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Dormancy-linked Population Structure of Weedy Rice (*Oryza* sp.)

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Abstract

Seed dormancy allows weedy rice (*Oryza* sp.) to persist in rice production systems. Weedy and wild relatives of rice (*Oryza sativa* L.) exhibit different levels of dormancy, which allows them to escape weed management tactics, increasing the potential for flowering synchronization, and therefore gene flow, between weedy *Oryza* sp. and cultivated rice. In this study, we determined the genetic diversity and divergence of representative dormant and nondormant weedy *Oryza* sp. groups from Arkansas. Twenty-five simple sequence repeat markers closely associated with seed dormancy were used. Four populations were included: dormant blackhull, dormant strawhull, nondormant blackhull, and nondormant strawhull. The overall gene diversity was 0.355, indicating considerable genetic variation among populations in these dormancy-related loci. Gene diversity among blackhull populations (0.398) was higher than among strawhull populations (0.245). Higher genetic diversity was also observed within and among dormant populations than in nondormant populations. Cluster analysis of 16 accessions, based on Nei's genetic distance, showed four clusters. Clusters I, III, and IV consisted of only blackhull accessions, whereas Cluster II comprised only strawhull accessions. These four clusters did not separate cleanly into dormant and nondormant populations, indicating that not all markers were tightly linked to dormancy. The strawhull groups were most distant from blackhull weedy *Oryza* sp. groups. These data indicate complex genetic control of the dormancy trait, as dormant individuals exhibited higher genetic diversity than nondormant individuals. Seed-dormancy trait contributes to population structure of weedy *Oryza* sp., but this influence is less than that of hull color. Markers unique to the dormant populations are good candidates for follow-up studies on the control of seed dormancy in weedy *Oryza* sp.

Introduction

Rice (*Oryza sativa* L.) has been shown to have originated from its wild relative, brownbeard rice (*Oryza rufipogon* Griffiths), which after many domestication events and hybridization of wild rice with cultivated rice, further gave rise to weedy rice (*Oryza* sp.) (Londo et al. 2006). Weedy *Oryza* sp. is widespread in the southern U.S. rice-producing states and continues to be a major constraint to production wherever it occurs. It belongs to the same species as cultivated rice (*Oryza sativa* L.) and is highly competitive with the crop (Burgos et al. 2006). Although some weedy *Oryza* sp. biotypes share many phenotypic traits compared with cultivated rice, other biotypes have phenotypically distinct traits such as a black-colored hull, presence of awn, high to intermediate shattering, greater height, and early to late flowering (Federici et al. 2001; Gealy et al. 2002; Suh et al. 1997; Vaughan et al. 2001). Competition results in significant yield losses and reduction of rice grain quality (Ottis et al. 2005; Shivrain et al. 2009). Hence, to make *Oryza* sp. management sustainable, it is important to understand the physiological characteristics of populations.

Among its weedy traits, seed dormancy is one of the major important factors for persistence of weedy *Oryza* sp. Seed dormancy can allow weedy *Oryza* sp. to persist in the soil for up to 10 yr (Goss and Brown 1939; Teekachunhatean 1985). The ability to predict dormancy level is important for improved weed management, as it allows prediction of infestation levels of various weedy species, thereby enabling farmers to adopt appropriate weed management techniques (Grundy and Mead 2000). Our current work contributes to characterization of seed dormancy genes, which eventually should help in breeding preharvest sprouting resistance in rice varieties to eliminate preharvest sprouting problems in Southeast Asia (Dong et al. 2003).

The length of dormancy in weedy *Oryza* sp. is affected by storage temperature and afterripening time. In previous experiments, it was determined that the optimum afterripening time for weedy *Oryza* sp. to release dormancy is 90 d, and the optimum germination temperature is 35 C (Tseng et al. 2013). The hull also plays an important role in imposing seed dormancy. In weedy *Oryza* sp., the blackhull ecotype is generally more dormant than the strawhull ecotype

(Do Lago 1983; Tseng et al. 2013). Our study on variation in weedy *Oryza* sp. seed dormancy (Tseng et al. 2013) revealed that afterripening time and germination response to incubation temperature differed both among and within weedy *Oryza* sp. accessions. In this paper, a population represents multiple plants of the same ecotype from the same field. One field can be infested with multiple weedy *Oryza* sp. ecotypes. The mean germination capacity (GC) of weedy *Oryza* sp. populations at 35 and 15 C was 84% to 100% and 44% to 97%, respectively. Blackhull populations showed 3% to 11% lower germination than strawhull accessions at all incubation temperatures and required an afterripening time at least 30 d longer to release dormancy. In addition, blackhull weedy *Oryza* sp. showed a higher inter- and intrapopulation variation in dormancy than strawhull. The phenotypic diversity, specifically maturation period and seed dehiscence, among both blackhull and strawhull weedy *Oryza* sp. populations is high (Shivrain et al. 2010a). Knowing this, we evaluated the genetic diversity of dormancy-related loci among and within weedy *Oryza* sp. populations. Because of the large variability in seed dormancy variation among weedy *Oryza* sp. populations, we hypothesized that the genetic diversity of seed dormancy-linked loci may even be high among populations of the same hull color. Therefore, the objective of this study was to determine the genetic diversity among and within the Arkansas weedy *Oryza* sp. populations with respect to selected dormancy-linked loci.

