

**Population-Structure Analysis of Red Rice in Arkansas: DNA Marker Evidence for Gene Flow between Rice and Red Rice**

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**ABSTRACT**

Red rice is a troublesome weed problem in Arkansas rice fields and numerous biotypes are present. Outcrossing between rice and red rice occurs at low rates, resulting in unusual plant types, and can complicate weed management efforts. STRUCTURE (STR) analysis of DNA SSR marker data is useful to infer population structure, to assign individuals to different populations, and to identify hybrids. Thus, this procedure was used to evaluate the genetic backgrounds of numerous red rice types that, based on visual traits, apparently had developed from natural outcrossing with rice. STR analysis of suspected red rice crosses obtained from a multi-county, multi-state area yielded little evidence that genetic markers initially associated with rice were retained in red rice populations at high levels over time. Additional studies involving a larger number of markers or markers specifically associated with rice cultivars (e.g. semidwarfism or herbicide resistance) may be necessary to confirm these preliminary findings. In contrast to the aforementioned results, STR analysis clearly showed that a group of red rice plants obtained from Mississippi Co., Ark., partially shared a genetic background with both long-grain commercial rice and red rice. They probably resulted from a recent cross between the two plant types. These studies demonstrate that STR analysis can be used to identify and characterize red rice crosses in some cases, and that it could be useful as a diagnostic tool.

**INTRODUCTION**

Rice and red rice belong to the same species (*Oryza sativa*) and they can intercross at low rates. Outcrossing can occur with either plant type serving as pollen donor (Chen

et al., 2004; Gealy and Estorninos, 2007; Zhang et al., 2003). Hybrids can differ in coloration and plant type, depending on the red rice parent (Gealy et al., 2006). Gealy and Estorninos (2007) recently used SSR analysis to confirm that reciprocal outcrossing rates between U.S. red rice and commercial long-grain rice in controlled field plots over a five-year period averaged 0.26% with red rice as pollen donor and 0.056% with rice as pollen donor. Results from related studies have demonstrated that red rice accessions that appeared to have been derived from crosses between red rice and long grain rice were, in fact, such crosses (Estorninos et al., 2006; Gealy et al., 2005). However, the extent to which progeny of such crosses can introgress into red rice populations and remain there is not well understood.

A model-based Bayesian cluster analysis (STRUCTURE; STR) (Pritchard et al., 2000) of SSR DNA marker data from rice and red rice can be used to evaluate the genetic backgrounds of red rice populations of interest. Such analyses can be used to infer population structure, assign individuals to sub populations, and to study hybrid populations. Thus, the goal of this research was to employ STR to discern parental backgrounds of numerous red rice populations that, based on physical traits, were presumed to be rice x red rice crosses.

## **PROCEDURES**

### **Multi-County/Multi-State Study**

In order to evaluate the evidence of long-term gene flow/introgression between commercial rice and red rice in Arkansas, seeds from more than 400 red rice accessions were obtained from farm samples and grown at Stuttgart, Ark., as described previously (Estorninos et al., 2006). In one or more field studies (unpublished data), a small fraction of these red rice types exhibited traits consistent with progeny of known hybrids (e.g. as in Gealy et al., 2006). Thus, four such subgroups, consisting of at least 10 individuals each (Table 1), were chosen for DNA analysis. These were 1) plants that were unusually short (Lhts; ~100 to 116 cm) compared to normal red rice plants (typically 130 to 167 cm); 2) plants with brief heading periods (Lhds; ~3 to 5 days from initiation of heading to completion) that are common in commercial rice cultivars and of shorter duration than those of typical red rice types (~6 to 12 days); 3) plants with very short awns (Sawn; ~0.5 to 1 cm) that are typical of certain hybrid progeny; and 4) plants with various combinations (Comb) of the traits described above. Red rice accessions chosen at random (Rndm) as being representative of ordinary red rice types, and several long-grain commercial rice cultivars, were included for comparison as standards.

