

7-2015

The Impact of Herbicide-Resistant Rice and Hybrid Rice Technology on Weedy Rice Evolution

Vijay Singh
University of Arkansas, Fayetteville

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The Impact of Herbicide-Resistant Rice and Hybrid Rice Technology on Weedy Rice Evolution

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Crop, Soil, and Environmental Sciences

By

Vijay Singh
CCS Haryana Agricultural University
Masters of Science in Agronomy, 2008

July 2015
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council

Nilda R. Burgos, Ph.D
Dissertation Director

Andy Pereira, Ph.D
Committee member

Edward E. Gbur, Ph.D
Committee member

David R. Gealy, Ph.D
Committee member

Robert C. Scott, Ph.D
Committee member

ABSTRACT

Weedy rice (*Oryza sativa* L.) competes aggressively with rice, reducing yields and grain quality. Clearfield™ rice, is nontransgenic herbicide-resistant (HR) rice introduced in 2002 to control weedy rice, has resulted in ALS-resistant weedy rice due to gene flow. Volunteers of Clearfield™ rice (F₂) accelerate the HR weedy rice evolution by acting as agents of gene flow. Weedy rice (89) collected from 11 counties in Arkansas were screened for resistance in a field experiment in Stuttgart (2011). Seventy-nine percent of accessions were resistant to imazethapyr and harbored S₆₅₃N mutation. These HR weedy accessions were outcrosses of Clearfield™ rice and weedy rice. Out of 727 characterized plants, nearly 70% of the HR weedy rice flowered at the same time as that of Clearfield™ rice with greatly reduced seed shattering in progenies of some accessions (15 to 87%). Kernels of 20% of the parent accessions had segregating white and red bran color. Two of these parent accessions (goldhull) were homozygous for white bran, with 100% white-bran progenies. Winter-flood reduced the germination of rice seed by 40-50% after 130-160 d of burial. Hybrid rice seed had higher capability to survive the winter (13-53% viability) than inbred rice seed (8-27%). Therefore, hybrid rice is expected to leave more volunteer rice in the field than inbred rice. Fields with cropping history of hybrid rice had higher volunteer rice infestation (20%) than fields planted only with inbred rice (5.6%). The total grain yield of rice was reduced by 0.4% for every 1% increase in volunteer rice density, averaged over cultivars. The 1000-kernel weight, kernel length-width ratio, %protein, %amylose, and head rice yield were affected by volunteer rice density. Various herbicides were tested for efficacy on volunteer rice at SEREC, Rohwer and RREC, Stuttgart, Arkansas. Application of pyroxasulfone (0.12 kg ha⁻¹) in the fall fb 2,4-D (1.12 kg ha⁻¹) 35 d pre-plant caused minimal (6%) crop injury and did not reduce yield. This treatment resulted in better control of volunteer rice (73%) than

pyroxasulfone alone at 0.12 kg ha⁻¹ applied in fall (64%). Pyroxasulfone is not currently labeled for fall application prior to rice planting in the spring.

ACKNOWLEDGEMENTS

I am very grateful to God for giving me opportunity to meet wonderful people in course of my learning process. I believe that along with academic knowledge, people around in one's life influence the thought process and bring positive modulation in attitude towards scientific world.

Dr. Nilda R. Burgos, who gave me opportunity to study under her expertise. I would like to express my deepest gratitude for her excellent guidance, care and support. I am honored to have her as my Ph.D 'Guru'. Apart from academic and professional life, I thank her for giving us family, away from home.

Dr. David R. Gealy, who guided me and always there to resolve issues and simplify tasks for my research studies. I thank him for his wonderful friendship and giving time to make me understand plant physiology concepts.

Dr. Robert C. Scott, who shared his knowledge and kept me updated about current trends in Arkansas rice production. I thank him for motivating me to expand my horizons. I will always look up to him as an expert in weed science and extension.

Dr. Andy Pereira, who willingly helped me in understanding the basics of plant genomics, and provided me excellent atmosphere and opportunity to work in his laboratory. His contribution is instrumental in completion of my molecular studies.

Dr. Edward E. Gbur, who taught me statistics concept and provided his expertise in handling research datasets. I thank him for trusting my statistical skills, giving me opportunity to experiment with JMP[®] and enhancing my confidence in analysis part.

Dr. Leopoldo Estorninos, who shared his professional experiences and helped me in field studies like an elder brother. I thank him for all his support and encouragement.

Mr. Larry Earnest, who not only helped in establishing rice experiments in Rohwer, AR but also participated in my personal and professional development by giving me experience of managing resources and machinery at research station. I thank him for his motivation, and setting an emotional bond.

Mr. Red Burgos, who gave me moral support, love and encouragement through last 4 years. He was the one who always cheered us in our stressful days.

Mr. Howard Black, who was more than willing to help me in Stuttgart, AR. For me, he was always a phone call away which I greatly appreciate. Also, I would like to thank him for tremendous help in characterization of red rice and maintenance of key experiments.

I would like to thank **Reiofeli Salas, Seth Abugho, Husain Tahir** and **Te Ming Tseng** for not only assisting in field experiments but also for being such great friends and colleagues. I thank and appreciate Virginia Boyett and Supratim Basu for helping me in molecular studies.

Apart from this, I would like to thank Pâmela Carvalho de Lima, Silvana Spaniol Fin, Josiane Argenta, Sirichai Sathuwijarn, Fernando Remirez, Coy Batoy, George Botha, Ana Roso, Fernando Martini, and Chris Rouse (Weedy Physiology Laboratory, University of Arkansas) for their assistance in germination assay and maintenance of field plots. Thanks to Meagon Garner, Linda Martin (SEREC, Rohwer) for providing their help and support whenever I needed. Finally, I would like to thank National Science Foundation (NSF) for funding my research.

DEDICATION

This work is dedicated to my family. I thank my wife (Shilpa Singh) and my parents (Mrs. and Dr. Samar Singh) for their love and support throughout the degree program. I also acknowledge the help and contribution of Shilpa Singh as she was involved in many of my research experiments being a Research Assistant in Weed Physiology laboratory, U of A. I would like to thank my brother (Amit Singh) and sister-in-law (Manisha Singh) for their love and trust which kept me moving.

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Singh, V., Burgos, N., Singh, S., Abugho, S., Earnest, L., Gbur, E., Scott, R., 2015. Herbicide and winter-flood treatments for controlling volunteer rice off-season. *Crop Protection*.

CHAPTER I

Introduction

Introduction

Weedy rice is a troublesome weed in many parts of the world. Weedy rice includes a wide range of ecotypes belonging to numerous species. The wild species *Oryza barthii* and *O. longistaminata* or weedy ecotypes from cultivated *O. glaberrima* are among the worst weeds in West Africa whereas *O. granulata*, *O. officinalis*, *O. rufipogon* and *O. nivara* are weedy or wild species in South-East Asian countries (Olofsdotter et al. 2000). Red rice is one of the most common weedy relative of cultivated rice (*Oryza sativa* L.) that competes aggressively with rice in southern US (Diarra et al. 1985; Ferrero 2001; Shivrain et al. 2009). Weedy rice in the southern US generally has red pericarp (Gealy et al. 2003; Gross et al. 2010; Smith 1981) and almost all are of the indica subgroup (Londo and Schaal 2007; Reagon et al. 2010). In the southern US, weedy red rice reduces yields from <5% to 100% (Burgos et al. 2014; Diarra et al. 1985; Shivrain et al. 2009) which results into large economic losses (Gealy et al. 2002; Lee et al. 2011), contaminates rice grains (Ottis et al. 2005) and is classified as a noxious weed in the United States (APHIS 2015; Burgos et al. 2008). Weedy rice shows a wide variability of anatomical, biological and physiological features (Shivrain et al. 2010; Tang et al. 1997; Vaughan et al. 2001). Weedy rice has many weedy traits which make it difficult to control. The primary weedy traits of weedy rice are seed shattering, seed dormancy, vigorous vegetative growth, high competitive ability, seed longevity (Goss and Brown 1939; Noldin 1995) and ability to emerge from greater soil depths (Gealy et al. 2000), which contribute to its persistence. The shattering of weedy rice seeds before crop harvest allows the weedy rice to disperse and dormancy (Tseng et al. 2013) allows it to persist for a long time. Weedy rice seed could remain dormant in the soil for up to ten years in the United States (Goss and Brown 1939, 1940) and three years in Brazil (Leitao et al. 1972).

Among 10 prevalent weeds in rice production in the southern US, including barnyardgrass (*Echinochloa crus-galli* L.), weedy rice caused the highest yield reduction in rice (about 80%) after season-long competition (Smith 1988). Weedy rice competes for production inputs; increases weed control costs, reduces yield and grain quality, and reduces economic returns (Burgos et al. 2006). In the past, before the introduction of herbicide-resistant (HR) rice technology, most of the weedy rice has been controlled by broad-spectrum herbicides (stale seed bed technique) and cultural practices in crop rotations with soybean, maize and grain sorghum (Burgos et al. 2008). It has been reported that, 40% of the rice fields in Arkansas were planted continuously with rice for at least 5 years (Shivrain et al. 2010). The monoculture perpetuates weedy red rice, and infestation increases every year. Since 2002, weedy rice could be selectively controlled in the southern US in an imazethapyr-resistant Clearfield™ (CL) rice production system (Gealy et al. 2003; Burgos et al. 2008). These cultivars were obtained through induced mutations of the *ALS* (acetolactate synthase) gene (Tan et al. 2005). Imazethapyr herbicide inhibits the *ALS* enzyme that catalyses the first step in the biosynthesis of branched chain amino acids - valine, leucine and isoleucine (Mazur et al. 1987). The mutant *als* gene in Clearfield™ rice makes it resistant to imazethapyr and other imidazolinone (IMI) herbicides. The first two Clearfield™ rice cultivars commercialized in 2002 were ‘CL121’ and ‘CL141’ which harbor G₆₅₄E (glycine to glutamic acid) mutation (Tan et al. 2005). Due to low yield potential and lower resistance of these cultivars to imazethapyr herbicide these were soon replaced by ‘CL161’ in 2003 (McClain 2003). This new cultivar has S₆₅₃N (serine to asparagine) mutation (Tan et al. 2005) which makes it 32% more resistant to imazethapyr compared with CL121 and CL141 (Avila et al. 2005).

