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Risk assessment of the transfer of imazethapyr herbicide resistance from Clearfield rice to red rice

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RISK ASSESSMENT OF THE TRANSFER OF IMAZETHAPYR HERBICIDE RESISTANCE FROM CLEARFIELD RICE TO RED RICE

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ABSTRACT

Potential outcrossing between Clearfield rice and red rice will have a direct impact on the management and long-term usefulness of imazethapyr technology for rice weed control. The principal objective of this research was to determine the rate and agronomic consequences for outcrossing between Clearfield rice and red rice in south Louisiana. Collection and analysis of red rice samples across two years and 24 commercial locations indicated that red rice infestation after imazethapyr application differed substantially at different Clearfield locations. Straw hull and awnless red rice was the principal biotype observed for both years. Red rice populations possessed extensive variation for agronomic traits such as plant height, panicle length, tillers/plant, seeds/plant, seed set and grain weight. Outcrossing occurred from all Clearfield rice varieties ('CL121', 'CL141' and 'CL161') to red rice. An average outcrossing frequency of 0.163% was observed in red rice samples collected in 2002 with a range of 0.017% to 0.583%. A four-fold increase in outcrossing frequency of 0.679% was found in red rice samples collected in 2003 with two locations exhibiting outcrossing > 1%.

Outcrossing frequency did not correlate with any agronomic trait from the red rice samples across two years. Imazethapyr resistance was generally controlled by a single dominant gene, except in some F_2 populations where significant deviations from expected resistant/susceptible ratios were detected. Similar results were observed in F_2 populations for segregation of pubescent/glabrous leaves.

F_1 hybrids between Clearfield rice and red rice in general did not show increased fitness in flowering characteristics over Clearfield rice, as most hybrids did not flower or produce seeds in the field. However, increased fitness in F_1 hybrids, derived from red rice samples collected in 2002, was detected over Clearfield rice for plant height, tillers/plant, and panicles/plant.

Enhanced fitness in F_1 hybrids from red rice samples collected in 2003 over Clearfield rice was exhibited for plant height, panicle length, spikelets/panicle, and panicles/plant. Results from this study indicate that outcrossing between Clearfield and red rice will occur rapidly at rates that warrant early-season field scouting and a rotation scheme for Clearfield rice to prolong usefulness of the imazethapyr technology.

CHAPTER 1 INTRODUCTION

1.1 The Importance of Rice

Rice is one of the most important crops in the world with 53,324,898 ha planted in 2003 producing 588,563,933 Mt and 3.37 Mt/ha (Anonymous 2003c). Rice provides 60% of daily caloric needs for countries that depend upon rice as their primary staple food (Khush, 2003). Moreover, rice provides nutrient value, such as carbohydrate, protein, calcium, iron, zinc, and vitamin B6 (Anonymous 1999). In the United States, rice is planted in Louisiana, Arkansas, Texas, California, Mississippi and Missouri with 1,212,860 ha planted in 2003 (Anonymous 2003c). United States rice production not only satisfies the requirement of the domestic market, but also exports ~ 40% of the total to major rice consuming areas around the world (Anonymous 2003b).

1.2 Effects of the Weedy Red Rice on Commercial Rice Production

Red rice (*Oryza sativa* L.), with the same genus and species as cultivated rice (Gianessi et al., 2002), is a troublesome weed in most rice growing regions in the world (Fisher and Ramirez, 1993), and a noxious pest for rice production in the southern United States (Oard et al., 2000). U.S. red rice biotypes consist primarily of awnless straw hull and black hull types, but brown hull, golden hull (Noldin et al., 1999), and gray hull biotypes (Constantin, 1960) have also been observed in other regions of the world. Red rice decreases the yield of cultivated rice due to its competition for sunlight, water and nutrients, and presence of red pericarp from the weed in the milling process reduces market value of the commercial product (Dilday et al., 1990). Other deleterious characteristics of red rice include seed shattering and dormancy (Lago, 1982), resulting in a seed bank with prolonged viability in soil that ultimately will affect production and quality of the commercial crop.

1.3 Outcrossing in Other Crops

Genetic engineering brings about a new approach to rapidly improve specific characteristics of elite varieties, such as herbicide and insect resistance. Gene flow (outcrossing) is the introduction of genetic material from one population of a species to another, thereby changing the composition of the gene pool of the receiving population (Anonymous 2005). Outcrossing between a crop and its weedy or wild relatives has caused concern about not only weed control, but also ecological conservation (Rhymer and Simberloff, 1996). Natural hybridization can be common between cultivated varieties and weedy forms, although the frequency depends upon many factors such as different genetic backgrounds and environments. Intermediate phenotype indicated the occurrence of hybridization between the world's most important food crops, barley (*Hordeum vulgare* L.), finger millet (*Eleusine coracana* L.) and their wild relatives. Substantial phenotypic and molecular evidence demonstrates the existence of outcrossing between important food crops in the world including wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), grain sorghum (*Sorghum bicolor* L.), dry and green and string beans (*Phaseolus vulgaris* L.), rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.) and sugarcane (*Saccharum officinarum* L.), and their wild relatives (reviewed by Ellstrand et al., 1999).

Various phenotypic markers, such as the widely used resistance to glufosinate or glyphosate herbicides in transgenic rapeseed (Bechie et al., 2003; Darmency et al., 1998), and resistance to the imidazolinone herbicide family in a sunflower mutant (Massinga et al., 2003) were used to evaluate outcrossing events between crops and their wild or weedy relatives. Molecular markers for these outcrossing studies included random amplified polymorphic DNA (RAPD) marker (Amand, 2000; Guadagnuolo et al., 2001; Jorgensen et al., 1994; Linder et al.,

1998; Whitton et al., 1997), and "simple sequence repeat" (SSR) markers have proven invaluable for analysis of outcrossing (Chase et al., 1996; Guadagnuolo et al., 2001; Innan et al., 1997). SSR markers consist of a tandem repeat sequence of 1-5 nucleotides (Wu and Tankley, 1993) that are co-dominant, highly polymorphic, and abundant in plant genomes (Ishii et al., 2001; Queller et al., 1993).

Various outcrossing studies have evaluated distance from pollen donor to pollen recipient (Arias et al., 1994; Arriola et al., 1996; Ray et al., 2003; Rognli et al., 2000), wind direction (Amand, 2000), amount of pollen produced (Darmency et al., 1998; Halfhill et al., 2004; Marchis et al., 2003) and genetic factors under different experimental designs from small plot to production fields (Lavigne et al., 1998). The frequency of outcrossing, dependent upon different genetic backgrounds (Baranger et al., 1995), decreased in a non-linear pattern with the increase of distance from pollen donor to recipient (Arias et al., 1994; Arriola et al., 1996; Rognli et al., 2000). Isolated distances of 100 m, 1000 m and 1557 m were suggested from cultivated sorghum to johnsongrass (Arriola et al., 1996), from cultivated sunflower to weedy sunflower (Arias et al., 1994) and in commercial alfalfa seed production (Amand, 2000), respectively. Wind direction during the flowering period affected the frequency of outcrossing and the highest rate of outcrossing was detected in the direction of prevailing winds (Rognli et al., 2000). Moreover, high densities of pollen provided ample opportunities for outcrossing (Guadagnuolo et al., 2001; Lefol et al., 1996; Rognli et al., 2000).

Fitness of F_1 or F_2 populations derived from rapeseed and *B. rapa* (Hauser et al., 1998a; Hauser et al., 1998b), showed high or low characteristics for different agronomic traits under different planting patterns (Hauser et al., 2003). Similar results were obtained between a yellow crookneck squash (*Cucurbita. pepo ssp. ovifera*. var. *ovifera*) and free living populations of

Cucurbita pepo (Spencer et al., 2001). However, no significant differences in fitness were found between hybrids of cultivated sorghum (*Sorghum bicolor*) and johnsongrass (*Sorghum halepense*) and the commercial sorghum varieties (Arriola et al., 1997).

1.4 Outcrossing among Rice Plants

Like other crops mentioned above, spontaneous hybridization also exists among cultivated rice and wild or weedy relatives, even though rice is considered a self-pollinating crop. Natural hybrids have been detected for the following: African cultivated rice *Oryza glabberima Steud*. and its weedy relative *O. breviligula Chev.et Roehr*, Asian cultivated rice *O. sativa* L. and its weedy relative *O. perennis Moench* (Oka and Morishima, 1971), cultivated rice and modern hybrids (Rong et al., 2004), and cultivated rice and the wild relative *Oryza rufipogon* (Chen et al., 2004; Song et al., 2004), and varieties and red rice (Langevin et al., 1990) using phenotypic and/or molecular data. Similarly, outcrossing may occur between transgenic lines, and weedy or wild relatives.

Herbicide resistant transgenic lines expressing the *bar* gene were used to detect outcrossing with their non-transgenic counterparts related to distance from pollen donor to pollen recipient, wind direction factors (Messenguer et al., 2001), and to evaluate maximal outcrossing frequencies with red rice (Chen et al., 2004; Zhang et al., 2003). The frequency of outcrossing between cultivated rice, transgenic lines and their weedy or wild relatives was affected by different genotypes (Chen et al., 2004; Langevin et al., 1990; Rong et al., 2004; Zhang et al., 2003), and the rate of outcrossing between cultivated rice and wild relatives was affected by wind direction with the largest rate occurring in the direction of prevailing winds (Song et al., 2004).

The findings of Song et al., 2004 were in contrast to those of Messenguer et al., (2001) who used a circle plot design between transgenic rice and its non-transgenic counterpart. The performance of F_1 or F_2 populations relative to parents can be variable for certain agronomic traits, including those relating to hybrid vigor or fitness. (Langevin et al.,1990; Oard et al., 2000; Song et al., 2004; Zhang et al., 2003). The expression of the bar transgene in hybrid progeny was controlled by single dominant gene (Oard et al., 2000; Zhang et al., 2003), except for certain progenies exhibiting abnormal genetic segregation (Oard et al., 2000). Segregation for pubescent or glabrous leaves also followed Mendelian inheritance in the hybrid progeny (Zhang et al., 2003).

1.5 Clearfield Rice

Clearfield rice varieties, developed and released by the LSU AgCenter, were produced from the progeny between elite Louisiana rice varieties and Ethyl Methane Sulfonate (EMS) induced mutant (93AS3510) that are resistant to the imazethapyr herbicide (Croughan, 1994). 'CL121' and 'CL141' were developed from the crosses of Cocodrie and the 93AS3510, and Maybelle and the 93AS3510, respectively. 'CL161' was derived from a mutated Cypress plant (Wenefrida et al., 2004). The CL121 and CL141 varieties were commercially released in Louisiana and Arkansas in 2002 with 2% CL121 and <1% CL141 of the total acreage in Louisiana, respectively (Anonymous 2002a). In 2003, the second generation variety CL161 was released with 7% of the total rice planting in Louisiana (Anonymous 2003a) that increased to 22% of total rice area in Louisiana in 2004 (Anonymous 2004). CL161 showed higher level of imazethapyr tolerance compared with CL121 and CL141 (Anonymous 2002c), and was similar to Cocodrie in yield.

1.6 The Objectives

The objectives of this research are to: (1) evaluate the frequency of outcrossing from Clearfield rice to red rice through pollen dispersal at 24 commercial Clearfield locations over a two year sampling period, (2) characterize agronomic traits of red rice populations among twelve locations each year, (3) detect and evaluate potential associations between outcrossing frequency and various agronomic traits in different red rice biotypes, and (4) determine genetic control of imazethapyr resistance in F_2 populations developed by crosses between red rice and Clearfield rice.

CHAPTER 2 LITERATURE REVIEW

2.1 Red Rice as a Troublesome Weed

Red rice (*Oryza sativa* L.) is a noxious weed that coexists with cultivated rice in paddy fields in many rice producing areas of the world (Fisher and Ramirez, 1993). This weed is one of the most problematic pests in rice production in the southern United States (Oard et al., 2000) that competes for nutrients, water and sunlight with cultivated rice to limit productivity and quality (Dilday et al., 1990). The decease in grain quality of the harvested crop is mainly attributed to the pericarp and poor milling yields of red rice. Red rice in commercial fields consists of highly polymorphic individuals (Galli, 1991), that include straw hull and black hull as the main biotypes with golden hull and brown hull forms being less frequent (Noldin et al., 1999). Gray hull and golden hull biotypes have also been observed in certain red rice populations (Constantin, 1960). Generally, red rice is characterized as having pubescent, light green leaves and pubescent seeds (Diarra et al., 1985), but red rice collected from Louisiana and Mississippi exhibited dark green, glabrous leaves (Noldin et al., 1999). Red rice has been described as taller than elite semi-dwarf varieties (Langevin et al., 1990), although wide variation for plant height in red rice population does exist in commercial rice fields. Moreover, the characteristics of seed shattering and seed dormancy in red rice populations (Dilday et al., 1990), and the close relationship with commercial rice have made effective and economic weed control difficult (Gianessi et al., 2002).

2.2 Ecological Concerns from Use of Transgenic Plants

Genetic engineering of commercial rice may involve the insertion of a foreign gene into elite varieties with a wide spectrum of resistance to herbicides. However, the potential transfer of the foreign gene into a weed population through pollen dispersal has raised concerns about the

utilization of this technology (Ellstrand, 1988). This phenomenon, the introduction of genetic material from one population of a species to another, thereby changing the composition of the gene pool of the receiving population, is referred to as gene flow or outcrossing (anonymous 2005). For example, in one early study by Lavigne et al. (1998), rapeseed (*Brassica napus*) resistant to oxynil (Ioxynil/Bromoxynil) herbicide was produced by the pollination of transgenic rapeseed coding for a nitrilase enzyme. Therefore, risk assessment of transgene escape through pollen dispersal has become important for release of commercial transgenic crops including allogamous species like maize and sugar beet and self-pollinated crops with a high outcrossing rate such as rapeseed (Messenguer et al., 2001),

2.3 Hybridization in Other Crops Except for Rice

2.3.1 Hybridization among Traditional Crops and Wild or Weedy Relatives

The potential of some crops to hybridize with wild related species has been demonstrated (Lavigne et al., 2002), such as cultivated corn (Doebley, 1984), cotton (Brubaker et al., 1993; Brubaker and Wendel, 1994; Wendel and Percy, 1990; Wendel et al., 1992), and hybrids have been observed in diverse genera such as *Brassica* (Baranger et al., 1995; Darmency, 1994), *Beta* (Boudry et al., 1993; Santoni and Berville, 1992), and *Helianthus* (Linder et al., 1998). Heiser (1954, 1976) and Rieseberg et al. (1988) documented the occurrence of intraspecific and interspecific hybridization in *Helianthus annuus* and its close relatives. Ray et al. (2003) identified natural cross-pollination in soybean under field conditions. One soybean variety with white flowers acted as a pollen recipient while a second variety with purple flowers functioned as a phenotypic marker for screening natural hybrids. The rate of natural cross-pollination decreased with the increase of distance relative to pollen source from each side of the field, and the highest rate of 0.41% occurred at 0.9 m from the pollen donor while the lowest frequency of

0.03% occurred at 5.4 m from pollen source. Jorgensen et al. (1994) evaluated the spontaneous hybridization between rapeseed (*Brassica napus* L.) and weedy *Brassica campestris* (*B. campestris*) through phenotypic data, species-specific isozyme, random amplified polymorphic DNA (RAPD) marker, chromosome counting and the fertility of hybrids under different experimental designs. A 1:1 mixture of *B. campestris* and rapeseed was sown in small plots, and a hybridization rate of 13% and 9% were found from *B. campestris* and rapeseed, respectively. Compared with hybrid seeds in the two species, different seed characteristics existed with a wide range of seed size (1.25 mm to 2.0 mm) for hybrid seed in *B. campestris*, but only small size of seeds for hybrid seeds in rapeseed. In another experimental design, single plants of *B. campestris* were transplanted into winter and spring rapeseed fields, two to three weeks before the flowering of weedy *B. campestris* and cultivated rapeseed, respectively. A high hybridization rate of 93% and 56% were detected from *B. campestris* in winter and spring oilseed fields, respectively. Further analysis for the origin of hybrid seeds showed that the contribution of individual *B. campestris* plants to the frequencies of spontaneous hybridization differed among individual weedy plants in winter and spring rapeseed fields. Renno et al. (1997) detected outcrossing between cultivated and wild pearl millets under field conditions. The average frequency of 45% and 39% hybridization was detected from wild peal millet to two cultivated pearl millets. The frequency of hybridization was negatively correlated with the number of viable hybrids which could develop into seedlings and the germination rate of hybrid seeds. In contrast, the average frequency of hybridization from cultivated pearl millet was affected by the variation of pollen during flowering period which was different from the outcrossing frequency from wild pearl millet to cultivated pearl millet.

Recently, Rognli et al. (2000) used two grass meadow fescues (*Festuca pratensis* Huds.) with different homozygous allozymes at the *Gpi*-2 (glucose phosphate isomerase-2) locus to evaluate the effect of the distance from pollen recipient to pollen donor, the density of pollen recipient and wind direction during flowering period on outcrossing. The rate of outcrossing drastically decreased with the increase of distance from pollen recipient to pollen donor within the range of 75 m. Beyond that distance, the rate of outcrossing decreased very slowly. The highest rate of hybridization occurred at the prevailing wind direction during the flowering period. Another factor, the density of pollen recipient plants, also affected outcrossing estimates, showing the larger rate of outcrossing for pairs of plants compared with single plants. Arriola et al. (1996) investigated the incidence and rate of outcrossing from cultivated sorghum (*Sorghum bicolor*) to weedy johnsongrass (*Sorghum halepense*) in field plots at two locations in two consecutive years. The rate of hybridization varied at different distances and locations and years, and showed a decrease with the increase of distance from pollen source to pollen sink, with the total frequency of outcrossing of 2% detected across various distances and locations. Compared with the results between two years, the rate of hybridization in the second year was higher than that in the first year, and hybrids detected up to 100 m distance from cultivated sorghum to weedy johnsongrass. A minimal isolation distance of 1500 meters was needed in alfalfa seed production based on the distance and direction of alfalfa pollen dispersal using species-specific isozyme and DNA (RAPD) markers in alfalfa (Amand, 2000). A 1000 m isolated distance was necessary for cultivated sunflower to minimize or avoid outcrossing from domesticated sunflowers to weedy sunflowers (Arias et al., 1994). A natural hybrid was detected from wheat (*Triticum aestivum* L.) to the wild sea barley (*Hordeum marinum s.str.* Huds.) grown 20-50 m apart. Wheat-specific RAPD and SSR markers were used in the detection of hybrid even if the

intermediate morphology between wheat and sea barley did not exist in the hybrid (Guadagnuolo et al., 2001).

Hybrids produced from outcrossing represent a terminal event, but do not provide any information about the initial development after pollination, such as pollen grain germination and the development of the fertilized ovary, and the relation between initial stages after pollination and the production of hybrids. Obviously, the study of these aspects will be helpful for predicting the frequency of outcrossing. Kerlan et al. (1992) studied pollen germination, pollen tube growth, pollen fertility and ovary development after pollination between rapeseed and five weedy and wild relatives. Pollen grain adhered to the surface of stigma of each female parent in all reciprocal combinations, and pollen germination from 24 to 48 hours showed no difference in any reciprocal combination. However, after 48 hours, the percentage of pollen germination showed differences in different combinations when rapeseed was used as the male or female. Pollen fertility varied considerably from 0 to 94%, and variation of fertility occurred with different florets in the same plant. Batra et al. (1990) investigated the germination of pollen grains on the surface and growth of pollen tubes from reciprocal controlled crosses between wild *Diplotaxis siifolia*, and three crop *brassicas*. Wild species as female and male parents showed different effects on the germination of pollen grain and the growth of pollen tube. The germination of pollen grain and the growth of pollen tube were normal. However, a post-fertilization barrier caused abortion of hybrid seeds when wild species was used as the female parent. On the other hand, the pollen tube failed to enter the stigma even if pollen grains germinated when wild species acted as male parents, indicating the presence of a strong pre-germination barrier. Carney et al. (1994) reported the growth of pollen for intra and interspecific hybridizations between two *Iris* species. The average length of pollen tube 3.5 hours after

fertilization in the *Iris fulva* maternal plant was not significantly different among self-pollination, intra and interspecific hybridizations, whereas significant differences existed between interspecific hybridization, and self-pollination and intraspecific hybridization with the *Iris hexagona* maternal plant.

2.3.2 Hybridization among Transgenic Crops and Wild or Weedy Relatives

Outcrossing between transgenic plants and their wild relatives has been studied by various researchers (Baranger et al., 1995; Chevre et al., 2000; Lavigne et al., 2002; Lefol et al., 1995 and 1996; Whitton et al., 1997). Marchis et al. (2003) used transgenic *Lotus corniculatus* plants expressing the asparagine synthetase gene (*asn*A) and β-glucuronidase gene (*uid*A), respectively, as pollen donors, and wild type plants of *Locus corniculatus*, *L. pedunculatus*, and *L. tennis* as pollen recipients, to identify the occurrence and rate of outcrossing under different planting area of pollen donors and recipients, and different distances from pollen recipients to pollen donors in different field designs at two locations. At the small planting area of pollen donors (1.5 × 1.2 m) and pollen recipients (0.9 × 0.5 m), a outcrossing rate of 11% was detected from transgenic *Locus corniculatus* plants expressing *asn*A to wild type plants of *Locus corniculatus* planted inside pollen donor plots, and the rate decreased with the increase of distance from pollen recipient. At the large planting area of pollen donors (4 × 3.5 m) and pollen recipients (1.4 × 0.6 m), the higher rate of outcrossing was detected at the same plot of pollen donor than the small planting area. Outcrossing occurred at longer distance from pollen recipient to pollen donor in the same species *Locus corniculatus* expressing *asn*A in the large planting area than small planting area. No outcrossing was found between and from *Locus corniculatus* to either *L. pedunculatus* or *L. tenuis* for transgenic plants expressing *uid*A gene for the two designs. Halfhill et al. (2004) evaluated the incidence and frequency of outcrossing from transgenic rapeseed

(*Brassica napus* L.) lines, containing green fluorescent protein (*gft*) and Bacillus thuringiensis (*bt*) genes, to their wild relatives, *Brassica rapa* (*B. rapa*) and *Raphanus raphanistrum* (*R. raphanistrum*). Wild relatives of rapeseed were planted inside or within or at the margin of different transgenic lines. The progenies derived from wild relatives were analyzed for expression of green fluorescent protein (GFP). In the planting pattern of high ratio (600:1) of rapeseed vs wild relative, the average frequency of outcrossing in *B. rapa* was 10% with the range of 3.97% to 22.02%. No outcrossing was found from transgenic lines to *R. raphanistrum*. In the design of low ratio (180:1) of rapeseed vs wild relative, the range from 0.08% to 3.24% of outcrossing was found. Under within and the margin designs, 37.2% and 5.2% outcrossing were detected in the seeds harvested from *B. rapa*, respectively. The frequency of outcrossing showed no significant difference among margin design.

An outcrossing study between transgenic rapeseed expressing the *pat* gene and wild radish was performed (Chevre et al., 2000). Only one hybrid was found from the seeds produced from isolated wild radish planted at the margin of the rapeseed field, and no hybrids from rapeseed to wild radish was produced. In contrast, outcrossing from wild radish to rapeseed occurred at all locations where rapeseed was grown and sampled, and high rates of outcrossing occurred with rapeseed plants adjacent to wild radish plants grown in the group or at the margin of the rapeseed field. The range of outcrossing was 2×10^{-5} to 5×10^{-4} across all rapeseed seedlings. Darmency et al. (1998) used transgenic rapeseed containing the *bar* gene, and a transgenic rapeseed line resistant to chlosulfuron to evaluate outcrossing to wild radish in different experimental designs and field trials. Seedlings developed from wild radish died due to the treatment of glufosinate, showing no hybrids produced from transgenic rapeseed to wild radish. No hybrids were found in a second planting pattern between transgenic rapeseed

expressing glufosinate resistance in two consecutive years. A separate experimental design with a 600:1 ratio of wild radish vs transgenic rapeseed expressing chlosulfuron resistance produced a 0.03% overall outcrossing frequency over three years. Lefol et al. (1996) evaluated the occurrence and rate of outcrossing between rapeseed and its wild relative hoary mustard (*Hirschfeldia incana*). In two experimental designs, transgenic rapeseed expressing the *bar* gene and *H. incana* at the ratio of 30:1 were planted in insect proof cages, and *H. incana* plants in one plant per 12 m^2 grown within 200 m^2 plot of chlorsulfuron-resistant rapeseed. No hybrids were produced from the seeds of *H. incana* under the cage condition, but an average of one hybrid per *H. incana* plant was found under field conditions. Brown et al. (1996) planted two transgenic rapeseed lines resistant to glufosinate herbicide and one non-transgenic rapeseed line as a pollen donor, and the weedy relative, field mustard (*Brassica rapa*), being highly self-incompatible, was used as the pollen recipient. A total of 134 hybrids were obtained from 3000 pollinations from rapeseed to *B. rapa*. All hybrids involved in the non-transgenic rapeseed line showed glufosinate resistance, whereas 97% of the hybrids showed glufosinate resistance. Only two of the F_1 hybrids were partially fertile with small numbers of seeds produced, and the others were completely sterile. Furthermore, the F_2 generation showed 60% fertility, and all plants were resistant to glufosinate herbicide.

In a separate study (Beckie et al., 2003), transgenic rapeseed expressing glufosinate or glyphosate resistance gene was used to detect the incidence and rate of outcrossing for multiple herbicide resistance sources. Rapeseed plants were collected at 0, 50, 100, 200, 400, 600 and 800 m along a transect perpendicular to the common border of the paired commercial rapeseed fields. One field consisted of transgenic rapeseed resistant to glyphosate, and another field consisted of transgenic rapeseed resistant to glufosinate at each of 11 sites. The range of outcrossing for two

transgenic rapeseed fields across all sites was 0.04% to 1.4%, and the longest distance where outcrossing occurred was 400 m. The rate of outcrossing was still affected by the distance, decreasing rapidly after 50 m from pollen donor to recipient. In a subsequent study, Warwick et al. (2003) evaluated outcrossing from transgenic *Brassica napus* L. resistant to glufosinate/glyphosate herbicide or expressing green inflorescent protein to four wild species: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L. and *Erucastrum gallicum* under greenhouse or field conditions. Outcrossing of 6.8% and 6.5% were detected from 13,423 and 15,850 seedlings developed from two *B. rapa* populations planted in a *B. napus* field resistant to glyphosate, and a 13.6% outcrossing rate was observed from 2,881 seedlings produced from one *B. rapa* population planted in the field of transgenic *B. napus*. Polowick et al. (2002) reported the occurrence and extent of outcrossing between transgenic pea to three non-transgenic pea varieties through leaf characteristics and *gus* gene expression in field plots. The rate of outcrossing was different for different pea varieties and was not evenly distributed across the plots. Scheffler et al. (1993) showed that the frequency of outcrossing for peas was 4.8% in the center of non-transgenic plots and decreased with the increase of the distance from pollen donor to recipient and sharply decreased over a 12 m distance. Lavigne et al. (1998) and Umbeck et al (1991) investigated pollen dispersal distance and genetic background effects on outcrossing in rapeseed and cotton, respectively. Approximately 50% of the pollen produced by an individual transgenic rapeseed plant fell within 3 meters, and the probability of fertilization afterwards decreased slowly along a negative exponential of the distance. A consistent and significant reduction in pollen dissemination occurred as the distance from the test plot increased (Lavigne et al., 1998). Outcrossing from transgenic cotton to common commercial cotton occurred from 5 % to less than 1 % at 7 m from the test plots. Less than 1% of pollen dispersal still occurred in

the remaining border rows over a distance of 25 m (Umbeck et al., 1991). Murray et al. (2002) studied outcrossing with wild oats resistant to the acetyl-CoA carboxylase inhibitor that were surrounded by susceptible wild oat plants in a hexagonal planting pattern. The range of outcrossing from resistant wild oat to susceptible biotypes was 0.08 to 0.05% at low and high densities. Distance from pollen donor to recipient showed different influences on the rate of outcrossing at different planting densities and fields. Significant differences were only observed at high densities.

Ritala et al. (2002) detected a 3% outcrossing rate in male sterile lines of barley up to a 50 m distance from donor to recipient. Male sterile rapeseed was used to determine the hybridization between wild hoary mustard to rapeseed. A total of 1.5 to 26 hybrids per hoary mustard plant were produced in the insect-proof cages where male sterile rapeseed and hoary mustard were planted at the ratio of 16:16 and 4:16, whereas 0.36 hybrids per hoary mustard was produced in male sterile rapeseed and hoary mustard in field plots (Lefol et al., 1996). The same male sterile system was also used as a female parent to identify outcrossing from another wild relative of rapeseed, wild radish. The ratio of 1:1 and 1:2 for wild radish vs rapeseed male sterile variety was planted in cages in two consecutive years, respectively. Seeds from wild radish plants were collected and sown in the greenhouse. Phenotypic data, such as hairiness, leaf shape intermediate morphology, and molecular data, such as isozyme characteristic bands were collected to show that 40% and 86% hybrids were produced based on the total seedlings of wild radish seeds in two years, respectively (Darmency et al., 1998). Baranger et al. (1995) used male sterile lines of rapeseed as a female parent and a wild species as male parent to show that the frequency of hybridization was significantly affected by the genotypes of rapeseed. Eber et al.

(1994) reported substantially different hybridization rates of male-sterile rapeseed that were planted in different plot patterns.

2.4 Hybridization in the Genus *Oryza*

2.4.1 Hybridization among Non-transgenic Rice and Wild or Weedy Relatives

Outcrossing between different cultivated rice and red rice species has been reported. Natural hybrids between the African cultivated rice *Oryza glabberima Steud* and its weedy relative *O. breviligula Chev.et Roehr* as well as the Asian species *O. sativa* L. and its weedy relative *O. perennis Moench* (Chu and Oka, 1970; Morishima et al., 1961; Oka and Chang, 1961; Oka and Morishima, 1971) have been successfully produced. Langevin et al. (1990) reported a wide outcrossing range of 1%-52% with commercial varieties and red rice. A total of 100 red rice plants were collected from each of four replicate rice fields in six variety fields. Five seeds from each red rice plant were collected, and a total of 12,000 seeds from all six varieties were planted to identify hybrids through morphological characteristics and specific enzyme bands for red rice and the six varieties. Natural outcrossing from variety to red rice occurred with all six varieties, and a high frequency of 52% was found in the red rice population harvested from the late maturing variety 'Nortai'. Rong et al. (2004) detected natural hybrids between two extensively planted rice varieties in Yunnan province, China; one traditional rice variety 'Huangkenyuo' and one hybrid rice variety 'Shanyou-63' in different intercropping patterns. Different planting patterns did not significantly affect the rate of outcrossing in any one of two varieties, but asymmetric outcrossing was detected in the traditional variety (0.04%) vs 0.18% in hybrid variety. Chen et al. (2004) observed outcrossing from cultivated rice 'Minghui 63' to the wild rice *Oryza rufipogon* (*O. rufipogon*) in different planting patterns. Song et al. (2004) showed the highest detected outcrossing rate of 2.94% occurred with a circle planting design of 3

m distance from donor to recipient. The longest distance that hybrids were detected was 43 m. Pollen source may be a key factor for the occurrence of outcrossing from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrical* Host.). A range of 1% to 7% outcrossing was detected from wheat to three-jointed goatgrass populations in wheat fields, but no hybrids between wheat and jointed goatgrass were produced in the greenhouse (Guadagnuolo et al., 2001).

