Genetic Variation Within and Among Lowland Switchgrass Cultivars as Revealed with AFLP Polymorphisms

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ABSTRACT

Switchgrass (*Panicum virgatum* L.) has gained wider attention due to its recognition and use as a model herbaceous crop species for bioenergy production. Genetic diversity information in lowland switchgrass cultivars can help to specify cultivars to be used in the breeding programs aiming for hybrid vigor. The objective of this research was to analyze genetic variation within and among five lowland switchgrass cultivars using amplified fragment length polymorphism (AFLP) markers. AFLP polymorphisms indicated the presence of high genetic variation within lowland switchgrass cultivars with 'Alamo' exhibiting the highest genetic variation and 'Performer' the lowest. The Nei's genetic diversity parameters revealed the lowest genetic distance between cultivars 'Alamo' and 'Cimarron' and the highest value between cultivars 'Alamo' and 'Kanlow'. 'Alamo' and 'Cimarron' were clustered together while 'BoMaster', 'Kanlow', and 'Performer' were grouped into the other cluster. In addition, there were clusters with mixed genotypes. The findings of this study can be used to select diverse lines as parents for heterosis and inbreeding studies.

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1. Introduction

Switchgrass (*Panicum virgatum* L.) is a highly polymorphic and wind pollinated polyploid species with disomic inheritance (Liu & Wu, 2012; McLaughlin & Kszos, 2005; Nielsen, 1944; Okada et al., 2010; Taliaferro, 2002). Lowland and upland are two ecotypes in switchgrass and the ploidy level in switchgrass has been reported from diploid (2n=2x=18) to duodecaploid (2n=12x=108) (Adhikari, Anderson, Klatt & Wu, 2015; Nielsen, 1944). Ploidy level in switchgrass is characteristic of ecotype. The lowland ecotypes are tetraploid (2n=4x=36) but the upland ecotypes can be tetraploid (2n=4x=36) or octaploid (2n=8x=72) or very rarely hexaploids (2n=6x=54) (Narasimhamoorthy, Saha, Swaller & Bouton, 2008; Nielsen, 1944). Aneuploidy has been reported to be more common in higher ploidy levels, i.e., octaploid (86.3%) than in lower ploidy levels, i.e., tetraploids (23.2%) (Costich, Friebe, Sheehan, Casler & Buckler, 2010). Switchgrass has a reference genome, assembled for the cultivar Alamo (AP13), which is approximately 1,165.7 Mb in size and includes 98,935 complete genes (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvirgatum_er).

Genetic diversity is the result of selection, mutation, migration, genetic drift and/or recombination (de Vicente & Fulton, 2003). Variation can be evaluated on phenotypic and/or genotypic levels. Genotypic variation is evaluated at the level of DNA molecules responsible for transmitting genetic information (de Vicente & Fulton, 2003). DNA markers simply refer to a DNA sequence in the genome that can be used to genotype individuals or a population. The markers are selected based on the nucleic acid hybridization (RFLP), PCR (RAPD, AFLP, SSR), single base-pair change [single nucleotide polymorphisms (SNP)], array hybridization [diversity arrays technology (DArT)], and restriction site associated DNA (RAD).

Different molecular markers have been used in the switchgrass diversity studies. They include random amplified polymorphic DNA (RAPD) (Casler, Stendal, Kapich & VOgel, 2007; Gunter, Tuskan & Wullschleger, 1996; Nageswara-Rao, Soneji Kwit & Stewart, 2013), restriction fragment length polymorphism (RFLP) (Missaoui, Paterson & Bouton, 2006), expressed sequence tag-simple sequence repeat markers (EST-SSRs)

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(Cortese, Honig, Miller & Bonos, 2010; Huang, Bughrara, Zhang, Bales-Arcelo & Bin, 2011; Narasimhamoorthy et al., 2008), amplified fragment length polymorphism (AFLP) (Todd et al., 2011), simple sequence repeats (SSR) (Zalapa et al., 2011), sequence-related amplified polymorphism (SRAP) (Huang et al., 2011) and a network-based single nucleotide polymorphism (SNP) (Lu et al., 2013). SNP markers were used in the switchgrass genetic diversity analysis to identify seven population groups that corresponded to ecotype, ploidy, and geographic distribution (Evans et al., 2017). AFLP markers can delineate between upland and lowland ecotypes and related plants according to broad geographic regions (Todd, Wu, Wang & Samuels, 2011). SSR markers were used to delineate diversity between ecotypes and between ploidy levels (Zhang et al., 2011). RFLP markers were used to determine extensive diversity between lowland tetraploid cultivar 'Alamo' (AP13) and upland tetraploid cultivar 'Summer' (VS16) and to develop linkage maps (Missaoui, Paterson & Bouton, 2005, 2006).

The information on the extent of diversity in lowland cultivars will help determine the specific cultivars to be used in future crop improvement programs to develop potentially high yielding switchgrass cultivars. The immediate benefit of such diversity information will be in the development of advanced inbreds [selfing generations 5 to 6 (S5 to S6)] which can be used to produce hybrids for harnessing hybrid vigor. Bhandari, Nayak, Dalid & Sykes (2017) found up to 23% high parent heterosis from a cross between selections of divergent populations of switchgrass. Inbred populations can be utilized in the development of linkage maps (Liu, Wu, Wang & Samuels, 2012) and identification of quantitative trait loci (QTL) associated with agronomic, quality and disease traits. The QTL information can then be used in the marker assisted selection in switchgrass breeding.

Polymerase chain reaction (PCR) is a simple, automated technique for repeated copying of a short DNA molecule (Conner & Hartl, 2004). AFLP is a PCR based dominant marker and used in genetic research, DNA fingerprinting, and genetic engineering (Vos et al., 1995). It is a highly sensitive method for detecting polymorphisms in DNA. Unlike SNP genotyping, AFLP genotyping lack the information regarding the DNA sequence, which hinders in comparative genome analysis such as co-regulation of markers or orthologous gene studies using common techniques such as nucleotide BLAST. However, AFLP is still a proven method that at low cost without specialized equipment can generate many bands. It has been successful at accessing genetic diversity and linkage mapping in many crops around the world (Meudt & Clarke, 2007). Because information on genetic diversity with outcrossing lowland switchgrass populations is generally lacking, this study aimed to analyze genetic diversity among and within five lowland switchgrass cultivars using AFLP. The cultivars included 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer'.

2. Study Methods

2.1 Plant Materials and Genomic DNA Extraction

Plant materials consisted of 384 plants from five lowland tetraploid cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Performer', and 'Kanlow'. Seventy-six plants from the cultivar 'Performer' and 77 plants from each of the remaining four cultivars were seed-propagated and transplanted in individual 10-cm plastic pots with SUN-GRO Metro-Mix 200 series soil (Sun Gro Horticulture, WA) in a greenhouse at the Agronomy Research Station, Oklahoma State University, Stillwater, OK. The study began in July 2011 and completed in December 2013. Genomic DNA samples were extracted from leaf tissues using Zymo Research ZR Plant/Seed DNA Kit™ (Zymo Research Corporation, CA). DNA quality was checked with 1% agarose gel electrophoresis. The DNA samples were diluted to a final concentration of 100 ng μL^{-1} before enzyme digestion.

In the study for genetic variation among five cultivars, a total of 64 plants were used including 12 plants from cultivar 'Performer' and 13 plants from each of the remaining four cultivars (Fig. 1). The within genetic variation was studied separately for each of the five cultivars with 64 plants in each of them. The decision to use the above-mentioned numbers were based on capacity of polyacrylamide gel which can accommodate 64 sample lanes and two additional size marker lanes in a LI-COR 4300 DNA Analyzer.

2.2 AFLP Analysis

AFLP analysis was performed following Vos et al. (1995), with minor modifications (Todd et al., 2011; Wu, Taliaferro, Bai & Anderson, 2005). In the first step, the genomic DNA was double-digested with EcoRI and MSeI restriction enzymes and the DNA fragments were ligated to oligonucleotide AFLP adapters. The ligated DNA fragments were pre-amplified by PCR using a primer combination based on adapter sequences. In the second step, 12 AFLP selective primer combinations (Table 1) were used for selective amplification. The EcoRI primers were labeled with either IRD-700 or IRD-800 infrared fluorescence dye. The number of polymorphic bands (loci) considered appropriate for genetic variation in switchgrass is >400 (Todd et al., 2011). Accordingly, 12 selective primer pairs were used to generate >400 amplification products (polymorphic loci). All PCRs were conducted in an Applied Biosystems 2720 thermocycler (Applied Biosystems Inc., IL). In the third step, approximately one microliter of selectively amplified PCR products were loaded on a 0.25 mM thick 6.5% (w/v) polyacrylamide gel with 66 wells in a LI-COR 4300 DNA Analyzer (LI-COR Inc., Lincoln, NE) and run in 1x TBE buffer at 1500 V for 2.5 h. Standard DNA size markers (50-700 bp) (LI-COR Inc., Lincoln, NE) were loaded on first and last lanes to determine the size of the selectively amplified fragments in the final gel image. A total of 36 gels including 6 gels for among cultivar genetic variation and 30 gels (6 gels for each of the five cultivars) for within cultivar genetic variation were run.

2.3 Data Analysis

AFLP bands throughout the gel profile were visually scored as present (1), absent (0), and ambiguous (9). The scoring is repeated at least twice for all gel profiles to accurately collect data. The bands were scored between ~75 and 500 bp. The binary data matrix was recorded in a Microsoft Excel data sheet. Numerical Taxonomy System version 2.0 (NTSYSpc 2) program (Rohlf, 1998) was used to analyze the data. Each gel gave two images based on IRD-700 or IRD-800 infrared fluorescence dye. Data from six gels (12 images) were used for among-cultivar variation study. Data from six gels (12 images) for each of the five cultivars were separately analyzed for the within-cultivar variation analysis. A total of 72 gel images, including 12 gel images for among genetic variation and 60 gel images for within genetic variation, were scored and analyzed. In NTSYSpc 2 program, SIMQUAL module was used to compute genetic similarity coefficients (SC). The cluster analysis was based on unweighted pair-group method with arithmetic mean (UPGMA) within the SAHN module. DCENTER module was used for the principle coordinate analysis.

Table 1. Polymorphic band information with 12 different AFLP selective amplification primer pairs for five cultivars together (among cultivars) and within each of the five cultivars separately.

		Pre- and selective amplification primers*															
		e-ACC-m-CAT	e-ACG-m-CAT	e-AAC-m-CTC	e-ACG-m-CTC	AAG-m-CAA	e-AGG-m-CAA	e-AAG-m-CTA	e-AGC-m-CTA	e-ACC-m-CTG	e-ACT-m-CTG	e-ACA-m-CAG	e-ACG-m-CAG	Total bands	Percentage (%)	Average bands	Standard deviation
Among cultivars	Total bands	53	51	63	51	43	51	58	50	65	47	60	50	642	100	54	7
	Polymorphic	45	44	44	42	35	49	48	43	55	41	56	47	549	85.5	46	6
	Monomorphic	8	7	19	9	8	2	10	7	10	6	4	3	93	14.5	8	4
Alamo	Total bands	96	82	68	58	52	50	41	35	67	63	60	45	717	100	60	17
	Polymorphic	90	79	52	53	48	50	40	31	62	60	47	36	648	90.4	54	17
	Monomorphic	6	3	16	5	4	0	1	4	5	3	13	9	69	9.6	6	5
BoMaster	Total bands	54	42	36	43	45	44	40	47	50	45	50	47	543	100	45	5
	Polymorphic	53	40	35	43	36	42	28	38	42	42	43	38	480	88.4	40	6
	Monomorphic	1	2	1	0	9	2	12	9	8	3	7	9	63	11.6	5	4
Cimarron	Total bands	65	54	60	59	53	56	54	42	50	47	47	43	630	100	53	7
	Polymorphic	53	47	47	47	43	48	44	35	41	36	36	32	509	80.8	42	6
	Monomorphic	12	7	13	12	10	8	10	7	9	11	11	11	121	19.2	10	2
Kanlow	Total bands	48	44	47	46	57	24	60	52	56	59	47	42	582	100	49	10
	Polymorphic	48	44	47	46	48	24	47	41	54	54	39	42	534	91.8	45	8
	Monomorphic	0	0	0	0	9	0	13	11	2	5	8	0	48	8.2	4	5
Performer	Total bands	48	36	56	46	50	48	54	58	54	52	52	41	595	100	50	6
	Polymorphic	35	29	33	32	38	41	47	43	38	31	34	21	422	70.9	35	7
	Monomorphic	13	7	23	14	12	7	7	15	16	21	18	20	173	291	14	6

* e, preamplification primer of EcoRI (GACTGCGTACCAATTC); m, preamplification primer of MseI (GATGAGTCCTGAGTAA)

Table 2. Similarity coefficient comparison for five lowland switchgrass cultivars based on similarity coefficient tables.