Recently, Clearfield™ rice hybrids have also been commercialized in the southern US (Shivrain et al. 2009; Salassi and Deliberto 2010). CL hybrids yield higher than CL inbred varieties (Walker et al. 2008) and fetch more economic returns while also allowing control of weedy rice (Salassi and Deliberto 2010). Imidazolinone-resistant rice, offers an opportunity to effectively control weedy rice with little effect on crop safety (Steele et al. 2002). Pollen-mediated gene flow resulted in evolution of weedy rice populations. (Gealy et al. 2003; Shivrain et al. 2007). In rare case, HR weedy rice has evolved from hybridization with Clearfield™ rice (Rajguru et al., 2005). Growing herbicide-resistant rice varieties in proximity with sexually compatible *Oryza* relatives provides an opportunity for gene flow to the weedy populations (Langevin et al. 1990; Olofsdotter et al. 2000; Gealy et al. 2003) and ultimately, leads to the evolution of HR weedy populations. The transfer of HR rice genes via pollen flow to diverse weedy red rice populations complicates long term weedy red rice management strategies (Gealy 2005; Zhang et al. 2006; Shivrain et al. 2008).

The frequent occurrence of weed populations resistant to ALS inhibitors is due to the widespread usage of these herbicides that exerts strong selection pressure. Resistance to imidazolinone herbicides and has been attributed to many factors including differential metabolism (Little and Shaner 1991; Masson and Webster 2001; Tranel and Wright 2002; Yu et al. 2009; Yu and Powles 2013), foliar absorption and translocation (Shaner and Robson 1985; Little and Shaner 1991; Ballard et al. 1995; Yu et al. 2009) and target site point mutations (Heap 2015; Yu et al. 2010; Yu and Powles 2013). In most reported cases, evolved resistance to ALS inhibitor herbicides is due to reduced sensitivity of acetolactate synthase enzyme (Devine and Eberlein 1997; Saari et al. 1994; Tranel and Wright 2002; Yu et al. 2009; Yu and Powles 2013) caused by point mutations (Yu and Powles 2013) within discrete conserved domains of the *ALS*

gene. Point mutations could occur due to deletion-insertion or substitution of one (Single Nucleotide Polymorphism) or multiple nucleotides that changes the amino acid sequence (Tranel and Wright 2002; Wright et al. 1998). Several such substitutions conferring resistance to ALS-inhibiting herbicides have been identified in various plant species (Tranel et al. 2015).

Synchronization in flowering between rice cultivars and weedy rice increases the probability of pollen-mediated gene flow and introgression of herbicide-resistant trait into weedy rice resulting in reduced efficacy of Clearfield™ rice technology for controlling the weed (Shivrain et al. 2007; Gealy et al. 2003). The majority of weedy rice plants shatter seeds easily (Delouche et al. 2007; Constantin 1960). Seed shattering helps weedy rice proliferate and spread; dormancy prolongs its existence in the cropping system. Unlike weedy rice, the rice varieties are selected for minimal seed shattering but if any cultivar lodges and shatters seeds in the field due to unfavorable weather conditions, some of these seeds can survive the winter and become volunteer rice in the following season.

Volunteer rice (*Oryza sativa*), which germinates from shattered seeds of the previous crop, is a common problem in the rice paddy (Gealy 2005; Warwick and Stewart 2005; Sudianto et al. 2013). Volunteer rice is considered as weedy rice when it is morphologically and phenologically different from cultivated rice. Volunteer plants from Clearfield™ rice are a concern as these act as an agent of gene flow from Clearfield™ rice to weedy rice (Sudianto et al. 2013). Volunteers from Clearfield™ inbred rice, are not weedy but volunteers from Clearfield™ hybrid rice are problematic because these are segregating in emergence trait and will increase the window for gene flow to weedy rice populations (Scott 2009). The volunteers from hybrid rice also segregate into several weedy type plants of variable productivity, competing with cultivated rice, resulting in yield loss. When Clearfield™ rice and weedy rice

outcross, the F1 progenies are large, vigorous, late-maturing, and eventually develop into more diverse weedy plants (Gealy et al. 2006; Shivrain et al. 2007). These outcrosses cannot be controlled selectively with imazethapyr because of the introgression of herbicide-resistance trait in these populations.

Therefore, HR weedy rice and volunteer rice can become a serious problem in rice production. In order to continue using the HR technology, it is important to understand the evolutionary changes in morphological and physiological characteristics of the HR weedy populations. Testing of escaped weedy red rice populations for resistance to ALS herbicides and characterizing these plants would be helpful in effective management of HR weedy rice. Understanding the effect of cropping scenarios on rice grain quality, volunteer rice infestation and weedy red rice evolution, would provide an insight into the consequences of recent shift from non-hybrid rice to hybrid rice system on weedy rice management strategies.

Experiments were designed with the following objectives:

1. Evaluate the effect of volunteer rice density on rice grain yield and quality
2. Evaluate the herbicide and winter flood treatments for off-season control of volunteer rice
3. Evaluate the overwintering potential of hybrid and non-hybrid seed
4. Characterize the weedy traits in ALS-resistant weedy red rice populations
5. Evaluate the introgression of resistance-conferring *ALS* mutations and hybridization between HR rice and weedy red rice populations

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CHAPTER II

Review of Literature

Review of literature

Rice (*Oryza sativa* L.) is the world's single most important food crop and a primary food source for more than a third of the world's population. There are two major cultivated rice groups - *O. sativa indica* and *O. sativa japonica*. *Japonicas* are further divided into *javanica* (tropical) and *japonica* (temperate) (Khush, 1997). *O. sativa japonica* is the prominent and widely cultivated rice in the US (Mackill and McKenzie, 2003) which is distinct from *indica* and *aus* cultivars (Caicedo et al., 2007; Garris et al., 2005; Reagon et al., 2010). *Indica* and *aus* cultivars have never been cultivated in the US (Mackill and McKenzie, 2003; Moldenhauer et al., 2004; Lu et al., 2005). Rice cultivation in the U.S. began in South Carolina and Georgia about 300 years ago and then moved to the southern U.S. (Stubbs et al., 1904; Craigmiles, 1978). Rice production plays a vital role in agri-industry of the southern U.S. and is produced mainly in Arkansas, California, Louisiana, Mississippi, Missouri, and Texas. Arkansas produces nearly 43% of the total U.S. rice production (Hardke, 2014). Apart from high yielding cultivars, effective weed control is a major factor that contributes to high rice yields. Poor weed management and high weed pressure can lead to 85% of rice yield losses (Smith, 1983). Worldwide loss in yield of rice due to weeds has been estimated around 10 percent of total production (Moody, 1991). Particularly, in the U.S., annual rice yield and quality losses are estimated up to 15% (Smith, 1979). The major rice weeds in the southern U.S. are *Echinochloa crus-galli*, *E. colona*, *Cyperus difformis*, *C. rotundus*, *C. esculentus*, *C. iria*, *Urochloa platyphylla*, *Leptochloa filiformis*, *Digitaria sanguinalis*, *Oryza sativa*, *Eleusine indica*, *Fimbristylis littoralis*, *Ischaemum rugosum*. *Echinochloa crus-galli* is the most troublesome weed of rice in Arkansas (Norsworthy et al., 2007; 2012). The major rice production area in the southern U.S. is under direct-seeded rice; therefore, such rice production systems face serious problems of weeds which are closely related in biology and morphology to rice, like weedy rice.

Weedy rice includes a wide range of ecotypes belonging to numerous species. The wild species *Oryza barthii* and *O. longistaminata* or weedy ecotypes from cultivated *O. glaberrima* are among the worst weeds in West Africa whereas *O. granulata*, *O. officinalis*, *O. rufipogon* and *O. nivara* are weedy or wild species in South-East Asian countries (Olofsson et al., 2000). *O. glumaepatula*, is a subtype of *O. rufipogon* from America; it is closer to *O. barthii* than the Asian species. *O. glumaepatula* may have evolved independently, or from African ancestors, over a long period (Aggarwal et al., 1999). *Oryza sativa*, *O. rufipogon*, *O. nivara* and *O. longistaminata* share AA genome along with *O. nivara* and *O. longistaminata* (Olofsson et al., 1999).