2.4.2 Hybridization among Transgenic Rice and Wild Rice or Weedy Relatives

Like the studies of outcrossing among non-rice transgenic crops and wild or weedy relatives, herbicide resistance is a suitable phenotype to study outcrossing between transgenic rice plants and their wild or weedy relatives. Messenguer et al. (2001) evaluated the influence of distance from pollen recipient to pollen donor and wind direction on the rate of outcrossing between transgenic lines expressing the *bar* gene and their non-transgenic counterparts. In normal side by side plot design, outcrossing rapidly decreased from 0.08% at a 2 m distance to 0% at 2.4 m distance, as judged by glufosinate treatment and molecular markers. The highest rate of outcrossing (0.53%) occurred in a plot design with 1 m interval between an inner non-transgenic circle to an outer transgenic circle. The lowest outcrossing of 0.01% occurred in another circle design with 5 m intervals between the outer circle of transgenic lines and inner circle of non-transgenic materials. Zhang et al. (2003) used transgenic line CPB6 as pollen donor and red rice as pollen recipient to evaluate the rate of outcrossing. In small plots with a 50: 50 mixture of transgenic and red rice, an outcrossing frequency of 0.33% was determined by incidence of glufosinate resistance, pubescent leaves, and confirmed by molecular analysis. No outcrossing from transgenic line to red rice was detected, presumably due to height differences between transgenic and red rice lines. Chen et al. (2004) detected a outcrossing of 0.011% to

0.046% from transgenic lines expressing the *bar* gene to different biotypes of red rice that was dependant on flowering date and height between pollen donor and recipient.

2.5 Identification of Hybrids through Molecular Analysis

Whitton et al. (1997) surveyed two variety-specific RAPD markers in 2,700 progeny in a naturally occurring population of wild *Heliaanthus annuus* over five generations following a single generation of hybridization with the variety. Outcrossing was detected in the first generation and variety allele frequencies did not significantly decline over four subsequent generations. Linder et al. (1998) also used species-specific RAPD markers to investigate the introgression from cultivated sunflower to three wild sunflower populations which were adjacent to cultivated sunflower for 20 to 40 years. The individuals of these wild sunflowers analyzed had at least one of these RAPD markers present, indicating the existence of gene introgression from cultivated sunflower to wild sunflower populations. Using 6-phosphogluconate dehydrogenase isozyme, Arias et al. (1994) obtained data for incidence and rate of outcrossing from sunflower varieties into wild populations. The distance from variety to wild population affected the rate of outcrossing in that the highest outcrossing of 27 % occurred within a 3 m of distance between domesticated and wild forms. A low 2% outcrossing rate was detected at a 1,000 m distance between variety and wild population. Arriola et al. (1996) utilized an isozyme marker to demonstrate gene escape from Sorghum *bicolor* to *S. halepense*, johnsongrass, and the frequency was related to the distance between Sorghum *bicolor* and *S. halepense*. Combining isozyme analysis with fertility and phenotype of progeny from *Chenopodium berlandieri,* considered as a weed in *C. quinoa* fields, Wilson et al. (1993) showed that over 30% of the progeny produced from seeds of *C. berlandieri* were hybrids.

2.6 Fitness of F_1 and F_2 Hybrid Populations

2.6.1 The Performance of F_1 or F_2 Populations Derived from Non-rice Crops

Fitness of the first and subsequent generations of crop-weed hybrids is a key parameter to predict likelihood of gene introgression. Fitness of hybrids between *B. napus* and weedy *Brassica rapa* was inferred based on the comparison of seed production per plant of hybrid and the two parents under different conditions (Hauser et al. 2003). Hybrids between *B. napus* and *B. rapa* (F_1), two parents and a backcross generation (F_1× *B. rapa*) were planted in pure stands and in different mixtures at low, medium and high densities. The F_1 hybrids exhibited higher seed production in mixtures than in pure stands. In contrast, the parents produced more seed in pure vs. mixed stands. In an earlier study, Hauser et al. (1998) investigated the basis of high and low fitness of hybrids between *B. napus* and *B. rapus* relative to different fitness components. The reciprocal crosses *B. napus* and *B. rapa* (Bn × Br), *B. rapa* and *B. napus* (Br × Bn), and *B. napus* and *B. napus* (Bn × Bn), and *B. rapa* and *B. rapa* (Br × Br) were used to analyze the combination of fitness components, the proportion of pollinated flowers developing into pods, the number of fully developed seeds on maternal plants, the proportion of fully developed seeds, the number of pods per F_1 generation plant, and the number of seeds per pod on F_1 plants. The reciprocal crosses Br × Bn and Bn × Br showed higher fitness than Br × Br, and Bn × Bn produced the highest fitness. When the components of fitness parameters were considered, the high fitness in the reciprocal crosses of Br × Bn was mainly attributed to the number of pods per F_1 plant, whereas the reduced fitness in Br × Bn and Bn × Br compared to Br × Br was mainly due to the number of seeds per pod in the F_1 generation. Maternal effect on the fitness of the progenies of four backcross populations was detected by Hauser et al. 1998. Mason et al. (2003) demonstrated that a second backcross generation involving *B. rapa* with transgenic *B. napus* did not possess

any fitness advantages relative to the original *B. rapa* line. Zhu et al. 2004 found different inheritance patterns of GFP and Bt transgenes among four backcross with *B. rapa* and *B napa* that were dependent upon the specific transgenic line being evaluated. Arriola et al. (1997) showed that hybrids between sorghum and weedy johnsongrass showed no significant increase or decrease in flowering date, panicle production per plant, seed production per panicle, pollen viability, tiller production or biomass. Snow et al. (2001) demonstrated that hybrids between weedy radish (*Raphanus raphanistrum*) × cultivated radish (*R. xsativus*) showed delayed flowering date, produced smaller number of seeds per fruit with lower pollen viability compared to the weedy parent.

2.6.2 The Performance of F_1 or F_2 Hybrid Rice Populations

Langevin et al. (1990) compared morphological characteristics between natural hybrids of cultivated rice varieties and red rice. Seeds of red rice were collected in the fields of six rice varieties, four fields for each variety. Hybrids exhibited stronger vegetative vigor than red rice. Hybrids produced in five cultivated rice fields produced greater height and more tillers than red rice. Oard et al. (2000) evaluated the performance of F_2 populations from controlled crosses between transgenic lines containing the b*ar* gene, non-transgenic counterparts and red rice at two locations under small plot field conditions. At the Ben Hur Farm, Baton Rouge, Louisiana, seed germination rate 1 week after harvest did not significantly differ among red rice (control), 'Cypress' (CP) and all F_2 populations derived from the cross between CP or transgenic Cypress (tCP) or 'Bengal' or transgenic Bengal (tBG) and red rice. Similarly, the trait showed no significant differences among tCP, tBG and BG. However, significant difference existed between any of red rice (control), CP and F_2 populations, and any of tCP, tBG and BG. For seed germination rate 8 weeks after harvest, significant differences were observed between any two F_2

populations (tCP × Red #1 and tCP × Red #4) and any other F_2 populations, red rice (control) and parents. No significant differences were found between F_2 population tCP × Red #1 and tCP × Red #4. The lack of significant differences was also found among red rice (control), parents and F_2 populations except for the two F_2 populations stated above. Another agronomic trait, plant height was significantly different between F_2 populations in reciprocal cross. At the Hope, Arkansas, seed number per panicle was not significantly different among red rice (control), parents except for red rice (parent) and F_2 populations. Significant difference existed between the red rice parent and any of others. With regard to seed weight per panicle, no significant difference was found among red rice (parent), one F_2 population (red rice #1× tCP) and BG. Similarly, no significant difference existed among F_2 populations except for F_2 (red rice #1× tCP) and parents except for red rice (parent) and BG. Moreover, herbicide resistance in F_2 populations segregated with one or two-gene Mendelian inheritance, although abnormal segregation was detected in some crosses.

Recently, Zhang et al. (2003) used the same transgenic Cypress (tCP6) with the *bar* gene and red rice to evaluate the performance of F_1 and F_2 populations under small plot conditions. The fitness of F_1 hybrids was reduced relative to parents due to reduced tiller number per plant, grain number per panicle, seed setting rate along with extreme late maturity. With regard to F_1 hybrids between one purple line and red rice, the same tendency was observed, namely, low fitness of F_1 hybrids between the transgenic line and red rice. In F_2 populations from the natural hybrid CPB6 × red rice, plant height showed no significant difference from red rice, but significant differences were found between CPB6 and corresponding F_1 hybrid. Panicle length was not significantly different from CPB6, but differences were detected between red rice and the F_1 hybrid. Hybrids did not differ in spikelet number per panicle, grain number per panicle or

seed setting rate. Glufosinate resistance exhibited a 3:1 segregation ratio for resistance vs susceptibility, indicating glufosinate resistance was controlled by one single dominant gene. Similar results were found for segregation of pubescent vs. glabrous leaf. In addition to agronomic performance of F_1 or F_2 population developed from a cross between cultivated rice, a transgenic line and red rice, the fitness of F_1 hybrids between cultivated rice to the wild relative *O. rufipogon* was compared with two parents (Song et al. 2004). F_1 hybrids were obtained by controlled crosses of *O. rufipogon* as pollen recipient and Minghui-63 as the pollen donor. Agronomic traits of germination rate, vegetative and reproductive growth were measured. The F_1 exhibited significantly higher seed germination rate, seedling survival rate, tiller number per plant, pollen viability and seed setting rate than both two parents, whereas no significant differences were found for flowering date or spikelet number per panicle. For plant height and panicle length, the F_1 was not significantly different from *O. rufipogon*, but higher than Minghui-63. Overall, the F_1 hybrid showed stronger fitness than the two parents for seed germination and reproductive traits, but no significant differences were observed in vegetative growth or composite fitness relative to the parents.

The previous outcrossing studies between crop and weedy relatives will undoubtedly provide valuable information concerning the utilization of transgenic resources and the conservation of valuable wild germplasm. However, most studies do not reflect actual commercial production situations. Rice germplasm resistant to imazethapyr herbicide was originally selected from the rice breeding line AS3510 treated with ethyl methane sulfonate (EMS) mutagenesis by Dr. Tim Croughan, Rice Station, Crowley, Louisiana (Anonymous 2002b). The progeny from the cross of EMS mutant (93AS3510) and Cocodrie was selected through three generations to create ‘CL121’. The initial cross of EMS mutant (93AS3510) and

Maybelle was backcrossed with Maybelle for additional two times, and then the progeny was selected for three generations to produce ‘CL141’. These two varieties were released commercially in 2002 for the first time in Louisiana and Arkansas. CL121 is a long-grain variety with 94 cm plant height, 7262.6 kg/ha grain yield, and is susceptible to sheath blight disease. Clearfield 141 is also a long-grain variety, susceptible to sheath blight, with plant height and yield at 109.2 cm and 7514.8 kg/ha, respectively. ‘CL161’, an EMS-derived mutant of Cypress, produces ~343 kg/ha higher grain yield than Clearfield 121 (Anonymous 2001) with greater resistance to imazethapyr herbicide than Clearfield 121 and 141.

CHAPTER 3 OUTCROSSING FROM CLEARFIELD RICE TO RED RICE UNDER FIELD CONDITIONS

3.1 Introduction

Spontaneous outcrossing is common between crops and their wild relatives (reviewed by Ellstrand et al., 1999) such as barley (*Hordeum vulgare* L.), finger millet (*Eleusine coracana* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), sorghum (*Sorghum bicolor* L.), dry and green and string beans (*Phaseolus vulgaris* L.), rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.) and sugarcane (*Saccharum officinarum* L.). Natural hybrids have been found from African cultivated rice *Oryza glabberima Steud* and its weedy relative *O. breviligula Chev.et Roehr*, Asian cultivated rice *O. sativa* L. and its weedy relative *O. perennis Moench* (Oka and Morishima, 1971) and wild relative *O. glaberrima* (Ellstrand et al., 1999), traditional variety and modern hybrid (Rong et al., 2004), and cultivated rice and the wild relative *Oryza rufipogon* (*O. rufipogon*) (Chen et al., 2004; Song et al., 2004).

Rong et al. (2004) detected asymmetric outcrossing between one traditional rice variety 'Huangkenong' and one modern hybrid rice 'Shanyou-63' under different interplanting patterns. The frequency of outcrossing from Shanyou-63 to Huangkenong was 0.04%, whereas 0.18% outcrossing rate was observed from Huangkenong to Shanyou-63. The frequency of outcrossing between interplanting patterns for any one of the two varieties showed no significant difference. Chen et al. (2004) and Song et al. (2004) used the widely planted rice variety 'Minghui-63' in southern China to evaluate outcrossing to the wild relative *O. rufipogon* in different planting patterns. Similarly, Song et al. (2004) evaluated three different planting designs and their effects on outcrossing. A "central population" design showed that the frequency of outcrossing was influenced by wind direction with the highest rate occurring at the direction of the prevailing

wind during the rice flowering period. However, no effect of pollen size on outcrossing was found. An "encircled population" design combined with the central population design indicated that location of the pollen recipient had no effect on outcrossing. In a "uni-directional population" design, distance from pollen donor to pollen recipient influenced outcrossing, and the longest distance of 43.2 m in the study did detect outcrossing events. The highest rate (2.94%) of outcrossing occurred at an encircled population design with a 3.6 m interval distance between pollen donor and pollen recipient.

When outcrossing from transgenic lines expressing herbicide resistance as pollen donor to a non-transgenic counterpart was considered, the same effects of distance and wind direction as in the study above were found in a "side by side" design of transgenic rice line expressing bar gene and non-transgenic material (Messenguer et al., 2001)**.** Similarly, the same effect was also detected in circle designs with 1 m intervals from the outer circle of a transgenic line to the inner circle of non-transgenic material.

Red rice (*Oryza sativa*), the same genus and species as cultivated rice (Gianessi et al., 2002), exhibits seed shattering (Dilday et al., 1990) and dormancy characteristics (Lago, 1982). These features make red rice a troublesome weed in most rice planting regions in the world (Fisher and Ramirez, 1993), and is considered a noxious pest for rice production in the southern U.S. (Oard et al., 2000).

Studies of outcrossing between cultivated rice and red rice (Langevin et al., 1990), and transgenic rice and red rice (Zhang et al., 2003) have been conducted. Langevin et al. (1990) found natural hybridization from six traditional rice varieties to red rice to range from 1% to 52%. The highest rate of outcrossing occurred from the late season variety 'Nortai' to red rice, indicating that overlapping in flowering time between variety and red rice affected the frequency

of outcrossing. Zhang et al. (2003) detected a 0.33% outcrossing from red rice to a transgenic line expressing the *bar* gene under small research plot conditions. No outcrossing was detected from the transgenic line to red rice.

Phenotypic and molecular markers have been used in the identification of hybrids between cultivated rice and weedy or wild relatives (Chen et al., 2004; Rong et al., 2004; Song et al., 2004; Zhang et al., 2003). RAPD markers were used to detect hybrids between a single red rice biotype and transgenic herbicide tolerant rice (Zhang et al., 2003). SSR markers were used to identify hybrids between cultivated rice and *O. rufipogon* (Chen et al., 2004 and Song et al., 2004), and between two different cultivated varieties (Rong et al., 2004).

Clearfield rice tolerant to imazethapyr herbicide was produced from the progeny between elite Louisiana rice varieties and ethyl methane sulfonate (EMS) induced mutants (Croughan, 1994) by the LSU AgCenter. 'CL121' and 'CL141' as the first generation of Clearfield rice varieties were first released in Arkansas and Louisiana in 2002 (Anonymous 2002a). 'CL161' as the second generation of Clearfield rice was released in 2003 (Anonymous 2003). Clearfield rice, especially CL161, are being planted with increasing acreage both in Louisiana and other U.S. rice planting regions, In 2004, CL161 was planted on 110,574 acres and 470,000 acres in Louisiana and USA, respectively (personal communication, Dr. Steve Linscombe). However, no comprehensive study concerning outcrossing from Clearfield rice to red rice in commercial fields has been conducted. The objectives of this study are to: (1) determine the frequency of outcrossing from Clearfield rice to red rice through pollen dispersal at 24 commercial Clearfield locations over a two year sampling period, (2) characterize agronomic traits of red rice populations among twelve locations each year, (3) detect and evaluate potential associations between outcrossing frequency and agronomic traits of red rice populations.

3.2 Materials and Methods

3.2.1 Plant Materials

3.2.1.1 Clearfield Rice Locations

In 2002, ten commercial locations planted with CL121 and two locations with CL 141 were selected for outcrossing studies. The twelve Clearfield sites in 2002 were located from 30.06438°N to 30.46575°N in latitude, and from 92.32355°W to 93.04704°W in longitude. Ten locations with CL161, one location with CL121 and one location with Clearfield hybrid 'CLXL8', were selected for the second year study (2003). The twelve Clearfield locations in 2003 were the range from 30.06438°N to 30.46575°N in latitude, and from 92.33813°W to 92.9546°W in longitude. The selection of Clearfield locations was based on the number of red rice. Clearfield was chosen if over 100 red rice plants can be collected in the field.

3.2.1.2 Data Collection

The first flowering date and visual estimates of percent red rice infestation were recorded for all locations across both years. Data for the following traits in 2002 and 2003 were recorded for 100 randomly-selected red rice plants and 10 Clearfield plants at each location: plant height, measured at maturity from the soil to the tip of panicle of main stem, panicle length, seeds per panicle, seeds per plant, spikelets/panicle, seeds per panicle divided by spikelets/panicle, and 100 grain weight calculated by 100 x seed weight/plant divided by seeds/plant. Harvested panicles were dried at 50^{o}C for four days to ~ 12 percent moisture. All seeds were put into paper bags, stored at 4^{0}C in the dark until planting the following year. Red rice seeds were collected from the ratoon crop at location 2002-11.

Controlled crosses between Clearfield rice (CL121 and CL161) and red rice were made by hand emasculation in the greenhouse in 2002. Fifteen red rice plants each were obtained from

locations 2002-6 and 2002-9, as well as the South Farm of the Rice Station, Crowley, Louisiana. Seeds of CL121 and CL161 were provided by Dr. Linscombe, Rice Station. The hybrids and subsequent F_2 populations will be used for fitness analysis.

3.2.1.3 Experimental Design

The identification and evaluation of putative hybrids produced from pollen transfer between Clearfield and red rice in 2002 and 2003 was conducted at the Ben Hur Farm, Baton Rouge, Louisiana. The field layout was 73.2 m x 30.5 m, including 30 tiers, with each measuring 2.4 m x 30.5 m. Seeds from a single red rice plant were planted in a single 2.4 m row. On May 6, 2003, all seeds from red rice samples collected in 2002 were planted with Cocodrie and CL121 as controls every 20 rows. The seedlings of 27 F_1 populations from controlled crosses between Clearfield rice and red rice were transplanted.

On April 15, 2004, $\leq$ 3.5 g of red rice seeds from each plant harvested in 2003 were sown in a single-row plot in the same experimental setup as in the previous year. Cocodrie, CL121, CL161 and CLXL8 served as controls every 20 rows. A total of 44 F_2 populations derived from natural red rice-Clearfield hybrids produced from red rice samples collected in 2002 were also planted in 2004.

Imazethapyr herbicide at 140g/ha, two times the labeled rate, was applied using a backpack sprayer at the two-three leaf stage June 6, 2003, during the first year and May 26, 2004 for the second year. The same rate of imazethapyr was applied again 19 to 20 days later. Arrosolo (5.046kg/ha), Command (0.448kg/ha), and Permit (0.07kg/ha) herbicides were also applied on May 24, 2004. After herbicide applications, a 13-13-13 fertilizer formulation was applied at a rate of 235 kg/ha.

Just before the first imazethapyr application, the number of rice seedlings in each row was determined. Eight days after the second imazethapyr treatment, healthy, green rice plants were counted in each row (Figure 3.1). Nylon bags were placed over panicles of imazethapyr-tolerant red rice and Clearfield plants for seed harvest.

Figure 3.1 Imazethapyr-tolerant red rice plants and Clearfield controls 20 days after the second 2X imazethapyr treatment, Ben Hur Farm, Louisiana, 2004

3.2.2 Statistical Analysis-Duncan Comparison, Correlation Tests

3.2.2.1 PROC GLM (SAS 9.0 edition) was used to for statistical evaluation of plant height, tiller number, panicle length, seeds/panicle, spikelets/panicle, seed set rate and 100-grain weight.

3.2.2.2 Biplot and Cluster analyses (SAS 9.0 edition) were used in each year to find key agronomic traits contributing to differences among the 12 red rice populations.

3.2.2.3 PROC CORR (SAS 9.0 edition) was used to determine the correlation between the frequency of outcrossing from Clearfield rice to red rice and agronomic traits, and to evaluate the correlation among agronomic traits.

3.2.3 Molecular Marker/Chemical Analyses for Detection of Putative Red Rice-Clearfield Hybrids

3.2.3.1 DNA Purification

For CL121, CL141, red rice (control) and natural hybrids derived from red rice samples collected in 2002, total genomic DNA was extracted from young leaves, following the procedure described by Oard and Dronavalli (1992). Briefly, 10-50 mg of leaf tissue was ground into a fine powder in a 1.5 ml eppendorf tube containing liquid nitrogen. The grinding tool was an autoclaved wooden applicator stick. A total of 600 µl of extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol) was added to the tube. The tube was boiled for 10-15 minutes. Subsequently, the tube was centrifuged at 14,000 rpm at 4^{o}C for 15 minutes. The supernatant (450 µl) was transferred into a new tube. Forty-five µl of 10 M ammonium acetate and 1 ml of 95% ethanol were added to the new tube. Centrifugation was carried out as described above. The supernatant was discarded, and the tube was dried for 30 minutes at room temperature. The DNA pellet was resuspended in 50 µl of TE buffer.

Total genomic DNA was extracted from 30 rice plants from each of five F_2 populations derived from natural hybrids #2, #15, #34, #38 and #70 produced in 2002 and 58 of 327 hybrids in 2003 using the UltraClean Plant DNA Isolation Kit (*MO BIO Laboratories*, Inc.).

3.2.3.2 Simple Sequence Repeat (SSR) Markers

SSR markers were used to identify putative red rice-Clearfield hybrids for this study. Four markers (RM215, RM234, RM251 and RM253) were selected based on previous identification of red rice, rice, and hybrid populations using microsatellite markers (Gealy et al., 2002), while

the fifth marker (RM180) was selected based on suggestions of Dr. David Gealy, USDA-Stuttgart, Arkansas (Table 3.1).

Table 3.1 SSR markers used to detect Clearfield-red rice hybrids

SSR marker	Sequence
RM180	Forward: CTACATCGGCTTAGGTGTAGCAACACG
	Reverse: ACTTGCTCTACTTGTGGTGAGGGACTG
RM215	Forward: CAAAATGGAGCAGCAAGAGC
	Reverse: TGAGCACCTCCTTCTCTGTAG
RM234	Forward: ACAGTATCCAAGGCCCTGG
	Reverse: CACGTGAGACAAAGACGGAG
RM251	Forward: GAATGGCAATGGCGCTAG
	Reverse: ATGCGGTTCAAGATTCGATC
RM253	Forward: TCCTTCAAGAGTGCAAAACC
	Reverse: GCATTGTCATGTCGAAGCC

3.2.3.3 Polymerase Chain Reaction (PCR)

For the 2002 data analysis, the Li-Cor 4200 system was used to resolve and evaluate PCR products. The template used in the PCR reactions was genomic DNA from CL121, CL141, red rice (control) and natural hybrids derived from red rice samples collected in 2002. The M13 sequence was linked to the 5' end of the forward primer, and M13 primer was ligated to the IRDye 700. PCR reactions were carried out in a thermocycler (iCycler, Bio Rad). A 20 µl reaction consisted of 1 × PCR reaction buffer, 2.5 mM $MgCl_2$, 200 nM forward and reverse primers, 1 µM M13 primer, 200 µM dNTP, 0.5 unit of Taq polymerase and 30 ng genomic DNA. The thermocycle profile consisted of a denaturation step at 94^{o}C for 4 min followed by 35 cycles of 45 s at 94^{o}C, 45 s at 55^{o}C and 1 min at 72^{o}C. A final extension step was performed for 5 min at 72^{o}C. After completion of the PCR, 4 µl of stop solution was immediately added to each reaction. Before loading into the gel, PCR products were denatured at 94^{o}C for 4 min.

For 2003 data analysis, a 6% native polyacrylamide gel electrophoresis was used to separate PCR products using the MegaGel (C.B.S Scientific Company) electrophoresis system.

Templates used in PCR reaction were genomic DNA from CL121, CL161, CLXL8 F_2 plant (pubescent leaf), CLXL8 F_2 plant (glabrous leaf), red rice (control), natural hybrids derived from red rice samples collected in 2003, and F_2 populations produced from natural hybrids produced from red rice samples collected in 2002. The total reaction volume was 30 µl. All components in this reaction system consisted of 1 × PCR reaction buffer, 2.5 mM $MgCl_2$, 200 nM forward and reverse primers, 200 µM dNTP, 0.75 unit of Taq polymerase and 50 ng genomic DNA. The reaction conditions, such as time and temperature for pre-denaturation, denaturation, annealing, extension and final extension were the same as PCR reactions for the Li-Cor 4200 system described above.

3.2.3.4 Acetohydroxy Acid Synthase (AHAS) Activity

AHAS is the enzyme catalyzing the synthesis of amino acids valine, leusine and isoleusine. Imazethapyr herbicide inhibits the activity of AHAS so that the three amino acids above can not be synthesized. The gene encoding AHAS in Clearfield rice has been mutated by chemical mutagenesis to render Clearfield rice tolerant to imazethapyr herbicide. Analysis of AHAS activity for Clearfield rice, red rice and natural hybrids derived from red rice samples collected in 2002 were carried out by the BASF Corporation.

3.3 Results and Discussions

3.3.1 Outcrossing from Clearfield Rice to Red Rice in 2002

3.3.1.1 Red Rice Biotypes and Infestation in Clearfield Rice

Table 3.2 shows biotypes and percent red rice infestation at 12 Clearfield locations in 2002. From 1200 red rice plants sampled, straw hull and awnless biotypes were the most common (1024) while the black hull biotype was the least with only two biotypes. The number of other biotypes was 28 (awns), 90 (black hull, awns), 16 (brown hull) and 40 (brown hull, awns). When

red rice populations at the 12 locations were considered separately, straw hull and awnless were the main biotypes at 11 of 12 locations. Black hull and awn was main biotype (74%) at the remaining location. In general for all locations, the black hull was associated with presence of awns.

Red rice infestation, as estimated from visual observation, was highly variable across different Clearfield locations. The highest infestation of red rice with 50% occurred at location 2002-9, and the lowest infestation of 0.8% was found at location 2002-2. The range of red rice infestation across 12 Clearfield locations was due to many factors, such as seed bank of red rice in the soil, water management, and general weed control practices.

Table 3.2 Percent infestation and numbers of biotypes of red rice at 12 commercial Clearfield locations in southwest Louisiana, 2002

		Biotypes					
		Straw hull		Black hull		Brown hull	
Location	Infestation of red rice (%)*	Awn	Awnless	Awn	Awnless	Awn	Awnless
2002-1. Denison	10	0	100	0	0	0	0
2002-2. Hoppe	0.8	0	94	6	0	0	0
2002-3. Tibadeaux	1.5	0	100	0	0	0	0
2002-4. Habit	2.3	0	100	0	0	0	0
2002-5. SoileauI	3	6	87	3	0	3	1
2002-6. SoileauII	2.5	1	95	2	0	0	2
2002-7. Brunnel	0.9	0	99	0	0	1	0
2002-8. Leonard	6	1	92	4	2	1	0
2002-9. Habetz	50	4	55	0	0	28	13
2002-10. Britt	10	1	97	1	0	1	0
2002-11. Hensgens	1	0	100	0	0	0	0
2002-12. Erol Lounsberry	5	15	5	74	0	6	0
Total		28	1024	90	2	40	16

*Infestation of red rice percentage estimated by visual observation

3.3.1.2 Agronomic Traits of Red Rice and Clearfield Rice

Table 3.3 shows the agronomic data collected for plant height, panicle length, tillers/plant, spikelets/panicle, seed set rate, 100 grain weight and seeds/plant of Clearfield rice and red rice at 12 locations in 2002. Extensive variation for all traits was observed except for 100 grain weight. For example, the tallest red rice plants (mean = 158.3 cm) occurred for red rice plants at location

2002-5 while the shortest plants (mean = 112.7 cm) were found for red rice plants at location 2002-2. There was no significant difference in plant height for red rice among locations 2002-3, 2002-4, 2002-11 and 2002-12 or between locations 2002-4 and 2002-7, or between location 2002-8 and 2002-9. The longest mean panicle length (27.1 cm) was found for red rice plants at location 2002-6 while the shortest (22.2 cm) occurred for red rice plants at location 2002-1. No significant difference for average panicle length was found in red rice among locations 2002-4, 2002-7, 2002-10, 2002-11 and 2002-12, and the same tendency occurred between locations 2002-5 and 2002-8. Red rice at location 2002-8 produced the largest number of tillers/plant (3) with no significant difference at locations 2002-11 and 2002-12. The smallest value for this trait (1.1) occurred for red rice plants at locations 2002-9 and 2002-10 with no significant difference from red rice at locations 2002-1, 2002-2, 2002-3, 2002-4 and 2002-6.

Red rice at location 2002-8 produced the greatest mean number of spikelets/panicle (138.6) and location 2002-1 produced the smallest value (65.1). No significant differences in this trait were found between locations 2002-9 and 2002-10, or among locations 2002-2, 2002-3, 2002-4, 2002-5 and 2002-7. The highest seed set rate (85.16%) for red rice occurred at location 2002-6 and the lowest value (54.15%) was found at location 2002-2. No significant differences existed for red rice among locations 2002-3, 2002-4, 2002-5, 2002-10 and 2002-11. The highest number of seeds/plant (337.1) was found at location 2002-8, while the lowest value (51.2) was detected for red rice at location 2002-9. No significant differences were found for red rice plants at locations 2002-3, 2002-4, 2002-5, 2002-6, 2002-7, 2002-10 and 2002-12.

Variation for flowering date for Clearfield rice and red rice occurred at the 12 locations in 2002. At 9 of 12 locations, red rice exhibited the same first date of flowering as Clearfield rice. At other three locations, red rice flowered 7 to 10 days later than Clearfield rice.

