AFLP GENETIC VARIATION, INBREDS DEVELOPMENT, AND QTL LOCALIZATION FOR PLANT HEIGHT IN LOWLAND SWITCHGRASS

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Abstract: Switchgrass (*Panicum virgatum* L.) has gained wider attention due to its recognition as a model herbaceous crop species for bioenergy production. The objectives of this research were to analyze genetic variation among and within five lowland switchgrass cultivars using amplified fragment length polymorphism (AFLP) markers; to develop (i) S_3 inbreds from S_2 populations and (ii) S_4 inbreds from S_3 populations using a bagging method; and to analyze phenotypic variation for biomass and plant height and to localize QTLs associated with the plant height. AFLP polymorphisms indicated the presence of high genetic variation within lowland switchgrass cultivars. 'Alamo' exhibited the highest genetic variation and 'Performer' had the lowest. The Nei's genetic diversity parameters revealed the lowest genetic distance between cultivars 'Alamo' and 'Cimarron' and highest value between cultivars 'Alamo' and 'Kanlow'. Using 195 S_2 inbreds, 279 S_3 inbreds and 224 S_4 inbreds were produced by bagging and confirmed with simple sequence repeat (SSR) markers. Two lowland switchgrass mapping populations field established at Perkins and Stillwater, OK were deployed in the plant height associated QTL experiment. Large genetic variation existed for plant biomass and height within the two populations. Plant height was positively correlated with biomass yield. Twenty-one QTLs were identified on 11 linkage groups, including nine of the QTL markers were detected in the selfed population and remaining 12 QTL markers were identified in the hybrid population. The findings of this research and the advanced inbreds developed in these experiments would be useful for future plant breeding and genetic improvement programs in lowland switchgrass.

TABLE OF CONTENTS

CHAPTER I

GENERAL INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial, warm season, multipurpose crop species for bioenergy production, soil and water conservation, and livestock production. It can also be used in the generation of electricity (Greenwell et al., 2013) and hydrogen fuels (Zhang et al., 2004). The two ecotypes, based on edaphic adaptation and plant morphology, include upland and lowland. The upland ecotypes are based on upland sites receiving occasional or frequent droughts; and the lowland ecotypes are based on sites subjected to seasonally wet soils (Casler, 2012). Both upland and lowland ecotypes exhibit morphological variations, however, the lowland ecotypes are in general larger in size (Porter, 1966). Switchgrass can also be further grouped into leafy or stemmy morphotypes (Bhandari et al., 2014). Switchgrass can grow in mesic to wet prairies, on dry slopes, open oak or pine woodlands, shores, river banks, and brackish marshes (Barkworth et al., 2007). According to Brunken and Estes (1975), the upland ecotype

occurs in tallgrass prairie and the lowland ecotype in riverine grasslands. The morphological and physiological variation in switchgrass is closely associated with climatic factors (Casler, 2012). The adaptation of switchgrass along north-south range is dependent on photoperiod (Casler, 2012). Switchgrass is native to North America with large morphological diversity and wide adaptation area (Parrish and Fike, 2005). The plant adaptation ranges of switchgrass include the eastern side of Rocky Mountains, from southern Canada through the United States to Mexico, Cuba, Bermuda, and Costa Rica, and possibly an introduction in Argentina (Barkworth et al., 2007).

Zhang et al., (2011) identified primary centers of diversity for switchgrass in the eastern and western Gulf Coast regions. They indicated that migration, drift, and selection have resulted into adaptive radiation in switchgrass. They concluded that this adaptive radiation created regional gene pools within each of the main taxa (Zhang et al., 2011). They estimated that both upland-lowland divergence and 4x-to-8x polyploidization within switchgrass began approximately 1.5-1 M ybp and that subsequent ice age cycles have resulted in gene flow between ecotype lineages and between ploidy levels. They inferred that gene flow has resulted in "hot spots" of genetic diversity in the southeastern USA and along the Atlantic Seaboard (Zhang et al., 2011).

The plants of switchgrass are self-incompatible and highly outcrossing in nature (Liu et al., 2014). Interestingly, some lowland switchgrass genotypes are self-compatible (Liu et al., 2012). The basic chromosome number (x) for switchgrass is x=9 (Calser, 2012). Switchgrass is a multiploid crop species and ploidy levels ranging from diploid (2n=2x=18) to duodecaploid (2n=2x=108) are available from past study reports (Nielson, 1944). The lowland cultivars were tetraploids and the upland cultivars were tetraploids or octaploids,

with hexaploids rare (Nielsen, 1944; Narasimhamoorthy et al., 2008). Tetraploid lowland switchgrass exhibits disomic inheritance (Okada et al., 2010; Liu et al., 2012). Aneuploidy has also been reported in switchgrass (Costich et al., 2010).

LITERATURE REVIEW

Botany of Switchgrass

Switchgrass (*Panicum virgatum* L.) belongs to Kingdom Plantae (Plants), Subkingdom Tracheobionta (Vascular plants), Superdivision Spermatophyta (Seed plants), Division Magnoliophyta (Flowering plants), Class Liliopsida (Monocotyledons), Subclass Commelinidae, Order Cyperales, and Family Poaceae (Grass family) (USDA-NRCS, 2014). Hitchcock and Chase (1951) have provided detailed botanical description of switchgrass plants. Switchgrass plants generally have green or glaucous color, form large bunches, and develop numerous scaly creeping rhizomes. The plants are erect, tough and have hard culms. The height ranges from 1 to 2 m., and rarely to 3 m. The tillers have glabrous sheaths. The leaf blades are 10 to 60 cm long, 3 to 15 mm wide, and are flat, glabrous, or sometimes pilose above near the base, rarely pilose all over. The panicle length varies from 15 to 50 cm. The panicle is open and sometimes diffuse. The spikelets are 3.5 to 5 mm long and acuminate. It has clasping first glume, two-thirds to three-fourths in length compared to the spikelet, and are acuminate or cuspidate. The fruit is narrowly ovate and the margins of the lemma inrolled only at the base (Hitchcock and Chase, 1951).

Seed Size and Seedling Development

Switchgrass inflorescence bears very small seeds that remain dormant after harvest (Bransby, 2009). Dormancy can be overcome by aging, treatment with water, chilling

temperatures or storing it in warm condition (Bransby, 2009). Due to small seed size, seedlings are slow to develop and susceptible to weed competition in the beginning establishment phase (Bransby, 2009). The full potential yield is realized only in the third year from the field planting/establishment and the second year yield is about two thirds of the full yield (Bransby, 2009). Initially, higher germination rates, early shoot growth, and early adventitious root growth were observed in seedlings from heavy seeds compared to light seeds (Smart and Moser, 1998). However, after 8 to 10 wk as two or more adventitious roots form, the seed size no longer affects establishment and growth (Smart and Moser, 1998).

Genetic Diversity

Selection, mutation, migration, genetic drift and/or recombination lead to genetic diversity in plants (De Vicente and Fulton, 2004). The knowledge of genetic diversity is very important in crop improvement programs. Based on the knowledge of genetic diversity, heterotic groups are identified to be used in the crop development experiments. Genetic diversity in switchgrass can be evaluated using different molecular markers such as random amplified polymorphic DNA (RAPD) (Nageswara-Rao et al., 2013; Casler et al., 2007; Gunter et al., 1996), restriction fragment length polymorphism (RFLP) (Missaoui et al., 2003; Missaoui et al., 2006), expressed sequence tag-simple sequence repeat markers (EST-SSRs) (Narasimhamoorthy et al., 2008; Cortese et al., 2010; Huang et al., 2011), 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).

Molecular Markers

(1) AFLP: AFLP marker technique, first introduced by Vos et al. (1995), represents a combination of RFLP and PCR (Weising et al., 2005). Five steps as described by Chial (2008) are: Step 1: Genomic DNA is digested with restriction enzymes MseI and EcoRI. Step 2: Restriction fragments are ligated to MseI adaptor and EcoRI adaptor with DNA ligase. Ligation provides a series of DNA fragments. Step 3: In order to selectively amplify a smaller number of genomic DNA fragments, the procedure uses primer sets complementary to the MseI or EcoRI adaptor sequences starting at their 5′ ends with additional upto three unique nucleotides following the end of the original MseI or EcoRI recognition site. Step 4: In most cases, one of the two primers (typically the EcoRI primer) is radioactively or fluorescently labeled that enables easy detection of PCR reaction products. High resolution electrophoresis is also available to separate the DNA fragments based on the size and overall negative charge. Step 5: Analysis of DNA banding pattern can be done manually or by automated approaches.

Advantages of AFLP: Advantages of AFLP include high genomic abundance, considerable reproducibility, generation of many bands per reaction (Kumar et al., 2009). AFLP markers can disclose a high number of polymorphic markers by a single reaction (Vos et al., 1995; Kumar et al., 2009). No prior sequence knowledge is required in producing AFLP bands (Vos et al., 1995, Blears et al., 1998). AFLP marker system can be used for DNA samples of any origin or complexity (Blears et al., 1998). It is an extremely efficient technique as it produces numerous bands on a gel for simultaneous analysis (Blears et al., 1998). AFLP technique allows simultaneous amplification of multiple genomic DNA fragments with high specificity and reproducibility (Chial, 2008).

Disadvantages of AFLP: AFLP marker requires purified, high molecular weight DNA (Kumar et al., 2009). The dominance of alleles and possible non-homology of co-migrating fragments belonging to different loci are other disadvantages (Kumar et al., 2009). AFLP marker system is dominant in nature and hence the Hardy-Weinberg equilibrium evaluation becomes impossible (Campbell et al., 2003). AFLP can only detect dominant genetic markers and hence it cannot confirm whether an individual is homozygous or heterozygous for a given marker (Chial, 2008). The assumption of band homology instead of being demonstrated by sequence analysis may hinder the reliability of AFLP (Campbell et al., 2003). In detection of immigrant individuals in the human population, AFLP markers may not provide enough information compared to codominant marker methods (Campbell et al., 2003).

(2). SSR: Simple sequence repeats (SSRs), also known as microsatellites or short tandem repeats (STRs), are PCR based molecular markers. SSRs are DNA fragments consisting of tandemly repeating one to five nucleotide units arranged throughout the genome of most eukaryotes (Kumar et al., 2009).

Advantages: Advantages include codominance of alleles, high genomic abundance in eukaryotic species, random distribution throughout the genome (Kumar et al, 2009). SSR has high reproducibility and the SSR analysis does not require high quality DNA (Kumar et al., 2009). If the size ranges of alleles of different loci do not overlap, SSR can be multiplexed during PCR or gel electrophoresis (Kumar et al., 2009). SSRs are highly polymorphic and abundant sequences in most eukaryotic genomes (Hayden and Sharp, 2001). Being a co-dominant marker, SSR can report whether the individual is homozygous or heterozygous for given loci.

Disadvantages: For previously unstudied species, development of adequate primer sequences becomes cost expensive (Kumar et al., 2009). Mutations in primer annealing sites may lead to occurrence of null alleles (absence of amplification of PCR product) resulting into erroneous genotype scoring (Kumar et al., 2009). The other disadvantage is appearance of stutter bands which are artifacts resulting from DNA slippage during PCR amplification (Kumar et al., 2009).

(3). RAPD: Random amplified polymorphic DNA (RAPD) is a PCR-based marker system. RAPD markers (pronounced 'rapid') was first proposed by Williams et al. in 1990. It uses primers of arbitrary nucleotide sequence to access random genomic DNA segments and reveals polymorphisms (Williams et al., 1990). It amplifies target or random DNA segments enzymatically with arbitrary primers (Kumar et al., 2009). RAPDs are the DNA fragments generated by PCR amplification using short synthetic oligonucleotide primers (generally 10 bp) of random sequence(Kumar et al., 2009). The oligonucleotides are act as both forward and reverse primers, and usually can amplify fragments from 1 to 10 genomic sites simultaneously (Kumar et al., 2009).The final amplified products (generally 0.5-5 kb size range) are separated on agarose gels containing ethidium bromide and viewed under ultraviolet light (Kumar et al., 2009).

Advantages: Low quantity of template DNA (usually 5-50 ng per reaction) can be successfully used for RAPD (Kumar et al., 2009). The procedure is quick and easy to assay (Kumar et al., 2009). No sequence data for primer construction are needed because of commercial availability of random primers (Kumar et al., 2009). They have high genomic abundance and are randomly distributed in entire genome (Kumar et al., 2009).

Disadvantages: Being dominant markers, RAPDs have limitations in use as markers for mapping (Kumar et al., 2009). They have low reproducibility (Kumar et al., 2009) and require highly standardized experimental procedures as they are highly sensitive to reaction conditions (Kumar et al., 2009). Generally, purified, high molecular weight DNA is required in the analysis (Kumar et al., 2009). RAPDs have the inherent problems of reproducibility that make them unsuitable markers for transference or comparison of results among different research teams working on similar species and subjects (Kumar et al., 2009).

(4). SNP: SNPs are single-base pair positions, at which different sequence alternatives (alleles) exist in population genomes (Weising et al., 2005). In SNPs, the sequence variation is based on single base substitution at a particular position (Weising et al., 2005). In general, SNPs are biallelic markers and are highly useful for chip-based microarray technology (reviewed by Weising et al., 2005). During screening for SNPs, genomic DNA from several related test organisms is PCR amplified using either a specific pair of primers flanking a known sequence or by arbitrary priming (Weising et al., 2005). The recognition of single base substitutions can be assessed by their impact on the mobility of single-stranded DNA (ssDNA) molecules in single-strand conformation polymorphisms (SSCP) gels (Weising et al., 2005). The sequencing of PCR fragments that are polymorphic among the test organism is performed and the SNP is localized.

Advantages: SNPs are abundant, genetically stable, and agreeable to high-throughput automated analysis (Heaton et al., 2002). They are fast and are good for semi-automatic multiplex typing (reviewed by Krawczak, 1999). Hybridization assays of SNPs analysis brought forth the sophisticated typing systems such as high-density oligonucleotide microarrays (“DNA-chips”) (cited in Krawczak, 1999).

Disadvantages: The technique is cost expensive. SNP informativity may vary among populations significantly (Krawczak, 1999; Heaton et al., 2002). The allelic diversity is limited to the four possible nucleotides for an SNP and hence a give SNP can never exceed 75% heterozygosity, or gene diversity (Krawczak, 1999).

(5). VNTR: The term variable number of tandem repeats (VNTR), also known as minisatellites, was first introduced by Jeffery et al. (1985) (cited in Kumar et al., 2009). VNTRs consist of chromosomal regions that contain 10-50 base motif tandem repeat units, flanked by conserved DNA restriction sites (Kumar et al., 2009).

Advantages: High level of polymorphism and high reproducibility are advantages (Kumar et al., 2009).

Disadvantages: Schlotterer (2004) has questioned the random distribution of minisatellites across the genome (cited in Kumar et al., 2009).

Linkage and QTL Mapping

Missaoui et al. (2005) reported combined restriction fragment length polymorphism (RFLP) based linkage map from two outbred and genetically different parents Alamo AP13 (a tetraploid lowland cultivar) and Summer VS16 (a tetraploid upland cultivar). Okada et al. (2010) reported the first complete linkage maps of two switchgrass genotypes: Kanlow (as female parent) and Alamo (as male parent) using a full-sib population of 238 plants with use of SSR and STS markers. Liu et al. (2012) reported a complete, longest and most dense linkage map from 18 linkage groups in an inbred lowland switchgrass population using mostly codominant SSR markers. Various software packages are available for linkage and QTL mapping such as Join Map 4 (for linkage map construction), Map QTL 6 (for QTL

analysis), and many other software packages. Dong (2014) reported QTLs associated with reproductive maturity in switchgrass. D. Serba et al (2014) recently published QTLs underlying biomass yield and plant height in switchgrass.

Inbreds and Hybrids in Switchgrass

In nature, switchgrass is highly self-incompatible and highly outcrossing species but selfing can be enforced manually. A simple paper bag can be used for bagging to self switchgrass as is generally practiced in maize crop. Liu and Wu (2012) reported occurrence of high self-fertility in one lowland switchgrass NL94. Inbreeding depression is the unwanted side of selfing in outcrossing species. According to Charlesworth and Willis (2009), inbreeding depression is mainly caused by the cumulative effects of deleterious mutations at many loci, with probability of contribution from overdominance at a few loci. The intercrossing of surviving lines produces superior hybrids compared to their parents and frequently surpass the best parent values for several traits (Charlesworth and Willis, 2009). Inbreeding techniques can be employed to develop superior homozygous lines to be used as heterotic groups to produce hybrids. Hybrids can be obtained by simple bagging of selected parents together. The mechanism of self-incompatibility favors crossing inside the bag. The seeds can be harvested separately from each parent as mother and the other as father. The confirmation of inbreeding or crossing can be done by paternity test using codominant molecular markers such as simple sequence repeats (SSRs). SSRs have been successfully used in paternity test for inbred confirmation switchgrass (Todd et al., 2011). Martinez-Reyna and Vogel (2002) reported emasculation and pollination technique for hybridization in switchgrass. Commercially, hybrid cultivars are not yet available.

Selected Lowland Cultivars

'Alamo' was found at George West, TX and maintained by the Plant Genetic Resources Conservation Unit, Griffin. Burns et al. (2008a and 2008b) reported the development and registration of tow lowland cultivars 'BoMaster' and 'Performer' for the South eastern U.S. region. 'Cimarron' was developed as a synthetic cultivar by polycrossing seven elite clonal parents of Alamo origin (Wu and Taliaferro, 2008). 'Kanlow' was collected from a lowland site near Wetumka, OK.

Biofuels

According to Energy Independence and Security Act of 2007 (EISA, 2007), "conventional biofuel" means renewable fuel which is ethanol derived from corn starch; "advanced biofuel" means a renewable fuel, other than ethanol derived from corn starch, which are at 50% less than baseline lifecycle greenhouse gas (GHG) emissions; "biomass-based diesel" means renewable fuel which is biodiesel that is at 50% less than baseline lifecycle GHG emissions; and "cellulosic biofuel" means renewable fuel derived from any cellulose, hemicellulose or lignin that is derived from renewable biomass which is at 60% less than baseline lifecycle GHG emissions. Based on the genetic materials and agronomic technology available in 2000 and 2001, the average dry biomass yield of switchgrass was reported in the range 5.2-11.1 Mg ha^{-1} with resulting average net energy yield (NEY) of 60 GJ $ha^{-1}y^{-1}$ (Schmer et al., 2008). The renewable energy yield was 540% of the non-renewable energy consumed in the production of switchgrass (Schmer et al., 2008)

AFLP based genetic variation analysis is limited with regard to exclusive focus on lowland cultivars of switchgrass. Selfing can be enforced by bagging switchgrass panicle, however

there is no previous study available regarding development of inbred lines at the third generation (S3) and the fourth generation (S4) which can be used later to produce hybrids. More QTL work is needed to better understand genetic structure for many agronomic traits including plant height that can contribute in biomass yield and quality. Therefore, the objectives of this study were (i) to analyze genetic diversity among and within five lowland switchgrass cultivars ('Alamo', 'BoMaster', 'Cimarron', 'Kanlow', and 'Performer') using AFLP, (ii) to develop inbred lines at the third and the fourth generation by comparison of SSR alleles between offspring and the maternal parent, and (iii) to analyze phenotypic variation for biomass yield and plant height, and to localize QTLs associated with the plant height based on linkage maps developed in the two OSU populations (one being derived from selfing a northern lowland genotype 'NL94 LYE 16x13', and another from crossing 'NL94 LYE 16x13' and 'SL93 7x15').

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CHAPTER II

GENETIC VARIATION WITHIN AND AMONG LOWLAND SWITCHGRASS CULTIVARS AS REVEALED WITH AFLP POLYMORPHISMS

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a highly polymorphic and wind pollinated polyploid species with disomic inheritance (Nielson, 1944; Taliaferro, 2002; McLaughlin and Kszos, 2005; Okada, 2010; Liu and Wu, 2012). The ploidy level in switchgrass ranges from diploid (2n=2x=18) to duodecaploid (2n=12x=108) (Nielson, 1944). The two ecotypes in switchgrass are lowland and upland. 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 et al., 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 et al., 2010).

Genetic diversity is the result of selection, mutation, migration, genetic drift and/or recombination (De Vicente and Fulton, 2004). 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 and Fulton, 2004). Molecular markers are very useful tools to study genetic variation in many plants including switchgrass. Molecular markers generate a unique pattern of the DNA fragments of each individual arranged in a gel lane according to the fragment's molecular weight (base pairs). The pattern can be read as a visible DNA finger print. DNA Fingerprint refers to generation of distinct DNA fragments from a single DNA sample (Chial, 2008). These fragment patterns are related to genotypic information and hence are useful to calculate extent of genetic diversity in plants.

Molecular markers used in the evaluation of switchgrass diversity include random amplified polymorphic DNA (RAPD) (Gunter et al., 1996; Casler et al., 2007; Nageswara-Rao et al., 2013), restriction fragment length polymorphism (RFLP) (Missaoui et al., 2003; Missaoui et al., 2006), expressed sequence tag-simple sequence repeat markers (EST-SSRs) (Narasimhamoorthy et al., 2008; Cortese et al., 2010; Huang et al., 2011), 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). AFLP markers delineated upland and lowland ecotypes and also related plants according to broad geographic regions (Todd et al., 2011). Missaoui et al. (2006) used RFLP markers and determined extensive diversity between lowland tetraploid cultivar 'Alamo' (AP13) and upland tetraploid cultivar

‘Summer’ (VS16) (Missaoui et al., 2003). These cultivars AP13 and VS16 were later used to construct a linkage map (Missaoui et al., 2005).

The information on the extent of diversity in lowland cultivars will help to determine the specific cultivars to be used in the future breeding and crop improvement programs to develop potentially high yielding varieties of switchgrass. 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, development of linkage maps, identification of QTLs associated with biomass yield and different biomass yield related attributes, and QTLs associated with seed production. The QTL information can then be used in the marker assisted selection in switchgrass breeding.

Many of the recent molecular markers are based on polymerase chain reaction (PCR). PCR is a simple, automated technique for repeated copying of a short DNA molecule (Conner and Hartl, 2004). AFLP is a PCR based dominant marker and used in genetic research, DNA fingerprinting, and genetic engineering (Vos et al., 1995). It was first developed by Keygene company in Netherlands in 1990. It is a highly sensitive method for detecting polymorphisms in DNA. The technique was originally described by Vos et al. (1995).

Study on AFLP analysis for genetic diversity is limited with regard to exclusive consideration for lowland cultivars of switchgrass. Therefore, the objective of this experiment is to analyze genetic diversity among and within five lowland switchgrass

cultivars using AFLP. The cultivars included ‘Alamo’, ‘BoMaster’, ‘Cimarron’, ‘Kanlow’, and ‘Performer’.

MATERIALS AND METHODS

Plant Materials and Genomic DNA Extraction

Plant materials consisted of 384 plants from five lowland cultivars ‘Alamo’, ‘BoMaster’, ‘Cimarron’, ‘Performer’, and ‘Kanlow’. Seventy-six plants from the cultivar ‘Performer’ and 77 plants from each of the remaining four cultivars (Table 1) 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. Genomic DNA samples were extracted from healthy leaf tissues for each plant using Zymo Research ZR Plant/Seed DNA Kit™ (Zymo Research Corporation, CA) according to the manufacturer’s instructions. DNA quality was checked with 1% agarose gel electrophoresis and GelDoc-It™ TS Imaging System (UVP, Upland, CA). DNA quantity was measured in a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The DNA samples were diluted to a final concentration of 100 ng μL^{-1} before enzyme digestion.

In the study for genetic variation among the five cultivars, a total of 64 plants were used including 12 plants from the cultivar ‘Performer’ and 13 plants from each of the remaining four cultivars. The within genetic variation was studied separately for each of the five cultivars. A total of 64 plants from each of the five aforementioned cultivars were used. The decision to use the above mentioned numbers were based on capacity of

polyacrylamide gel which accommodates 64 sample lanes and two additional size marker lanes in a LI-COR 4300 DNA Analyzer.

AFLP Analysis

AFLP analysis was performed according to Vos et al. (1995), with minor modifications as described by Wu et al. (2005) and Todd et al. (2011). In the first step, the genomic DNA was double digested with *Eco*RI and *MSe*I 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 2.1) were used for selective amplification. The *Eco*RI 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 (Communication with Dr. Yanqi Wu). 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 the first and the 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.

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 collect data accurately. 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.

Analysis of molecular variance (AMOVA), Nei's (1972) based genetic diversity calculation, Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) were performed using GenAlEx 6 (Peakall and Smouse, 2006, 2012). AMOVA and Nei's distance calculation were performed in among variation data and 'I', 'He', and 'uHe' were computed separately for each of the five cultivars (Table 2.7). AFLP bands initially scored as present (1), absent (0), and ambiguous (9) 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 (1972) distance.

RESULTS AND DISCUSSIONS

A total of 384 plants grown in greenhouse at Oklahoma State University were used in the experiment. Table 2.1 shows polymorphic band information 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 2.1). 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 are shown in Table 2.2 and the tables for each of the cultivars separately are not provided but summary of those tables are provided in Table 2.3.

'Alamo' exhibited the highest genetic variation (coefficient of variation=9.53) and 'Performer' exhibited the lowest (coefficient of variation=4.21) (Table 2.3). Analysis using five cultivars together showed 'A4' from 'Alamo' and 'P4' from 'Performer' were the most divergent (similarity coefficient=0.60) (Table 2.3). The average similarity coefficient ranged from 0.76 to 0.82 indicating the presence of high genetic variation among switchgrass genotypes.

The cluster analysis in AFLP variation among five cultivars generated a dendrogram with a small cluster (L) that included genotypes P4, P6, and P8 from cultivar 'Performer' and big cluster (M) which included the remaining 61 genotypes (Fig. 2.1). A genotype C4 from cultivar 'Cimarron' was observed separate from rest of the individuals

in that 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. 2.7) mostly consistent with the clusters generated from the cluster analysis. The principal coordinate 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 coordinate analysis are mostly congruent for AFLP variation within each of the five cultivars ‘Alamo’, ‘BoMaster’, ‘Cimarron’, ‘Kanlow’, and ‘Performer’ (Fig. 2.2-2.6 and Fig. 2.8-2.12). In these five cultivars, the first principal coordinate explained 11.80, 9.38, 8.09, 7.98, and 11.4% variations, respectively while the second principal coordinate explained 5.90, 6.27, 5.61, 5.72, and 4.33% variations, respectively.

Mantel test results are shown in Table 2.4. 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% (Table 2.5).

Nei's genetic diversity revealed the lowest genetic distance between cultivars 'Alamo' and 'Cimarron' and highest value between cultivars 'Alamo' and 'Kanlow' (Table 2.6). 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 2.7).

The cultivars 'Alamo' and 'Kanlow' were developed from wild germplasm sources. 'Alamo' (PI 422006) was the cultivar collected from George West, TX (USDA, GRIN) and 'Kanlow' was initially collected in 1957 at a lowland site near Wetumka, OK (USDA, GRIN). 'Kanlow' (PI 421521) accession was developed as a cultivar by a cooperative effort of Kansas AES and Plant Science Research Division, 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 of seven elite clonal parents in 2001 at Oklahoma State University (Wu and Taliaferro, 2009). The selection of parent plants for 'Cimarron' was based on the evaluation of biomass yield of their half-sib families (Wu and Taliaferro, 2009). The dendrogram and two dimensional plot showed 'Alamo' and 'Cimarron' in the same group exhibiting the genetic relatedness consistent with the pedigree information.

'BoMaster' and 'Performer' switchgrass cultivars were developed by North Carolina Agricultural Research Service, NC (Burns et al., 2008a, 2008b). Both 'BoMaster' (Reg. No. CV-248, PI 645256) and 'Performer' (Reg. No. CV-247) 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. In terms of geographic location, ‘Alamo’ and ‘Cimarron’ cultivars belong to relatively southern parts of USA when compared with ‘Kanlow’, ‘BoMaster’, and ‘Performer’. Self-incompatibility and inter-cultivar gene flow are characteristics of switchgrass and hence the clusters of mixed genotypes can also be possible.

CONCLUSIONS

The presence of high genetic variation was observed within lowland switchgrass cultivars. The highest genetic variation was observed in ‘Alamo’ while the lowest variation was observed in ‘Performer’. ‘A4’ from ‘Alamo’ and ‘P4’ from ‘Performer’ were the most divergent genotypes. ‘Alamo’ and ‘Cimarron’ were grouped 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 study would be useful for future plant breeding and crop improvement programs in switchgrass.

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LIST OF TABLES

LIST OF FIGURES

Table 2.1. 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	e-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	29.1	14	6

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

Table 2.2. Similarity coefficients among five lowland switchgrass cultivars. Each plant genotype ID 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 2.2. Similarity coefficients among five lowland switchgrass cultivars. Each plant genotype ID 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 2.2. Similarity coefficients among five lowland switchgrass cultivars. Each plant genotype ID 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 2.3. 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 2.4. Mantel's test. Criteria for goodness of fit of the dendrogram to dissimilarity matrix: $r \geq 0.90$ very good fit, $0.9 > r \geq 0.80$ good fit, $0.80 > r \geq 0.70$ poor fit, and $r < 0.70$ very poor fit (Rohlf, 1998).

Tests for association	Among cultivars	Alamo	BoMaster	Cimarron	Kanlow	Performer
Matrix correlation (r) (= normalized Mantel statistic Z)	0.77	0.95	0.96	0.89	0.76	0.82
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 2.5. Analysis of molecular variance (AMOVA) for AFLP variation among five cultivars ‘Alamo’, ‘BoMaster’, ‘Cimarron’, ‘Kanlow’, and ‘Performer’.

Sources of variation	df	Sum of squares	MS	Est. Var.	%
Between cultivars	4	980.53	245.13	13.34	15%
Within cultivar	59	4388.81	74.39	74.39	85%
Total	63	5369.34		87.73	100%

Table 2.6. 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

Table 2.7. 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

Fig. 2.1. 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.

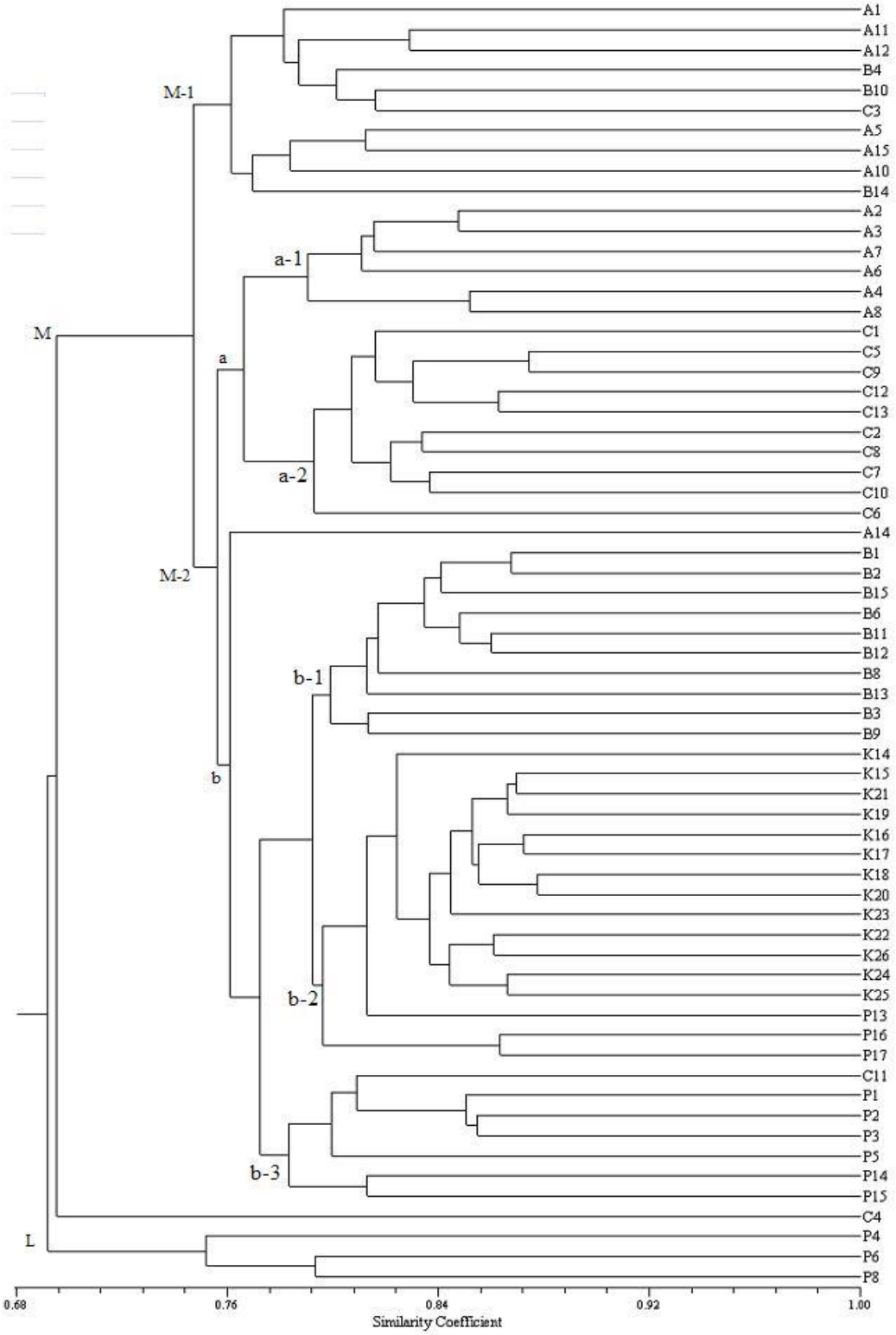


Fig. 2.2. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar ‘Alamo’. A represents cultivar ‘Alamo’.