Materials and Methods

Plant Materials

Thirty-two nondormant and 26 dormant weedy *Oryza* sp. accessions, equally representing blackhull and strawhull ecotypes, were selected (Tseng et al. 2013). These plants were harvested between July and August 2008 from 17 fields across 9 counties, namely, Arkansas, Chicot, Craighead, Jackson, Lee, Lincoln, Lonoke, Mississippi, and Prairie. An accession represents a weedy *Oryza* sp. plant of a particular ecotype collected from a rice field. To confirm the dormancy category, seeds of all accessions were incubated at 30 C for 28 d. The germination assay was conducted as described in Tseng et al. (2013). From this germination assay, eight nondormant (≥80% GC) and eight dormant (≤20% GC) populations, equally representing strawhull and blackhull ecotypes, were selected (Table 1). Three accessions per population were included to assess the genetic diversity within populations. Three accessions per population were used, because the majority of populations had at least three accessions. The 16 populations were assigned to 4 groups: dormant blackhull (D-BH), nondormant blackhull (ND-BH), dormant strawhull (D-SH), and nondormant strawhull (ND-SH).

DNA Extraction

From the germination assay, a germinated seed from each accession of the nondormant populations was planted in the greenhouse, and leaf tissues were harvested from each plant at the 3-leaf stage. For the dormant populations, DNA was extracted from a firm and nongerminated seed from each accession. Total genomic DNA was extracted from leaf tissues and seeds using a modified hexadecyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1990). Briefly, 0.05 g of leaf tissue or a single dehulled seed was placed in 2-ml collection microtubes (Qiagen, Hilden, Germany) containing two stainless-steel beads (Qiagen). Each collection microtube had 500 µl of CTAB extraction buffer

Table 1. List of weedy *Oryza* sp. populations used in this study showing respective group, zone, and county of collection.[a]

Group	Population[b]	Accessions per population	Awn type	Ecotype	Dormancy trait	County	Zone
D-BH	CHI08D-DBH	3	Awned	BH	D	Chicot	Delta
	LON08F-DBH	3	Awned	BH	D	Lonoke	Grand Prairie
	CRA08B-DBH	3	Awned	BH	D	Craighead	White River
	ARK08C-DBH	3	Awned	BH	D	Arkansas	Grand Prairie
D-SH	ARK08B-DSH	3	Awnless	SH	D	Arkansas	Grand Prairie
	CRA08B-DSH	3	Awnless	SH	D	Craighead	White River
	LEE08C-DSH	3	Awnless	SH	D	Lee	Delta
	MIS08D-DSH	3	Awnless	SH	D	Mississippi	Delta
ND-BH	CRA08B-NDBH	3	Awnless	BH	ND	Craighead	White River
	JAC08B-NDBH	3	Awned	BH	ND	Jackson	White River
	PRA08C-NDBH	3	Awned	BH	ND	Prairie	Grand Prairie
	LIN08C-NDBH	3	Awned	BH	ND	Lincoln	Delta
ND-SH	CRA08B-NDSH	3	Awnless	SH	ND	Craighead	White River
	JAC08A-NDSH	3	Awnless	SH	ND	Jackson	White River
	LON08B-NDSH	3	Awnless	SH	ND	Lonoke	Grand Prairie
	PRA08B-NDSH	3	Awnless	SH	ND	Prairie	Grand Prairie

[a]Abbreviations: BH, blackhull; D, dormant; ND, nondormant; SH, strawhull.
[b]County codes: ARK, Arkansas; CHI, Chicot; CRA, Craighead; JAC, Jackson; LEE, Lee; LIN, Lincoln; LON, Lonoke; MIS, Mississippi; PRA, Prairie; letter before hyphen indicates field code.

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(containing 100 mM Tris-HCl, 20 mM EDTA, 2 M NaCl, 2% CTAB, 2% polyvinylpyrrolidone-40, 1 mM phenanthroline, and 0.3% β-mercaptoethanol) added. The sample was then homogenized using a MM400 mixer mill (Retsch, Haan, Germany) at 30 Hz for 2 min. After an equal volume of phenol:chloroform: isoamyl alcohol (25:24:1) was added to each tube, the mixture was incubated at 55 C for 45 min and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to a new 1.5-ml centrifuge tube (Eppendorf) containing an equal volume of absolute isopropanol, mixed by inverting the tube, and incubated overnight at -80 C. DNA was then pelleted by centrifugation at 12,000 rpm for 10 min. The DNA pellet was washed with absolute ethanol, air-dried, and resuspended in 30 ml of 1X TE (containing 10 mM Tris-HCl and 1 mM EDTA). The genomic DNA was quantified using a NanoDrop 2000c spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE), diluted to 100 ng/µl with deionized water, and used as a template in PCR.

Microsatellite DNA Amplification

For PCR, 25 simple sequence repeat (SSR) primers distributed across four chromosomes were used (Table 2). PCR was carried out in 25-µl reaction mixtures containing 100 ng/µl DNA and 0.4 mM dNTPs each, 25 µ/ml Taq DNA polymerase (New England Biolabs, Ipswich, MA), 3 mM $MgCl_2$, and 1 µM each of forward and reverse primers. The PCR profile started at 94 C for 5 min, followed by 35 cycles of denaturation at 94 C for 1 min, annealing at 55 C for 1 min, and extension at 72 C for 2 min. A final extension of 72 C for 7 min was included. The PCR products were electrophoresed in a 6% denaturing polyacrylamide gel at 180 V for 70 min. Gels were stained with ethidium bromide, and bands were photographed.