DNA extraction and SSR marker analysis generally were performed as described previously (Estorninos et al., 2006). Amplified PCR products from 19 SSR markers that were distributed among all 12 chromosomes were visualized on an ABI 3730 (or an ABI 3700) automated DNA sequencer using DNA isolated from leaf tissues and analyzed in Genemapper software. The markers were RM5, RM154, RM234, RM124, RM210, RM174, RM231, RM232, RM512, RM206, RM215, RM488, RM283, RM484, RM317, RM408, RM146, RM133, and RM253. To determine the population structure of these

suspected crosses, a model-based Bayesian cluster analysis was performed using all red rice accessions and rice cultivars (Pritchard et al., 2000). STR software can identify true crosses as having a shared genetic background (i.e., subpopulations) with one or more red rice types and rice cultivars.

### **Mississippi Co., Ark., Study**

A group of 15 accessions, obtained from Mississippi Co., Ark., in 2005 that consisted of variable red rice-like plant types that apparently represented a segregating population derived from a cross between red rice and rice (Estorninos et al., 2006), was included in these studies as an example of a population recently developed from outcrossing. Plant types included MS-1, pink-purple stem, rough leaf, pink awn; MS-2, purple stem, rough-smooth leaf, short green awn; MS-3, purple stem, rough leaf, short awn; MS-4, green stem, smooth leaf, awnless; MS-5, purple stem, rough leaf, pink awn; MS-6, purple stem, rough leaf, very short-awn/awnless; MS-7, green stem, smooth leaf, very short-awn/awnless; MS-8, green stem, smooth leaf, awnless; MS-9, green stem, smooth leaf, green awn; MS-10, purple stem, rough leaf, pink awn; MS-11, purple stem, rough leaf, pink awn; MS-12, purple stem, smooth leaf, green awn; MS-13, purple stem, rough leaf, pink awn; MS-14, purple stem, rough leaf, pink awn; MS-15, purple stem, rough-smooth leaf, green awn. Plant types also varied with respect to heading date and seed coat color (not shown). Overall, this combination of traits is indicative of a segregating population ( $F_2$  or later generation) derived from a rice x red rice cross (Gealy et al., 2006). STR analysis was performed generally as described above except that nine SSR markers were used. They were RM167, RM253, RM219, RM234, RM180, RM215, RM224, RM206, and RM220.

## **RESULTS AND DISCUSSION**

### **Multi-County/Multi-State Study**

Five independent runs in the STR software using  $k$  values (hypothetical number of subpopulations) from 3 to 6 showed the highest number of accessions assigned to a specific cluster with a probability higher than 80% was obtained with  $k = 6$ , thus indicating the presence of complex relationships among accessions. Using the clustering diagrams with  $k = 6$ , the subpopulations identified by STR largely corresponded to pools originating from commercial rice, and red rice with normal-length awns, very short awns, or without awns (Fig. 1).

Known hybrids (e.g., 'RT XL8', 'CL 161' x red rice, and 'Kaybonnet' (KBNT) x STGS) showed the expected shared genetic background encompassing alleles from both parents in a first-generation cross (Fig. 1). There was essentially no evidence of a shared genetic background between any of the four groups of putative rice x red rice crosses or the standard red rice types and the long-grain cultivars tested (Fig. 1). However, red rice accession 1022\_02 Lhds (Fig. 1; 4<sup>th</sup> entry from bottom) may share a subpopulation

(*k*5) with the commercial rice, STBN (Starbonnet; no longer grown). There also was no evidence of significantly shared genetic backgrounds between these red rice accessions and a group of cultivars historically grown in the southern U.S., such as long-grains ('Rexoro', 'Newbonnet', 'Lemont', 'LaGrue', 'Gulfmont', 'Drew', 'Dawn', 'Cypress', 'Bluebonnet', and 'Carolina Gold'), medium-grains ('Zenith', 'Saturn', 'Nato', 'Mars', and 'Bengal'), as well as several japonica cultivars ('M-204' and 'Koshihikari') (Lu et al., 2005; data not shown). However, the red rice groups appeared to share common alleles with indica rice germplasm (e.g., 'TeQing', data not shown).