3.3.1.3 Analysis of Red Rice Populations

3.3.1.3.1 Cluster Analysis

Figure 3.2 shows the cluster dendrogram based on agronomic traits where the red rice populations were grouped into three clusters. The first cluster consisted of red rice populations at locations 2002-8, 2002-11 and 2002-12. The second cluster contained red rice populations at locations 2002-5 and 2002-6. The third cluster consisted of red rice populations at locations 2002-1, 2002-2, 2002-3, 2002-4, 2002-7, 2002-9 and 2002-10. Based on the first two important principal component analyses (PCA) in Biplot analysis (Figure 3.4), three clusters could be formed. The first cluster consisted of red rice populations at locations 2002-8 and 2002-11. The second cluster contained red rice populations at locations 2002-5 and 2002-6. The third cluster were formed by red rice populations at locations 2002-1, 2002-2, 2002-3, 2002-4, 2002-7, 2002-9, 2002-10 and 2002-12. The two red rice populations in the first cluster produced high number of seeds/plant, and showed approximate mean value for plant heights of the 12 red rice populations. The two red rice populations in the second cluster showed tall plants among the 12 red rice populations. However, red rice populations at locations 2002-7 and 2002-12 were separated due to the third important PCA, seed set rate. The large difference of seed set rate existed between red rice populations at locations 2002-7 and 2002-12. Moreover, the red rice population at location 2002-12 possessed mean value of seeds/plant, the third highest number of seeds/plant among the 12 red rice populations. So, the red rice population at location 2002-12

Table 3.3 Mean values of agronomic traits of Clearfield rice and red rice at 12 locations in southwest Louisiana, 2002

Trait		Location											
		2002-1	2002-2	2002-3	2002-4	2002-5	2002-6	2002-7	2002-8	2002-9	2002-10	2002-11	2002-12
Plant height (cm)	Clearfield rice	81.6^{g}	81.6^{g}	82.6^{fg}	80.3^{g}	103.5^{b}	110.5^{a}	87de	92.5^{c}	83.5^{efg}	102.2^{b}	86.3^{def}	88.9^{d}
	Red rice	113.8^{g}	112.7^{g}	119.5^{f}	121.3^{ef}	158.3^{a}	144.4^{b}	122.8^{e}	135.2^{dc}	132.5^{d}	136.3^{c}	119^{f}	119.7^{f}
Panicle length (cm)	Clearfield rice	18.6^{cde}	19.1^{bcde}	17.6^{e}	19.6^{abc}	20.9^{a}	20.7^{a}	17.9^{de}	19.8^{bac}	19.3^{abcd}	20.6^{ab}	20.3^{ab}	19.7^{abc}
	Red rice	22.2^{f}	23.8^{de}	23.8^{de}	24.6^{c}	26.4^{b}	27.1^{a}	24.6^{c}	26^{b}	23.5^{e}	24.4^{cd}	25^{c}	25^{c}
No. of tillers/plant	Clearfield rice	10.5^{a}	5.5^{de}	8.5^{abc}	5.2^{de}	6.7^{bcd}	7.5^{bcd}	6.3^{cd}	9^{ba}	6.9^{bcd}	3.2^{e}	6.8^{bcd}	7.3^{bcd}
	Red rice	1.3^{de}	1.5^{cd}	1.4^{cde}	1.4^{cde}	1.7^{c}	1.2^{de}	2.6^{b}	3^{a}	1.1^{e}	1.1^{e}	2.8^{ab}	2.7^{ab}
No. of spikelets/panicle	Clearfield rice	110.5^{cd}	107.1^{cd}	108.4^{cd}	124.4^{cd}	97.9^{d}	165.1^{b}	102^{d}	96.1^{d}	121.1^{cd}	204.2^{a}	117.2^{cd}	132.4^{c}
	Red rice	65.1^{f}	109.1^{c}	110.8^{c}	109.4^{c}	104^{c}	122^{b}	104.8^{c}	138.6^{a}	88.7^{d}	88.7^{d}	121.9^{b}	77.87^{e}
Seed set rate (%)	Clearfield rice	85.65^{ef}	86.95^{de}	80.97^{gh}	89.31^{cd}	92.66^{ab}	88.72^{cde}	93.64^{a}	83.82^{gf}	82.21^{g}	78.29^{h}	90.31^{bc}	66.76^{i}
	Red rice	82.87^{a}	54.15^{f}	78.19^{bc}	77.76^{c}	78.13^{bc}	85.16^{a}	58.82^{e}	82.04^{ba}	57.23^{ef}	75.23^{c}	74.75^{c}	70.05^{d}
100 grain weight (g)	Clearfield rice	2.42^{bcd}	2.51^{ab}	2.15^{f}	2.59^{a}	2.47^{bc}	2.38^{cd}	2.51^{ab}	2.2^{f}	2.23^{f}	2.24^{ef}	2.33^{de}	2.17^{f}
	Red rice	2.49^{a}	2^{a}	2.01^{a}	1.76^{a}	2.11^{a}	2.31^{a}	2.7^{a}	1.83^{a}	1.83^{a}	2.41^{a}	1.79^{a}	2.13^{a}
No. of seeds/plant	Clearfield rice	960.9^{a}	481.7^{b}	716.8^{b}	577.2^{b}	597.1^{b}	1090^{a}	598.7^{b}	687.4^{b}	589.3^{b}	505.8^{b}	708.4^{b}	625.3^{b}
	Red rice	65.1^{d}	80.1^{d}	114.1^{c}	113.7^{c}	138.1^{c}	123^{c}	132.3^{c}	337.1^{a}	51.2^{d}	123.2^{c}	232.2^{b}	138.9^{c}
Flowering date of red rice relative to Clearfield rice		Same	10 days later	Same	7 days later	Same	Same	Same	Same	Same	Same	Same	10 days later

Means followed by the same letter within rows are not significantly different (α=0.05), Duncan's Multiple Range Test.

was grouped into the first cluster by the Biplot analysis. Figure 3.3 showed that two red rice populations in the second cluster were located at the same geographical site. Red rice populations in the other two clusters were not related to geographical location.

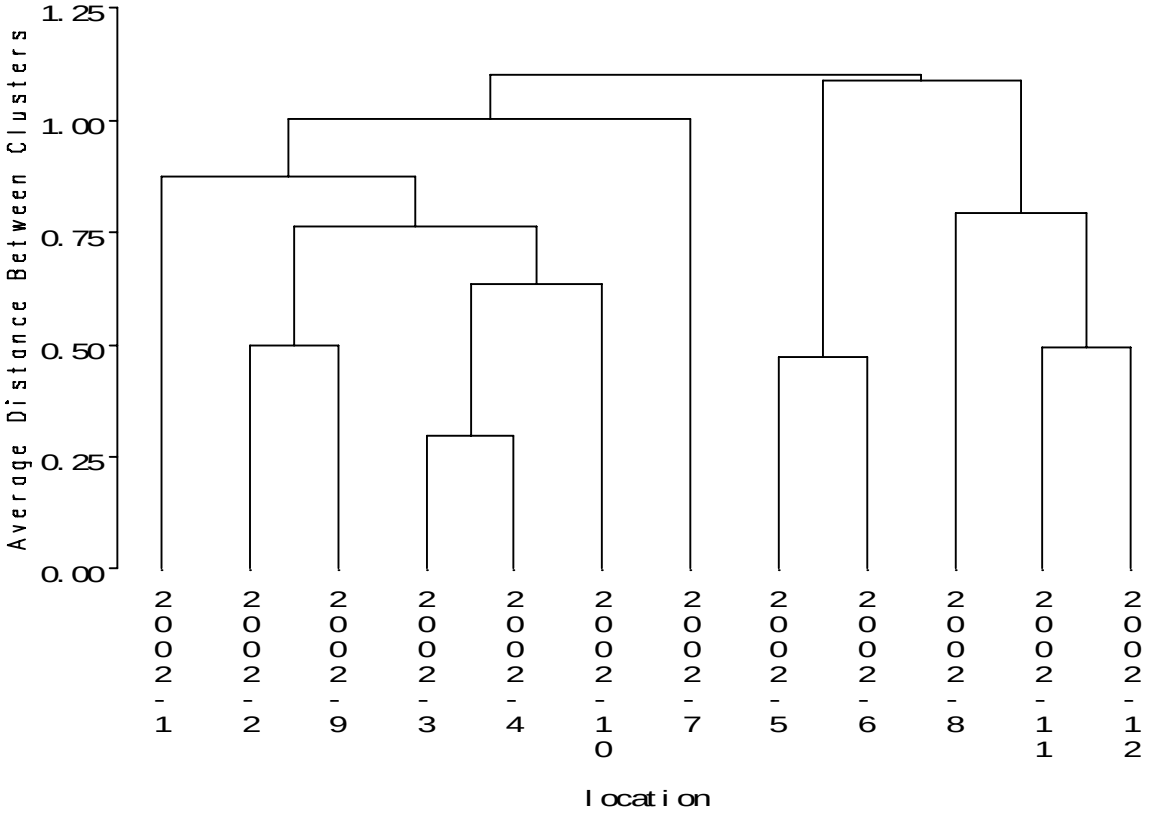

Figure 3.2 UPGMA cluster analysis of agronomic traits collected from 12 commercial Clearfield sites in southwest Louisiana, 2002. Refer to Figure 3.3 for location of sites.

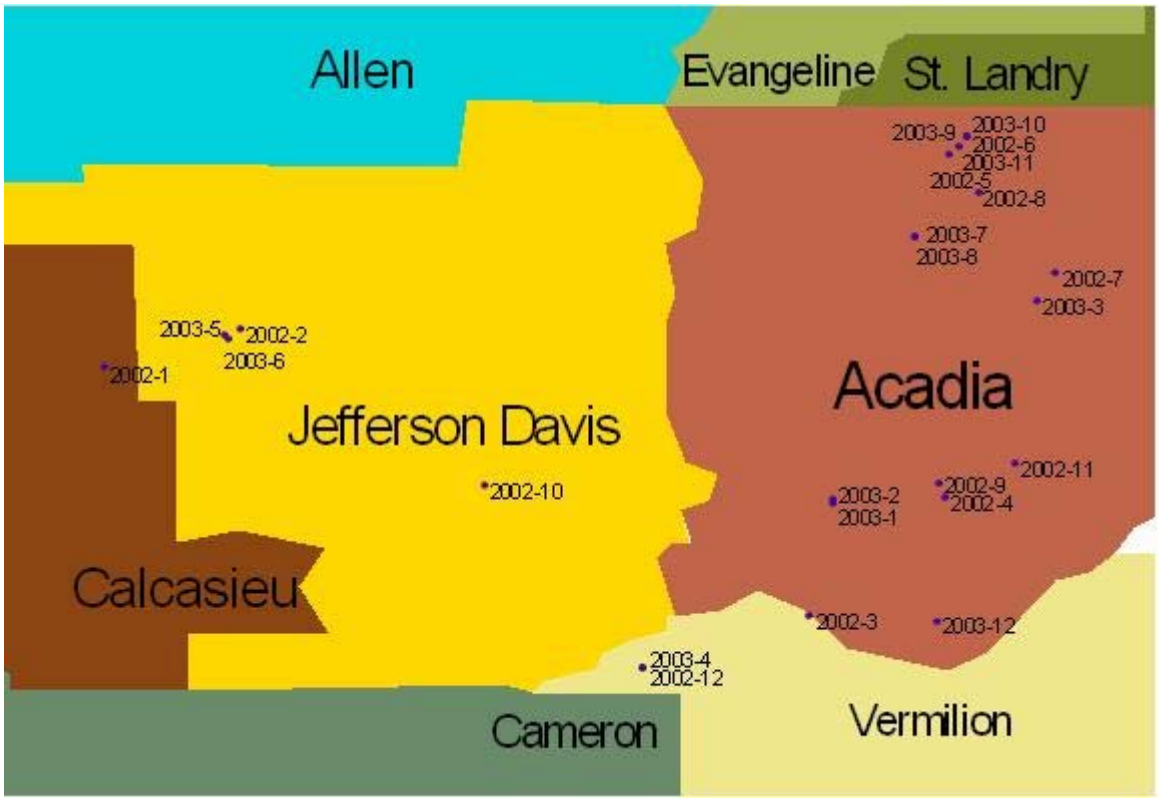

Figure 3.3 Locations of 24 commercial Clearfields sites in 2002 and 2003.

3.3.1.3.2 Biplot Analysis

Biplot analysis (SAS Institute, ver. 9) is a graphical tool to depict variables and observations in a single plot or two-dimensional graph. Horizontal and vertical lines typically reflect the first two principal components, respectively, after data reduction. The first principal component (x axis) is the most discriminating and usually highly correlated with trait mean values. The second principal component (y axis) is the next most discriminating trait. The characteristics of observations are dependent upon the projections of the observations on the horizontal and vertical axes. Figure 3.4 shows biplot analysis based on agronomic traits as variables, and red rice populations at 12 locations as observations. Seeds/plant was the most significant variable separating the twelve environments since its vector was closely aligned with the first PCA axis. Indeed, seeds/plant explained over 95% of the variation in the red rice populations across the twelve environments. The second most important trait for discriminating

environments was plant height which paralleled the second PCA axis and explained an additional 3.1% of the variation. Seed set rate was the third most important source of variation for the red rice populations. The other three variables, panicle length, 100 grain weight and tillers/plant produced little or no effect on the variation of red rice populations because they were located near the point of origin. Red rice populations at location H produced the highest number of seeds per plant and height essentially equal to the mean height across all twelve locations. On the contrary, the red rice population at location E was the tallest, but possessed seeds/plant close to the mean of the twelve locations. Red rice population at location I produced the least number of seeds/plant.

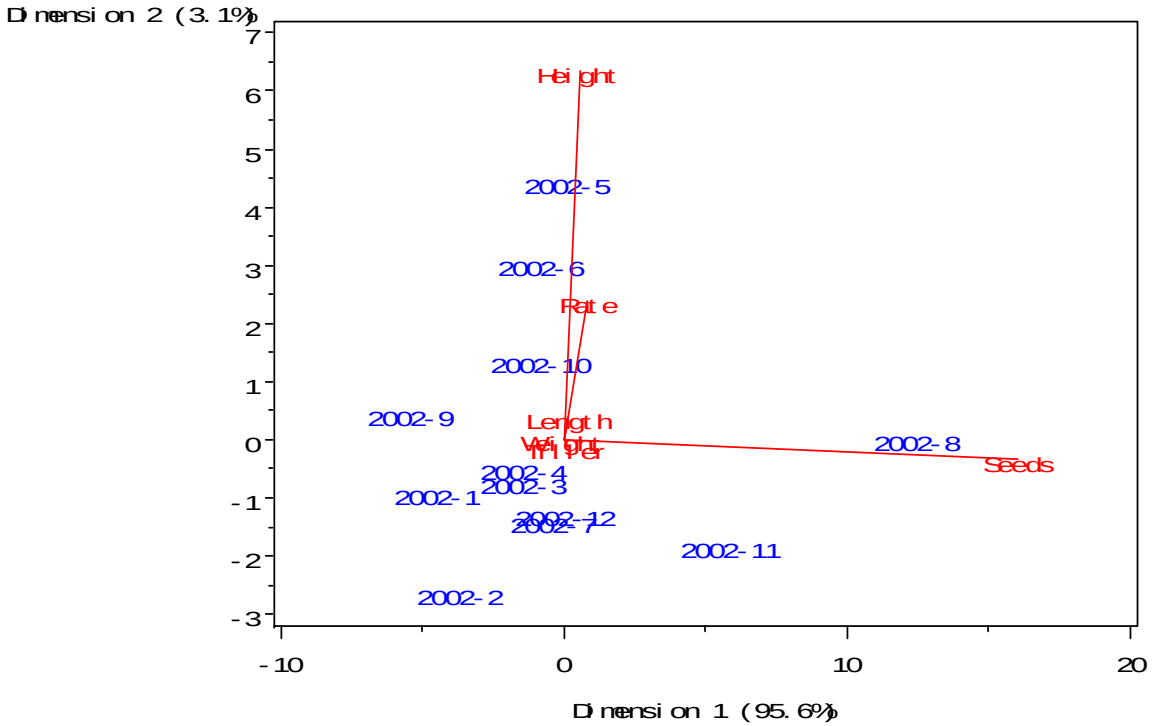

Figure 3.4 Biplot analysis of agronomic traits from red rice populations collected from 12 commercial Clearfield locations in Southwest LA, 2002. Height: plant height, tiller: tillers/plant, length: panicle length, rate: seed set rate, weight: 100 grain weight, seeds: seeds/plant.

3.3.1.4 Incidence and Frequency of Outcrossing

3.3.1.4.1 Hybrid Characteristics

A total of 81 imazethapyr-tolerant plants were found in the 2002 study at the Ben Hur Farm. All putative red rice-Clearfield hybrids produced pubescent leaves and showed seed shattering characteristics. Five exhibited purple pigmentation on the leaf margins. The majority (64/81) did not flower during the field season that ended in October 2002. Details for additional agronomic traits are given in Chapter 5.

3.3.1.4.2 Molecular Data

3.3.1.4.2.1 SSR

Table 3.4 shows DNA band characteristics using SSR marker RM180. CL121 and CL141 produced the same PCR 127bp amplified fragment. One red rice plant possessed a DNA fragment at 127bp. The plant was confirmed to be hybrid between red rice and Clearfield rice because the segregation of pubescent/glabrous leaves was also observed in field. A total of 5 DNA bands (178bp, 190bp, 196bp, 199bp and 214bp) were amplified from the remaining 22 red rice plants collected from locations 2002-8, 2002-10, 2002-11 and 2002-12. Eight of 22 red rice plants possessed one DNA fragment, suggesting that the RM180 locus in these red rice plants was homozygous. The other 14 red rice plants were heterozygous at the RM180 locus because two DNA fragments were amplified from each of the red rice plants.

For 81 putative hybrids from Clearfield rice to red rice, two plants produced a single DNA fragment derived from Clearfield rice. Inspection of seed shape demonstrated that the two plants were not produced directly from Clearfield rice. One plant had one additional DNA fragment in addition to two DNA fragments, one from Clearfield rice and the other from red rice. All others

exhibited amplified products from Clearfield rice and red rice, suggesting that they were hybrids between red rice and Clearfield rice.

3.3.1.4.2.2 AHAS Activity

Analysis of AHAS activity of red rice plants tolerant to imazethapyr was performed by BASF using the half-plate *in vitro* AHAS assay method (Table 3.5). AHAS relative or cross resistance to different families of AHAS inhibitors (imazethapyr, imazaquin, chlorsulfuron and AC2990169) was used to determine target-based or non-target based resistance. In addition, the mutation type of imazethapyr-tolerant plants was evaluated. All plants indicated a target-based (AHAS locus) tolerance to imazethapyr. Three responses of AHAS activity were observed. Seventy two imazethapyr-tolerant red rice plants showed an AHAS response pattern similar to a heterozygous 93AS3510-like mutation. Five imazethapyr-tolerant plants (#3, #38, #40, #47 and #82) showed AHAS response patterns similar to the homozygous 93AS3510 or a PWC16-like mutation. Four plants (#61, #64, #71 and #76) exhibited a distinct enzyme response pattern.

3.3.1.4.3 Outcrossing Analysis

Table 3.6 shows the frequency of outcrossing from Clearfield rice to red rice at 12 locations in 2002. No hybrids were detected at locations 2002-7, 2002-8, 2002-9 and 2002-11. The remaining 8 locations showed an extensive range of outcrossing that varied from 0.017% to 0.583%. A total of 81 red rice plants tolerant to imazethapyr were found in 46,629 red rice seedlings. The average outcrossing rate across locations was 0.163%. CL141 as a parent produced higher outcrossing frequencies than CL121. The two highest rates were observed in field with CL141. Moreover, outcrossing was detected in one ratoon crop from location 2002-11 where two hybrids were found among 1,700 red rice plants.

Table 3.4 Identification of red rice-Clearfield hybrids produced from red rice samples collected in 2002 using SSR marker RM180

Plant No.	Material	Plant ID Number, Ben Hur Farm, 2003	Location	Allele 1	Allele 2 and allele 3
01	CL121		1	127	
02	CL141		5	127	
03	red rice 1	84	8		178
04	red rice 2	96	8	127	
05	red rice 3	97	8		178, 196
06	red rice 4	98	8		178
07	red rice 5	99	8		178, 196
08	red rice 6	100	8		178, 196
09	red rice 7	101	8		178, 196
10	red rice 8	102	8		178, 196
11	red rice 9	103	8		178
12	red rice 10	104	10		178, 196
13	red rice 11	105	10		178, 196
14	red rice 12	106	11		178, 199
15	red rice 13	107	11		178, 199
16	red rice 14	108	11		178, 199
17	red rice 15	85	12		178, 196
18	red rice 16	87	12		190
19	red rice 17	88	12		190
20	red rice 18	89	12		190
21	red rice 19	90	12		190, 214
22	red rice 20	91	12		190, 214
23	red rice 21	92	12		190
24	red rice 22	94	12		178, 196
25	red rice 23	95	12		190
26	rice plant 1 tolerant to imazethapyr	1	1	127	178
27	rice plant 2 tolerant to imazethapyr	2	1	127	178
28	rice plant 3 tolerant to imazethapyr	3	2	127	178
29	rice plant 4 tolerant to imazethapyr	4	2	127	193
30	rice plant 5 tolerant to imazethapyr	5	2	127	178
31	rice plant 6 tolerant to imazethapyr	6	2	127	178
32	rice plant 7 tolerant to imazethapyr	7	2	127	178
33	rice plant 8 tolerant to imazethapyr	8	2	127, 134	190
34	rice plant 9 tolerant to imazethapyr	9	2	127	193
35	rice plant 10 tolerant to imazethapyr	10	2	127	193
36	rice plant 11 tolerant to imazethapyr	11	2	127	178
37	rice plant 12 tolerant to imazethapyr	12	2	127	178
38	rice plant 13 tolerant to imazethapyr	13	3	127	178

Table 3.4 (continued)

Plant No.	Material	Plant ID Number, Ben Hur Farm, 2003	Location	Allele 1	Allele2 and allele3
39	rice plant 14 resistant to imazethapy	14	3	127	178
40	rice plant 15 tolerant to imazethapyr	15	3	127	178
41	rice plant 16 tolerant to imazethapyr	16	3	127	178
42	rice plant 17 tolerant to imazethapyr	17	3	127	178
43	rice plant 18 tolerant to imazethapyr	18	3	127	178
44	rice plant 19 tolerant to imazethapyr	19	3	127	190
45	rice plant 20 tolerant to imazethapyr	20	3	127	178
46	rice plant 21 tolerant to imazethapyr	21	3	127	190
47	rice plant 22 tolerant to imazethapyr	22	3	127	190
48	rice plant 23 tolerant to imazethapyr	23	3	127	190
49	rice plant 24 tolerant to imazethapyr	24	3	127	178
50	rice plant 25 tolerant to imazethapyr	25	3	127	190
51	rice plant 26 tolerant to imazethapyr	26	4	127	190
52	rice plant 27 tolerant to imazethapyr	27	4	127	178
53	rice plant 28 tolerant to imazethapyr	28	4	127	178
54	rice plant 29 tolerant to imazethapyr	29	4	127	178
55	rice plant 30 tolerant to imazethapyr	30	4	127	190
56	rice plant 31 tolerant to imazethapyr	31	4	127	178
57	rice plant 32 tolerant to imazethapyr	32	4	127	178
58	rice plant 33 tolerant to imazethapyr	34	4	127	ND*
59	rice plant 34 tolerant to imazethapyr	35	4	127	190
60	rice plant 35 tolerant to imazethapyr	37	5	127	178
61	rice plant 36 tolerant to imazethapyr	38	5	127	181
62	rice plant 37 tolerant to imazethapyr	39	5	127	181
63	rice plant 38 tolerant to imazethapyr	40	5	127	181
64	rice plant 39 tolerant to imazethapyr	41	5	127	181
65	rice plant 40 tolerant to imazethapyr	42	5	127	181
66	rice plant 41 tolerant to imazethapyr	43	5	127	178
67	rice plant 42 tolerant to imazethapyr	44	5	127	190
68	rice plant 43 tolerant to imazethapyr	45	5	127	181
69	rice plant 44 tolerant to imazethapyr	46	6	127	181
70	rice plant 45 tolerant to imazethapyr	47	6	127	181
71	rice plant 46 tolerant to imazethapyr	48	6	127	181
72	rice plant 47 tolerant to imazethapyr	49	6	127	181
73	rice plant 48 tolerant to imazethapyr	50	6	127	175
74	rice plant 49 tolerant to imazethapyr	51	6	127	181
75	rice plant 50 tolerant to imazethapyr	52	6	127	181
76	rice plant 51 tolerant to imazethapyr	53	6	127	181
77	rice plant 52 tolerant to imazethapyr	54	6	127	181
78	rice plant 53 tolerant to imazethapyr	55	6	127	181

Table 3.4 (continued)

Plant No.	Material	Plant ID Number, Ben Hur Farm, 2003	Location	Allele 1	Allele 2 and allele 3
79	rice plant 54 tolerant to imazethapyr	56	6	127	175
80	rice plant 55 tolerant to imazethapyr	57	6	127	175
81	rice plant 56 tolerant to imazethapyr	58	6	127	184
82	rice plant 57 tolerant to imazethapyr	59	6	127	175
83	rice plant 58 tolerant to imazethapyr	60	6	127	175
84	rice plant 59 tolerant to imazethapyr	61	6	127	178
85	rice plant 60 tolerant to imazethapyr	62	6	127	178
86	rice plant 61 tolerant to imazethapyr	63	6	127	178
87	rice plant 62 tolerant to imazethapyr	64	6	127	181
88	rice plant 63 tolerant to imazethapyr	65	6	127	175
89	rice plant 64 tolerant to imazethapyr	66	6	127	172
90	rice plant 65 tolerant to imazethapyr	67	6	127	175
91	rice plant 66 tolerant to imazethapyr	68	6	127	175
92	rice plant 67 tolerant to imazethapyr	69	6	127	178
93	rice plant 68 tolerant to imazethapyr	70	6	127	178
94	rice plant 69 tolerant to imazethapyr	71	6	127	178
95	rice plant 70 tolerant to imazethapyr	72	6	127	178
96	rice plant 71 tolerant to imazethapyr	73	6	127	181
97	rice plant 72 tolerant to imazethapyr	74	6	127	178
98	rice plant 73 tolerant to imazethapyr	75	6	127	181
99	rice plant 74 tolerant to mazethapyr	76	6	127	181
100	rice plant 75 tolerant to imazethapyr	77	6	127	178
101	rice plant 76 tolerant to imazethapyr	78	6	127	175
102	rice plant 77 tolerant to imazethapyr	79	6	127	175
103	rice plant 78 tolerant to imazethapyr	80	10	127	178
104	rice plant 79 tolerant to imazethapyr	81	12	127	178
105	rice plant 81 tolerant to imazethapyr	82	11 ratoon	127	ND*
106	rice plant 81 tolerant to imazethapyr	83	11 ratoon	127	178

ND*: no DNA fragment found.

Table 3.5 AHAS activity for imazethapyr tolerant red rice plants from red rice samples collected in 2002 using four AHAS inhibitors (data provided by BASF Corp.)

		Inhibitors			
No. of red rice plant	Response to imazethapyr treatment	Imazethapyr	Imazaquin	Chlorsulfuron	AC299016
1	R*	R*	R*	S†	S†
2	R	R	R	S	S
3	R	R	R	S	S
4	R	R	R	S	S
5	R	R	R	S	S
6	R	R	ND*	S	S
7	R	R	R	S	S
8	R	R	R	S	S
9	R	R	R	S	S
10	R	R	R	S	S
11	R	R	R	S	S
12	R	R	R	S	S
13	R	R	R	S	S
14	R	R	R	S	S
15	R	R	R	S	S
16	R	R	R	S	S
17	R	R	ND*	S	S
18	R	R	R	S	S
19	R	R	R	S	S
20	R	R	R	S	S
21	R	R	R	S	S
22	R	R	R	S	S
23	R	R	R	S	S
24	R	R	R	S	S
25	R	R	R	S	S
26	R	R	R	S	S
27	R	R	R	S	S
28	R	R	R	S	S
29	R	R	R	S	S
30	R	R	R	S	S
31	R	R	ND*	S	S
32	R	R	R or S	S	S
34	R	R	R	S	S
35	R	R	ND*	S	S
37	R	R	R	S	S
38	R	R	R	S	S
39	R	R	R	S	S
40	R	R	R	S	S
41	R	R	R	R or S	S
42	R	R	ND*	S	S
43	R	R	R	S	S
44	R	R	R	S	S
45	R	R	R	S	S
46	R	R	R	S	S
47	R	R	R	S	S
48	R	R	R	S	S
49	R	R	R	S	S
50	R	R	R	S	S
51	R	R	R	S	S
52	R	R	R	R or S	S
53	R	R	R	R or S	S
54	R	R	R	S	S
55	R	R	R	S	S
56	R	R	R	S	S
57	R	R	R	S	S
58	R	R	R	S	S
59	R	R	R	S	S
60	R	R	R	S	S
61	R	R	R	S	S
62	R	R	R	S	S
63	R	R	R	S	S

Table 3.5 (continued)

No. of red rice plant	Response to imazethapyr treatment	Inhibitors			
		Imazethapyr	Imazaquin	Chlorsulfuron	AC299016
64	R	R	R	S	R or S
65	R	R	R	S	S
66	R	R	R	S	S
67	R	R	R	S	S
68	R	R	R	S	S
69	R	R	R	S	S
70	R	R	R	S	S
71	R	R	R	S	S
72	R	R	R or S	S	S
73	R	R	R	S	S
74	R	R	R	S	S
75	R	R	R	S	S
76	R	R	R	S	S
77	R	R	R	S	S
78	R	R	R	S	S
79	R	R	R	S	S
80	R	R	R	S	S
81	R	R	R	S	S
82	R	R	R	S	S
83	R	R	R	S	S
85 (Normal red rice)	S	S	S	S	S
Total	82				

R*: tolerant, S†: susceptible
Imazethapyr, Imazaquin, Chlorsulfuron and AC2990169 are four different herbicides

Table 3.6 Outcrossing frequency between red rice and Clearfield rice at 12 commercial locations, southwest Louisiana, 2002

Location	No. of seeds sown	No. of plants emerged	Germination rate (%)	No. of Imazethapyr-tolerant plants
2002-1. Denison	6510	3697	57	2 (0.054%)
2002-2. Hoppe	8009	2393	30	10 (0.418%)
2002-3. Tibadeaux	11409	4597	40	13 (0.283%)
2002-4. Habit	11372	5424	48	9 (0.289%)
2002-5. Soileau I	13810	3119	22	9 (0.464%)
2002-6. Soileau II	12304	7326	59	34 (0.583%)
2002-7. Brunnel	13229	8726	70	0
2002-8. Leonard	33707	1706	5	0
2002-9. Habetz	5080	487	10	0
2002-10. Britt	12316	2924	24	1 (0.034%)
2002-11. Hensgens	23216	1624	7	0
2002-12. Lounsberry	13894	5884	42	1 (0.017%)
Ratoon (Location 2002-11)	15603	1722	11	2 (0.116%)
Total	180459	46629		81 (0.163%)

Values in parentheses represent the percentage of outcrossing calculated by 100×No. of imazethapyr tolerant plants/No. of plants emerged.

3.3.1.4.4 Correlation among Agronomic Traits

Table 3.7 shows correlations among various traits including the frequency of outcrossing.

The frequency of outcrossing had no significant correlation with any agronomic traits analyzed.

However, significant positive association was detected between plant height and panicle length. Similarly, a significant positive correction was found between tillers/plant and seeds/plant.

Table 3.7 Correlation between outcrossing frequency and agronomic traits for red rice samples Collected in 2002

	Plant height	No. of tillers	Panicle length	Seed set rate	Grain weight	No. of seeds/plant
Outcrossing frequency	0.37907 0.2243	-0.47123 0.122	0.43294 0.1598	0.21025 0.5119	-0.05044 0.8763	-0.2696 0.3968
Plant height		-0.14563 0.6516	0.7171 0.0087	0.32893 0.2965	0.03534 0.9132	0.18299 0.5692
No. of tillers			0.31448 0.3195	-0.03489 0.9143	-0.11293 0.7268	0.7717 0.0033
Panicle length				0.3591 0.2516	-0.11339 0.7257	0.53815 0.0711
Seed set rate					-0.00998 0.9754	0.36922 0.2375
Grain weight						-0.3172 0.3151

Value: p value. Significant correlation existed between traits if p value was less than $\alpha=0.05$.

3.3.2 Outcrossing between Red Rice and Clearfield Rice in 2003

3.3.2.1 Biotypes and Infestation of Red Rice at 12 Locations

Table 3.8 shows biotypes and infestation levels of red rice at 12 Clearfield locations in 2003. Straw hull, black hull and brown hull red rice, including awn and awnless biotypes were observed in 2003. Moreover, one golden hull and awn red rice was observed. Other biotypes (straw hull and awn red rice, black hull and awn or awnless red rice, brown hull and awn or awnless red rice) were considered as minor biotypes. At location 2003-3, black hull and awn red rice was the predominant biotype.