Data Analysis

The individual bands were scored as codominant markers using Cross Checker 2.91 (Buntjier 1999). Because the number of bands produced by the SSR markers ranged from 1 to 9, the bands were scored as binary characters to maintain the allelic information. Thus, data were entered into a binary matrix as discrete variables (1 for presence and 0 for absence of the band), and this data matrix was used to compute the allelic frequencies, observed alleles (n_a), effective alleles (n_e), number of alleles per locus (A), percentage of polymorphic loci (P), genetic distance (D), Shannon's index (I), and Nei's gene diversity (h), using POPGENE software v. 1.32 (Yeh et al. 1999). The values of genetic distance were used to conduct cluster analysis with a UPGMA (unweighted pair group method with arithmetic mean) algorithm, and a dendrogram was constructed using the program TREEVIEW v. 1.52 (Page 1996). A one-way ANOVA and LSD t-test ($P < 0.05$) were conducted to compare gene diversity values of SSR markers among and within weedy *Oryza* sp. populations and groups using JMP for Windows software (v. 10.0.0; SAS Institute, Cary, NC).

Results and Discussion

Marker Analysis

A total of 102 alleles with an average of 4.12 alleles per locus (ranging from 60 to 650 bp) was generated by the 25 SSR primers (Table 3). The highest number of alleles was scored at the locus RM564 (9 alleles) and the lowest number (1 allele) was scored at the loci RM28662 and RM28682. The highest level of gene diversity was detected at the locus RM28665 ($h = 0.486$; Table 3), and the lowest level of gene diversity was detected at locus RM28656 ($h = 0.153$; Table 3). The allelic frequency data indicated two monomorphic loci (RM28656 and RM28682) in the D-BH group, while loci RM5672 was monomorphic in both the D-SH and ND-SH groups (Table 4). The ND-BH group, however, showed the highest polymorphism, with all loci being polymorphic. Marker RM28656 was unique to blackhull, while marker RM5672 was unique to strawhull weedy *Oryza* sp. Among the D-BH populations, 92% of loci were polymorphic (Table 4). In addition, one rare allele, RM25 (140 bp), was observed exclusively in the D-BH populations (unpublished data). Among the ND-BH populations, all loci were polymorphic (Table 4), and one rare allele, RM28621 (290 bp), was observed (unpublished data). Twenty-four out of 25 loci were polymorphic among the D-SH populations and among the ND-SH populations (Table 4). No rare alleles were observed exclusively in the D-SH or ND-SH population.

Seed dormancy is a hereditary trait that varies from one genotype to another, and this variation is dependent on the environment (Baskin and Baskin 1998; Gu et al. 2004; Hori et al. 2010; Li et al. 2006; Ye et al. 2010). Many studies have used molecular approaches to investigate seed dormancy mechanisms in various species, including wild and cultivated rice and weedy *Oryza* sp. (Cai and Morishima 2000; Gu et al. 2004, 2011; Lang 1996; Li and Foley 1997). Most of these studies involved identification of quantitative trait loci (QTLs) associated with seed dormancy. QTLs are regions in the genome that are associated with complex traits, in this case, seed dormancy. A study using 245 restriction fragment length polymorphism markers was able to detect 5 QTLs linked to seed dormancy in cultivated rice (Lin et al. 1998). These QTLs accounted for about 48% of the total phenotypic variation and were located on chromosomes 3, 5, 7, and 8. Furthermore, qPHS-7 was found to be associated with after-ripening time, whereby increasing the afterripening period released the qPHS-7–linked dormancy in rice. In wild rice, three QTLs, namely grm 1.1 (in chromosome 1), grm 4.1 (in chromosome 4), and grm 6.1 (in chromosome 6) were identified to be associated with decreased seed germination (Thomson et al. 2003). Gu et al. (2004) used 151 rice microsatellite markers distributed across 12 chromosomes to identify seed dormancy QTLs in an EM93-1/SS18-2 cross, where EM93-1 is a nondormant rice cultivar, while SS18-2 is a dormant weedy *Oryza* sp. accession from Thailand. Four of the QTLs, qSD-4, qSD-6, qSD-8, and qSD-12, were located on chromosomes 4, 6, 8, and 12, respectively, while qSD-7-1 and qSD-7-2 were located on chromosome 7. Locus qSD-12 was associated with the after-ripening period, whereas locus qSD-7-1 was found to be linked to red pericarp color and was later found to be responsible for increasing abscisic acid production, which in turn induces seed dormancy (Gu et al. 2011). Markers used in this study were selected from the abovementioned studies based on their high association with seed dormancy in weedy *Oryza* sp. (Table 2). We found two markers that were able to discriminate between blackhull and strawhull weedy *Oryza* sp. However, none of the markers were found to be unique to dormant or nondormant weedy *Oryza* sp. alone. This is contrary to the findings of Gu et al. (2004), who reported that all these markers were tightly linked to dormant weedy *Oryza* sp. This difference may be due to the difference in biotypes used. The materials used in the current study were from field populations in Arkansas, USA, while Gu et al. (2004) used a pure line derived by crossing a nondormant strawhull rice (EM93-1) with dormant blackhull weedy *Oryza* sp. (SS18-2) originating from Thailand. Although we did not find any rare alleles to discriminate dormant

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Table 2. List of 25 rice SSR markers used for DNA amplification in this study.