It is possible that the unusual red rice phenotypes that were tested in these studies may have arisen from preexisting genetic diversity within the red rice populations. It is also possible that rice alleles, which may have been transferred to red rice plants through intercrossing, were subsequently lost from these populations due to selection pressure. Analysis of numerous additional markers, including those specifically associated with commercial rice cultivars (e.g., markers for the SD-1 semidwarfing gene), may be more informative than the markers used in this test, and are being investigated.

### **Mississippi Co., Ark., Study**

Using the clustering diagrams with *k* = 4, the subpopulations identified by STR largely corresponded to pools originating from commercial rice (*k* = 1 and 2), black-hull awned red rice standards such as TX4 and redrice\_8 (*k* = 3), and the awnless red rice standard StgS (*k* = 4) (Fig. 2). However, all of the red rice standards, particularly 11D\_RR, shared subpopulations to some degree with the commercial rice standards. As would be expected in a segregating population derived from a red rice x rice cross, some red rice accessions were composed of subpopulations more indicative of rice (e.g., MS-12, 13, and 14), others were more indicative of red rice (e.g., MS-8, 9, 10, and 11), and others were indicative of both rice and red rice (e.g., MS-2, 6, and 7). Thus, STR analysis in combination with physical traits suggests that these Mississippi Co. plants had been derived from a cross between long-grain rice and an awned red rice similar to 11D\_RR, TX4std, or redrice\_8 (Fig. 2). More than one initial cross or involvement of an awnless red rice similar to StgS is also possible.

### **SIGNIFICANCE OF FINDINGS**

These results have confirmed the effectiveness and efficiency of STR analysis in the evaluation and interpretation of DNA markers for the purpose of identification and subsequent management of rice x red rice crosses in farm fields. Results from these analyses suggest that, in combination with physical traits, STR could be highly useful in monitoring outcrossing and gene flow dynamics between red rice and rice. STR revealed little evidence of the presence of rice DNA markers in red rice accessions that were hypothesized to have been derived from outcrossing events far in the past. This suggests either that the accessions in question were not actually crosses, or that a large portion of the rice DNA originally present in the cross had been lost over time so that it was not easily detected by the small number of markers used.

## ACKNOWLEDGMENTS

This research was partially funded by the Arkansas Rice Research and Promotion Board. Thanks to Howard Black for technical assistance and Melissa Jia for DNA analysis.

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**Table 1. Phenotypic and geographic descriptions of the red rice accessions obtained from a multi-county, multi-state area, as compared to rice and red rice standards.<sup>2,3,4,5</sup>**

Sample name (Rice or red rice)	Awns	Hull color	Leaf texture	County	Spread (days)	Height (cm)	Emg-head (days)	Phenotypic grouping
1013_01	N	S	R	Jackson	8.3	104	70.0	Lhts
1064_01	N	S	R	Independence	9.0	114	95.7	Lhts
1092_01	N	S	R	Randolph	5.5	116	82.5	Lhts
1151_01	N	S	R	Poinsett	6.7	107	95.7	Lhts
1156_01	N	S	R	Woodruff	7.3	107	96.7	Lhts
1167_01	Y	B	R	Faulkner	6.0	115	104.3	Lhts
1176_01	Y	B	R	Faulkner	6.0	116	103.3	Lhts
1226_02	N	S	R	Clay	7.3	108	95.3	Lhts
1287_02	Y	B	R	Lee	8.0	112	103.7	Lhts
1290_02	N	S	R	Randolph	9.0	101	90.0	Lhts
1043_01	Y-SHR	S	R	Lincoln	4.7	124	83.0	Sawn
1099_02	Y-SHR	Br	R	Phillips	6.0	121	85.0	Sawn
1111_01	Y-SHR	S	R	Woodruff	7.7	130	88.7	Sawn
1124_01	Y-SHR	S	R	White	5.3	132	83.7	Sawn
1147_02	Y-SHR	S	R	Prairie	8.5	132	88.0	Sawn
1183_01	Y-SHR	S	R	Chicot	12.3	130	88.3	Sawn
1202_02	Y-SHR	Br	R	Jefferson	4.7	146	79.7	Sawn
1218_02	Y-SHR	S	R	St Francis	6.3	131	89.0	Sawn
1254_02	Y-SHR	S	R	Jefferson	4.7	146	79.7	Sawn
1300_02	Y-SHR	Br	R	Dunklin, Mo.	4.7	132	85.0	Sawn
1312_02	Y-SHR	S	R	Morehouse, La.	8.7	133	87.0	Sawn
1022_02	Y	B	R	White	5.0	141	88.7	Lhds
1052_02	Y	B	R	Lee	3.3	160	93.0	Lhds
1078_01	Y	B	R	Jackson	4.0	114	103.3	Lhds
1096_01	Y	B	R	Arkansas	3.3	118	101.7	Lhds
1131_01	N	S	R	Cross	4.0	143	87.0	Lhds
1148_02	Y-SHR	S	R	Prairie	3.3	126	82.3	Lhds
1157_02	Y-SHR	S	R	Desha	3.7	127	83.7	Lhds
1186_01	Y-SHR	S	R	Drew	3.7	136	84.3	Lhds