	Among cultivars	**Alamo**	**BoMaster**	**Cimarron**	**Kanlow**	**Performer**
Average	0.76	0.79	0.82	0.79	0.76	0.82
Standard deviation	0.05	0.08	0.07	0.05	0.04	0.03
Maximum	0.88	0.89	0.98	0.90	0.88	0.90
Minimum	0.60	0.41	0.48	0.60	0.59	0.69
Coefficient of variation	5.96	9.53	8.03	5.70	5.82	4.21
Maximum between	K18 and K20	A33 and A36; and A35 and A36	B74 and B75	C27 and C28	K39 and K40	P56 and P57; P65 and P69; P76 and P77
Minimum between	A4 and P4	A9 and A72	B5 and B45	C23 and C50	K7 and K88	P30 and P49; P30 and P51; P30 and P57; P30 and P61; P30 and P64

Table 3. Summary of Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) for five different cultivars of lowland switchgrass.

	I		**He**		**uHe**	
Cultivar	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Alamo	0.425	0.008	0.277	0.006	0.280	0.006
BoMaster	0.412	0.010	0.269	0.007	0.271	0.007
Cimarron	0.373	0.010	0.243	0.007	0.245	0.007
Kanlow	0.444	0.009	0.292	0.007	0.294	0.007
Performer	0.345	0.011	0.227	0.008	0.229	0.008

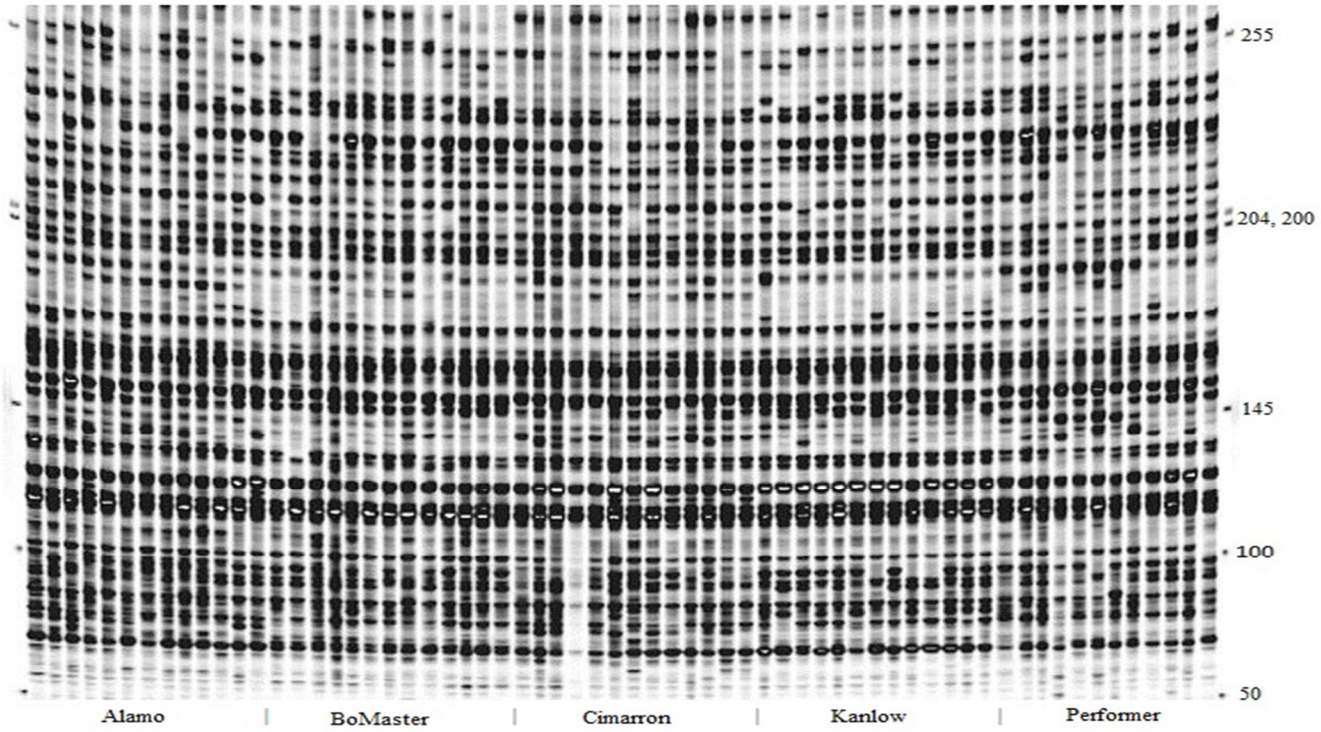

Figure 1. AFLP fingerprints generated for five lowland switchgrass cultivars ('Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer') using primer combination e-ACC-m-CTG. The fragment size (bp) is indicated on the right and the cultivars are indicated at the bottom of the image.

Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) were computed separately for each of the five cultivars; analysis of molecular variance (AMOVA) and Nei's genetic distance (Nei 1972) calculation were performed in among-variation data. GenAlEx 6 (Peakall & Smouse, 2012, Peakall & Smouse, 2006) software was used for these computations. AFLP bands initially scored as present (1), absent (0), and ambiguous (9) for NTSYSpc 2 were converted into present (1), absent (0), and ambiguous/missing (-1) for calculations in GenAlEx 6. AMOVA was performed to partition variation between cultivars. Pairwise genetic distance in different cultivars was computed using Nei's distance (Nei, 1972).

3. Results

Table 1 shows polymorphic band data with 12 different selective amplification primer pairs used in the experiment. In the analysis among five cultivars together, 85.5% of bands were polymorphic (Table 1). In the analysis within each of the five cultivars separately, polymorphic band percentages ranged from a minimum of 70.9% in 'Performer' to a maximum of 91.8% in 'Kanlow'. Similarity coefficients from analysis among five cultivars were given in Table S1 and the summary of similarity coefficients, for among cultivars and for each of the five cultivars, was provided in Table 2.

'Alamo' exhibited the highest within-cultivar genetic variation (coefficient of variation=9.53) and 'Performer' exhibited the lowest within-cultivar genetic variation (coefficient of variation=4.21) (Table 2). Analysis using five cultivars together showed 'A4' from 'Alamo' and 'P4' from 'Performer' were the most divergent (similarity coefficient=0.60) (Table 2 and Table S1). The average similarity coefficient ranged from 0.76 to 0.82 indicating the presence of high genetic variation among switchgrass genotypes.

The cluster analysis on AFLP variation among five cultivars generated a dendrogram with big cluster (m) which included 61 genotypes from different cultivars and a small cluster (n) that included genotypes P4, P6, and P8 from cultivar 'Performer' (Fig. 2). A genotype C4 from cultivar 'Cimarron' was observed separate from the rest of the individuals in the big cluster. The cluster m produced a cluster (m-1) of mixed genotypes from 'Alamo', 'BoMaster', and 'Cimarron' and a cluster (m-2) with two sub-clusters (a and b). The sub-cluster 'a' included cultivars 'Alamo' (a-1) and 'Cimarron' (a-2) while the sub-cluster 'b' included 'BoMaster' (b-1), 'Kanlow' (b-2), and 'Performer' (b-3). In the sub-cluster 'b', 'BoMaster' and 'Kanlow' were genetically more similar. The two-dimensional plot from principal coordinates analysis produced groupings (Fig. S1) mostly consistent with the clusters generated from the cluster analysis. The principal coordinates analysis revealed that the first principal coordinate explained 10.34% variation and the second principal coordinate explained 7.85% variation. The dendrograms from cluster analysis and two-dimensional plots from principal coordinates analysis are mostly congruent for AFLP variation within each of the five cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer' (Figs. S2, S3, S4, S5, S6, S7, S8, S9, S10, S11). In these five cultivars, the first principal coordinate explained 11.80, 9.38, 8.09, 7.98, and 11.40% variations, respectively,

while the second principal coordinate explained 5.90, 6.27, 5.61, 5.72, and 4.33% variations, respectively.

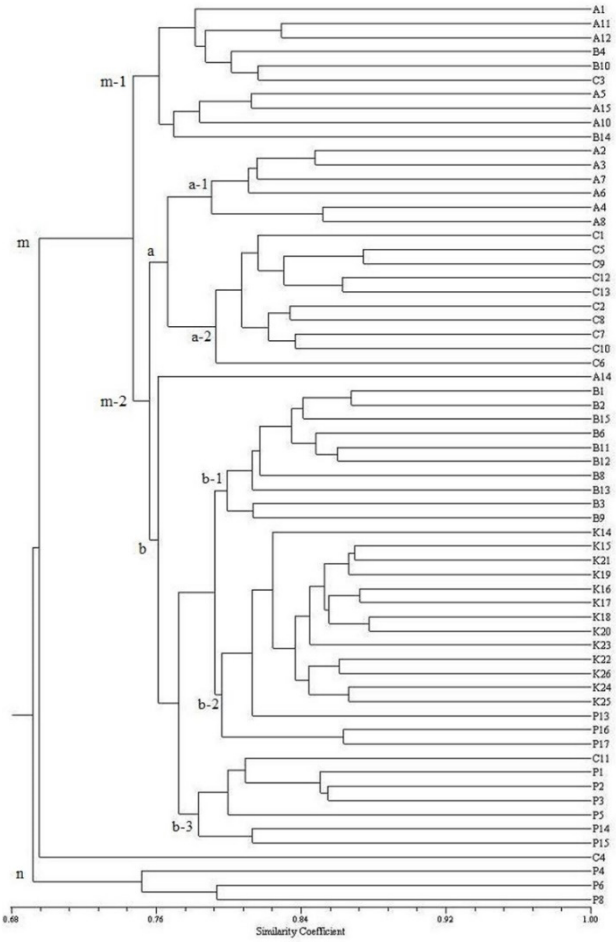

Figure 2. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation among five lowland switchgrass cultivars. A, B, C, K, and P represent cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer', respectively.

Mantel test results are shown in Table S2. The goodness of fit of the dendrograms to the original dissimilarity matrices (i.e., similarity coefficient table) was poor for among-cultivars (analysis of five cultivars together) and for 'Kanlow', however, the dendrograms were not significantly different from dissimilarity matrices ($P = 1 > 0.05$ in both cases). The dendrograms were a good or a very good fit to the dissimilarity matrices for each of 'Alamo', 'BoMaster', 'Cimarron', and 'Performer'.