Marker	Locus	Chromosome[a]	Forward (5' to 3') and reverse (5' to 3') sequence	SSR start	SSR end	Predicted product size	Anneal temperature	Reference
				——————bp——————			C	
RM220	qSD1	1	F-gaaatgcttcccacatgtct R-ggaaggtaactgtttccaac	4424458	4424495	127	55	Akagi et al. 1996
RM252	qSD4	4	F-atgacttgatcccgagaacg R-ttcgctgacgtgataggttg	8997573	8997594	216	55	Temnykh et al. 2000
RM564	qSD4	4	F-atgcagaggattggcttgag R-catggccttgtgtatgcatc	4966447	4966488	228	55	Temnykh et al. 2000
RM118	qSD7	7	F-cacatcctccagcgacgccgag R-ccaatcggagccaccggagagc	24488142	24488167	156	67	Temnykh et al. 2000
RM5672	qSD7-1	7	F-tgcccaatatagaggcaacc R-caccctacaaggaaacaagc	6413197	6413217	209	50	McCouch et al. 2002
RM180	qSD7-1	7	F-accttgctctacttgtggtgagggactg R-ctacatcggcttaggtgtagcaacacg	5036853	5036876	110	55	Temnykh et al. 2000
RM270	qSD12	12	F-tgcgcagtatcatcggcgag R-ggccgttggttctaaaatc	22181892	22181957	108	55	Akagi et al. 1996
RM28595	qSD12	12	F-gcccaatcatcttgcatctt R-tacaacgcaccccatctgta	24625121	24625154	240	55	Gu et al. 2008
RM28603	qSD12	12	F-caccaatctccgccattact R-cattggactcacctggaagg	24750565	24750588	207	55	Gu et al. 2008
RM28607	qSD12	12	F-ggcagctcaacccttttcatag R-agaactagagagatgagaaaggaaag	24833611	24833632	250	55	Gu et al. 2008
RM28608	qSD12	12	F-actacaatatggggcggatg R-tgtgtattagtttccatatggtcttca	24834751	24834770	248	55	Gu et al. 2008
RM28621	qSD12	12	F-gccaaaaggtcagggttaca R-cacagtcgaattgcaaagga	25009516	25009541	249	55	Gu et al. 2008
RM28638	qSD12	12	F-ctgaagagctgcgagaatcc R-ccatcctgcctctagcatgt	25137026	25137052	288	55	Gu et al. 2008
RM28642	qSD12	12	F-gtacctctccacccatcgac R-agctgctgagaacacaatcg	25188806	25188827	237	55	Gu et al. 2008
RM28643	qSD12	12	F-ccgatgttgagacaaggtga R-tgggggttgtactcttctcc	25192137	25192156	190	55	Gu et al. 2008
RM28645	qSD12	12	F-acgcagcatgtaggagaggt R-gggcgccagtattagtgttg	25234768	25234788	231	55	Gu et al. 2008
RM28651	qSD12	12	F-cggaactgccgtttattcat R-cttcctggcttcaactctgg	25310459	25310488	250	55	Gu et al. 2008
RM28652	qSD12	12	F-tctcaattgcactccatcca R-aacaacattctctgcaattttcc	25343437	25343456	219	55	Gu et al. 2008
RM28656	qSD12	12	F-tccgattataccattgtattcgtt R-gcacacagtggaagtacgtttg	25420162	25420227	363	55	Gu et al. 2008
RM28659	qSD12	12	F-ccatcgaaagatgtgtggaa R-aaacgcatgcagaagaacct	25459387	25459407	220	55	Gu et al. 2008
RM28661	qSD12	12	F-cgcgtgttgtatggtttcac R-acagtgactttggccgtgtt	25497465	25497486	169	55	Gu et al. 2008
RM28662	qSD12	12	F-gttttaaggcccccatcatt R-ttggagctgattttggagtttt	25502723	25502770	195	55	Gu et al. 2008
RM28664	qSD12	12	F-tgggaagcagaagagtttttg R-gccttagcttctccctgctt	25510147	25510170	243	55	Gu et al. 2008
RM28665	qSD12	12	F-ctcaaggacgtgtggaacg R-tgcagatggtgaggaagttg	25522353	25522373	220	55	Gu et al. 2008
RM28682	qSD12	12	F-tctcctctgcatcacaatcaa R-tctccgagagggtacgtgtc	25804426	25804503	192	55	Gu et al. 2008

[a]Chromosomal location of markers with respect to rice.

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Table 3. Allele number, gene diversity, and Shannon's index of the 25 SSR markers.

Marker	Locus	Chromosome[a]	Allele number (*A*)	Gene diversity (*h*)	Shannon's information index (*I*)
RM220	qSD1	1	6	0.349	0.528
RM252	qSD4	4	7	0.289	0.459
RM564	qSD4	4	9	0.380	0.563
RM118	qSD7	7	7	0.418	0.606
RM5672	qSD7-1	7	3	0.307	0.478
RM180	qSD7-1	7	8	0.243	0.404
RM270	qSD12	12	2	0.354	0.536
RM28595	qSD12	12	2	0.274	0.444
RM28603	qSD12	12	8	0.410	0.595
RM28607	qSD12	12	5	0.349	0.529
RM28608	qSD12	12	3	0.386	0.571
RM28621	qSD12	12	7	0.383	0.568
RM28638	qSD12	12	4	0.337	0.506
RM28642	qSD13	12	3	0.431	0.619
RM28643	qSD14	12	3	0.377	0.562
RM28645	qSD15	12	2	0.336	0.517
RM28651	qSD16	12	4	0.296	0.468
RM28652	qSD17	12	2	0.305	0.483
RM28656	qSD18	12	2	0.153	0.287
RM28659	qSD19	12	2	0.352	0.537
RM28661	qSD20	12	2	0.411	0.600
RM28662	qSD21	12	1	0.458	0.650
RM28664	qSD22	12	7	0.438	0.625
RM28665	qSD23	12	3	0.486	0.679
RM28682	qSD24	12	1	0.187	0.334

[a]Chromosomal location of markers with respect to rice.

and nondormant plants of the strawhull ecotype, we found two alleles, RM28621 (140 bp) and RM28682 (290 bp) that can be used to distinguish between dormant and nondormant plants, respectively, of the blackhull ecotype. To this date, there is no information on alleles that are specific to dormant or nondormant blackhull weedy *Oryza* sp. These rare alleles are of immense importance, as they can be used to ascertain whether a blackhull weedy *Oryza* sp. population infesting a rice field is dormant or not.