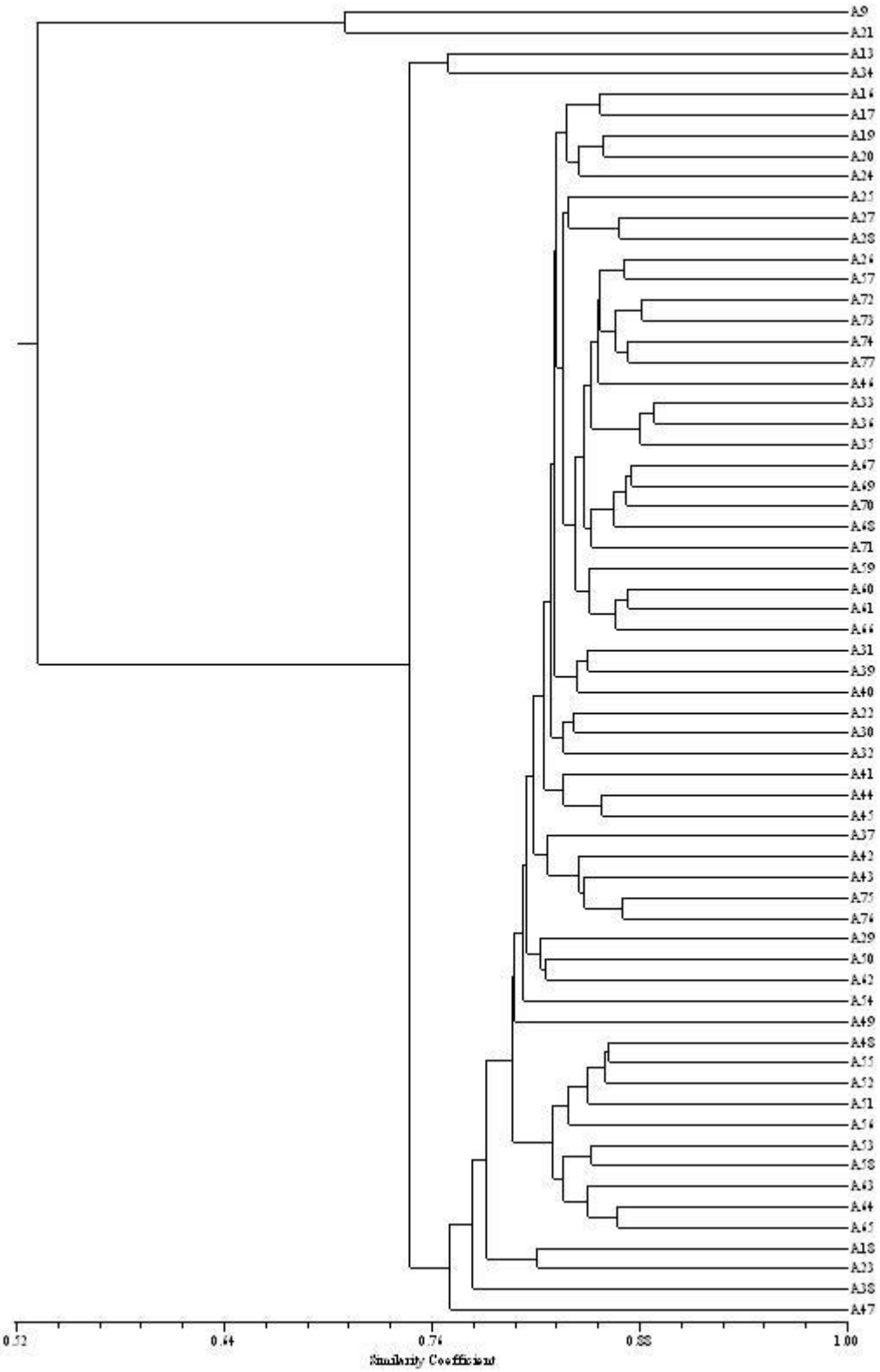


Fig. 2.3. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar ‘BoMaster’. B represents cultivar ‘BoMaster’.

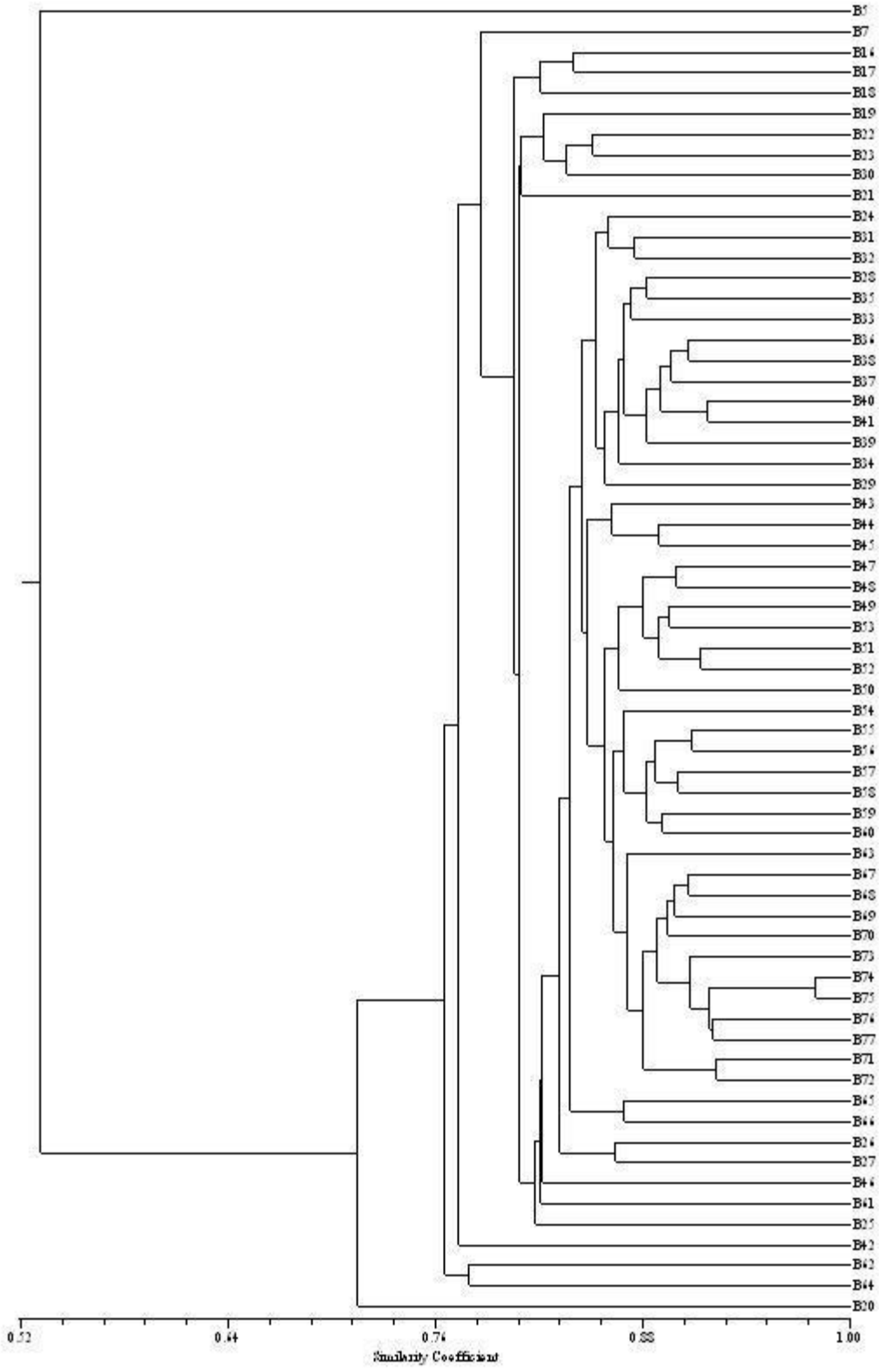


Fig. 2.4. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar ‘Cimarron’. C represents cultivar ‘Cimarron’.

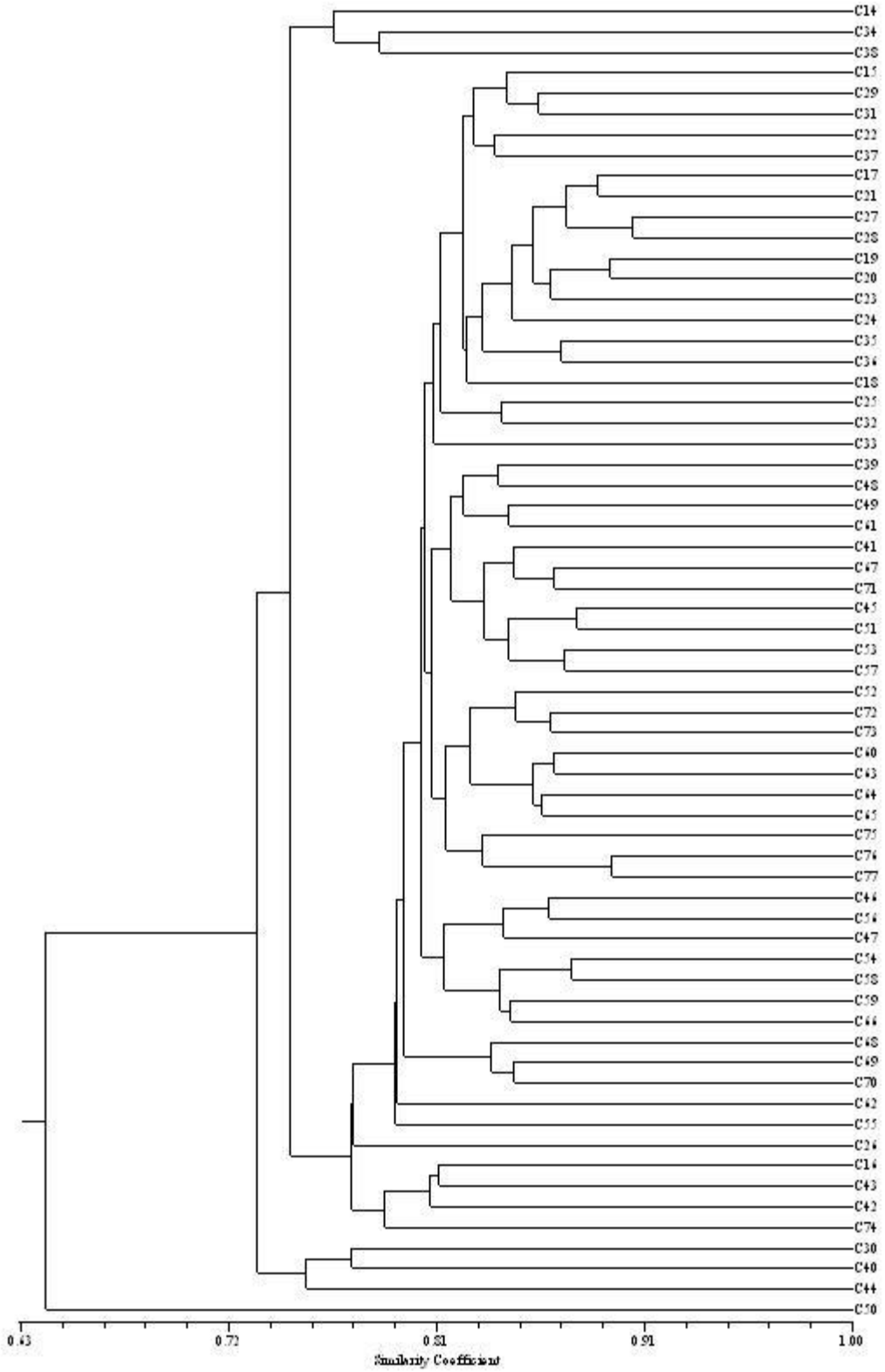


Fig. 2.5. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar ‘Kanlow’. K represents cultivar ‘Kanlow’.

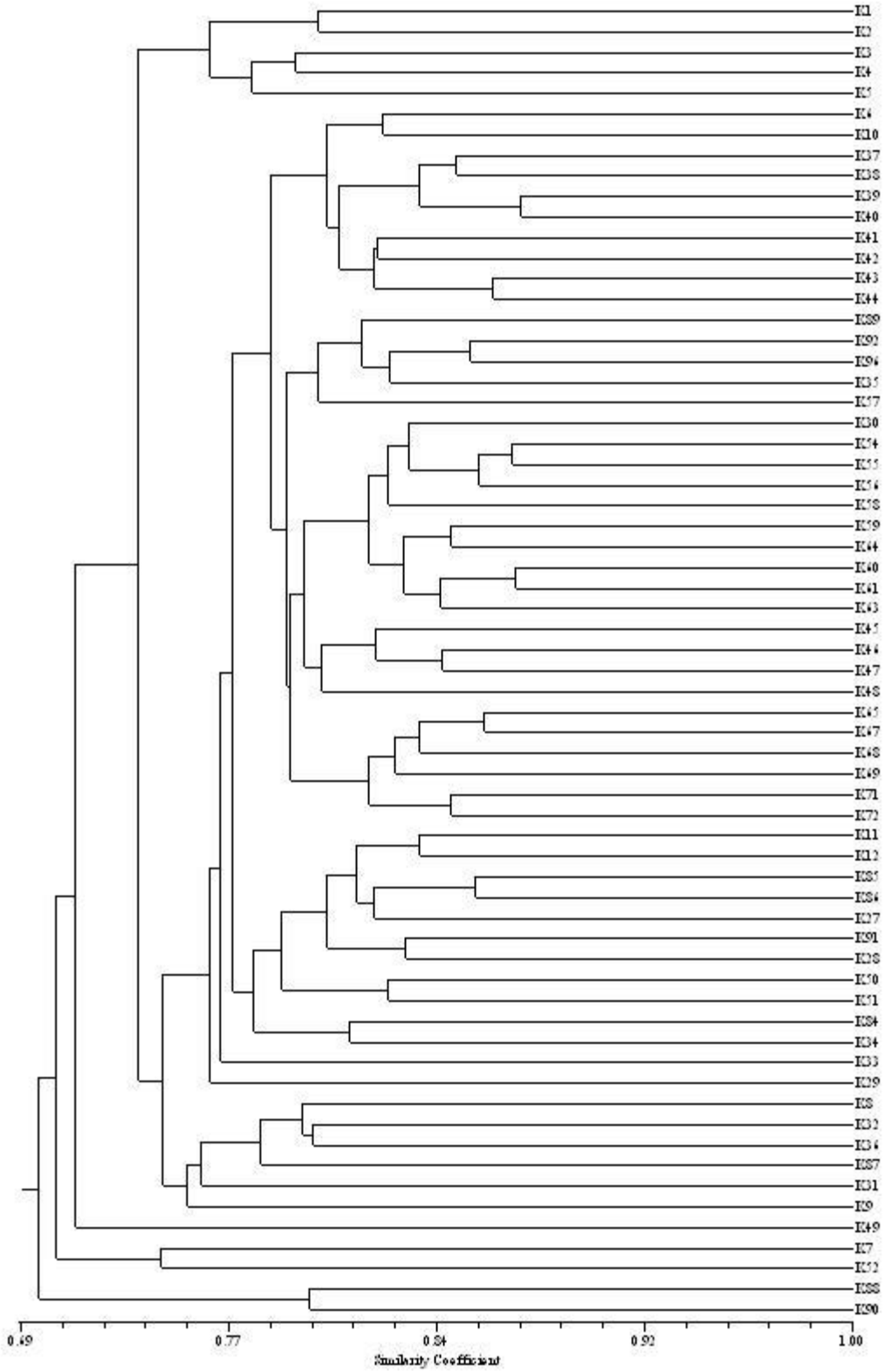


Fig. 2.6. UPGMA tree of similarity coefficients (dendrogram) obtained from AFLP variation within cultivar ‘Performer’. P represents cultivar ‘Performer’.

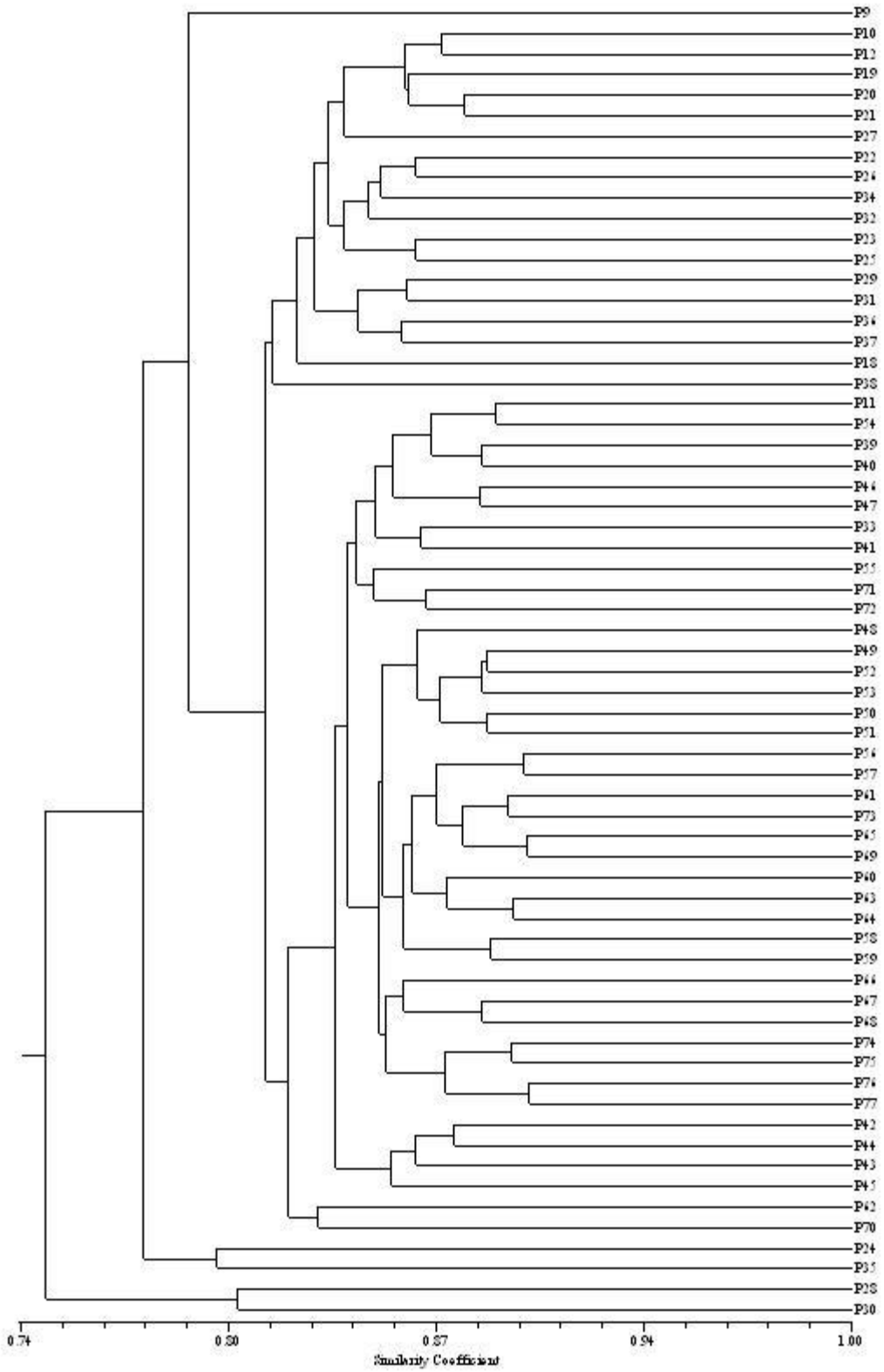


Fig. 2.7. Principal coordinates analysis indicating two major groupings in AFLP variation among five cultivars. PC-1 and PC-2 are two major principal coordinate axes.

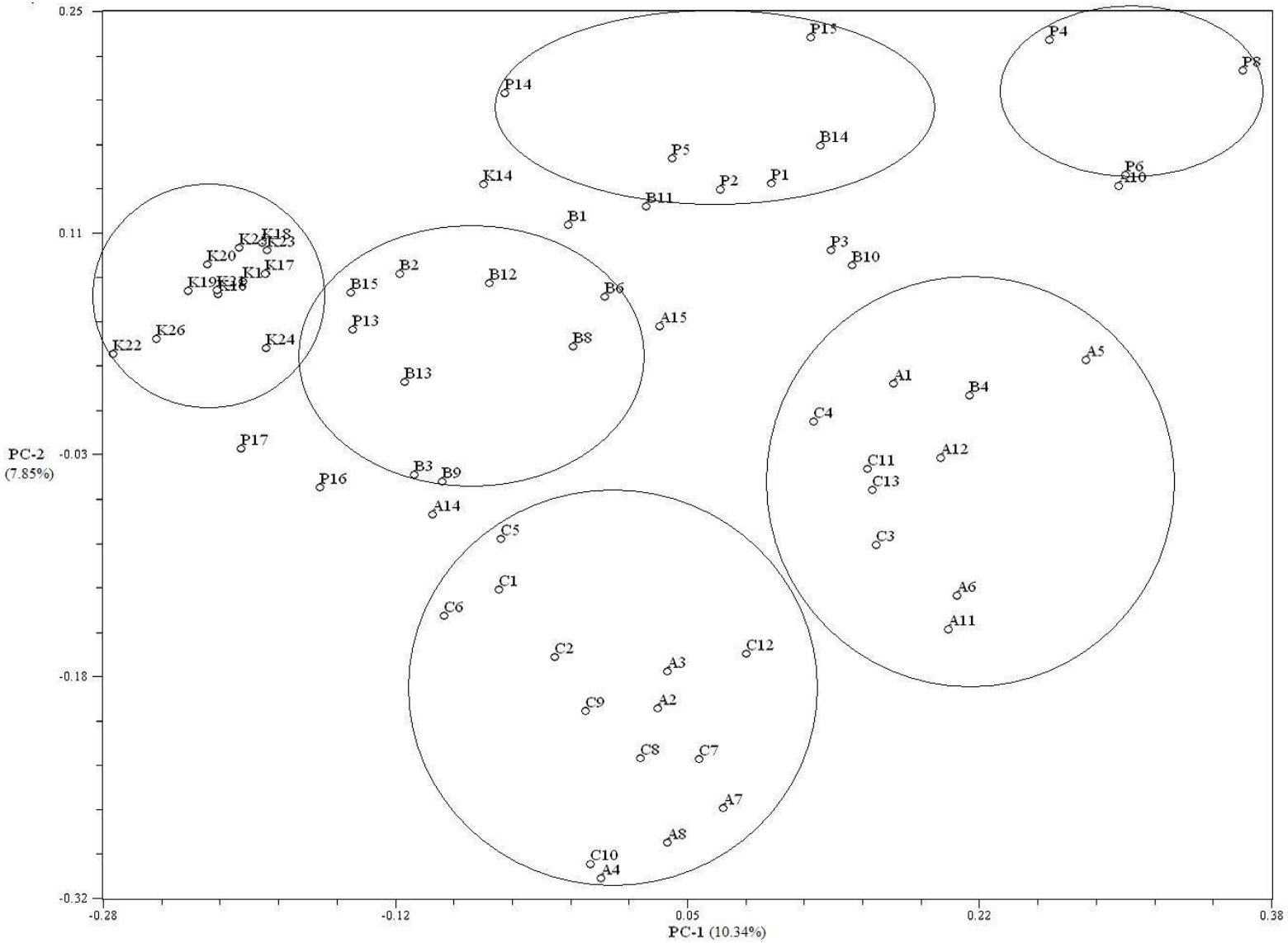


Fig. 2.8. Principal coordinates analysis in ‘Alamo’. PC-1 and PC-2 are two major principal coordinate axes.

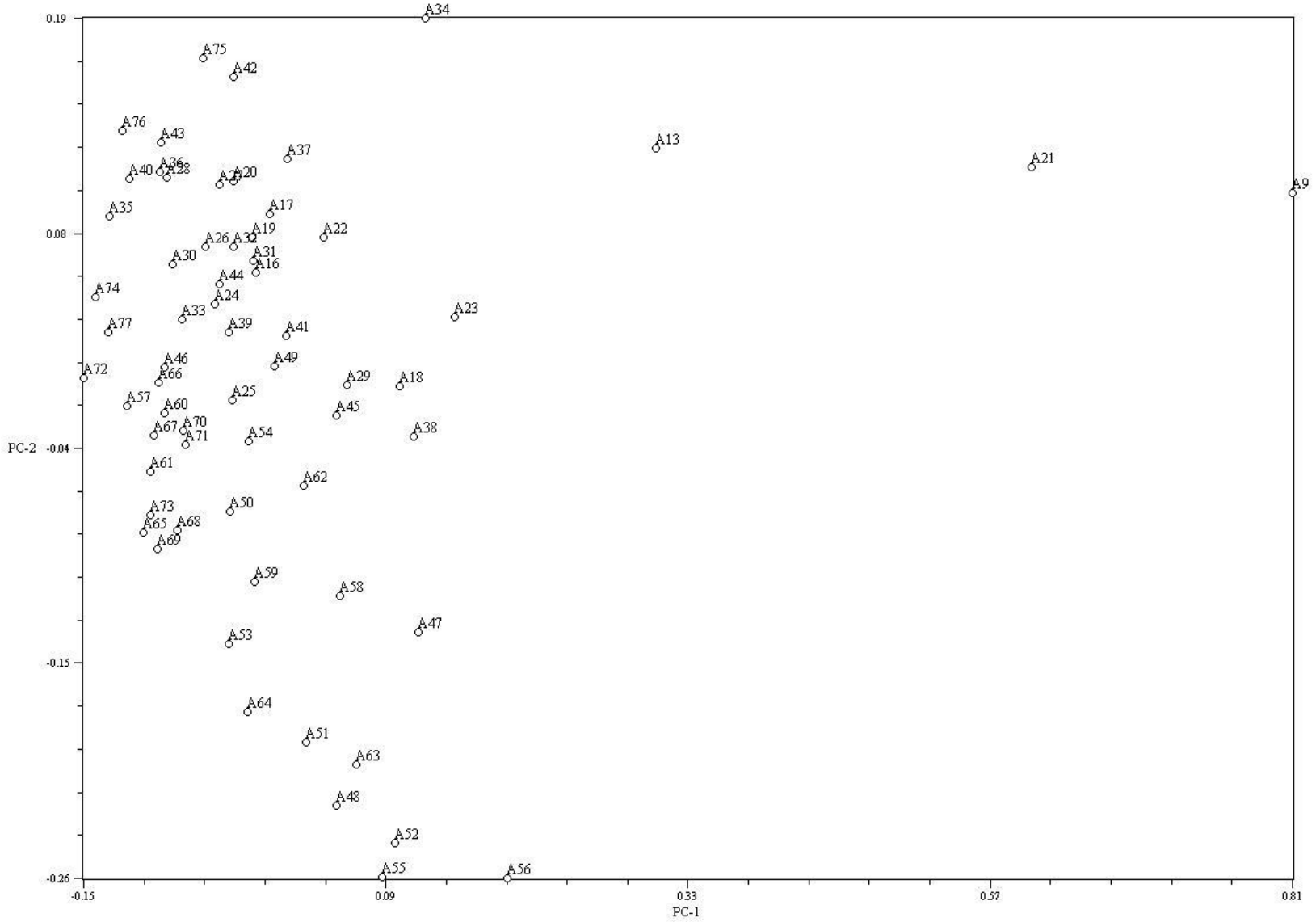


Fig. 2.9. Principal coordinates analysis in ‘BoMaster’. PC-1 and PC-2 are two major principal coordinate axes.

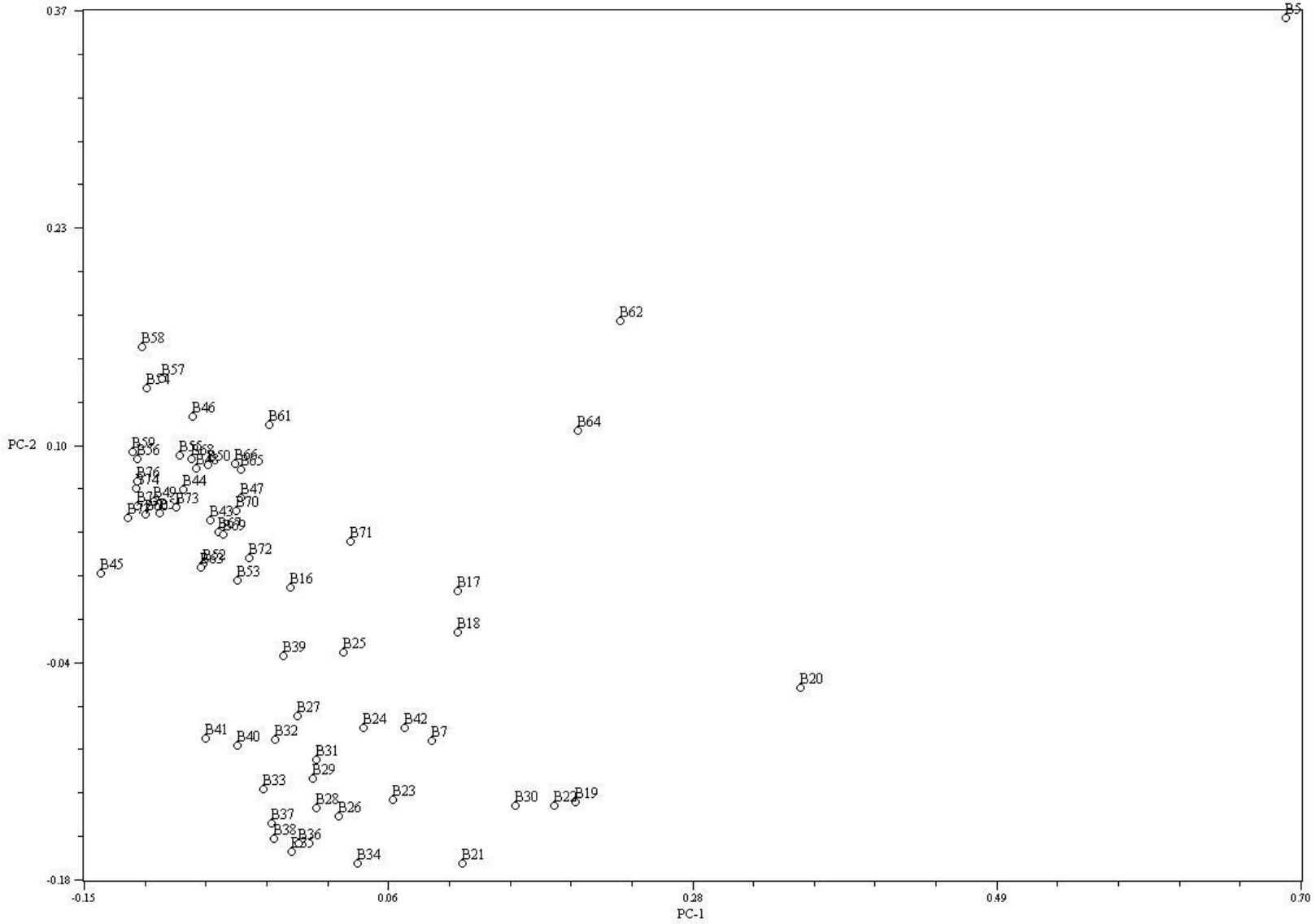


Fig. 2.10. Principal coordinates analysis in ‘Cimarron’. PC-1 and PC-2 are two major principal coordinate axes.

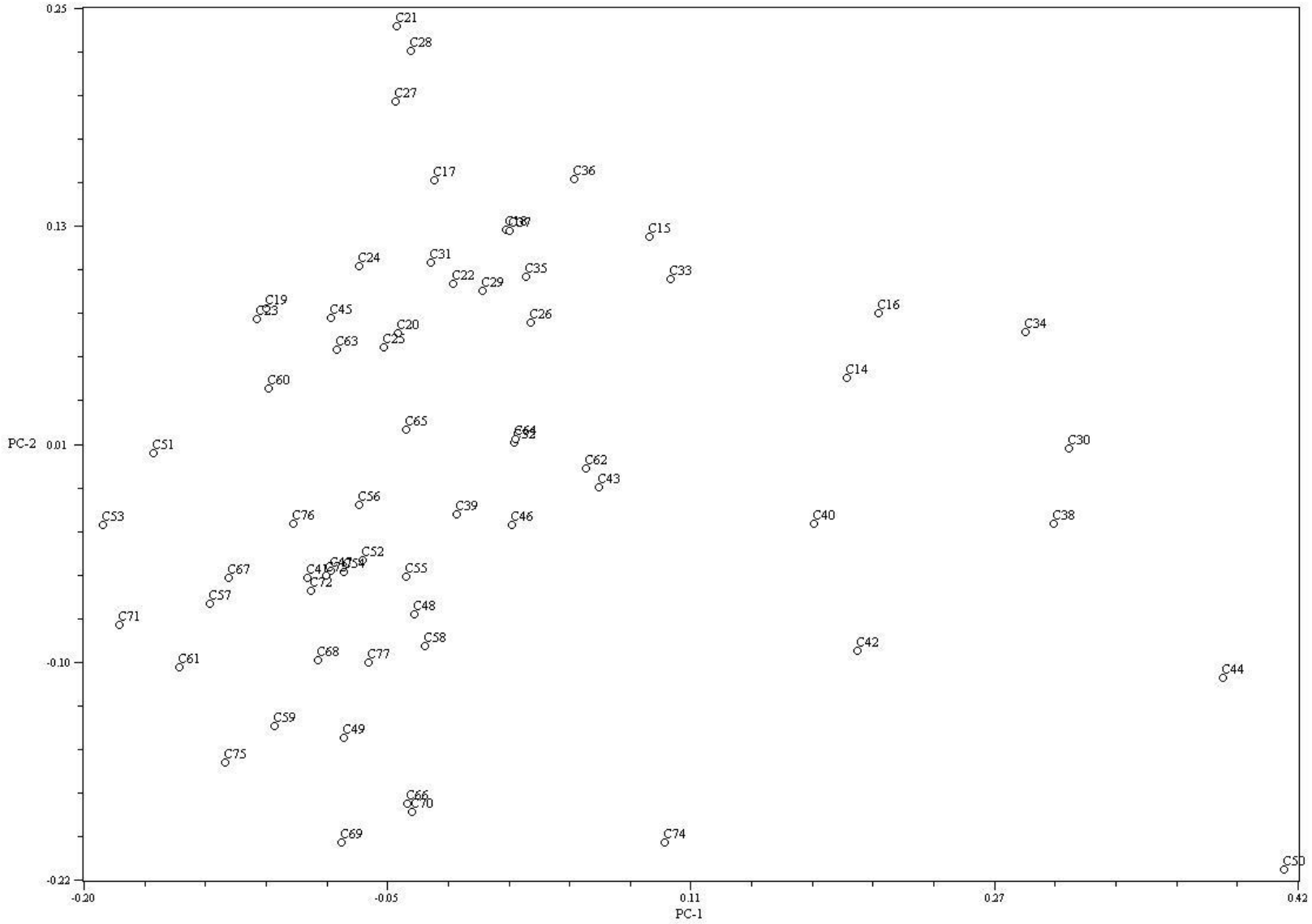


Fig. 2.11. Principal coordinates analysis in ‘Kanlow’. PC-1 and PC-2 are two major principal coordinate axes.