Weedy rice in the southern US

Weedy rice in southern US belongs to the *indica* subgroup (Londo and Schaal, 2007; Reagon et al., 2010). However, all of the cultivated rice belongs to the *japonica* subgroup (Moldenhauer et al., 2004; Reagon et al., 2010). Weedy rice might have originated from hybridization of Asian rice, *O. sativa*, and the wild ancestor *O. rufipogon* (Ellstrand, 2003) or reversion of the domestication process (Londo and Schaal, 2007). Londo and Schaal (2007) selected twenty-one microsatellite loci to cover the rice genome; one locus per chromosome. Structure analysis indicated that cultivated *O. sativa* varieties (*indica*, *aus* and *japonica* subgroups) are the result of the domestication process from wild rice whereas strawhull (SH) weedy rice and blackhull (BHA) weedy rice (pre-dominantly present in the US) have arisen through de-domestication from *indica* and *aus*, respectively. It was also indicated that strawhull and blackhull weedy rice in the US might not share weedy traits due to high genetic distance between them ($F_{st} = 0.7872$) (Londo and Schaal, 2007). These findings were corroborated by other studies (Caicedo et al. 2007; Gross et al., 2010; Reagon et al., 2010). Reagon et al. (2010)

sequenced a total of 48 fragments from 111 randomly chosen sequenced tagged sites (STS), 4 loci for each chromosome. InStruct analyses grouped SH weedy rice individuals with *indica*. All SH accessions had the same cytotype, which was also the most frequent in *indica* (60%) and *O. rufipogon* (53%) and was found in all of the *O. rufipogon* and *O. nivara* accessions that shared greater than 50% membership with *indica*. Similarly, BHA cytotype was most common in tropical *japonica* (63%), and rare in *indica* (20%) and *O. rufipogon* (7%).

Weedy rice with white kernels have been observed in Southern America and Asia, but all weedy rice in North America have red bran (Delouche et al., 2007). Thus, weedy rice in the US is collectively called 'red rice' (*O. sativa*) (Gealy et al., 2003; Gross et al., 2010; Smith, 1981). Red bran is a dominant trait of weedy red rice (Gealy et al., 2003), which is inherited from the wild ancestor. Sweeney et al. (2006) reported that white bran (*rc* allele) is the mutant (nonfunctional) version of the ancestral *O. rufipogon* (*Rc*) red allele. Weedy red rice in the US carries (*Rc*) red allele. Recently, a red-bran variant of the long-grain cultivar 'Wells' in Arkansas was discovered, which raised concerns over the genetic purity of this cultivar (Brooks et al., 2008). Microsatellite DNA fingerprinting indicated that red pericarp in 'Wells' was due to (*Rc-g*) red allele (Brooks et al., 2008), which is another dominant wild type allele evolved through natural mutation within *rc* allele. This could be an example of a reversion process from a domesticated trait to the wild trait. However, the process of reversion was proved wrong by Gross et al. (2010) who showed that reversion of domestication alleles does not account for the red pigmentation of weedy red rice kernels and haplotypes characterizing the weedy red rice are either absent or very rare in cultivated rice. These findings strengthen the hypothesis of origin of weedy red rice through hybridization between Asian rice (*O. sativa*) and wild rice (*O. rufipogon*), rather than just sole de-domestication from cultivars

Weedy red rice was introduced as contaminant in rice seed in the US from Asia (Craigmiles, 1978). Weedy red rice infestation in the US was first reported in 1846 (Allston, 1846). It was declared a problem in the early 20th century (Vincenheller, 1906) and has become the second most troublesome weed after barnyardgrass (Burgos et al., 2008; Norsworthy et al., 2007). In 2006 more than 60% of rice production area in Arkansas was infested with weedy rice (Burgos et al., 2008). The annual economic loss from weedy rice infestation was estimated at \$275 ha⁻¹ in Arkansas (Burgos et al., 2008). Up to 100% yield losses have been reported due to weedy rice competition and seed contamination resulted in reduced grain quality (Burgos et al., 2014; Diarra et al., 1985; Kwon et al., 1991; Ottis et al., 2005).

Physiologically, weedy rice is similar to cultivated rice which grows taller and generally, has more tillers and panicles per plant (Noldin et al., 1999). Shivrain et al. (2006) reported that weedy rice plants ranged from 40 cm to 180 cm in plant height and generally, 15-65% taller than cultivated rice (Do Lago, 1982; Shivrain et al., 2006). Ninety percent of the weedy rice populations were reported to flower 98 days after planting (DAP) with flowering range of 70 to 130 DAP (Shivrain et al., 2006). Strawhull and blackhull weedy rice are two prominent hull types in in the US (Gealy et al., 2002; Reagon et al., 2010). More recently, Tseng (2013) also reported that the majority of weedy rice ecotypes in Arkansas are strawhull and blackhull. However, many intermediate hull types (brownhull, goldhull, grayhull) were also observed (Burgos et al., 2014; Shivrain et al., 2008; 2010; Vaughan et al., 2009). Blackhull ecotypes in general, are taller (139 cm) than strawhull (133 cm) ecotypes (Shivrain et al., 2010). Blackhull and brownhull weedy rice ecotypes flower later (101 DAP) than strawhull ecotype (96 DAP) whereas cultivated rice flower 90-93 DAP (Do Lago, 1982; Shivrain et al., 2004). Blackhull ecotypes also have more tillers (102/plant), and higher yield potential (949 g/plant) than

strawhull ecotypes (tillers = 85/plant; yield = 608 g/plant). Thus the blackhull ecotypes are more competitive while the strawhull ecotypes have the ability to mature earlier than cultivated rice (Shivrain et al., 2009, 2010).

In general, weedy rice seeds mature asynchronously and shatter before rice harvest which is very common and thus, add to soil seed bank (Cao et al., 2006; deWet, 1975; Ladizinsky, 1985). Once deposited in soil seed bank, it can remain dormant for several years (Goss and Brown, 1939; Vidotto and Ferrero, 2000; Tseng et al., 2013) and can emerge from greater depths (Gealy et al., 2000). Due to irregular germination patterns, weedy rice would be difficult to control by cultural means in a rice crop. The increase in weedy rice infestation in the fields led to the development of Clearfield™ rice production technology which allows growers to use the imidazolinone herbicide to control 95 to 100% of the weedy rice (Steele et al., 2002; Ottis et al., 2004; Levy et al., 2006).

Clearfield™ (CL) rice technology

Clearfield™ (CL) rice is non-transgenic herbicide-resistant rice technology (Tan et al., 2005), introduced in 2002 (Williams et al., 2002). Clearfield™ rice varieties have resistance to imidazolinone herbicides such as imazethapyr. Imazethapyr herbicide inhibits the ALS (Acetolactate synthase) enzyme that catalyzes the first step in the biosynthesis of branched chain amino acids - valine, leucine and isoleucine (Mazur et al., 1987). The mutant *als* gene in Clearfield™ rice makes it resistant to imazethapyr and other imidazolinone (IMI) herbicides. Clearfield™ rice cultivars were developed from US cultivar 'AS3510' by treating it with mutagen 'EMS' (ethyl methanesulfonate) and screening with imazethapyr to obtain a mutant line (Croughan, 2001). The mutant line was hybridized with Cocodrie and Maybelle, which produced

'CL121' and 'CL141' as the first Clearfield™ rice cultivars (Tan et al., 2005). These two Clearfield™ rice cultivars commercialized in 2002 harbored G₆₅₄E (glycine to glutamic acid) mutation (Tan et al. 2005). Due to lower resistance of these cultivars to imazethapyr and low yield potential, these were soon replaced with 'CL161' in 2003 (McClain, 2003) and other recently released Clearfield™ rice cultivars. These new cultivars have S₆₅₃N (serine to asparagine) mutation (Tan et al., 2005) which makes them more resistant to imazethapyr compared with CL121 and CL141 by about 30% points (Avila et al., 2005). Therefore, with the introduction of superior Clearfield™ rice cultivars with higher resistance and higher grain yield, this technology rapidly gained acceptance among farmers (Shivrain et al., 2006).

Gene flow from Clearfield™ rice to weedy rice

Late-season weedy rice escapes are a serious concern in Clearfield™ rice (Meins et al., 2003; Shivrain et al., 2009; Zhang et al., 2006) because of the potential of Clearfield™ rice to outcross with escaped weedy rice plants (Gealy et al., 2003). Natural hybridization between cultivated rice and weedy rice plants contributes to the diversity of weedy rice and hybridization between weedy rice ecotypes (strawhull and blackhull), which is causing further diversification of weedy rice (Londo and Schaal, 2007). Weedy rice is an autogamous species with an extremely low outcrossing rate and restricted pollen-mediated gene flow (Gealy et al., 2003; Chen et al., 2004). The main factor affecting outcrossing between cultivated rice and weedy red rice could be the potential overlap of flowering period. In general, outcrossing rates between rice and red rice are lower than 1% (Gealy et al., 2003; Shivrain et al., 2007). Such a low frequency of hybridization and introgression plays an important role in the long-term evolution of weedy rice populations (Cao et al., 2006). The rate of outcrossing between CL cultivars and weedy rice

depends on both the geographic and environmental conditions. The outcrossing rate of weedy rice with CL161 was significantly higher (0.0028%) than with CL121 (0.0012%) (Shivrain et al., 2007). Shivrain et al. (2007) detected outcrossing between ClearfieldTM rice (CL161) and weedy rice in Arkansas up to 6 m which was the farthest limit of the experiment, but hypothesised that beyond 6 m outcrossing rate will diminish drastically. However, Song et al. (2003) reported outcrossing between 'Minghui-63' and *O. rufipogon* up to 43.2 m under field conditions in China. Zhang et al. (2006) reported outcrossing rates of 0.1% to 3.2% between ClearfieldTM rice cultivars and weedy rice. The higher outcrossing rates allow more opportunities for natural hybridization and introgression among weedy rice individuals and with cultivated rice (Cao et al., 2006). Once the ClearfieldTM cultivars outcross with weedy rice, crop genes may introgress into weedy populations within a few generations (Gealy et al., 2003) with selection pressure from imidazolinone herbicides favoring the proliferation of weedy outcrosses. Therefore, hybridization may contribute to adaptive evolution, and specifically weedy plant evolution (Ellstrand and Schierenbeck, 2000).