Genetic Diversity among Oryza sp. Ecotypes

Gene diversity (*h*) and Shannon's index (*I*) are methods most commonly used for measuring genetic variation (Nei 1978). Gene diversity is a measure of expected heterozygosity, while Shannon's index is a measure of degree of uncertainty in determining the species an individual would belong to if randomly picked from a group of species. Higher values of gene diversity and Shannon's index would indicate higher genetic diversity. A positive correlation of genetic diversity with weedy *Oryza* sp. groups, ecotype, dormancy trait, populations, and county of collection ($P < 0.05$; Figures 1 and 2; Table 4) was observed. Overall, a substantial level of genetic diversity was detected among *Oryza* sp. populations across the dormancy loci (mean $h = 0.355$, $I = 0.534$).

Among the two primary ecotypes, the blackhull groups possessed the highest level of gene diversity and Shannon's index ($h = 0.398$, $I = 0.584$) when compared among themselves (Figure 1; Table 4). In contrast, the strawhull group showed lower levels of genetic diversity ($h = 0.245$, $I = 0.370$) among populations. Also, among the four dormancy categories, the blackhull groups D-BH and ND-BH showed the highest genetic diversity ($h = 0.367$, $I = 0.539$, and $h = 0.365$, $I = 0.530$, respectively). The ND-SH group of populations were least diverse ($h = 0.218$, $I = 0.332$) (Figure 1; Table 4). Among the blackhull groups, gene diversity within populations ranged from 0.092 to 0.244 for the D-BH group and from 0.039 to 0.187 for the ND-BH group (Figure 2; Table 3). The highest level of gene diversity was found within the CHI08D-DBH and CRA08B-NDBH populations of the D-BH and ND-BH groups, respectively, whereas the lowest gene diversity was found within the CRA08B-DBH and PRA08C-NDBH populations of the D-BH and ND-BH groups, respectively. The gene diversity within populations ranged from 0.139 to 0.196 for the D-SH group and from 0.065 to 0.174 for the ND-SH group. The highest level of gene diversity was within the LEE08C-DSH and CRA08B-NDSH populations of the D-SH and ND-SH groups, respectively, whereas the lowest gene diversity was within the MIS08D-DSH and JAC08A-NDSH populations of the D-SH and ND-SH groups, respectively.

Gene diversity among weedy *Oryza* sp. accessions found in this study ($h = 0.355$) was similar to gene diversity of the overall genome ($h = 0.407$) reported by Shivrain et al. (2010b). These results also complement previous observations on high variability in seed dormancy among and within weedy *Oryza* sp. populations (Do Lago 1983; Veasey et al. 2004). The high level of gene diversity among blackhull weedy *Oryza* sp. compared with the strawhull weedy *Oryza* sp. may be because blackhull is more closely related to wild rice (Londo and Schaal 2007; Vaughan et al. 2001), and the wild rice group displays higher gene diversity compared with cultivated and weedy *Oryza* sp. groups (Londo and Schaal 2007). Also, blackhull weedy *Oryza* sp., in general, shows higher phenotypic and genetic variation compared with strawhull weedy *Oryza* sp. (Burgos et al. 2014; Shivrain et al. 2010a, 2010b). Thus, it is not surprising that the blackhull weedy *Oryza* sp. populations are more genetically diverse than the strawhull types. Because the majority of the accessions were awned blackhull and awnless strawhull, our data conform to previous findings of Shivrain et al. (2010b), wherein the awned blackhull group had higher gene diversity (0.337) than the awnless strawhull group (0.239) with respect to genome-wide markers. These findings also support the high intrapopulation variation in dormancy in blackhull weedy *Oryza* sp. observed in our previous studies (Tseng et al. 2013).

Genetic Diversity among Dormant and Nondormant Oryza sp.

The mean gene diversity and Shannon's index among individuals for the dormant populations was higher (mean $h = 0.177$, $I = 0.254$), than for the nondormant populations (mean $h = 0.120$,

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Table 4. Genetic diversity among weedy *Oryza* sp. populations by groups based on polymorphisms of the 25 SSR markers.[a]

Group	Populations per group	Ecotype	Dormancy trait[a]	Observed alleles (n_a)	Effective alleles (n_e)	Gene diversity (h)	Shannon's information index (I)	Monomorphic marker	Percent polymorphic loci (P)
D-BH	4	BH	D	1.90	1.66	0.364	0.529	RM28656, RM28682	92
D-SH	4	SH	D	1.67	1.42	0.239	0.356	RM5672	96
ND-BH	4	BH	ND	1.93	1.63	0.366	0.539	—	100
ND-SH	4	SH	ND	1.67	1.36	0.217	0.331	RM5672	96
Blackhull	8	BH	—	2.00	1.69	0.397	0.583	RM28656	96
Strawhull	8	SH	—	1.75	1.42	0.245	0.370	RM5672	96
Dormant	8	—	D	1.98	1.60	0.353	0.526	—	100
Nondormant	8	—	ND	1.96	1.55	0.331	0.500	—	100

[a]Abbreviations: BH, blackhull; D, dormant; ND, nondormant; SH, strawhull.