AMOVA analysis carried out in the data from AFLP variation among five lowland switchgrass cultivars partitioned variation between cultivars at 15% as estimated variances and degrees of freedom (df) between cultivars, within cultivar, and total were 13.34 and 4, 74.39 and 59, and 87.73 and 63, respectively. Nei's genetic diversity revealed the lowest genetic distance between cultivars 'Alamo' and 'Cimarron' and the highest value between cultivars 'Alamo' and 'Kanlow' (Table S3). Shannon's information index (I), expected heterozygosity (He), and unbiased heterozygosity (uHe) calculated separately for each of the five cultivars revealed higher values for 'Kanlow' and 'Alamo' compared to the other three cultivars (Table 3).

4. Discussion

The morphological and physiological variation in switchgrass is closely associated with climatic factors and the adaptation along north-south range is dependent on photoperiod (Casler, 2012). Switchgrass is a native crop from North America with a large morphological diversity and wider adaptation (Parrish & Fike, 2005). The cultivars 'Alamo' and 'Kanlow' were developed from wild germplasm sources. 'Alamo' (PI 422006) was the cultivar collected from George West, TX (U.S. National Plant Germplasm System, 2014) and 'Kanlow' was initially collected in 1957 at a lowland site near Wetumka, OK (U.S. National Plant Germplasm System, 2014). 'Kanlow' (PI 421521) accession was developed as a cultivar by a cooperative effort of Kansas Agricultural Experiment Station (AES) and Plant Science Research Division, Agricultural Research Service (ARS) and was released in 1963.

The original ancestor of cultivar 'Cimarron' was primarily from 'Alamo'. 'Cimarron' was developed as a synthetic cultivar by polycrossing seven elite clonal parents in 2001 at Oklahoma State University (Wu, 2014). The selection of parent plants for 'Cimarron' was based on the evaluation of biomass yield of their half-sib families (Wu, 2014). The dendrogram and two-dimensional plot showed 'Alamo' and 'Cimarron' in the same group exhibiting the genetic relatedness consistent with the pedigree information. It appears that lowland switchgrass can be divided into two broad populations, including those from the south related to 'Alamo' like 'Cimarron' and the northern populations related to 'Kanlow' such as 'BoMaster' and 'Performer'. It was between these populations, 'Alamo' and 'Kanlow', that midparent heterosis was found and one of the crosses showed 23% high parent heterosis (Bhandari et al., 2017).

'BoMaster' and 'Performer' switchgrass cultivars were developed by North Carolina Agricultural Research Service, NC. Both 'BoMaster' (Reg. No. CV-248, PI 645256) (Burns, Godshalk & Timothy, 2008a) and 'Performer' (Reg. No. CV-247) (Burns, Godshalk & Timothy, 2008b) switchgrass cultivars were developed through three cycles of selection from a selected group of 161 lowland switchgrass plants that represented 11 different germplasm sources which included 'Kanlow'. The method in the development of these cultivars was recurrent half-sib selection. The selection for both cultivars was based on dry matter yield and in vitro dry matter digestibility. 'BoMaster' was selected for dry matter yield (Burns et al., 2008a) and 'Performer' was for in vitro dry matter digestion (Burns et al., 2008b) during the cultivar development. Similarly, the dendrogram and the two-dimensional plot showed 'Kanlow', 'BoMaster', and 'Performer' in the same group. The seven population groups identified in a genetic diversity analysis include Upland West, Upland East, Upland North, Upland Montane, Lowland North, Lowland South, Lowland Central, and admixed (Evans et al., 2017), indicating the genetic diversity across North-South and across East-West. 'Alamo', 'Cimarron', and 'Kanlow' were originated in George West in Texas, Stillwater in Oklahoma, and Wetumka in Oklahoma, respectively. 'BoMaster' and 'Performer' both were

originated in Raleigh in North Carolina. The variation among these cultivars can also be attributed to the switchgrass genetic diversity across North-South and East-West locational gradients. Self-incompatibility and inter-cultivar gene flow, the characteristics of switchgrass, can also be the possible attributing factors for the clusters of mixed genotypes. We have used genetic distance based on AFLP markers as the basis to separate cultivars as heterotic. However, a further study may be required to determine if AFLP genetic distance is correlated with heterosis.

5. Conclusions and Recommendations

Lowland tetraploid switchgrass cultivars showed a high level of genetic variation. 'Alamo' showed the highest genetic variation while 'Performer' showed the lowest. The plant 'A4' from 'Alamo' and the plant 'P4' from 'Performer' were the most divergent genotypes. 'Alamo' and 'Cimarron' were clustered together while 'BoMaster', 'Kanlow', and 'Performer' were grouped into the other cluster. In addition, there were clusters with mixed genotypes as well. The findings of this research would be useful for future plant breeding and genetic improvement programs in lowland switchgrass. The results can be used for the selection of diverse lines as parents for heterosis and inbreeding studies.

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Figure S1. Principal coordinates analysis for AFLP variation among five cultivars. A, B, C, K, and P represent cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer', respectively. PC-1 and PC-2 are two major principal coordinate axes.

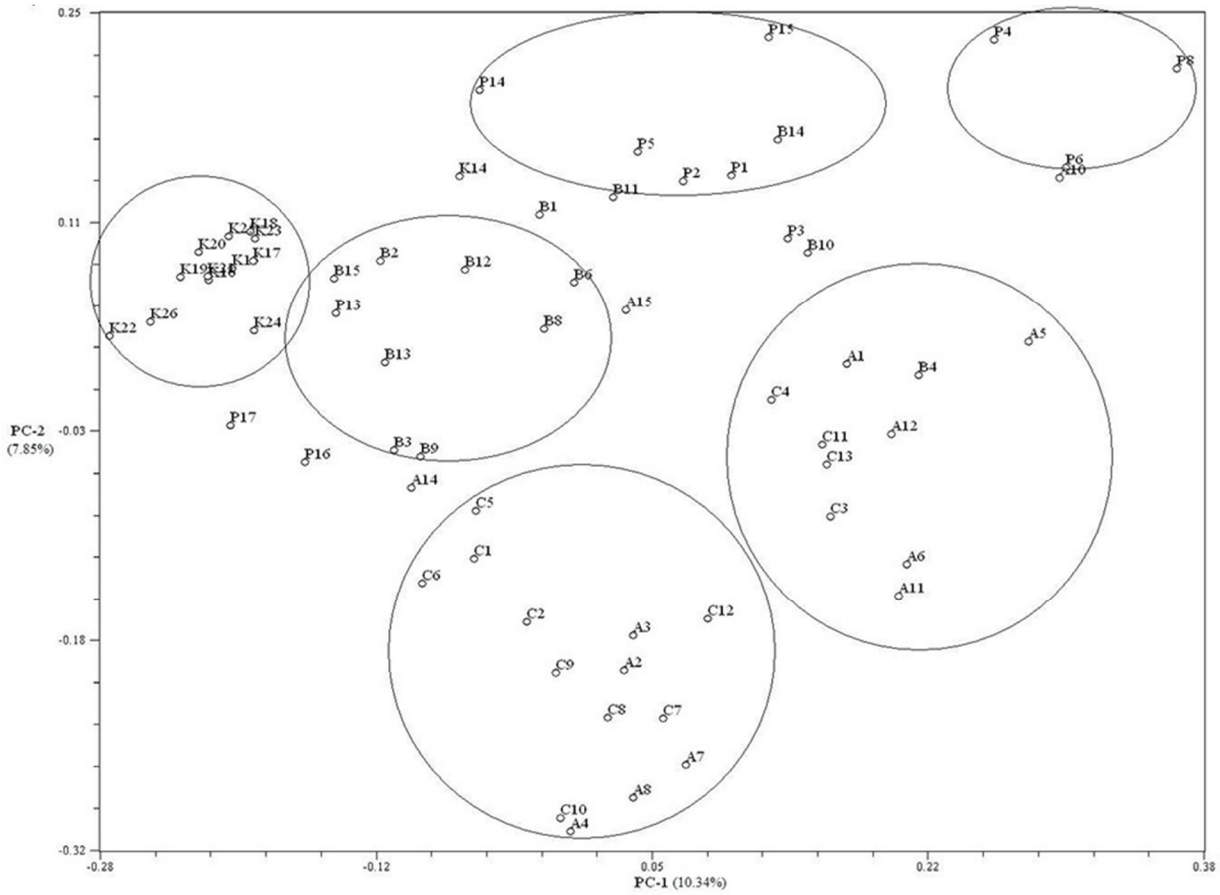

Figure S2. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar 'Alamo'. A represents cultivar 'Alamo'.

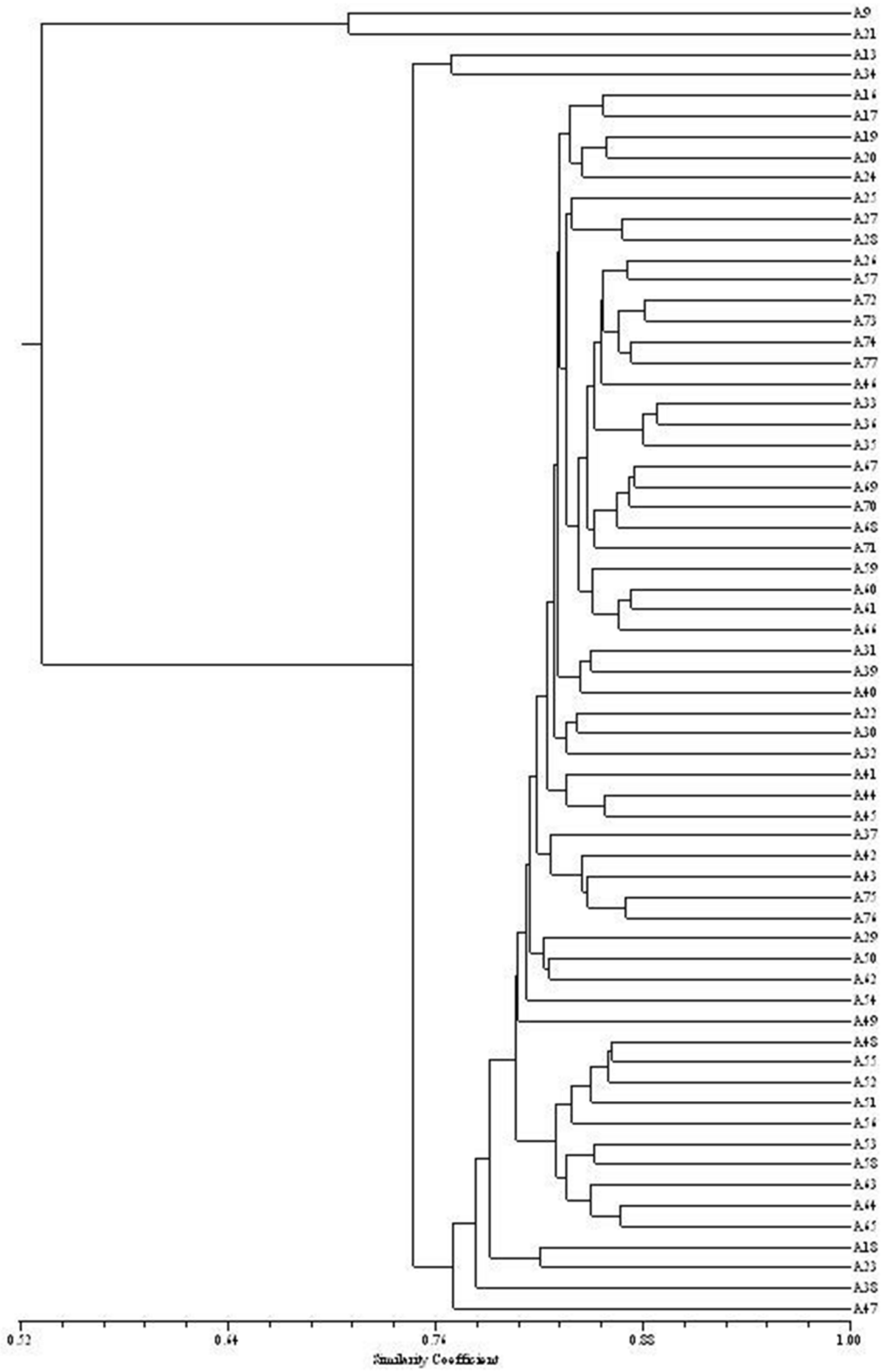

Figure S3. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar 'BoMaster'. B represents cultivar 'BoMaster'.