Resistance-conferring point mutations in *ALS* gene

Five different families of herbicides belong to the ALS group- (1) pyrimidinyl thiobenzoate (PTBs), (2) sulfonylureas (SUs), (3) imidazolinones (IMIs), (4) triazolopyrimidine (TPs) and (5) sulfonyl aminocarbonyl triazolinones (SCTs) (Shimizu et al., 2002; Tranel and Wright, 2002; Kawai et al., 2007). Inhibition of ALS leads to plant starvation of essential amino acids valine, leucine and isoleucine resulting in plant death. The resistance mechanism of ALS inhibitor herbicides is mainly due to altered target site and in some cases, rapid or enhanced metabolism of herbicide (Christopher et al., 1992; Hidayat and Preston, 2001; Tranel and

Wright, 2002). In altered target-site mechanism, the binding ability of ALS herbicides is reduced due to point mutation in *ALS* gene which changes amino acid in a target enzyme (Yu et al., 2013). There are six resistance-conferring target-site mutations in the *ALS* gene: Ala₁₂₂, Pro₁₉₇, Ala₂₀₅, Asp₃₇₆, Trp₅₇₄, and Ser₆₅₃ (Tranel and Wright, 2002; Whaley et al., 2004). The mutations at amino acid positions Ala₁₂₂, Ala₂₀₅, Ser₆₅₃ result in resistance to imidazolinone herbicides; Pro₁₉₇ confers resistance to sulfonylureas but not to imidazolinones; Trp₅₇₄ confers resistance to both SUs and IMIs. Also, S₆₅₃N, G₆₅₄E and A₁₂₂T mutations have been used in developing the herbicide-resistant rice through genetic transformation techniques (Croughan et al., 1997; Sebastian et al., 1989; Tan et al., 2005).

The first two Clearfield™ rice cultivars commercialized (CL121 and CL141) harbored G₆₅₄E (glycine to glutamic acid) mutation (Tan et al., 2005). Another resistant line was developed (PWC16) which harbored S₆₅₃N (serine with asparagine), mutation at position 653; was used to develop 'CL161' and 'CLXL8' which were commercialized in 2003 (Webster and Masson, 2001; Gealy et al., 2003; Tan et al., 2005). In the Clearfield™ rice production system, hard to control weedy rice can be managed easily. But due to continuous use of Clearfield™ rice technology, the more use of imidazolinone herbicides has introduced the selection pressure for these herbicide, and weedy rice being the weedy relative of rice, has evolved resistance to IMIs. Rajguru et al. (2005) found four mutations (Ser₆₅₃Asn, Ser₁₈₆Pro, Lys₄₁₆Glu, and Leu₆₆₂Pro) in *ALS* gene of HR weedy rice relative to the susceptible rice cultivars which made those weedy strawhull rice resistant to IMI herbicides. It is expected that over a long period of time *ALS* gene in weedy rice evolve some mutations which would create diversity among their populations. Apart from selection pressure from herbicide, another reason for resistance development in weedy rice could be gene flow from Clearfield™ rice to weedy relatives that would result in HR

weedy populations (Sales et al., 2008). Sales et al., (2008) identified six base polymorphisms in the *ALS* gene of susceptible and resistant weedy rice, three of which resulted in amino acid substitutions (Glu₆₃₀Asp, Gly₆₅₄Glu, Val₆₆₉Met). The most common mutations reported in HR weedy rice populations in the US are Ser₆₅₃Asn (S₆₅₃N) and Gly₆₅₄Glu (G₆₅₄E). These mutations are present in commonly grown ClearfieldTM rice varieties. The area planted with ClearfieldTM rice varieties harboring the G₆₅₄E mutation (CL121 and CL141) is reduced in US in the last decade. In 2004, CL121, CL141 and CL161 were planted in approximately 19% of rice acres, but CL121 and CL141 are no longer planted in Arkansas recently. The HR weedy rice reported in recent studies in Arkansas (Shivran et al., 2007; Sales et al., 2008), all harbored the S₆₅₃N mutation, not G₆₅₄E. In Greece, HR weedy rice populations carry the S₆₅₃N mutation that is the same mutation in the ClearfieldTM cultivar grown in the country (Kaloumenos et al., 2013). Another mutation, A₁₂₂T, is frequently reported from Argentina, Bolivia, Brazil, Paraguay, Uruguay and other countries in south and Central America (Roso et al., 2010). The A₁₂₂T mutation is present in the imidazolinone HR rice cultivar 'PUITÁ INTA CL', which is commercialized in these regions. Apart from the mutation in CL cultivars novel *ALS* mutations were also reported. A novel mutation Val₆₆₉Met was found in weedy rice populations from Arkansas, USA which indicates that prolonged use of IMI herbicides can result in *ALS*- resistant red rice (Sales et al., 2008).

Herbicide resistance-conferring mutations in the *ALS* gene of weedy rice can be verified with molecular markers (Roso et al., 2010; Rajguru et al., 2005; Sales et al., 2008; Kadaru et al., 2008). The rapid development of molecular markers provides an effective tool for studying genetic diversity and population differentiation of plant species (Parker et al., 1998; O'Hanlon et al. 2000). Molecular techniques can identify the mechanism of resistance caused by known

mutation (Corbett and Tardif 2006). Molecular markers like simple sequence repeats (SSRs), also referred to as microsatellites, are frequently used to study genetic diversity and evolutionary relationships. These markers are robust, exhibit high allelic variation, and are distributed throughout the *Oryza* genome (McCouch et al. 1997). SSR markers can be used to detect outcrossing between weedy rice and rice cultivars due to their co-dominant nature (Gealy et al., 2002). Eighteen microsatellite markers were tested by Gealy et al. (2002) to detect weedy rice and rice outcrosses; four (RM215, RM234, RM251, and RM253) produced one or more alleles that were present only in weedy rice or cultivated rice. SSR markers can differentiate medium-grain and long-grain temperate japonica cultivars also. RM180 marker allowed detection of outcrosses of weedy red rice with CL161 ClearfieldTM rice (Shivrain et al., 2007). Zhang et al. (2006) also used RM180, together with RM234 and RM253 markers, to detect 81 and 327 weedy rice hybrids in 2003 and 2004, respectively. Microsatellite markers can be used to identify the cultivated rice parents of outcrossed weedy rice (Gealy et al., 2002). Along with that, SNP (Single Nucleotide Polymorphism) markers can be used to identify polymorphism in *ALS* gene of weedy rice responsible for resistance to imidazolinone herbicides (Kadaru et al., 2008). Resistance conferring SNPs can be detected by use of SNAP markers (single nucleotide-amplified polymorphism) (Roso et al., 2010). The SNAP marker is the modified SNP marker and consists of an additional mismatched nucleotide within the third base from the 3' end of the SNP primer such that only one allele will amplify in PCR (Drenkard et al., 2000) and this modified allele-specific PCR procedure for assaying SNPs can easily discriminate between the specific and nonspecific alleles.

Seed dormancy in weedy rice

Seed dormancy is one of the principal weedy characteristics of weedy rice that enables its long persistence in soil. Dormant seed is viable seed that does not germinate under favorable conditions (Bewely, 1997; Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is a favorable and adaptive trait for weedy species because it allows weeds to escape control measures and persist in the soil seedbank. Factors like temperature, after-ripening period and ecotypes of red rice play a major role in seed dormancy (Diarra et al., 1985; Noldin et al., 1999; Vidotto and Ferrero, 2000). Weedy rice is highly variable in its dormancy and ranged from 26-100% when after-ripened for 12 d at room temperature (Do Lago, 1982) and can stay dormant and viable for up to 10 years in the soil (Goss and Brown, 1939). The seed maturity at harvest and temperature during after ripening affect the rate of seed dormancy (Roberts, 1961, 1965; Cohn and Hughes, 1981). The after-ripening facilitates the release of seed dormancy in all plants in general including weedy rice (Oliveira, 1992). Franco et al. (1997) observed that after-ripening at room temperature, release dormancy in both weedy rice (60-120 d and cultivated rice (90-120 d). The after-ripening process in rice with 11% moisture content occurred rapidly at 20-30 C (Cohn and Huges, 1981). In weedy rice, after-ripening at 25 C was faster between 6-14% moisture content and severe inhibition of after-ripening occurred at >18% and < 5% moisture content (Leopold et al., 1988). In another study, when the weedy rice seeds were incubated at 30° C in Petri-plates wrapped in aluminum foil in dim green light, the dormancy gradually decreased and was completely released at 25 days (Gianinetti and Cohn, 2008; Moreno, 1990). In most of the weedy rice populations (9 SH, 7 BHA) collected from Southern-Arkansas, dormancy was released at 90 days after-ripening (Tseng et al., 2013). Moreno, (1990) conducted a study on two rice cultivars (Labelle and Nato) and two weedy rice (strawhull and blackhull) ecotypes at 5 days

after-ripening and found that the germination of both cultivars was 8 and 4 % respectively, whereas for weedy rice was 7 and 5 % respectively. A variation in germination of the two cultivars and two weedy rice ecotypes was observed at 5 d and 25 d after-ripening. The germination of 'Labelle' and 'Nato' at 5 days was 94 and 9% whereas at 25 d, germination of Nato increased to 99%. In weedy rice, the germination increased from 92% to 99% from 5 days to 25 d after-ripening. In another study, in weedy rice ecotypes 84-100% germination was observed after-ripened for 60 d when incubated at 35° C (Tseng et al., 2013). Temperature also, plays an important role in variability in seed dormancy. The high variation in germination was observed (44-97%) at 15° C among weedy rice populations whereas no germination was observed at 1° C. Variation in dormancy between the major ecotypes of weedy rice - strawhull and blackhull ecotypes were observed irrespective of the temperature conditions. Blackhull populations had lower germination capacity than strawhull weedy rice populations (Tseng et al., 2013) and blackhull weedy rice required longer after-ripening time to release dormancy.