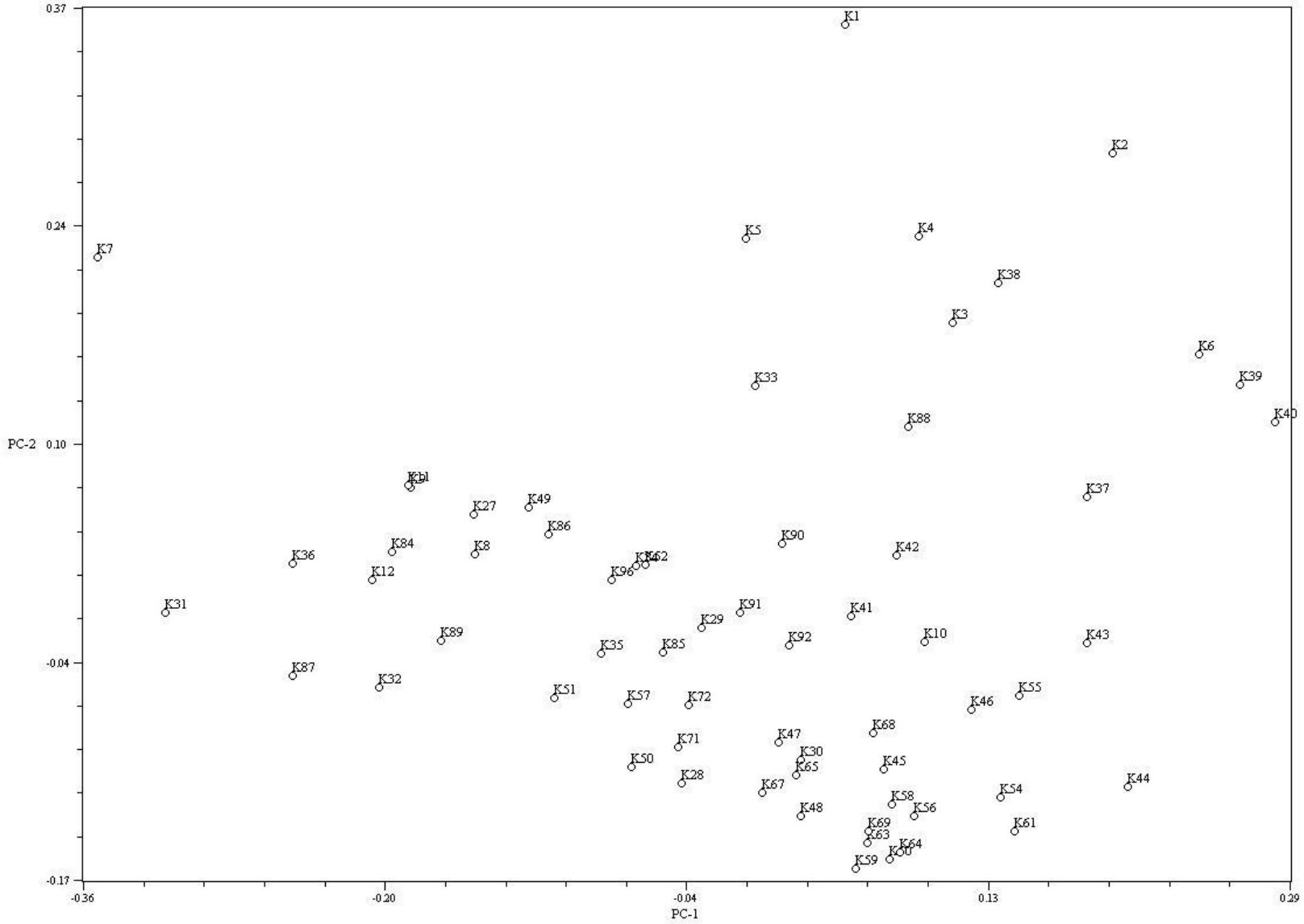
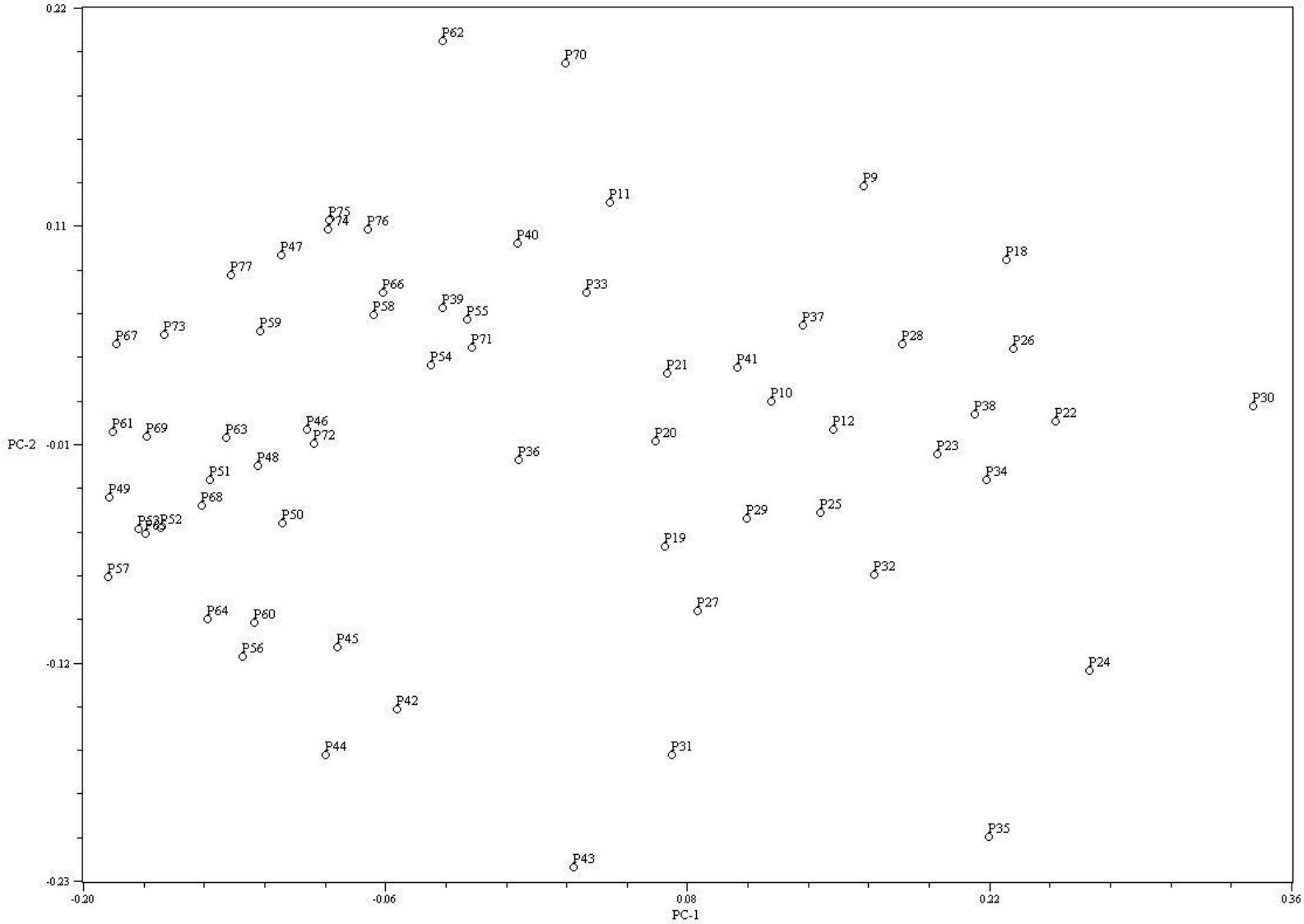


Fig. 2.12. Principal coordinates analysis in ‘Performer’. PC-1 and PC-2 are two major principal coordinate axes.



CHAPTER III

INBREDS DEVELOPMENT IN LOWLAND SWITCHGRASS ASSISTED WITH SSR MARKERS

INTRODUCTION

Self-Incompatibility in Switchgrass

Switchgrass (*Panicum virgatum* L.) is a perennial, self-incompatible, and highly outcrossing grass species pollinated by wind. It produces very little or no seed upon self-pollination (Taliaferro and Hopkins, 1996; Martinez-Reyna and Vogel, 2002) and has disomic inheritance (Okada, 2010; Liu and Wu, 2012). Ploidy level, a characteristic of ecotype, ranges from diploid (2n=2x=18) to duodecaploid (2n=12x=108) (Nielson, 1944). Most of the switchgrass plants are tetraploid or octaploid (Hopkins et al., 1996; Martinez-Reyna and Vogel, 2002). The lowlands are tetraploid but the uplands can be tetraploid or octaploid or very rarely hexaploids (Narasimhamoorthy et al., 2008; Nielsen, 1944).

Taliaferro and Hopkins (1996) reported a crossability (calculated as seed set divided by florets and expressed as percentage) of 0.06% for the cross between octaploid (female) and tetraploid (male) but no seed from reciprocal crosses (cited in Martinez-Reyna and Vogel, 2002). In a cross in switchgrass, seeds are harvested from one of the parents while the seeds harvested from remaining parent represent seeds from reciprocal cross. Martinez-Reyna and Vogel (2002) reported that there should be a strong genetic barrier that prevents inter-ploidy gene flow. Martinez-Reyna and Vogel (2002) reported self-compatibility for tetraploid parents at 0.35% and octaploid parents at 1.39%, and the reports were consistent with previous findings by Talbert et al. (1983) and Taliaferro and Hopkins (1996) as cited in Martinez-Reyna and Vogel (2002).

Two different types of self-incompatibility available in flowering plants are sporophytic self-incompatibility (SSI) and gametophytic self-incompatibility (GSI). Switchgrass possesses GSI with two multiallelic loci S and Z which segregate independently (Martinez-Reyna and Vogel, 2002). When both S and Z alleles of a pollen grain are matched in the recipient pistil, the pollen grain becomes incompatible (Baumann et al., 2000). Depending on the genotypes, the degree of compatibility can be either 0, 50, 75 or 100%. For example, a cross between a plant with genotypes $S_{1.1}Z_{1.2}$ and $S_{1.2}Z_{1.2}$ will show the pollen donor with 75% compatible pollen grains and the reciprocal with 50% compatible pollen grains (Baumann et al., 2000).

Martinez-Reyna and Vogel (2002) showed three seed forms in selfing switchgrass, in addition to unfertilized ovaries: (i) small seed with limited endosperm development, (ii) small seed, generally in brownish color with shriveled endosperm, and (iii) normal seed. The first two abnormal mechanisms are responsible for post-

fertilization incompatibility in switchgrass (Martinez-Reyna and Vogel, 2002) and this incompatibility is independent of the pre-fertilization barriers imposed in matings (Martinez-Reyna and Vogel, 2002). Plant processes including fertilization and seed development are controlled by genetic and environmental factors. Martinez-Reyna and Vogel (2002) obtained 17 crosses [9 crosses in tetraploids ('Kanlow' and 'Summer') and 8 crosses in octaploids ('Pathfinder' and 'IL62')] and their respective reciprocal crosses. In each of the crosses, they obtained compatible and incompatible pollens. Pollen was assumed to be incompatible when a pollinated floret had no embryo developed (Martinez-Reyna and Vogel, 2002) and the failure of embryo development could reflect post-zygotic environmental and genetic effects (Martinez-Reyna and Vogel, 2002). When an embryo developed without normal endosperm, pollen was considered compatible but it reflected post-fertilization incompatibility. If normal seed was produced, then pollen was compatible and post-fertilization incompatibility was absent. The results of Martinez-Reyna and Vogel (2002) indicated (1) prefertilization incompatibility in switchgrass was under gametophytic control, and (2) the involvement of more than one locus in incompatibility determination. From these study results, Martinez-Reyna and Vogel (2002) showed switchgrass with S-Z incompatibility system similar to the other members of the Poaceae. In interploidy crosses made by Martinez-Reyna and Vogel (2002), (1) seed obtained was small and shriveled from tetraploid plant as female, and (2) seed obtained was small with floury endosperm from octaploid plant as female. These results were consistent with past study reports (reviewed by Martinez-Reyna et al., 2002). In summary, Martinez-Reyna and Vogel (2002) reported (1) self-pollinated switchgrass showed presence of maximum expression of prefertilization incompatibility and absence

of postfertilization incompatibility, (2) cross pollination expressed both prefertilization and post fertilization incompatibility, (3) Interploidy crosses showed maximum expression of postfertilization incompatibility, but presence of prefertilization incompatibility was also possible. The study of Martinez-Reyna and Vogel (2002) indicated existence of pre-fertilization incompatibility in switchgrass which was similar to the S-Z system previously reported in other members of Poaceae (reviewed by Martinez-Reyna and Vogel (2002). Their study also indicated presence of postfertilization incompatibility system that inhibited interploidy crossings to develop mature seeds (octaploids and tetraploids).

Overcoming Self-incompatibility in Switchgrass

Bagging inflorescences of switchgrass plants can produce inbred lines. However, study on biochemical and molecular mechanisms of breakdown of self-incompatibility (SI) by bagging are still unknown. In perennial ryegrass, additional loci independent of S and Z have been reported to cause the breakdown of SI (Thorogood et al., 2005; Aguirre, 2013). In perennial ryegrass species, self-fertility (SF) is monogenetically inherited and dominant (Aguirre, 2013). Jenkin (1931) concluded that self-fertility was genotype-dependent and it should be possible to produce fully self-fertile plants (cited in Thorogood and Hayward, 1991). In switchgrass, such study reports are not available yet to our information. High temperatures can induce pseudo-compatibility and can be used to overcome SI (reviewed by Thorogood and Hayward, 1991) in perennial ryegrass. Thorogood and Hayward (1991) opine that it is possible to use optimal temperatures to achieve pseudocompatibility for inbred seed production. As a safe alternative to temperature treatment, Thorogood and Hayward (1991) further brings idea of application

of sprayable compounds. Switchgrass at present does not have any study from that sort of perspectives to our information. Matsubara (1984), to overcome self-incompatibility, applied three kinds of plant hormones, sucrose, three kinds of amino acids, and two kinds of vitamins to test the cultivars (cvs.) Honbashi-taibyo Minowase (H-Mino) and Minowase (Mino) of *Raphanus sativus*. Practicality and feasibility study of such approach is not available in switchgrass yet to our information.

Selfing in Switchgrass

Selfing is beneficial in the production of superior genotypes for selection (McClosky et al., 2013). In subsequent generations, selfing decreases additive genetic variation within the lines while it increases additive genetic variation between the lines (McClosky et al., 2013; Cornish, 1990). With the combination of advanced inbred lines and QTL localization, cultivar development cycles can also be shortened. McClosky et al. (2013), based on their simulation study, reported that fully inbred candidates for potential commercialization can be identified as early as the F_4 generation (McClosky et al., 2013).

Kenna et al. (1991), in their study of inbreeding gamagrass (*Tripsacum dactyloides* L.), stated that inbreeding can be useful in exposing recessive alleles existing at low frequencies and in the development of inbred lines. Yield improvement (heterosis) can be obtained by intercrossing inbred strains (Charlesworth and Willis, 2009). Classical genetic studies and modern molecular evolutionary approaches at present indicate that the presence of recessive deleterious mutations in populations is the main reason for inbreeding depression and heterosis (Charlesworth and Willis, 2009). Although the extreme low survival and fertility rates of individuals are observed in experimentally

produced inbred lines and the lines may even go extinct (Charlesworth and Willis, 2009), the intercrossing of surviving lines produces the hybrids that often possess better qualities than their parents and frequently surpass the best parent values for several characters (Charlesworth and Willis, 2009). Therefore inbreeding practices in switchgrass can also be useful to reduce unfit alleles and to increase favorable alleles.

Cross pollinated plants generally suffer from severe inbreeding depression but hybrid vigor (heterosis) is generally restored upon crossing (Bernardo, 2002). Crossing of two inbreds results in a single cross (hybrid) which is 100% heterozygous at the loci that are different between the two inbreds (Bernardo, 2002). Since the progeny obtained from the hybrids would suffer from inbreeding depression, new hybrid seed is required for each planting season (Bernardo, 2002). An alternative is a synthetic cultivar which is produced by intermating six or more inbreds and planting the resulting seeds as the cultivar (Bernardo, 2002). Synthetics minimize the inbreeding depression resulting from open-pollination in a hybrid while exploiting some amount of hybrid vigor (Bernardo, 2002).

Homozygosity is achieved much faster in disomic inheritance compared to tetrasomic inheritance (Liu and Wu, 2012). Knowledge of self-incompatibility helps in effective utilization of germplasms of a species in a breeding program (Martinez-Reyna and Vogel, 2002). Switchgrass is highly self-incompatible grass species in open pollinating conditions, however selfing can be enforced by bagging.

The occurrence of high self-fertility (self-compatibility) has been previously reported in one lowland switchgrass plant, ‘NL94 LYE 16×13’ (NL94) confirmed with

simple sequence repeat-based molecular markers (Liu and Wu, 2012). Self-compatibility is useful in the development of inbred lines for use in producing hybrid cultivars (Liu and Wu, 2012). Todd (2011) reported the development of S1 and S2 inbreds by bagging panicles of selected switchgrass plants. Although selfing can be enforced by bagging switchgrass panicles, there is no previous study available regarding development of advanced inbred lines (S3 and S4) that can be used later to produce hybrids. Therefore, the objectives of this study were to develop (i) S_3 inbreds from confirmed S_2 populations by comparison of SSR alleles between offspring (S_3) and the maternal parent (S_2), and (ii) S_4 inbreds from confirmed S_3 populations.

MATERIALS AND METHODS

Field Preparation and Plant Materials

The experimental fields were located at the Oklahoma State University Agronomy Research Station in Stillwater, OK. The original parent population established in May 16, 2000 was used in the study. The soil type for the nursery plots of original S_0, S_1, S_2, S_3, and S_4 plants was Kirkland silt loam. Initially, by selfing of the selected parent plants in the original S_0 parent population, S_1 population was obtained and established at Agronomy Research Station. The S_1 population was used in the development of S_2 inbred population by Todd (2011). The confirmed S_2 inbreds developed by Todd (2011) were used in the present study as a continuation for the development of the third and the fourth generation inbred lines (S_3 and S_4). The plants belonged to one of the two lowland cultivars ‘Alamo’ and ‘Kanlow’. The pedigree information of S2, S3, and S4 plants was

provided in Table 3.2. The layouts for S2, S3, S3 additional, and S4 fields were shown in Figs. 3.1 – 3.4.

Bagging, Seed Harvesting and Cleaning

The inflorescences of confirmed inbred plants were paper bagged (Lawson 17.1 cm x15.9 cm x12.1 cm x 39.4 cm No. GB504) before the anthesis. S_2 and S_3 plants were bagged in September of 2010 and 2012 respectively. The bags with inflorescence inside were tied at the base of the inflorescence and anchored to an iron pole using a metal wire. After about one month from bagging, mature inflorescences in the bag were collected manually for each plant for seed harvest and stored at room temperature for 3 to 4 weeks. The seeds were then separated from the panicles by using rubbing boards and sieves. The seeds were cleaned in a South Dakota Seed Blower (Seedburo Equipment Co., IL) to get rid of empty seeds, contaminating seeds (weed seeds), all light materials and chaffs leaving only heavy and healthy seeds (Fig. 3.6). The clean seeds were then put in labeled paper bags.

Prechilling, Greenhouse Growing, and Field Planting

For each sample, seed counts ≤ 30 were pre-chilled in Petri dishes. Two layers of tissue paper (C-Fold Towels, Scott Brand of Kimberly-Clark Professional) cut in circle shape were put in a Petri dish (60x15 mm diameter by height) (VWR, Denver, CO). The paper layers were moistened with Millipore water (tap water can also be used) and seeds were put over the paper. The seeds were then covered by another two layers of tissue papers. The cover plate of the Petri dish was put and sealed with paraffin paper membrane. The prepared seed samples in Petri dishes were then stored at 4^{o}C for 2

weeks. The remaining seed samples were stored in the cold storage. The pre-chilled seeds were germinated in the greenhouse. Finally, the confirmed inbreds were transplanted in the field. Fig. 3.7-10 show seeds germinated in rectangular plastic pots in greenhouse, seeds covered with plastic cover to conserve moisture, putative S_3 seedlings growing in rectangular plastic pots, and putative S_3 seedlings growing in conetainers after being transplanted from plastic pots.

DNA Extraction for Both S3 and S4 Seedlings

Prechilled seeds were germinated in 4-inch rectangular 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 germinated seedlings were later transferred to conetainers. Healthy leaf tissues were collected from parent plants from the field and progeny seedlings from the greenhouse. Genomic DNA samples were extracted from healthy leaf tissues for each plant using the CTAB method (Doyle and Doyle, 1990) with some modifications. DNA quality was checked with 1% agarose gel electrophoresis and GelDoc-It™ TS Imaging System (UVP, Upland, CA) and the DNA quantity was measured in a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The extracted DNA samples were adjusted to a final concentration of 10 ng μl^{-1} for PCR reactions.

PCR, Gel electrophoresis, and SSR Marker Scoring

SSR analysis was performed according to Wu and Huang (2008). The PCR reactions were performed on 96-well plates on 2720 Thermal Cyclers (Applied Biosystems, CA). The SSR PCR reaction mixtures (total volume 10.5 µl) consisted of 1.5

µl of 10 ng μl^{-1} template DNA, 7.35 µl of nuclease free water, 1 µl of 10× standard reaction buffer, 0.2 µl of 10 mM of dNTP, 0.05 µl of 5U μl^{-1} Taq DNA polymerase (enzyme), 0.2 µl of 1 µM SSR forward primer, 0.2 µl of 1 µM SSR reverse primer, 0.2 µl M13 forward primer labeled with fluorescent dye either in 700 or 800 nm. The PCR cycling parameters were set for 5 min at 94 ºC, 14 cycles each of 20 s at 94 ºC, 1 min at 58 ºC and 30 s at 72 ºC, followed by 28 cycles each of 20 s at 94 ºC, 1 min at 55 ºC and 30 s at 72 ºC, and finally an extension of 10 min at 72 ºC. To each PCR reaction, 5 µl Blue Stop Solution (95% formamide, 25 mM EDTA, and 2% bromophenol blue) was added (making the total volume 15.5 µl), mixed thoroughly, and then denatured in thermal cycler for 3 min at 94°C. In order to load two plates in a single gel, a 700 nm dye labeled plate and a 800 nm dye labeled plate were mixed thoroughly and pooled the contents together in a single plate. The contents were then loaded into wells of 6.5% KB plus polyacrylamide gel solution on a LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE) and allowed to run for 1 hr 45 min to separate amplified fragments of SSR alleles. Table 3 shows SSR primer pairs (PP) used in inbreeding confirmation for S3 families of switchgrass developed by Wang et al. (2011). The gel images of SSR markers were scored visually for each DNA sample by uploading the gel image on SAGA Generation 2 Lite (LI-COR Biosciences, Lincoln, NE).

RESULTS

The total number of plants in the S_2 field was 544 which included 195 confirmed inbreds and 349 plants that were not inbreds (Todd, 2011). Inbred confirmation was done by comparing parent-offspring identification using SSR markers. A total of 195 S_2 inbreds included 45 ‘Alamo’ plants and 150 ‘Kanlow’ plants. In the S_2 field, one plant

was found dead and seven plants did not produce any inflorescence. Therefore a total of 187 S_2 plants out of 195 plants were bagged in September and harvested in October, 2010 to constitute putative S3 inbred seeds. Fig. 3.5. shows selfing of S_2 plants in the field by paper bagging to get S_3 inbreds. The S_3 inbreds developed from these plants included 279 plants, of which 66 were from 'Alamo' plants and 213 originally from 'Kanlow' plants. From 229 S_3 plants in the field, 224 S_4 inbreds were developed including 119 from 'Alamo' and 105 of 'Kanlow' (Table 3.1). The other 50 S_3 plants were not used for further selfing. Table 3.3 provides information on SSR primer pairs used in inbreeding confirmation. For inbreeding confirmation of S_3 progeny, a single SSR primer pair procedure was followed with six primer pairs while for inbreeding confirmation of S_4 progeny, a duplex SSR procedure was used with 8 primer pairs (Table 3.3 and Table 3.4). Duplex SSR was time efficient and reduced the time required for SSR work by half compared to the single SSR procedure.

CONCLUSIONS

The experiment demonstrated that S_3 and S_4 inbreds were developed in lowland switchgrass using bagging and confirmed with SSR markers. Using 195 S_2 inbreds, 279 S_3 inbreds and 224 S_4 inbreds were produced. Inbreds developed in this way can be used for future breeding and crop improvement programs to exploit heterosis upon hybridization of genetic complementary and heterotic inbreds.

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LIST OF TABLES

LIST OF FIGURES

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Table 3.1. Inbreds developed in the second generation (S_2), the third generation (S_3), and the fourth generation (S_4).

	Plants count		
Inbreds	Alamo	Kanlow	Total
S_2	45	150	195
S_3	41	188	229
S_3 (additional)	25	25	50
S_4	119	105	224

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/115	Ku/115/1	1	1	Ku/115/1/1	1			
Ku	Ku/115	Ku/115/2	2	57	Ku/115/2/2	2	4		
Ku	Ku/115	Ku/115/2	2		Ku/115/2/3	3			
Ku	Ku/115	Ku/115/2	2		Ku/115/2/4	4			
Ku	Ku/115	Ku/115/2	2		Ku/115/2/5	5			
Ku	Ku/115	Ku/115/2	2		Ku/115/2/7	6			
Ku	Ku/115	Ku/115/3	3	11	Ku/115/3/1	7			
Ku	Ku/115	Ku/115/4	4	82	Ku/115/4/1	8	8		
Ku	Ku/115	Ku/115/4	4		Ku/115/4/2	9	2		
Ku	Ku/115	Ku/115/4	4		Ku/115/4/5	10			
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11	10	Ku/115/4/6/1	1
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11		Ku/115/4/6/2	2
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11		Ku/115/4/6/3	3
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11		Ku/115/4/6/4	4
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11		Ku/115/4/6/5	5
Ku	Ku/115	Ku/115/4	4		Ku/115/4/6	11		Ku/115/4/6/6	6
Ku	Ku/115	Ku/115/5	5	111	Ku/K115/5/1	12	38	Ku/K115/5/1	7
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/2	8
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/3	9
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/4	10
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/5	11
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/6	12
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/7	13
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/8	14
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/9	15
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/10	16
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/11	17
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/12	18
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/1	12		Ku/K115/5/13	19
Ku	Ku/115	Ku/115/5	5		Ku/K115/5/2	13			
Ku	Ku/116	Ku/116/1	7	212	Ku/116/1/11	14			
Ku	Ku/116	Ku/116/1	7		Ku/116/1/1	1K			
Ku	Ku/116	Ku/116/1	7		Ku/116/1/2	2K			
Ku	Ku/116	Ku/116/1	7		Ku/116/1/7	3K			
Ku	Ku/116	Ku/116/1	7		Ku/116/1/8	4K			
Ku	Ku/116	Ku/116/1	7		Ku/116/1/10	5K			
Ku	Ku/116	Ku/116/4	10	166					
Ku	Ku/116	Ku/116/13	14	38	Ku/116/13/1	15			
Ku	Ku/116	Ku/116/13	14		Ku/116/13/2	16			
Ku	Ku/116	Ku/116/13	14		Ku/116/13/4	17	3	Ku/116/13/4/1	20
Ku	Ku/116	Ku/116/13	14		Ku/116/13/5	18			
Ku	Ku/116	Ku/116/13	14		Ku/116/13/6	19			
Ku	Ku/116	Ku/116/13	14		Ku/116/13/7	20	3		
Ku	Ku/116	Ku/116/13	14		Ku/116/13/8	21			
Ku	Ku/121	Ku/121/3	20	29					

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/121	Ku/121/4	21	33	Ku/121/4/6	22			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/7	23	12	Ku/121/4/7/1	21
Ku	Ku/121	Ku/121/4	21		Ku/121/4/7	23		Ku/121/4/7/2	22
Ku	Ku/121	Ku/121/4	21		Ku/121/4/7	23		Ku/121/4/7/3	23
Ku	Ku/121	Ku/121/4	21		Ku/121/4/8	24			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/9	25			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/10	26			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/1	6K			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/2	7K			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/3	8K			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/4	9K			
Ku	Ku/121	Ku/121/4	21		Ku/121/4/5	10K			
Ku	Ku/133	Ku/133/1	34	87	Ku/133/1/2	27	1		
Ku	Ku/133	Ku/133/1	34		Ku/133/1/4	28			
Ku	Ku/133	Ku/133/1	34		Ku/133/1/5	29			
Ku	Ku/133	Ku/133/1	34		Ku/133/1/6	30			
Ku	Ku/133	Ku/133/1	34		Ku/133/1/7	31			
Ku	Ku/133	Ku/133/1	34		Ku/133/1/8	32			
Ku	Ku/134	Ku/134/2	36	2					
Ku	Ku/133	Ku/133/2	103	148	Ku/133/2/2	33			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/3	34			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/4	35			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/5	36	2		
Ku	Ku/133	Ku/133/2	103		Ku/133/2/6	37			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/10	38	1		
Ku	Ku/133	Ku/133/2	103		Ku/133/2/12	39			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/13	40	20		
Ku	Ku/133	Ku/133/2	103		Ku/133/2/14	41			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/15	42			
Ku	Ku/133	Ku/133/2	103		Ku/133/2/17	43			
Ku	Ku/146	Ku/146/6	42	23	Ku/146/6/4	44			
Ku	Ku/146	Ku/146/6	42		Ku/146/6/10	45			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/1	46			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/2	47			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/4	48			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/5	49			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/6	50			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/7	51			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/8	16K			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/9	17K			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/10	18K			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/13	19K			
Ku	Ku/146	Ku/146/22	110		Ku/146/22/14	20K			
Ku	Ku/152	Ku/152/2	113		Ku/152/2/1	52			
Ku	Ku/152	Ku/152/2	113		Ku/152/2/2	53			

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/146	Ku/146/16	43	6					
Ku	Ku/154	Ku/154/4	44	16	Ku/154/4/2	54			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/3	55			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/4	56			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/5	57			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/6	58			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/7	11K			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/8	12K			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/9	13K			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/10	14K			
Ku	Ku/154	Ku/154/4	44		Ku/154/4/11	15K			
Ku	Ku/154	Ku/154/10	47	81	Ku/154/10/1	59			
Ku	Ku/154	Ku/154/10	47		Ku/154/10/2	60			
Ku	Ku/154	Ku/154/10	47		Ku/154/10/3	61			
Ku	Ku/154	Ku/154/10	47		Ku/154/10/4	62			
Ku	Ku/154	Ku/154/10	47		Ku/154/10/6	63			
Ku	Ku/154	Ku/154/10	47		Ku/154/10/8	64	32		
Ku	Ku/154	Ku/154/13	49	18	Ku/154/13/1	65			
Ku	Ku/154	Ku/154/13	49		Ku/154/13/2	66			
Ku	Ku/116	Ku/116/18	100	1					
Ku	Ku/146	Ku/146/21	109	0					
Ku	Ku/146	Ku/146/22	110	150					
Ku	Ku/146	Ku/146/23	111	9					
Ku	Ku/152	Ku/152/2	113	6					
Ku	Ku/216	Ku/216/2	116	6	Ku/216/2/1	67	4		
Ku	Ku/216	Ku/216/2	116		Ku/216/2/2	68			
Ku	Ku/221	Ku/221/5	122	13					
Ku	Ku/221	Ku/221/6	123	66	Ku/221/6/1	69			
Ku	Ku/221	Ku/221/6	123		Ku/221/6/2	70			
Ku	Ku/221	Ku/221/6	123		Ku/221/6/4	71			
Ku	Ku/221	Ku/221/6	123		Ku/221/6/5	72			
Ku	Ku/221	Ku/221/6	123		Ku/221/6/6	73			
Ku	Ku/221	Ku/221/6	123		Ku/221/6/7	74			
Ku	Ku/221	Ku/221/11	128	8					
Ku	Ku/221	Ku/221/12	129	26	Ku/221/12/1	75			
Ku	Ku/221	Ku/221/13	129		Ku/221/12/2	76			
Ku	Ku/221	Ku/221/14	129		Ku/221/12/3	77			
Ku	Ku/221	Ku/221/15	129		Ku/221/12/4	78	4		
Ku	Ku/241	Ku/221/16	132	47	Ku/241/3/1	79	0		
Ku	Ku/241	Ku/221/17	132		Ku/241/3/2	80	0		
Ku	Ku/241	Ku/221/18	132		Ku/241/3/4	81	182	Ku/241/3/4/1	25
Ku	Ku/241	Ku/221/19	132		Ku/241/3/5	82	16		
Ku	Ku/241	Ku/221/20	133	9					
Ku	Ku/241	Ku/221/21	134	40	Ku/241/5/1	83			
Ku	Ku/241	Ku/221/22	134		Ku/241/5/2	84			

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/241	Ku/221/23	134		Ku/241/5/3	85			
Ku	Ku/241	Ku/221/24	134		Ku/241/5/5	86			
Ku	Ku/241	Ku/221/25	134		Ku/241/5/7	87	6		
Ku	Ku/241	Ku/221/26	134		Ku/241/5/8	88			
Ku	Ku/241	Ku/221/27	135	27	Ku/241/6/1	89			
Ku	Ku/241	Ku/221/28	135		Ku/241/6/2	90			
Ku	Ku/241	Ku/221/29	135		Ku/241/6/3	91			
Ku	Ku/241	Ku/221/30	135		Ku/241/6/4	92			
Ku	Ku/241	Ku/221/31	135		Ku/241/6/5	93			
Ku	Ku/241	Ku/221/32	135		Ku/241/6/6	94			
Ku	Ku/241	Ku/221/33	135		Ku/241/6/7	95			
Ku	Ku/241	Ku/221/34	135		Ku/241/6/8	96	8		
Ku	Ku/241	Ku/221/35	137	70	Ku/241/8/3	97			
Ku	Ku/241	Ku/221/36	137		Ku/241/8/4	98			
Ku	Ku/241	Ku/221/37	137		Ku/241/8/5	99			
Ku	Ku/241	Ku/221/38	137		Ku/241/8/6	100			
Ku	Ku/241	Ku/221/39	137		Ku/241/8/7	101	13		
Ku	Ku/241	Ku/221/40	137		Ku/241/8/8	102	3		
Ku	Ku/241	Ku/221/41	137		Ku/241/8/9	103			
Ku	Ku/241	Ku/221/42	137		Ku/241/8/10	104	41	Ku/241/8/10/1	27
Ku	Ku/241	Ku/221/43	137		Ku/241/8/10	104		Ku/241/8/10/2	28
Ku	Ku/241	Ku/221/44	137		Ku/241/8/10	104		Ku/241/8/10/3	29
Ku	Ku/241	Ku/221/45	137		Ku/241/8/10	104		Ku/241/8/10/4	30
Ku	Ku/241	Ku/221/46	138	47	Ku/241/9/1	105			
Ku	Ku/241	Ku/221/47	138		Ku/241/9/2	106			
Ku	Ku/241	Ku/221/48	138		Ku/241/9/3	107			
Ku	Ku/241	Ku/221/49	138		Ku/241/9/6	108			
Ku	Ku/241	Ku/221/50	138		Ku/241/9/7	109	10		
Ku	Ku/241	Ku/221/51	138		Ku/241/9/9	110			
Ku	Ku/241	Ku/221/52	138		Ku/241/9/10	111			
Ku	Ku/241	Ku/221/53	138		Ku/241/9/12	112	132	Ku/241/9/12/1	31
Ku	Ku/241	Ku/221/54	138		Ku/241/9/13	113			
Ku	Ku/241	Ku/221/55	138		Ku/241/9/14	114			
Ku	Ku/241	Ku/221/56	139	432	Ku/241/10/1	115	402		
Ku	Ku/241	Ku/221/57	140	58					
Ku	Ku/241	Ku/221/58	143	13	Ku/241/14/2	116			
Ku	Ku/241	Ku/221/59	144	181	Ku/241/15/1	117	6	Ku/241/15/1/1	33
Ku	Ku/241	Ku/221/60	144		Ku/241/15/3	118	480	Ku/241/15/3/1	34
Ku	Ku/241	Ku/221/61	144		Ku/241/15/3	118		Ku/241/15/3/2	35
Ku	Ku/241	Ku/221/62	144		Ku/241/15/3	118		Ku/241/15/3/3	36
Ku	Ku/241	Ku/221/63	144		Ku/241/15/3	118		Ku/241/15/3/4	37
Ku	Ku/241	Ku/221/64	144		Ku/241/15/3	118		Ku/241/15/3/5	38
Ku	Ku/241	Ku/221/65	144		Ku/241/15/3	118		Ku/241/15/3/6	39
Ku	Ku/241	Ku/221/66	144		Ku/241/15/3	118		Ku/241/15/3/7	41
Ku	Ku/241	Ku/221/67	144		Ku/241/15/3	118		Ku/241/15/3/8	42