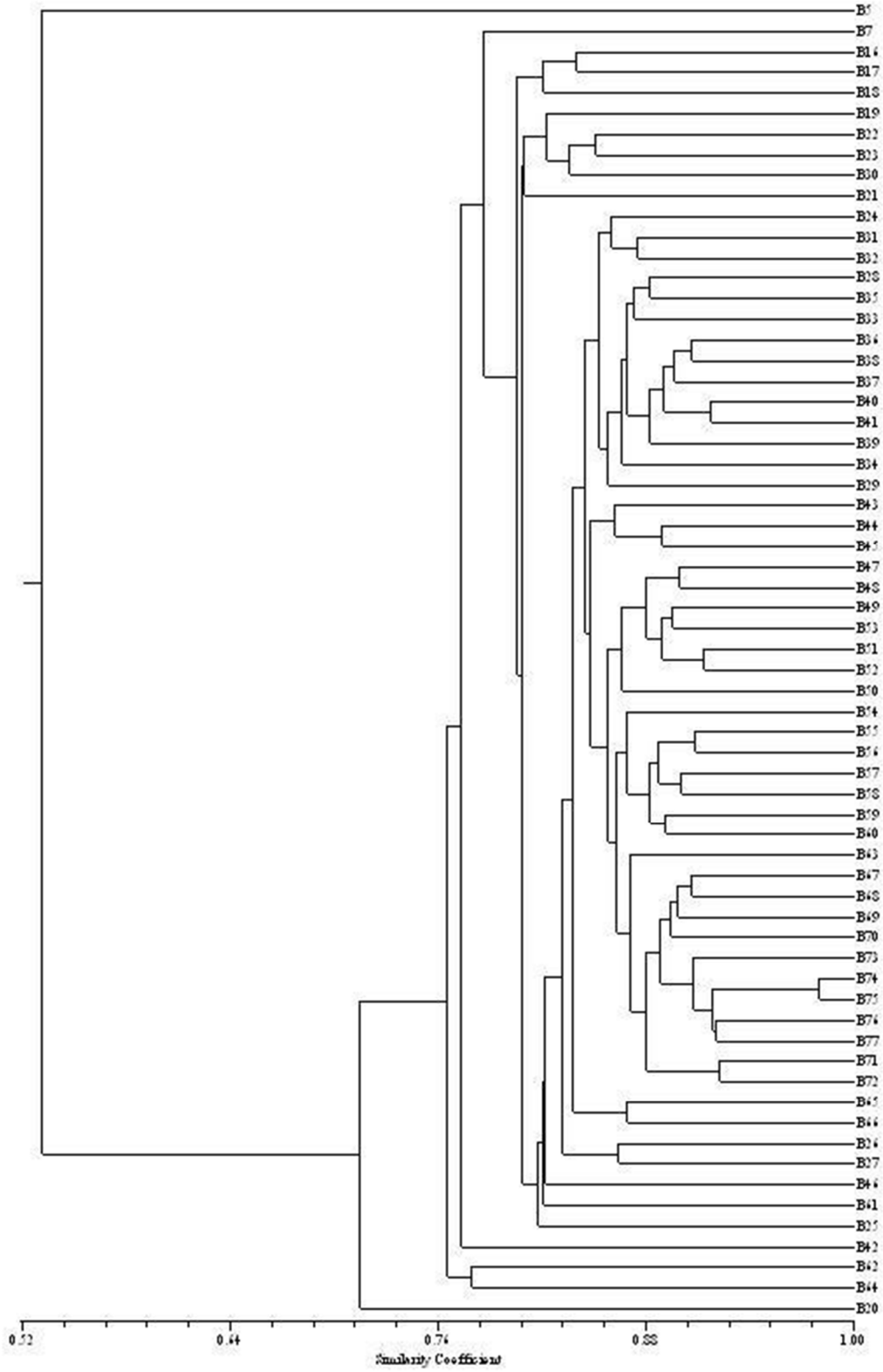

Figure S4. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar 'Cimarron'. C represents cultivar 'Cimarron'.

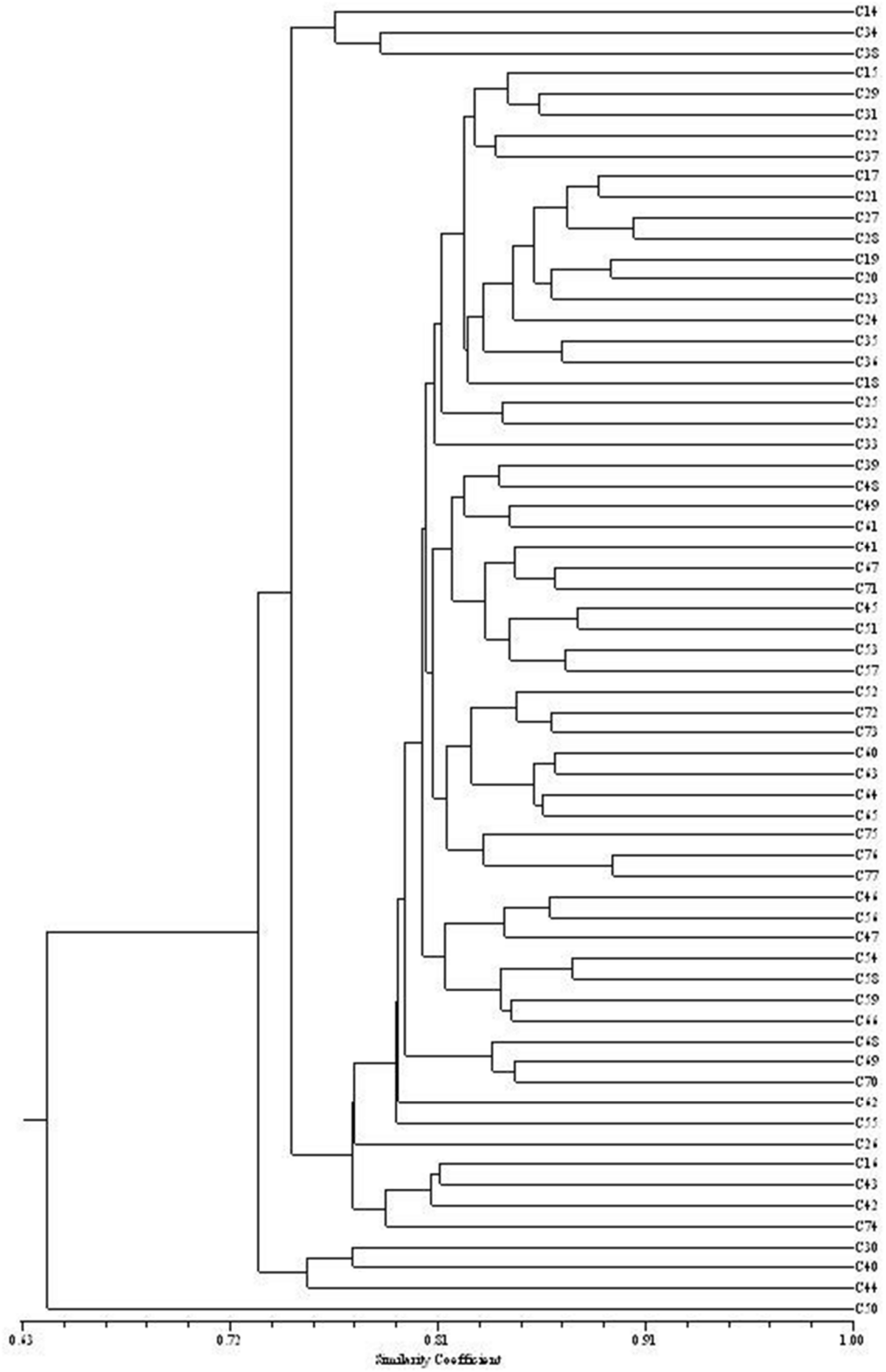

Figure S5. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar 'Kanlow'. K represents cultivar 'Kanlow'.

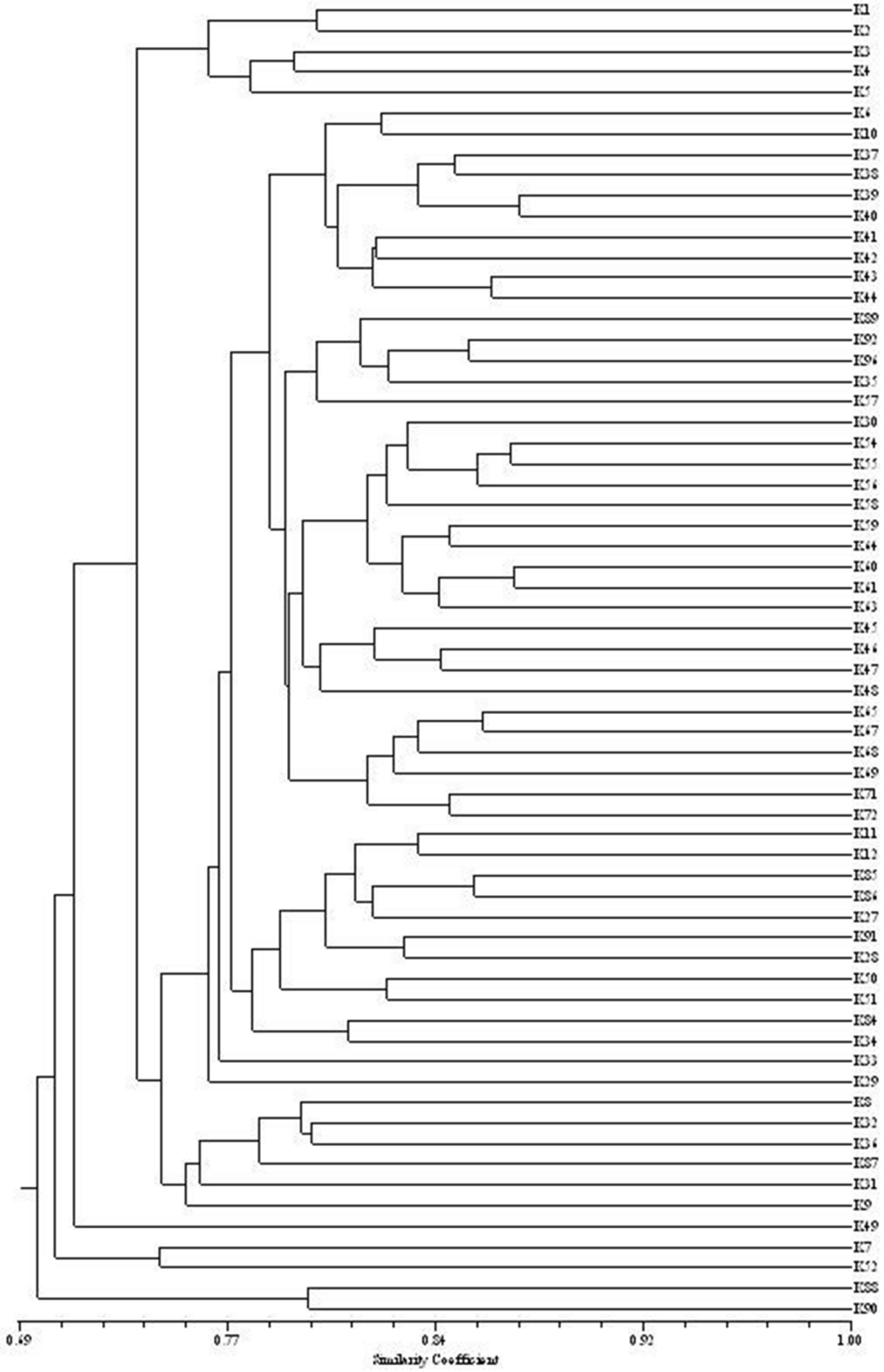

Figure S6. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar 'Performer'. P represents cultivar 'Performer'.

