de la Sierra	10	40º 51' 49'' N	1º 51' 04'' W	1203

	HU1	Huesca	Almunia de San Juan	10	41º 56' 25'' N	0º 15' 55'' E	427
	HU2	Huesca	Monte Palau	10	42º 01' 49'' N	0º 16' 30'' E	521
	HU3	Huesca	El Grado	10	42º 07' 51'' N	0º 13' 34'' E	357
	HU4	Huesca	Mirador de Mipanas	10	42º 12' 12'' N	0º 12' 44'' E	544
	HU5	Huesca	Escanilla	10	42º 16' 51'' N	0º 11' 46'' E	463
	HU6	Huesca	Morillo de Tou	30	42º 22' 30'' N	0º 09' 13'' E	516
	HU7	Huesca	Escalona	10	42º 29' 53'' N	0º 08' 44'' E	576
	HU9	Huesca	Nueno	10	42º 17' 05'' N	0º 25' 29'' W	826
	J3	Jaén	Mata-Bejid	10	37º 41' 18'' N	3º 29' 31'' W	1065
	J4	Jaén	Pass of Tiscar	10	37º 47' 12'' N	3º 02' 20'' W	1154
	J6	Jaén	National Park 'Sierra de Segura'	10	38º 01' 59'' N	2º 51' 57'' W	677
	J7	Jaén	El Tranco	10+30	38º 07' 23'' N	2º 47' 47'' W	687
	MA1	Madrid	Patones	30	40º 53' 17'' N	3º 26' 59'' W	837
	ME1	Mallorca	Mahón (Island of Menorca)	30	39º 53' 41'' N	4º 16' 13'' E	37
	SG1	Segovia	Moral de Hornuez	10	41º 27' 56'' N	3º 36' 39'' W	1109
	SG2	Segovia	Carabias	10	41º 26' 12'' N	3º 40' 00'' W	1157
	Z2	Zaragoza	Puerto Cavero	30	41º 23' 32'' N	1º 33' 55'' W	771
	Z3	Zaragoza	Torralba de los Frailes	10	40º 58' 13'' N	1º 39' 28'' W	1134
CRF-INIA	LP2 (NC050401)	Las Palmas	Betancuria (Island of Fuerteventura)	30	28º 25' N	14º 03' W	396
	LP6 (NC050440)	Las Palmas	Teguise (Island of Lanzarote)	20	29º 04' N	13º 31' W	283
	TE1 (NC050335)	Tenerife	Buenavista N (Island of Tenerife)	30	28º 21' N	16º 51' W	499
	TE4 (NC050363)	Tenerife	Agulo (Island of Gomera)	20	28º 11' N	17º 11' W	202
	TE5 (NC050368)	Tenerife	Vallehermoso (Island of Gomera)	30	28º 11' N	17º 14' W	456

*: Cytotype of derived lines that have been used in this study.

Alt: Altitude above sea level.

TABLE S2.
Description of Brachypodium molecular marker loci used in this study. Chromosomal location, amplification range, and polymorphism data are referred to *B. distachyon* (2n = 10) lines.

Marker locus	Repeat unit	Forward Primer	Reverse Primer	Chromosomal location	Amplification range (bp)	No. of Alleles	Major Allele Frequency	Gene Diversity	Heterozygosity
ALB165	ata	TTCGTGGTTCAACAACATGG	ATTTGCCCCACAAATGGTTA	Bd1: 35,998,570	150-195	14	0.2812	0.8672	0
ALB311	ga	CCTAACAGCTTCCGTCTCCA	CGTCGTCTTCAGGTCTTTCC	Bd5: 18,580,928	223-227	3	0.6094	0.4878	0
BdSSR330	ND	GTCTCCTCGGGATCCAACTT	CCTTCCCCGTCTTCTCTTCT	Bd2: 52,344,096	174-176	2	0.7969	0.3237	0
R2-3-ABI	ND	TCCGGATCTCGATCTCAAAC	CGTCGACAAGCTCAAGAAGC	Bd3: 59,072,368	191-204	4	0.6406	0.4818	0

TABLE S3.

SSR profiles in the Brachypodium lines examined. Distinct lines derived from the same population are named by different numbers following the population code. The catalogue number (NC) of lines established from CRF-INIA accessions is noted in brackets. For the lines whose ALB165-SSR amplification products have been analyzed in agarose gels, the corresponding lane in Figure 2 is noted.