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/241	Ku/221/68	144		Ku/241/15/3	118		Ku/241/15/3/9	43
Ku	Ku/241	Ku/221/69	144		Ku/241/15/3	118		Ku/241/15/3/10	44
Ku	Ku/241	Ku/221/70	144		Ku/241/15/3	118		Ku/241/15/3/11	45
Ku	Ku/241	Ku/221/71	144		Ku/241/15/3	118		Ku/241/15/3/12	46
Ku	Ku/241	Ku/221/72	144		Ku/241/15/3	118		Ku/241/15/3/13	47
Ku	Ku/241	Ku/221/73	144		Ku/241/15/4	119	243	Ku/241/15/4/1	52
Ku	Ku/241	Ku/221/74	144		Ku/241/15/5	120	1		
Ku	Ku/241	Ku/221/75	144		Ku/241/15/8	121	216	Ku/241/15/8/1	55
Ku	Ku/241	Ku/221/76	144		Ku/241/15/8	121		Ku/241/15/8/2	56
Ku	Ku/241	Ku/221/77	144		Ku/241/15/8	121		Ku/241/15/8/3	57
Ku	Ku/241	Ku/221/78	144		Ku/241/15/8	121		Ku/241/15/8/4	58
Ku	Ku/241	Ku/221/79	144		Ku/241/15/8	121		Ku/241/15/8/5	60
Ku	Ku/241	Ku/221/80	144		Ku/241/15/8	121		Ku/241/15/8/6	61
Ku	Ku/241	Ku/221/81	144		Ku/241/15/8	121		Ku/241/15/8/7	63
Ku	Ku/241	Ku/221/82	144		Ku/241/15/8	121		Ku/241/15/8/8	64
Ku	Ku/241	Ku/221/83	144		Ku/241/15/8	121		Ku/241/15/8/9	66
Ku	Ku/241	Ku/221/84	144		Ku/241/15/8	121		Ku/241/15/8/10	67
Ku	Ku/241	Ku/221/85	144		Ku/241/15/8	121		Ku/241/15/8/11	69
Ku	Ku/241	Ku/221/86	144		Ku/241/15/9	122	184		
Ku	Ku/241	Ku/221/87	144		Ku/241/15/10	123	155		
Ku	Ku/241	Ku/221/88	144		Ku/241/15/12	124	51		
Ku	Ku/241	Ku/221/89	145	39	Ku/241/16/1	125	1		
Ku	Ku/241	Ku/221/90	145		Ku/241/16/3	126	27	Ku/241/16/3/1	81
Ku	Ku/241	Ku/221/91	145		Ku/241/16/4	127			
Ku	Ku/241	Ku/221/92	145		Ku/241/16/5	128			
Ku	Ku/241	Ku/221/93	145		Ku/241/16/8	129	15	Ku/241/16/8/1	83
Ku	Ku/241	Ku/221/94	145		Ku/241/16/8	129		Ku/241/16/8/2	84
Ku	Ku/241	Ku/221/95	145		Ku/241/16/8	129		Ku/241/16/8/3	85
Ku	Ku/241	Ku/221/96	146	55	Ku/241/17/4	130			
Ku	Ku/241	Ku/221/97	146		Ku/241/17/5	131	1		
Ku	Ku/241	Ku/221/98	146		Ku/241/17/6	132	10	Ku/241/17/6/1	86
Ku	Ku/241	Ku/221/99	146		Ku/241/17/6	132		Ku/241/17/6/2	87
Ku	Ku/241	Ku/221/100	146		Ku/241/17/6	132		Ku/241/17/6/3	88
Ku	Ku/241	Ku/221/101	146		Ku/241/17/7	133	8	Ku/241/17/7/1	89
Ku	Ku/241	Ku/221/102	146		Ku/241/17/7	133		Ku/241/17/7/2	90
Ku	Ku/241	Ku/221/103	146		Ku/241/17/7	133		Ku/241/17/7/3	91
Ku	Ku/241	Ku/221/104	146		Ku/241/17/8	134	12	Ku/241/17/8/1	92
Ku	Ku/241	Ku/221/105	146		Ku/241/17/8	134		Ku/241/17/8/2	93
Ku	Ku/241	Ku/221/106	146		Ku/241/17/8	134		Ku/241/17/8/3	94
Ku	Ku/241	Ku/221/107	146		Ku/241/17/8	134		Ku/241/17/8/4	95
Ku	Ku/241	Ku/221/108	146		Ku/241/17/8	134		Ku/241/17/8/5	96
Ku	Ku/241	Ku/221/109	146		Ku/241/17/9	135			
Ku	Ku/241	Ku/221/110	146		Ku/241/17/10	136			
Ku	Ku/241	Ku/221/111	146		Ku/241/17/11	137	24	Ku/241/17/11/1	97
Ku	Ku/241	Ku/221/112	146		Ku/241/17/11	137		Ku/241/17/11/2	98

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/241	Ku/221/113	146		Ku/241/17/11	137		Ku/241/17/11/3	102
Ku	Ku/241	Ku/221/114	146		Ku/241/17/11	137		Ku/241/17/11/4	103
Ku	Ku/241	Ku/221/115	146		Ku/241/17/11	137		Ku/241/17/11/5	104
Ku	Ku/241	Ku/221/116	146		Ku/241/17/11	137		Ku/241/17/11/6	105
Ku	Ku/241	Ku/221/117	146		Ku/241/17/11	137		Ku/241/17/11/7	106
Ku	Ku/241	Ku/221/118	146		Ku/241/17/11	137		Ku/241/17/11/8	107
Ku	Ku/241	Ku/221/119	146		Ku/241/17/11	137		Ku/241/17/11/9	108
Ku	Ku/241	Ku/221/120	150	191	Ku/241/21/1	138	938	Ku/241/21/1/1	111
Ku	Ku/241	Ku/221/121	150		Ku/241/21/1	138		Ku/241/21/1/2	112
Ku	Ku/241	Ku/221/122	150		Ku/241/21/1	138		Ku/241/21/1/3	113
Ku	Ku/241	Ku/221/123	150		Ku/241/21/1	138		Ku/241/21/1/4	115
Ku	Ku/241	Ku/221/124	150		Ku/241/21/4	139	536		
Ku	Ku/241	Ku/221/125	151	34	Ku/241/22/1	140	14		
Ku	Ku/241	Ku/221/126	151		Ku/241/22/2	141			
Ku	Ku/241	Ku/221/127	151		Ku/241/22/3	142	66	Ku/241/22/3/1	122
Ku	Ku/241	Ku/221/128	151		Ku/241/22/3	142		Ku/241/22/3/2	124
Ku	Ku/241	Ku/221/129	151		Ku/241/22/3	142		Ku/241/22/3/3	125
Ku	Ku/241	Ku/221/130	151		Ku/241/22/4	143			
Ku	Ku/241	Ku/221/131	151		Ku/241/22/5	144			
Ku	Ku/241	Ku/221/132	151		Ku/241/22/6	145			
Ku	Ku/241	Ku/221/133	151		Ku/241/22/8	146	95	Ku/241/22/8/1	128
Ku	Ku/241	Ku/221/134	151		Ku/241/22/8	146		Ku/241/22/8/2	129
Ku	Ku/241	Ku/221/135	151		Ku/241/22/8	146		Ku/241/22/8/3	130
Ku	Ku/241	Ku/221/136	151		Ku/241/22/8	146		Ku/241/22/8/4	131
Ku	Ku/241	Ku/221/137	151		Ku/241/22/9	147			
Ku	Ku/241	Ku/221/138	151		Ku/241/22/10	148	148		
Ku	Ku/241	Ku/221/139	151		Ku/241/22/11	149			
Ku	Ku/241	Ku/221/140	151		Ku/241/22/12	150	2		
Ku	Ku/241	Ku/221/141	151		Ku/241/22/13	151	40	Ku/241/22/13/1	137
Ku	Ku/241	Ku/221/142	151		Ku/241/22/14	152	44		
Ku	Ku/241	Ku/221/143	151		Ku/241/22/15	153			
Ku	Ku/241	Ku/221/144	152	1					
Ku	Ku/241	Ku/221/145	153	92					
Ku	Ku/241	Ku/221/146	156	6					
Ku	Ku/241	Ku/221/147	157	12					
Ku	Ku/241	Ku/221/148	158	130	Ku/241/29/6	154			
Ku	Ku/241	Ku/221/149	158		Ku/241/29/7	155			
Ku	Ku/241	Ku/221/150	158		Ku/241/29/8	156	10	Ku/241/29/8/1	140
Ku	Ku/241	Ku/241/29	158		Ku/241/29/2	21K			
Ku	Ku/241	Ku/241/29	158		Ku/241/29/3	22K			
Ku	Ku/241	Ku/241/29	158		Ku/241/29/5	23K			
Ku	Ku/241	Ku/241/29	158		Ku/241/29/9	24K			
Ku	Ku/241	Ku/241/29	158		Ku/241/29/10	25K			
Ku	Ku/241	Ku/221/151	159	9					
Ku	Ku/241	Ku/221/152	161	22					

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/241	Ku/221/153	162	49					
Ku	Ku/241	Ku/221/154	163	230					
Ku	Ku/241	Ku/221/155	164	3					
Ku	Ku/241	Ku/221/156	165	32					
Ku	Ku/241	Ku/221/157	167	55					
Ku	Ku/241	Ku/221/158	168	52					
Ku	Ku/241	Ku/221/159	169	32					
Ku	Ku/241	Ku/221/160	171	6					
Ku	Ku/241	Ku/221/161	172	101	Ku/241/43/1	157	20		
Ku	Ku/241	Ku/221/162	172		Ku/241/43/2	158	1		
Ku	Ku/241	Ku/221/163	173	172	Ku/241/44/1	159	20		
Ku	Ku/241	Ku/221/164	173		Ku/241/44/2	160			
Ku	Ku/241	Ku/221/165	174	55					
Ku	Ku/241	Ku/221/166	175	6					
Ku	Ku/241	Ku/221/167	176	8					
Ku	Ku/241	Ku/221/168	177	2					
Ku	Ku/241	Ku/221/169	178	10					
Ku	Ku/241	Ku/221/170	179	104	Ku/241/50/1	161			
Ku	Ku/241	Ku/221/171	180	40	Ku/241/51/1	162			
Ku	Ku/241	Ku/241/52	181	3					
Ku	Ku/241	Ku/241/53	182	32					
Ku	Ku/241	Ku/241/54	183	5					
Ku	Ku/241	Ku/241/55	184	52					
Ku	Ku/241	Ku/241/57	186	16					
Ku	Ku/241	Ku/241/58	187	1					
Ku	Ku/241	Ku/241/59	188	1					
Ku	Ku/241	Ku/241/60	189	60					
Ku	Ku/241	Ku/241/61	190	35					
Ku	Ku/241	Ku/241/62	191	11					
Ku	Ku/241	Ku/241/63	192	41					
Ku	Ku/241	Ku/241/64	193	34					
Ku	Ku/241	Ku/241/65	194	3					
Ku	Ku/241	Ku/241/66	195	1					
Ku	Ku/241	Ku/241/67	196	102					
Ku	Ku/241	Ku/241/70	199	81					
Ku	Ku/241	Ku/241/72	201	35					
Ku	Ku/241	Ku/241/73	202	5					
Ku	Ku/241	Ku/241/74	203	33					
Ku	Ku/241	Ku/241/75	204	3					
Ku	Ku/241	Ku/241/76	205	4					
Ku	Ku/241	Ku/241/78	207	1					
Ku	Ku/241	Ku/241/79	208	5					
Ku	Ku/241	Ku/241/80	209	145					
Ku	Ku/241	Ku/241/81	210	28					
Ku	Ku/241	Ku/241/83	212	33					

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/241	Ku/241/84	213	33	Ku/241/84/1	163			
Ku	Ku/241	Ku/241/84	213		Ku/241/84/2	164	50		
Ku	Ku/241	Ku/241/85	214	60					
Ku	Ku/241	Ku/241/86	215	33	Ku/241/86/1	165	9		
Ku	Ku/241	Ku/241/86	215		Ku/241/86/2	166	113	Ku/241/86/2/1	145
Ku	Ku/241	Ku/241/86	215		Ku/241/86/3	167			
Ku	Ku/241	Ku/241/88	217	41	Ku/241/88/1	168	141	Ku/241/88/1/1	156
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/2	157
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/3	159
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/4	162
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/5	163
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/6	164
Ku	Ku/241	Ku/241/88	217		Ku/241/88/1	168		Ku/241/88/1/7	165
Ku	Ku/241	Ku/241/88	217		Ku/241/88/2	169			
Ku	Ku/241	Ku/241/89	218	48					
Ku	Ku/241	Ku/241/90	219	51					
Ku	Ku/241	Ku/241/92	221	30					
Ku	Ku/241	Ku/241/93	222	189	Ku/241/93/1	170	1		
Ku	Ku/241	Ku/241/93	222		Ku/241/93/2	171	56	Ku/241/93/2/1	168
Ku	Ku/241	Ku/241/93	222		Ku/241/93/2	171		Ku/241/93/2/2	170
Ku	Ku/241	Ku/241/93	222		Ku/241/93/2	171		Ku/241/93/2/3	172
Ku	Ku/241	Ku/241/93	222		Ku/241/93/2	171		Ku/241/93/2/4	176
Ku	Ku/241	Ku/241/94	223	234	Ku/241/94/1	172			
Ku	Ku/241	Ku/241/96	225	252	Ku/241/96/1	173			
Ku	Ku/241	Ku/241/96	225		Ku/241/96/2	174			
Ku	Ku/241	Ku/241/96	225		Ku/241/96/4	175			
Ku	Ku/241	Ku/241/96	225		Ku/241/96/5	176	11		
Ku	Ku/241	Ku/241/96	225		Ku/241/96/6	177	1		
Ku	Ku/241	Ku/241/96	225		Ku/241/96/7	178			
Ku	Ku/241	Ku/241/96	225		Ku/241/96/8	179	3	Ku/241/96/8/1	178
Ku	Ku/241	Ku/241/97	226	110	Ku/241/97/1	180	15		
Ku	Ku/241	Ku/241/97	226		Ku/241/97/2	181			
Ku	Ku/241	Ku/241/97	226		Ku/241/97/4	182	56		
Ku	Ku/241	Ku/241/99	228	21					
Ku	Ku/241	Ku/241/100	229	210					
Ku	Ku/241	Ku/241/101	230	41					
Ku	Ku/241	Ku/241/102	231	134					
Ku	Ku/241	Ku/241/103	232	78					
Ku	Ku/241	Ku/241/104	233	6					
Ku	Ku/241	Ku/241/106	235	2					
Ku	Ku/241	Ku/241/107	236	62					
Ku	Ku/241	Ku/241/108	237	12					
Ku	Ku/241	Ku/241/109	238	3					
Ku	Ku/241	Ku/241/110	239	3					
Ku	Ku/256	Ku/256/127	304	33					

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Ku	Ku/256	Ku/256/128	305	5					
Ku	Ku/336	Ku/336/13	331	140					
Au	Au/114	Au/114/5	336	21	Au/114/5/1	183			
Au	Au/114	Au/114/5	336		Au/114/5/2	184	3		
Au	Au/114	Au/114/5	336		Au/114/5/3	185	170	Au/114/5/3/1	180
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/2	181
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/3	182
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/4	183
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/5	184
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/6	185
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/7	186
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/8	187
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/9	188
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/10	189
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/11	190
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/12	191
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/13	192
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/14	194
Au	Au/114	Au/114/5	336		Au/114/5/3	185		Au/114/5/3/15	195
Au	Au/114	Au/114/5	336		Au/114/5/6	186	19	Au/114/5/6/1	196
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/2	197
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/3	198
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/4	199
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/5	200
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/6	202
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/7	203
Au	Au/114	Au/114/5	336		Au/114/5/6	186		Au/114/5/6/8	205
Au	Au/114	Au/114/5	336		Au/114/5/7	187	28	Au/114/5/7/1	206
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/2	207
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/3	208
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/4	209
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/5	210
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/6	211
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/7	212
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/8	213
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/9	214
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/10	215
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/11	216
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/12	217
Au	Au/114	Au/114/5	336		Au/114/5/7	187		Au/114/5/7/13	218
Au	Au/114	Au/114/5	336		Au/114/5/9	188	17	Au/114/5/9/1	220
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/2	221
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/3	222
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/4	223
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/5	224

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/6	225
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/7	226
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/8	227
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/9	228
Au	Au/114	Au/114/5	336		Au/114/5/9	188		Au/114/5/9/10	229
Au	Au/114	Au/114/7	337	18	Au/114/7/11	189			
Au	Au/114	Au/114/7	337		Au/114/7/1	1A			
Au	Au/114	Au/114/7	337		Au/114/7/2	2A			
Au	Au/114	Au/114/7	337		Au/114/7/3	3A			
Au	Au/114	Au/114/7	337		Au/114/7/4	4A			
Au	Au/114	Au/114/7	337		Au/114/7/10	5A			
Au	Au/116	Au/116/2	340	456	Au/116/2/1	190	10	Au/116/2/1/1	231
Au	Au/116	Au/116/2	340		Au/116/2/2	191			
Au	Au/116	Au/116/2	340		Au/116/2/9	192	5		
Au	Au/116	Au/116/2	340		Au/116/2/12	193			
Au	Au/116	Au/116/2	340		Au/116/2/4	6A			
Au	Au/116	Au/116/2	340		Au/116/2/5	7A			
Au	Au/116	Au/116/2	340		Au/116/2/6	8A			
Au	Au/116	Au/116/2	340		Au/116/2/7	9A			
Au	Au/116	Au/116/2	340		Au/116/2/8	10A			
Au	Au/116	Au/116/3	341	15					
Au	Au/116	Au/116/10	346	9					
Au	Au/116	Au/116/16	350	2					
Au	Au/122	Au/122/9	356	2					
Au	Au/125	Au/125/4	397	6					
Au	Au/125	Au/125/6	398	4					
Au	Au/135	Au/135/18	404	2					
Au	Au/135	Au/135/27	406	2					
Au	Au/135	Au/135/28	407	3					
Au	Au/143	Au/143/3	411	2					
Au	Au/145	Au/145/2b	459	13	Au/145/2b/1	194			
Au	Au/151	Au/151/4	415	98	Au/151/4/3	195	236	Au/151/4/3/1	232
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/2	233
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/3	234
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/4	235
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/5	236
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/6	237
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/7	238
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/8	239
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/9	240
Au	Au/151	Au/151/4	415		Au/151/4/3	195		Au/151/4/3/10	241
Au	Au/151	Au/151/4	415		Au/151/4/5	196			
Au	Au/151	Au/151/4	415		Au/151/4/8	197	19	Au/151/4/8/1	243
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/2	244
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/3	245

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/4	246
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/5	247
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/6	248
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/7	249
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/8	250
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/9	251
Au	Au/151	Au/151/4	415		Au/151/4/8	197		Au/151/4/8/10	252
Au	Au/151	Au/151/4	415		Au/151/4/9	198	8	Au/151/4/9/1	253
Au	Au/151	Au/151/4	415		Au/151/4/9	198		Au/151/4/9/2	254
Au	Au/151	Au/151/4	415		Au/151/4/10	199	9	Au/151/4/10/1	255
Au	Au/151	Au/151/4	415		Au/151/4/10	199		Au/151/4/10/2	256
Au	Au/151	Au/151/4	415		Au/151/4/10	199		Au/151/4/10/3	257
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/1	258
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/2	259
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/3	260
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/4	261
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/5	262
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/6	263
Au	Au/151	Au/151/4	415		Au/151/4/11	200		Au/151/4/11/7	264
Au	Au/151	Au/151/4	415		Au/151/4/12	201	16	Au/151/4/12/1	265
Au	Au/151	Au/151/4	415		Au/151/4/12	201		Au/151/4/12/2	266
Au	Au/151	Au/151/4	415		Au/151/4/12	201		Au/151/4/12/3	270
Au	Au/151	Au/151/4	415		Au/151/4/15	202			
Au	Au/151	Au/151/4	415		Au/151/4/17	203	12		
Au	Au/151	Au/151/4	415		Au/151/4/20	204	6	Au/151/4/20/1	273
Au	Au/151	Au/151/4	415		Au/151/4/21	205			
Au	Au/151	Au/151/4	415		Au/151/4/1	11A			
Au	Au/151	Au/151/4	415		Au/151/4/2	12A			
Au	Au/151	Au/151/4	415		Au/151/4/4	13A			
Au	Au/151	Au/151/4	415		Au/151/4/6	14A			
Au	Au/151	Au/151/4	415		Au/151/4/7	15A			
Au	Au/225	Au/225/3	422	5					
Au	Au/225	Au/225/14	423	3					
Au	Au/246	Au/246/7	430	1					
Au	Au/255	Au/255/3	434	4					
Au	Au/255	Au/255/5	435	2					
Au	Au/255	Au/255/11	437	4					
Au	Au/111	Au/111/1	439	1					
Au	Au/116	Au/116/18	441	4					
Au	Au/116	Au/116/19	442	21					
Au	Au/116	Au/116/20	443	49					
Au	Au/146	Au/146/1	461	75					
Au	Au/146	Au/146/2	462	13					
Au	Au/152	Au/152/3	473	3					
Au	Au/152	Au/152/11	481	2					

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Au	Au/152	Au/152/31	501	14	Au/152/31/1	206			
Au	Au/152	Au/152/31	501		Au/152/31/2	207			
Au	Au/152	Au/152/31	501		Au/152/31/4	208	2	Au/152/31/4/1	274
Au	Au/152	Au/152/31	501		Au/152/31/5	209			
Au	Au/152	Au/152/31	501		Au/152/31/6	210	30	Au/152/31/6/1	278
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/2	280
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/3	281
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/4	282
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/5	283
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/6	284
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/7	285
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/8	286
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/9	287
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/10	289
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/11	291
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/12	292
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/13	293
Au	Au/152	Au/152/31	501		Au/152/31/6	210		Au/152/31/6/14	294
Au	Au/152	Au/152/31	501		Au/152/31/7	211	1		
Au	Au/152	Au/152/41	511	25	Au/152/41/1	212	24	Au/152/41/1/1	296
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/2	297
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/3	298
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/4	299
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/5	300
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/6	301
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/7	302
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/8	303
Au	Au/152	Au/152/41	511		Au/152/41/1	212		Au/152/41/1/9	305
Au	Au/152	Au/152/41	511		Au/152/41/4	213			
Au	Au/152	Au/152/41	511		Au/152/41/5	214			
Au	Au/152	Au/152/41	511		Au/152/41/7	215			
Au	Au/152	Au/152/41	511		Au/152/41/8	216			
Au	Au/152	Au/152/41	511		Au/152/41/9	217			
Au	Au/152	Au/152/41	511		Au/152/41/11	218			
Au	Au/255	Au/255/1	432	9	Au/255/1/6	219			
Au	Au/255	Au/255/1	432		Au/255/1/1	21A			
Au	Au/255	Au/255/1	432		Au/255/1/2	22A			
Au	Au/255	Au/255/1	432		Au/255/1/3	23A			
Au	Au/255	Au/255/1	432		Au/255/1/4	24A			
Au	Au/255	Au/255/1	432		Au/255/1/5	25A			
Au	Au/152	Au/152/41	511		Au/152/41/2	16A			
Au	Au/152	Au/152/41	511		Au/152/41/3	17A			
Au	Au/152	Au/152/41	511		Au/152/41/10	18A			
Au	Au/152	Au/152/41	511		Au/152/41/12	19A			
Au	Au/152	Au/152/41	511		Au/152/41/14	20A			

Table 3.2. Pedigree information for the second generation (S_2), the third generation (S_3), and the fourth generation (S_4) inbreds (contd.).

S_0	S_1	S_2	S_2 field plant #	Seed progeny from S_2 (putative S_3)	S_3	S_3 field plant #	Seed progeny from S_3 (putative S_4)	S_4	S_4 field plant #
Au	Au/152	Au/152/20	490	15	Au/152/20/1	220			
Au	Au/152	Au/152/20	490		Au/152/20/5	221	30	Au/152/20/5/1	308
Au	Au/152	Au/152/20	490		Au/152/20/5	221		Au/152/20/5/2	309
Au	Au/152	Au/152/20	490		Au/152/20/5	221		Au/152/20/5/3	311
Au	Au/152	Au/152/20	490		Au/152/20/5	221		Au/152/20/5/4	313
Au	Au/152	Au/152/20	490		Au/152/20/5	221		Au/152/20/5/5	317
Au	Au/152	Au/152/20	490		Au/152/20/5	221		Au/152/20/5/6	318
Ku	Ku/241	Ku/241/41	170		Ku/241/41/2	222			
Ku	Ku/241	Ku/241/41	170		Ku/241/41/3	223			
Au	Au/135	Au/135/6	398		Au/135/6/1	224			
Ku	Ku/241	Ku/241/6	135		Ku/241/6/9	225			
Au	Au/114	Au/114/5	336		Au/114/5/14	226	24	Au/114/5/14/1	329
Au	Au/114	Au/114/5	336		Au/114/5/14	226		Au/114/5/14/2	331
Au	Au/114	Au/114/5	336		Au/114/5/14	226		Au/114/5/14/3	333
Au	Au/114	Au/114/5	336		Au/114/5/14	226		Au/114/5/14/4	334
Au	Au/114	Au/114/5	336		Au/114/5/14	226		Au/114/5/14/5	335
Au	Au/114	Au/114/5	336		Au/114/5/14	226		Au/114/5/14/6	338
Ku	Ku/133	Ku/133/2	103		Ku/133/2/9	227			
Ku	Ku/241	Ku/241/81	210		Ku/241/81/1	228			
Ku	Ku/241	Ku/241/85	214		Ku/241/85/2	229	250		
Au	Au/246	Au/246/4	522	1					
Au	Au/225	Au/225/1	526	35					
Au	Au/246	Au/246/2b	531	2					
Au	Au/246	Au/246/3b	532	2					
Au	Au/311	Au/311/2	539	15					

Table 3.3. SSR primer pairs used in inbreeding confirmation for S_3 and S_4 families of switchgrass.

S.N.	Primer pair	Repeat motif	Primer Sequence (5'→3')	Expected size (bp)	Melting point (°C)	SSR type	SSR band size position (bp)
1	PVCA 201-202	(AC)9	F: GCATCTCATGGTTGGTGTTC	154	58.9	Single	169-199
			R: TCCAAGAGAGAAAGGTGAGTTG		58.6		
2	PVGA 1549-1550	(GAA)6	F: AGTAAGCCGCAGACAGGAAT	269	59.0	Duplex	255-316
			R: ACAAATATCCAGCAGGGAGG		59.0		
3	PVGA 1963-1964	(GA)9-(AG)6	F: TATAGGTGGATCCCCACTCG	197	59.8	Duplex	191-215
			R: TTATTGGATGGGCTCCTCTC		59.1		
4	PVGA 2015-2016	(CT)13	F: CCTTGCTCCACTGTCTCAAA	288	59.0	Single	274-289
			R: CCCATCTTGGACAGACCTTT		59.0		
5	PVGA 2025-2026	(AG)19	F: CACCCCTTGGTTCTTGTTTT	181	58.9	Single	157-191
			R: AACACAGCAGCATCATAGCC		58.9		
6	PVCAG 2187-2188	(GCA)7	F: TGGTGGGCACTACACAGAGT	158	59.2	Duplex	156-183
			R: TTGGTAGGTGTTGCCTTTCA		59.2		
7	PVCAG 2207-2208	(GCT)8-(CTG)5	F: TGAAGTGCTTGAGGAACTGG	215	59.0	Duplex	219-244
			R: GTAGTCATAGCCCAAGCCGT		59.2		
8	PVCAG 2269-2270	(CAG)8	F: CTACCAGTGCTGTGGCAGTT	231	59.0	Duplex	214-248
			R: GTGGATACACCAGTGTGGGA		59.2		
9	PVCAG 2279-2280	(GCT)8	F: GCAGTATGAGGCCAATGCTA	227	58.9	Duplex	225-246
			R: TCTGCTTTGATTGGTTGCTC		59.0		
10	PVCAG 2285-2286	(CAG)8	F: GCCAATATGCTGGACATCAC	345	59.0	Single	353
			R: GCTATGGTGGAGCATAACGA		58.7		
11	PVCAG 2289-2290	(TGC)5	F: ATGATCTTCAGGGGAAAACG	171	59.0	Single/Duplex	163-188
			R: CAGCACTGCAACCCTAATTG		59.3		
12	PVCAG 2361-2362	(AGC)8	F: AGTGTCCCGTTGACATGAGA	259	59.1	Duplex	265-277
			R: GTTTGCATTCGTGCCTAAAG		58.4		
13	PVAAG 3367-3368	(ACA)29	F: AGACCCACACCCACGATAAT	250	59.1	Single	193-235
			R: GTTACCAATGCGGTTTTCCT		59.0		

Source: Wang et al. (2011)

Table 3.4. Combinations of primer pairs used in the duplex SSR.

S.N.	Primer pairs in duplex	SSR band size position (bp)
1	PVGA 1549-1550	255-316
	PVCAG 2187-2188	156-183
2	PVCAG 2207-2208	219-244
	PVCAG 2289-2290	163-188
3	PVCAG 2279-2280	225-246
	PVGA 1963-1964	191-215
4	PVCAG 2361-2362	265-277
	PVCAG 2269-2270	214-248

Source: Wang et al. (2011)

Fig. 3.1. The S_2 field layout at Oklahoma State University Agronomy Research Station. The numbers inside shaded cells were inbreds and the remaining were not inbreds. Each row (extending from East to West) could accommodate a maximum of 57 plants with plant to plant distance of 0.60 m. The ten rows were maintained at a row to row distance of 1.05 m.

57	56	55	54	53	52	51	50	49	48	47	46	45	44	43	42	41	40	39	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
114	113	112	111	110	109	108	107	106	105	104	103	102	101	100	99	98	97	96	95	94	93	92	91	90	89	88	87	86	85	84	83	82	81	80	79	78	77	76	75	74	73	72	71	70	69	68	67	66	65	64	63	62	61	60	59	58
171	170	169	168	167	166	165	164	163	162	161	160	159	158	157	156	155	154	153	152	151	150	149	148	147	146	145	144	143	142	141	140	139	138	137	136	135	134	133	132	131	130	129	128	127	126	125	124	123	122	121	120	119	118	117	116	115
228	227	226	225	224	223	222	221	220	219	218	217	216	215	214	213	212	211	210	209	208	207	206	205	204	203	202	201	200	199	198	197	196	195	194	193	192	191	190	189	188	187	186	185	184	183	182	181	180	179	178	177	176	175	174	173	172
285	284	283	282	281	280	279	278	277	276	275	274	273	272	271	270	269	268	267	266	265	264	263	262	261	260	259	258	257	256	255	254	253	252	251	250	249	248	247	246	245	244	243	242	241	240	239	238	237	236	235	234	233	232	231	230	229
										332	331	330	329	328	327	326	325	324	323	322	321	320	319	318	317	316	315	314	313	312	311	310	309	308	307	306	305	304	303	302	301	300	299	298	297	296	295	294	293	292	291	290	289	288	287	286
389	388	387	386	385	384	383	382	381	380	379	378	377	376	375	374	373	372	371	370	369	368	367	366	365	364	363	362	361	360	359	358	357	356	355	354	353	352	351	350	349	348	347	346	345	344	343	342	341	340	339	338	337	336	335	334	333
446	445	444	443	442	441	440	439	438	437	436	435	434	433	432	431	430	429	428	427	426	425	424	423	422	421	420	419	418	417	416	415	414	413	412	411	410	409	408	407	406	405	404	403	402	401	400	399	398	397	396	395	394	393	392	391	390
503	502	501	500	499	498	497	496	495	494	493	492	491	490	489	488	487	486	485	484	483	482	481	480	479	478	477	476	475	474	473	472	471	470	469	468	467	466	465	464	463	462	461	460	459	458	457	456	455	454	453	452	451	450	449	448	447
																544	543	542	541	540	539	538	537	536	535	534	533	532	531	530	529	528	527	526	525	524	523	522	521	520	519	518	517	516	515	514	513	512	511	510	509	508	507	506	505	504

N ↑

Source: The S_2 field layout and inbreds information was provided by Dr. James Todd (Todd, 2011).

Fig. 3.2. The S_3 field layout at Oklahoma State University Agronomy Research Station. The numbers inside shaded cells are inbreds (all plants are inbreds). Each row (extending from East to West) could accommodate a maximum of 57 plants with plant to plant distance of 0.60 m. The five rows were maintained at a row to row distance of 1.05 m.

57	56	55	54	53	52	51	50	49	48	47	46	45	44	43	42	41	40	39	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
114	113	112	111	110	109	108	107	106	105	104	103	102	101	100	99	98	97	96	95	94	93	92	91	90	89	88	87	86	85	84	83	82	81	80	79	78	77	76	75	74	73	72	71	70	69	68	67	66	65	64	63	62	61	60	59	58
171	170	169	168	167	166	165	164	163	162	161	160	159	158	157	156	155	154	153	152	151	150	149	148	147	146	145	144	143	142	141	140	139	138	137	136	135	134	133	132	131	130	129	128	127	126	125	124	123	122	121	120	119	118	117	116	115
228	227	226	225	224	223	222	221	220	219	218	217	216	215	214	213	212	211	210	209	208	207	206	205	204	203	202	201	200	199	198	197	196	195	194	193	192	191	190	189	188	187	186	185	184	183	182	181	180	179	178	177	176	175	174	173	172
																																																								229

N ↑

Fig. 3.3. The additional S_3 field layout at Oklahoma State University Agronomy Research Station. The combination of number and letter inside shaded cell represent the inbred plant number and associated cultivar. These plants were not used to produce S_4 plants. The layout was designed in order to facilitate inter-cultivar hybridization [between 'Alamo' (A) and 'Kanlow' (K)]. Each row (extending from East to West) could accommodate a maximum of 25 plants with plant to plant distance of 1.20 m. The two rows were maintained at a row to row distance of 0.30 m.

25A	24A	23A	22A	21A	20A	19A	18A	17A	16A	15A	14A	13A	12A	11A	10A	9A	8A	7A	6A	5A	4A	3A	2A	1A
25K	24K	23K	22K	21K	20K	19K	18K	17K	16K	15K	14K	13K	12K	11K	10K	9K	8K	7K	6K	5K	4K	3K	2K	1K

Fig. 3.4. The S_4 field layout at Oklahoma State University Agronomy Research Station. The numbers inside shaded cells were inbreds and the remaining were not inbreds. Each row (extending from North to South) could accommodate a maximum of 40 plants with plant to plant distance of 0.60 m. The nine rows were maintained at a row to row distance of 1.05 m.