Seed dormancy in rice and weedy rice has been studied extensively by using different molecular techniques (Gu et al., 2004, 2011; Lin et al., 1998). Many QTLs (Quantitative trait loci) have been identified associated with seed dormancy. Five QTLs associated with seed dormancy in cultivated rice have been identified which were located on chromosome 3, 5, 7 and 8 and accounted for 48% of phenotypic variation (Lin et al., 1998). BILs (backcross inbred lines) of a cross between 'Nipponbare' and 'Kasalath' were developed and seeds from 98 BC₁F₅ were collected on the 40th day after heading and a germination was set up at 30°C under 100% humid conditions for seven days (Lin et al., 1998). Phenotypic variations explained by each QTL ranged from 6.7% to 22.5% and the five putative QTLs explained about 48% of the total phenotypic variation in the BC₁F₅ lines.

Many studies have indicated that the presence of red pericarp (bran), which is governed by *Rc* allele is associated with high levels of dormancy, and high levels of seed shattering in weedy rice (Vaughan et al., 2001; Gealy et al., 2003; Delouche et al., 2007). Genetic mapping of weedy rice from Thailand, localized a QTL to *Rc* locus which govern pericarp pigmentation in weedy rice (Gu et al., 2004). Due to continuous natural hybridization between cultivated rice and red rice, loss of pericarp pigmentation as a result of outcrossing can be disadvantageous to weedy rice populations in terms of reduced dormancy.

Seed shattering in weedy rice

Seed shattering is one of the important weedy traits. Seed dispersal through shattering helps weedy rice to enhance their presence in the seed bank and spread to new areas (Harlan and DeWet, 1965). Abscission layer cells (a barrier of thin-walled parenchyma *cells*) play a major role in shattering of weedy rice seeds. As seeds mature, cells at the abscission layer degrade, allowing the grain to detach from the mother plant (Jin and Inouye, 1982). In general, *indica* cultivars shatter more than *japonica* rice (Konishi et al., 2006). Numerous genes and QTLs appear to be involved in the differentiation of abscission layer cells. The major QTLs reported for seed shattering in rice were *sh4* and *qSH1* (Li et al., 2006; Konishi et al., 2006) and one recessive shattering gene *OsCPL1* (Ji et al., 2009). In general, mutation in *Sh4* protein inhibits the normal development of an abscission layer, which results in reduced seed shattering (Li et al., 2006), whereas inactivation of *OsCPL1* gene enhances the development of the abscission layer and seed shattering in rice (Ji et al., 2009). A single nucleotide polymorphism (SNP) in shattering QTL, *qSH1*, regulates the expression of homeobox protein. The reduction in expression by this SNP at the provisional abscission layer prevents shattering in rice (Konishi et al., 2006). SNP in *qSH1* is also responsible for reduced seed shattering in *japonica* cultivars. In

the absence of this SNP in *qSH1* QTL of *indica* cultivars, plants exhibit relatively strong seed shattering, whereas some *japonica* cultivars do not shatter at all. Reagon et al., (2010) found one non-shattering weedy rice accession among a group of weedy rice studied for morpho-physiological characteristics. The non-shattering weedy rice accession in their study could have mixed ancestry. Non-shattering weedy rice accessions might have acquired additional non-shattering alleles through hybridization with cultivated rice. *Sh4* and *qSH1* were reported to have played role in the domestication of cultivated rice from wild rice (*O. rufipogon*) and regulate 69% of the seed shattering in *japonica* and *indica* subspecies (Konishi et al., 2006; Li et al., 2006). However, Zhu et al. (2012) indicated that *sh4* played a minor role in the domestication of Asian rice and unidentified loci are responsible for domestication of cultivated rice. Recently, Nunes et al. (2013) presented evidence that *qSH1* and *sh4* genes are not associated with seed shattering in weedy rice. They evaluated the expression of 6 genes related to cell wall development (*Os09g0530200*, *Os11g0462100*, *Os04g04480900*, *Os02g0170900*, *OsXTH8* and *OsCel9D*), 3 known genes or QTLs related to seed shattering (*qSH1*, *sh4* and *OsCPL1*) and 6 putative seed shattering gene reported by Huang et al., (2010) in two weedy rice accessions and two rice cultivars ('Lacassine' and 'Batatais'). Evaluation of gene expression revealed that the genes *OsCPL1* and *OsXTH8* are related with the occurrence of high seed shattering in weedy rice and the expression of the *OsCel9D* gene is associated with reduction of seed shattering. Despite high diversity between cultivated rice and weedy rice ecotypes, all sampled rice and weedy rice accessions possessed similar or identical alleles at *sh4* locus, suggesting that the domestication-associated non-synonymous substitution (G/T) in the second exon of *sh4* (Li et al., 2006) is not sufficient for loss of shattering (Nunes et al., 2013). Seed shattering and dormancy were also reported to be affected by the genes which govern red bran in weedy red rice. The presence of

red pericarp (bran) is associated with high levels of dormancy, and high levels of seed shattering (Vaughan et al., 2001; Gealy et al., 2003; Delouche et al., 2007). This implies that loss of red pericarp color in weedy red rice can reduce seed shattering which is also the basis of domestication theory of rice. Hybridization between cultivated rice, wild and weedy rice populations can impact the population dynamics of weedy rice (Gressel and Valverde, 2009; Gealy et al., 2003; Zhu et al., 2012). The continuous exchange of pollen between cultivated rice and weedy rice could transfer the shattering genes among these populations. Transfer of non-shattering gene from cultivated rice to weedy rice would reduce seed shattering in weedy rice and therefore, reduce the soil seed bank. On the other hand, the introgression of shattering genes to cultivated rice would increase the grain shattering of rice cultivars, reduce harvested rice, and increase the volunteer rice problem.

What is volunteer rice?

Volunteer rice is a crop stand which emerges from shattered seeds of the previous crop (Gealy, 2005; Warwick and Stewart, 2005; Sudianto et al., 2013). Volunteer rice is the most common problem in paddy where crop rotation is not practiced. Normally, volunteer rice germinates either before winter (Oct.-Nov.) or in spring (Apr-May) (Kumar et al., 2008; Sudianto et al., 2013). It contaminates the rice seed and increases economic losses in succeeding crops. Volunteer rice is a weed when it is morphologically and phenologically different from rice cultivar and has lower grain yield and quality. Volunteers from hybrid rice are segregating plants, which compete with the crop, but could have low yield and low grain quality, which may reduce the overall productivity of a given field and quality of harvested grain. HR volunteer rice could be a similar problem as that of HR weedy rice. Volunteers from a HR rice crop (e.g.

Clearfield riceTM) could serve as agents for gene flow from rice cultivars to the natural weedy rice populations (Gealy et al., 2003; Gressel and Valverde, 2009; Shivrain et al., 2007; Sudianto et al., 2013). These outcrosses give rise to diverse weedy populations which compete with rice, contaminate the rice seed during harvest and increase economic losses.

Impact of winter-flood on volunteer rice seed viability

In rice growing areas of the U.S., winter flooding is a widespread practice wherein rice fields are flooded from the start of autumn following rice harvest until the spring before tillage operations (Fogliatto et al., 2010). Winter-flood improves rice straw decomposition which acts as an alternative strategy to the burning of rice residue (van Groenigen et al., 2003). Winter-flood also decreases the number of tillage passes needed to prepare the field for planting (Bird et al., 2002). Water management is especially important for weed control in rice production (Caton et al., 2002) because water-saturated soil limits the germination and growth of many weeds. Flooding at 7-14 DAS reduced *C. difformis* plant heights by at least half compared to no-flood conditions. Most weedy rice seedlings are killed with winter-flood and low winter temperatures, and thus, do not need to be sprayed with broad spectrum herbicides, such as glyphosate (Valverde, 2005), as commonly required with the conventional stale seed bed. The germination of weeds is inhibited under anaerobic soil conditions (Bird et al., 2002; Caton et al., 2002). The viability of cultivated rice seed was reduced by 10 times under flooded conditions compared with no-flood overwintering (Baek and Chung, 2012). In a field study of seed overwintering at soil surface in South Korea, weedy rice was compared with cultivated rice (Baek and Chung, 2012). Weedy rice had higher viability (92.7%) compared with cultivated rice (4.3%) without any flood treatment. In the second year, weedy rice and cultivated rice were

subjected to winter-flood and dry conditions, where weedy rice showed higher viability with (90%) and without winter-flood (61%) compared with cultivated rice. Cultivated rice had 5% viability in no flood and 0.5% viability in winter-flood condition. Similarly, Fogliatto et al., (2010) conducted a field experiment in Southern US with weedy rice over 2 years with 3 treatments - winter-flood in a field without any flooding history, winter-flood in a field which was flooded in previous year and no-flood treatment. In the first year, the treatment with no-flood history had higher weedy rice germination compared with field which was flooded over winter in previous year. However, no difference was observed in these treatments in the second year owing to similar flooding history in the first year. Overall, winter-flood reduced the weedy rice density up to 95% compared with conventional no-flood system (27%). Collectively, these studies demonstrate that in rice-growing areas of temperate climate, winter-flooding alone is an effective strategy for reducing the viability of shattered seeds in the field to minimize volunteer rice and weedy rice infestation.