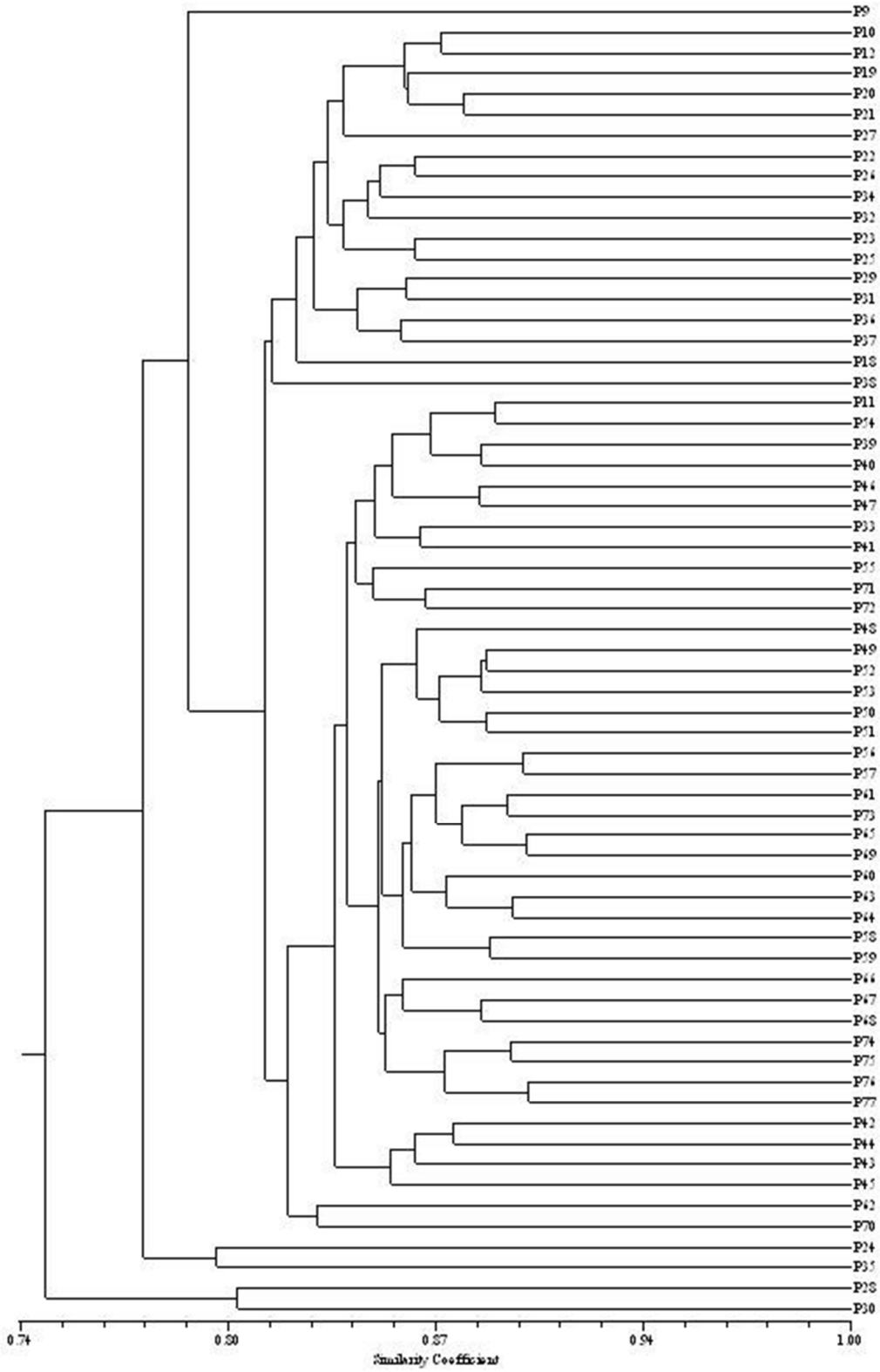

Figure S7. Principal coordinates analysis in 'Alamo'. PC-1 and PC-2 are two major principal coordinate axes.

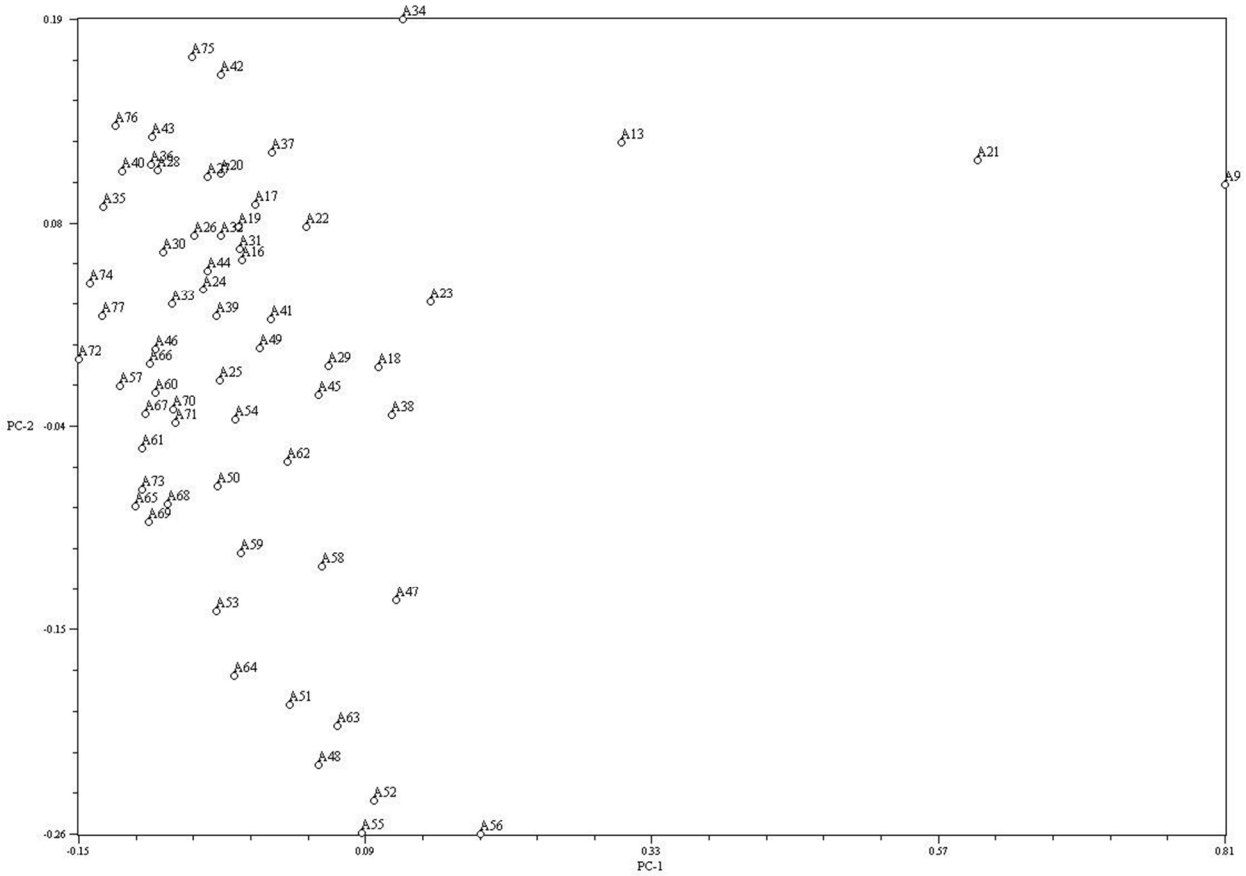

Figure S8. Principal coordinates analysis in 'BoMaster'. PC-1 and PC-2 are two major principal coordinate axes.

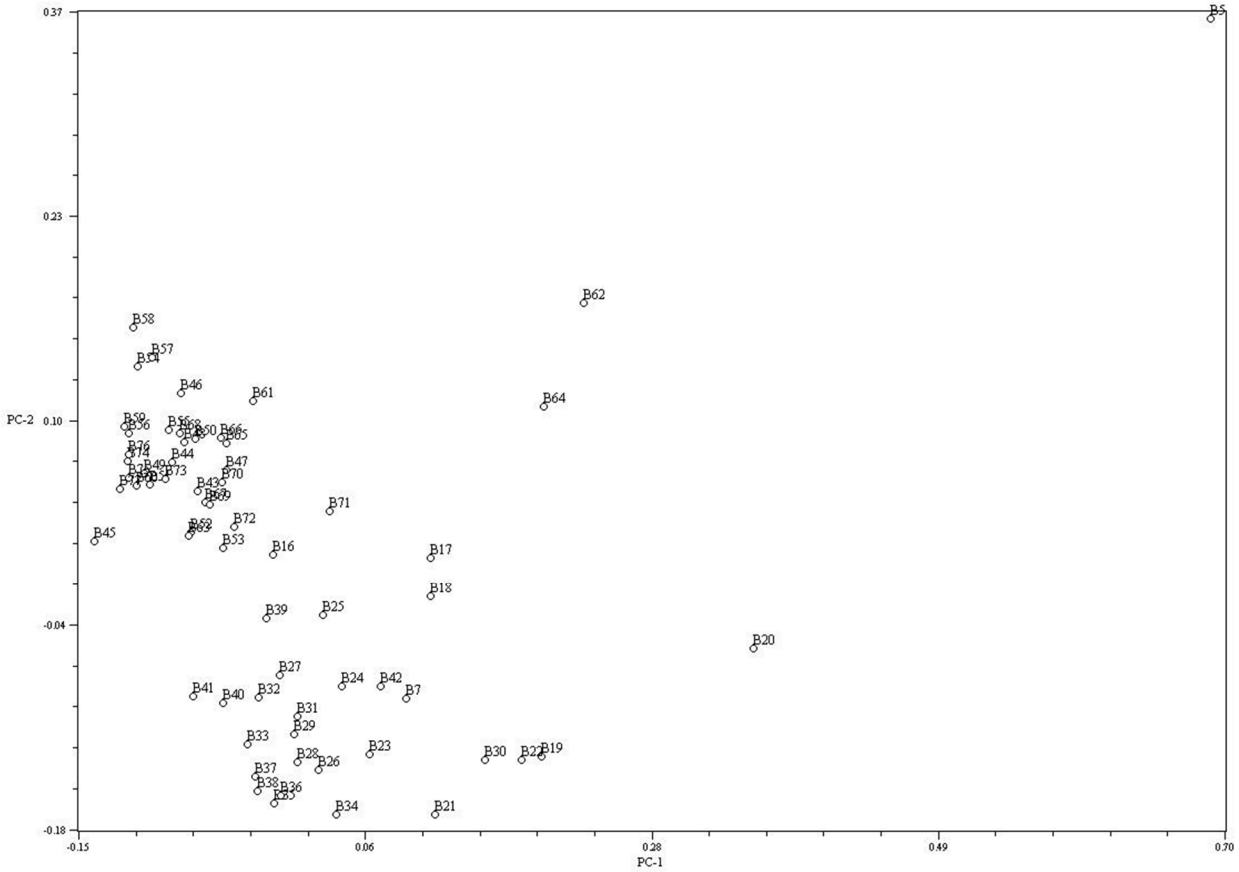

Figure S9. Principal coordinates analysis in 'Cimarron'. PC-1 and PC-2 are two major principal coordinate axes.