N ↑

40	80	120	160	200	240	280	320	
39	79	119	159	199	239	279	319	
38	78	118	158	198	238	278	318	
37	77	117	157	197	237	277	317	
36	76	116	156	196	236	276	316	
35	75	115	155	195	235	275	315	
34	74	114	154	194	234	274	314	
33	73	113	153	193	233	273	313	
32	72	112	152	192	232	272	312	
31	71	111	151	191	231	271	311	
30	70	110	150	190	230	270	310	
29	69	109	149	189	229	269	309	
28	68	108	148	188	228	268	308	
27	67	107	147	187	227	267	307	
26	66	106	146	186	226	266	306	
25	65	105	145	185	225	265	305	
24	64	104	144	184	224	264	304	
23	63	103	143	183	223	263	303	
22	62	102	142	182	222	262	302	
21	61	101	141	181	221	261	301	
20	60	100	140	180	220	260	300	
19	59	99	139	179	219	259	299	339
18	58	98	138	178	218	258	298	338
17	57	97	137	177	217	257	297	337
16	56	96	136	176	216	256	296	336
15	55	95	135	175	215	255	295	335
14	54	94	134	174	214	254	294	334
13	53	93	133	173	213	253	293	333
12	52	92	132	172	212	252	292	332
11	51	91	131	171	211	251	291	331
10	50	90	130	170	210	250	290	330
9	49	89	129	169	209	249	289	329
8	48	88	128	168	208	248	288	328
7	47	87	127	167	207	247	287	327
6	46	86	126	166	206	246	286	326
5	45	85	125	165	205	245	285	325
4	44	84	124	164	204	244	284	324
3	43	83	123	163	203	243	283	323
2	42	82	122	162	202	242	282	322
1	41	81	121	161	201	241	281	321

Fig. 3.5. The plants in the S_2 field being paper bagged for selfing. The seeds obtained from these plants constitute putative S_3 inbreds.

Fig. 3.6. The South Dakota Seed Blower assembly for seed cleanig.

Fig. 3.7. The seeds being germinated in a rectangular plastic pots in greenhouse.

Fig. 3.8. Seeds being covered with plastic cover to conserve moisture.

Fig. 3.9. The putative S3 inbred seedlings growing in rectangular plastic pots.

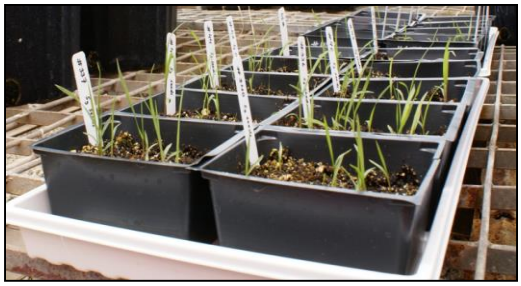

Fig. 3.10. The putative S_3 seedlings growing in conetainers after being transplanted into individual conetainers from plastic pots.

Fig. 3.11. The fourth generation (S_4) inbreds growing in the field at Oklahoma State University Agronomy Research Station.

CHAPTER IV

QTL LOCALIZATION FOR PLANT HEIGHT IN LOWLAND SWITCHGRASS

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a model cellulosic herbaceous feedstock species selected for biofuels production by the United States Department of Energy (DOE) in 1991 (Wright and Turhollow, 2010). It is a perennial, C_4, highly polymorphic, self-incompatible and wind pollinated polyploid species exhibiting disomic inheritance (Nielson, 1944; Taliaferro, 2002; McLaughlin and Kszos, 2005; Okada, 2010; Liu and Wu, 2012; Liu and Wu, 2014). The base chromosome number in switchgrass is x=9. Switchgrass ploidy level ranging from diploid (2n=2x=18) to duodecaploid (2n=12x=108) has been reported (Nielson, 1944). The lowland and upland ecotypes are two dominant phenotypic groups in switchgrass (Zhang et al., 2011). The lowland ecotypes are exclusively tetraploid (2n=4x=36) while the upland ecotypes are tetraploid (2n=4x=36) or octaploid (2n=8x=72) with reportedly rare hexaploids (2n=6x=54) (Zhang et al., 2011; Narasimhamoorthy et al., 2008; Nielsen, 1944). Aneuploidy was reportedly

more common in octaploids (86.3%) than in tetraploids (23.2%) (Costich et al., 2010).

Biomass yield and plant height are quantitative traits. Height is an important yield component that influences biomass yield significantly. Past studies reported that biomass yield was strongly and positively correlated with plant height (Lemus et al., 2002; Das et al., 2004; Sripathi et al., 2013). Lemus et al. (2002) analyzed correlations among traits including selected agronomic traits and cellwall components on 20 upland switchgrass populations over four years (1998-2001) in southern Iowa. They observed significant positive correlation of biomass yield and plant height (r=0.85; $P < 0.0001$). In addition, they observed positive correlation of cellulose with plant height (r=0.52; $P < 0.05$). Das et al. (2004) studied genetic variability and trait relationships in half-sib families of switchgrass at Perkins, OK and Stillwater, OK. They reported significant variation in biomass yield among half-sib progeny families in each of the three populations SU (southern upland) C3, NU (northern upland) C3, and SL (southern lowland) C0 populations. Besides significant effect of genotype (i.e., population), Das et al. (2004) also reported the significant effect of location on biomass yield ($P < 0.01$). They reported small positive correlation of biomass yield and tiller length at 0.03 for Perkins, OK but the value was not significant. Sripathi et al., (2013) reported positive correlation based on study conducted in greenhouse conditions (r=0.76; $P < 0.001$) and in field experiment (r=0.82; $P < 0.001$) at Oklahoma State University. Bhandari et al. (2011) reported narrow-sense heritability estimates for lowland switchgrass biomass yield at 0.17, 0.14, and 0.24, based on half-sib families, full-sib/half-sib families, and midparent-progeny regression, respectively. They also reported narrow-sense heritability estimates for

lowland switchgrass plant height at 0.14, 0.53, and >1.0, based on full-sib/half-sib families, parent-progeny regression, and half-sib families, respectively.

Linkage mapping studies in switchgrass have been previously reported by Missaoui et al. (2005), Okada et al. (2010), and Liu et al. (2012, 2013). Missaoui et al. (2005) constructed a restriction fragment length polymorphism (RFLP) linkage map in a population derived from two outbred parents 'Alamo' AP13 (a tetraploid lowland genotype) and 'Summer' VS16 (a tetraploid upland genotype). Okada et al. (2010) constructed complete linkage maps of two lowland switchgrass genotypes based on SSR and STS markers. Their mapping population consisted of 238 full-sib F_1 progeny of a cross between selected genotypes of switchgrass 'Kanlow' as the female parent and 'Alamo' as the male parent. They also assessed the degree of preferential pairing and the structure of the tetraploid genome. Liu et al. (2012) constructed a complete genetic map of 18 linkage groups in an inbred lowland switchgrass population derived from selfing a heterozygous parent using SSR markers. The study also revealed a one-to-one relationship between nine switchgrass homeologous groups and nine foxtail millet chromosomes.

Serba et al. (2014), recently reported detection of QTLs for biomass yield and plant height in switchgrass based on parental linkage maps constructed using the AP13 x VS16 population, which was used by Missaoui et al. (2005). They identified four QTLs for biomass yield across ten environments and five for plant height across eight environments. They also reported more than 30 QTLs for each of the two traits in single environments and more than 50 epistatic QTLs in each trait. More work is needed to better understand genetic structure for these two and many other traits contributing to

biomass yield and quality. We have developed two mapping populations, one being derived from selfing a northern lowland genotype 'NL94 LYE 16x13', and another from crossing 'NL94 LYE 16x13' and 'SL93 7x15'. Obviously, our mapping populations were different from the populations used by Serba et al. (2014). Accordingly, the objectives of the present study were to analyze phenotypic variation for biomass and plant height, and to localize QTLs associated with the plant height based on linkage maps developed in the two OSU populations.

MATERIALS AND METHODS

Plant Materials

Two mapping populations, including a first-generation selfed population of 'NL94 LYE 16x13' (NL94) and a hybrid population derived from NL94 (♀) x 'SL93 7x15' (SL93) (♂) were used in this study. The NL94 plant was originally selected from the Oklahoma State University (OSU) northern lowland (NL) breeding population growing in a low yield environment (LYE), while SL93 was selected from OSU southern lowland (SL) breeding population (Liu and Wu, 2012; Wu, 2014). Those two parents were each grown in 30 cm diameter pots in a greenhouse at the OSU Agronomy Research Station in the summer of 2007 (Liu and Wu, 2012). Two pots, one pot each from the two parents, were moved to a large growth chamber in the OSU Controlled Environmental Research Laboratory in October 2007, just before anthesis (Liu and Wu, 2012). A total of 456 progeny were obtained from NL94 parent and 44 from SL93, and later Liu and Wu

(2012) identified 279 selfed progeny constituting the selfed population and 177 hybrids forming the hybrid population.

Experimental Design, Establishment and Management

To collect phenotypic data, two field trials were established in 2011, one at the OSU Cimarron Valley Research Station, Perkins (PKS) and the other at the OSU Agronomy Research Station, Stillwater (STW), OK. Soil types for PKS and STW were Teller fine sandy loam and Kirkland silt loam, respectively. The experimental design used at both locations was a randomized complete block with three replications. Each replication constituted 443 plots, encompassing 265 plots of the selfed population, 176 of the hybrid population, and two parents. Each plot consisted of three ramets of one genotype. The spacing between two neighboring rows and two adjacent plants in a row was 107 cm. To minimize border effects, border rows were maintained in each location.

The plants were transplanted in STW and PKS on May 16-17 and June 1-7, 2011 respectively (Dong, 2014). The fields were sprayed with 1.12 kg Atrazine (6-chloro-N-ethyle-N-isopropyle-1,3,5-triazine-2,4-diamine), 1.12 kg Surflan (Oryzalin: 3,5-dinitro-N^4N^4-dipropylsulfanilamide), and 0.007 kg Escort (Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] sulfonyl] benzoate) a.i. per ha immediately after transplanting (Dong, 2014). In March of 2012 and 2013, the fields were applied with 2.24 kg Atrazine, 2.24 kg Surflan, and 4.4 kg Roundup (Glyphosate: N-(phosphonomethyl) glycine) a.i. per ha before the greening up of switchgrass. Sufficient soil moisture was maintained at both locations by irrigation for two weeks after the transplanting. No fertilizer was applied in the establishment year 2011 (Dong, 2014). To facilitate data collection works, white

posts were put on the west end of each field at every 10 rows interval in the month of March. During active growth period of switchgrass in May, the fields were applied with urea at 67.2 Kg N/ha and the weedy plants and contaminants were removed by spot-spraying of Roundup or hand-weeding.

Field Data Collection and Analysis

Plant height was measured from the base of a plant to the top of its panicle. Plant height (cm) measurement was carried out prior to the harvest of plant biomass and after plants became dormant. The plants were cut at 10 cm height from the ground surface using a Single Row Silage Chopper (John Deere, Moline, Illinois) in the winters of 2012 and 2013 for biomass yields. Three tiller samples collected from each plot were weighed for fresh weight, dried at 55°C in a forced air oven for 3 to 7 d, and again weighed for dry weight to calculate dry matter percent. The statistical data analysis for phenotypic traits, plant biomass yield and plant height was carried out using SAS software, Version 9.4 of the SAS System (SAS Institute Inc., 2014).

Linkage Maps

DNA extraction, PCR amplification, SSR markers and genotyping analysis were previously completed by Liu and Wu (2012) for the linkage map construction. QTL mapping for selfed population obtained from NL94 was carried out using the linkage map previously developed by Liu and Wu (2012). QTL mapping for the hybrid population obtained from NL94 (♀) and SL93 (♂) was carried out using the linkage map developed by Dong (2014). Dong (2014) previously used the same data to identify QTLs associated with reproductive maturity associated in lowland switchgrass populations.

QTL Analysis Procedures

QTL analysis was carried out separately for each of the two population types, selfed and hybrids. In each population, the analysis was carried out separately for each environment (a combination of year and location). Software program MapQTL 6 (Van Ooijen, 2009) was used in the data analysis using a map file, a locus genotype file, and a quantitative trait file all in text format. For the selfed population, locus genotype file was converted into data format of F_2 population type (Dong, 2014). CP population type was used for the hybrid population. Quantitative trait files were prepared for each of the four environments by taking mean of each plot averaged over three replications. Both interval mapping (IM) and multiple QTL model (MQM) mapping employed in our analysis used regression algorithm. The other calculation options in QTL analysis included fit dominance for F2 (IM) as yes, mapping step size 1, maximum number of neighboring markers 5, maximum number of iterations 200, functional tolerance value 10^{-8}, $P = 0.02$ for automatic cofactor selection and number of permutations 1,000. In the initial analysis IM was used and putative cofactor marker loci were selected. LOD threshold to detect significant QTL at 95% confidence level was determined by the permutation test with the number of permutations set at1,000. The permutation test was considered to compute more accurate threshold LOD for QTL detection (Van Ooijen, 1999). Genome wide (GW) LOD threshold computed by permutation test was used in the analysis. The permutation test was considered to avoid the problem of non-normal data (Van Ooijen, 1999) and hence normality test was not carried out in this analysis. Besides, the IM procedure (including MQM, ACS) is considered quite robust against deviations from normality (Van Ooijen, 2009). After IM, using putative cofactors, MQM was performed.

After the first MQM, automatic cofactor selection (ACS) was performed. The MQM and ACS analyses were performed many times with each time excluding non-significant cofactors and adding newly detected QTL associated markers as cofactors until stable cofactors were obtained. The reliable QTLs were obtained in the final MQM analysis.

RESULTS AND DISCUSSIONS

Biomass yield and plant height both showed significant variation among genotypes in both the selfed and hybrid populations (Table 4.1). In general, year, location, and replication had significant effect for biomass yield and plant height with an exception that location was non-significant for biomass yield in the selfed population. The year*location interaction was significant for the biomass yield in both populations while it is significant for plant height in the selfed population only. The year*genotype interaction was significant only for the plant height in both populations but not for the biomass yield. The location*genotype interaction was significant for both traits in both populations but the year*location*genotype interaction was not significant for both traits (Table 4.1). Biomass yield of hybrid population was more than three times of the selfed population and plant height was also higher for the hybrid population (Table 4.2). Coefficient of variation (CV) was calculated as a ratio of standard deviation to population mean and expressed as percentage. It was used to compare variation of biomass yield and plant height. Yield variation was higher compared to plant height in both population types (Table 4.2).

Both biomass yield and plant height were significantly different among the plant genotypes in each environment (Table 4.3). Biomass yield and plant height were higher

in 2013 compared to 2012 in PKS whereas the results were not same in STW (Table 4.4). The critical growth period in switchgrass includes months of May, June, July, and August and any sharp departure from the normal rainfall and solar radiation activities in these months can impact plant growth (Makaju et al., 2013). In our study, the rainfall in May, June, and July for year 2012 was excessively lower compared to 30-yr means (Table 4.5). The year 2012 was the second year and 2013 was the third year of establishment of switchgrass in this study. In general, switchgrass production in the second year of establishment is about 70% of its full potential and production in the third and subsequent years is at full capacity. Biomass yield values were higher in STW than PKS in both populations, however, the difference does not exceed LSD values (Table 4.4). Biomass yield was higher in PKS in 2013 in both populations. Plant height was taller in PKS than STW in each environment (Table 4.4). Stillwater had more soil moisture deficit in upper 40.6 cm soil than Perkins when compared to an average for 15 years (1999-2013) for both 2012 and 2013 (Mesonet, 2014). The soil moisture deficit was observed for all active growing months May, June, and July in 2012, while deficit was observed for June to July in 2013 (Mesonet, 2014).

Biomass yield was positively correlated with plant height for both the selfed population (r=0.39, $P < 0.0001$) and the hybrid population (r=0.41, $P < 0.0001$). The estimated regression lines of the biomass yield on the plant height for the selfed and the hybrid populations showed that an increase of 1 cm plant height leads to an increase of biomass yield by 4 g/plant in the selfed population and by 8 g/plant in the hybrid population (Figs. 4.1 and 4.2). Similarly, the plant height explained 15% and 17% variation in the biomass yields in the selfed and the hybrid populations, respectively. The

correlation analysis by Serba et al. (2014) indicated 20% biomass variation accounted by plant height, and this can be compared with our result of 17% in the hybrid population. In the correlation analysis for each environment separately, significantly positive correlation was observed in each environment (Table 4.6 and Figs. 4.3 – 4.10). The histograms for biomass yield and plant height in the selfed population showed most of the distribution of progeny values were towards left side of parental values indicating effect of inbreeding depression (Fig. 4.11). In contrast, the histograms for biomass yield and plant height in the hybrid population indicated most of progeny values were towards right side of both parents which showed hybrid vigor (Fig. 4.12).

Detection of plant height associated QTLs

Our study revealed 21 QTLs, including nine in the selfed population and 12 in the hybrid population, were associated with switchgrass height in the two mapping populations (Tables 4.7 and 4.8). Nine QTLs detected in the selfed population belonged to six linkage groups and 12 in the hybrid population belonged to six linkage groups as well. Overall 11 linkage groups were associated with QTLs detection as revealed in both the populations. Phenotypic variance for switchgrass height explained by individual QTLs ranged from 4.8 to 14.4% (Tables 4.7 and 4.8). Fig. 4.13.1-9 show QTLs detected in the selfed population and Figs. 4.14.1-11 show QTLs detected in the hybrid population.

Serba et al. (2014) performed QTL analysis across all environments with explained phenotypic variation ranging from 5.1 to 12.0% and in each of the environments with explained variation ranging from 4.3 to 17.4%. Their across all

environment analysis produced additive effects ranging from -8.3 to 6.7 cm plant^{-1}. In our study for the selfed population, additive effects ranged from -8.9 to 5.1 cm plant^{-1}. Out of nine QTLs in the selfed population, two QTLs indicated positive additive effects and remaining seven QTLs were associated with negative additive effects. Serba et al. (2014) used LOD threshold as 2 and reported QTLs in LGs 1b, 4a, 4b, 5a, 5b, 6b, 7a, 9a, and 9b in the female (lowland cultivar 'AP13') map and in LGs 1a, 1b, 2b, 3b, 4a, 4b, 5a, 6a, and 9b in the male (upland cultivar 'VS16') map. Serba et al. (2014) indicated that there may be non-correspondence of the QTLs between the female (lowland ecotype) and the male (upland ecotype) maps.

In the selfed population, only one QTL was identified on LG 4 in 2012 (2012-STW environment) (Fig. 4.13.1). In 2013, three QTLs on LGs 1b, 2a, 9b were identified from data collected in the field trial at Perkins, OK (Fig. 4.13.2-4). In the same year, five QTLs were localized on LGs 1b, 2a, 7a, 9a & 9b from the trial at Stillwater, OK (Fig. 4.13.5-9). It appears significant QTLs detected at each environment explained 7.2% (2012-STW) to 37.2% (2013-STW) phenotypic variation. In 2013, the QTL was identified exactly at the same marker interval on LG 2a across two locations. However, QTLs on LGs 1b and 9b at the two locations were distant although on the same LGs.

In the hybrid population, a total of 12 QTLs were detected at three environments (2012-STW, 2013-PKS, and 2013-STW). In 2012, seven QTLs were identified, collectively accounting for 62.3% variation in switchgrass height in the trial at Stillwater. Only one QTL was identified in the trial at Perkins, OK in 2013. In 2013, four QTLs were recognized explaining 38.3% of total variation for the trait from the trial at Stillwater. The 12 QTLs were localized on LGs, 1b, 2a, 3a&b, 5b, 6b, 7a, and 9b,

respectively. The QTL on 1b was identified in the same marker interval in both years at Stillwater, OK.

CONCLUSIONS

Two lowland switchgrass mapping populations were deployed in this experiment. Large genetic variation existed for plant biomass and height within the two populations. This study confirmed that plant height was significantly correlated with biomass yield in lowland switchgrass. Twenty-one QTLs were identified on 11 LGs in this study. Nine of the QTL markers were detected in the selfed population and remaining 12 QTL markers were detected in the hybrid population. These markers tightly linked to the QTLs have potential to be used in marker assisted selection for crop improvement programs in switchgrass.

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LIST OF TABLES

LIST OF FIGURES

Table 4.1. ANOVA for the biomass yield (g/plant) and plant height (cm) for each of the selfed and the hybrid populations using GLM procedure.

	Selfed population				Hybrid population			
Sources of variation	df	Biomass yield	df	Plant height	df	Biomass yield	df	Plant height
Year	1	****	1	****	1	****	1	****
Location	1	NS†	1	****	1	****	1	****
Genotype	264	****	264	****	175	****	175	****
Replication	2	***	2	****	2	****	2	****
Year*Location	1	****	1	****	1	****	1	NS
Year*Genotype	264	NS	263	****	175	NS	175	***
Location*Genotype	258	****	257	****	175	****	175	**
Year*Location*Genotype	257	NS	251	NS	175	NS	175	NS

**** Significant at the 0.0001 probability level.

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

† Non-significant at the 0.05 probability level.

Table 4.2. Summary for the biomass yield (g/plant) and plant height (cm) for each of the selfed and the hybrid populations.

	Selfed Population		Hybrid Population	
Parameter	Biomass yield	Plant height	Biomass yield	Plant height
NL94 (P1)	979.75	206.33	979.75	206.33
SL93 (P2)			1258.58	203.17
Population mean	400.99	184.25	1468.70	212.21
LSD(0.05)	211.42	12.73	367.64	13.17
R^2	0.53	0.80	0.66	0.84
CV(%)	65.85	8.63	31.26	7.75

Table 4.3. ANOVA for the biomass yield and plant height in Perkins, OK (PKS) and Stillwater, OK (STW) from 2012 to 2013.

Sources of variation	Selfed population								Hybrid population							
	Biomass yield				Plant height				Biomass yield				Plant height			
	df	2012	df	2013	df	2012	df	2013	df	2012	df	2013	df	2012	df	2013
PKS																
Genotype	258	****	258	****	256	****	254	****	175	****	175	****	175	****	175	****
Replication	2	NS†	2	**	2	****	2	****	2	****	2	****	2	****	2	****
STW																
Genotype	263	****	264	****	263	****	262	****	175	****	175	****	175	****	175	****
Replication	2	***	2	*	2	****	2	**	2	**	2	*	2	NS	2	NS

**** Significant at the 0.0001 probability level.

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

† Non-significant at the 0.05 probability level.

Table 4.4. Summary of the biomass yield and plant height in Perkins, OK (PKS) and Stillwater, OK (STW) from 2012 to 2013.

		Selfed population				Hybrid population			
		Biomass yield		Plant height		Biomass yield		Plant height	
Location	Parameter	2012	2013	2012	2013	2012	2013	2012	2013
PKS	NL94 (P1)	922.33	1277.00	203.67	225.00	922.33	1277.00	203.67	225.00
	SL93 (P2)					1332.00	1783.33	206.33	242.33
	Population mean	275.08	538.32	169.57	213.11	1366.98	2028.51	205.70	248.80
	LSD	419.54	638.64	32.59	24.72	767.84	1018.41	34.67	21.40
	R^2	0.46	0.46	0.62	0.69	0.60	0.52	0.56	0.63
	CV	95.07	73.94	11.98	7.23	34.98	31.26	10.50	5.36
	RMSE	261.52	398.05	20.31	15.41	478.15	634.18	21.59	13.32
	SE	10.48	16.14	0.99	0.84	26.86	32.35	1.16	0.78
	Maximum	3511	3757	313	292	3223	4611	329	312
	Minimum	1	12	86	125	4	110	9	179
	N	750	732	717	693	528	528	526	521
STW	NL94 (P1)	886.67	833.00	181.00	215.67	886.67	833.00	181.00	215.67
	SL93 (P2)					1021.33	897.67	159.67	204.33
	Population mean	401.32	392.86	162.93	193.14	1398.95	1080.35	175.82	218.89
	LSD	299.17	236.36	20.26	18.51	547.11	429.71	23.96	20.49
	R^2	0.59	0.56	0.63	0.72	0.50	0.51	0.59	0.63
	CV	46.47	37.51	7.75	5.98	24.35	24.77	8.49	5.83
	RMSE	186.51	147.35	12.63	11.54	340.69	267.59	14.92	12.76
	SE	8.43	6.48	0.61	0.64	17.11	13.63	0.83	0.74
	Maximum	2226	1351	220	250	2782	3458	220	265
	Minimum	3	14	107	130	25	126	115	140
	N	781	784	765	767	528	528	526	526

Table 4.5. Monthly total precipitation at Perkins and Stillwater, OK from 2012 to 2013 compared with 30-yr average (1981 – 2010).

	Perkins, OK			Stillwater, OK		
Month	2012	2013	30-yr mean	2012	2013.0	30-yr mean
January	2.4	4.5	3.4	2.4	2.5	3.4
February	6.1	8.4	4.3	7.4	7.9	4.2
March	11.5	1.4	8.0	10.0	2.8	8.0
April	12.9	13.0	8.8	15.6	13.5	8.9
May	2.8	17.8	13.8	2.8	15.8	13.5
June	7.4	10.5	12.6	5.5	10.0	12.2
July	0.7	15.4	7.4	0.2	14.1	7.7
August	8.6	12.1	7.0	6.7	6.5	7.6
September	3.4	4.9	10.1	2.8	4.3	10.1
October	2.2	6.4	8.4	1.5	4.8	8.2
November	1.7	3.0	6.4	1.1	4.1	6.2
December	1.5	2.2	4.7	1.1	1.6	4.6
Total	61.1	99.5	94.8	57.3	88.0	94.6

Source: http://www.mesonet.org/index.php/weather/monthly_rainfall_table

Table 4.6. The Pearson correlation coefficient (r) between biomass yield and plant height. N is the number of observations used in the calculation.

		Selfed Population		Hybrid Population	
Year	Location	r	N	r	N
2012	PKS	0.41****	717	0.38****	526
2012	STW	0.29****	765	0.43****	526
2013	PKS	0.33****	693	0.20****	521
2013	STW	0.33****	767	0.30****	526

**** Significant at the 0.0001 probability level.

Table 4.7. QTL positions and associated QTL markers detected in the selfed population.

								Mean plant height (cm)						
Year	Location	Group	Position	Locus	Left Locus	Right Locus	LOD	A	H	B	Variance	PVE† (%)	Additive	Dominance
2012	STW	4a & 4b	37.6	nfsg-36	sww1918_220	PVCA-949/950	4.2	158.0	167.5	164.2	85.5	7.2	-3.1	6.4
2013	PKS	2a	39.3	nfsg-52	PVCA-327/328_130	PVCAG-2623/2624	6.0	194.1	202.9	211.8	113.9	9.8	-8.9	-0.1
2013	PKS	9b	62.3	sww-466	nfsg-262	nfsg-202	5.6	196.8	209.5	209.0	113.9	9.1	-6.1	6.6
2013	PKS	1b	35.6	PVCAG-2361/2362	PVGA-1947/1948	PVCA-179/180	4.6	208.1	207.8	197.8	113.9	7.2	5.1	4.8
2013	STW	2a	38.4		PVCA-327/328_130	PVCAG-2623/2624	5.9	177.1	186.0	193.2	65.4	6.5	-8.1	0.8
2013	STW	9b	91.0	PVCAG-2487/2488	PVGA-1843/1844	PVGA-1351/1352	4.9	180.5	180.9	192.0	68.0	5.5	-5.8	-5.4
2013	STW	9a	101.9		sww-2285	PVAAG-3027/3028	4.5	177.4	192.6	194.6	65.9	4.8	-8.6	6.5
2013	STW	1b	60.1		PVGA-1401/1402	PVGA-1735/1736	11.6	188.1	199.1	183.9	66.7	14.4	2.1	13.1
2013	STW	7a	9.9		sww-1742	PVAAG-3253/3254	5.4	181.9	180.1	192.3	67.4	6.0	-5.2	-7.0

† Phenotypic variance explained

Table 4.8. QTL positions and associated QTL markers detected in the hybrid population.

								Mean plant height (cm)					
Year	Location	Group	Position	Locus	Left locus	Right locus	LOD	ac{00}	ad{00}	bc{00}	bd{00}	Variance	PVE† (%)
2012	STW	3b	17.3	PVGA-1957/1958	PVCAG-2393/2394	PVGA-1201/1202	5.1	182.5	177.6	188.0	179.7	64.2	5.5
2012	STW	7a	28.1		sww-2167	PVGA-2139/2140	8.4	181.9	170.3	181.9	172.6	63.5	9.5
2012	STW	2a	92.4		nfsg-052	sww-2545	11.6	183.4	171.7	176.1	165.4	60.2	13.1
2012	STW	9b	56.5		nfsg-200	PVCA-7/8	8.5	182.1	198.6	177.8	186.1	62.8	9.5
2012	STW	3a	63.9	sww-530	PVAAG-2857/2858	PVCA-687/688	5.5	182.5	174.4	186.5	185.3	64.2	5.9
2012	STW	1b	18.2		sww-177	PVCA-179/180	9.1	182.5	192.2	183.2	172.4	63.6	10.4
2012	STW	1b	47.5		PVGA-1401/1402	PVCAG-2361/2362	7.5	182.3	190.5	185.4	200.9	63.7	8.4
2013	PKS	3a	80.4		PVCAG-2297/2298	nfsg-035	4.8	250.8	251.7	264.3	259.2	116.6	11.6
2013	STW	7a	11.0	PVAAG-2881/2882	PVAAG-3051/3052	PVGA-1969/1870	7.5	233.7	222.1	230.7	223.3	72.8	12.9
2013	STW	5b	39.7		PVAAG-3163/3164	PVCAG-2153/2154	6.0	234.4	230.4	222.9	224.1	72.5	9.9
2013	STW	6b	4.8	sww-1889	PVAAG-3017/3018	PVGA-2081/2082	4.4	233.1	225.0	233.2	226.5	81.2	8.0
2013	STW	1b	16.2	sww-177	sww-2320	sww-177	4.6	233.7	242.1	230.2	236.7	72.8	7.5

† Phenotypic variance explained

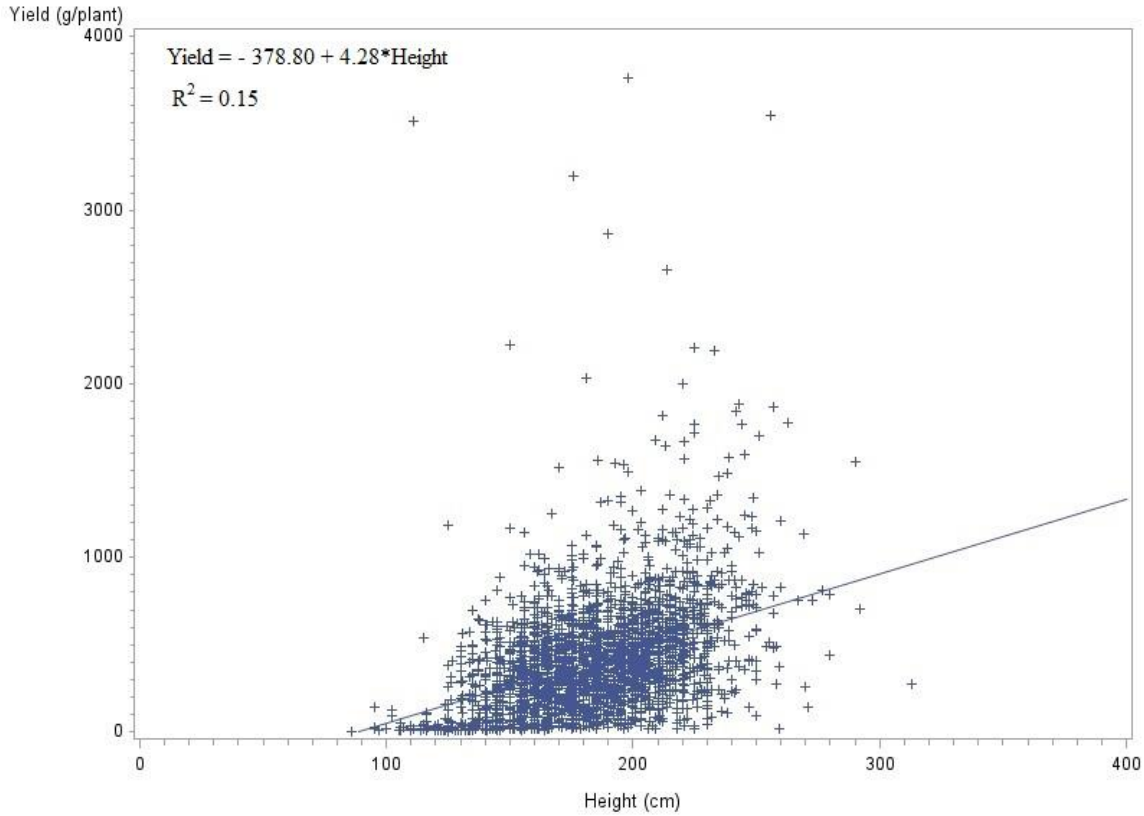

Fig.4.1. The observed values and estimated regression line of biomass yield (g/plant) on plant height (cm) for the selfed population across all environments.

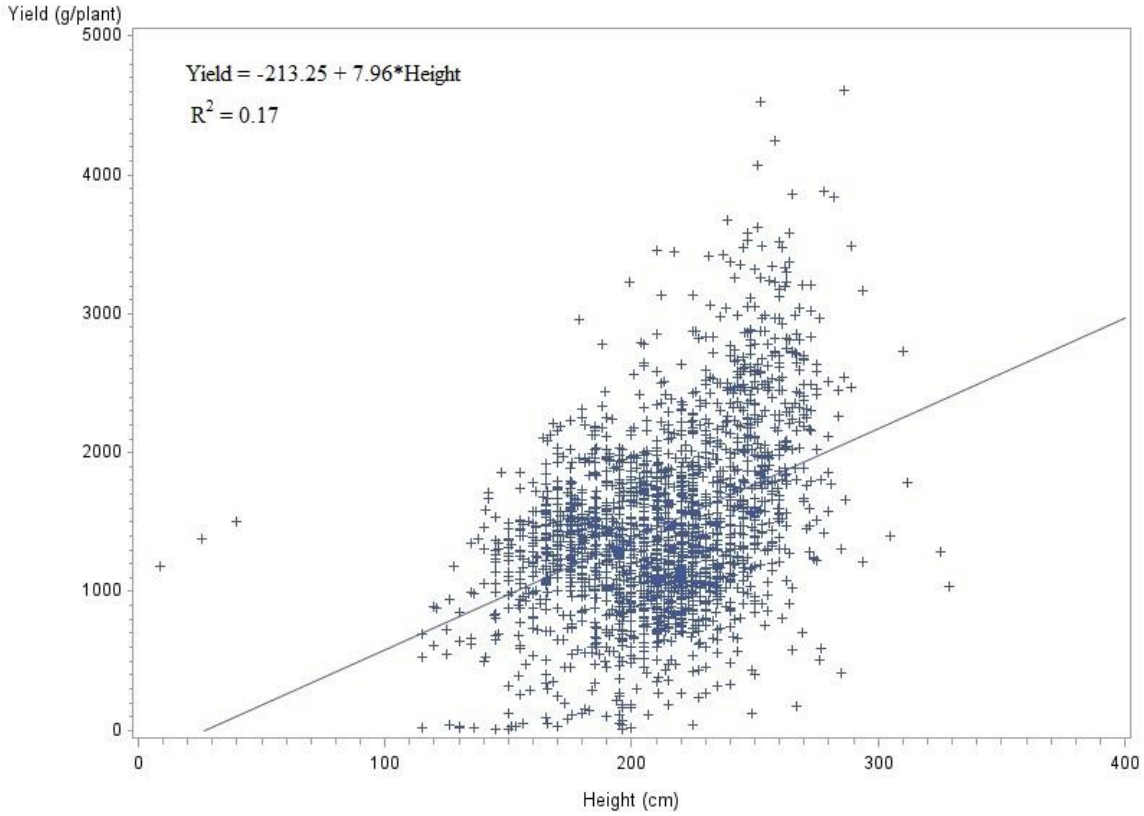

Fig.4.2. The observed values and estimated regression line of biomass yield (g/plant) on plant height (cm) for the hybrid population across all environments.