		SSR marker			
cytotype	**line**	**ALB165**	**ALB311**	**BdSSR330**	**R2-3-ABI**
	Bd21	157	223	176	204
2n=10	BdUPM_AB1.2	174	225	174	191
	BdUPM_AB1.4	177	225	176	202
	BdUPM_AB3.3	171 [3]	225	176	204
	BdUPM_AB3.5	163	225	174	204
	BdUPM_BA6.4	186	227	176	202
	BdUPM_BA6.6	160	227	174	204
	BdUPM_CU1.4	186	227	174	204
	BdUPM_CU1.6	150	227	174	202
	BdUPM_CU2.2	183	225	174	204
	BdUPM_CU2.6	157	227	174	204
	BdUPM_CU3.5	166	227	174	204
	BdUPM_CU3.6	160	227	174	204
	BdUPM_CU4.3	157	225	174	202
	BdUPM_CU4.6	163	227	174	204
	BdUPM_CU5.3	157	225	174	204
	BdUPM_CU5.5	166	225	174	204
	BdUPM_CU6.1	157	227	174	204
	BdUPM_CU6.4	183	227	174	204
	BdUPM_GR1.4	195	225	176	204
	BdUPM_GR1.5	160 [2]	227	176	202
	BdUPM_GR2.1	166	225	176	202
	BdUPM_GR2.6	180	225	176	204
	BdUPM_GR3.4	174	227	174	204
	BdUPM_GR3.6	180	225	176	202
	BdUPM_GR6.3	150	227	174	202
	BdUPM_GR6.4	153	223	174	202
	BdUPM_GU1.5	157	225	174	204
	BdUPM_GU1.6	174	225	176	204
	BdUPM_GU2.1	153	225	176	204
	BdUPM_GU2.4	157	225	176	204
	BdUPM_GU3.1	157	225	174	204
	BdUPM_GU3.5	157	225	174	204
	BdUPM_GU4.3	157	225	174	204
	BdUPM_GU4.5	166	225	174	204
	BdUPM_GU5.1	157	225	174	204
	BdUPM_GU5.6	171	225	174	204
	BdUPM_GU6.1	153	227	174	204
	BdUPM_HU1.5	153	227	174	204
	BdUPM_HU1.6	157	227	174	204

	BdUPM_HU2.1		160			225			174		204
	BdUPM_HU2.5		163			225			174		202
	BdUPM_HU3.4		174			225			174		204
	BdUPM_HU3.5		160			227			174		202
	BdUPM_HU4.5		186			227			174		202
	BdUPM_HU4.6		160			227			174		202
	BdUPM_HU5.5		157			227			174		204
	BdUPM_HU7.1		168			227			174		202
	BdUPM_HU7.6		166			225			174		204
	BdUPM_HU9.1		157			225			174		204
	BdUPM_HU9.2		157			225			174		200
	BdUPM_J3.3		163			225			174		202
	BdUPM_J3.6		186			225			174		204
	BdUPM_J4.2		157			225			176		204
	BdUPM_J4.3		171			225			174		202
	BdUPM_J6.2		174			225			174		202
	BdUPM_J6.5		174			225			174		204
	BdUPM_J7.2		174			227			176		202
	BdUPM_J7.6		183			225			174		204
	BdUPM_SG1.2		153			225			174		202
	BdUPM_SG1.5		153			225			174		202
	BdUPM_SG2.1		157	[1]		225			174		204
	BdUPM_SG2.3		157			225			174		204
	BdUPM_Z3.2		157			227			174		204
	BdUPM_Z3.6		160			227			174		202
2n=20	BsUPM_AL1.1	137				231		168		189	
	BsUPM_AL5.2	139		[4]		231		168		189	
	LP6.1 (NC050440)*	137		[5]		231		168		189	
	TE4.3 (NC050363)*	139		[6]		231		168		189	
2n=30	BhUPM_AB3.7	137	163		223	231		168	174	189	202
	BhUPM_AL1.2	141	163		225	231		168	174	189	202
	BhUPM_AL5.3	137	163		223	231		168	176	189	202
	BhUPM_BA3.2	137	157		225	231		168	174	189	202
	BhUPM_CC3.2	137	163		223	231		168	174	189	202
	BhUPM_GR5.1	137	163		223	231		168	176	189	202
	BhUPM_HU6.2	137	163		223	231		168	174	189	202
	BhUPM_J7.1	139	163		223	231		168	176	189	204
	BhUPM_J7.5	137	163		223	231		168	174	189	204
	BhUPM_MA1.2	137	171	[9]	227	231		168	174	189	202
	BhUPM_ME1.1	137	166	[8]	223	231		168	174	189	202
	BhUPM_Z2.2	137	163		227	231		168	174	189	202
	LP2.1 (NC050401)*	137	163		223	231		168	176	189	202
	TE1.1 (NC050335)*	137	163	[7]	223	231		168	176	189	202
	TE5.1 (NC050368)*	137	163		223	231		168	176	189	202