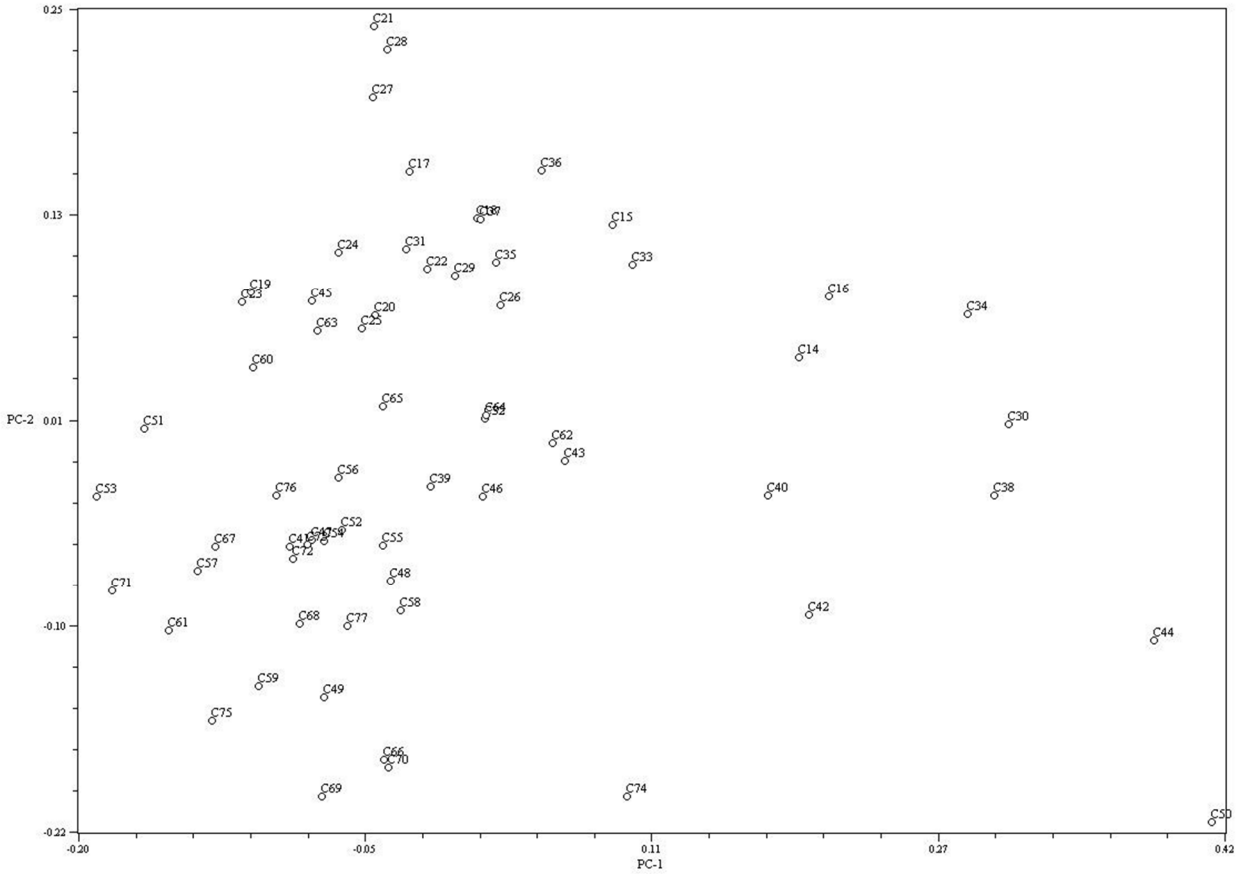

Figure S10. Principal coordinates analysis in 'Kanlow'. PC-1 and PC-2 are two major principal coordinate axes.

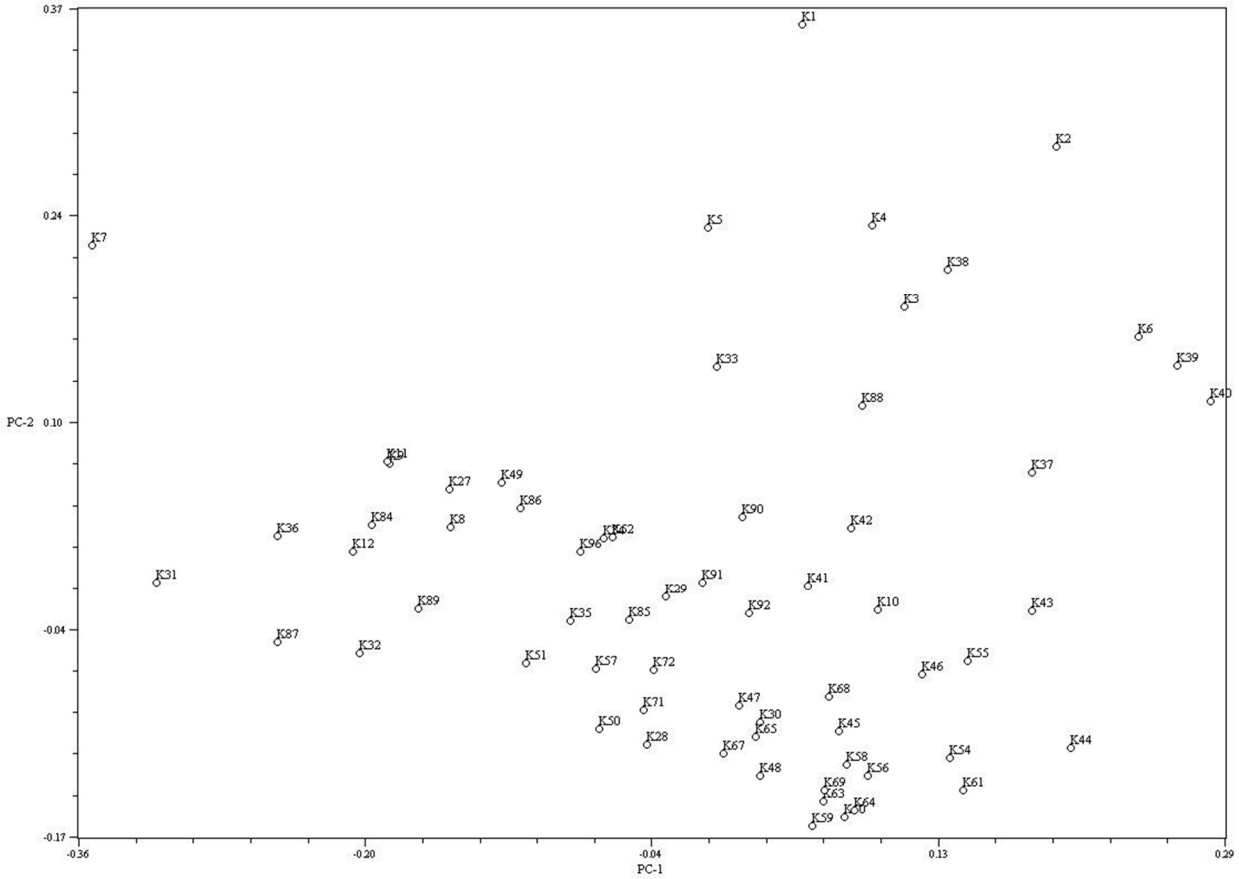

Figure S11. Principal coordinates analysis in 'Performer'. PC-1 and PC-2 are two major principal coordinate axes.

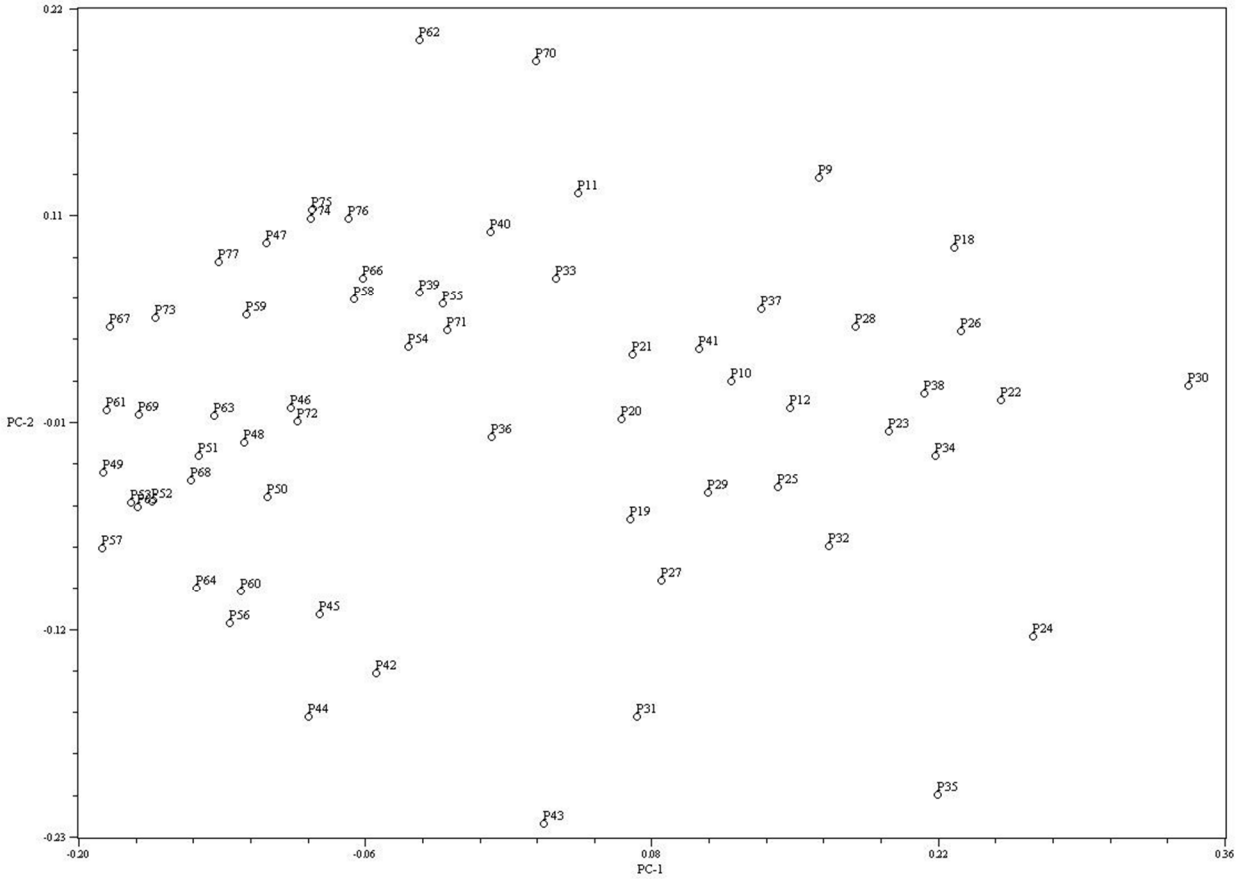

Table S1. Similarity coefficients among five lowland switchgrass cultivars. The ID for each plant genotype was denoted by a combination of letter and number. A, B, C, K, and P represented cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer', respectively (contd.)