continued

**Table 1. Continued**

Sample name (Rice or red rice)	Awns	Hull color	Leaf texture	County	Spread (days)	Height (cm)	Emg-head (days)	Phenotypic grouping
1271_02	Y-SHR	Br	R	Lincoln	2.7	115	83.7	Lhds
1406_02	Y-SHR	S	R	Woodruff	3.0	125	84.0	Lhds
1430_02	Y-SHR	S	R	Coahoma, Miss.	3.7	126	85.3	Lhds
1034_01	Y	B	R	St Francis	4.3	167	94.3	Comb
1042_01	Y	S	R	Jefferson	4.3	153	95.7	Comb
1043_02	Y	B	R	Monroe	5.0	159	94.0	Comb
1060_01	Y	B	R	Craighead	4.7	161	94.3	Comb
1111_02	N	S	R	Desha	5.0	128	94.3	Comb
1115_01	Y	B	R	Lawrence	4.3	160	94.7	Comb
1142_01	Y	B	R	Lonoke	5.0	122	102.3	Comb
1160_01	N	S	R	Morehouse, La.	4.3	142	92.3	Comb
1194_01	Y	B	R	Faulkner	4.7	127	105.0	Comb
1243_02	N	S	R	Morehouse, La.	5.0	142	92.0	Comb
1349_02	Y	B	R	Arkansas	5.0	135	103.0	Comb
1418_02	N	S	R	Jefferson	5.0	150	91.7	Comb
1431_02	Y	Br	R	East Carroll, La.	4.3	151	96.7	Comb
1039_02	N	S	R	St Francis	10.3	141	85.0	Rndm
1046_02	N	S	R	Phillips	8.0	139	86.0	Rndm
1061_01	Y	B	R	Butler	6.0	152	91.0	Rndm
1105_02	N	S	I	East Carroll, La.	9.0	148	83.3	Rndm
1132_01	N	S	R	Yell	9.7	146	84.7	Rndm
1154_01	Y	B	R	Desha	7.3	152	90.0	Rndm
1187_01	N	S	R	Ripley	9.0	145	85.7	Rndm
1263_02	N	S	R	Stoddard, Mo.	8.3	132	86.7	Rndm
1288_02	N	S	R	White	10.7	140	80.3	Rndm
1358_02	N	S	R	Craighead	8.7	154	84.0	Rndm
CL161 x RR	N	S	R	---	---	---	---	StdRxRR hyb
Kaybonnet x LA3	Y	S	R	---	---	---	---	StdRxRR hyb
Kaybonnet x StgS	N	S	R	---	---	---	---	StdRxRR hyb
RR x CL121	N	S	R	---	---	---	---	StdRxRR hyb

continued

**Table 1. Continued.**

Sample name (Rice or red rice)	Awns	Hull color	Leaf texture	County	Spread (days)	Height (cm)	Emg-head (days)	Phenotypic grouping
11D	Y	S	R	Arkansas	---	---	---	StdAwnedRR
LA3	Y	S	R	LA	6.7	158	99.0	StdAwnedRR
StgB	Y	B	R	Arkansas	7.0	165	104.7	StdAwnedRR
StgS	N	S	R	Arkansas	11.0	144	89.0	StdAwnlessRR
CL121	N	S	S	---	---	---	---	StdRice
CL161	N	S	S	---	---	---	---	StdRice
Cypress	N	S	S	---	3.7	104	90.0	StdRice
Kaybonnet	N	S	S	---	2.7	119	91.3	StdRice
RTXL8	N	S	R	---	---	---	---	StdRice Hyb
Starbonnet	N	S	S	---	5.3	125	96.3	StdRice
Wells	N	S	S	---	---	---	---	StdRice