Different levels of red rice infestation were found across Clearfield locations that ranged from 0.1% at locations 2003-2, 2003-3, and 2003-9 to 20% at location 2003-10. Compared with the 2002 study, a lower infestation level at Clearfield sites was generally found in 2003. Reasons for the differences could depend upon, among other things, different Clearfield varieties, used in different years as well as cultivation practices and level of imazethapyr weed control.

Table 3.8 Infestations and biotypes of red rice at 12 commercial Clearfield locations in southwest Louisiana, 2003

		Biotypes							
		Golden hull		Straw hull		Black hull		Brown hull	
Location	Infestation of red rice (%)*	Awn	Awnless	Awn	Awnless	Awn	Awnless	Awn	Awnless
2003-1. Bubba Houpaurr I	1.0	0	0	13	74	0	0	8	5
2003-2. Bubba Houpaurr II	0.1	0	0	2	98	0	0	0	0
2003-3. Hudley	0.1	0	0	18	82	0	0	0	0
2003-4. Erol Lounsberry	10.0	1	0	9	21	63	0	6	0
2003-5. Jimmy Hoppe I	0.5	0	0	8	81	9	0	0	2
2003-6. Jimmy Hoppe II	0.5	0	0	13	65	9	0	13	0
2003-7. Kimfrey	0.5	0	0	20	77	0	0	2	1
2003-8. Kimfrey	0.5	0	0	39	53	3	1	2	2
2003-9. Rockett I	0.1	0	0	8	92	0	0	0	0
2003-10. Rockett II	20.0	0	0	4	96	0	0	0	0
2003-11. Rockett III	5.0	0	0	14	74	1	1	10	0
2003-12. Tibadeaux	1.0	0	0	0	99	0	1	0	0
Total		1		148	912	85	3	41	10

*Infestation of red rice (%) estimated by visual observation

3.3.2.2 Agronomic Traits of Red Rice and Clearfield Rice

Table 3.9 shows agronomic traits of Clearfield rice and red rice at 12 locations in 2003. Unlike 2002, red rice samples had significant differences in all agronomic traits analyzed in 2003. Red rice at location 2003-9 produced the tallest plants (mean=142.7 cm), showing a significant difference compared to all other locations. The shortest plants (111.4 cm) were found at location 2003-7. No differences in plant height were observed for red rice plants at locations 2003-4, 2003-5, 2003-6 and 2003-8 with the same tendency occurring between locations 2003-3 and 2003-10. Red rice produced the longest panicle length (24.4 cm) at location 2003-9 and the shortest (19.9 cm) at location 2003-5, but no differences were detected for red rice plants among locations 2003-1, 2003-3, 2003-7 and 2003-11. Panicles were of similar length at locations 2003-2, 2003-6 and 2003-12.

Red rice at location 2003-7 produced more tillers/plant than all other locations, but no significant difference existed for red rice plants among locations 2003-5, 2003-7, 2003-8, 2003-11 and 2003-12. Red rice at location 2003-1 produced fewer tillers than red rice at other locations, but no significant difference was found for red rice among locations 2003-1, 2003-4 and 2003-9 or among locations 2003-2, 2003-3, 2003-4, 2003-5, 2003-6 and 2003-10. Red rice at location 2003-10 produced the greatest mean number of spikelets/plant (106.6), and produced the fewest (63.7) at location 2003-5. No significant difference was found in spikelets/plant for red rice among locations 2003-1, 2003-2, 2003-3, 2003-4, 2003-5, 2003-7 and 2003-11.

The highest seed set rate (82.1%) occurred at location 2003-11 with no significant differences at locations 2003-9 and 2003-10. The lowest seed set rate (59.63%) was observed at location 2003-3 with significant differences found at all other locations. No significant difference existed for this trait among locations 2003-1, 2003-2, 2003-4 and 2003-7, as well as locations 2003-5, 2003-6, 2003-8 and 2003-9. The heaviest 100 grain weight of 2.18 g occurred at location 2003-10, but no significant difference was found for 100 grain weight among locations 2003-4, 2003-5, 2003-9 and 2003-10. Red rice at location 2003-7 showed the lightest 100 grain weight (1.86 g) that was significantly different from all other locations. No significant difference in this trait was found for red rice among locations 2003-1, 2003-3, 2003-6, 2003-8 and 2003-11.

A total of 149 seeds per plant was the highest value detected at location 2003-10 with no significant differences from locations 2003-8 and 2003-12. The lowest value for seeds/plant (63.7) was observed at location 2003-3, and no significant differences were found for red rice among locations 2003-1, 2003-2, 2003-3 and 2003-4. The same tendency of no significant difference in seeds/plant occurred for red rice among locations 2003-5, 2003-6, 2003-7 and 2003-11. The same first flowering date for Clearfield rice and red rice was observed at 9 of 12

locations (Table 3.9). Red rice flowered 5 to l5 days later than Clearfield rice at the remaining three locations.

3.3.2.3 Comparison of Red Rice Populations among the 12 Locations

3.3.2.3.1 Cluster Analysis

Cluster analysis (Figure 3.5) showed that red rice populations across 12 Clearfield locations could be grouped into three clusters. The first cluster consisted of red rice populations from locations 2003-9 and 2003-10. The second cluster contained red rice populations from locations 2003-1, 2003-2, 2003-3 and 2003-4. The third cluster included red rice populations from locations 2003-5, 2003-6, 2003-7, 2003-8, 2003-11 and 2003-12. Biplot analysis (Figure 3.6) also showed that 12 red rice populations could be grouped into the same three clusters as cluster dendrogram shown. The two key important discriminating variables to the variation of 12 red rice populations determined red rice populations in the first cluster. The red rice populations at locations 2003-9 and 2003-10 possessed tall plants and high number of seeds/plant among 12 red rice populations. Red rice populations in the second cluster produced the least number of seeds/plant among 12 red rice populations. The number of seeds/plant for the four red rice populations showed no significant difference (Table 3.9). Moreover, Figure 3.3 showed that the two red rice populations in the first cluster belonged to the same geographical site. The second cluster consisted of two red rice populations at the same geographical site. The third cluster was formed by red rice populations at four geographical sites with two, two, one and one red rice populations, respectively.

3.3.2.3.2 Biplot Analysis

Similar to Biplot analysis for red rice populations collected in 2002, seeds/plant was also the first important discriminating variable to the variation of red rice populations in twelve

Table 3.9 Mean values of agronomic traits of Clearfield rice and red rice at 12 locations in southwest Louisiana, 2003

		Locations											
Traits		2003-1	2003-2	2003-3	2003-4	2003-5	2003-6	2003-7	2003-8	2003-9	2003-10	2003-11	2003-12
Plant height (cm)	Clearfield rice	88.3^{g}	98.5^{e}	93.4^{f}	97.1^{e}	109.3^{ab}	112.2^{a}	82.2^{h}	103.6^{cd}	107.1^{bc}	100^{de}	91.4^{fg}	98.3^{e}
	Red rice	117.4^{d}	117.6^{d}	129.8^{b}	125.3^{c}	122.1^{c}	125.2^{c}	111.4^{e}	122.2^{c}	142.7^{a}	128.8^{b}	112.6^{e}	116.1^{d}
Panicle length (cm)	Clearfield rice	19^{de}	18.8^{de}	19.2^{cde}	18.7^{de}	20.2^{c}	23.5^{a}	18.2^{e}	19.7^{cd}	21.5^{b}	22^{b}	21.3^{b}	18.9^{de}
	Red rice	20.8^{fgh}	22.1^{de}	21^{fg}	22.9^{cd}	19.9^{h}	22.3^{cde}	20.6^{fgh}	23.3^{bc}	24.4^{a}	24^{ab}	20^{gh}	21.5^{ef}
No. of tillers/plant	Clearfield rice	4.9^{e}	5.7^{cde}	6.4^{bcde}	9.6^{a}	7.8^{abcd}	10.1^{a}	5.4^{de}	7.7^{abcd}	5.1^{e}	5.3^{de}	8^{abc}	8.9^{ab}
	Red rice	1.3^{f}	1.7^{cde}	1.7^{cde}	1.6^{def}	2^{abc}	1.8^{bcd}	2.2^{a}	2.1^{ab}	1.4^{ef}	1.8^{bcd}	2.2^{ab}	2.1^{ab}
No. of spikelets/panicle	Clearfield rice	96.9^{def}	104.7^{de}	104.3^{de}	81.7^{ef}	80.3^{f}	152.9^{a}	113.7^{cd}	108.2^{cd}	130.2^{bc}	143^{ab}	118.7^{cd}	94.6^{def}
	Red rice	72.8^{cd}	65^{d}	70^{cd}	70.4^{cd}	63.7^{d}	77.5^{c}	70.3^{cd}	89.2^{b}	101.6^{a}	106.6^{a}	65.3^{d}	96.4^{ab}
Seed set rate (%)	Clearfield rice	85.92^{a}	76.25^{c}	82.83^{ab}	81.38^{abc}	79.58^{bc}	78.2^{bc}	81.59^{ab}	82.8^{ab}	81.3^{abc}	80.15^{bc}	80.95^{abc}	78.66^{bc}
	Red rice	73.57^{efg}	72.52^{fg}	59.63^{h}	72.16^{fg}	76.37^{cde}	78.24^{bcd}	70.98^{g}	77.23^{bcde}	79.87^{abc}	80.36^{ab}	82.1^{a}	75.9^{def}
100 grain weight (g)	Clearfield rice	2.2^{bc}	2.03^{e}	2.2^{bc}	2.18^{cd}	2.3^{a}	2.36^{a}	2.33^{a}	2.31^{a}	2.08^{e}	2.14^{d}	2.24^{bc}	2.25^{b}
	Red rice	2.05^{bc}	1.97^{cd}	2.05^{b}	2.17^{a}	2.15^{a}	2.04^{bcd}	1.86^{e}	1.99^{bcd}	2.15^{a}	2.18^{a}	2.01^{bcd}	1.96^{d}
No. of seeds/plant	Clearfield rice	411.7^{d}	443.3^{d}	554.2^{bcd}	631.9^{bcd}	492.3^{cd}	1177^{a}	498.7^{cd}	669.9^{bc}	522.4^{bcd}	580.4^{bcd}	728.4^{b}	598^{bcd}
	Red rice	69.3^{f}	74.7^{ef}	63.7^{f}	72^{f}	85.6^{def}	108^{bcd}	97.6^{cde}	130.9^{ab}	111.1^{bc}	149^{a}	106.4^{cd}	148.4^{a}
Flowering date of red rice relative to Clearfield rice		Same	Same	5 days later	7 days later	Same	Same	15 days later	Same	Same	Same	Same	Same

Means followed by the same letter in rows are not significantly different (α=0.05), Duncan's Multiple Range Test.

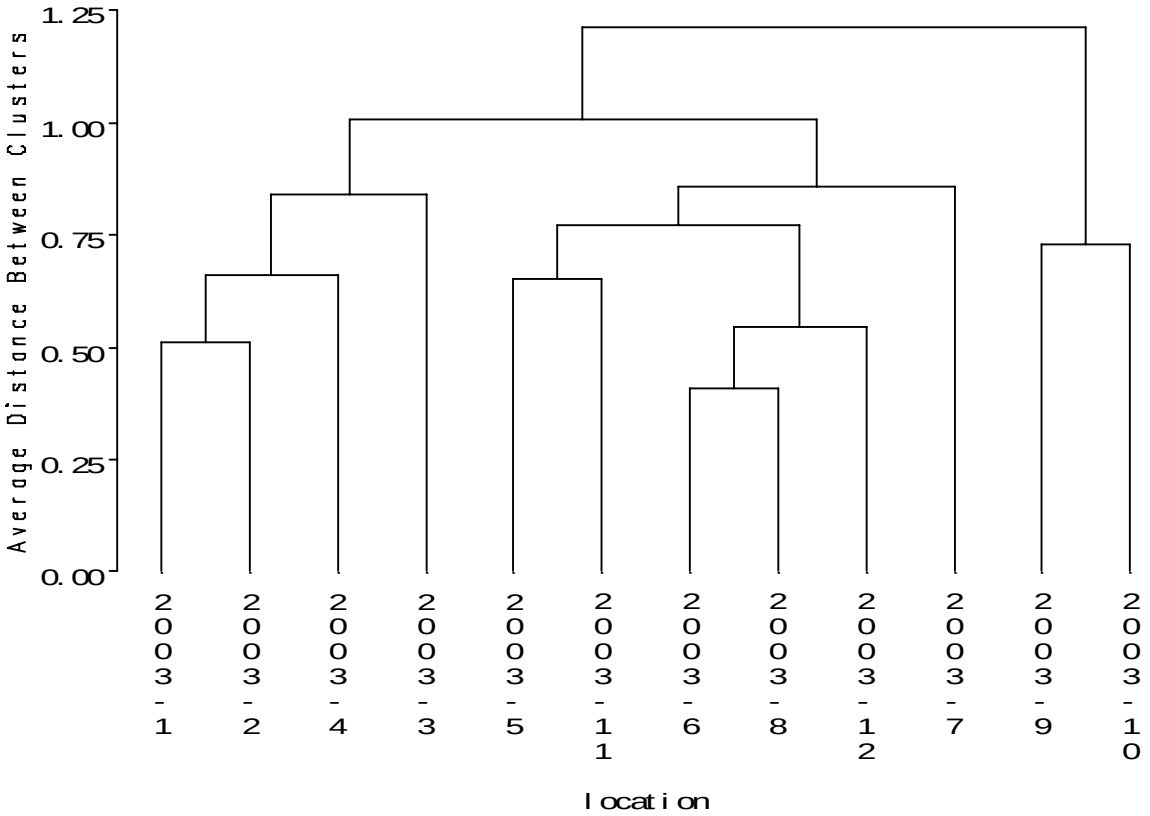

Figure 3.5 UPGMA cluster analysis of agronomic traits collected from 12 commercial Clearfield sites in southwest Louisiana, 2003. Refer to Figure 3.3 for location of sites.

environments. Seeds/plant explained 90.2% variation of red rice populations across twelve environments. Plant height was the second important discriminating factor to the variation of red rice populations at 12 locations. It illustrated 7.6% variation of the red rice populations at 12 locations. With regard to the first important discriminating factor, red rice populations at location 2003-10 produced the highest number of seeds/plant, and the second tallest plant among the12 red rice populations. On the other hand, the red rice population at location 2003-3 produced the fewest number of seeds/plant among the 12 red rice populations. For the second important discriminating factor, the red rice population at location 2003-9 produced the tallest plant, and highest number of seeds/plant among the 12 red rice populations. Red rice population at location 2003-7 showed the shortest plant height across the12 red rice populations (Figure 3.6).

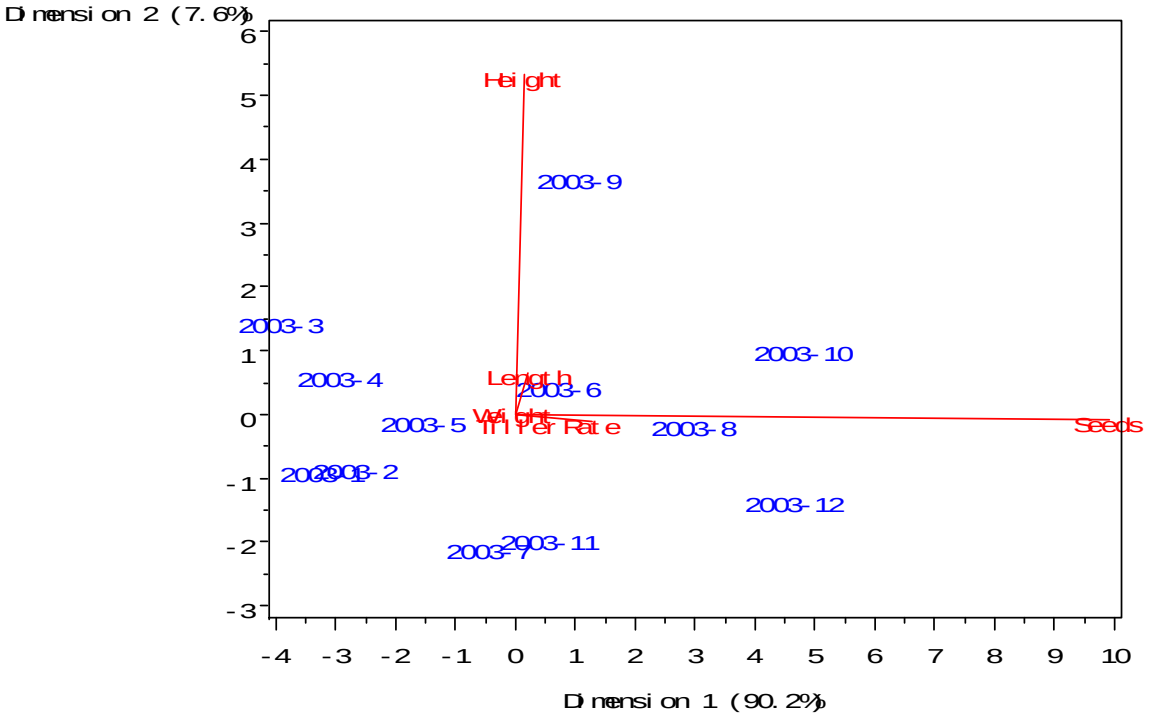

Figure 3.6 Comparison of red rice populations collected from 12 commercial Clearfield locations in southwest Louisiana, 2003 using biplot analysis. Height: plant height, tiller: tillers, length: panicle length, rate: seed set rate, weight: 100 grain weight, seeds: seeds/plant.

3.3.2.4 Incidence and Frequency of Outcrossing

3.3.2.4.1 Hybrid Characteristics

A total of 327 hybrids from Clearfield rice to red rice at 12 locations were detected from 48,127 red rice seedlings by imazethapyr treatment. The majority (87%) of the 327 hybrids were derived from red rice plants with the awnless straw hull biotype. The remainder were derived from red rice plants with awn, black hull, and brown hull and awn biotypes. Like natural hybrids from Clearfield rice to red rice derived from red rice samples collected in 2002, all 327 hybrids exhibited pubescent leaves. Some plants (51) produced purple leaf margins. Most hybrids (246) did not flower in the field during the field season that was terminated at the end of September, 2004. Three hybrids from locations 2003-1, 2003-2 and 2003-3 exhibited shorter plant heights (62 cm to 85 cm) compared with other natural hybrids (99 cm to 142 cm). Figure 3.7 shows one of the three hybrids in the field. Other agronomic traits will be described in detail in chapter 5.

3.3.2.4.2 Molecular Data (SSR)

Table 3.10 shows PCR results using red rice-Clearfield putative hybrids and five F_2 populations as templates. The red rice-Clearfield putative hybrids were derived from red rice samples collected in 2003. The five F_2 populations were developed from red rice-Clearfield hybrids found in red rice samples collected in 2002. Using marker RM180, a 127bp DNA fragment was amplified from CL121, and CL161. In addition, the 127bp fragment was detected in one glabrous CLXL8 F_2 plant, and the 127bp and a 142bp fragment were observed in one pubescent CLXL8 F_2 plant. Amplified DNA fragments with a range of 169bp to 217bp were detected in 36 red rice control plants from 12 locations. Three red rice plants were heterozygous at the RM180 locus. The two alleles in the three red rice plants were from Clearfield rice and red rice, indicating that the three red rice plants were hybrids between Clearfield rice and red rice.

Fifty of 58 imazethapyr tolerant red rice plants contained DNA fragments from both Clearfield rice and red rice. One tolerant red rice plant showed two DNA fragments only from red rice, and three had a DNA fragment amplified from Clearfield rice. A 124bp DNA fragment was amplified in RM180 locus from one tolerant red rice plant, and a 130bp DNA fragment was found in another tolerant red rice plant. The remaining two tolerant red rice plants possessed the 124bp DNA fragment, and the other fragments from red rice. F_2 plants showed segregation in deviation from the genetic ratio of 3:1. Moreover, additional alleles were also found in F_2 plants. One natural red rice-Clearfield hybrid, # 38, possessed only one DNA band at RM180 from Clearfield rice. Three F_2 plants did not show any amplification.

Using marker RM234, Clearfield rice possessed a 154bp DNA fragment, and red rice control plants possessed DNA fragments with a range of 156bp – 184bp. Thirty two of thirty six red rice plants showed one DNA fragment, and one possessed two DNA fragments. The same three red rice plants as stated above possessed two DNA fragments, one from Clearfield rice and the other from red rice. The heterozygous status in the three red rice control plants further confirmed that the three red rice plants were hybrids between Clearfield rice and red rice. With regard to 58 imazethapyr-tolerant red rice plants, 53 showed heterozygous at RM234 locus, one allele from Clearfield rice and the other from red rice. Two of 58 red rice tolerant plants showed two DNA fragments at this locus only from red rice. The remaining three plants possessed a 152bp DNA fragment, and the other alleles from red rice. Similar to the amplification using the marker RM180, 150 F_2 plants showed heterozygous and homozygous in RM234 locus. Additional alleles were detected in F_2 plants. In addition, no amplification using the marker RM234 was observed in one F_2 plant.

Using marker RM 253, a 151bp DNA fragment was amplified in Clearfield rice, and 139bp, 157bp, 159bp and 161bp DNA fragments were found in 36 red rice control plants. One of 58 imazethapyr tolerant red rice plants possessed one DNA band from Clearfield rice. The others showed two alleles, one from Clearfield rice and the other from red rice. Similar to the amplification using the markers RM180 and RM234, 150 F_2 plants derived from five F_1 red rice-Clearfield hybrids, still possessed additional alleles at RM253 locus besides heterozygous and homozygous F_2 plants at this locus were found. Moreover, no DNA fragments were found in two F_2 plants after PCR reaction using the marker RM253.

In summary, the markers RM180, RM234 and RM253 identified red rice-Clearfield hybrids and five F_2 populations based on DNA fragment characteristic of red rice and Clearfield rice. Eight putative F_1 hybrids were not confirmed by PCR amplification using the RM180, but six of them were confirmed to be hybrids through the markers RM234 and RM253, and the other two only through the marker RM253. Similarly, five putative F_1 hybrids were not determined to be hybrids using the marker RM234, but three of them were detected to be true hybrids through the other two primers, and two only through the marker RM253. One putative F_1 hybrid using the marker RM253 was not confirmed to be red rice-Clearfield hybrid, but also demonstrated by the other two primers. F_2 plants showed segregation in the RM180, RM234 and RM253 loci. The proportion of homozygous plants vs heterozygous plants deviated from the genetic ratio of 3:1. Additional DNA fragments were found in F_2 plants in addition to the two DNA fragments detected in corresponding F_1 plants. No amplification occurred in the same one F_2 plant using the three primers. Moreover, DNA fragment characteristic did not relate with leaf types (pubescent and glabrous). Plants with glabrous leaves consisted of DNA bands both from Clearfield rice and

red rice. Figure 3.8 shows DNA band characteristics of 20 natural hybrids from CL161 to red rice using marker RM180. All 20 hybrids possessed two DNA alleles at the RM180 locus, one from CL161 (127bp) and one from red rice (175bp).

Figure 3.7 Red rice-Clearfield hybrids, Ben Hur Farm, Baton Rouge, Louisiana, 2004

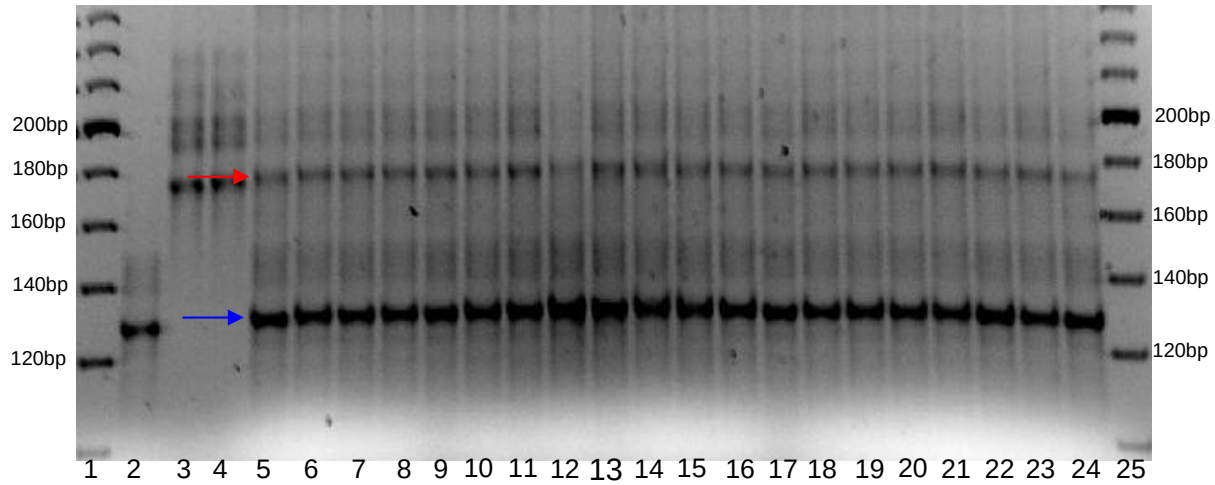

Figure 3.8 Detection of hybridization between CL161 and red rice using SSR marker RM180. Lane 1 and 25, marker; lane 2, CL161(127bp); lane 3 and 4, red rice (175bp); lane 5-24, natural hybrids with heterozygous banding pattern, one from CL161 and one from red rice labeled with blue and red arrow, respectively.

Table 3.10 Detection of natural red rice-Clearfield putative hybrids produced from red rice samples collected in 2003 and F_2 populations developed from red rice-Clearfield hybrids produced from red rice samples collected in 2002 using SSR markers

Entry	Material	Leaf type		SSR markers		
		P*	G†	RM180	RM234	RM253
1	CL121		Yes	127	154	151
2	CL161		Yes	127	154	151
3	CLXL8 F_2 plant	Yes		127, 142	154	151
4	CLXL8 F_2 plant		Yes	127	154	151
5	Red rice	Yes		169	166	159
6	Red rice	Yes		217	166	139
7	Red rice	Yes		169	168	139
8	Red rice	Yes		169	168	157
9	Red rice	Yes		127, 169	154, 168	157
10	Red rice	Yes		169	168	157
11	Red rice	Yes		169	158	139
12	Red rice	Yes		181	168	139
13	Red rice	Yes		181	158	157
14	Red rice	Yes		169	166	157
15	Red rice	Yes		169	166	157
16	Red rice	Yes		169	168	157
17	Red rice	Yes		169	168	157
18	Red rice	Yes		172	168	157
19	Red rice	Yes		127, 172	154, 168	157
20	Red rice	Yes		175, 196	156, 166	139
21	Red rice	Yes		172	170	159
22	Red rice	Yes		187, 208	170	159
23	Red rice	Yes		172	170	161
24	Red rice	Yes		172	170	161
25	Red rice	Yes		172	170	161
26	Red rice	Yes		127, 172	154, 172	161
27	Red rice	Yes		175	174	161
28	Red rice	Yes		175	174	161
29	Red rice	Yes		175	174	161
30	Red rice	Yes		175	174	161
31	Red rice	Yes		175	174	161
32	Red rice	Yes		175	174	161
33	Red rice	Yes		175	178	159
34	Red rice	Yes		178	178	159
35	Red rice	Yes		190	172	159
36	Red rice	Yes		190	178	161
37	Red rice	Yes		178	178	161
38	Red rice	Yes		178	178	161
39	Red rice	Yes		178	184	161
40	Red rice	Yes		181	184	161
41	2003 natural hybrid	Yes		124	152, 158	151, 161
42	2003 natural hybrid	Yes		127, 175	154, 172	151, 161
43	2003 natural hybrid	Yes		127, 175	154, 172	151, 159
44	2003 natural hybrid	Yes		127, 175	154, 172	151, 159
45	2003 natural hybrid	Yes		127, 175	154, 174	151, 159
46	2003 natural hybrid	Yes		127, 175	154, 172	151, 161
47	2003 natural hybrid	Yes		127	154, 162	151, 161
48	2003 natural hybrid	Yes		127, 187	152, 172	141, 151
49	2003 natural hybrid	Yes		127, 175	154, 162	151, 159
50	2003 natural hybrid	Yes		130	154, 172	141, 151
51	2003 natural hybrid	Yes		127, 187	154, 162	141, 151
52	2003 natural hybrid	Yes		127, 187	154, 172	139, 151
53	2003 natural hybrid	Yes		127, 187	154, 170	139, 151
54	2003 natural hybrid	Yes		127, 181	154, 170	151, 159
55	2003 natural hybrid	Yes		127, 181	154, 170	151, 159
56	2003 natural hybrid	Yes		127, 181	154, 172	151, 161
57	2003 natural hybrid	Yes		175, 196	154, 172	151, 161
58	2003 natural hybrid	Yes		124, 178	154, 174	151, 161
59	2003 natural hybrid	Yes		127, 175	154, 174	151, 161
60	2003 natural hybrid	Yes		127, 178	154, 172	151, 161
61	2003 natural hybrid	Yes		124, 175	152, 172	151, 161
62	2003 natural hybrid	Yes		127, 178	154, 172	151, 157
63	2003 natural hybrid	Yes		127, 175	154, 174	151, 159
64	2003 natural hybrid	Yes		127, 178	158, 174	151, 159
65	2003 natural hybrid	Yes		127, 175	154, 172	145, 151

Table 3.10 (continued)