$I = 0.171$) (Figure 1). Also, when comparing among populations, the dormant group showed higher diversity ($h = 0.353$, $I = 0.526$) than the nondormant group ($h = 0.331$, $I = 0.500$) (Table 4). Higher genetic diversity is known to increase the longevity or fitness of many plant and animal species (Danzmann et al. 1986; Ledig 1986; Wills 1981). Greater levels of genetic diversity help a population adapt to a wide range of environmental changes, thus allowing them to persist or dominate. The dormant weedy *Oryza* sp. Populations, which show higher gene diversity than the non-dormant populations, are expected to persist longer in the soil. It is therefore very important to adopt an intensive weedy *Oryza* sp. management strategy in locations infested with a dormant weedy *Oryza* sp. ecotype to prevent new deposits of weedy *Oryza* sp.

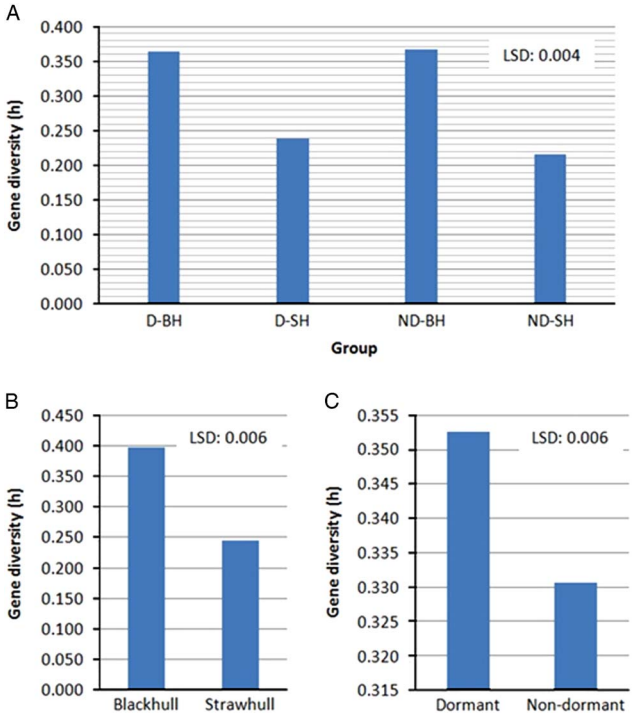

Figure 1. Variation in gene diversity among (A) weedy *Oryza* sp. groups, (B) ecotypes, and (C) dormancy types. Abbreviations: D-BH, dormant blackhull; D-SH, dormant strawhull; ND-BH, nondormant blackhull; ND-SH, nondormant strawhull. Gene diversity values are among all weedy *Oryza* sp. accessions within each group.

Effect of County of Origin on Genetic Diversity

Among the nine counties represented by the populations, the Chicot County populations showed the highest genetic diversity among individuals ($h = 0.244$, $I = 0.350$), while the Jackson County and Lincoln County populations showed the lowest genetic diversity among their individuals (mean $h = 0.092$, $I = 0.131$, and $h = 0.070$, $I = 0.100$, respectively) (Table 5). The high genetic diversity of weedy *Oryza* sp. populations from Chicot County is not yet understood. One theory is that Chicot county, being at the border of major rice-producing states (Arkansas, Mississippi, and Louisiana), embodies the confluence of a significant volume of rice grains and the seed-distribution hub across these states. Louisiana and Mississippi have very diverse weedy *Oryza* sp. populations (Constantin 1960; Do Lago 1983). Thus, Chicot County also acts as the gateway for interchange of weedy *Oryza* sp. seeds being moved along with rice grain. The geographical zones, however, had no significant impact on genetic diversity of the weedy *Oryza* sp. populations, implying that the evolution of dormancy trait is not localized to a particular zone in Arkansas.

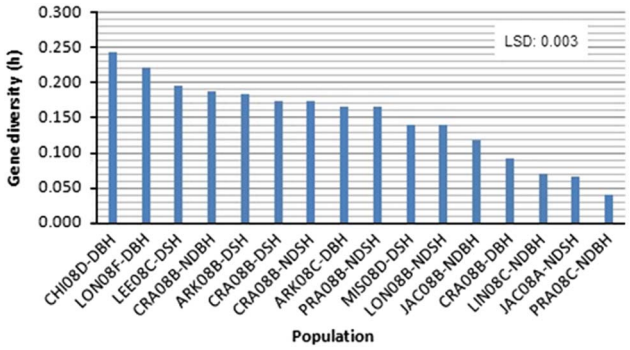

Figure 2. Variation in mean gene diversity among weedy *Oryza* sp. populations based on 25 SSR markers linked to four dormancy loci. Gene diversity values are among three *Oryza* sp. accessions within each population. Please refer to Table 1 for population codes.