<sup>z</sup> Key to terms: Awns: Y = long awn, N = no awn, Y-Shr = very short awn (< 0.5 cm). Hull Color: S = strawhull, B = blackhull, Br = brownhull. Leaf Texture: R = rough, S = smooth, I = intermediate. County of Origin (State of Arkansas unless otherwise noted). Spread=number of days from first heading to final heading; Height=plant height; Emg-Head = average number of days from emergence to heading. Phenotypic grouping: Lhts=very short plants, Lhds=low heading spread [i.e., all heading occurs within a few days], Sawn=short awns, Comb=combination of various plant types as defined for Lhds and/or Lhts, Rndm=randomly selected red rice standards included for comparison, StdR<sup>x</sup>RR hyb=standard hand-crossed rice x red rice hybrid, StdAwnedRR=standard awned red rice type, StdAwnlessRR=standard awnless red rice type, StdRice=standard long-grain rice cultivar, StdRice Hyb=standard commercial rice hybrid.

<sup>y</sup> Max. / min. values from all red rice accessions in field nursery averaged over 3 reps were 12.3 / 3.0 days spread, 167 / 101 cm height, and 105 / 70 days emergence to heading.

<sup>c</sup> --- Indicates data not available.



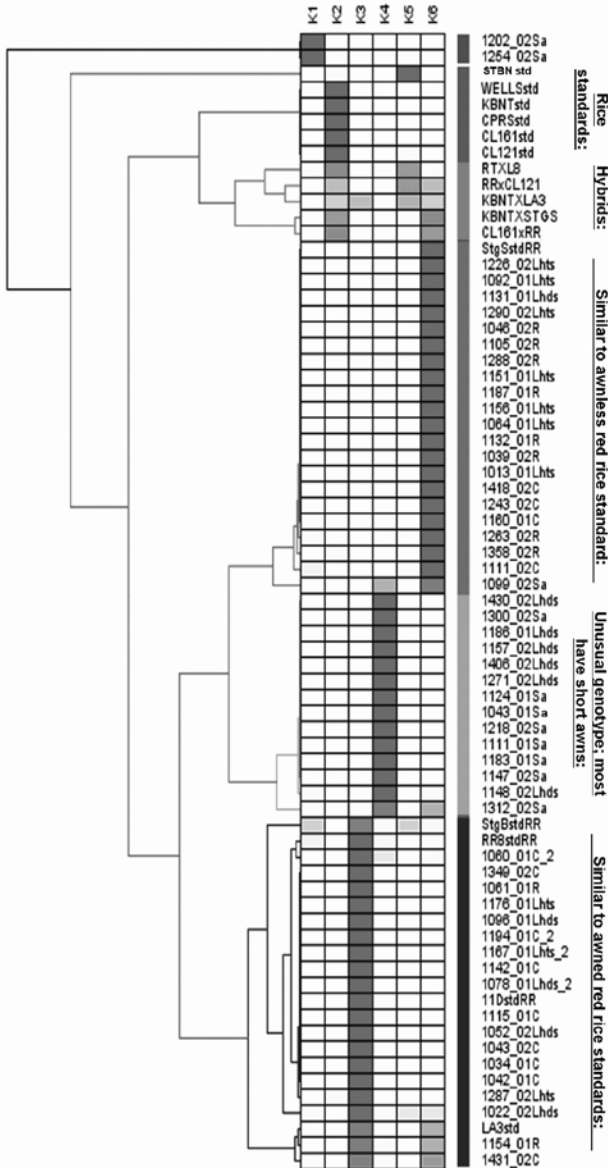
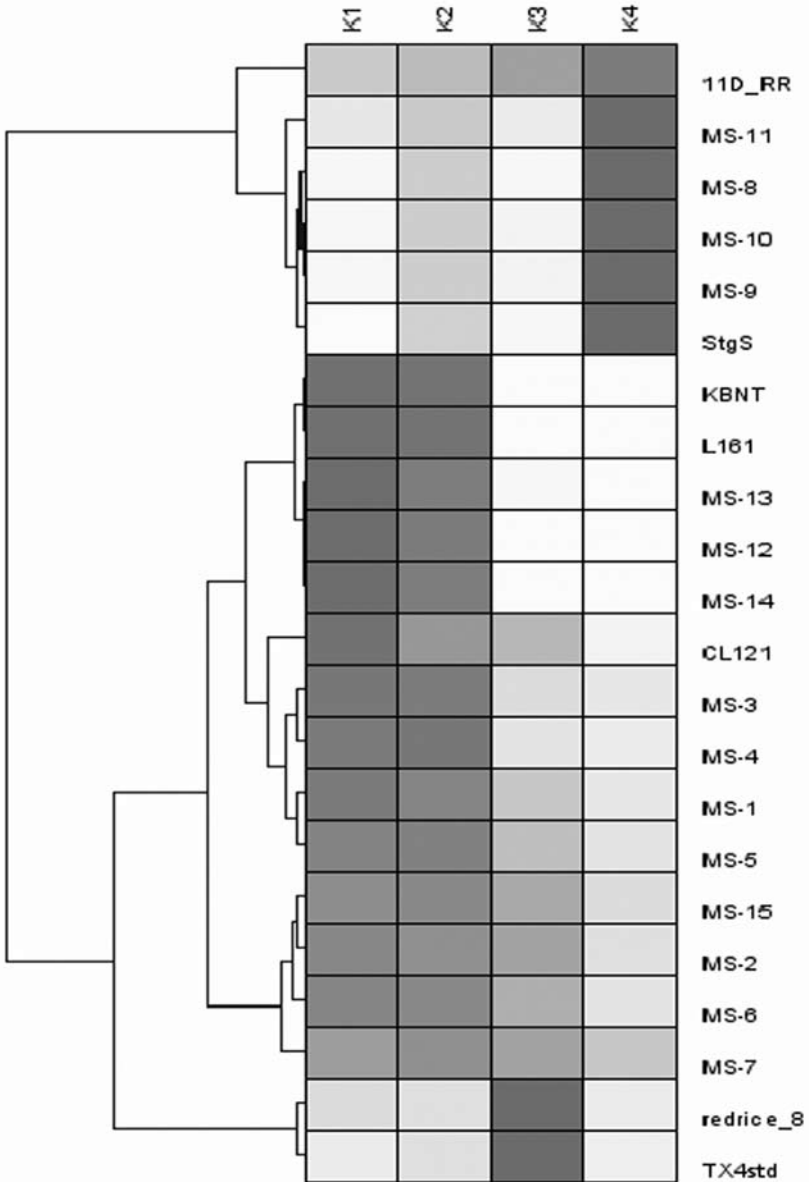


Fig. 1. Population structure of genotypes from a multi-county, multi-state collection of red rice accessions based on 19 SSR markers. More than one shaded box within a row indicates that the accession or cultivar consists of more than one genetic subpopulation ( $k$  value). Those with only one shaded box consist of a single identifiable subpopulation. Accessions or cultivars that are shaded in the same columns share the same subpopulation. Groupings of genotypes were based on six possible genetic backgrounds ( $k1-6$ ) using model-based clustering analysis.



**Fig. 2. Population structure of a red rice collection from Mississippi Co., Ark., thought to have resulted from a recent cross between rice and red rice. Groupings of genotypes were based on four possible genetic backgrounds (*k*1-4) using model-based clustering analysis. Rice cultivars: KBNT, Kaybonnet; L161, CL 161; and CL 121. Red rice standards: 11D\_RR, AR awned red rice; StgS, Stuttgart awnless red rice; redrice\_8, AR awned red rice #8; and TX4std, TX awned red rice. Mississippi Co. red rice types: MS-1 to MS-15.**