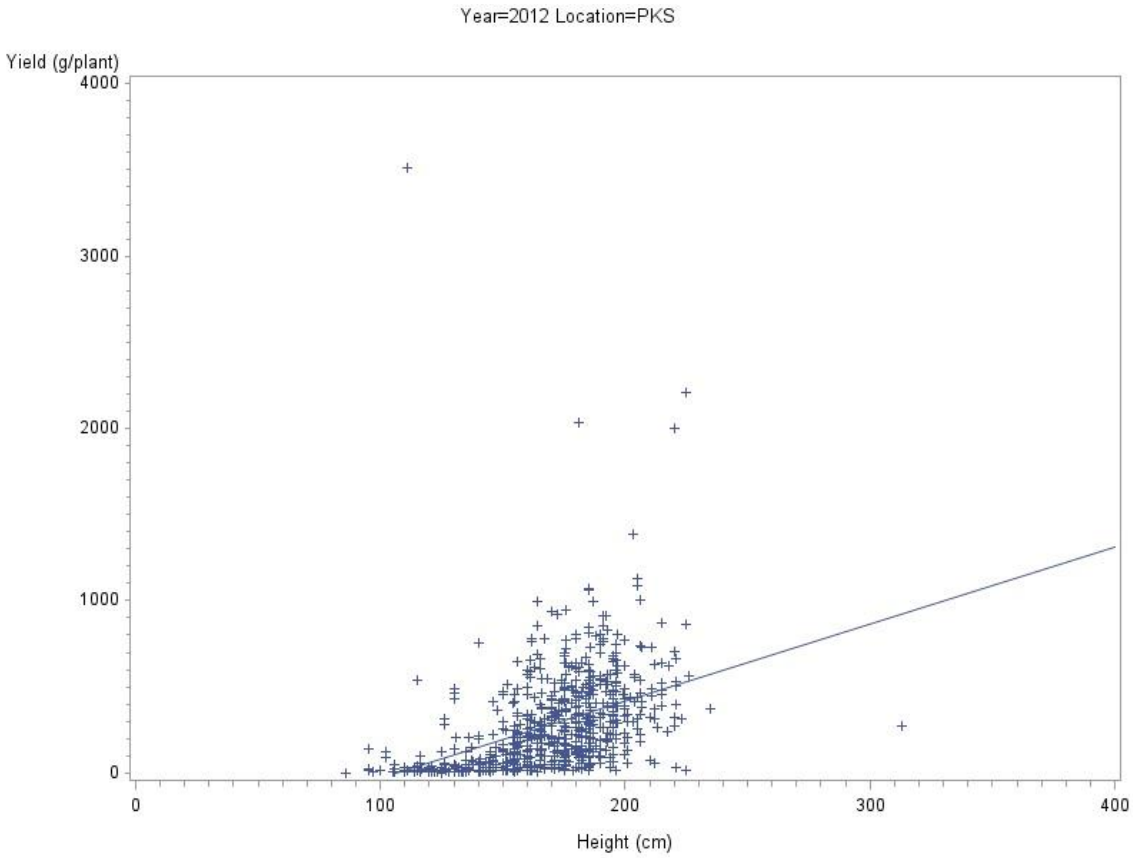

Fig. 4.3. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the selfed population in 2012 at Perkins, OK.

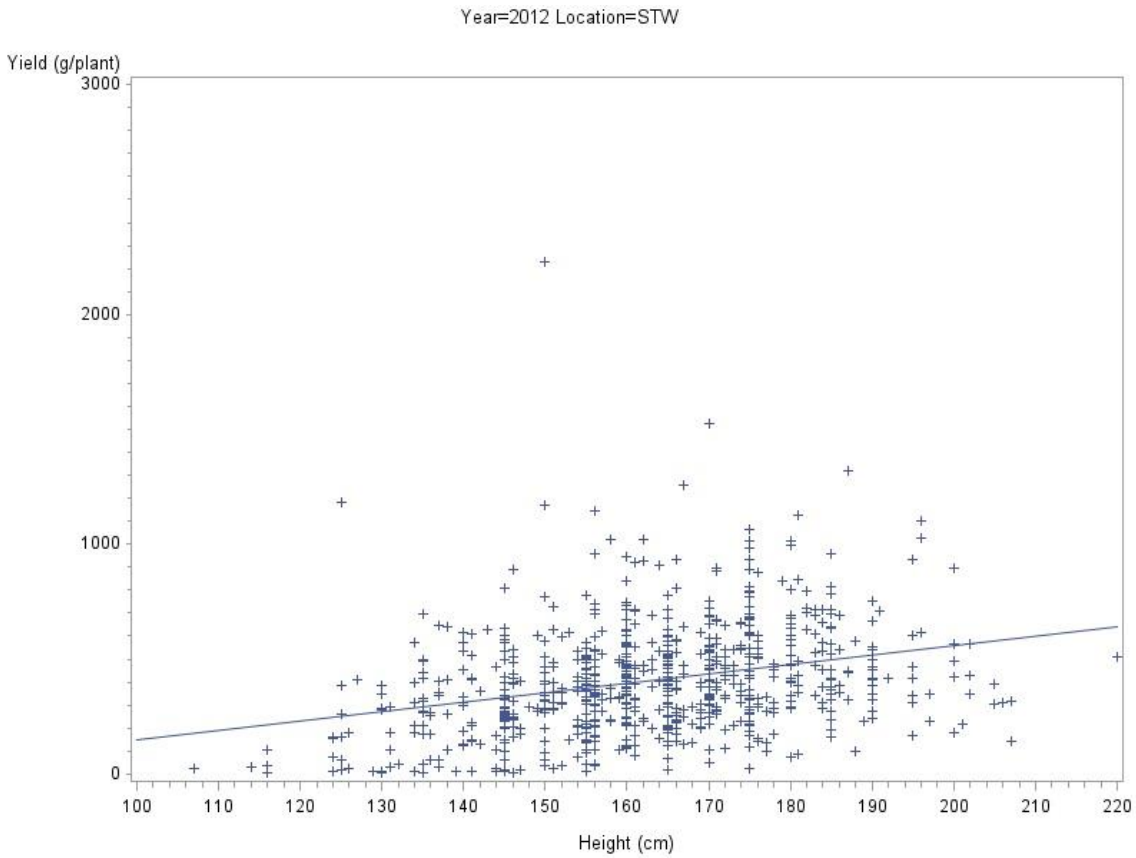

Fig. 4.4. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the selfed population in 2012 at Stillwater, OK.

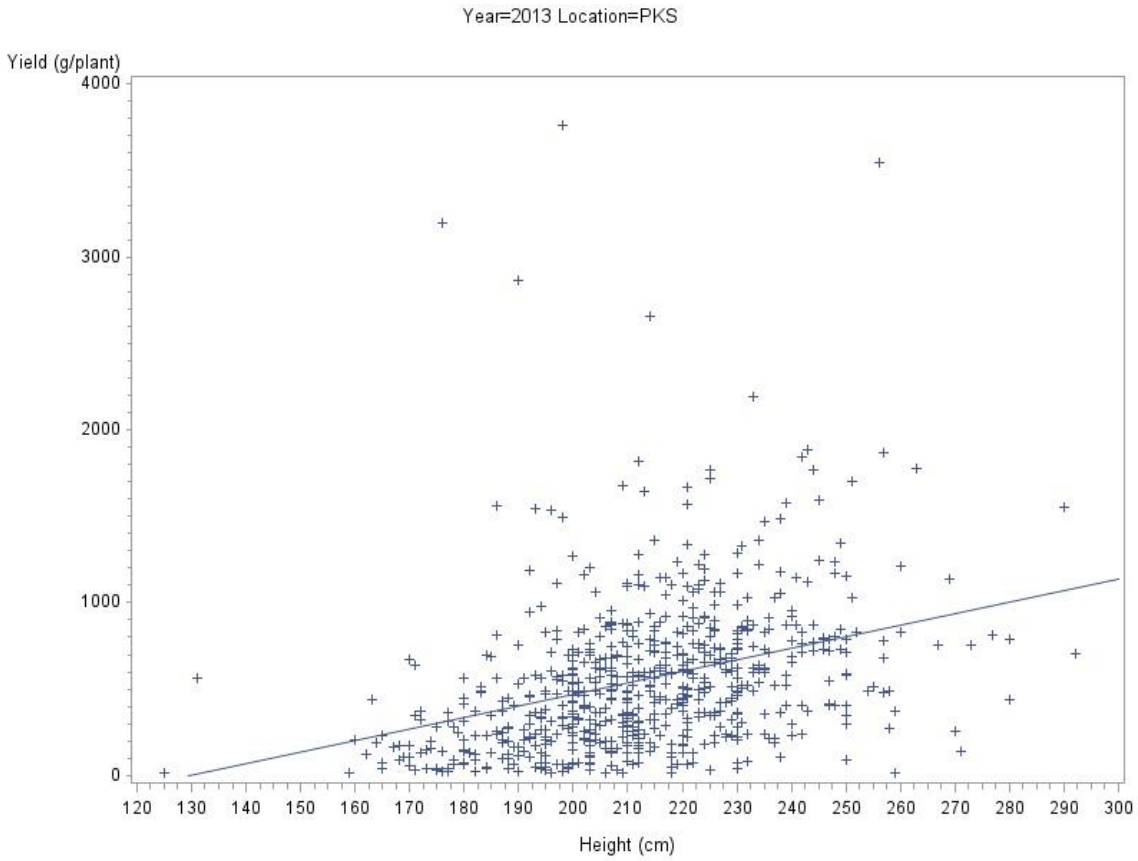

Fig. 4.5. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the selfed population in 2013 at Perkins, OK.

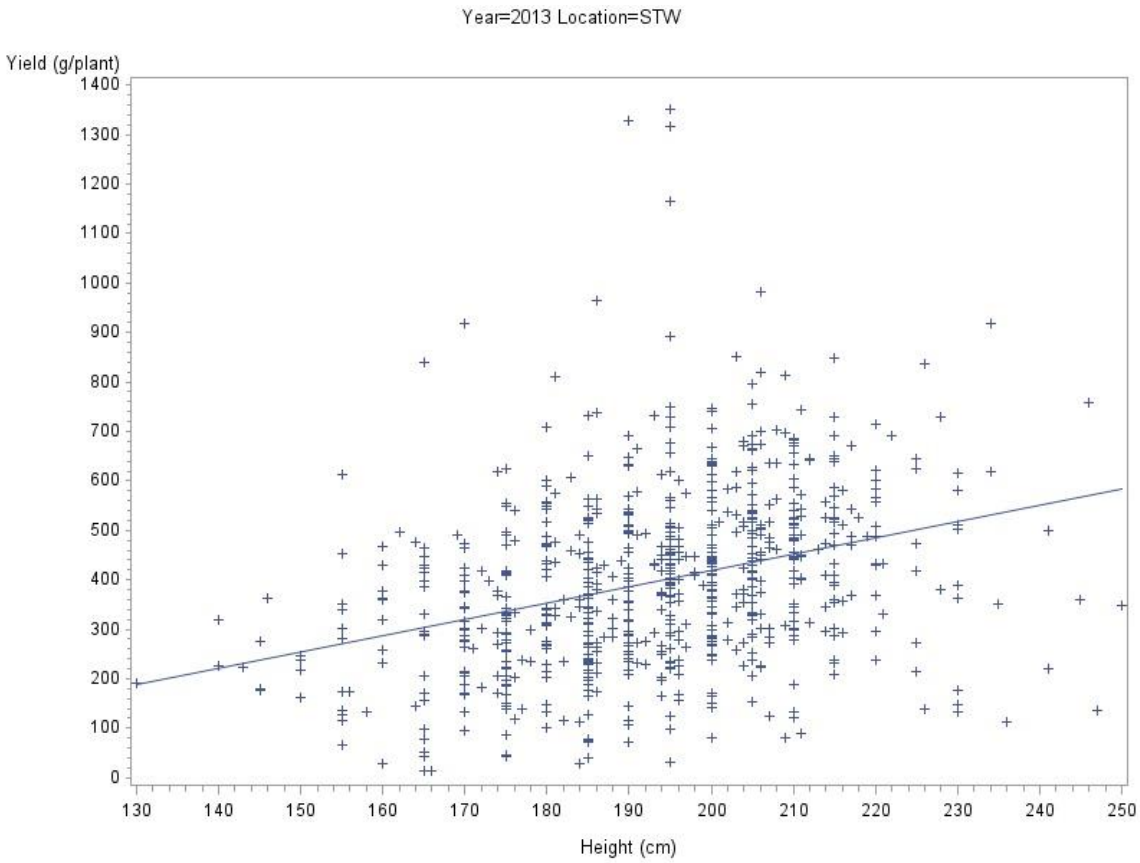

Fig. 4.6. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the selfed population in 2013 at Perkins, OK.

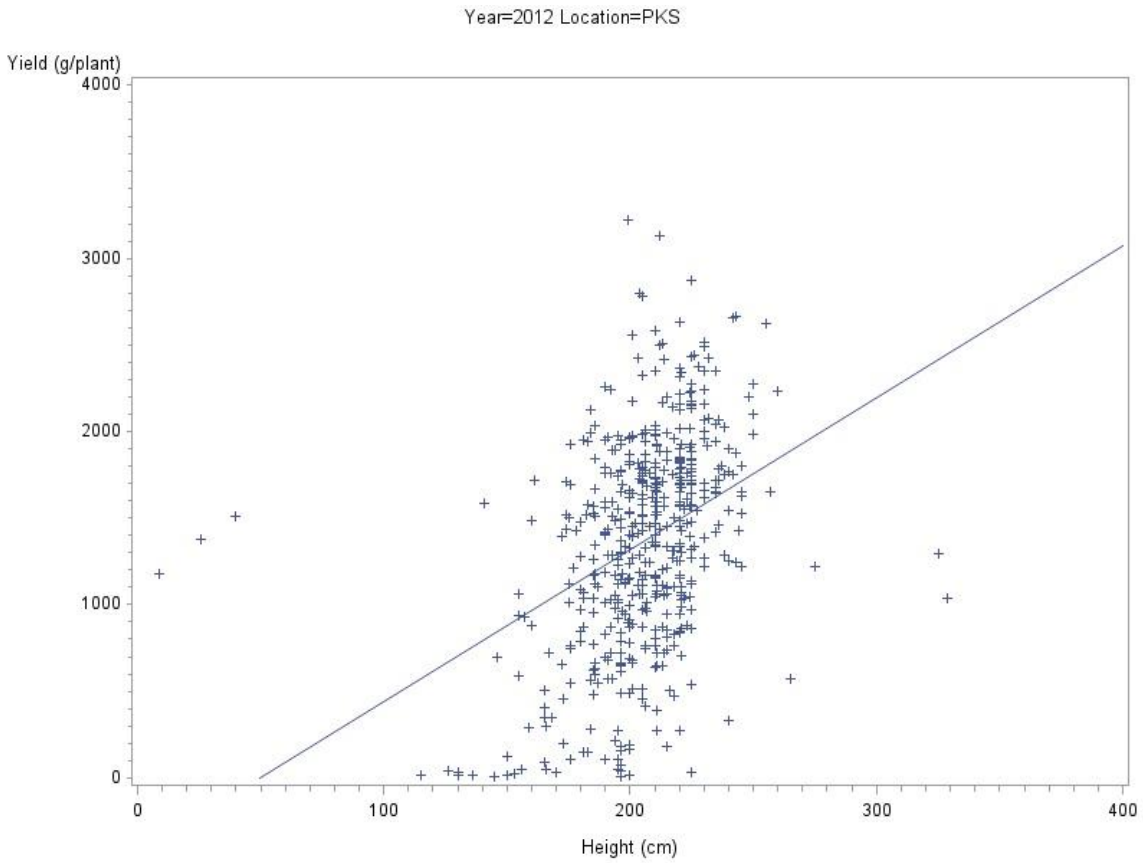

Fig. 4.7. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the hybrid population in 2012 at Perkins, OK.

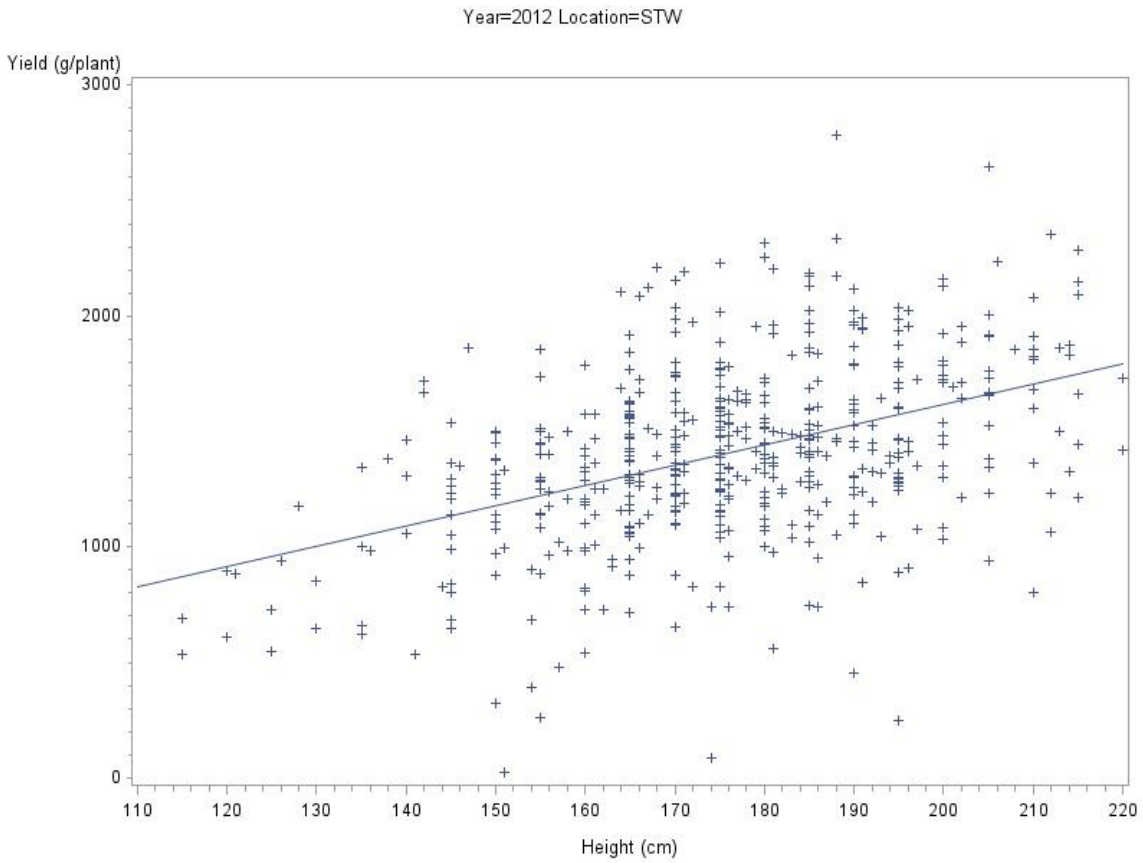

Fig. 4.8. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the hybrid population in 2012 at Stillwater, OK.

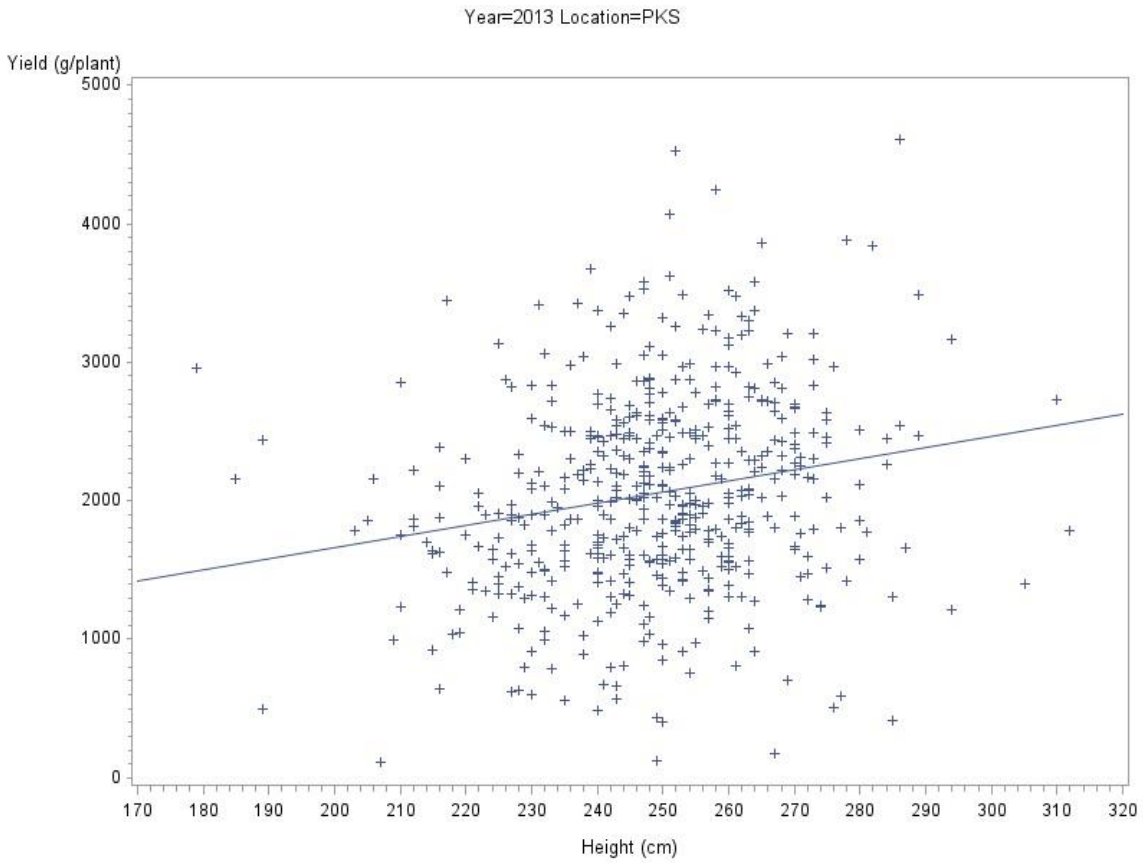

Fig. 4.9. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the hybrid population in 2013 at Perkins, OK.

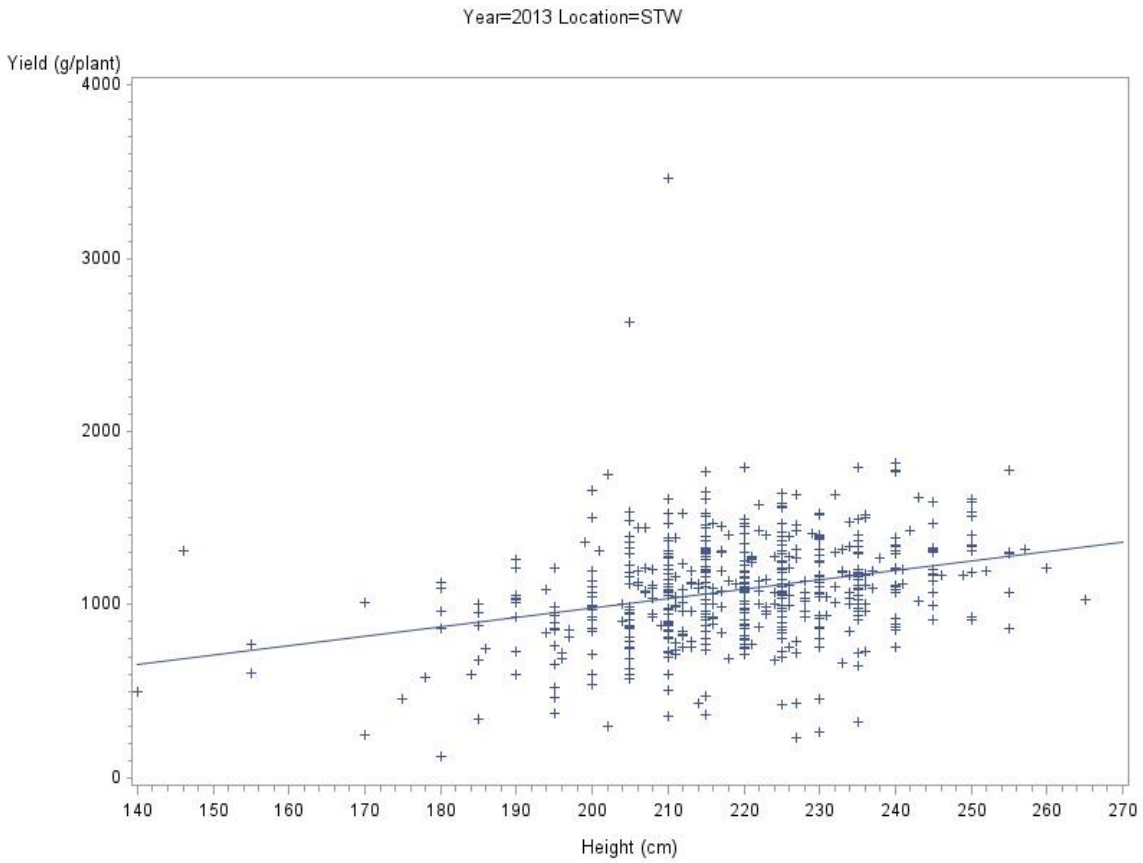

Fig. 4.10. The observed values and estimated regression line of the biomass yield (g/plant) on plant height (cm) for the hybrid population in 2013 at Stillwater, OK.

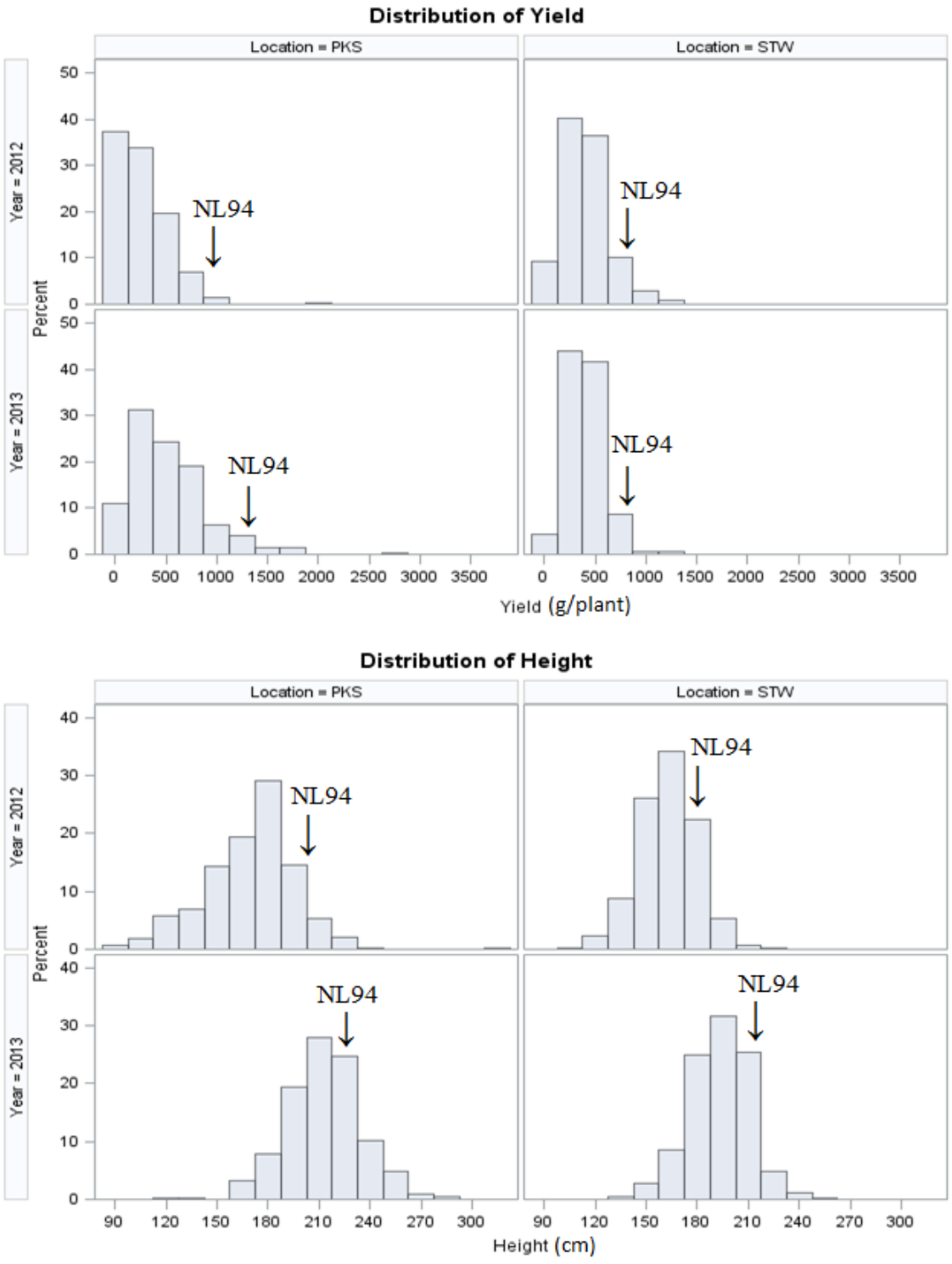

Fig. 4.11. Distribution of biomass yield and plant height in the selfed population with the parent NL94 for four environments. Each environment is a year and location combination. PKS represents Perkins, OK and STW represents Stillwater, OK.

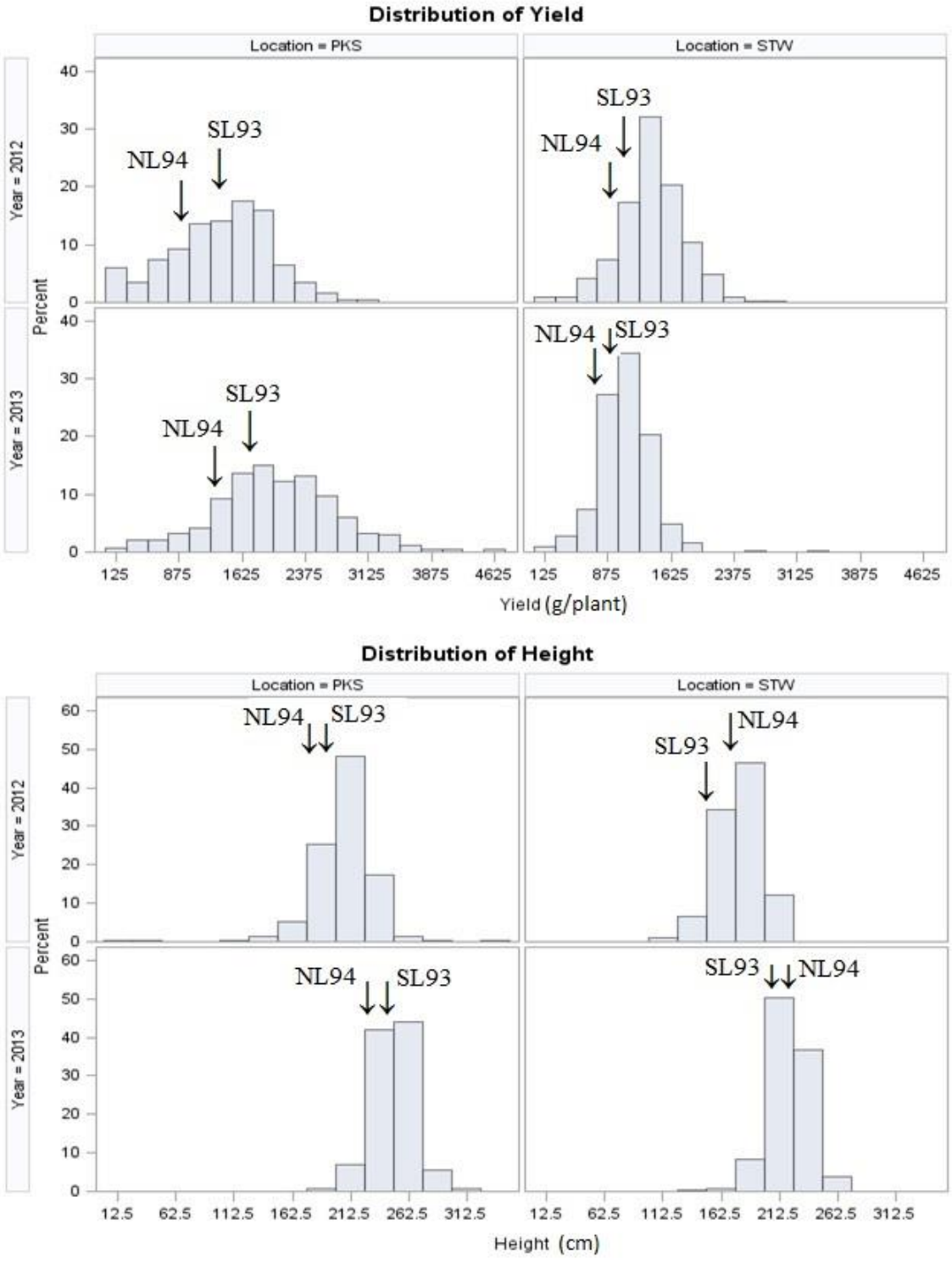

Fig. 4.12. Distribution of biomass yield and plant height in the hybrid population with parents NL94 and SL93 in four environments. Each environment is a year and a location combination. PKS represents Perkins, OK and STW represents Stillwater, OK.