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Table 5. Genetic diversity within the 16 populations based on polymorphisms of 25 SSR markers.[a]

Population[b]	Ecotype	Dormancy trait	County	Zone	Gene diversity (h)[c]	Mean gene diversity	Shannon's information index (I)	Effective alleles (n_e)	Percent polymorphic loci (P)
CHI08D-DBH	BH	D	Chicot	Delta	0.244	0.181	0.350	1.439	72
LON08F-DBH	BH	D	Lonoke	Grand Prairie	0.222		0.318	1.400	72
CRA08B-DBH	BH	D	Craighead	White River	0.092		0.131	1.165	48
ARK08C-DBH	BH	D	Arkansas	Grand Prairie	0.166		0.237	1.298	76
ARK08B-DSH	SH	D	Arkansas	Grand Prairie	0.183	0.173	0.262	1.329	68
CRA08B-DSH	SH	D	Craighead	White River	0.174		0.250	1.314	68
LEE08C-DSH	SH	D	Lee	Delta	0.196		0.281	1.353	76
MIS08D-DSH	SH	D	Mississippi	Delta	0.139		0.200	1.251	68
CRA08B-NDBH	BH	ND	Craighead	White River	0.187	0.103	0.268	1.337	76
JAC08B-NDBH	BH	ND	Jackson	White River	0.118		0.169	1.212	64
PRA08C-NDBH	BH	ND	Prairie	Grand Prairie	0.039		0.056	1.071	20
LIN08C-NDBH	BH	ND	Lincoln	Delta	0.070		0.100	1.126	36
CRA08B-NDSH	SH	ND	Craighead	White River	0.174	0.136	0.250	1.314	72
JAC08A-NDSH	SH	ND	Jackson	White River	0.065		0.094	1.118	40
LON08B-NDSH	SH	ND	Lonoke	Grand Prairie	0.139		0.200	1.251	56
PRA08B-NDSH	SH	ND	Prairie	Grand Prairie	0.166		0.237	1.298	68

[a]Abbreviations: B, blackhull; D, dormant; ND, nondormant; SH, strawhull.
[b]County codes: ARK, Arkansas; CHI, Chicot; CRA, Craighead; JAC, Jackson; LEE, Lee; LIN, Lincoln; LON, Lonoke; MIS, Mississippi; PRA, Prairie; letter before hyphen indicates field code.
[c]Gene diversity values are among three plants within each population.

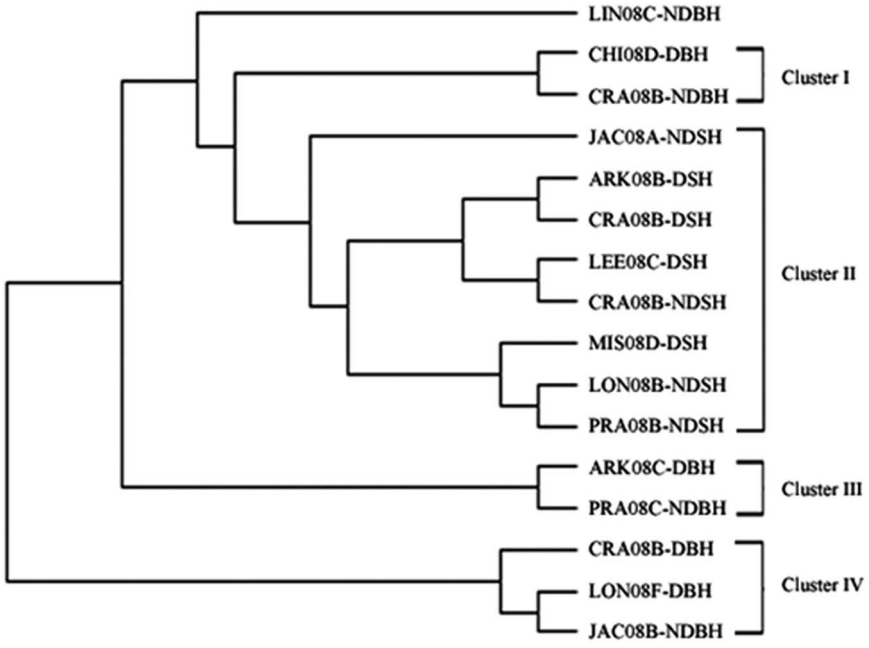

Figure 3. UPGMA (unweighted pair group method with arithmetic mean)-based dendrogram of 16 weedy *Oryza* sp. populations based on polymorphisms of 25 SSR markers, using Nei's (1972) genetic distance. Please refer to Table 1 for population codes.

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Table 6. Genetic distance (*D*) (Nei 1972) within and between weedy *Oryza* sp. groups based on 25 SSR markers.

Group description	Group name[a]	*D*
Dormant blackhull	D-BH	0.357
Dormant strawhull	D-SH	0.112
Nondormant blackhull	ND-BH	0.507
Nondormant strawhull	ND-SH	0.133
Dormant blackhull vs. nondormant strawhull	D-BH vs. D-SH	0.323
Dormant blackhull vs. nondormant blackhull	D-BH vs. ND-BH	0.417
Dormant blackhull vs. nondormant strawhull	D-BH vs. ND-SH	0.353
Dormant strawhull vs. nondormant blackhull	D-SH vs. ND-BH	0.339
Dormant strawhull vs. nondormant strawhull	D-SH vs. ND-SH	0.137
Nondormant strawhull vs. nondormant blackhull	ND-SH vs. ND-BH	0.340
Dormant vs. nondormant	D vs. ND	0.311
Blackhull vs. strawhull	BH vs. SH	0.339

[a]Abbreviations: B, blackhull; D, dormant; ND, nondormant; SH, strawhull.