Potential herbicides for volunteer rice control

Grass herbicides with good residual activity would be the potential alternate option for volunteer rice control apart from winter-flood if the herbicides do not cause injury to the rice crop. Herbicides like 2,4-D, sulfentrazone, pyroxasulfone, fluometuron, and flumioxazin are potential alternatives where residual activity of 2,4-D lasts for a few weeks and sulfentrazone remain in the soil up to one year. 2,4-D is a postemergence (POST) broadleaf weed control herbicide but has some residual activity on grass weeds. It is used as pre-plant herbicide in corn, sweet corn and rice (4 weeks before planting) and preemergence PRE in corn because it also has some activity when applied to the soil. It belongs to the synthetic auxin group of herbicides

available in both ester and amine forms. Its soil half-life values for both amine and ester forms have been estimated at 10 d (Vogue et al., 1994). In general, 2,4-D ester has low water solubility and thus, leaches less in flooded conditions than does the more water soluble amine form (Walters, 1999). Water temperature, soil pH and organic matter impact the degradation of 2,4-D. Dissipation (95%) of the initial concentration (DT95) of 2,4-D in paddy rice soil, rain-fed lowland rice, and bareground soil was estimated at 15, 26, and 20 d, respectively (Johnson et al., 1995). The amine salt form of 2,4-D rapidly hydrolyzes in soil and water to form 2,4-D acid (Tomlin, 2006). Microbial decomposition breaks down 2,4-D acid into CO₂, 2,4-DCP and other non-toxic forms (Ghassemi et al., 1981; Walters, 1999).

Flumioxazin belongs to the N-phenylphthalimide group and is registered as PRE, and POST, treatment for the control of broadleaf weeds and some grasses in soybean, sweet potato, cotton, dry beans, field corn, and sugarcane (Askew et al., 1999; Scott et al., 2001). It is tank-mixed with glyphosate in preplant burndown applications to provide residual activity and enhance desiccation of glyphosate-tolerant species. In general, flumioxazin quickly dissipates in soil and has a half-life of 10 to 25 days. The residual activity of flumioxazin is greatly affected by water content in soil. Higher water content decreases the binding of herbicide molecules to the soil (Clay and Koskinen, 1990; Ferrell et al., 2005). Only 24% of flumioxazin was detected 90 days after application in clayey soil (sand =14.4%, silt =35.3%, clay = 50.3%) (Alister et al., 2008). In a carryover study in Brazil on clayey textured soil, it was observed that the herbicide had no adverse effect on grain sorghum 6 months after application in a soybean field (Dan et al., 2010). The persistence of flumioxazin in southern US soils depends upon percentage of clay and soil organic matter content with higher adsorption in clay soils (Ferrell et al. 2005). Low organic matter and clay content results in rapid dissipation of flumioxazin.

Sulfentrazone belongs to the triazolinone group of herbicides with activity on broadleaf and grassy weeds in soybean, sugarcane, sunflower and many vegetables. It controls giant foxtail 97 to 100%, yellow nutsedge 96 to 98%, common waterhemp 97 to 98%, common cocklebur 91 to 94%, and ivyleaf morningglory 100% (Krausz and Young 2009). In potato (*Solanum tuberosum*), sulfentrazone (0.14 to 0.28 kg/ha) and flumioxazin (0.035 to 0.07 kg/ha) provided excellent broadleaf weed control at all the rates tested, whereas grass control increased as rate increased (Wilson et al. 2002). Higher rates of sulfentrazone (0.28 kg/ha) also control goosegrass and large crabgrass, but weak on common ragweed (58%) (Bailey et al., 2009). Its bioactivity is more intense in clayey soils and in soils containing high levels of organic matter (Reddy and Locke, 1998; Ohmes and Mueller, 2007). In soybean, when applied at 1.2 kg ai ha⁻¹, it can persist in soil for more than 539 days (Blanco et al. 2012). In beetroot crop, a longer crop rotation interval is required as injuries were observed at 302 days after application (Szmigielski et al., 2009). Vivian et al. (2006) reported that sulfentrazone persisted up to 467 days at 0-10 cm soil depth at the dosage of 0.9 kg ha⁻¹. Sulfentrazone (0.28 kg ha⁻¹) has very good grass control in potato but its residual activity may have negative effect on succeeding crop. Sulfentrazone applied in the fall reduced the stand of rice planted the following spring, but did not affect rice yield because of the ability of rice to compensate for low population density by producing more tillers when provided with optimum fertilizer and other crop production inputs (Zeng and Shannon, 2000). Sulfentrazone degradation in soil is enhanced in no-till systems where microbial activity is high, which results in reduced efficacy under such conditions (Locke and Bryson, 1997; Geier et al., 2006; Mahoney et al., 2014).

Pyroxasulfone belongs to the pyrazole group of herbicides, (Tanetani et al., 2009) and is labeled for corn, cotton and soybean. The high variation in half-life of pyroxasulfone was

attributed to soil moisture and clay content. The half-life (DT_{50}) of pyroxasulfone in soil was estimated to be at 71 days in loam soil, TN (Mueller and Steckel, 2011) and 104-134 days in Nunn fine clay loam soil, CO (Westra et al., 2014). In fine clay loam soil with 452 mm rainfall, the half-life (DT_{50}) of pyroxasulfone was estimated at 134 d; with higher rainfall (731mm) DT_{50} was 104 d (Westra et al. 2014). This indicates that pyroxasulfone would persist in soil longer if there is less soil water such as in non-flooded soil over the winter.

Volunteer rice impact on rice grain yield and quality

Grain quality is an important consideration in rice production and there is an increasing demand for improvements in grain quality characteristics. As rice (*Oryza sativa* L.) is one of the staple food crops therefore, the volume and grain quality are primary concerns in rice production. Grain quality is estimated by many parameters including milling ratio; head rice recovery; uniformity of grain shape, size, and color; and cooking quality (Septiningsih et al., 2003; Aluko et al., 2004). Contamination of the rice grain with other grains of various sizes and characteristics makes it inferior in quality and loses market value. Volunteer rice plants are the shattered seeds of the previous rice cultivars, are major contaminant in the field. Volunteer rice plants, when harvested with rice crop reduce rice milling quality if found in milling samples above threshold levels. After harvest, rough rice is cleaned, and de-hulled before actual milling when the bran is removed from rice seed. Head rice are the kernels which are at least three-fourth of the original length and plays a major role in determining the yield of brown and milled rice which together are assessed for rice market value and the milling quality (Webb, 1980; Juliano and Bechtel, 1985; Unnevehr et al., 1992; Aluko et al., 2004). Several other characteristics such as chalkiness, percentage of broken kernels, and undesirable grain color also reduce the

commercial value of the cultivated rice. (Bautista et al, 2009). Chalkiness in the rice grain deteriorates the strength of the kernel and tends to break easily during milling (Nagato, 1962; Rani et al., 2006). Basutkar et al. (2014) reported that milled rice yield, head rice yield, and head rice chalkiness of commingled samples vary according to the weighted average of the head rice yield of each cultivar and head rice chalkiness values, respectively. Apart from chalkiness, physical dimensions (Chen et al., 1999; Webb, 1980) and moisture content (Andrews et al., 1992; Reid et al., 1998) have been shown to affect rice milling performance.

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CHAPTER III

Herbicide and Winter Flood Treatments for Controlling Volunteer Rice Off-Season

Abstract

Field experiments were conducted to study the efficacy of 12 herbicide treatments for volunteer rice control with, or without, winter-flooding in Stuttgart and Rohwer, Arkansas, USA over two years (2012-13 and 2013-14). Herbicides were applied either in the fall or at 35 d prior to planting rice in the spring. Commercially harvested ClearfieldTM long-grain inbred rice 'CL152' was used as volunteer rice seed, broadcasted and lightly incorporated in October, 2012 and 2013. 'Jupiter' (medium-grain inbred, conventional rice) was planted in May as the rice crop. Winter-flood was initiated soon after the fall herbicide treatments were applied and terminated in February. Winter-flood reduced volunteer rice germination by 34% in 2013 and by 40% in 2014. Some fall herbicide treatments, without winter flood, generally caused more injury to the rice crop planted in the spring than the winter-flooded treatments. Fall application of pyroxasulfone (0.12 kg ha⁻¹), flumioxazin (0.14 kg ha⁻¹), and sulfentrazone (0.34 kg ha⁻¹) as well as pre-plant application of pyroxasulfone (0.12 kg ha⁻¹) and 2,4-D (2.24 kg ha⁻¹), had lower volunteer rice infestation, averaged over flood treatments. However, 2,4-D applied pre-plant (2.24 kg ha⁻¹), sulfentrazone in the fall (0.34 kg ha⁻¹) and pyroxasulfone pre-plant (0.12 kg ha⁻¹) injured the rice crop by 20%, 23%, and 47%, respectively. Fall application of pyroxasulfone (0.12 kg ha⁻¹) followed by a lower rate of 2,4-D (1.12 kg ha⁻¹) 35 d pre-plant caused minimal (6%) crop injury and did not reduce yield. This treatment provided better volunteer rice control (73%) than pyroxasulfone alone at 0.12 kg ha⁻¹ applied in the fall (64%). To evaluate the overwintering potential of hybrid and non-hybrid volunteer seeds, these seed types were planted at three depths (0, 7.5, 15 cm) in flooded and non-flooded conditions in an outdoor, buried-pot experiment at Stuttgart and Rohwer over 2 years. Winter-flood reduced rice germination by 50% in 2013-14 and 40% in 2014-15 (averaged over seed type and depth), after 160 d and 130 d of

burial, respectively. After the winter, the viability (germinable + dormant) of hybrid seed was higher (13 and 53%) than that of non-hybrid seed (8 and 27%).