	A1	A2	A3	A4	A5	A6	A7	A8	A10	A11	A12	A14	A15	B1	B2	B3	B4	B6	B8	B9	B10	B11	B12	B13	B14	B15	C1	C2	C3	C4	C5	C6
A1	1.00																															
A2	0.74	1.00																														
A3	0.77	0.85	1.00																													
A4	0.69	0.79	0.80	1.00																												
A5	0.77	0.74	0.73	0.73	1.00																											
A6	0.77	0.81	0.82	0.78	0.79	1.00																										
A7	0.72	0.81	0.82	0.81	0.72	0.81	1.00																									
A8	0.72	0.77	0.79	0.85	0.71	0.80	0.79	1.00																								
A10	0.78	0.69	0.70	0.65	0.81	0.74	0.69	0.67	1.00																							
A11	0.78	0.78	0.79	0.75	0.74	0.80	0.79	0.73	0.74	1.00																						
A12	0.79	0.77	0.79	0.76	0.78	0.81	0.78	0.75	0.77	0.83	1.00																					
A14	0.72	0.76	0.77	0.78	0.72	0.72	0.73	0.77	0.67	0.70	0.73	1.00																				
A15	0.76	0.77	0.76	0.74	0.81	0.76	0.75	0.73	0.76	0.73	0.76	0.79	1.00																			
B1	0.75	0.76	0.76	0.74	0.75	0.76	0.74	0.72	0.73	0.75	0.78	0.77	0.79	1.00																		
B2	0.76	0.76	0.77	0.74	0.74	0.76	0.75	0.73	0.73	0.74	0.78	0.76	0.80	0.87	1.00																	
B3	0.73	0.74	0.76	0.75	0.72	0.73	0.73	0.76	0.68	0.73	0.74	0.76	0.75	0.80	0.81	1.00																
B4	0.75	0.72	0.74	0.71	0.76	0.76	0.73	0.71	0.74	0.74	0.79	0.71	0.73	0.78	0.76	0.77	1.00															
B6	0.78	0.77	0.77	0.74	0.75	0.77	0.75	0.74	0.74	0.76	0.79	0.73	0.77	0.84	0.84	0.82	0.79	1.00														
B8	0.73	0.75	0.79	0.74	0.75	0.76	0.74	0.74	0.70	0.72	0.78	0.76	0.78	0.81	0.84	0.79	0.79	0.81	1.00													
B9	0.73	0.74	0.74	0.75	0.74	0.73	0.74	0.78	0.70	0.71	0.74	0.76	0.76	0.76	0.79	0.81	0.76	0.81	0.81	1.00												
B10	0.80	0.75	0.78	0.73	0.79	0.78	0.74	0.74	0.80	0.77	0.81	0.74	0.78	0.82	0.81	0.78	0.81	0.84	0.79	0.81	1.00											
B11	0.76	0.74	0.76	0.74	0.77	0.76	0.74	0.73	0.77	0.75	0.80	0.76	0.78	0.83	0.84	0.78	0.77	0.86	0.82	0.80	0.84	1.00										
B12	0.78	0.75	0.75	0.75	0.73	0.76	0.74	0.75	0.74	0.75	0.78	0.74	0.77	0.82	0.85	0.79	0.76	0.84	0.81	0.82	0.81	0.86	1.00									
B13	0.73	0.76	0.75	0.73	0.70	0.73	0.73	0.73	0.69	0.71	0.74	0.73	0.74	0.80	0.81	0.81	0.74	0.83	0.79	0.80	0.78	0.80	0.83	1.00								
B14	0.75	0.73	0.73	0.68	0.77	0.77	0.69	0.67	0.77	0.72	0.77	0.71	0.77	0.78	0.78	0.74	0.75	0.80	0.75	0.73	0.80	0.80	0.79	0.80	1.00							
B15	0.72	0.77	0.78	0.75	0.71	0.76	0.75	0.74	0.70	0.73	0.77	0.78	0.79	0.82	0.86	0.80	0.73	0.84	0.81	0.79	0.77	0.84	0.83	0.82	0.80	1.00						
C1	0.75	0.75	0.76	0.76	0.73	0.75	0.77	0.76	0.72	0.75	0.76	0.75	0.76	0.77	0.80	0.77	0.73	0.79	0.78	0.80	0.76	0.78	0.81	0.76	0.72	0.79	1.00					
C2	0.77	0.77	0.78	0.80	0.76	0.76	0.75	0.79	0.70	0.74	0.80	0.76	0.77	0.78	0.79	0.80	0.74	0.78	0.79	0.83	0.78	0.76	0.80	0.78	0.73	0.78	0.81	1.00				
C3	0.78	0.77	0.79	0.74	0.78	0.77	0.78	0.74	0.74	0.78	0.82	0.73	0.77	0.77	0.77	0.74	0.79	0.78	0.73	0.75	0.82	0.77	0.76	0.74	0.77	0.74	0.78	0.82	1.00			
C4	0.66	0.70	0.71	0.68	0.70	0.70	0.71	0.67	0.67	0.69	0.73	0.66	0.72	0.71	0.71	0.66	0.67	0.70	0.72	0.68	0.69	0.72	0.70	0.69	0.70	0.73	0.71	0.72	0.71	1.00		
C5	0.75	0.75	0.75	0.75	0.77	0.74	0.75	0.73	0.73	0.75	0.78	0.77	0.79	0.81	0.83	0.78	0.74	0.77	0.79	0.78	0.77	0.79	0.79	0.76	0.72	0.78	0.84	0.83	0.79	0.75	1.00	
C6	0.72	0.73	0.75	0.76	0.71	0.72	0.74	0.75	0.70	0.71	0.75	0.77	0.75	0.76	0.79	0.77	0.72	0.76	0.76	0.81	0.75	0.76	0.77	0.77	0.72	0.77	0.79	0.81	0.75	0.69	0.84	1.00

Table S1. Continue ….

Table S1. Similarity coefficients among five lowland switchgrass cultivars. The ID for each plant genotype was denoted by a combination of letter and number. A, B, C, K, and P represented cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer', respectively (contd.)

	A1	A2	A3	A4	A5	A6	A7	A8	A10	A11	A12	A14	A15	B1	B2	B3	B4	B6	B8	B9	B10	B11	B12	B13	B14	B15	C1	C2	C3	C4	C5	C6
C7	0.74	0.76	0.77	0.77	0.73	0.75	0.77	0.77	0.70	0.76	0.74	0.72	0.74	0.76	0.75	0.77	0.75	0.76	0.74	0.77	0.74	0.74	0.77	0.75	0.72	0.73	0.78	0.80	0.78	0.65	0.80	0.78
C8	0.74	0.80	0.82	0.79	0.76	0.77	0.80	0.78	0.72	0.76	0.77	0.76	0.76	0.77	0.78	0.76	0.75	0.76	0.76	0.78	0.76	0.77	0.78	0.77	0.73	0.75	0.81	0.83	0.79	0.69	0.81	0.79
C9	0.73	0.77	0.80	0.80	0.75	0.76	0.79	0.77	0.69	0.75	0.77	0.78	0.78	0.78	0.79	0.76	0.73	0.75	0.78	0.77	0.75	0.77	0.76	0.75	0.72	0.77	0.81	0.83	0.79	0.76	0.87	0.80
C10	0.72	0.75	0.75	0.80	0.73	0.76	0.75	0.79	0.65	0.74	0.73	0.76	0.73	0.70	0.73	0.75	0.71	0.73	0.75	0.80	0.74	0.72	0.74	0.74	0.67	0.73	0.80	0.82	0.75	0.66	0.79	0.79
C11	0.80	0.76	0.77	0.75	0.80	0.80	0.75	0.75	0.81	0.79	0.81	0.74	0.79	0.78	0.78	0.75	0.76	0.78	0.77	0.77	0.81	0.78	0.79	0.75	0.76	0.75	0.79	0.81	0.80	0.69	0.80	0.77
C12	0.74	0.77	0.78	0.78	0.78	0.78	0.79	0.76	0.74	0.79	0.79	0.74	0.78	0.77	0.79	0.76	0.76	0.77	0.78	0.77	0.79	0.78	0.79	0.75	0.74	0.75	0.82	0.83	0.83	0.73	0.81	0.78
C13	0.77	0.78	0.78	0.75	0.81	0.79	0.75	0.74	0.76	0.78	0.80	0.74	0.80	0.80	0.78	0.74	0.76	0.78	0.78	0.74	0.80	0.80	0.77	0.73	0.78	0.76	0.79	0.80	0.83	0.74	0.83	0.75
K14	0.75	0.76	0.78	0.72	0.74	0.74	0.74	0.70	0.74	0.74	0.77	0.75	0.80	0.81	0.82	0.77	0.74	0.80	0.76	0.76	0.80	0.81	0.81	0.78	0.79	0.81	0.77	0.76	0.79	0.69	0.78	0.74
K15	0.73	0.73	0.74	0.73	0.73	0.71	0.71	0.74	0.70	0.69	0.73	0.78	0.79	0.79	0.83	0.78	0.70	0.77	0.77	0.79	0.74	0.79	0.80	0.76	0.73	0.80	0.76	0.78	0.73	0.69	0.81	0.79
K16	0.73	0.75	0.78	0.74	0.72	0.73	0.75	0.73	0.69	0.71	0.76	0.78	0.79	0.79	0.82	0.78	0.70	0.77	0.79	0.78	0.76	0.78	0.79	0.77	0.73	0.81	0.77	0.77	0.75	0.69	0.80	0.77
K17	0.74	0.75	0.77	0.73	0.71	0.75	0.73	0.72	0.70	0.71	0.76	0.78	0.78	0.80	0.84	0.78	0.71	0.76	0.80	0.78	0.76	0.80	0.81	0.76	0.74	0.82	0.78	0.78	0.75	0.71	0.81	0.77
K18	0.74	0.75	0.78	0.74	0.71	0.75	0.74	0.72	0.70	0.73	0.77	0.78	0.79	0.83	0.84	0.79	0.73	0.81	0.80	0.79	0.80	0.81	0.82	0.81	0.76	0.83	0.78	0.79	0.77	0.68	0.79	0.78
K19	0.74	0.74	0.76	0.75	0.70	0.72	0.74	0.74	0.69	0.70	0.75	0.76	0.77	0.82	0.85	0.80	0.71	0.82	0.79	0.81	0.77	0.80	0.83	0.80	0.75	0.84	0.81	0.81	0.76	0.69	0.80	0.79
K20	0.74	0.74	0.76	0.75	0.72	0.74	0.72	0.73	0.69	0.71	0.74	0.76	0.78	0.80	0.83	0.79	0.71	0.80	0.80	0.79	0.76	0.80	0.82	0.78	0.75	0.81	0.79	0.78	0.73	0.68	0.79	0.78
K21	0.73	0.77	0.78	0.75	0.74	0.73	0.74	0.72	0.69	0.70	0.75	0.81	0.80	0.81	0.84	0.79	0.71	0.77	0.80	0.81	0.77	0.81	0.81	0.79	0.75	0.82	0.78	0.81	0.75	0.71	0.82	0.79
K22	0.72	0.74	0.76	0.75	0.70	0.72	0.72	0.73	0.67	0.70	0.73	0.77	0.75	0.78	0.81	0.79	0.70	0.77	0.78	0.78	0.75	0.76	0.80	0.77	0.73	0.79	0.79	0.79	0.74	0.66	0.82	0.78
K23	0.75	0.75	0.75	0.73	0.73	0.73	0.71	0.72	0.72	0.70	0.73	0.78	0.78	0.80	0.81	0.77	0.71	0.77	0.77	0.78	0.78	0.78	0.81	0.77	0.74	0.79	0.80	0.78	0.74	0.68	0.80	0.76
K24	0.73	0.76	0.78	0.77	0.71	0.75	0.75	0.75	0.68	0.74	0.76	0.76	0.78	0.80	0.82	0.78	0.70	0.79	0.78	0.77	0.77	0.79	0.79	0.81	0.76	0.82	0.78	0.80	0.74	0.70	0.78	0.74
K25	0.74	0.76	0.77	0.73	0.71	0.72	0.74	0.72	0.71	0.73	0.75	0.77	0.78	0.82	0.83	0.79	0.72	0.80	0.78	0.80	0.78	0.79	0.81	0.81	0.76	0.84	0.77	0.79	0.76	0.71	0.80	0.77
K26	0.71	0.72	0.74	0.71	0.69	0.69	0.72	0.70	0.66	0.69	0.72	0.75	0.76	0.77	0.79	0.76	0.70	0.76	0.76	0.77	0.72	0.77	0.79	0.76	0.74	0.80	0.79	0.76	0.72	0.67	0.79	0.76
P1	0.77	0.74	0.75	0.71	0.78	0.78	0.73	0.71	0.76	0.75	0.77	0.73	0.80	0.82	0.80	0.76	0.76	0.80	0.77	0.76	0.81	0.81	0.80	0.76	0.77	0.77	0.79	0.78	0.79	0.70	0.79	0.76
P2	0.77	0.73	0.76	0.72	0.78	0.75	0.71	0.70	0.74	0.75	0.79	0.74	0.78	0.79	0.79	0.75	0.77	0.77	0.78	0.77	0.81	0.78	0.79	0.75	0.76	0.77	0.77	0.77	0.79	0.67	0.76	0.75
P3	0.76	0.73	0.76	0.74	0.79	0.77	0.73	0.74	0.74	0.75	0.79	0.74	0.78	0.83	0.78	0.75	0.78	0.79	0.78	0.77	0.79	0.78	0.80	0.76	0.76	0.76	0.75	0.79	0.78	0.69	0.76	0.74
P4	0.67	0.63	0.68	0.60	0.72	0.70	0.64	0.63	0.72	0.66	0.71	0.65	0.67	0.69	0.67	0.63	0.70	0.67	0.68	0.65	0.70	0.71	0.67	0.62	0.70	0.66	0.65	0.65	0.67	0.69	0.66	0.63
P5	0.73	0.72	0.74	0.70	0.72	0.74	0.71	0.70	0.72	0.72	0.74	0.73	0.76	0.78	0.78	0.74	0.72	0.79	0.77	0.76	0.80	0.78	0.77	0.74	0.74	0.78	0.76	0.77	0.74	0.69	0.75	0.71
P6	0.74	0.70	0.73	0.69	0.81	0.76	0.69	0.70	0.76	0.73	0.79	0.70	0.75	0.74	0.72	0.69	0.77	0.74	0.76	0.72	0.79	0.76	0.73	0.68	0.79	0.71	0.70	0.72	0.74	0.68	0.72	0.69
P8	0.69	0.63	0.65	0.61	0.76	0.72	0.63	0.64	0.75	0.68	0.71	0.62	0.68	0.72	0.68	0.64	0.72	0.68	0.70	0.66	0.73	0.71	0.68	0.64	0.71	0.65	0.65	0.68	0.70	0.66	0.67	0.62
P13	0.73	0.75	0.77	0.75	0.73	0.75	0.73	0.75	0.69	0.71	0.75	0.78	0.78	0.77	0.81	0.78	0.70	0.80	0.78	0.80	0.77	0.78	0.80	0.80	0.74	0.81	0.77	0.79	0.75	0.68	0.78	0.77
P14	0.74	0.73	0.75	0.68	0.73	0.72	0.70	0.70	0.73	0.73	0.78	0.78	0.80	0.81	0.81	0.75	0.71	0.79	0.81	0.78	0.79	0.82	0.81	0.75	0.74	0.82	0.76	0.76	0.75	0.71	0.78	0.73
P15	0.72	0.70	0.71	0.66	0.76	0.70	0.67	0.65	0.74	0.70	0.75	0.72	0.79	0.77	0.76	0.71	0.71	0.75	0.75	0.72	0.78	0.78	0.76	0.72	0.80	0.77	0.71	0.71	0.73	0.70	0.74	0.69
P16	0.73	0.78	0.78	0.73	0.71	0.73	0.76	0.72	0.66	0.75	0.72	0.78	0.75	0.78	0.79	0.78	0.70	0.76	0.77	0.80	0.75	0.74	0.77	0.77	0.71	0.78	0.78	0.78	0.76	0.65	0.78	0.75
P17	0.68	0.76	0.76	0.74	0.68	0.70	0.74	0.70	0.64	0.72	0.71	0.76	0.75	0.77	0.80	0.76	0.69	0.77	0.76	0.76	0.74	0.76	0.77	0.78	0.71	0.81	0.78	0.77	0.73	0.68	0.76	0.75