Entry	Material	Leaf type		SSR markers		
		P*	G†	RM180	RM234	RM253
66	2003 natural hybrid	Yes		127, 175	154, 170	145, 151
67	2003 natural hybrid	Yes		127, 175	162, 174	145, 151
68	2003 natural hybrid	Yes		127, 175	154, 170	143, 151
69	2003 natural hybrid	Yes		127, 175	154, 170	143, 151
70	2003 natural hybrid	Yes		127, 175	154, 170	143, 151
71	2003 natural hybrid	Yes		127, 175	154, 172	143, 151
72	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
73	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
74	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
75	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
76	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
77	2003 natural hybrid	Yes		127, 175	154, 172	143, 151
78	2003 natural hybrid	Yes		127	154, 174	143, 151
79	2003 natural hybrid	Yes		127, 172	154, 174	143, 151
80	2003 natural hybrid	Yes		127, 175	154, 174	143, 151
81	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
82	2003 natural hybrid	Yes		127, 172	154, 172	143, 151
83	2003 natural hybrid	Yes		127, 175	154, 172	141, 151
84	2003 natural hybrid	Yes		127, 172	154, 172	151, 159
85	2003 natural hybrid	Yes		127	154, 172	151, 157
86	2003 natural hybrid	Yes		127, 175	154, 174	143, 151
87	2003 natural hybrid	Yes		127, 172	154, 174	143, 151
88	2003 natural hybrid	Yes		127, 175	154, 174	143, 151
89	2003 natural hybrid	Yes		127, 175	154, 170	143, 151
90	2003 natural hybrid	Yes		127, 169	154, 170	143, 151
91	2003 natural hybrid	Yes		127, 169	154, 168	143, 151
92	2003 natural hybrid	Yes		127, 169	154, 168	141, 151
93	2003 natural hybrid	Yes		127, 169	154, 166	141, 151
94	2003 natural hybrid	Yes		127, 169	154, 166	141, 151
95	2003 natural hybrid	Yes		127, 169	154, 166	151, 159
96	2003 natural hybrid	Yes		127, 169	154, 168	151, 159
97	2003 natural hybrid	Yes		127, 169	154, 170	151
98	2003 natural hybrid	Yes		127, 166	154, 170	141, 151
99	2002 hybrid 2	Yes		127, 169	154, 170	151, 161
100	F_2 population-2-1		Yes	127	154, 168	141, 151
101	F_2 population-2-2	Yes		127, 169	154, 168	143, 151
102	F_2 population-2-3	Yes		127, 169	154, 168	141, 151
103	F_2 population-2-4	Yes		127, 169	154, 186	159
104	F_2 population-2-5	Yes		175, 196	154, 186	141, 151
105	F_2 population-2-6	Yes		127, 169	154, 170	141, 151
106	F_2 population-2-7	Yes		127, 169	154, 170	141, 151
107	F_2 population-2-8		Yes	127	154, 186	141, 151
108	F_2 population-2-9	Yes		127, 169	154, 170	143, 151
109	F_2 population-2-10		Yes	127	154, 170	143, 151
110	F_2 population-2-11	Yes		127, 169	154, 186	143, 151
111	F_2 population-2-12	Yes		175, 199	154, 172	143, 151
112	F_2 population-2-13	Yes		175, 199	174	159
113	F_2 population-2-14	Yes		127, 169	154, 174	151, 159
114	F_2 population-2-15	Yes		127	154, 174	151, 159
115	F_2 population-2-16	Yes		127, 172	154, 174	151, 159
116	F_2 population-2-17	Yes		127, 172	154, 174	151, 159
117	F_2 population-2-18		Yes	127, 172	154, 192	159, 169
118	F_2 population-2-19	Yes		127, 172	174	151, 159
119	F_2 population-2-20		Yes	127, 178	154, 162	151, 159
120	F_2 population-2-21		Yes	127	154, 194	151, 159
121	F_2 population-2-22		Yes	175	178	151, 159
122	F_2 population-2-23	Yes		127, 175	156, 176	151, 159
123	F_2 population-2-24	Yes		127, 175	156, 164	151, 159
124	F_2 population-2-25	Yes		175	154, 174	159, 169
125	F_2 population-2-26	Yes		127	154, 174	151, 159
126	F_2 population-2-27	Yes		127, 178	154, 174	159, 169
127	F_2 population-2-28	Yes		127, 175	154, 174	151, 161
128	F_2 population-2-29	Yes		131, 178	154, 164	151, 161
129	F_2 population-2-30		Yes	130, 178	154, 164	151, 163
130	2002 hybrid 15	Yes		130, 178	154, 170	151, 159
131	F_2 population-15-1	Yes		178	154, 170	151, 159
132	F_2 population-15-2		Yes	130, 178	154, 170	151, 159
133	F_2 population-15-3	Yes		127, 178	154, 170	151, 159

Table 3.10 (continued)

Entry	Material	Leaf type		SSR markers		
		P*	G†	RM180	RM234	RM253
134	F_2 population-15-4	Yes		127, 178	154, 170	161
135	F_2 population-15-5		Yes	127, 178	154, 170	151, 161
136	F_2 population-15-6		Yes	127, 178	154, 164	151, 161
137	F_2 population-15-7		Yes	127, 178	168	151, 161
138	F_2 population-15-8	Yes		127, 178	170	151, 161
139	F_2 population-15-9	Yes		127, 193	154, 162	151, 161
140	F_2 population-15-10	Yes		175	154, 170	151, 161
141	F_2 population-15-11	Yes		127, 175	154, 170	151, 161
142	F_2 population-15-12		Yes	127, 169	154, 170	151, 161
143	F_2 population-15-13		Yes	127, 175	154, 170	151, 161
144	F_2 population-15-14	Yes		127, 178	154, 162	151, 161
145	F_2 population-15-15	Yes		127, 175	170	151, 161
146	F_2 population-15-16	Yes		127, 175	154, 170	151, 161
147	F_2 population-15-17	Yes		127, 178	154, 164	151, 161
148	F_2 population-15-18	Yes		127, 175	154, 172	151, 161
149	F_2 population-15-19		Yes	169, 193	154, 164	151, 161
150	F_2 population-15-20		Yes	127, 175	154, 162	157
151	F_2 population-15-21	Yes		127, 175	156, 164	157
152	F_2 population-15-22	Yes		127, 175	154, 164	157
153	F_2 population-15-23		Yes	127, 142	154, 164	
154	F_2 population-15-24	Yes		199	154, 192	157
155	F_2 population-15-25	Yes				
156	F_2 population-15-26	Yes		127, 172	154, 192	157
157	F_2 population-15-27	Yes		127, 190	154, 192	151, 157
158	F_2 population-15-28		Yes	127, 172	154, 172	151, 157
159	F_2 population-15-29	Yes		127, 190	154, 192	157
160	F_2 population-15-30	Yes		127, 172	154, 192	143, 151
161	2002 hybrid 34	Yes		127, 190	154, 174	143, 151
162	F_2 population-34-1	Yes		127, 190	154, 186	143, 151
163	F_2 population-34-2	Yes		127, 187	154, 172	143, 151
164	F_2 population-34-3	Yes			154, 172	143, 151
165	F_2 population-34-4	Yes		127, 187	154, 188	143, 151
166	F_2 population-34-5		Yes	127	154, 172	141, 151
167	F_2 population-34-6	Yes		127, 187	154, 172	141, 151
168	F_2 population-34-7	Yes		127, 187	154, 172	141, 151
169	F_2 population-34-8	Yes		127, 184	154, 188	141, 151
170	F_2 population-34-9	Yes		127, 184	154, 170	151, 157
171	F_2 population-34-10		Yes	127	170	151, 157
172	F_2 population-34-11	Yes		127, 184	154, 172	151, 157
173	F_2 population-34-12	Yes		127	154, 186	151, 157
174	F_2 population-34-13	Yes		127, 193	154, 188	151, 157
175	F_2 population-34-14	Yes		127, 181	154, 188	151, 157
176	F_2 population-34-15	Yes		127, 184	154, 174	151, 159
177	F_2 population-34-16	Yes			154, 172	151, 159
178	F_2 population-34-17	Yes		127, 190	154, 186	151, 159
179	F_2 population-34-18	Yes		127, 187	154, 172	151, 159
180	F_2 population-34-19		Yes	127, 190	154, 172	151, 159
181	F_2 population-34-20	Yes		190	168	151, 159
182	F_2 population-34-21	Yes		187	154, 168	151, 159
183	F_2 population-34-22	Yes		184	154, 168	151, 159
184	F_2 population-34-23	Yes		124, 184	170	151, 161
185	F_2 population-34-24	Yes		127, 154	170	151, 161
186	F_2 population-34-25	Yes		124, 187	170	151, 161
187	F_2 population-34-26	Yes		193	168	141, 151
188	F_2 population-34-27	Yes		127	154, 168	151, 161
189	F_2 population-34-28	Yes		124, 187	154, 184	151, 159
190	F_2 population-34-29	Yes		127	154, 170	151, 159
191	F_2 population-34-30		Yes	127	154, 170	151, 161
192	2002 hybrid 38	Yes		127	154, 176	151, 161
193	F_2 population-38-1		Yes	175, 196	154, 190	151, 159
194	F_2 population-38-2	Yes		127, 187	154, 174	141, 151
195	F_2 population-38-3	Yes		127, 166	154, 186	141, 151
196	F_2 population-38-4		Yes	127, 190	154, 176	141, 151
197	F_2 population-38-5		Yes	127, 166	154, 176	141, 151
198	F_2 population-38-6	Yes		127, 181	154, 178	141, 151
199	F_2 population-38-7	Yes		127, 166	154, 178	143, 151
200	F_2 population-38-8		Yes	127	154, 178	143, 151
201	F_2 population-38-9		Yes	127, 193	154, 192	151, 159

Table 3.10 (continued)

Entry	Material	Leaf type		SSR markers		
		P*	G†	RM180	RM234	RM253
202	F_2 population-38-10	Yes		127, 169	160	151, 159
203	F_2 population-38-11	Yes		127, 193	154, 182	151, 161
204	F_2 population-38-12	Yes		127	154, 182	143, 151
205	F_2 population-38-13	Yes		127, 199	154, 182	143, 151
206	F_2 population-38-14	Yes		127, 199	154, 184	143, 151
207	F_2 population-38-15		Yes	127	154, 188	143, 151
208	F_2 population-38-16	Yes		127, 175	154, 178	143, 151
209	F_2 population-38-17	Yes		127	154, 178	143, 151
210	F_2 population-38-18	Yes		127, 199	160	143, 151
211	F_2 population-38-19	Yes		127, 199	154	151, 159
212	F_2 population-38-20	Yes		127, 175	176	141, 151
213	F_2 population-38-21	Yes		127	154, 180	141, 151
214	F_2 population-38-22		Yes	127	178	141, 151
215	F_2 population-38-23	Yes		127, 175	154, 162	151, 159
216	F_2 population-38-24	Yes		127	154, 160	141, 151
217	F_2 population-38-25	Yes		127	164, 198	143, 151
218	F_2 population-38-26		Yes	127, 172	154, 162	141, 151
219	F_2 population-38-27		Yes	127, 178	154, 160	141, 151
220	F_2 population-38-28	Yes		127, 202	154, 160	141, 151
221	F_2 population-38-29		Yes	127, 175	154, 170	151, 159
222	F_2 population-38-30	Yes		127, 175	170, 180	139, 149
223	2002 hybrid 70	Yes		127, 199	154, 162	151, 163
224	F_2 population-70-1	Yes		127, 196	170, 180	151, 159
225	F_2 population-70-2	Yes		127, 196	154	151, 161
226	F_2 population-70-3		Yes	127, 199	154, 162	151, 163
227	F_2 population-70-4		Yes	127, 196	154, 170	151, 161
228	F_2 population-70-5	Yes		127	170, 180	151, 159
229	F_2 population-70-6		Yes	127, 196	154, 172	151, 159
230	F_2 population-70-7	Yes		127, 178	182	159, 169
231	F_2 population-70-8	Yes		127, 196	154, 172	151, 159
232	F_2 population-70-9	Yes		175	154, 164	151, 159
233	F_2 population-70-10		Yes	127, 190	154, 164	151, 159
234	F_2 population-70-11	Yes		127, 199	154, 174	151, 159
235	F_2 population-70-12		Yes	127, 196	154, 162	151, 169
236	F_2 population-70-13	Yes		127, 196	154, 174	151, 161
237	F_2 population-70-14	Yes		127, 175	154, 174	151, 161
238	F_2 population-70-15	Yes		127, 193	154, 174	151, 161
239	F_2 population-70-16	Yes		127, 193	154, 172	151, 161
240	F_2 population-70-17	Yes		127, 193	174	151, 161
241	F_2 population-70-18	Yes		178	154, 174	151, 159
242	F_2 population-70-19	Yes		127	172, 184	151, 159
243	F_2 population-70-20		Yes	175	154, 172	151, 163
244	F_2 population-70-21	Yes		175	154, 172	151, 163
245	F_2 population-70-22	Yes		127, 190	154, 172	143, 151
246	F_2 population-70-23	Yes		127, 187	172	151, 161
247	F_2 population-70-24		Yes	127, 187	154, 170	151, 159
248	F_2 population-70-25	Yes		127, 184	154, 184	151, 159
249	F_2 population-70-26	Yes		127, 169	154, 172	151, 159
250	F_2 population-70-27		Yes	127, 178	154, 184	151, 159
251	F_2 population-70-28	Yes		127, 178	154, 184	151, 159
252	F_2 population-70-29	Yes		127, 175	154, 184	159, 169
253	F_2 population-70-30		Yes	166	154, 184	151, 159

P*: pubescent leaf. G†: glabrous leaf.

3.3.2.4.3 Outcrossing Analysis

Table 3.11 shows the frequency of outcrossing at 12 Clearfield locations in 2003. No hybrids were detected from ~ 3,700 red rice seedlings derived from location 2003-4. Outcrossing occurred across the remaining locations from 0.09% to 3.2%. A total of 327 red rice plants tolerant to imazethapyr were detected among 48,127 red rice seedlings. The average outcrossing over all locations was 0.679%. The result of outcrossing showed that CL121, CL161 and CLXL8

had potential to transfer imazethapyr resistance to red rice. Compared with the results of outcrossing in 2002, a higher outcrossing frequency was found in red rice samples collected in 2003 that may be due to different Clearfield varieties.

Table 3.11 Outcrossing frequency between red rice and Clearfield rice at 12 commercial locations, southwest Louisiana, 2003

Location	No. of seeds planted	No. of plants emerged	Germination rate (%)	No. of imazethapyr tolerant plants (outcrossing frequency)*
2003-1. Bubba Houpaurr	6193	815	13.16	1 (0.123%)
2003-2. Bubba Houpaurr	6969	2634	37.8	85 (3.227%)
2003-3. Erol Lounsberry	5991	3310	55.25	50 (1.511%)
2003-4. Hundley	6996	3784	54.09	0
2003-5. Jimmy Hoppe I	7889	4665	59.13	4 (0.086%)
2003-6. Jimmy Hoppe II	8147	4117	50.53	24 (0.583%)
2003-7. Kimfrey	9077	1690	18.62	5 (0.296%)
2003-8. Kimfrey	11082	4098	36.98	24 (0.586%)
2003-9. Rockett	9742	6542	67.15	17 (0.26%)
2003-10. Rockett	11010	6828	62.02	44 (0.644%)
2003-11. Rockett	9138	4626	50.62	45 (0.973%)
2003-12. Tibadeaux	10858	5018	46.21	28 (0.558%)
Total	103092	48127		327 (0.679%)

*Values in parentheses represent the percent of outcrossing, and were obtained by 100×No. of imazethapyr tolerant plants/No.of plants emerged.

3.3.2.4.4 Correlation between Traits

Table 3.12 shows correlation values among traits including outcrossing frequency. The result showed no significant correlation between outcrossing frequency and any agronomic trait. Like the 2002 correlation analysis, a significant positive correlation existed between plant height and panicle length at $\alpha=0.05$ level. Unlike the correlation result in 2002, significantly positive correlations were found between plant height and grain weight, and seed set rate and seeds/plant. Moreover, a significant negative correlation was observed between plant height and tillers/plant.

Straw hull and awnless red rice or black hull red rice as main biotypes was reported in a previous study (Galli 1991). In the present study, straw hull and awnless red rice, and black hull

and awn red rice as main biotypes were observed in eleven and one of twelve Clearfield locations in red rice collections across two years, respectively. Minor groups, namely brown hull

Table 3.12 Correlation between outcrossing frequency and agronomic traits, and associations among agronomic traits for red rice samples collected in 2003.

	Plant height	No. of tillers	Panicle length	Seed set rate	Grain weight	No. of seeds/plant
Outcrossing frequency	-0.14258	0.01291	-0.02846	-0.28092	-0.32316	-0.21935
	0.6585	0.9682	0.93	0.3764	0.3056	0.4934
Plant height		-0.57955	0.70349	0.01345	0.7057	0.04913
		0.0483	0.0107	0.9669	0.0103	0.8795
No. of tillers			-0.37427	0.16134	-0.55699	0.47095
			0.2307	0.6164	0.0599	0.1223
Panicle length				0.28762	0.42083	0.42351
				0.3647	0.1731	0.1701
Seed set rate					0.21651	0.63256
					0.4991	0.0273
Grain weight						-0.04128
						0.8986

Value: p value
Significant correlation existed if p value was less than $\alpha=0.05$.

and awn/awnless, straw hull and awn, black hull and awnless, and golden hull red rice, were also observed.

Noldin et al. (1999) reported that red rice produced more tillers and panicles, and taller plants than rice cultivars based on the investigation of vegetative traits of sixteen red rice ecotypes and three rice cultivars. Our study showed that red rice possessed taller plants, but less tillers and panicles than Clearfield rice. The difference in tiller and panicle characteristics may be due to imazethapyr application in Clearfield production. Red rice infestations were different in different Clearfield locations. This could be due to many factors, such as field conditions in previous years and the application of imazethapyr herbicide.

Outcrossing occurred from Clearfield rice to red rice, including CL121, CL141, CL161 and CLXL8. Natural red rice-Clearfield hybrids were found in geographical sites from 30.06438°N to 30.46575°N in latitude, and from 92.35436°W to 93.04704°W in longitude in Clearfield

planted in 2002. In Clearfield planted in 2003, outcrossing occurred in geographical sites from 30.06438°N to 30.46575°N in latitude, and from 92.3913°W to 92.95332°W in longitude. Outcrossing occurred in a wider geographical region in 2002 than in 2003. Outcrossing frequencies were different in two years with higher rate in the second year. CL161 was planted at ten locations in the second year. CL121 was planted at ten locations in the first year. The difference of outcrossing frequency may be caused by different Clearfield cultivars. This finding was also reflected on the difference of outcrossing frequency from CL121/CL141 planted in same growth season to red rice. Taller plants in CL141 may contribute the higher rate of outcrossing from CL141 to red rice than that from CL121 to red rice. Less than 1% average rate of outcrossing detected in two years was consistent with previous studies on outcrossing among rice cultivar and its weedy and wild relatives (Chen et al., 2004; Messenguer et al., 2001; Rong et al., 2004; Song et al., 2004; Zhang et al., 2003). However, ~1.5% and ~3.2% outcrossing frequencies were found in two locations in the second year. Outcrossing frequencies higher than 1% have been reported from cultivated rice to *Oryza rufipogon* (Song et al., 2004), traditional rice cultivar to hybrid rice (Rong et al., 2004) and cultivated rice to red rice (Langevin et al., 1990). The flowering habits of hybrids (Rong et al., 2004) and *Oryza rufipogon* (Song et al., 2004), and hybridization beyond one generation (Langevin et al., 1990) were postulated to explain the relatively high outcrossing events.

To predict outcrossing frequency from Clearfield rice to red rice under commercial field conditions, a correlation analysis of outcrossing frequency with easily identified agronomic traits was conducted. No significant correlation was found between outcrossing frequency and any agronomic traits among the sampled red rice biotypes. Nevertheless, plant maturity and flowering date were two crucial characteristics that showed extensive variation among the red

rice biotypes sampled across 24 locations. This fact alone indicates that red rice had maintained sufficient genetic variability to flower during the same time period as Clearfield and other commercial varieties.

The cluster and biplot analyses were used to separate red rice populations into groups based on the performance of agronomic traits of red rice populations. The purpose of the analyses was to compare outcrossing frequency between different clusters. The cluster dendrogram and biplot analyses separated 12 red rice populations into three groups within each of the two years. For red rice sample collected in 2002, one group possessed the highest outcrossing frequencies. However, no consistent pattern was detected for the other two groups, and the same tendency was observed for red rice biotypes collected in 2003. These results indicate that individual commercial locations will exhibit specific outcrossing frequencies and that tendencies for outcrossing between red and commercial rice cannot be predicted based on geographical location. Instead, other factors such as variety, date of planting, herbicide and water management, and crop rotation schemes will likely have a greater impact on incidence of outcrossing and long-term management of the Clearfield technology.

In summary, straw hull and awnless red rice was main red rice biotype for red rice plants collected from 12 Clearfield locations each of two years. If red rice plants from each Clearfield location were independently considered, straw hull and awnless red rice as main biotype was found at 11 of 12 locations for two years. In the other location across the two years, black hull and awned was main biotype. Extensive variation in red rice was detected for plant height, panicle length, tillers/plant, seeds/plant, seed set and 100 grain weight at each location for both years. Of the agronomic traits considered, plant height exhibited the largest effect on variation among the red rice population. Moreover, the same first flowering dates of red rice and

Clearfield rice were observed at 9 of 12 locations each year. This indicates that variation in pollination times of red rice biotypes should allow ample opportunity to hybridize with Clearfield and other commercial varieties. Outcrossing from Clearfield rice to red rice occurred for both years. However, no red rice-Clearfield hybrids were found at 4 locations in 2002, or at one location in 2003. Outcrossing frequencies were also different across two years. In 2002, a 0.163% outcrossing frequency was detected in comparison with the 0.679% outcrossing frequency observed in 2003. The different rate of outcrossing in two years indicated that CL121, CL141 and CL161 may have different outcrossing rates with red rice, although this would have to be verified by additional experiments. Based on results from this study, it can be concluded that outcrossing of weedy red rice will occur readily within a short period of time with Clearfield rice. For long-term management of Clearfield technology, rotation with Round-Up Ready soybean is one example to minimize the consequence of hybrids between red rice and commercial rice. Application of imazethapyr herbicide should be carried out each time that Clearfield is planted, and early-season monitoring of fields for potential outcrossing should become a standard management practice for Clearfield technology.

CHAPTER 4 GENETIC ANALYSIS OF F_2 POPULATIONS DEVELOPED FROM HYBRIDS BETWEEN CLEARFIELD RICE AND RED RICE

4.1 Introduction

With the commercial release of transgenic crops, the escape of transgenes to wild relatives has brought about ecological concern because further backcrossing of the transgene with wild relatives may result in the persistence of transgene in wild populations (Hoffman, 1990; Snow, 2002). Genetic stability in successive generations within wild relatives may play a role in successful introgression of transgenes (Scheffler and Dale, 1994). Mendelian inheritance is one criterion to evaluate genetic behavior of transgenes in successive generations after the initial crop-weed hybrid has occurred (Zhu et al., 2004).

The inheritance of random amplified polymorphic DNA (RAPD) markers in a backcross generation between *Brassica campestris* and the cross between *B. napus* and *B. campestris* was determined by Mikkelsen et al., 1996. Thirty three RAPD markers were transferred in the backcross generation at different frequencies. Thirty markers fitted the expected 1:1 segregation ratio while three markers showed abnormal segregations with the presence of one specific marker in the highest frequency (91%) and one in the lowest frequency (26%). Metz et al. (1997) studied inheritance of the herbicide resistance *bar* gene in backcrosses between *B. napus* expressing the transgene and two weedy accessions of *B. rapa*. In the first backcross generation (BC_1), the expected segregation ratio of 1:1 was found with the first accession. However, significant deviation from the 1:1 segregation ratio was detected with the second accession for BC_2, BC_3 and BC_4 generations. In another study, Zhu et al. (2004) evaluated genetic behavior of the green fluorescent protein (GFP) gene in crosses between transgenic *B. napus* lines and three wild accessions. The expected segregation ratio of 1:1 for the *gfp* gene was detected in the BC_1, but an erratic pattern of inheritance was observed in the BC_2 to BC_4 generations. The inheritance

of herbicide resistance in F_2 populations between transgenic rice lines and red rice was assessed (Oard et al., 2000; Zhang et al., 2003). Oard et al. (2000) found that 3:1, 9:7 and 7:9 segregation ratios of tolerant plants vs susceptible plants under herbicide treatment in F_2 populations between red rice and transgenic Cypress and Bengal lines expressing bar gene, indicating that herbicide resistance was controlled by one or two genes acting in a Mendelian fashion. However, abnormal segregation in herbicide resistance was also detected in some F_2 populations. Using red rice and the same transgenic Cypress line from the study of Oard et al. 2000, Zhang et al. (2003) evaluated F_2 populations derived from naturally occurring red rice-transgenic hybrids and from controlled reciprocal crosses between red rice and the transgenic line. A 3:1 segregation ratio for herbicide resistance was found in all F_2 populations derived from crosses between red rice and the transgenic Cypress. Moreover, genetic analysis of pubescent/glabrous leaves showed normal Mendelian inheritance.

The objectives of this study are to: (1) determine the inheritance of imazethapyr resistance in F_2 populations derived from natural red rice-Clearfield hybrids in 2002, (2) determine genetic control of pubescent/glabrous leaves in the F_2 populations developed from red rice-Clearfield hybrids in 2002 and (3) determine the inheritance of pubescent/glabrous leaves in F_2 populations produced from controlled crosses between Clearfield rice and red rice.

4.2 Materials and Methods

4.2.1 Pubescent/Glabrous Leaves

4.2.1.1 Plant Materials

The experimental material consisted of 44 F_2 populations derived from natural red rice-Clearfield hybrids, and 27 F_2 populations produced from control crosses between Clearfield rice and red rice. Forty four natural hybrids were obtained from red rice sample at locations 2002-1,

2002-2, 2002-3, 2002-4, 2002-5, 2002-6, 2002-10 and one ratoon crop at location 2002-11. The number of hybrids from the locations mentioned above was 1, 6, 11, 1, 8, 15, 1 and 1, respectively. Hybrids that produced at least five or more grams of seeds were planted while seeds from those hybrids that produced less than five grams were stored at 4^0C. Four populations of 27 F_2 populations developed from controlled crosses between Clearfield rice and red rice were not used in genetic analysis due to low numbers of emerged plants.

4.2.1.2 Experimental Design

Field evaluation of the F_2 populations was carried out at the Ben Hur Farm, Baton Rouge, Louisiana, 2004. The research plot area was 73.2 m x 30.5 m. The area was divided into 30 tiers with 2.4 m x 30.5 m for each tier that consisted of 80 rows with 0.36 m spacing between rows. The F_2 populations were planted on April 15, 2004. Arrosolo (5.046kg/ha), Command (0.448kg/ha), and Permit (0.07kg/ha) herbicides were applied on May 24, 2004 to maintain the area weed-free. A 13-13-13 fertilizer formulation was applied at a rate of 235.4 kg/hectare on May 20 and June 20, 2004. The pubescent/glabrous leaves were recorded for each F_2 population just before flowering, and the χ^2 test was used to test good-to fit for a 3:1, 15:1, 9:7 and 63:1 models.

4.2.2 The Inheritance of Imazethapyr Herbicide Resistance in F_2 Populations

4.2.2.1 Plant Material

The experimental material included three pooled natural red rice-Clearfield hybrid F_2 populations, and CL121 and CL161 Clearfield varieties as controls. Each of the three pooled F_2 populations was developed from an equal mixture of seeds from four natural red rice-Clearfield F_1 hybrid plants. Three pooled populations, hereafter referred to as F_2-1, F_2-2 and F_2-3, were obtained from red rice sample at locations 2002-2, 2002-3 and 2002-5.

4.2.2.2 Experimental Design

A study was performed in the greenhouse, Louisiana State University, Baton Rouge, Louisiana, August to September, 2004. Figure 4.1 shows the layout for growth and evaluation of the F_2 populations and control varieties. Seeds from the hybrid populations and the controls were soaked in distilled water for 24 hours, and pre-germinated on wet paper towels for 30 hours at 28°C in the dark. Four hundred seeds from each F_2 population and controls were germinated and placed individually into a SC-10 Super cell (Stuewe & Sons, Inc) with 3.8 cm diameter and 21 cm depth that was randomly assigned among four groups for further growth and development in the greenhouse. Rice seedlings from each group were treated at the two to three-leaf stage with 1X, 2X, 3X or 4X rates of imazethapyr herbicide, equal to 70, 140, 210 and 280 g/ha. The same treatments were applied 19 days later to the same corresponding groups.

Figure 4.1 Experimental greenhouse layout for F_2 populations derived from natural red rice-Clearfield hybrids, Louisiana State University, Baton Rouge, Louisiana, August to September, 2004

4.2.2.3 Identification of Visual Damage Symptom

Fourteen days after the second imazethapyr treatment, the number of tolerant and susceptible plants was recorded. Susceptible plants were considered those that died 28 days after treatment or did not produce new, green emerging leaves. The remaining plants were scored as tolerant plants. A goodness-to-fit χ^2 test was carried out to determine genetic control of imazethapyr resistance for the three pooled F_2 populations.

4.3 Results and Discussions

4.3.1 Inheritance of Pubescent/Glabrous Leaves

Table 4.1 represents the inheritance of pubescent/glabrous leaves in F_2 populations derived from natural red rice-Clearfield hybrids in 2002. The pubescent leaf was dominant over glabrous leaf which is consistent with previous studies (Oard et al., 2000; Zhang et al., 2003). Thirty five of 44 F_2 populations showed a 3:1 segregation ratio of pubescent leaves vs. glabrous leaves, indicating that this character was controlled by a single dominant Mendelian factor. Nine of 44 F_2 populations showed abnormal segregation for this trait. Similar to the expected genetic ratio of 3:1, the nine F_2 populations showed significant deviation from the expected genetic ratios of 9:7, 15:1 and 63:1 in the segregation of pubescent leaves vs glabrous leaves.

Table 4.2 represents the genetic behavior of pubescent/glabrous leaves in F_2 populations derived from controlled crosses between Clearfield rice and red rice. Twenty one of 23 F_2 populations showed the expected 3:1 segregation ratio of pubescent vs glabrous leaves. The remaining two populations showed abnormal segregation, not only from the expected genetic ratio of 3:1, but also from the expected ratios of 9:7, 15:1 and 63:1 in this trait.

The data from this study indicate in general that the pubescent/glabrous leaf trait segregated as a single dominant factor in F_2 populations, including F_2 populations derived from natural red

Table 4.1 Segregation of pubescent/glabrous leaves in F_2 populations derived from natural red rice-Clearfield hybrids produced from red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2004

			No. of plants				
F_2 population	Location	Total No. of plants	P*	G†	Expected genetic ratio	$\chi 2$ value	Probability
2	1	126	87	39	3:1	2.381	0.10-0.25
3	2	38	31	7	3:1	0.877	0.25-0.50
4	2	27	22	5	3:1	0.605	0.25-0.50
8	2	92	67	25	3:1	0.232	0.50-0.75
9	2	52	36	16	3:1	0.923	0.25-0.50
10	2	25	17	8	3:1	0.653	0.25-0.50
11	2	43	33	10	3:1	0.054	0.75-0.90
15	3	356	273	83	3:1	0.539	0.25-0.50
16	3	29	21	8	3:1	0.103	0.50-0.75
17	3	167	128	39	3:1	0.242	0.50-0.75
18	3	31	22	9	3:1	0.269	0.50-0.75
19	3	104	71	33	3:1	2.513	0.10-0.25
20	3	78	62	16	3:1	0.838	0.25-0.50
21	3	721	534	187	3:1	0.337	0.50-0.75
22	3	86	68	18	3:1	0.76	0.50-0.75
23	3	22	16	6	3:1	0.061	0.75-0.90
24	3	255	184	71	3:1	1.099	0.25-0.50
25	3	70	58	12	3:1	2.305	0.10-0.25
34	4	78	67	11	3:1	4.855	0.025-0.05
38	5	159	123	36	3:1	0.4717	0.25-0.50
39	5	43	34	9	3:1	0.38	0.50-0.75
40	5	55	41	14	3:1	0.006	0.90-0.95
41	5	118	100	18	3:1	5.977	0.01-0.025
42	5	121	99	22	3:1	3	0.05-0.10
43	5	119	87	32	3:1	0.227	0.50-0.75
44	5	153	128	25	3:1	4.705	0.025-0.05
45	5	165	125	40	3:1	0.05	0.75-0.90
46	6	17	16	1	3:1	3.314	0.05-0.10
47	6	10	7	3	3:1	0.1333	0.50-0.75
57	6	117	92	25	3:1	0.823	0.25-0.50
61	6	31	24	7	3:1	0.097	0.75-0.90
62	6	42	32	10	3:1	0.032	0.75-0.90
63	6	17	15	2	3:1	1.588	0.10-0.25
64	6	82	63	19	3:1	0.049	0.75-0.90
69	6	121	102	19	3:1	5.578	0.01-0.025
70	6	174	147	27	3:1	8.345	<0.005
71	6	46	39	7	3:1	2.348	0.10-0.25
75	6	279	235	44	3:1	12.675	<0.005
76	6	184	152	32	3:1	5.681	0.01-0.025
77	6	223	186	37	3:1	8.408	<0.005
78	6	187	142	45	3:1	0.087	0.75-0.90
79	6	41	32	9	3:1	0.203	0.50-0.75
80	10	101	82	19	3:1	2.063	0.10-0.25
83	11 ratoon	209	175	34	3:1	8.499	<0.005

P*: pubescent leaf, G†: glabrous leaf

rice-Clearfield hybrids in 2002 and F_2 populations derived from controlled crosses between Clearfield rice and red rice.