1. Introduction

Volunteer rice (*Oryza sativa* L.) is a common problem in the rice paddy, which emerges from shattered seeds of the previous crop (Gealy, 2005; Warwick and Stewart, 2005; Sudianto et al., 2013). In temperate regions volunteer rice germinates either before winter (October-November) or in the spring (April-May) (Kumar et al., 2008; Sudianto et al., 2013). Volunteer rice is a weed when it is morphologically and phenologically different from, and has low grain yield and quality compared with cultivated rice. Volunteers from hybrid rice are segregating plants, which compete with the crop, but could also have low yields and low grain quality *per se*, thereby reducing the overall productivity of a given field and the overall quality of harvested grain. Therefore, it is expected that the volunteer rice problem would be less with conventional cultivars compared with hybrid rice or weedy rice. Volunteers from a herbicide-resistant rice crop (e.g. Clearfield riceTM) could serve as agents for gene flow from rice cultivars to the natural weedy rice populations through outcrossing, especially in the case of the ClearfieldTM hybrids (Gealy et al., 2003; Gealy, 2005; Gressel and Valverde, 2009; Shivrain et al., 2007; Sudianto et al., 2013). Rice is primarily self-pollinating, and typically, <1% cross pollination occurs between rice cultivars and weedy populations (Gealy et al., 2003; Cao et al., 2006; Shivrain et al., 2007). Once introgressed, crop genes can persist in the weedy populations indefinitely (Ellstrand et al., 1999; Gealy et al., 2003). These outcrosses give rise to populations of weedy type plants which compete with rice, contaminate the rice seed during harvest and increase economic losses. The amount of crop seed left in the field depends primarily on the harvesting efficiency. Furthermore, rice cultivars differ in seed shattering trait, which also impacts the amount of seed dropped in the field. Shattering is one of the most important seed dispersal mechanisms in plants. Early and high level of seed shattering is a common trait among the diverse types of weedy rices (Delouche et al. 2007). The proportion of shattered seed that will become volunteers in the following

cropping season is impacted by many factors. Shattered rice seeds can be consumed by predators, killed by adverse climatic conditions, germinate, or remain dormant to enrich the seed bank (Vidotto et al., 2001). The environmental conditions experienced by seed after dispersal or shattering and storage (Cohn and Hughes, 1981; Ferrero, 2003) can affect seed dormancy or germination (Fogliatto et al., 2011). The longevity of weedy rice seed increases with depth of burial (Goss and Brown 1939, 1940; Delouche et al., 2007) and buried seeds in flooded soil last longer than those buried in non-flooded soil (Noldin et al., 2006). Cultivated rice seed has a short life span in soil (less than 2 years) (Goss and Brown, 1939, 1940) compared with weedy rice (Noldin et al., 2006; Fogliatto et al., 2011), but the crop seeds stay viable long enough to be a problem in the succeeding season.

To minimize volunteer rice, Arkansas farmers have been practicing: (1) winter-flood or fallow without flood, (2) stale seed bed technique using glyphosate and tillage, (3) planting the same cultivars over several years to avoid contamination by dissimilar crop seeds, and (4) burning of residue after rice harvest (R.C Scott, Extension Weed Scientist, 2013 pers. Communication). Volunteer rice from conventional cultivars can be controlled with acetolactate synthase (ALS) inhibitor herbicides in Clearfield™ rice, but there is no chemical option during the rice growing season for controlling ALS-resistant volunteer rice from Clearfield™ rice. Soil-applied herbicides with good residual activity for grass control would be a potential alternative but, the efficacy of such herbicides for controlling volunteer rice (or weedy rice) and its residual effect on the succeeding rice crop are not known. This research was conducted to (1) assess the effects of fall- and pre-plant-applied herbicides on volunteer rice population in the succeeding rice crop and how this is impacted by winter-flooding; (2) determine the effect of fall- and pre-

plant-applied herbicides on the rice crop; and (3) compare the overwintering potential of hybrid and non-hybrid rice seeds.

2. Materials and Methods

2.1. Experiment 1. Off-season control of volunteer rice

To evaluate the effect of off-season herbicide treatments and winter flooding on volunteer rice germination, experiments were conducted at the Rice Research and Extension Center (RREC), Stuttgart (34°27'54.8"N, 91°23'58.4" W) and the Southeast Research and Extension Center (SEREC), Rohwer, (34°48'07.8"N, 91°17'11.6" W), Arkansas, USA between 2012 and 2014. The soil at RREC was a DeWitt silt loam (fine smectitic, thermic, Typic Albaqualfs) with 1.2% organic matter and a pH of 5.8. The soil at SEREC was Sharkey clay with <1% organic matter and a pH of 7.2. The experimental units were in a split-plot arrangement within a randomized complete block design with three replications at each location. The main factor was winter-flood (two levels: winter-flood and no flood) and the sub-factor was herbicide and dose combinations (12 levels: fall and spring pre-plant applications). In both years, the field was prepared by mid-October. ClearfieldTM rice 'CL152' was broadcasted over the field (175 kg ha⁻¹) and lightly incorporated (Fig 1, Table 1). Levees were formed to separate the flooded and non-flooded treatments, prior to herbicide application. For fall and pre-plant application, the herbicides were applied using a CO₂ backpack sprayer fitted with 4 flat fan nozzles (Tee Jet 11002) spaced 48 cm apart, delivering 187 L ha⁻¹ of spray volume at 276 kPa boom pressure. Each bay was covered with a fine mesh netting to prevent seed loss by predation. Winter-flood treatment was initiated two weeks after the fall herbicide application and was continued until February in the following year (Fig 1). The pre-plant herbicide treatments were applied in

March, 35 d prior to planting of rice (Table 1). Rice 'Jupiter' was drill-seeded at 100 kg ha⁻¹ in April in zero-till conditions. Permanent flood was established at the four- to five-leaf stage of rice. Phosphorous (P₂O₅) and potassium (K₂O) were applied pre-flood at 110 kg ha⁻¹ and 30 kg ha⁻¹, respectively. Nitrogen (N) fertilizer was applied twice to provide 100 and 50 kg ha⁻¹ N immediately before permanent flooding and at panicle initiation, respectively. For general weed control in rice, clomazone + quinclorac (0.67 kg ai ha⁻¹ + 0.56 kg ai ha⁻¹), preemergence (PRE) and propanil + thiobencarb (4.5 kg ai ha⁻¹ + 3.4 kg ai ha⁻¹), postemergence (POST) were applied to the whole field at both locations. Bensulfuron-methyl POST (0.12 kg ha⁻¹) was applied to control aquatic weeds (*Heteranthera limosa* and *Sagittaria montevidensis*) at RREC. Standard agronomic and pest management practices were implemented during the growing season and the crop was harvested with a combine in October in both years. The herbicide treatments which resulted in severe injury on rice in the first year were replaced with sequential application of promising herbicide treatments in the second year (Table 2). The total volunteer rice reduction was estimated with reference to the respective average of non-treated plots in winter-flood and no-flood treatments.

2.2. Data recorded

Volunteer rice germination was recorded from a 1-m² quadrat, randomly located in each plot at 18 WAS (weeks after sowing), 20 WAS, 28 WAS and 30 WAS (Fig. 1). Neither volunteer rice nor weed emergence occurred before 28 WAS due to unfavorable weather conditions (Fig.2). Crop injury and rice stand count was recorded 3 weeks after rice 'Jupiter' planting (29 WAS). Yield and grain moisture (%) was recorded at harvest.

2.3. Data analysis

Data were subjected to analysis of variance (ANOVA) using SAS (v.9.3) (Table 3, 4). Locations were considered as random effect and blocks were nested within location. Flooding and herbicide treatments were considered as fixed effects. The data were pooled over two locations and analyzed separately by year. Means were separated using Fisher's protected LSD at $P \leq 0.05$. The mean and variance models are described as follows:

$$\text{Mean} = \mu + f + h + (f \times h)$$

$$\text{Var} = \text{var}(l) + \text{var}[b(l)] + \text{var}[f \times b(l)] + \text{var}(R_E)$$

Where: f is the flood treatment, h is herbicide, b is block, l is the location and R_E is the residual error.

2.4. Experiment 2. Overwintering potential of hybrid and non-hybrid rice seeds

This experiment was conducted during the 2013-14 and 2014-15 winter season at the same sites as the volunteer rice control experiment. At each location within a year, the experiment was arranged in a three factor-factorial randomized complete block design (RCBD) with three replications. Two hundred seeds of 'Jupiter' (inbred rice) and 'CLXL745' (ClearfieldTM hybrid rice) were placed at the surface, or at depths of 7.6 cm and 15 cm in pots (d = 20 cm) filled with field soil. Each depth treatment was set-up in separate pots that were either flooded or not flooded during the winter. In total, there were 12 combinations of flood, seed placement depth, and seed type. The pots were buried up to the top 10 cm from the lid to facilitate flooding of designated treatments and at the same time allowing the seed environment to equilibrate with that of the soil profile. The flood treatment was initiated on November 29 and removed on February 28 in both years (2014 and 2015).

Holes were bored into the non-flooded pots, before burial, to allow drainage of rain water and equilibration of soil moisture and gases inside the pot and the soil environment. All pots were covered with a metal mesh to prevent seed loss by predation. Seeds buried at various depths were placed between two sheets of fine nylon mesh to facilitate extraction of seeds at termination of the study. The mesh was covered with thin layer of soil before placement of seeds. After placement of seeds in the pots, all pots were then buried in the spot from where soil was extracted to fill the pots. Water was maintained in the flooded pots at 6-8 cm up to February 28, in both years (2014 and 2015). The seeds were extracted on May 10, 2014 in year 1 and April 8, 2015 in year 2 after recording seed germination in the field. The excavated seeds were washed with tap water and placed in 9 cm-diameter Petri-dishes containing moistened filter paper and incubated for 12 days at 30° C in the dark. The germinated seeds were then counted and discarded. The viability of remaining seeds was determined using the tetrazolium (2,3,5 triphenyltetrazolium chloride) test (Overaa, 1984; Noldin et al., 2006). Seed embryos were cut longitudinally, immersed in a staining solution (1% tetrazolium) in Petri dishes, and incubated at 30° C for three hours. Seeds that were firm with pink or red embryos were considered alive and dormant. The fraction of dormant seed was calculated based on 200 seeds.