Table S1. Continue ….

Table S1. Similarity coefficients among five lowland switchgrass cultivars. The ID for each plant genotype was denoted by a combination of letter and number. A, B, C, K, and P represented cultivars 'Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer', respectively.

	C7	C8	C9	C10	C11	C12	C13	K14	K15	K16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	P1	P2	P3	P4	P5	P6	P8	P13	P14	P15	P16	P17
C7	1.00																															
C8	0.83	1.00																														
C9	0.80	0.84	1.00																													
C10	0.84	0.83	0.82	1.00																												
C11	0.79	0.79	0.78	0.79	1.00																											
C12	0.80	0.83	0.83	0.80	0.82	1.00																										
C13	0.80	0.79	0.85	0.78	0.82	0.86	1.00																									
K14	0.73	0.76	0.76	0.72	0.80	0.77	0.79	1.00																								
K15	0.75	0.77	0.78	0.74	0.77	0.76	0.77	0.82	1.00																							
K16	0.73	0.77	0.79	0.74	0.75	0.75	0.76	0.83	0.86	1.00																						
K17	0.74	0.77	0.80	0.74	0.77	0.77	0.78	0.83	0.84	0.87	1.00																					
K18	0.74	0.77	0.79	0.73	0.79	0.77	0.77	0.84	0.83	0.85	0.85	1.00																				
K19	0.74	0.78	0.76	0.74	0.78	0.77	0.76	0.83	0.87	0.84	0.86	0.87	1.00																			
K20	0.74	0.76	0.78	0.74	0.76	0.77	0.75	0.82	0.85	0.86	0.86	0.88	0.86	1.00																		
K21	0.75	0.80	0.81	0.75	0.77	0.78	0.78	0.84	0.87	0.85	0.86	0.86	0.87	0.86	1.00																	
K22	0.74	0.76	0.77	0.76	0.75	0.75	0.73	0.83	0.85	0.85	0.85	0.84	0.85	0.87	0.86	1.00																
K23	0.73	0.75	0.76	0.74	0.78	0.78	0.76	0.82	0.86	0.84	0.83	0.84	0.82	0.86	0.87	0.85	1.00															
K24	0.74	0.75	0.77	0.74	0.76	0.77	0.78	0.82	0.82	0.83	0.82	0.84	0.83	0.84	0.83	0.84	0.84	1.00														
K25	0.72	0.77	0.77	0.72	0.76	0.76	0.77	0.83	0.83	0.84	0.84	0.86	0.85	0.84	0.86	0.85	0.85	0.87	1.00													
K26	0.73	0.76	0.76	0.74	0.71	0.73	0.72	0.78	0.81	0.80	0.81	0.82	0.81	0.83	0.83	0.86	0.83	0.84	0.85	1.00												
P1	0.77	0.75	0.75	0.72	0.82	0.79	0.80	0.80	0.78	0.77	0.77	0.81	0.80	0.79	0.79	0.76	0.78	0.77	0.80	0.75	1.00											
P2	0.76	0.77	0.77	0.73	0.80	0.78	0.78	0.81	0.78	0.78	0.77	0.79	0.77	0.79	0.79	0.78	0.79	0.78	0.78	0.76	0.85	1.00										
P3	0.79	0.78	0.75	0.74	0.81	0.77	0.78	0.81	0.77	0.76	0.76	0.78	0.76	0.77	0.78	0.75	0.77	0.77	0.77	0.73	0.85	0.85	1.00									
P4	0.62	0.66	0.65	0.62	0.68	0.64	0.72	0.70	0.66	0.69	0.70	0.68	0.67	0.66	0.68	0.64	0.67	0.65	0.66	0.64	0.72	0.71	0.73	1.00								
P5	0.71	0.72	0.73	0.71	0.77	0.73	0.75	0.78	0.75	0.77	0.77	0.77	0.79	0.76	0.78	0.74	0.77	0.77	0.78	0.72	0.83	0.79	0.81	0.73	1.00							
P6	0.70	0.73	0.71	0.69	0.75	0.73	0.78	0.75	0.72	0.70	0.70	0.70	0.68	0.70	0.71	0.69	0.71	0.70	0.71	0.67	0.77	0.80	0.79	0.76	0.77	1.00						
P8	0.67	0.66	0.67	0.65	0.74	0.68	0.73	0.70	0.66	0.63	0.67	0.66	0.65	0.65	0.66	0.62	0.65	0.64	0.65	0.61	0.77	0.74	0.78	0.74	0.73	0.79	1.00					
P13	0.75	0.75	0.75	0.76	0.76	0.77	0.76	0.81	0.80	0.82	0.80	0.82	0.84	0.81	0.83	0.82	0.81	0.82	0.81	0.78	0.79	0.79	0.77	0.66	0.78	0.73	0.66	1.00				
P14	0.72	0.75	0.77	0.71	0.76	0.75	0.77	0.79	0.79	0.81	0.82	0.82	0.80	0.80	0.83	0.77	0.81	0.80	0.82	0.79	0.79	0.84	0.79	0.73	0.81	0.76	0.71	0.81	1.00			
P15	0.69	0.70	0.73	0.66	0.74	0.74	0.76	0.77	0.72	0.75	0.75	0.75	0.72	0.74	0.76	0.71	0.74	0.73	0.74	0.70	0.78	0.80	0.78	0.70	0.76	0.77	0.74	0.76	0.81	1.00		
P16	0.77	0.78	0.78	0.79	0.75	0.76	0.75	0.79	0.77	0.81	0.81	0.79	0.80	0.78	0.82	0.83	0.79	0.80	0.81	0.80	0.76	0.79	0.78	0.65	0.77	0.69	0.63	0.81	0.80	0.73	1.00	
P17	0.73	0.76	0.76	0.77	0.71	0.77	0.73	0.77	0.75	0.79	0.78	0.81	0.80	0.79	0.82	0.81	0.77	0.80	0.80	0.80	0.75	0.76	0.76	0.61	0.76	0.67	0.60	0.81	0.78	0.75	0.86	1.00

Table S2. Mantel's test. Criteria for goodness of fit of the dendrogram to dissimilarity matrix: r ≥ 0.90 very good fit, 0.9 > r ≥ 0.80 good fit, 0.80 > r ≥ 0.70 poor fit, and r < 0.70 very poor fit (Rohlf, 1998).

Tests for association	Among cultivars	Alamo	BoMaster	Cimarron	Kanlow	Performer
Matrix correlation (r)	0.77	0.95	0.96	0.89	0.76	0.82
(= normalized Mantel statistic Z)						
Approximate Mantel t-test (t)	10.25	8.04	8.16	8.44	8.39	9.25
Probability random Z < observed Z (*P*)	1.00	1.00	1.00	1.00	1.00	1.00
Goodness of fit of the dendrogram to the original dissimilarity matrix	Poor	Very good	Very good	Good	Poor	Good

Table S3. Pairwise Nei's (1972) genetic distance in five lowland switchgrass cultivars.

	Alamo	BoMaster	Cimarron	Kanlow
BoMaster	0.051			
Cimarron	0.047	0.057		
Kanlow	0.089	0.061	0.072	
Performer	0.062	0.058	0.071	0.088