4.3.2 Imazethapyr Resistance Characteristics

4.3.2.1 Segregation

F_2-1, F_2-2 and F_2-3 showed segregation for tolerance to 4 concentrations of imazethapyr treatment, having no injured plants, slightly or moderately or severely affected plants, such as new, brown twisted leaf, and no emergence of a new, green leaf. With the increase of the rate of imazethapyr applied, the number of plants with no visible injury was reduced for each F_2 population. CL121 was only slightly affected at any concentration of imazethapyr treatment, through the whole treatment period, showing retarded plant height compared with controlled plants. CL161 showed almost no injury at any rate of imazethapyr treatment used.

4.3.2.2 The Genetic Behavior of Imazethapyr Herbicide Resistance

At the 2X imazethapyr treatment, F_2-1, F_2-2 and F_2-3 showed a 3:1 segregation ratio of tolerant plants vs susceptible plants, indicating that imazethapyr tolerance was controlled by a single Mendelian dominant gene. Similarly, two of three F_2 populations under the 1X imazethapyr treatment showed the expected segregation ratio of 3:1. All three F_2 populations at 3X and 4X imazethapyr treatments showed significant deviation from the expected genetic ratio of 3:1 (Table 4.3). The abnormal segregation was most likely due to presence of more susceptible plants at the 3X and 4X imazethapyr treatment levels than was expected. However, it is not known why the F_2-2 population showed significant deviation from expected genetic ratio under the 1X imazethapyr treatment as it also produced large numbers of susceptible plants.

Under the treatment of imazethepyr herbicide with proper concentration, the F_2 generation developed from red rice-Clearfield hybrids showed a simple Mendelion 3:1 genetic segregation

Table 4.2 Segregation of pubescent/glabrous leaves in F_2 populations derived from controlled crosses between Clearfield rice and red rice at Ben Hur Farm, Baton Rouge, Louisiana, 2004

		Agronomic traits					No. of plants				
F_2 population	Crosses	Red rice	Plant height (cm)	No. tillers/ plant	Panicle length (cm)	Total No. of Plants	P*	G†	Expected genetic ratio	$\chi 2$	Probability
X1	Red rice#4 * CL121	Red rice#4	124	7	23	30	22	8	3:1	0.044	0.75-0.90
X2	Red rice#10 * CL121	Red rice#10	110	14	24.2	15	13	2	3:1	1.089	0.25-0.50
X3	Red rice#11* CL121	Red rice#11	113	19	26.1	24	18	6	3:1	0	0.99-0.995
X4	Red rice#12 * CL121	Red rice#12	103	18	23.5	306	232	74	3:1	0.109	0.50-0.75
X5	Red rice#13 * CL121	Red rice#13	95	19	23.8	53	45	8	3:1	2.774	0.05-0.10
X6	Red rice#14 * CL121	Red rice#14	95	20	20.7	13	10	3	3:1	0.026	0.75-0.90
X7	Red rice#15 * CL121	Red rice#15	102	12	26	19	16	3	3:1	0.86	0.25-0.50
X8	Red rice#16 * CL121	Red rice#16	103	22	24.6	22	19	3	3:1	1.515	0.10-0.25
X9	Red rice#17 * CL121	Red rice#17	120	10	23.5	153	120	33	3:1	0.961	0.25-0.50
X10	Red rice#18 * CL121	Red rice#18	98	17	22.2	53	41	12	3:1	0.157	0.50-0.75
X12	Red rice#20 * CL121	Red rice#20	105	22	28	70	56	14	3:1	0.93	0.25-0.50
X13	Red rice#28 * CL121	Red rice#27	101	16	20.4	345	251	94	3:1	0.928	0.25-0.50
X14	Red rice#30 * CL121	Red rice#28	117	20	19.4	44	31	13	3:1	0.485	0.25-0.50
X15	Red rice#31 * CL121	Red rice#29	130	16	21.3	20	18	2	3:1	2.4	0.10-0.25
X16	Red rice#27 * CL121	Red rice#30	90	8	17.8	133	111	22	3:1	5.075	0.01-0.025
X17	Red rice#29 * CL121	Red rice#31	89	10	18.4	76	50	26	3:1	3.439	0.05-0.10
X21	CL 121 * red rice#34	Red rice#34				59	45	14	3:1	0.051	0.75-0.90
X22	CL 121 * red rice#35	Red rice#35	87	7	17	1245	931	314	3:1	0.032	0.75-0.90
X23	CL 121 * red rice#36	Red rice#36	96	8	19.5	34	29	5	3:1	1.922	0.10-0.25
X24	CL 121 * red rice#37	Red rice#37	107	12	17.4	52	42	10	3:1	0.923	0.25-0.50
X25	CL 161 * red rice#38	Red rice#38	115	26	21.6	24	18	6	3:1	0	0.99-0.995
X26	CL161 * red rice#39					366	295	71	3:1	6.124	0.01-0.025
X27	CL161 * red rice#40					410	312	98	3:1	0.263	0.50-0.75

P*: pubescent leaf, G†: glabrous leaf

Table 4.3 Segregation of imazethapyr resistance in the greenhouse for 1X-4X treatment levels in F_2 populations developed from red rice–Clearfield hybrids collected in 2002

			No. of plants				
Imazethapyr	F_2	Total No. of plants	R*	S†	Expected Genetic ratio	$\chi 2$	Probability
1X*	F_2-1	88	61	27	3:1	1.515	0.10-0.25
	F_2-2	102	63	39	3:1	9.529	<0.005
	F_2-3	103	71	32	3:1	2.023	0.10-0.25
2X*	F_2-1	104	74	30	3:1	0.821	0.25-0.50
	F_2-2	103	71	32	3:1	2.023	0.10-0.25
	F_2-3	102	80	22	3:1	0.641	0.25-0.50
3X*	F_2-1	102	59	43	3:1	4.69	0.025-0.05
	F_2-2	105	67	38	3:1	7.013	0.005-0.01
	F_2-3	99	59	40	3:1	12.529	<0.005
4X*	F_2-1	95	46	49	3:1	35.793	<0.005
	F_2-2	95	40	55	3:1	54.825	<0.005
	F_2-3	100	44	56	3:1	51.253	<0.005

R*: resistance, S†: susceptible
1X*, 2X*, 3X* and 4X*: 70, 140, 210, and 280 g ai/hectare, respectively.

ratio for imizathepyr tolerant vs susceptible plants. This pattern of inheritance was reported in all F_2 populations derived from controlled crosses and natural hybrids between transgenic Cypress and red rice (Zhang et al., 2003), and 60% of F_2 generations developed from controlled crosses between red rice and transgenic Bengal or transgenic Cypress for glufosinate herbicide tolerance (Oard et al., 2000). Similar inheritance patterns were also found in the first backcross generation of Chinese cabbage (*Brassica rapa* L.) and the hybrid between Chinese cabbage for phosphinothricin tolerance, and three *Brassica rapa* (Metz et al., 1997) and the hybrids between the three *Brassica rapa* and rapeseed expressing green fluorescent protein (*gfp*)-Bacillus thuringiensis (*bt*) transgenes for GFP-Bt expressing plants vs non-expressing plants (Zhu et al., 2004).

Pubescent/glabrous leaves in F_2 generations developed from natural red rice-Clearfield hybrids and control crosses between red rice and Clearfield rice showed the similar tendency. Three to one genetic segregation ratio was observed in 80% (35/44) of F_2 populations from natural red rice-Clearfield hybrids and 91% (21/23) F_2 populations from controlled crosses.

Other F_2 populations significantly deviated from 3:1 genetic segregation ratio. This result was consistent with that in F_2 population developed from the cross between red rice and transgenic Bengal for pubescent/glabrous leaves and two F_2 populations derived from the crosses of red rice and transgenic Bengal or Cypress for glufosinate resistance/susceptibility (Oard et al., 2000), and backcross generations of *Brassica pekinensis* and the hybrids between *Brassica pekinensis* and phosphinothricin tolerant rapeseed for phosphinothricin tolerant rapeseed plant vs susceptible plants (Metz et al., 1997), and the second, third and fourth backcross generations of three wild *Brassica rapa* and the hybrids between the three wild *Brassica rapa* and rapeseed expressing *gfp-bt* transgenes for GFP-Bt expressing plants vs non-expressing plants (Zhu et at., 2004). The possible reasons for the abnormal segregation were that the transgenes were inserted into C-chromosome of rapeseed (Metz et al., 1997; Zhu et al., 2004), were genetic silencing or genetic unstablility and maternal effect (Oard et al., 2000). However, pubescent/glabrous leaves did not produce from genetic engineering, but were innate characteristic possessed by different rice accessions. The reasons causing abnormal segregation in the F_2 generation in the present study need to be further identified.

In summary, a genetic ratio of 3:1 for pubescent vs glabrous leaves was found in 35 of 44 F_2 populations developed from natural red rice-Clearfield hybrids collected in 2002, and 21 of 23 F_2 populations derived from controlled crosses between CL121/CL161 and red rice. This suggested that the trait pubescent/glabrous leaf was controlled in most cases by a single dominant Mendelian gene, and pubescent leaf was dominant to glabrous leaf.

Imazethapyr herbicide resistance in F_2 populations showed 3:1 segregation for resistance plants vs susceptible plants for 2X imazethapyr treatments (140g a.i./ha). These results indicated that imazethapyr herbicide resistance was controlled in the red rice-Clearfield hybrids by a single

dominant Mendelian gene. Although deviation from the expected 3:1 genetic ratio was observed for a small proportion of the F_2 populations evaluated, the overall results suggest genetic control and inheritance of Newpath resistance behaved normally, a result that has practical and long-term implications for management of the Clearfield technology.

CHAPTER 5 THE PERFORMANCE OF F_1 AND F_2 POPULATIONS FROM THE CROSSES OF CLEARFIELD RICE AND RED RICE

5.1 Introduction

Spontaneous hybridization occurs between the most important food crops in the world, such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), rice (*Oryza sativa* L.) and their wild relatives (reviewed by Ellstrand et al., 1999). The hybrids produced through this process in wild populations may affect the phenotype or ecological characteristic of populations that leads to development of aggressive species and extinction of other species (Hauser et al., 1998a). Introgression of exotic characteristics depends in part on different components of fitness of hybrids and their successive generations (Hauser et al., 1998b). Reciprocal crosses of *B. napus* and *B. rapa* possessed high fitness in number of seed pods per F_1 plants, but low fitness in number of seeds per pod. On the contrary, no significant change in the fitness of hybrids between cultivated sorghum (*Sorghum bicolor*) and weedy johnsongrass (*Sorghum halepense*) was observed for various agronomic traits analyzed (Arriola et al., 1997). Similar results were demonstrated in the second backcross generation involving *B. rapa* with transgenic *B. napus*, showing no fitness advantage relative to *B. rapa* (Mason et al., 2003). Moreover, reduced fitness was observed in hybrids between weedy radish (*Raphanus raphanistrum*) and cultivated radish (*R. sativus*) compared to the parents for flowering date, number of seeds per fruit and pollen viability (Snow et al., 2001).

Rice is one of the most important cereal crops in the world (anonymous 2003c), and provides ~ 60% of caloric needs for countries that depend upon rice as a main food resource (Khush, 2003). Natural hybrids between cultivated rice and weedy red rice (Langevin et al., 1990), transgenic and weedy red rice (Zhang et al., 2003), and cultivated rice and the wild relative *Oryza rufipogon* (Chen et al., 2004; Song et al., 2004) have been observed. Langevin et

al. (1990) reported that hybrids between rice varieties and red rice possessed stronger vegetative vigor than red rice, showing taller plants with more tillers. In addition to the analysis of fitness for hybrids between cultivated rice and weedy relative, the fitness of hybrids between cultivated rice and wild relative was compared with two parents (Song et al., 2004). F_1 hybrids between the rice variety 'Minghui 63' and wild relative *Oryza rufipogon* showed higher fitness than two parents in seed set and tillers/plant. For plant height and panicle length, F_1 hybrids showed no increase or decrease in fitness relative to *Oryza rufipogon*, but higher fitness than the second parent. Oard et al. (2000) evaluated the fitness of F_2 populations from crosses of transgenic Cypress and Bengal lines, and red rice based on agronomic traits. F_2 populations produced taller plants than transgenic lines, but no significant difference in seeds/panicle relative to the two transgenic lines. With regard to seed weight per panicle, F_2 populations derived from crosses of transgenic Bengal and red rice showed no significant difference from the transgenic parent. Three of four F_2 populations produced from crosses of transgenic Cypress and red rice possessed higher seed weight/panicle than transgenic Cypress. Zhang et al. (2003) used the same transgenic Cypress line and red rice to produce F_2 populations. The F_2 populations showed no significant difference from red rice in plant height, but a significant difference from the transgenic line was detected for plant height. All F_2 populations showed significant difference from parents in seed set rate. The objectives of the present study are to compare fitness of F_1 hybrids between Clearfield rice and red rice, and F_2 populations developed from these F_1 hybrids relative to Clearfield rice.

5.2 Materials and Methods

5.2.1 Plant Materials

Experimental materials included 81 and 327 F_1 hybrids derived from red rice biotypes collected in commercial Clearfield sites in 2002 and 2003, respectively. Controlled crosses between red rice and Clearfield rice were made in a greenhouse, Louisiana State University, Baton Rouge, Louisiana, 2002. Forty-four F_2 populations were developed from red rice-Clearfield hybrids produced from red rice samples collected in 2002, and 27 F_2 populations from controlled crosses between Clearfield rice and red rice.

5.2.2 Field Trials

The study was performed at the Ben Hur Farm, Baton Rouge, Louisiana in 2003 and 2004. The field size 73.2 m x 30.5 m was evenly divided into 30 tiers, each 2.4 m x 30.5 m. Each tier consisted of 80 rows with 0.36 m spacing between two rows. All seeds from 100 individual red rice plants, collected from each of 12 commercial Clearfield locations in 2002, were planted on May 6, 2003. F_1 seedlings from controlled crosses between Clearfield rice and red rice were transferred into the field plots on May 13, 2003. Approximately 3.5 g of seeds from 100 individual red rice plants collected from each of 12 Clearfield locations in 2003 were planted at Ben Hur Farm on April 15, 2004. Seeds of 27 F_2 populations from controlled crosses between Clearfield rice and red rice were planted at the same time. F_2 seeds developed from 44 natural red rice-Clearfield hybrids were also planted. Fertilizer with a 13-13-13 formulation was applied twice at 35 days and 65 days after planting at a rate of 235.4 kg/ha. Arrosolo (5.046kg/ha), Command (0.448kg/ha), and Permit (0.07kg/ha) herbicides were applied on May 24, 2004.

A 2X imazethapyr herbicide application, equal to 140g /ha, was applied at the two to three leaf stage on June 6, 2003 for red rice samples collected in 2002, and May 6, 2004 for red rice

samples collected in 2003. A second treatment of imazethapyr at the same rate was applied 19 to 20 days later. Eighty one hybrids produced from red rice sample collected in 2002, 327 hybrids derived from red rice sample collected in 2003 were also the materials used.

5.2.3 Data Collection

Flowering date was recorded for F_1's, F_2's, and Clearfield parents that flowered before September 15 for each year. Nylon seed bags, 0.35 m X 0.40 m, were used to cover the panicles of flowering plants before the milk stage to prevent seed loss from bird damage and shattering. F_1 hybrid plants from red rice samples collected in 2002 were transferred into a greebhouse, Louisiana State University on September 15, 2003. AS in 2003, most of the F_1 hybrids (234 of 327) from red rice samples collected in 2003 did not flower in the field. Plant height was measured from the tip of the tallest panicle to the soil surface for flowering plants, and from leaf tip to soil surface for the plants not flowering in the field. Tillers/plant and panicles/plant were counted in the field. Panicles from each F_1 hybrid were harvested and placed into separate envelopes. Data for seeds/panicle, spikelets/panicle, seed set and 100 grain weight were also collected for each parent and population.

Approximately 100 panicles from each of 44 F_2 populations developed from natural red rice-Clearfield hybrids produced in red rice samples collected in 2002, and 13 F_2 populations developed from controlled crosses between Clearfield rice and red rice were collected and placed separately into paper bags after measuring plant height. Data for panicle length, seeds/panicle, spikelets/panicle, seed set and 100 grain weight were collected in the laboratory.

5.3 Results and Discussions

5.3.1 Flowering Date

Table 5.1 shows the flowering date of natural red rice-Clearfield hybrids derived from red

rice samples collected in two years, and controlled crosses between Clearfield rice and red rice. Sixteen of 81 hybrids from red rice samples collected in 2002 flowered from August 12 to August 29 in the field. The 16 plants originated from locations 2002-3, and 2002-4 and 2002-5 with 2, 7 and 7 plants, respectively. The first flowering date for Clearfield rice ranged from July 25 to August 2. Overlapping in flowering date between Clearfield rice and red rice-Clearfield hybrids was virtually nonexistent. Therefore, potential backcrossing of hybrids with Clearfield rice would occur at a very low probability. However, the hybrids will directly affect Clearfield production through competition of nutrition, water and sunlight. Moreover, the quality of Clearfield rice will decrease due to the mixture of hybrid seeds. On the other hand, red rice populations possessed extensive variation in flowering date, especially for black hull red rice. For example, black hull red rice was the main biotype at location 2002-12. The first flowering date of red rice was August 10 (the flowering date directly originated from field observation). Obviously, a late sowing time could affect the flowering date of red rice and Clearfield rice. This suggested that it is possible for red rice-Clearfield hybrids to further backcross with red rice under some conditions. Sixty five of 81 hybrids did not flower in the field. The range of first flowering date in the greenhouse for plants that did not flower in the field was from September 24 to October 7. The hybrids originated from all locations except 2002-7, 2002-8, 2002-9 and 2002-11. For the location 2002-11 ratoon crop, two hybrids were found.

Ninety three of 327 natural red rice-Clearfield hybrids from red rice samples collected in 2003 flowered in the field. The earliest flowering date was July 11, and the latest flowering date was August 22. Four of 93 flowering plants directly showed 50% tillers flowering. Twenty six of 93 flowering plants showed some panicles flowering, and some tillers did not head before September 15. Compared with 2002 results, the flowering hybrids in 2003 occurred at locations

2003-1, 2003-2, 2003-3, 2003-6, 2003-8, 2003-9, 2003-10 and 2003-11. CL121 flowered from July 9 to July 14. Only one hybrid found in CL121 field flowered on August 22, indicating that there was little chance for backcrossing between them. The probability of backcrossing of the hybrids with red rice in the field and the effect of the hybrids on commercial rice production are similar to the 16 flowering hybrids in 2002. CL161 and CLXL8 flowered in early July to early August. A high probability existed for backcrossing of the remaining 92 flowering hybrids with CL161 and CLXL8.

Similar to natural red rice-Clearfield hybrids, controlled crosses between Clearfield rice and red rice showed the same tendency in flowering time. Eight of 27 controlled crosses flowered in the field. Different hybrid combinations between Clearfield rice, CL121 and CL161, and different red rice plants, flowered at different dates, no matter whether Clearfield acted as a male or female parent. One reciprocal cross (X5 and X32) between CL121 and red rice also possessed different flowering dates.

Table 5.2 shows the flowering dates of 44 F_2 populations derived from natural red rice-Clearfield hybrids produced from red rice samples collected in 2002. The F_2 populations were divided into two sections based on flowering date. One section consisted of 10 F_2 populations. The F_1 hybrids that produced the 10 F_2 populations flowered in the field in the previous year. The remaining 34 F_2 populations developed from F_1 hybrids not flowering in the field were divided into the other group. All 10 F_2 populations flowered, and all tillers flowered from three populations. The earliest and latest flowering dates for the 10 F_2 populations were June 24 and August 11. Compared with the flowering date of their F_1 plants, earlier flowering times were detected in the F_2 populations. However, the planting date in the second year was 23 days earlier than in the first year. The remaining 34 F_2 populations all flowered in the field, but only 5

Table 5.1 Flowering date of natural hybrids from red rice samples collected in two years, and controlled crosses (F_1) at Ben Hur Farm, Louisiana , 2003 and 2004

Natural hybrids from red rice samples in 2002			Natural hybrids from red rice samples in 2003					Controlled crosses		
Field code	Location	First flowering date	Field code	Location	First flowering date	50% flowering date	Last flowering date	Field code	Combination	First flowering date
1	1	24-Sep	40	1	19-Jul	19-Jul	25-Jul	X1	Red rice#4*CL121	10-Oct
2	1	27-Sep	44	2	20-Jul	31-Jul	16-Aug	X2	Red rice#10* CL121	5-Oct
3	2	5-Oct	59	2	19-Jul	19-Jul	27-Jul	X3	Red rice#11* CL121	6-Oct
4	2	7-Oct	60	2	11-Jul	18-Jul	24-Jul	X4	Red rice#12* CL121	30-Jun
5	2	29-Sep	61	2	19-Jul	19-Jul	23-Jul	X5	Red rice#13*CL121	8-Oct
6	2	4-Oct	62	2	18-Jul	18-Jul	24-Jul	X6	Red rice#14*CL121	8-Sep
7	2	1-Oct	63	2	21-Jul	24-Jul	27-Jul	X7	Red rice#15*CL121	27-Aug
8	2	25-Sep	64	2	21-Jul	21-Jul	26-Jul	X8	Red rice#16*CL121	21-Oct
9	2	30-Sep	65	2	21-Jul	23-Jul	15-Aug	X9	Red rice#17*CL121	16-Jun
10	2	26-Sep	74	2	2-Aug	5-Aug		X10	Red rice#18*CL121	1-Oct
11	2	1-Oct	95	2	22-Jul	5-Aug		X11	Red rice#19*CL121	5-Oct
12	2	4-Oct	96	2	19-Jul	31-Jul	16-Aug	X12	Red rice#20*CL121	7-Oct
13	3	27-Sep	97	2	22-Jul	22-Jul	6-Aug	X13	Red rice#28*CL121	20-Jun
14	3	24-Sep	98	2	18-Jul	5-Aug	16-Aug	X14	Red rice#30*CL121	3-Sep
15	3	25-Aug	104	2	19-Jul	19-Jul	25-Jul	X15	Red rice#31*CL121	2-Oct
16	3	1-Oct	105	2	25-Jul	25-Jul	5-Aug	X16	Red rice#27*CL 161	2-Sep
17	3	2-Oct	106	2	24-Jul	24-Jul	4-Aug	X17	Red rice#29*CL 161	15-Jun
18	3	5-Oct	107	2	19-Jul	19-Jul	27-Jul	X19	CL121-1*red rice#32	7-Oct
19	3	7-Oct	108	2	22-Jul	27-Jul	2-Aug	X20	CL121*red rice#33	16-Jul
20	3	30-Sep	109	2	19-Jul	22-Jul	7-Aug	X21	CL121*red rice#34	1-Sep
21	3	18-Aug	122	2	10-Aug	16-Aug		X22	CL121*red rice#35	3-Oct
22	3	5-Oct	126	3	18-Jul	18-Jul	7-Aug	X23	CL121*red rice#36	11-Sep
23	3	28-Sep	127	3	18-Jul	18-Jul	29-Jul	X24	CL121*red rice#37	31-Aug
24	3	26-Sep	128	3	20-Jul	1-Aug	7-Aug	X25	CL161*red rice#38	9-Oct
25	3	3-Oct	129	3	20-Jul	29-Jul		X26	CL161*red rice#39	4-Jul
26	4	16-Aug	132	3	21-Jul	24-Jul	7-Aug	X27	CL161*red rice#40	4-Jul
27	4	24-Aug	133	3	21-Jul	24-Jul	5-Aug	X32	CL121 * Red rice#13	31-Aug
28	4	26-Aug	134	3	24-Jul	24-Jul	1-Aug			

Table 5.1 (continued)

Natural hybrids from red rice samples in 2002			Natural hybrids from red rice samples in 2003				
Field code	Location	First flowering date	Field code	Location	First flowering date	50% flowering date	Last flowering date
29	4	29-Aug	135	3	31-Jul	7-Aug	16-Aug
30	4	27-Sep	136	3	23-Jul	23-Jul	10-Aug
31	4	28-Aug	137	3	1-Aug	6-Aug	13-Aug
32	4	25-Aug	138	3	31-Jul	6-Aug	16-Aug
34	4	25-Aug	139	3	19-Jul	23-Jul	6-Aug
35	4	1-Oct	140	3	19-Jul	24-Jul	16-Aug
37	5	28-Sep	141	3	18-Jul	25-Jul	16-Aug
38	5	28-Aug	142	3	22-Jul	31-Jul	11-Aug
39	5	25-Sep	143	3	20-Jul	20-Jul	28-Jul
40	5	28-Aug	144	3	20-Jul	23-Jul	5-Aug
41	5	25-Aug	145	3	19-Jul	23-Jul	4-Aug
42	5	12-Aug	146	3	26-Jul	29-Jul	11-Aug
43	5	17-Aug	147	3	25-Jul	24-Jul	12-Aug
44	5	17-Aug	149	3	21-Jul	26-Jul	2-Aug
45	5	14-Aug	150	3	22-Jul	26-Jul	16-Aug
46	6	6-Oct	151	3	24-Jul	24-Jul	4-Aug
47	6	4-Oct	152	3	21-Jul	25-Jul	4-Aug
48	6	3-Oct	153	3	25-Jul	25-Jul	5-Aug
49	6	2-Oct	154	3	19-Jul	23-Jul	7-Aug
50	6	25-Sep	155	3	19-Jul	21-Jul	30-Jul
51	6	28-Sep	156	3	21-Jul	23-Jul	2-Aug
52	6	30-Sep	157	3	21-Jul	25-Jul	1-Aug
53	6	2-Oct	158	3	25-Jul	25-Jul	7-Aug
54	6	7-Oct	159	3	29-Jul	25-Jul	2-Aug
55	6	3-Oct	160	3	31-Jul	12-Aug	16-Aug
56	6	26-Sep	161	3	25-Jul	29-Jul	6-Aug
57	6	30-Sep	162	3	25-Jul	25-Jul	16-Aug
58	6	2-Oct	164	3	30-Jul	5-Aug	12-Aug

Table 5.1 (continued)

Natural hybrids from red rice samples in 2002			Natural hybrids from red rice samples in 2003				
Field code	Location	First flowering date	Field code	Location	First flowering date	50% flowering date	Last flowering date
59	6	27-Sep	169	3	28-Jul	30-Jul	16-Aug
60	6	5-Oct	170	3	25-Jul	31-Jul	5-Aug
61	6	6-Oct	171	3	30-Jul	30-Jul	16-Aug
62	6	28-Sep	172	3	1-Aug	1-Aug	11-Aug
63	6	5-Oct	173	3	24-Jul	24-Jul	6-Aug
64	6	25-Sep	174	3	27-Jul	29-Jul	7-Aug
65	6	26-Sep	175	3	31-Jul	6-Aug	16-Aug
66	6	29-Sep	202	6	22-Aug		
67	6	1-Oct	209	8	31-Jul	5-Aug	
68	6	2-Oct	211	8	28-Jul	6-Aug	
69	6	1-Oct	249	9	20-Jul	31-Jul	16-Aug
70	6	2-Oct	264	10	19-Jul	23-Jul	11-Aug
71	6	5-Oct	294	11	22-Jul	31-Jul	
72	6	7-Oct	295	11	30-Jul	5-Aug	
73	6	30-Sep	296	11	30-Jul	10-Aug	
74	6	26-Sep	297	11	21-Jul	31-Jul	
75	6	2-Oct	298	11	22-Jul	31-Jul	16-Aug
76	6	3-Oct	299	11	20-Jul	30-Jul	
77	6	30-Sep	303	11	30-Jul	14-Aug	
78	6	1-Oct	304	11	21-Jul	1-Aug	
79	6	2-Oct	305	11	22-Jul	6-Aug	
80	10	30-Sep	306	11	4-Aug	10-Aug	
81	12	27-Sep	307	11	3-Aug	16-Aug	
82	11 ratoon	2-Oct	308	11	23-Jul	5-Aug	
83	11 ratoon	4-Oct	310	11	23-Jul	5-Aug	
			311	11	28-Jul	4-Aug	
			312	11	22-Jul	31-Jul	16-Aug
			313	11	22-Jul	31-Jul	16-Aug

Table 5.1 (continued)

Natural hybrids from red rice samples in 2003

Field code	Location	First flowering date	50% flowering date	Last flowering date
315	11	30-Jul	4-Aug	19-Aug
316	11	22-Jul	31-Jul	
318	11	31-Jul	5-Aug	21-Aug
319	11	22-Jul	4-Aug	
321	11	6-Aug	11-Aug	
324	11	31-Jul	5-Aug	
325	11	31-Jul	5-Aug	
327	11	27-Jul	4-Aug	16-Aug
338	11	30-Jul	6-Aug	

The flowering date was recorded in the field if the date is before September 15. Otherwise, the flowering date was recorded in greenhouse.

Blank cell: no flowering

populations reached 50% flowering, and no population showed 100 % flowering of all tillers. The first flowering date for the 34 F_2 populations was from June 19 to July 14, and 50% flowering date was from July 25 to August 8.

Table 5.3 shows the flowering date of F_2 populations developed from controlled crosses between red rice and Clearfield rice. Like the grouping for F_2 populations derived from natural red rice-Clearfield hybrids, 27 F_2 populations were also divided into two groups. One group consisted of 8 F_2 populations. The F_1 hybrids that produce these F_2 populations all flowered in the field as the previous study. The remaining 19 F_2 populations were grouped together. Their F_1 hybrids did not flower in the field. The 8 F_2 populations produced tillers with 50% flowering except for F_2 populations X20-1 and X20-2 from controlled crosses that produced tillers with less than 50% flowering. Four of the 8 F_2 populations showed 100% tillers flowering. X4 and X9 F_2 populations possessed the earliest flowering date (June 28), whereas X17 F_2 population possessed the latest flowering date (August10). Of the remaining 19 F_2 populations, 4 populations showed less than 50% tillers flowering. Six populations possessed all tillers flowering. Nine populations showed more than 50% and less than 80% tillers flowering. The earliest flowering date (June23) occurred at X21 F_2 population, and the latest flowering date (August12) occurred at X22 F_2 population.