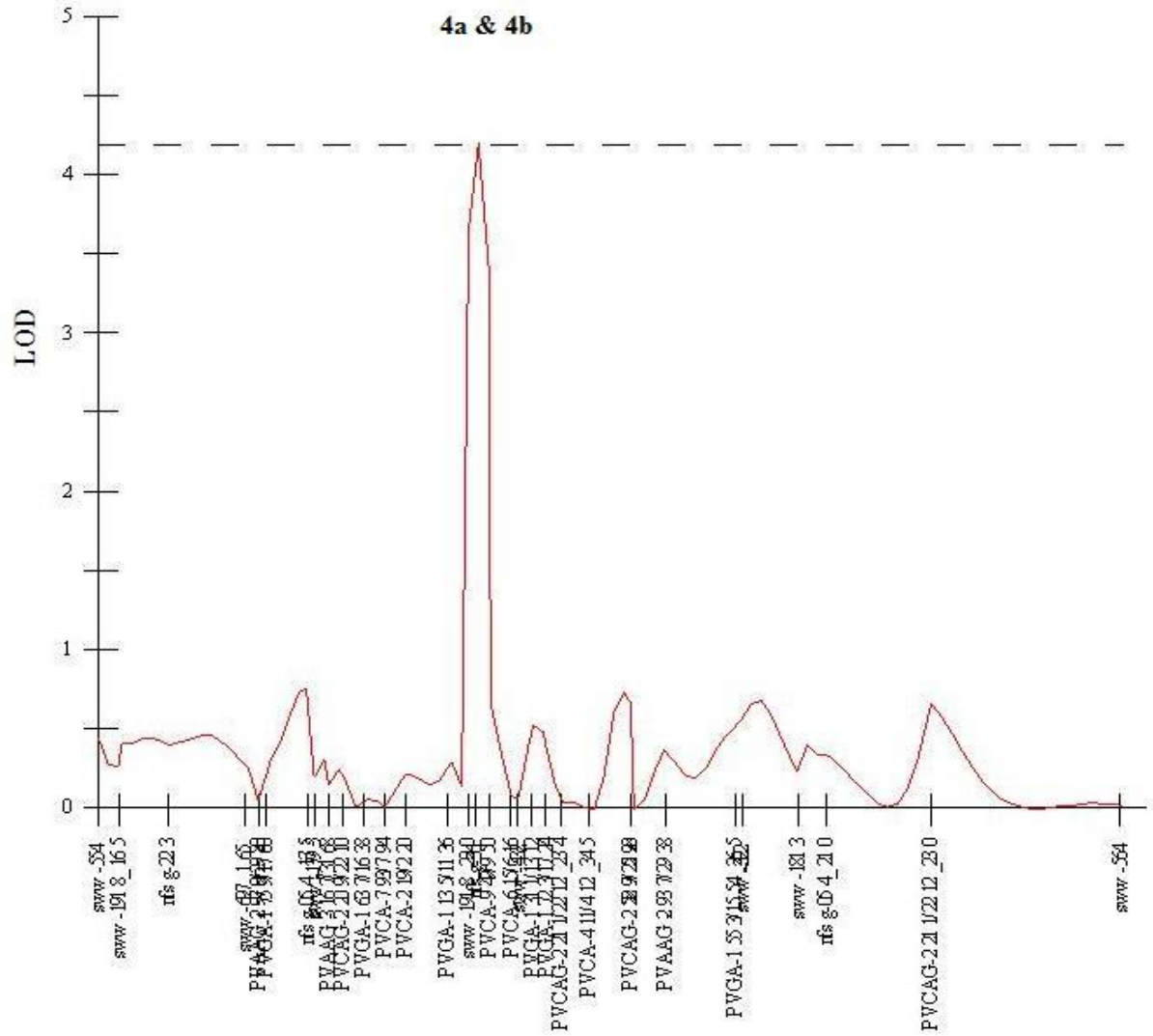

Fig. 4.13.1. QTL detected in linkage group 4a & 4b for the selfed population at Stillwater, OK in 2012.

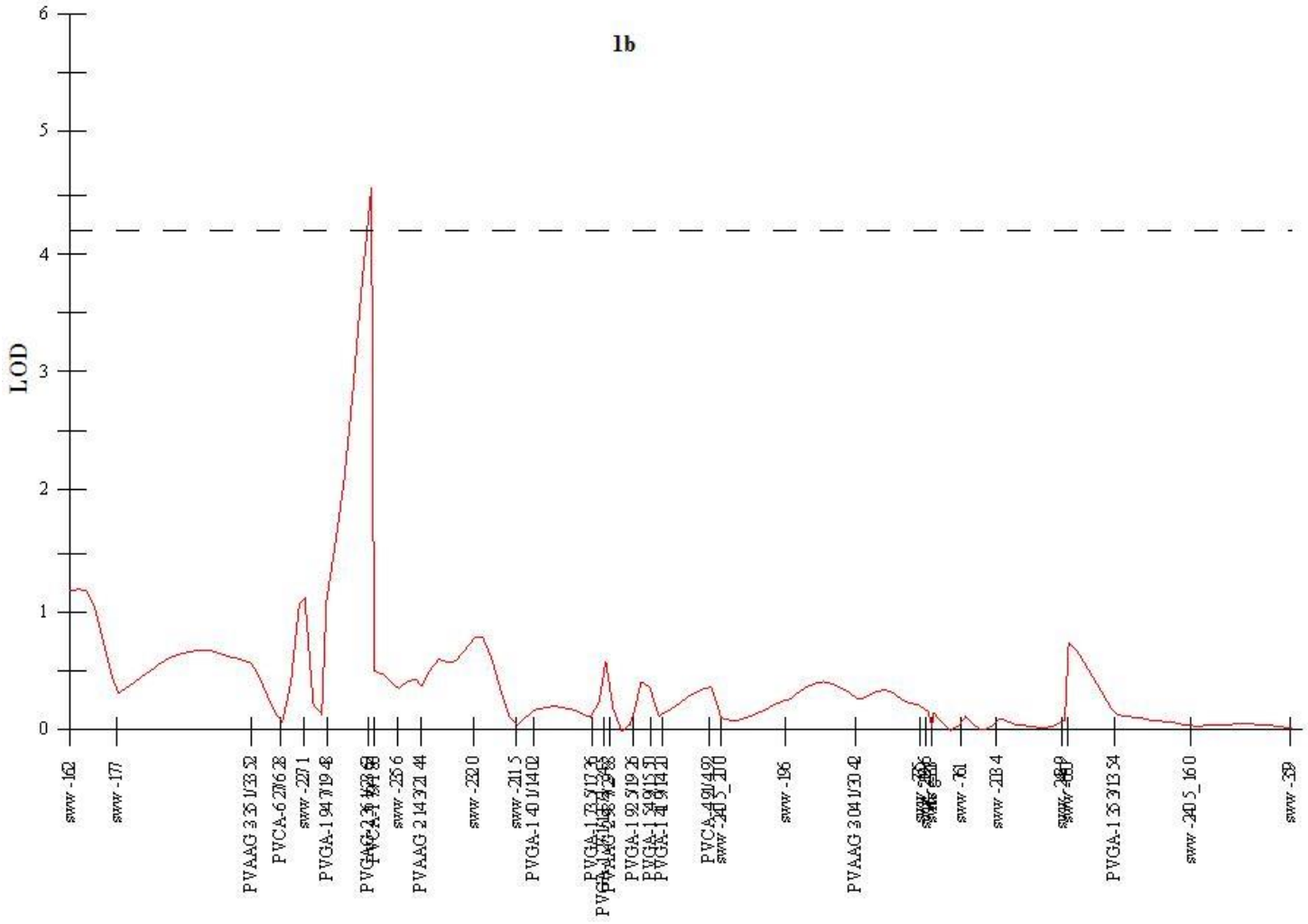

Fig. 4.13.2. QTL detected near in linkage group 1b for the selfed population at Perkins, OK in 2013

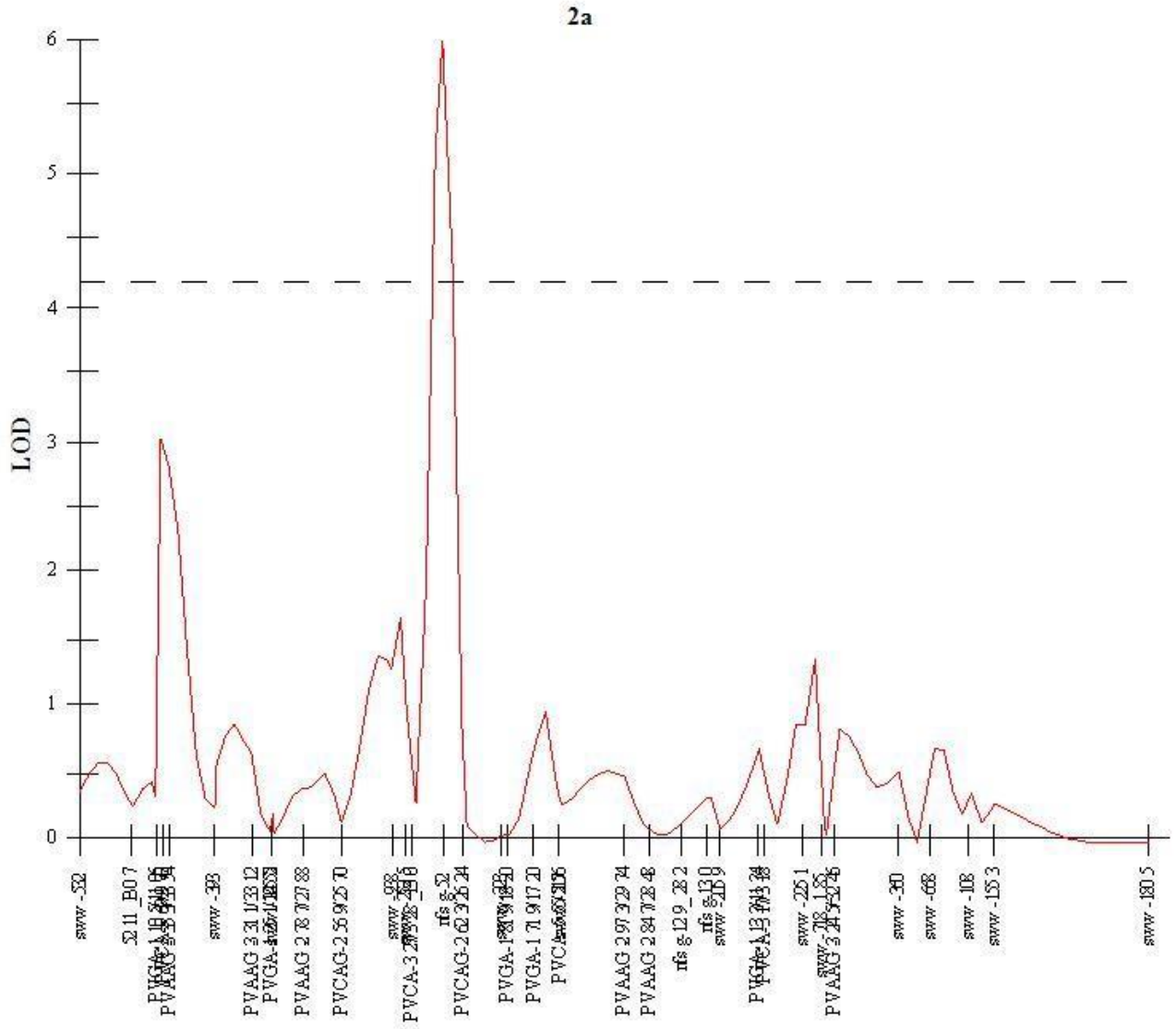

Fig. 4.13.3. QTL detected in linkage group 2a for the selfed population at Perkins, OK in 2013.

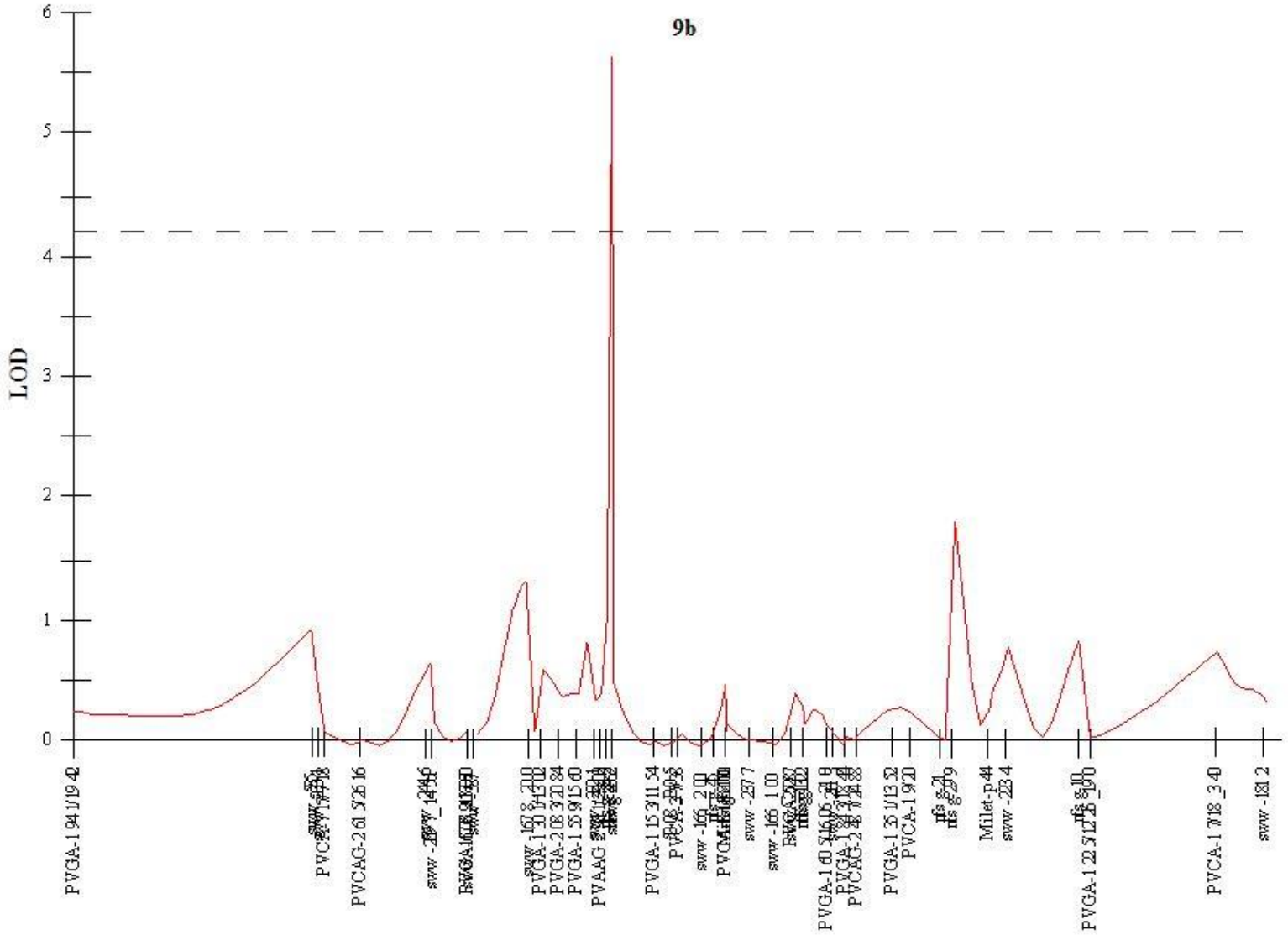

Fig. 4.13.4. QTL detected in linkage group 9b for the selfed population at Perkins, OK in 2013

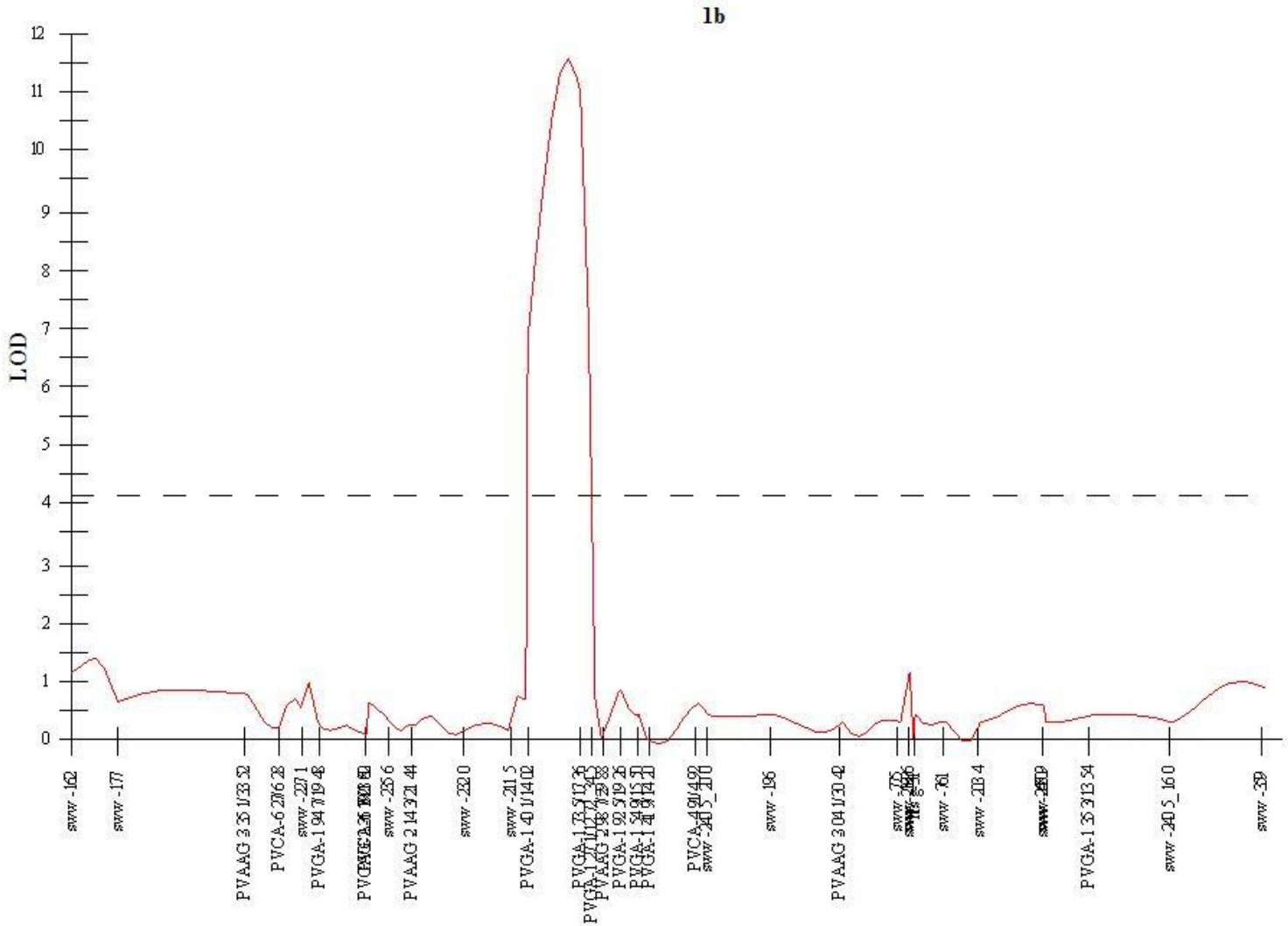

Fig. 4.13.5. QTL detected in linkage group 1b for the selfed population at Stillwater, OK in 2013.

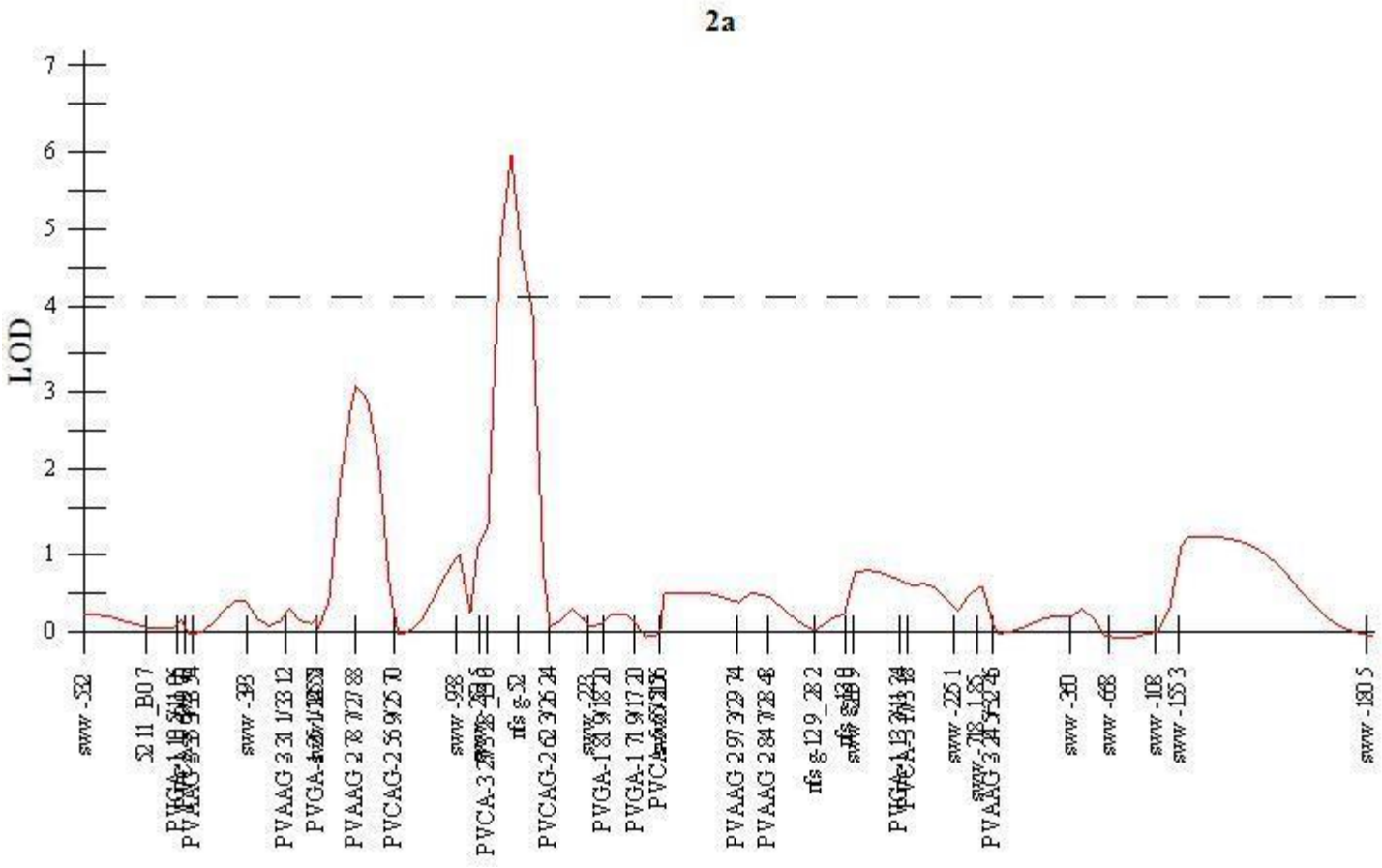

Fig. 4.13.6. QTL detected in linkage group 2a for the selfed population at Stillwater, OK in 2013.

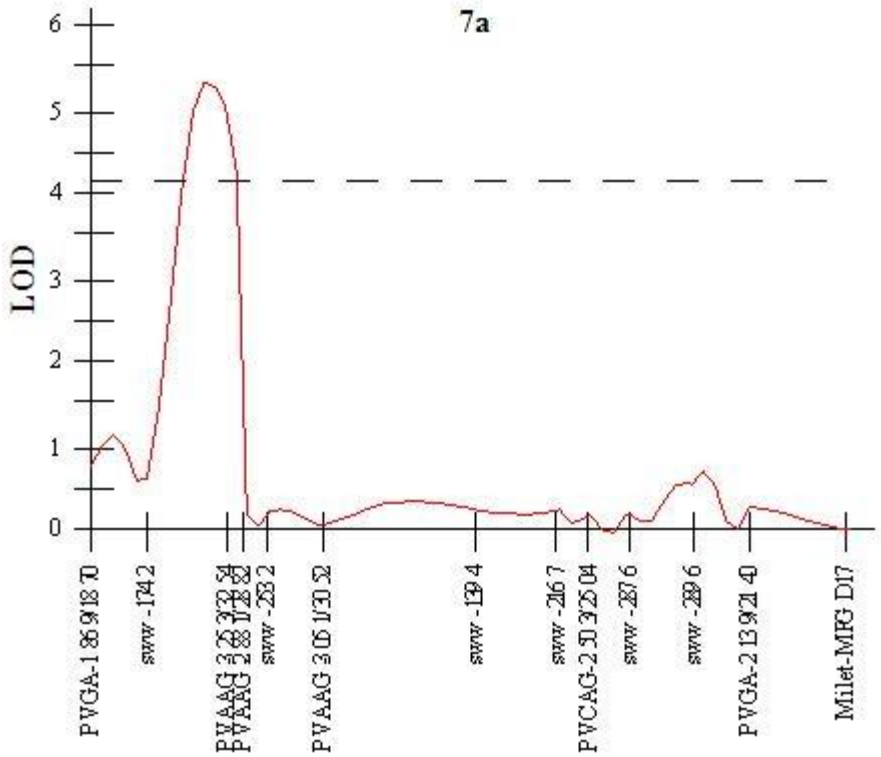

Fig. 4.13.7. QTL detected in linkage group 7a for the selfed population at Stillwater, OK in 2013

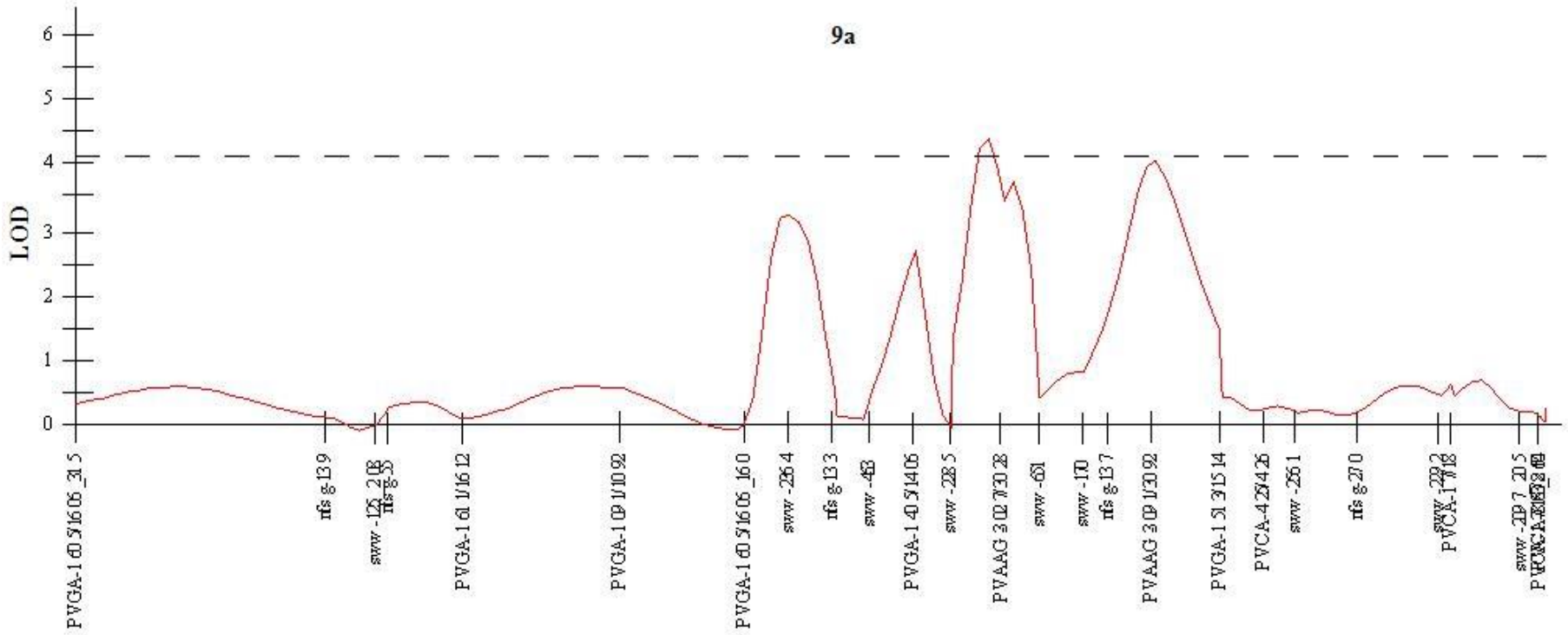

Fig. 4.13.8. QTL detected in linkage group 9a for the selfed population at Stillwater, OK in 2013.

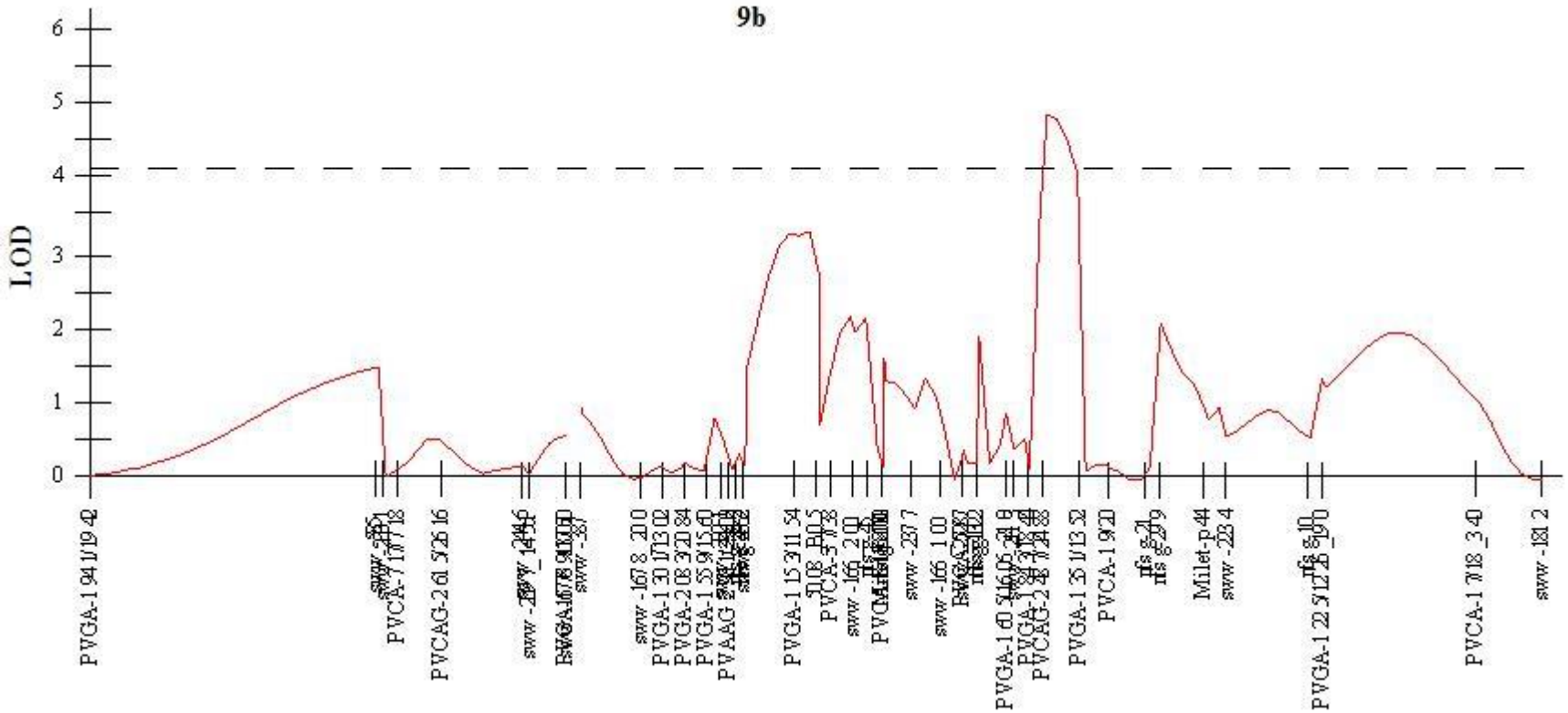

Fig. 4.13.9. QTL detected in linkage group 9b for the selfed population at Stillwater, OK in 2013.

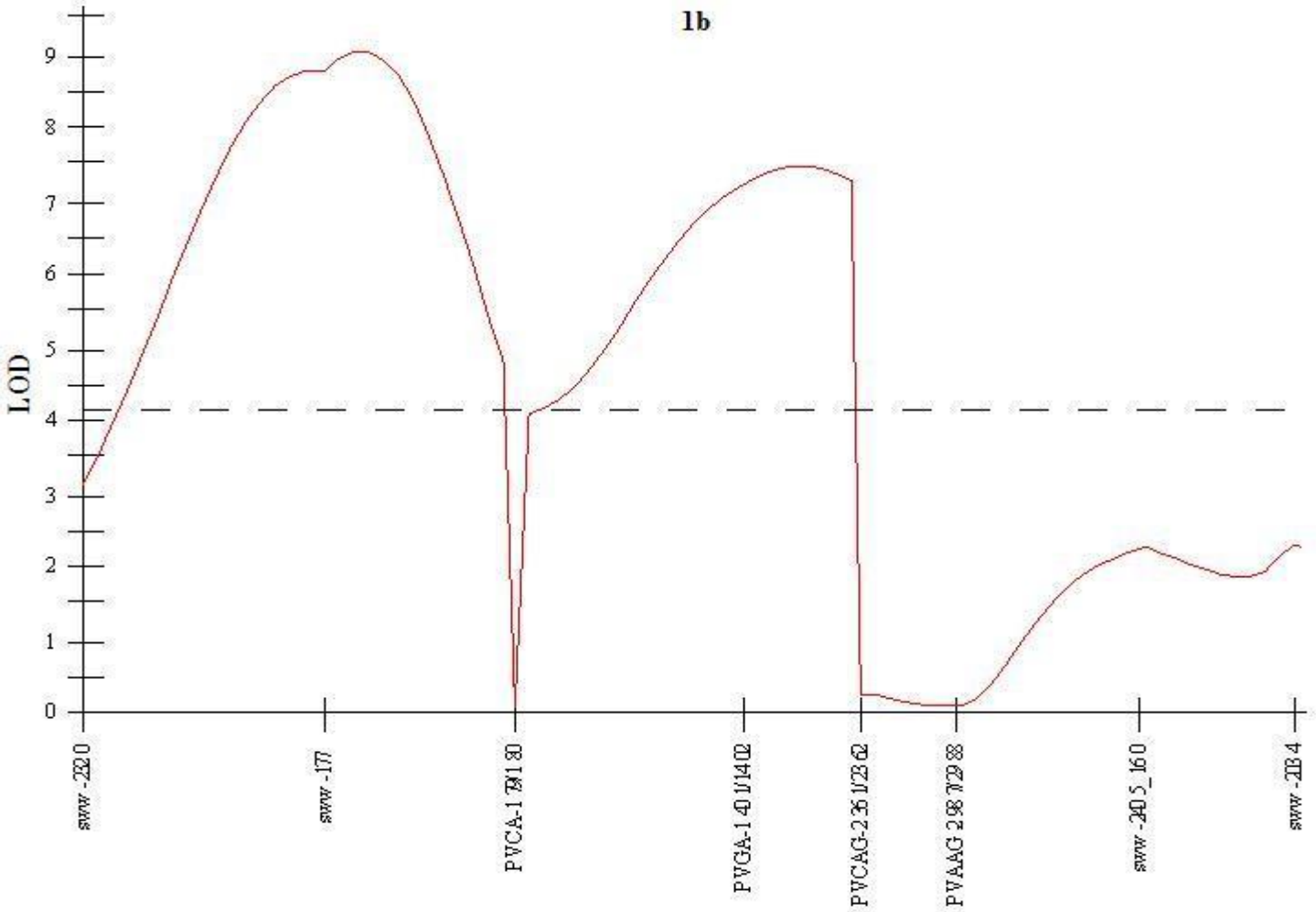

Fig. 4.14.1. QTL detected in linkage group 1b for the hybrid population at Stillwater, OK in 2012.