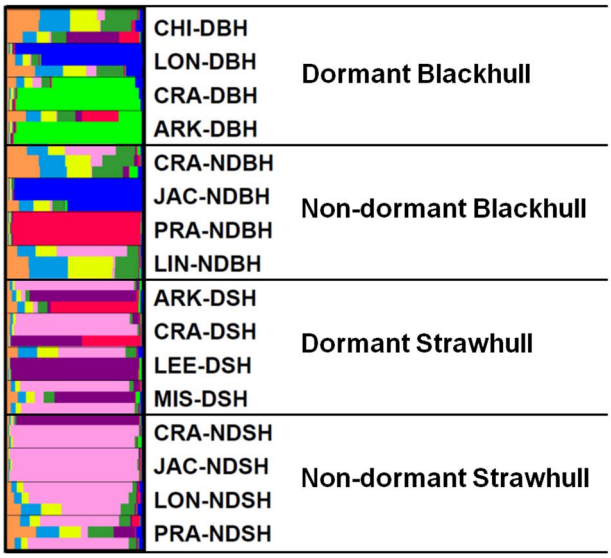

Figure 4. Population structure of 16 weedy *Oryza* sp. populations based on polymorphisms of 25 SSR markers. Please refer to Table 1 for population codes.

Cluster Analysis

The UPGMA-based dendrogram obtained from these data is shown in Figure 3. Populations were grouped into four clusters consisting of 15 out of 16 populations. The remaining population, LIN08C-NDBH, shared less similarity with other populations and was therefore not included in any cluster. Clusters I, III, and IV, comprised only blackhull ecotype, and included two, two, and three populations, respectively. Cluster II was the largest, composed of only strawhull populations. In this large cluster, there were two subclusters, one comprising mostly dormant populations, and the other comprising mostly nondormant populations. One population, JAC08A-NDSH, did not cluster with any population, as it was most distant from the other strawhull populations. Each of the three clusters, Clusters I, III, and IV, contained a mixture of populations with varying levels of dormancy and belonging to different counties of origin, thus indicating the markers were not tightly influenced by mircroenvironments associated with the field of origin. For example in Cluster IV, two out of the three populations, LON08F-DBH (from Lonoke county; dormant) and JAC08B-NDBH (from Jackson county; nondormant), were closely related to each other and thus clustered together. The genetic distance between the dormant and nondormant strawhull groups appeared to be lower ($D = 0.137$) than between the dormant and nondormant blackhull groups ($D = 0.417$) (Table 6). Genetic diversity was higher within the two blackhull groups ($D = 0.357$ and 0.507, respectively) than within the two strawhull groups ($D = 0.112$ and 0.133, respectively). The population structure graph clearly distinguishes the strawhull populations from the blackhull populations (Figure 4). The genetic structures of blackhull populations are admixed. No differences were observed between dormant and nondormant populations belonging to the same ecotype.

The populations fell into four clusters mainly based on hull color ecotype, indicating that hull color had a bigger influence on genetic clustering than dormancy trait. By extension, this indicates that the markers developed for dormancy trait were mostly related to hull color rather than dormancy. This finding is logical, because the mapping population used to develop the markers was derived from a cross between a nondormant strawhull and a dormant blackhull. Essentially, the cosegragation of markers with dormancy trait in that population could be confounded with segregation of genes controlling hull color. Dormant and nondormant weedy *Oryza* sp. populations of blackhull ecotype are more distinct than those of the strawhull ecotype. Also, plants of the blackhull ecotype had higher level of genetic variation among themselves than plants of the strawhull ecotype. Similar results reported by Gealy et al. (2002) indicated that the genetic distance of the blackhull group was higher ($D = 0.33$) than that of the strawhull group ($D = 0.20$). The strawhull and blackhull are genetically diverse groups with a genetic distance of 0.339. Similar findings have been reported in other studies in which the two major ecotypes, blackhull and strawhull, were shown to be genetically distinct from each (Burgos et al. 2014; Gealy et al. 2002; Londo and Schaal 2007; Shivrain et al. 2010b). However, the genetic distance values reported in previous studies on weedy *Oryza* sp. genetic diversity are generally higher than those reported in our study. This is because most of these studies used SSR markers nonspecific to dormancy loci and were distributed across the genome, in contrast to the dormancy-linked loci on just four chromosomes used in this study. This, however, indicates that the dormancy loci used in this study are less polymorphic compared with the genome-wide loci used for genetic diversity studies of weedy *Oryza* sp.

Weedy *Oryza* sp. populations used in this study exhibit high genetic diversity with respect to seed dormancy loci. This genetic diversity depends on the ecotype, dormancy level, and county of collection. The blackhull weedy *Oryza* sp. groups are most dormant and possess a higher level of genetic diversity than the strawhull weedy *Oryza* sp. groups. Genetic diversity is also higher in the dormant than in the nondormant group. Variation in genetic diversity was also observed among the nine counties, and populations from Chicot County showed the highest diversity. The high genetic variability of seed dormancy among and within weedy *Oryza* sp. populations presents a challenge for effective weedy *Oryza* sp. management in Arkansas. Rice producers should

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not grow rice consecutively for more than a year, especially if dormant weedy *Oryza* sp. has been detected in the field. This could prevent the dormant weedy *Oryza* sp. seeds from spreading and also reduce the weedy *Oryza* sp. soil seedbank. Moreover, the two alleles unique to dormant and nondormant blackhull populations, respectively, can be used in the identification of dormant and nondormant phenotypes among blackhull weedy *Oryza* sp. populations. These unique alleles can also be used in follow-up studies on molecular mechanisms involved in weedy *Oryza* sp. seed dormancy. Because of the weak association of some markers used in this study to seed dormancy, there is a need to develop new set of dormancy-linked markers using dormant and nondormant strawhull versus dormant and nondormant blackhull populations.

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