5.3.2 Other Agronomic Data

Table 5.4 shows the mean values for agronomic traits of the natural red rice-Clearfield hybrids found in red rice samples collected in 2002. Five hybrids did not produce seeds even if they were transferred to the greenhouse for growth and seed production. The remaining 76 hybrids included 37 F_1 natural hybrids from CL121 x red rice, and 39 natural hybrids from CL141 x red rice. The 37 natural red rice-CL121 hybrids were derived from red rice samples at

Table 5.2 Flowering date of F_2 populations derived from natural red rice-Clearfield hybrids from red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2004

F_2 population	First flowering date	50% flowering date	Last flowering date
2	30-Jun	5-Aug	
3	6-Jul	3-Aug	
4	24-Jun	25-Jul	
8	19-Jun		
9	28-Jun		
10	30-Jun		
11	2-Jul	5-Aug	
15	30-Jun	30-Jul	
16	14-Jul	6-Aug	
17	23-Jun		
18	28-Jun		
19	28-Jun		
20	2-Jul		
21	1-Jul	31-Jul	
22	6-Jul		
23	2-Jul		
24	22-Jun		
25	8-Jul		
34	12-Jul	25-Jul	7-Aug
38	20-Jun	20-Jul	
39	6-Jul		
40	6-Jul	25-Jul	11-Aug
41	24-Jun	25-Jul	
42	26-Jun	19-Jul	7-Aug
43	30-Jun	26-Jul	
44	1-Jul	26-Jul	
45	4-Jul	26-Jul	
46	11-Jul		
47	22-Jul		
57	28-Jun		
61	6-Jul		
62	10-Jul		
63	30-Jun		
64	5-Jul		
69	25-Jun		
70	24-Jun		
71	3-Jul	6-Aug	
75	20-Jun		
76	21-Jun		
77	21-Jun		
78	23-Jun	8-Aug	
79	1-Jul		
80	6-Jul		
83	30-Jun		

The numbers in the first column were the number of F_1 hybrids used to develop corresponding F_2 populations.
Blank cell: no flowering observed in the field up to September 15, 2002

Table 5.3 Flowering date of F_2 populations derived from controlled crosses between Clearfield rice and red rice at Ben Hur Farm, Louisiana, 2004

F_2 populations	Combinations	First flowering date	50% flowering date	Last flowering date
X1-1	Red rice#4*CL121	9-Jul	3-Aug	
X1-2		19-Jul		
X2	Red rice#10*CL121	12-Jul	31-Jul	
X3-1	Red rice#11*CL121	5-Jul	28-Jul	
X3-2		9-Jul	29-Jul	7-Aug
X3-3			6-Aug	
X4-1	Red rice#12*CL121	12-Jul	19-Jul	31-Jul
X4-2		2-Jul	28-Jul	2-Aug
X4-4		29-Jun	19-Jul	1-Aug
X4-5		8-Jul	28-Jul	2-Aug
X4-6		31-Jul	6-Aug	
X4-7		28-Jun	28-Jul	
X4-8		29-Jun	1-Aug	8-Aug
X4-9		6-Jul	22-Jul	1-Aug
X5-1	Red rice#13*CL121	29-Jun	19-Jul	
X5-2		27-Jun		
X5-3		17-Jul	27-Jul	
X6-1	Red rice#14*CL121	16-Jul		
X6-2		18-Jul		
X7-1	Red rice#15*CL121	4-Jul	18-Jul	
X7-2		10-Jul	13-Jul	
X7-3		30-Jun	18-Jul	
X8-1	Red rice#16*CL121	13-Jul	19-Jul	2-Aug
X8-2		22-Jul		
X8-3		30-Jun	30-Jul	
X9-1	Red rice#17*CL121	6-Jul	19-Jul	31-Jul
X9-2		28-Jun	19-Jul	30-Jul
X9-3		6-Jul	19-Jul	30-Jul
X9-4		4-Jul	18-Jul	31-Jul
X9-6		7-Jul	19-Jul	31-Jul
X9-7		2-Jul	19-Jul	1-Aug
X10-1	Red rice#18*CL121	29-Jun	30-Jul	
X10-2		8-Jul	30-Jul	
X10-3		18-Jul		
X11-1	Red rice#19*CL121	13-Jul		
X11-2		19-Jul	29-Jul	1-Aug
X12-1	Red rice#20*CL121	24-Jun	6-Aug	
X12-2		9-Jul	31-Jul	
X13-1	Red rice#28*CL121	19-Jul	28-Jul	9-Aug
X13-2		19-Jul	28-Jul	6-Aug
X13-3		18-Jul	28-Jul	2-Aug
X13-4		15-Jul	28-Jul	4-Aug
X13-5		16-Jul	31-Jul	6-Aug
X13-6		17-Jul	30-Jul	2-Aug
X13-7		20-Jul	28-Jul	2-Aug
X13-8		16-Jul	29-Jul	2-Aug
X13-10		15-Jul	27-Jul	6-Aug

Table 5.3 (continued)

F_2 populations	Combinations	First flowering date	50% flowering date	Last flowering date
X13-11		10-Jul	26-Jul	1-Aug
X14-1	Red rice#30*CL121	4-Jul		
X14-2		15-Jul		
X15	Red rice#31*CL121	16-Jul		
X16	Red rice#27*CL161	24-Jun	31-Jul	
X17-1	Red rice#29*CL161	15-Jul	29-Jul	
X17-2		18-Jul	31-Jul	2-Aug
X17-3		7-Jul	15-Jul	10-Aug
X19-1	CL121*red rice#32			
X19-2		16-Jul	6-Aug	
X20-1	CL121*red rice#33			
X20-2				
X20-3		8-Jul	30-Jul	
X21-1	CL121*red rice#34	28-Jun	18-Jul	
X21-2		8-Jul		
X21-3		23-Jun		
X22-1	CL121*red rice#35	15-Jul	28-Jul	6-Aug
X22-2		17-Jul	31-Jul	
X22-3		19-Jul	31-Jul	
X22-4		19-Jul	30-Jul	9-Aug
X22-5		17-Jul	29-Jul	
X22-6		16-Jul	30-Jul	9-Aug
X22-7		17-Jul	31-Jul	
X22-8		17-Jul	31-Jul	9-Aug
X22-9		15-Jul	3-Aug	12-Aug
X22-10		14-Jul	31-Jul	
X22-11		15-Jul	31-Jul	
X22-12		15-Jul	28-Jul	
X22-13		15-Jul	30-Jul	10-Aug
X-22-14		15-Jul	30-Jul	
X22-15		16-Jul	29-Jul	1-Aug
X22-16		15-Jul	30-Jul	
X22-17		18-Jul	28-Jul	
X22-18		18-Jul	30-Jul	
X22-19		18-Jul	28-Jul	9-Aug
X22-20		18-Jul	31-Jul	
X22-21		18-Jul	30-Jul	
X22-22		15-Jul	30-Jul	
X23-1	CL121*red rice#36	12-Jul	19-Jul	
X23-2		1-Jul	19-Jul	
X23-3		14-Jul	29-Jul	
X24-1	CL121*red rice#37	7-Jul	28-Jul	
X24-2		20-Jul	26-Jul	
X24-3		6-Jul	30-Jul	
X25	CL161*red rice#38	28-Jun		
X26-1	CL161*red rice#39	11-Jul	26-Jul	7-Aug
X26-2		14-Jul	29-Jul	
X26-3		12-Jul	31-Jul	4-Aug

Table 5.3 (continued)

F_2 populations	Combinations	First flowering date	50% flowering date	Last flowering date
X26-4		14-Jul	19-Jul	2-Aug
X27-1	CL161*red rice#40	14-Jul	30-Jul	
X27-2		9-Jul	28-Jul	
X27-3		14-Jul	28-Jul	9-Aug
X27-4		17-Jul	30-Jul	2-Aug
X27-5		18-Jul	27-Jul	2-Aug
X27-6		11-Jul	31-Jul	
X32	CL121*Red rice#13	30-Jul	5-Aug	

X1-1 and X1-2 were regarded as one F_2 (X1) population developed from two plants harvested separately from controlled cross X1. Other controlled crosses were the same.
Blank cell: no flowering

locations 2002-1, 2002-2, 2002-3, 2002-4, 2002-10, 2002-12, the ratoon crop at location 2002-11, and the 39 natural red rice-CL141 hybrids at locations 2002-5 and 2002-6. With regard to the 37 red rice-CL121 hybrids, heterosis over the CL121 parent was observed for plant height, tiller production and panicle production. On the other hand, CL121 exhibited an advantage in seeds/panicle and seed set rate. The hybrids showed no decreased or increased vigor in panicle length, spikelets/panicle and grain weight over CL121. When the hybrids were analyzed based on locations, the hybrids showed differences in comparison with CL121 in tillers/plant, spikelets/plant and panicle length. Some hybrids possessed hybrid vigor, but others showed no heterosis for tillers/plant, spikelets/plant and panicle length over CL121 (Table 5.5a). For the 39 red rice-CL141 hybrids, the hybrids showed heterosis for plant height, tillers/plant, panicles/plant and panicle length over CL141. CL141 produced greater seeds/plant, seed set rate and grain weight than the hybrids at α=0.05 level. No significant difference was found in spikelets/panicle between the hybrids and CL141. Similar to the hybrids from CL121 to red rice, significant differences were observed for some agronomic traits between the hybrids and CL141 when location factor was included in significant test. The hybrids showed no significant difference from CL141 in grain weight at one of two locations. On the contrary, the hybrids showed heterosis in spikelets/panicle over CL141 at one of two locations (Table 5.5b).

Tables 5.6a and 5.6b show mean values of agronomic traits for natural red rice-Clearfield hybrids in 2003. A total of 93 of 327 hybrids flowered, but one hybrid did not produce seeds before September 15, so 92 of 327 hybrids were used for the analyses of agronomic traits. The 92 natural hybrids were derived from red rice sample at locations 2003-1, 2003-2, 2003-3, 2003-8, 2003-9, 2003-10 and 2003-11. All the hybrids were produced from red rice * CL161. The hybrids showed hybrid vigor in plant height, panicles/plant, panicle length and spikelets/panicle, but no significant difference in seeds/panicle, seed set rate and grain weight over CL161. When the hybrids were compared with CL161 among different locations, the hybrids exhibited hybrid vigor for all agronomic traits at some locations except for seed set rate (Table 5.7).

Table 5.8 shows means of agronomic traits for controlled crosses between Clearfield rice and red rice. The controlled crosses included 22 hybrids between CL121 and red rice, and five hybrids between CL161 and red rice. With regard to the 22 hybrids between CL121 and red rice, the hybrids showed hybrid vigor in plant height and tillers/plant, but no decrease or increase in panicles/plant, panicle length and spikelets/panicle vs. CL121. However, CL121 produced higher seeds/panicle, seed set rate and 100 grain weight compared to the hybrids. The same tendency between CL121 and the red rice-CL121 hybrids was also exhibited in five hybrids between CL161 and red rice in all agronomic traits except for grain weight. No significant difference was found for grain weight between the hybrids and CL161 (Table 5.9).

Table 5.10 shows the average of agronomic traits of 44 F_2 populations derived from red rice-Clearfield hybrids produced from red rice samples collected in 2002. The 44 F_2 populations included 23 F_2 populations developed from natural hybrids from red rice x CL121 and 21 F_2 populations developed from natural hybrids between red rice x CL141. For the 23 F_2 populations, the hybrids showed hybrid vigor for plant height and panicles/plant over CL121, but showed a

Table 5.4 Means of agronomic traits of natural red rice-Clearfield hybrids (F_1) derived from red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2003

Hybrid no.	Plant height (cm)	No. tillers	No. panicle	Panicle length (cm)	No. spikelets/ panicle	No. seeds/panicle	Seed set rate (%)	100 grain weight (g)
1	101	81	16	19	122.6	3.1	2.56	1.44
2	107	53	28	27	262.5	31.6	12.04	2.29
3	97	71	35	21.7	107.5	11.7	10.93	1.88
4	99	61	27	20.7	112.6	11.6	10.3	1.91
5	91	42	5	15.6	75	6.0	8	1.19
6	101	73	7	14.4	69.1	1.6	2.27	1.29
7	105	72						
8	91	60	16	22.2	124	40.4	32.56	2.25
9	99	28	12	20.3	157.1	35.7	22.71	2.05
10	114	92	45	20.1	115.8	6.8	5.91	1.98
11	109	57	27	19.8	102.6	16.7	16.32	2.05
12	106	62	31	18.1	76.1	5.4	7.08	2.01
13	102	51	12	18.4	81.4	22.4	27.53	0.89
14	100	27	6	16.9	60.8	28.7	47.12	1.86
15	121	64	34	21.7	196.3	79.0	40.25	1.95
16	93	75	13	18.2	59.2	27.2	45.9	1.84
17	108	92	41	18.5	112.9	32.6	28.85	1.62
18	112	121	30	19.6	104.3	11.7	11.25	1.82
19	89	76	44	20.5	95	21.9	23.04	1.73
20	95	60	39	18.9	90.1	31.2	34.63	1.05
21	115	81	81	23.5	104.1	66.3	63.66	2.13
22	102	99	36	20.4	113.1	30.9	27.31	1.41
23	101	84	29	17.1	62.3	14.6	23.35	1.67
24	115	95	57	23.5	58.1	33.8	58.18	2.09
25	96	84	33	18	70.6	24.6	34.86	1.78
26	97	34	33	20.4	245.9	6.2	2.51	2.02
27	98	23	21	22.5	205.7	4.2	2.06	2.07
28	111	53	43	22.9	255.6	2.6	1.03	1.99
29	91	34	9	24.2	209.7	0.9	0.42	2.35
30	102.5	29	12	26.4	203.5	2.6	1.27	2.29
31	114	25	18	26.7	216.2	2.1	0.98	2.17
32	100.5	31	17	24.6	256.8	4.2	1.63	1.9
34	97	22	20	20.9	149.4	40.6	27.14	2.08
35	114	42	6	22.2	85.2	16.3	19.18	1.83
37	120	60	12	19.7	162.2	10.8	6.68	1.59
38	111.4	44	33	24.8	71.3	35.5	49.79	2.12
39	120	71	54	18.5	70.8	11.5	16.19	1.34
40	112	67	16	29.2	160.6	44.2	27.51	1.76
41	108	80	30	25.6	107	38.0	35.55	1.96
42	106	44	21	24.4	150.2	45.2	30.09	2.02
43	114	50	30	24.8	94.7	37.5	39.6	1.82
44	130	54	28	24.4	101.5	44.6	44	2.08
45	110	73	35	23.9	119.1	33.4	28.05	2.08

Table 5.4 (continued)

Hybrid no.	Plant height (cm)	No. tillers	No. panicle	Panicle length (cm)	No.spikelet/ panicle	No. seeds/plant	Seed set rate (%)	100 grain weight (g)
46	117	81	47	19.9	102.4	7.2	7.03	1.86
47	120	96	41	22.3	107	8.2	7.66	1.79
48	116	59	10	22.8	105.2	9.1	8.65	1.77
49	115	40	11	22.6	96.3	11.5	11.9	2.01
50	104	67	21	19.2	84.4	9.0	10.72	1.77
51	116	77	5	17.9	13.2	12.4	93.94	1.81
52	112	91	34	21.2	92.7	7.7	8.35	1.89
53	103	68	7	22	101.9	5.4	5.33	1.76
54	117	61	9	21	17.9	9.9	55.28	1.74
55	115	55	15	20.2	133.9	10.5	7.87	1.66
56	108	58						
57	114	63	37	22.6	152.6	27.7	18.17	1.8
58	124	44						
59	117	53	18	19.8	87.1	3.1	3.51	1.71
60	114	69						
61	119	77	28	24.2	60.4	16.9	27.99	1.87
62	117	72	22	22.1	91.2	23.7	26.02	1.9
63	112	65	35	21.6	90	11.1	12.35	1.86
64	111	36	36	20.8	103	22.4	21.71	1.86
65	114	30	9	19.9	83.1	14.1	16.98	2.08
66	112	22	5	19.8	115.8	16.2	13.99	1.89
67	113	44	7	21.8	97.7	25.4	26.02	1.76
68	117	36	6	20.8	89.3	18.2	20.34	1.67
69	120	53	38	22.2	110.1	28.5	25.91	1.85
70	112	36	35	21.9	98.6	38.2	38.76	1.98
71	113	42	27	22.4	116.1	22.5	19.39	1.78
72	116	53	11	19.9	107.5	11.3	10.48	1.73
73	121	41						
74	120	50	20	23.3	118.3	11.9	10.06	1.7
75	110	59	49	23.8	89.7	46.1	51.46	1.9
76	108	62	45	21.7	102.8	32.3	31.47	1.99
77	110	40	39	22.9	55.3	44.3	80.19	2
78	114	63	43	24.3	53.7	37.1	69.1	1.95
79	114	46	31	23	145.5	19.0	13.04	1.92
80	99	91	43	22.4	81.4	25.4	31.18	1.72
81	104	117	30	19.4	102.8	7.3	7.07	1.84
82	105	42	28	19.8	84.1	7.2	8.53	1.82
83	103	63	53	23	96.3	37.8	39.24	1.86
CL121	80	3.7	3.7	18	70	80	92.48	2.1
CL141	87	4.1	4.1	17.9	76.2	82.4	87.50	2.0

The hybrids underlined flowered in field before 09/15/2003.
Plant height for these flowering plants in the field was measured from stem base to panicle top, whereas plant height for those non-flowering plants was measured from stem base to tip of highest leaf.
All plants were transferred from the field to the greenhouse on September 15, 2003.
Blank cell: not applicable

Table 5.5a Mean values for agronomic traits for natural red rice-CL121 hybrids (F_1) produced from red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2003

Locations	Plant height	No. tillers /plant	No. panicles/ plant	Panicle length	No. spikelets/ panicle	No. seeds/ panicle	Seed set rate	Grain weight
2002-1	104[a]	67.0[bcd]	22.0[ab]	23.0[a]	192.6[a]	17.4[b]	7.30[c]	1.87[a]
2002-2	100.8[a]	60.7[bcd]	22.8[ab]	19.2[a]	104.4[b]	15.1[b]	12.90[bc]	1.85[a]
2002-3	103.8[a]	77.6[bc]	35.0[ab]	19.6[a]	92.9[b]	32.7[b]	35.84[b]	1.68[a]
2002-4	102.8[a]	32.6[de]	19.9[ab]	23.4[a]	203.1[a]	8.9[b]	6.25[c]	2.08[a]
2002-10	99[a]	91.0[ab]	43.0[a]	22.4[a]	81.4[b]	25.4[b]	31.18[bc]	1.72[a]
2002-12	104[a]	117.0[a]	30.0[ab]	19.4[a]	102.8[b]	7.3[b]	7.07[c]	1.84[a]
2002-11 ratoon	104[a]	52.5[cd]	40.5[a]	21.4[a]	90.2[b]	22.5[b]	23.89[bc]	1.84[a]
CL121	80.0[b]	3.7[e]	3.7[b]	18.0[a]	80.0[b]	70.0[a]	92.48[a]	2.1[a]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

Table 5.5b Mean values of agronomic traits for natural red rice-CL141 hybrids (F_1) produced from red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2003

Locations	Plant height	No. tillers /plant	No. panicles/ plant	Panicle length	No. spikelets/ panicle	No. seeds/ panicle	Seed set rate	Grain weight
5	114.6[a]	60.3[a]	28.8[a]	23.9[a]	115.3[a]	33.4[b]	30.83[b]	1.86[ab]
6	113.9[a]	56.6[a]	24.7[a]	21.6[b]	94.1[ab]	18.7[c]	25.12[b]	1.84[b]
CL141	87[b]	4.1[b]	4.1[b]	17.9[c]	82.4[b]	76.2[a]	87.50[a]	2.00[a]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

Table 5.6a Means of agronomic traits of natural red rice-Clearfield hybrids (F_1) derived from red rice samples collected in 2003 at Ben Hur Farm, Louisiana, 2004

Field code	Location	Plant height (cm)	Panicle length (cm)	No. seeds/panicle	No. spikelets/panicle	Seed set rate (%)	100 grain weight (g)
40	1	66	21.2	46.2	196.2	23.55	1.654
44	2	129	27.2	233.0	261.6	89.04	2.264
59	2	131	32	197.4	277.0	71.26	2.15
60	2	129	26.4	93.2	215.2	43.32	1.926
61	2	128	29.2	159.7	219.3	72.82	2.223
62	2	126	29.1	153.0	198.2	77.20	2.163
63	2	125	28.2	140.9	217.5	64.77	2.197
64	2	123	24	72.7	115.1	63.17	2.115
65	2	133	31.1	135.0	225.0	60.00	2.183
74	2	126	30.2	249.5	281.9	88.51	2.069
95	2	121	31.5	55.9	247.3	22.61	2.227
96	2	121	32.1	144.5	257.3	56.15	2.277
97	2	126	30.4	209.1	289.5	72.22	1.193
98	2	127	32.1	105.1	198.5	52.95	2.203
104	2	62	18.4	51.9	121.9	42.59	1.594
105	2	102	30	128.3	222.3	57.72	2.284
106	2	111	30.6	146.7	246.7	59.47	2.291
107	2	135	32	50.7	87.7	57.80	2.291
108	2	119	31.4	124.3	209.7	59.28	2.321
109	2	130	33.2	63.4	142.4	44.53	2.371
122	2	115	31.6	125.0	269.8	46.33	2.123
126	3	119	29.4	101.0	122.4	82.52	2.128
127	3	124	22.6	68.6	86.4	79.39	2.093
128	3	142	25.3	179.3	225.3	79.58	2.172
129	3	130	27.7	116.8	141.2	82.72	2.153
132	3	122	26.2	106.3	123.5	86.08	2.209
133	3	117	27	94.1	116.9	80.49	2.243
134	3	113	26.9	102.8	120.2	85.53	2.246
135	3	116	27.8	210.5	219.3	95.99	2.101
136	3	120	26.6	68.6	86.6	79.22	2.239
137	3	105	25.6	128.1	147.1	87.08	2.269
138	3	138	32	114.7	239.7	47.84	2.402
139	3	120	23.6	77.5	95.5	81.16	2.119
140	3	117	26.9	5.0	78.0	6.41	1.504
141	3	116	23.6	15.4	94.0	16.38	1.979
142	3	125	24.9	118.5	127.5	92.94	2.143

Table 5.6a (continued)

Field code	Location	Plant height (cm)	Panicle length (cm)	No. seeds/panicle	No. spikelets/panicle	Seed set rate (%)	100 grain weight (g)
143	3	85	23.3	130.5	144.5	90.31	1.626
144	3	118	29.1	140.1	151.5	92.48	2.148
145	3	124	27.9	73.7	88.7	83.09	2.199
146	3	130	26.6	163.4	183.4	89.09	2.192
147	3	125	26.1	91.6	101.6	90.16	2.135
149	3	122	26.3	106.4	122.4	86.92	2.166
150	3	122	28	178.3	200.1	89.13	2.154
151	3	132	27.7	134.5	148.3	90.70	2.251
152	3	134	27.1	80.2	97.6	82.17	2.264
153	3	126	24.5	83.0	108.6	76.42	2.039
154	3	121	24.2	76.7	88.1	87.06	2.152
155	3	122	22.7	43.5	54.1	80.41	2.108
156	3	121	26.4	73.6	100.3	73.33	2.095
157	3	118	27.8	133.8	162.2	82.49	2.055
158	3	121	26.4	79.4	111.4	71.29	2.212
159	3	126	25.9	48.3	62.1	77.76	2.145
160	3	119	26.2	167.4	185.4	90.29	2.149
161	3	117	27.1	130.4	142.8	91.32	2.131
162	3	115	25	75.5	92.1	81.97	2.102
164	3	133	24.5	122.9	145.7	84.35	2.064
169	3	128	26.2	129.6	139.0	93.24	2.101
170	3	108	24.7	90.9	115.1	78.98	2.093
171	3	132	23.6	105.6	120.6	87.56	2.158
172	3	117	24.8	113.5	141.1	80.44	2.174
173	3	118	24.2	75.9	85.7	88.56	2.093
174	3	127	26	91.3	104.5	87.37	2.116
175	3	126	26.7	166.1	184.5	90.03	2.142
209	8	120	27.8	142.1	204.1	69.62	2.067
211	8	100	26.6	93.6	179.2	52.24	2.084
249	9	119	30.4	105.1	175.9	59.76	2.23
264	10	116	24.2	66.0	95.2	69.32	2.55
294	11	126	31.1	161.2	261.2	61.72	2.204
295	11	122	31.3	152.8	234.8	65.08	2.265
296	11	121	32.5	148.7	270.5	54.98	2.371
297	11	117	30	122.6	202.8	60.45	2.158
298	11	115	29.2	115.6	178.6	64.73	2.139
299	11	117	28.7	81.4	163.6	49.76	2.334

Table 5.6a (continued)

Field code	Location	Plant height (cm)	Panicle length (cm)	No. seeds/panicle	No. spikelets/panicle	Seed set rate (%)	100 grain weight (g)
303	11	120	32.3	159.3	264.1	60.32	2.241
304	11	120	27.9	177.5	207.9	85.38	2.182
305	11	123	29.2	137.8	204.8	67.28	2.187
306	11	121	30.5	175.8	241.4	72.83	2.237
307	11	119	29.5	143.1	193.7	73.88	2.291
308	11	127	30.4	174.6	238.0	73.36	2.249
310	11	124	30.9	169.1	224.1	75.46	2.31
311	11	125	30.7	158.8	231.0	68.74	2.137
312	11	118	30.1	140.4	197.4	71.13	2.272
313	11	126	30.6	126.3	202.5	62.36	2.226
315	11	117	31.7	139.5	215.9	64.61	2.112
316	11	125	29.4	167.5	225.7	74.21	2.338
318	11	123	29.1	159.0	200.8	79.18	2.152
319	11	127	28.9	158.2	232.4	68.07	2.281
321	11	99	32	100.1	269.3	37.16	2.266
324	11	119	31	181.8	263.8	68.92	2.218
325	11	123	29.6	137.6	216.2	63.65	2.097
327	11	126	30.8	102.9	200.9	51.23	2.307
338	11	126	30	197.1	262.3	75.15	2.226
CL121		75.8	18.9	74.4	87.8	84.8	2.3
CL161		86	19.7	70.9	78.7	90.0	2.2
CLXL8		82.8	22.4	101.1	122.8	82.3	2

Five panicles per plant are sampled for analysis of agronomic traits except for #40, #60, #128, #150 and #156, and #144. Agronomic traits are investigated based on four panicles for the former five plants, three panicles for the last plant, respectively.

Table 5.6b Means of agronomic traits of natural hybrids (F_1) from Clearfield rice to red rice derived from red rice samples collected in 2003 at Ben Hur Farm, Louisiana, 2004

Field code	Location	Tiller production of non flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
40	1		72	
41	2	54		129
42	2	53		131
43	2	75		120
44	2		72	
45	2	54		111
46	2	52		125
47	2	43		129
48	2	29		131
49	2	15		126
50	2	38		132
51	2	32		128
52	2	53		127
53	2	69		125
54	2	72		117
55	2	67		128
56	2	89		131
57	2	75		123
58	2	77		133
59	2		41	
60	2		23	
61	2		13	
62	2		15	
63	2		15	
64	2		14	
65	2		34	
66	2	36		126
67	2	25		126
68	2	23		123
69	2	55		131
70	2	66		130
71	2	45		138
72	2	86		135
73	2	53		128
74	2		37	
75	2	55		132
76	2	54		130
77	2	46		120
78	2	53		126
79	2	45		132
80	2	50		134
81	2	55		133
82	2	24		127
83	2	41		128
84	2	18		130
85	2	27		138
86	2	48		124
87	2	85		133
88	2	37		128
89	2	51		132
90	2	70		128
91	2	72		127
92	2	87		124
93	2	82		120
94	2	49		128
95	2		35	
96	2		22	

Table 5.6b (continued)

Field code	Location	Tiller production of non flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
97	2		18	
98	2		37	
99	2	55		133
100	2	65		121
101	2	72		122
102	2	77		130
103	2	83		122
104	2		59	
105	2		29	
106	2		20	
107	2		43	
108	2		20	
109	2		52	
110	2	110		137
111	2	35		134
112	2	50		142
113	2	25		132
114	2	19		134
115	2	43		136
116	2	22		135
117	2	13		132
118	2	20		139
119	2	21		140
120	2	37		142
121	2	66		139
122	2		37	
123	2	50		136
124	2	45		141
125	2	47		130
126	3		42	
127	3		58	
128	3		85	
129	3		60	
130	3	102		122
131	3	103		120
132	3		54	
133	3		47	
134	3		42	
135	3		25	
136	3		76	
137	3		59	
138	3		101	
139	3		85	
140	3		50	
141	3		82	
142	3		23	
143	3		13	
144	3		53	
145	3		52	
146	3		62	
147	3		66	
148	3	81		126
149	3		48	
150	3		38	
151	3		36	
152	3		26	

Table 5.6b (continued)

Field code	Location	Tiller production of non-flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
153	3		39	
154	3		40	
155	3		22	
156	3		16	
157	3		41	
158	3		49	
159	3		44	
160	3		51	
161	3		44	
162	3		53	
163	3	86		102
164	3		44	
165	3	56		119
166	3	37		121
167	3	56		123
168	3	59		130
169	3		43	
170	3		30	
171	3		40	
172	3		33	
173	3		39	
174	3		34	
175	3		38	
176	5	88		125
177	5	85		124
178	5	91		120
179	5	82		148
180	6	142		113
181	6	99		109
182	6	89		107
183	6	95		131
184	6	134		119
185	6	69		126
186	6	39		109
187	6	45		123
188	6	22		125
189	6	81		119
190	6	82		117
191	6	36		120
192	6	37		115
193	6	54		132
194	6	101		118
195	6	105		134
196	6	125		115
197	6	52		115
198	6	102		112
199	6	114		120
200	6	136		124
201	6	85		103
202	6	128	9	
203	6	126		111
204	7	79		112
205	7	64		110
206	7	95		128
207	7	95		110
208	7	79		115

Table 5.6b (continued)

Field code	Location	Tiller production of non flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
209	8	81		117
210	8	128		115
211	8		55	
212	8	51		123
213	8	65		118
214	8	116		125
215	8	107		123
216	8	108		128
217	8	122		118
218	8	128		127
219	8	98		115
220	8	101		114
221	8	100		115
222	8	110		123
223	8	93		119
224	8	56		119
225	8	90		116
226	8	88		116
227	8	81		133
228	8	61		120
229	8	95		146
230	8	59		130
231	8	55		134
232	8	89		122
233	9	124		122
234	9	59		134
235	9	49		128
236	9	73		123
237	9	107		118
238	9	71		131
239	9	46		128
240	9	98		122
241	9	86		128
242	9	104		124
243	9	121		128
244	9	125		120
245	9	108		123
246	9	52		124
247	9	59		123
248	9	113		116
249	9		97	
250	10	116		117
251	10	94		133
252	10	69		122
253	10	66		130
254	10	26		138
255	10	38		128
256	10	36		140
257	10	70		129
258	10	84		125
259	10	111		125
260	10	102		133
261	10	103		124
262	10	107		130
263	10	97		123
264	10		71	

Table 5.6b (continued)

Field code	Location	Tiller production of non flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
265	10	86		122
266	10	31		117
267	10	72		128
268	10	52		117
269	10	90		115
270	10	107		113
271	10	77		122
272	10	45		145
273	10	35		139
274	10	14		140
275	10	21		136
276	10	37		126
277	10	50		138
278	10	19		133
279	10	31		133
280	10	33		139
281	10	67		136
282	10	90		133
283	10	110		115
284	10	71		118
285	10	61		129
286	10	15		125
287	10	33		130
288	10	40		133
289	10	19		128
290	10	33		127
291	10	34		124
292	10	58		124
293	10	109		116
294	11		92	
295	11		61	
296	11		46	
297	11		56	
298	11		59	
299	11		51	
300	11	52		127
301	11	80		121
302	11	93		117
303	11		68	
304	11		60	
305	11		55	
306	11		36	
307	11		56	
308	11		68	
309	11	69		127
310	11		62	
311	11		72	
312	11		52	
313	11		79	
314	11	115		128
315	11		75	
316	11		82	
317	11	93		121
318	11		53	
319	11		51	
320	11	101		114

Table 5.6b (continued)

Field code	Location	Tiller production of non flowering plants	Panicle production of flowering plants	Plant height for non-flowering plant
321	11		55	
322	11	126		128
323	11	111		122
324	11		80	
325	11		78	
326	11	104		128
327	11		84	
328	11	80		137
329	11	88		123
330	11	72		134
331	11	65		134
332	11	98		131
333	11	75		129
334	11	110		123
335	11	68		122
336	11	99		125
337	11	104		123
338	11		57	
339	12	70		123
340	12	49		124
341	12	27		121
342	12	70		117
343	12	59		114
344	12	58		117
345	12	74		124
346	12	68		131
347	12	111		127
348	12	119		134
349	12	153		116
350	12	68		119
351	12	76		122
352	12	137		116
353	12	132		120
354	12	124		111
355	12	119		110
356	12	97		111
357	12	74		120
358	12	29		125
359	12	64		119
360	12	79		115
361	12	87		122
362	12	85		126
363	12	69		128
364	12	57		127
365	12	71		136
366	12	101		124
CL121		5.2	5.2	
CL161		5	5	
CLXL8		4.4	4.4	