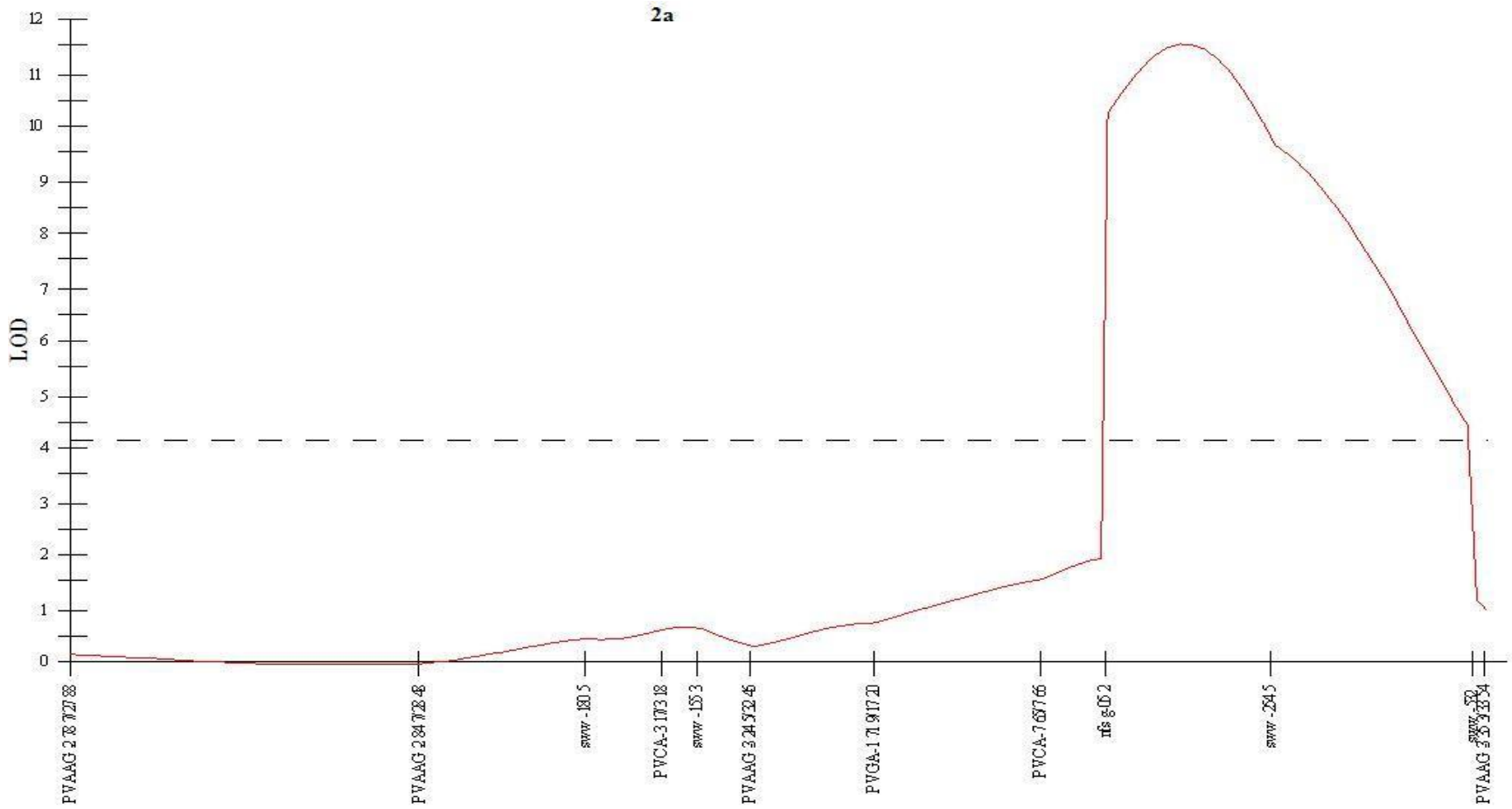

Fig. 4.14.2. QTL detected in linkage group 2a for the hybrid population at Stillwater, OK in 2012.

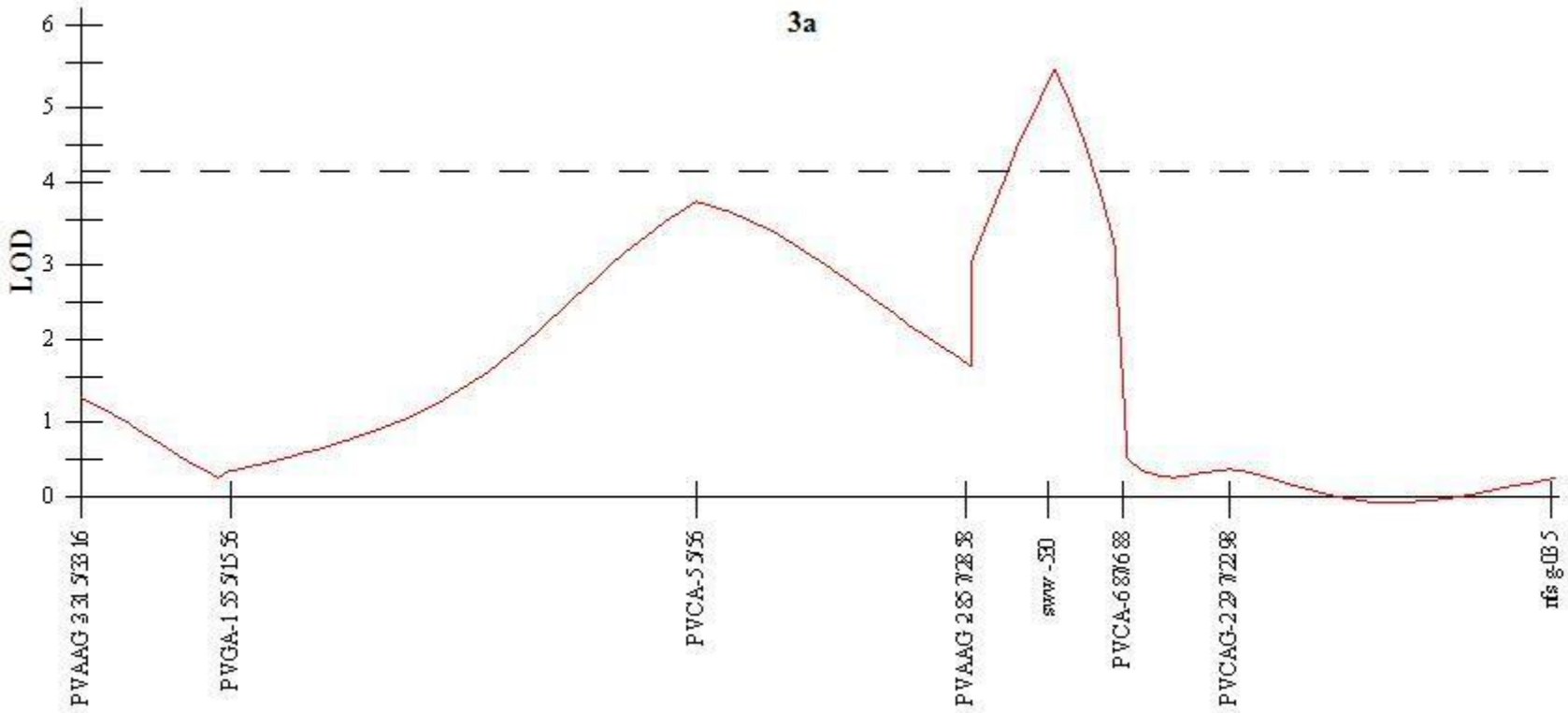

Fig. 4.14.3. QTL detected in linkage group 3a for the hybrid population at Stillwater, OK in 2012.

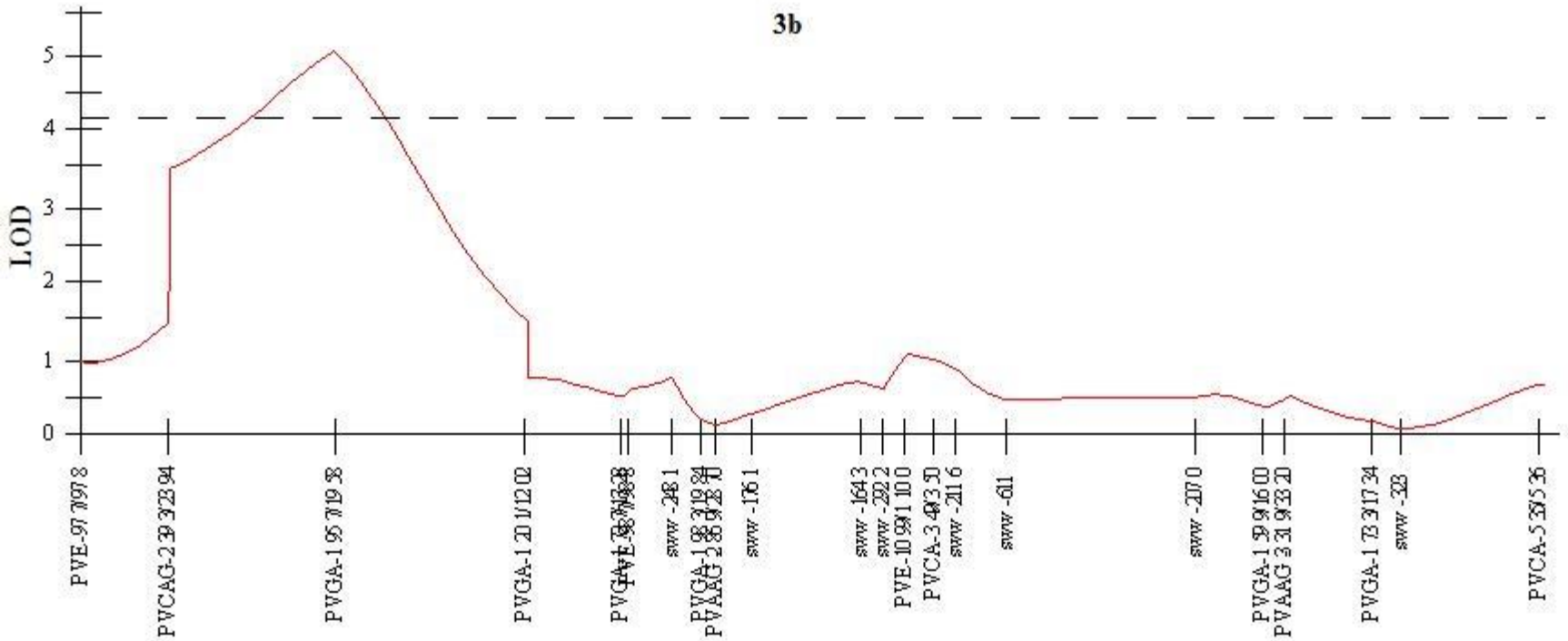

Fig. 4.14.4. QTL detected in linkage group 3b for the hybrid population at Stillwater, OK in 2012.

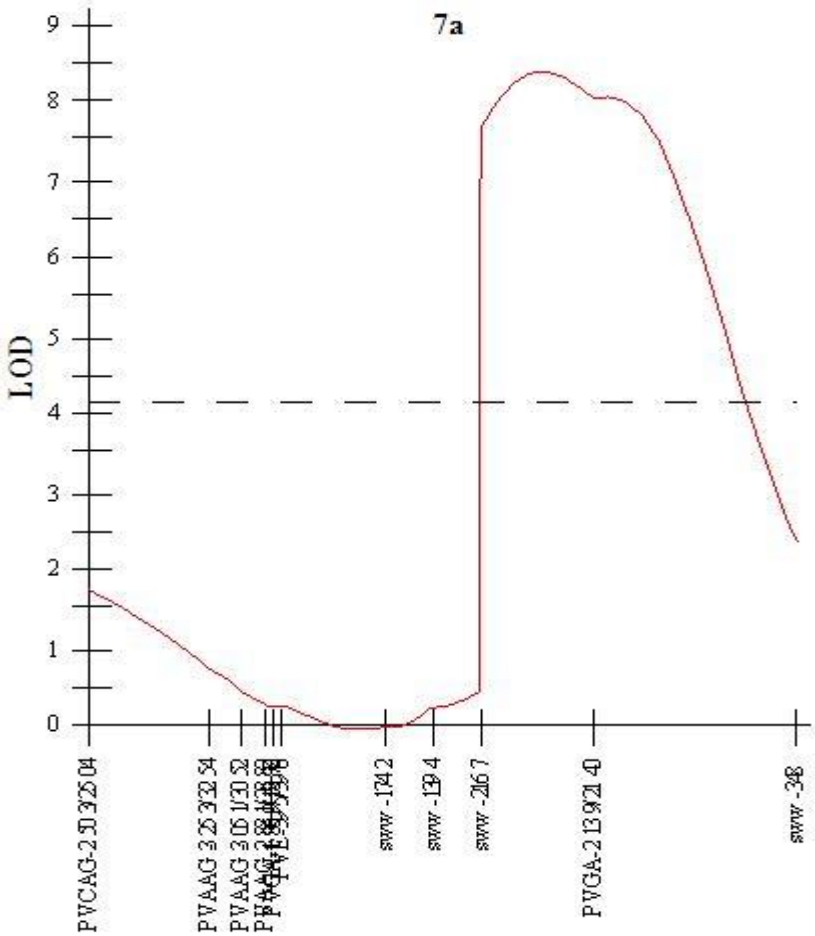

Fig. 4.14.5. QTL detected in linkage group 7a for the hybrid population at Stillwater, OK in 2012.

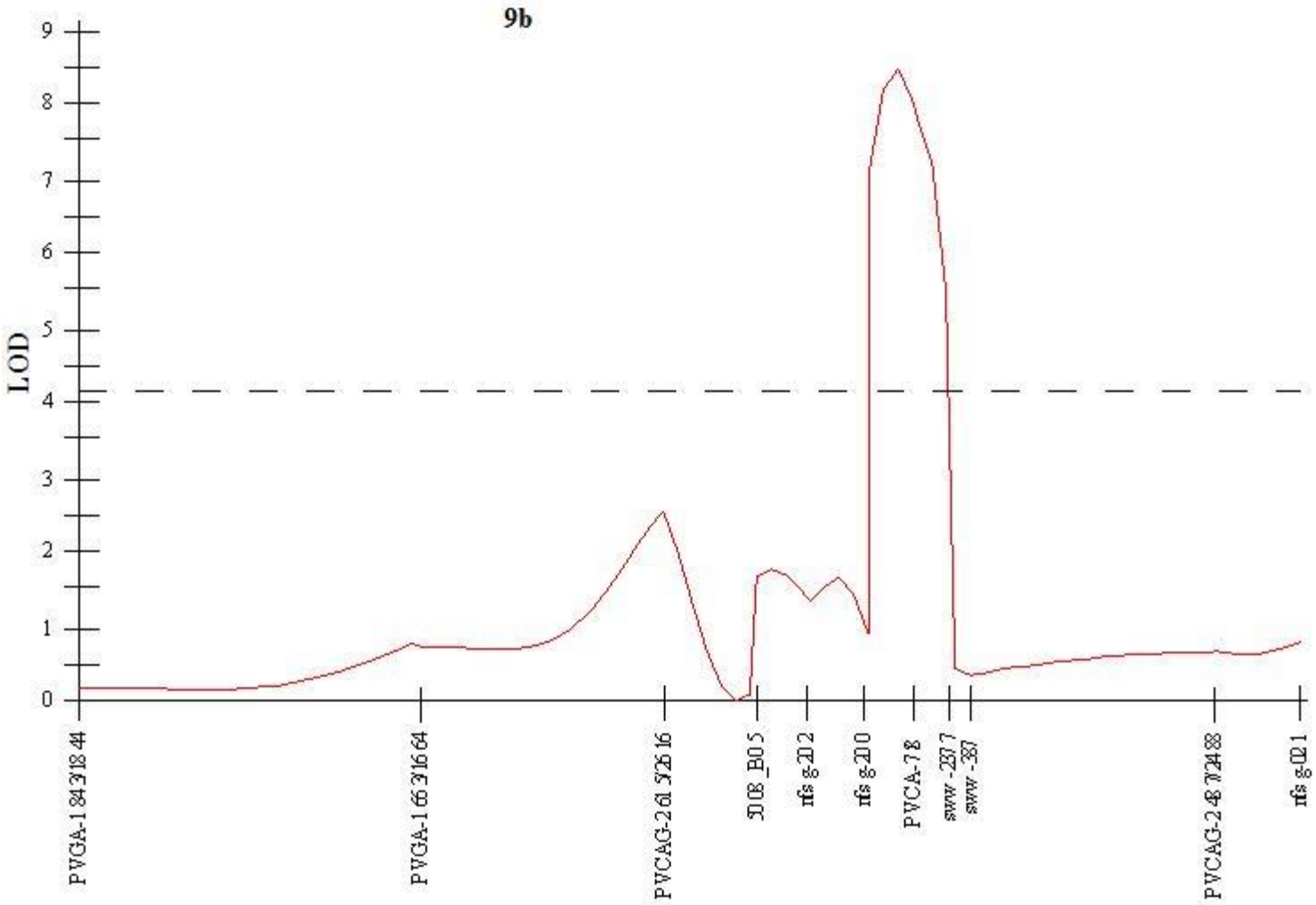

Fig. 4.14.6. QTL detected in linkage group 9b for the hybrid population at Stillwater, OK in 2012.

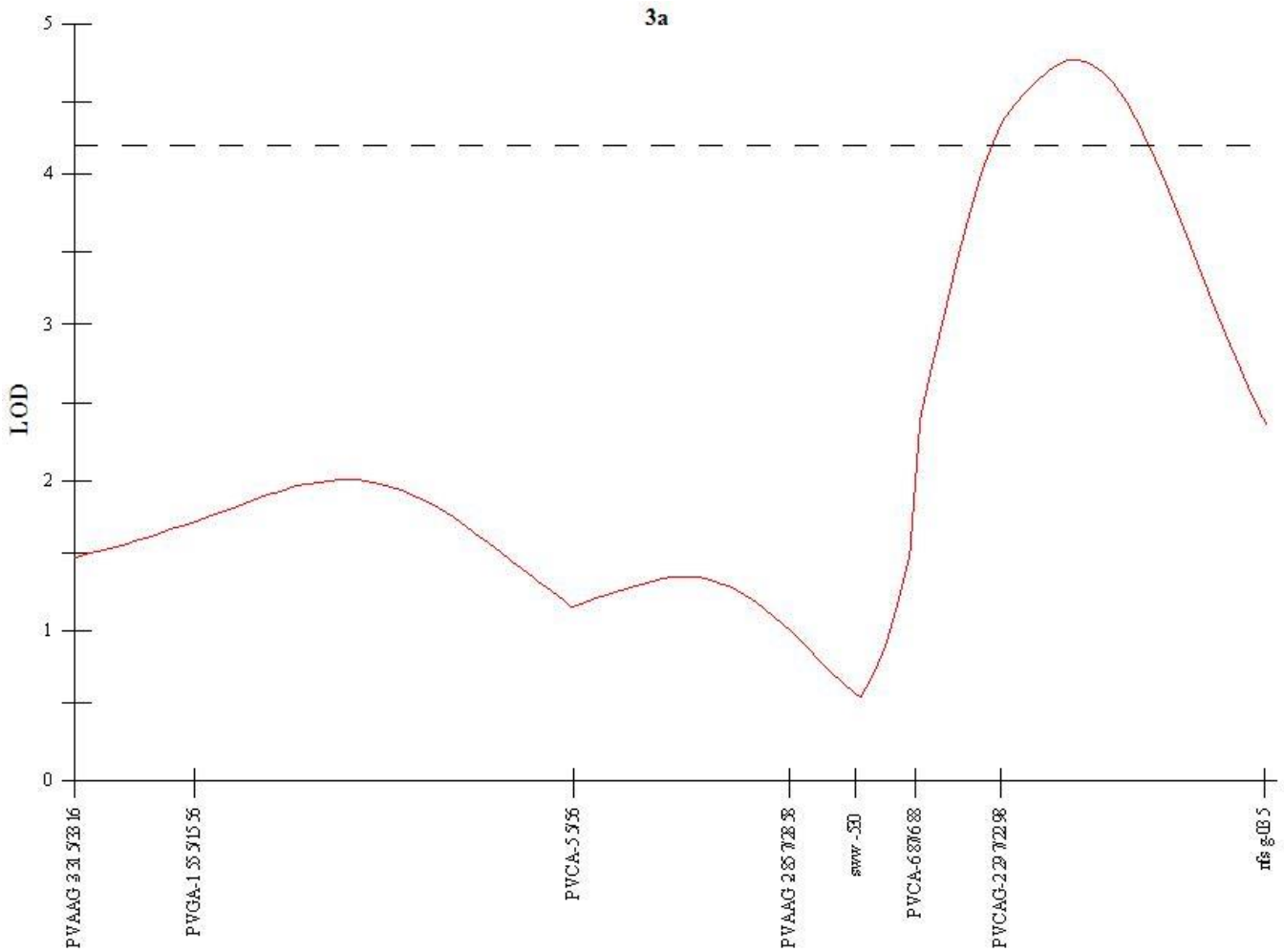

Fig. 4.14.7. QTL detected in linkage group 3a for the hybrid population at Perkins, OK in 2013.

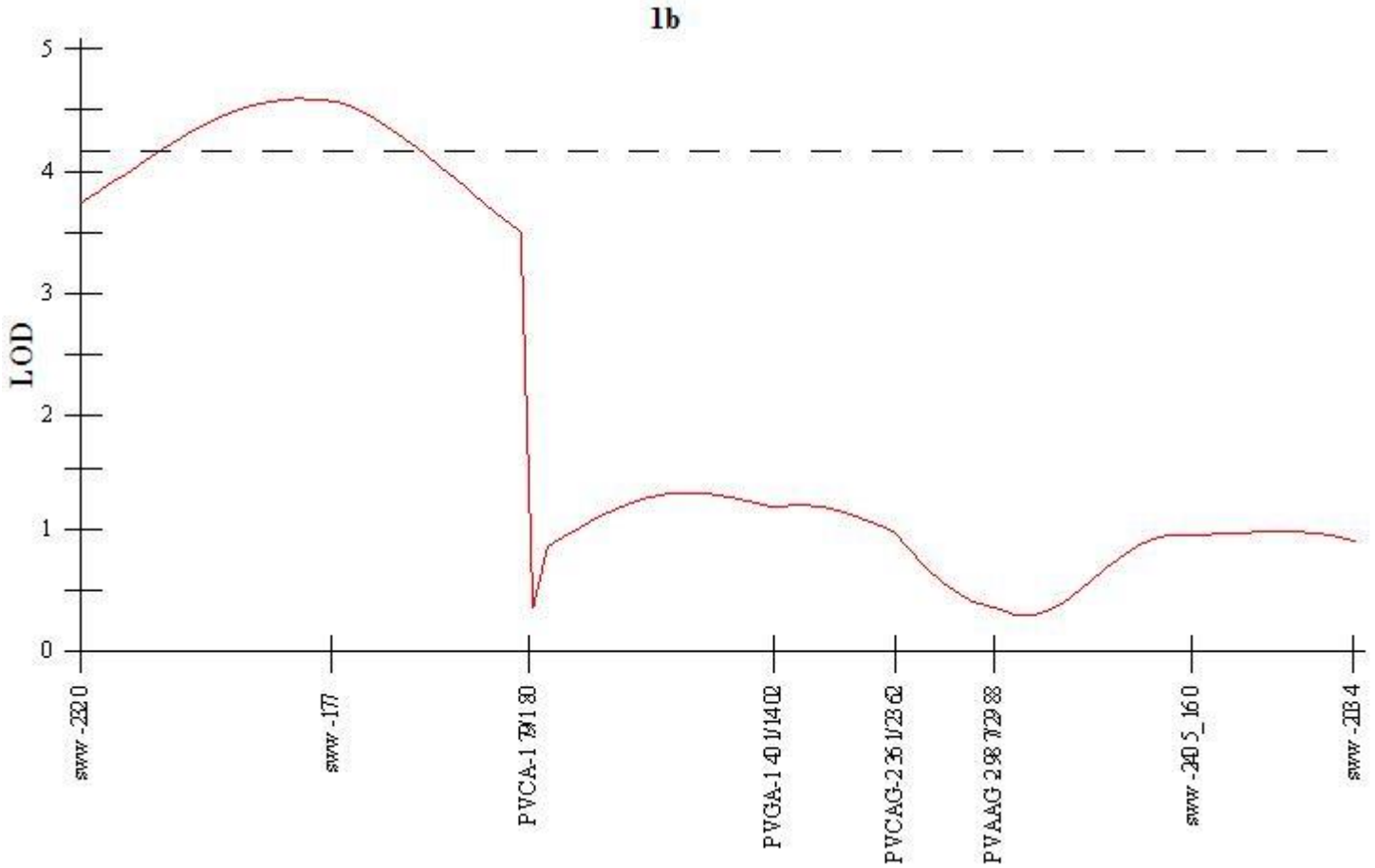

Fig. 4.14.8. QTL detected in linkage group 1b for the hybrid population at Stillwater, OK in 2013.

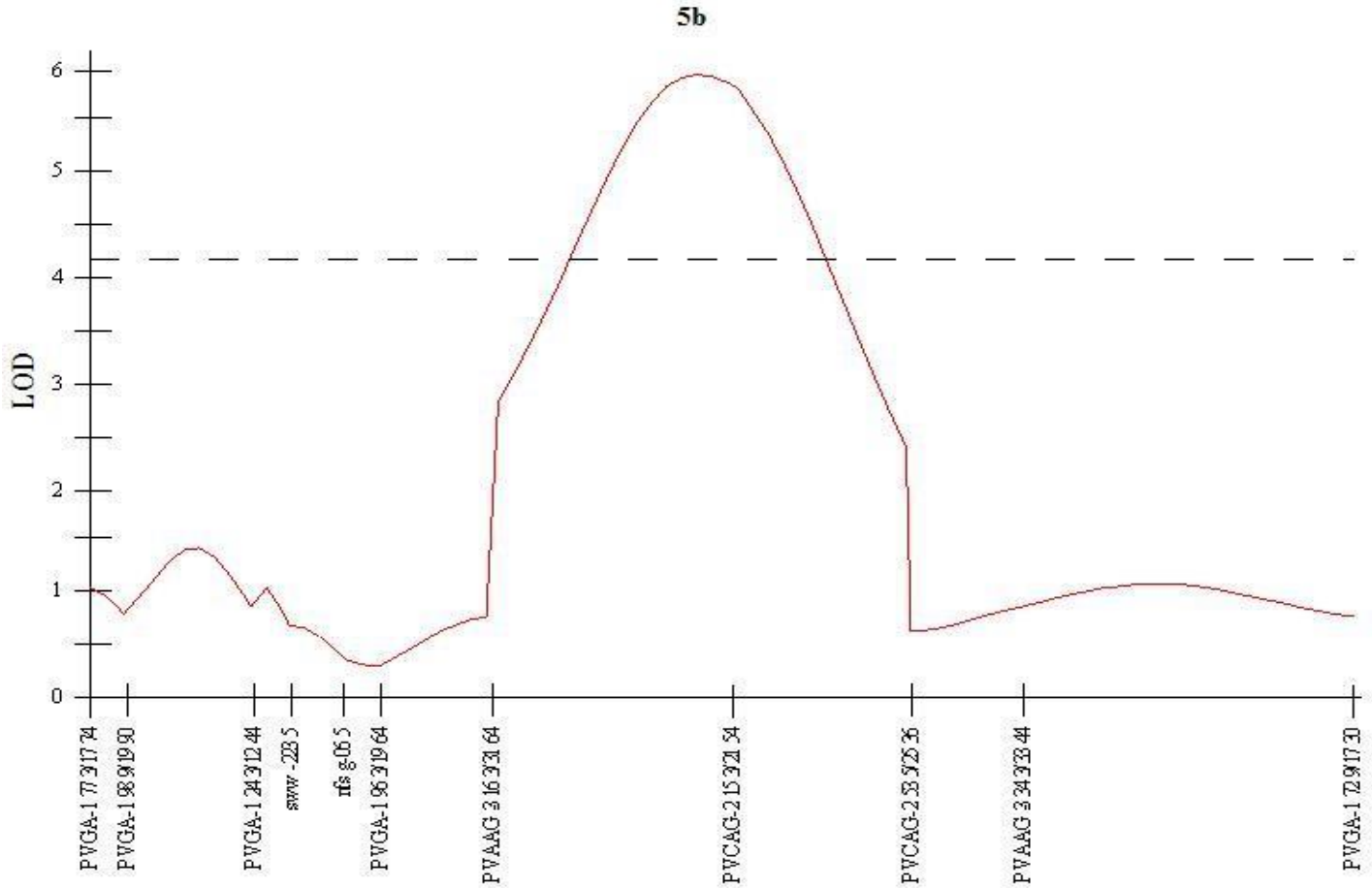

Fig. 4.14.9. QTL detected in linkage group 5b for the hybrid population at Stillwater, OK in 2013.

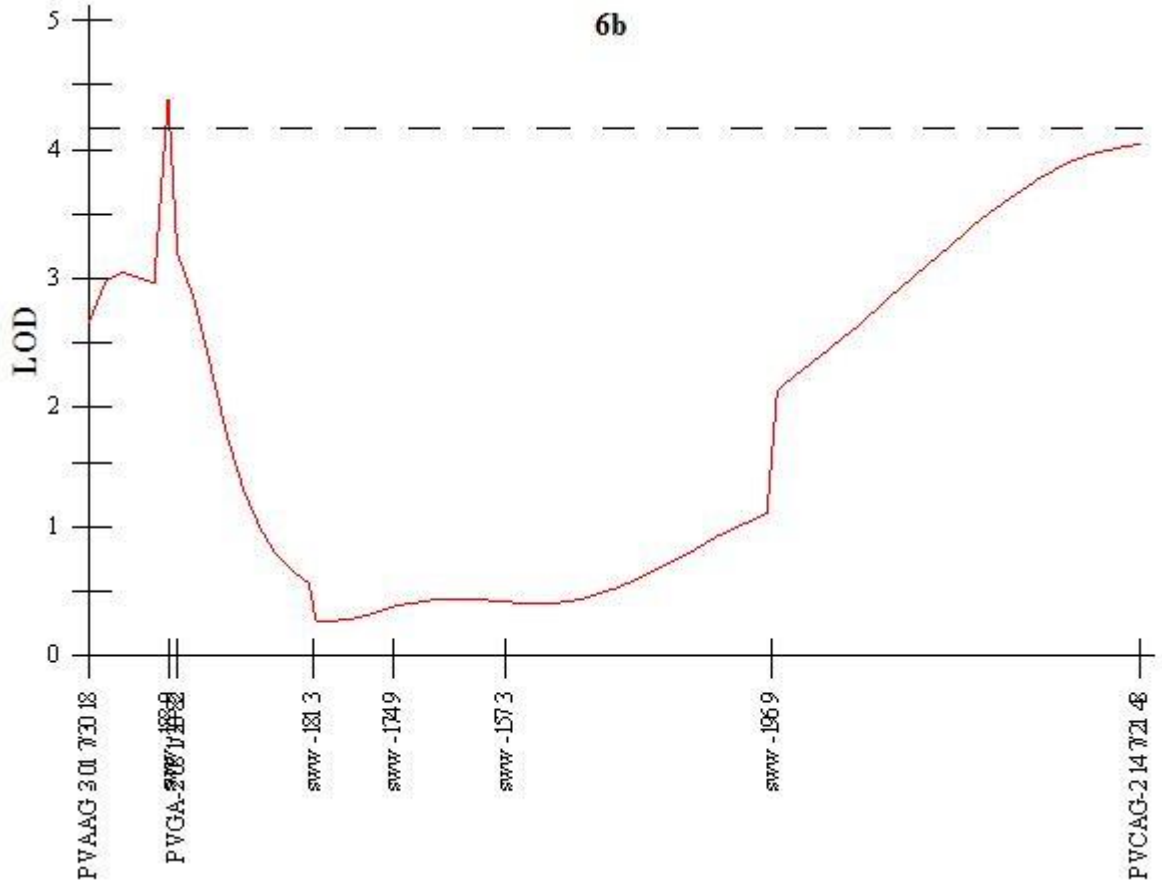

Fig. 4.14.10. QTL detected in linkage group 6b for the hybrid population at Stillwater, OK in 2013.

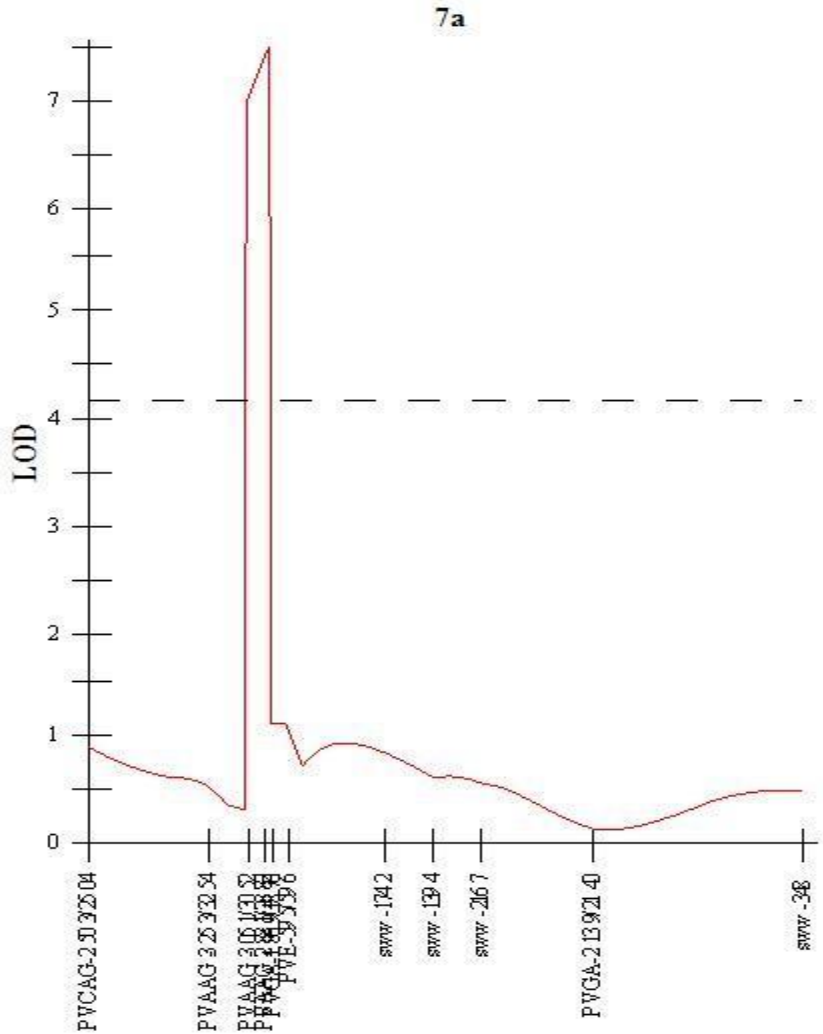

Fig. 4.14.11. QTL detected in linkage group 7a for the hybrid population at Stillwater, OK in 2013.

VITA

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