Blank cell: not applicable for data

Table 5.7 Mean values of agronomic traits for natural red rice-CL161 hybrids (F_1) derived from red rice samples collected in 2003 at Ben Hur Farm, Louisiana, 2004

Locations	Plant height	No. panicles/plant	Panicle length	No. seeds/panicle	No. spikelets/panicle	Seed set rate	Grain weight
2003-1	66.0^{b}	72.0^{ab}	21.2^{c}	46.2^{b}	196.2^{ab}	23.55^{b}	1.65^{c}
2003-2	121.0^{a}	31.8^{cd}	29.5^{a}	132.0^{ab}	215.2^{ab}	60.09^{a}	2.12^{b}
2003-3	121.7^{a}	47.2^{bc}	26.1^{ab}	105.1^{ab}	128.7^{bcd}	80.48^{a}	2.13^{b}
2003-8	110.0^{a}	32.0^{cd}	27.2^{ab}	117.9^{ab}	191.7^{ab}	60.93^{a}	2.08^{b}
2003-9	119.0^{a}	97.0^{a}	30.4^{a}	105.1^{ab}	175.9^{abc}	59.76^{a}	2.23^{ab}
2003-10	116.0^{a}	71.0^{ab}	24.2^{bc}	66.0^{ab}	95.2^{cd}	69.32^{a}	2.55^{a}
2003-11	121.0^{a}	63.5^{abc}	30.3^{a}	147.6^{a}	224.2^{a}	65.99^{a}	2.23^{ab}
CL161	85.8^{b}	5.0^{d}	19.7^{c}	70.9^{ab}	79.4^{d}	90.99^{a}	2.22^{ab}

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

Table 5.8 Means of agronomic traits for F_1 from controlled crosses between Clearfield rice and red rice at Ben Hur Farm, Louisiana, 2003. (Imazethapyr herbicide not applied)

Hybrid	Cross	Plant height (cm)	Tiller production	Panicle production	Panicle length (cm)	No. seeds/ panicle	No. spikelets/ panicle	Seed set rate (%)	100 grain weight (g)
X1	Red rice#4*CL121	129	54.5	5.0	21.8	19.0	116.7	16.15	2.005
X2	Red rice#10*CL121	136	47	9.0	20.7	19.0	92.0	20.65	1.830
X3	Red rice#11*CL121	132.3	49.7	6.0	21.0	24.4	97.3	25.14	1.767
X4	Red rice#12*CL121	133.9	41.2	8.0	21.1	29.9	60.0	50.21	2.057
X5	Red rice#13*CL121	176.2	23	7.0	14.8	14.1	40.1	34.38	1.680
X6	Red rice#14*CL121	170.6	23.8	3.0	19.7	21.3	107.0	21.41	1.760
X7	Red rice#15*CL121	169.3	35.5	5.3	19.4	25.0	104.6	18.37	1.747
X8	Red rice#16*CL121	190.4	18	6.3	14.6	5.8	29.7	19.06	1.870
X9	Red rice#17*CL121	129.6	26.2	5.4	18.3	23.3	40.0	58.72	2.021
X10	Red rice#18*CL121	195.2	19	6.3	13.2	20.2	36.1	51.34	1.903
X11	Red rice#19*CL121	199	29.5	2.5	10.5	10.2	21.9	50.08	1.755
X12	Red rice#20*CL121	169.6	14	4.5	26.8	46.7	186.0	24.36	1.885
X13	Red rice#28*CL121	149	54	7.7	17.4	24.0	45.6	55.55	2.065
X14	Red rice#30*CL121	179	19.4	5.5	19.7	21.6	54.9	32.66	2.005
X15	Red rice#31*CL121	158	35	2.0	30.0	54.5	235.0	23.19	1.970
X16	Red rice#27*CL161	175.4	28.2	14.0	26.7	69.0	171.6	40.22	1.860
X17	Red rice#29*CL161	142.4	73.2	7.6	16.3	13.8	34.0	39.28	1.764
X19	CF 121*red rice#32	157.6	31.4	2.0	15.4	2.5	24.9	10.06	1.835
X20	CF 121*red rice#33	140.8	32.2	1.3	11.9	14.2	32.7	28.30	1.747
X21	CL121*red rice#34	162.2	13.4	7.7	14.6	23.0	28.4	81.90	1.793
X22	CL121*red rice#35	136.6	69.4	13.8	17.0	24.1	39.7	61.01	2.100
X23	CL121*red rice#36	144.6	29.4	3.0	21.1	32.2	106.8	31.34	1.593
X24	CL121*red rice#37	149.8	20.6	5.0	17.9	18.8	50.8	25.34	1.760
X25	CL161*red rice#38	170	28.8	4.0	28.1	56.5	204.5	27.63	1.430
X26	CL161*red rice#39	125.5	50.5	15.5	19.0	30.0	74.0	47.40	1.938
X27	CL161*red rice#40	125.4	48	18.2	17.1	21.2	46.5	44.57	1.998
X32	CL121*Red rice#13	132	80	7.0	12.3	7.1	24.6	29.07	1.470
CL121		84	5.2	5.2	19.5	101	123.4	81.85	2.25
CL161		95	6.2	6.2	20.1	105	127	82.68	2.1

Table 5.9 Mean values of agronomic traits for controlled crosses between Clearfield rice and red rice (F_1) at Ben Hur Farm, Louisiana, 2003 (No Imazethapyr applied)

Material	Plant height	No. tillers /plant	No. panicles/ plant	Panicle length	No. spikelets/ panicle	No. seeds/ panicle	Seed set rate	Grain weight
F_1 between CL121 and red rice	156.4[a]	34.8[a]	5.6[a]	18.1[a]	123.4.0[a]	21.9[b]	34.92[b]	1.85[b]
CL121	84.0[b]	5.2[b]	5.2[a]	19.5[a]	71.6[a]	101.0[a]	81.85[a]	2.25[a]
F_1 between CL161 and red rice	147.7[a]	45.7[a]	11.9[a]	21.4[a]	106.1[a]	38.1[b]	39.82[b]	1.80[a]
CL161	95.0[b]	6.2[b]	6.2[a]	20.1[a]	127.0[a]	105.0[a]	82.68[a]	2.10[a]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

significant decrease in seeds/plant and seed set rate compared with CL121. No significant difference was found for the other agronomic traits between the F_2 populations and CL121. When the F_2 populations at different locations were separately analyzed, no significant difference was found in panicle length and seeds/panicle between the F_2 populations and CL121. The significant difference did not occur in seed set rate between all F_2 populations and CL121 (Table 5.11a). For the remaining 21 F_2 populations, the same tendency between the 23 F_2 populations and CL121 was also detected between the F_2 populations and CL141 in all agronomic traits. Moreover, the tendency did not change due to location (Table 5.11b).

Table 5.12 shows the means of agronomic traits of F_2 populations derived from controlled crosses between Clearfield rice and red rice. Both F_2 populations developed from the crosses between CL121 and red rice, and F_2 populations produced from the crosses between CL161 and red rice, showed the same tendency in all agronomic traits. All F_2 populations from the controlled crosses showed hybrid vigor in plant height and panicle length over CL121 and CL161, but no differences were found for the remaining traits (Table 3.13).

That hybrids between Clearfield rice and red rice flower in normal commercial Clearfield, and overlap in flowering date with Clearfield rice indicates the possibility of further outcrossing of the hybrids with Clearfield rice. Otherwise, there is no chance for hybridization between the

Table 5.10 Means of agronomic traits of F_2 populations developed from natural red rice-Clearfield hybrids found in red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2004

		Plant height (cm)			Panicle length (cm)			No. seeds/panicle			Spikelets/panicle		
F_2 population	No. samples	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
2	98	96.0	121	69	22.8	35.8	15.4	33.3	113	1	89.3	265	26
3	27	93.4	125	71	20.6	29.2	13.7	18.3	45	5	60.1	133	9
4	33	89.8	120	65	22.4	30.3	10.6	40.2	116	5	78.8	158	11
8	93	97.2	127	62	23.6	33.4	11.2	40.0	179	1	99.3	246	15
9	33	87.9	112	67	21.3	28.4	13.5	26.6	81	1	65.5	121	19
10	21	87.1	118	63	20.2	26.8	13.9	30.8	72	1	58.1	129	11
11	28	86.4	112	68	19.0	25.6	12.2	46.4	135	9	76.0	180	18
15	100	100.0	129	66	20.6	26.6	12.7	70.2	183	17	84.4	218	25
16	45	104.3	137	53	24.1	31.1	13.2	65.6	136	13	111.5	243	19
17	100	87.8	120	56	21.0	29.7	12.6	55.8	155	5	77.8	280	21
18	24	90.4	121	70	24.4	31.3	16.1	99.2	216	17	139.8	250	37
19	99	90.4	128	64	21.5	30.7	12.5	56.0	252	7	75.8	308	15
20	99	90.6	126	58	20.5	30.6	9.6	70.1	225	2	91.5	259	12
21	99	96.9	138	59	21.5	35.4	13.3	75.2	240	24	96.9	350	30
22	100	94.7	139	55	22.0	29.4	14.7	77.1	260	12	99.9	270	26
23	34	89.9	129	60	20.8	32.4	13.5	53.9	174	16	81.3	236	23
24	100	93.1	127	65	23.6	34.3	15.4	63.2	164	5	86.4	234	25
25	100	93.2	123	59	21.3	31.8	14	70.8	252	12	94.8	336	17
34	100	100.3	133	73	21.0	26	14.8	78.0	162	18	93.2	172	37
38	100	94.1	126	54	23.4	30.8	13	50.5	152	9	86.9	187	13
39	35	100.5	128	76	22.9	33.1	14.8	37.9	100	3	60.6	144	10
40	100	97.9	125	62	24.1	35.3	15.8	59.3	172	2	82.5	185	23
41	100	95.2	123	65	21.7	29.3	14.2	52.8	161	7	80.9	220	20
42	100	95.5	119	64	21.6	31.4	12.9	53.3	130	7	95.5	253	25
43	100	100.4	142	74	22.8	34.3	14.7	64.6	162	12	90.8	271	29
44	100	101.6	136	65	23.1	33.3	12.9	61.8	166	11	83.8	257	19
45	100	102.8	132	81	23.5	31.3	17.1	48.9	126	4	85.7	184	30
46	51	100.3	140	60	25.6	35.1	14.9	97.8	280	20	123.8	351	27
47	10	104.0	133	88	24.7	30.5	18.6	59.1	107	30	79.0	133	47

Table 5.10 (continued)

F_2 population	No. samples	Plant height (cm)			Panicle length (cm)			No. seeds/panicle			Spikelets/panicle		
		Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
57	100	93.5	129	60	22.3	32.2	12.7	57.5	218	9	84.2	285	19
61	49	95.1	121	56	21.3	32.2	11.9	47.3	123	7	69.1	181	10
62	67	95.4	126	62	22.3	30.2	12.3	43.6	198	1	100.4	287	28
63	35	89.3	113	62	20.9	26.7	14.4	42.6	87	12	71.4	268	19
64	100	85.1	117	55	20.4	29.6	9.8	44.2	184	2	73.6	228	12
69	100	92.7	127	60	21.3	31.6	10.8	59.8	172	4	85.9	280	19
70	98	93.6	135	74	20.9	29.7	13.3	53.5	161	5	81.5	221	28
71	98	91.5	135	63	21.6	32.1	10.9	65.1	285	2	87.2	325	12
75	100	100.9	128	73	23.5	32.7	12.7	63.1	179	3	97.0	214	18
76	100	95.0	140	58	21.0	29.8	11.8	57.8	209	8	88.2	276	18
77	100	93.7	136	61	20.9	30.2	11.6	60.8	185	8	82.7	245	18
78	99	92.8	131	60	21.7	32.8	11.9	49.6	156	7	79.1	182	18
79	70	92.0	118	62	21.4	30.7	13.2	49.3	205	7	77.3	239	16
80	100	94.5	127	55	22.3	31.8	11.9	68.6	249	2	100.4	305	9
83	100	97.7	135	70	22.1	30	13.7	63.3	170	12	80.5	186	20
CL121		75.8			18.9			74.4			87.8		
CL141		81			18.6			82			96		

Table 5.10 (continued)

		Seed set rate (%)			100 grain weight (g)		
F_2 population	No. samples	Mean	Maximum	Minimum	Mean	Maximum	Minimum
2	98	39.12	84.44	1.25	2.448	3.415	1.500
3	27	39.18	92.59	7.46	2.327	2.993	1.827
4	33	48.90	87.39	5.77	2.211	2.679	1.740
8	93	42.06	96.97	1.37	2.216	3.700	1.450
9	33	39.03	94.74	1.02	2.358	2.912	1.800
10	21	52.30	85.71	6.25	2.221	2.867	1.835
11	28	61.01	94.23	7.32	2.229	2.975	1.820
15	100	83.73	100.00	18.89	2.310	3.142	1.750
16	45	60.58	96.15	22.64	2.297	2.884	1.673
17	100	73.65	100.00	20.00	2.187	2.841	1.558
18	24	67.27	91.28	32.61	1.994	2.772	1.645
19	99	73.69	100.00	12.64	2.230	2.802	1.465
20	99	74.42	100.00	2.56	2.181	2.757	1.494
21	99	78.99	100.00	40.78	2.428	2.938	1.680
22	100	76.71	100.00	35.37	2.110	2.634	1.324
23	34	69.22	90.20	30.69	2.235	2.725	1.803
24	100	73.78	100.00	6.85	2.379	3.516	1.254
25	100	78.49	100.00	22.45	2.292	2.853	1.799
34	100	84.41	100.00	31.03	2.480	3.045	1.936
38	100	63.12	100.00	6.82	2.406	3.342	1.914
39	35	66.35	92.31	8.57	2.238	2.767	1.603
40	100	72.51	100.00	1.90	2.297	2.823	1.600
41	100	67.64	97.78	7.69	2.342	2.831	1.771
42	100	63.49	100.00	8.74	2.518	3.492	1.709
43	100	73.24	97.56	9.92	2.196	2.883	1.604
44	100	76.22	100.00	17.46	2.326	3.060	1.858
45	100	62.39	100.00	2.86	2.486	3.296	1.619
46	51	78.48	98.04	51.85	2.419	2.885	1.977
47	10	75.43	88.00	32.26	2.268	2.604	1.794

Table 5.10 (continued)

		Seed set rate (%)			100 grain weight (g)		
F_2 population	No. samples	Mean	Maximum	Minimum	Mean	Maximum	Minimum
57	100	70.36	100.00	27.27	2.248	2.897	1.724
61	49	69.09	100.00	33.70	2.372	2.959	1.888
62	67	49.59	94.74	1.14	2.198	2.844	1.472
63	35	64.22	92.00	13.43	2.136	2.543	1.526
64	100	63.18	97.14	5.26	2.277	2.968	1.729
69	100	70.65	97.37	7.14	2.362	3.068	1.820
70	98	66.63	96.77	9.68	2.372	2.959	1.843
71	98	72.13	97.62	3.70	2.290	3.200	1.571
75	100	67.23	100.00	1.73	2.466	3.035	0.743
76	100	65.60	96.97	15.09	2.383	2.966	1.847
77	100	72.41	97.56	15.07	2.275	3.075	1.458
78	99	63.77	96.88	7.27	2.213	2.857	1.290
79	70	65.72	95.74	22.44	2.194	2.700	1.648
80	100	66.83	100.00	6.10	1.998	2.745	0.884
83	100	79.29	100.00	20.00	2.230	2.751	1.602
CL121		84.79			2.30		
CL141		85.42			2.20		

Table 5.11a Mean values of agronomic traits for F_2 populations developed from natural red rice-CL121 hybrids found in red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2004

Locations	Plant height	Panicle length	No. spikelets/ panicle	No. seeds/panicle	Seed set rate	Grain weight
2002-1	96.0[a]	22.8[a]	89.3[a]	33.3[a]	39.12[d]	2.45[a]
2002-2	90.3[a]	21.2[a]	73.0[a]	33.7[a]	47.08[cd]	2.11[b]
2002-3	93.8[a]	21.9[a]	94.6[a]	68.8[a]	73.69[ab]	2.26[ab]
2002-4	100.3[a]	21.0[a]	93.2[a]	78.0[a]	84.41[a]	2.24[ab]
2002-10	92.8[a]	21.7[a]	79.1[a]	49.6[a]	63.77[bc]	2.48[a]
2002-12	92.0[a]	21.4[a]	77.3[a]	49.3[a]	65.72[abc]	2.21[ab]
2002-11 ratoon	96.1[a]	22.2[a]	90.5[a]	66.0[a]	73.06[ab]	2.19[ab]
CL121	75.8[b]	18.9[a]	87.8[a]	74.4[a]	84.79[ab]	2.30[ab]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

Table 5.11b Mean values of agronomic traits for F_2 populations developed from natural red rice-CL141 hybrids found in red rice samples collected in 2002 at Ben Hur Farm, Louisiana, 2004

Locations	Plant height	Panicle length	No. spikelets/ panicle	No. seeds/panicle	Seed set rate	Grain weight
2002-5	97.3[a]	22.8[a]	82.7[a]	53.1[b]	67.73[b]	2.33[a]
2002-6	95.6[a]	22.2[a]	86.2[a]	57.5[b]	68.24[b]	2.33[a]
CL141	81.0[b]	18.6[b]	96.0[a]	82.0[a]	85.42[a]	2.20[a]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

Table 5.12 Means of agronomic traits of F_2 populations from controlled crosses between Clearfield rice and red rice at Ben Hur Farm, Louisiana, 2004

F_2 population		No. samples	Plant height (cm)			Panicle length (cm)			No. seeds/panicle			No. spikelets/panicle		
			Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
X3	Red rice#11*CL121	100	101.2	139	61	24.0	32.1	13.7	64.1	179	1	132.9	276	41
X4	Red rice#12*CL121	100	104.9	147	68	24.6	36.4	16.3	95.2	271	17	123.9	281	42
X5	Red rice#13*CL121	100	101.7	132	57	22.4	30.3	11.7	84.1	214	3	114.6	319	24
X9	Red rice#17*CL121	100	117.9	169	71	24.3	35.4	15.2	81.8	191	18	96.7	223	26
X10	Red rice#18*CL121	90	91.3	115	56	20.9	29.1	10.7	62.6	158	9	83.0	185	12
X13	Red rice#28*CL121	100	118.9	150	85	23.4	30.8	13.4	80.2	182	21	91.9	194	21
X16	Red rice#27*CL161	100	106.8	145	55	24.4	34.9	14.8	87.1	246	4	124.7	270	33
X17	Red rice#29*CL161	100	125.2	162	80	23.9	37.8	16.1	81.0	175	23	88.1	183	24
X22	CL 121*red rice#35	100	92.6	130	51	21.4	31.5	10.7	76.7	221	6	105.6	306	12
X23	CL 121*red rice#36	100	92.6	130	51	21.4	31.5	10.7	76.7	221	6	105.6	306	12
X26	CL 161*red rice#39	99	117.6	146	87	22.6	32	14.7	66.2	178	20	89.8	247	31
X27	CL161*red rice#40	99	122.0	159	89	22.1	30.2	13.4	77.6	229	20	87.9	253	23
X32	CL121 * Red rice#13	31	103.1	127	77	24.2	29.3	15.5	149.7	312	29	183.0	372	36
CL121			75.8			18.9			74.4			87.8		
CL161			86			19.7			70.9			78.7		

Table 5.12 (continued)

F_2 population	No. samples	Seed sett rate (%)			100 grain weight (g)		
		Mean	Maximum	Minimum	Mean	Maximum	Minimum
X3	100	48.81	96.00	0.79	2.246	3.027	1.830
X4	100	78.38	100.00	12.78	2.460	3.258	1.864
X5	100	72.47	98.00	8.11	2.250	2.990	1.533
X9	100	86.59	100.00	51.40	2.488	3.180	1.817
X10	90	74.55	100.00	24.18	2.348	2.941	1.797
X13	100	88.28	100.00	36.36	2.646	3.540	1.891
X16	100	68.59	96.10	5.00	2.155	2.864	1.281
X17	100	92.19	100.00	45.16	2.364	2.846	1.238
X22	100	68.58	95.89	13.92	2.216	2.774	1.515
X23	100	68.58	95.89	13.92	2.216	2.774	1.515
X26	99	77.12	100.00	15.38	2.387	2.990	1.473
X27	99	88.40	100.00	47.44	2.489	3.121	1.973
X32	31	54.15	100.00	11.76	2.410	2.933	2.031
CL121		84.79			2.30		
CL161		90.00			2.20		

Table 5.13 Mean values of agronomic traits for F_2 populations derived from controlled crosses between Clearfield rice and red rice at Ben Hur Farm, Louisiana, 2004

Material	Plant height	Panicle length	No. spikelets/ panicle	No. seeds/panicle	Seed set rate	Grain weight
F_2 populations derived from the crosses between CL121 and red rice	102.7[a]	23.0[a]	115.2[a]	85.7[a]	71.15[a]	2.36[a]
CL121	75.8[b]	18.9[b]	87.8[a]	74.4[a]	84.79[a]	2.30[a]
F_2 populations derived from the crosses between CL161 and red rice	117.9[a]	23.3[a]	97.6[a]	78.0[a]	81.57[a]	2.35[a]
CL161	86.0[b]	19.7[b]	78.7[a]	70.9[a]	90.00[a]	2.20[a]

Means followed by the same letter in columns are not significantly different (α=0.05), Duncan's Multiple Range Test.

hybrids and Clearfield rice. Moreover, grain quality of Clearfield rice was deceased by the mixture of the hybrid seeds with Clearfield rice if they approximately mature at the same time. In a previous study, glufosinate resistant line Cypress-red rice hybrids showed extreme late and did not flower in normal field (Zhang et al., 2003). In this study, a small proportion (~30%) of natural red rice-Clearfield F_1 hybrids derived from red rice samples in two years flowered in the fields. Red rice-Clearfield hybrids found in red rice sample collected in 2002, including red rice-CL121 and red rice-CL141, first flower over 36 days and 32 days later than red rice-CL161 F_1 hybrids detected in red rice sample collected in 2003, respectively. Planting date in 2002 is 23 days later than that in 2003. Flowering date of F_1 hybrids may change due to different planting date. Further backcrossing of natural red rice-CL121, red rice-CL141 F_1 hybrids with Clearfield rice seems to be impossible because of their different flowering date. Controlled crosses between CL121 and red rice showed inconsistent result with natural red rice-CL121 hybrids in that early flowering date (June 15) was found. A high probability in further backcrossing of natural red rice-CL161 F_1 hybrids with CL161 existed due to overlapping extent of their flowering date.

F_2 plants developed from F_1 hybrids between red rice and Clearfield rice possessed a wide range of flowering date (over 40 days) from June to August, indicating that further backcrossing with Clearfield rice is possible. In addition to backcrossing, F_1 hybrids and F_2 plants, no matter

the plants flower or not, will directly affect commercial Clearfield production through the competition of nutrient, water and sunlight. Some plants including F_1 hybrids and F_2 plants can mature at approximate same time with commercial Clearfield rice. These seeds may mix into Clearfield rice seeds when commercial Clearfield rice is harvested.

The performance of agronomic traits in hybrids and the successive generations predicts the fate of characteristic transferred through hybridization at some extent (Hauser et al., 1998b). Hybrids between cultivated rice and red rice produced heterosis in plant height and the number of tillers over red rice (Langevin et al., 1990). Zhang et al (2003) found heterosis in hybrids between transgenic Cypress and red rice in plant height over both parents, and significant decrease in tillers/plant in the hybrids over both parents. Moreover, the hybrids showed significant decrease in spikelets/panicle and seeds/panicle over transgnic Cypress. Comparison of F_2 plants developed from the hybrids with transgenic Cypress showed that the same tendency between the F_1 hybrids and transgenic Cypress existed in plant height, spikelets/panicle and seeds/panicle. Similar result for F_2 plants developed from the crosses between transgenic Cypress and red rice over transgenic Cypress was found in plant height. However, no significant difference was detected in seeds/panicle between the F_2 plants and transgenic Cypress (Oard et al., 2000). In the present study, agronomic traits in F_1 hybrids and F_2 plants showed different characteristic due to Clearfield varieties and/or red rice. Red rice-CL121 F_1 hybrids produced heterosis in vegetative traits, such as plant height and tiller production, and less advantages in reproductive traits, for example, seeds/panicle and seed set rate over CL121. The same tendency between red rice-CL121 F_1 hybrids and CL121 was found in red rice-CL141 F_1 hybrids over CL141, controlled crosses between red rice and Clearfield rice over Clearfield rice. On the contrary, red rice-CL161 F_1 hybrids did not exhibit significant difference in seeds/panicle and

seed set rate over CL161. F_2 plants developed from natural red rice-CL121 and red rice-CL141 F_1 hybrids possessed heterosis in plant height and panicle length, but showed significantly decrease in seeds/panicle and seed set rate over CL121 and CL141, respectively. The same tendency in plant height and panicle length between the F_2 plants and Clearfield rice was found between F_2 plants developed from controlled crosses and Clearfield rice, but no significant difference was detected in seeds/panicle and seed set rate between F_2 plants developed from the controlled crosses and Clearfield rice.

In summary, 20-30% of the red rice-Clearfield hybrids produced from red rice samples collected in 2002 and 2003 did flower under small plot conditions. F_2 populations derived from natural and controlled crosses showed extensive variation in flowering date. This indicated the real possibility of outcrossing between the red rice-Clearfield hybrids with red rice under field conditions. Therefore, crop rotation should be adopted to avoid backcrossing. For other agronomic traits such as plant height, panicle length, spikelets/panicle, seeds/panicle, seed set and grain weight, F_1 hybrids and F_2 populations produced significantly different values from those of Clearfield rice. In general, the hybrids and F_2 populations produced heterosis or hybrid vigor for vegetative characteristics. However, F_1 hybrids between CL121/CL141 and red rice, and F_2 populations developed from the F_1 hybrids, showed a significant decease in reproductive traits such as seeds/panicle and percent seed set compared to the normal Clearfield rice varieties. F_1 hybrids between CL161 and red rice, and F_2 populations developed from the F_1 hybrids were not significantly different for seeds/panicle or percent seed set. These results indicate that red rice-Clearfield hybrids can express hybrid vigor for many characteristics except those directly measured for seed production. However, given the ability of red rice to cross with commercial sources, continued hybridization in the field over time could increase red rice seed production to

levels that approach elite varieties. A long-term approach for adequate red rice control is therefore necessary for maximum benefit of the Clearfield technology.

CHAPTER 6 SUMMARY

6.1 Red Rice Populations

Strawhull and awnless red rice were the main biotypes for red rice plants sampled in each of the two years. This indicated that high hybridization (100% for red rice sample in 2002 and 87% for red rice sample in 2003) occurred from Clearfield rice to red rice with this biotype. Red rice populations in two years showed variation in agronomic traits, such as plant height, tiller number, panicle length, spikelets/panicle, seeds/panicle, seed set and grain weight. This extensive variation in plant types suggest that outcrossing frequency may be different between Clearfield rice and different red rice populations, a result readily observed in the two year study. Red rice infestation showed extensive variability across different Clearfield locations possibly due to differences in cultivation practices and/or genetic composition of red rice populations. The same first flowering date existed between Clearfield rice and red rice at 9 of 12 Clearfield locations, indicating that chances for outcrossing at these sites were very likely.

6.2. Outcrossing

Outcrossing occurred with Clearfield varieties used, namely CL121, CL141, CL161 and CLXL8. To avoid outcrossing from Clearfield rice to red rice, crop rotation must be adopted. At one Clearfield location in 2002, hybrids were found in the ratoon crop, but not in the main crop. Growth and development of red rice and Clearfield rice may be synchronized after the first harvest, so practices to reduce outcrossing should also be implemented for the ratoon as well as the main crop. The average of outcrossing frequency was less than 1% for red rice samples in two years. However, 1.5% and 3.2% outcrossing occurred at two Clearfield locations in 2003. Such high outcrossing frequencies may be due to microclimates in the two Clearfield locations.

High levels of hybridization occurred from Clearfield rice to red rice with the straw-hull awnless biotype. Flowering characteristics of red rice with the straw-hull and awnless biotype are more adapted to produce hybrids with Clearfield rice than other biotypes.

6.3 Correlation among Outcrossing Frequency and Agronomic Traits

Outcrossing frequency in two years did not significantly correlate with plant height, tillers/plant, panicle length, seeds/plant, seed set or grain weight. Therefore, outcrossing from Clearfield rice to red rice can not be predicated from easily identifiable agronomic traits and that other measures such as crops rotation should be implemented to reduce outcrossing.

6.4. Genetic Characteristics of F_2 Populations

Pubescent/glabrous leaves segregated in F_2 populations, including F_2 populations derived from natural red rice-Clearfield hybrids and controlled crosses between Clearfield rice and red rice. A genetic ratio of 3:1 for pubescent leaves vs glabrous leaves was found at most F_2 populations. This suggested that pubescent leaf was dominant to glabrous leaf, and pubescent/glabrous leaf was controlled in most cases by a single dominant Mendelian gene. Similarly, imazethapyr resistance segregated in F_2 populations, and a genetic ratio of 3:1 was found in tolerant vs susceptible plants for the F_2 populations. Imazethapyr resistance was controlled by a Mendelian single dominant gene that informs rice weed scientists and breeders about red rice management strategies.

6.5. Fitness of F_1 and F_2 Populations

A small proportion of hybrids (20-30%) between Clearfield rice and red rice flowered in the field. This indicated that backcrossing of hybrids with Clearfield rice may occur. F_1 hybrids and the resulting F_2 populations possessed extensive variation in flowering date that insured a certain proportion of the hybrids would have opportunities for outcrossing with the commercial

crop. The same chances for outcrossing would occur for backcrossing to Clearfield in the subsequent generation. F_1 hybrids between Clearfield rice and red rice possessed heterosis in vegetative growth over Clearfield rice. F_1 hybrids between CL121/CL141 and red rice showed significant decrease in reproductive production, seeds/panicle and seed set over corresponding Clearfield rice, whereas F_1 hybrids between CL161 and red rice showed no significant difference in seeds/panicle and seed set over CL161. This indicated that genetic background affected the performance of F_1 hybrids, especially in reproductive production. The same tendency for agronomic traits between F_1 hybrids and Clearfield rice was found in F_2 populations. Like F_1 hybrids, genetic background was influential in the agronomic performance of F_2 populations.

In summary, different Clearfield varieties, cultivation practices and/or red rice biotypes contributed to the different infestation levels of red rice in all Clearfield locations. The same first flowering date between Clearfield rice and red rice indicated that outcrossing may occur, whereas extensive variation of agronomic traits in red rice populations indicated that different rates of outcrossing may exist at different Clearfield locations. Hybrids were found in all Clearfield varieties used at 9 of-11 Clearfield locations, indicating that outcrossing from Clearfield rice to red rice was not uncommon phenomenon in the commercial Clearfield production. High hybridization rates of Clearfield rice and the straw-hull awnless biotype is consistent with strawhull and awnless red rice as the main biotype in red rice samples collected during the two year study. Outcrossing frequency from Clearfield rice to red rice could not be predicated by agronomic traits of red rice populations, so crop rotation must be adopted to avoid hybridization between the cultivated and weedy rice. Long-term use of Clearfield technology will be possible for rice producers that employ sound agronomic practices that include

application of Newpath herbicide for each Clearfield site, early scouting of possible hybrid formation, and crop rotation to minimize weedy hybrid formation.

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VITA

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