2.5. Data analysis

The data were subjected to analysis of variance using JMP Pro (v.11) (Table 7). The location and its interaction effect with treatments was not significant so data were pooled over two locations within a year; therefore, the data were analyzed separately by year. The location effect was considered as random. Means were separated using Fisher's protected LSD at $P \leq 0.05$.

3. Results

3.1. Experiment 1. Off-season control of volunteer rice

There was no germination of volunteer rice after the fall herbicides were applied until 28 WAS due to freezing conditions in February-March. The temperature remained cool until May in both years (Fig 2). The volunteer rice population density was higher in 2014 than in 2013 as remnant seed of the rice crop from 2013 augmented the soil seed bank. The herbicide and winter-flood interaction effect was significant on the total volunteer rice germination recorded at 28 WAS and 30 WAS (Table 3). Contrast analysis of the non-herbicide, weed-free checks over flood treatments showed that winter-flood by itself reduced volunteer rice germination by 34% in 2013 and by 40% in 2014 (Fig. 3).

In 2012-13, the total volunteer rice germination was higher in winter-flood treatment with 2,4-D (2.24 kg ha⁻¹; fall), flumioxazin (0.14 kg ha⁻¹; fall), S-metolachlor (1.68 kg ha⁻¹; fall), and sulfentrazone (0.34 kg ha⁻¹; fall). The highest volunteer rice reduction of 81-85% was observed with pyroxasulfone at 0.12 kg ha⁻¹ applied in the fall or 35 d pre-plant (Table 5). This was followed by 2,4-D at 2.24 kg ha⁻¹ pre-plant. Fall herbicide treatments with winter-flood had no effect on the rice crop in terms of crop stand and yield. Pyroxasulfone pre-plant (0.12 kg ha⁻¹) was effective in reducing volunteer rice infestation, but caused the highest crop stand reduction. Significant stand reduction was also observed with 2,4-D (2.24 kg ha⁻¹; pre-plant) and S-metolachlor (1.68 kg ha⁻¹; fall). However, pyroxasulfone applied in the fall (0.12 kg ha⁻¹) and the lower rate of 2,4-D (1.12 kg ha⁻¹) applied pre-plant did not reduce crop stand. Fall-applied herbicides caused higher crop injuries in no-flood treatments compared with winter-flood treatments. Among the herbicides, pyroxasulfone pre-plant (0.12 kg ha⁻¹) caused the highest injury (47%) followed by sulfentrazone in the fall (0.34 kg ha⁻¹) (29%) and 2, 4-D pre-plant

(2.24 kg ha⁻¹) (16%) in 2012-13 averaged over winter-flood treatments (Table 5). Similarly, pyroxasulfone pre-plant reduced rice yield by 32% and sulfentrazone applied in the fall reduced yield by 25%. The pre-plant application of 2,4-D also resulted in less rice yield relative to the nontreated, weed-free check. Rice planted into plots sprayed with 2,4-D (2.24 kg ha⁻¹) 35 d prior to planting in the spring yielded less (6.9 t ha⁻¹) than rice in plots sprayed with 2,4-D at the same rate in the fall (8.2 t ha⁻¹). The lower rate of 2,4-D (1.12 kg ha⁻¹) applied either in the fall or 35 d pre-plant, did not reduce rice yield. Rice planted in plots sprayed with sulfentrazone in the fall (0.12 kg ha⁻¹) yielded similar to that of non-treated, weed-free rice. The lower rate of 2,4-D (1.12 kg ha⁻¹) applied in the fall or 35 d pre-plant in 2012-13, were not effective on volunteer rice and so were excluded in 2013-2014.

In 2013-14, the highest volunteer rice reduction of 69 – 73% was recorded in plots sprayed with 2,4-D preplant (2.24 kg ha⁻¹) and pyroxasulfone fb 2,4-D (0.12 fall fb 1.12 kg ha⁻¹ pre-plant) (Table 6). However, 2,4-D pre-plant at 2.24 kg ha⁻¹ caused 23% crop injury. The sequential application of pyroxasulfone (0.12 kg ha⁻¹; fall) and 2,4-D (1.12 kg ha⁻¹; pre-plant) did not cause any additional crop injury and provided 14% higher volunteer rice control compared to pyroxasulfone (0.12 kg ha⁻¹) alone. Fall application of sulfentrazone (0.34 kg ha⁻¹) and fluometuron (1.12 kg ha⁻¹) in non-flooded plots caused higher injury to the rice crop than application in flooded plots (Table 6). The lowest yields (5.8 t ha⁻¹ and 6.0 t ha⁻¹) were recorded in 2,4-D pre-plant (2.24 kg ha⁻¹) and sulfentrazone in the fall (0.34 kg ha⁻¹). Rice in plots treated with all other herbicides, yielded similar to rice in the non-treated weed-free plots (7.1 t ha⁻¹).

3.2. Experiment 2. Overwintering potential of hybrid and non-hybrid rice seeds

The effects of winter-flood, seed type, burial depth, and seed type x depth interaction on

germination capacity (GC) were significant in both years (Table 7). The germination of rice seed buried in winter-flooded soil was reduced by 50% in 2014 and 40% in 2015 (averaged over seed type and burial depth) after 160 d and 130 d of burial, respectively (Fig. 4). The overwintering capacity of rice was also influenced by seed type x depth interaction at both locations. The GC of hybrid seed 'CLXL745' placed at the soil surface was 4 times higher (27%) than that of non-hybrid seed 'Jupiter' (7%) in 2013-14 and 2 times higher (8%) in 2014-15 compared with non-hybrid (4%) (Table 9). The GC of non-hybrid seed placed on the soil surface and at 7.5 cm depth did not differ. The germination of hybrid seed decreased to 8% and 2% at 15 cm in 2013-14 and 2014-15, respectively, relative to germination of seeds placed on the soil surface. The non-hybrid seed germination was 1% at 15 cm depth in both years.

The dormancy of rice seed was influenced by all three factors (flood, seed type and depth), flood x depth and seed type x depth interaction in both years. No interaction effect of flooding x seed type was observed in either year (Table 7). Dormancy was higher in no-flood treatment (30 and 9%) than in winter-flood (22 and 6%) in 2013-14 and 2014-15, respectively (averaged over seed type and burial depth) (Fig. 4). Dormancy increased with depth in both years (Table 8). In both years (2013-14 and 2014-15), there was no difference in dormancy of seed placed at the surface and at 7.5 cm depth under winter-flood conditions. Dormancy increased to 29% and 8% at 15 cm depth compared with surface (16% and 4%, respectively) (Table 8). Under no-flood treatment, irrespective of seed type, the dormancy increased to 37% and 12% at 15 cm in 2013-14 and 2014-15 from 21% and 5% at the surface, respectively. Seed type x depth interaction showed a similar trend of increased dormancy with depth in case of both hybrid and non-hybrid seed, where hybrid seed dormancy was higher at each depth compared to non-hybrid seed in both years (Table 9).

4. Discussion

4.1. Influence of winter-flood on volunteer rice seed viability

In rice growing areas of the United States, winter flooding is a widespread practice consisting of flooding rice fields from the start of autumn following rice harvest until the spring before tillage operations (Fogliatto et al., 2010) where anaerobic soil conditions limit the germination and growth of many weeds including weedy rice (Bird et al., 2002; Caton et al., 2002). Winter-flood improves rice straw decomposition and an alternative to the commonly practiced burning of rice residue to clean the field, postharvest (van Groenigen et al., 2003). Our research showed that winter-flood alone can reduce the volunteer rice germination significantly compared with no-flood conditions. According to Baek and Chung, (2012), winter-flood reduced the viability of cultivated rice seed by 10 times, compared with no-flood overwintering. Similar findings on weedy rice were reported by Fogliatto et al. (2010), where winter-flood reduces the weedy rice seed density up to 95% compared with conventional no-flood system (27%). Collectively, these studies demonstrate that in rice-growing areas of temperate climate, winter-flood alone is an effective strategy for reducing the viability of shattered seeds in the field to minimize volunteer rice infestation. Where possible, winter-flooding should be a part of an integrated management system for weedy or volunteer rice.

4.2. Herbicide efficacy on volunteer rice

Herbicide adsorption and transport in the soil are important factors which determine the herbicide efficacy and its residual activity. Adsorption of herbicide molecules to soil depends upon the chemical structure of herbicide, soil texture, moisture and organic matter (Ferrell et al.,

2005). Likewise, the type of herbicide, application rate, herbicide persistence and mobility, rainfall, topography and climate are some of the factors affecting movement of herbicide in the soil (Wauchope, 1994; Westra et al., 2014).

2,4-D belongs to the synthetic auxin group of herbicides and is recommended as POST treatment for broadleaf weed control in turf and major grass crops. It is also used as pre-plant in corn, sweet corn and rice (4 weeks before planting) and PRE in corn because it also has some activity when applied to the soil. Its soil half-life values have been estimated at 10 d for the acid, diethylamine salt, and ester forms (Vogue et al., 1994). In general, 2,4-D ester is less likely to leach in flooded conditions due to low water solubility but the amine form may leach more due to its higher water solubility and polarity (Walters, 1999). The aerobic and anaerobic half-life of 2,4-D was estimated at 66 d and 312 d, respectively (Concha and Shepler, 1994). In water, the biodegradation of 2,4-D is affected by nutrient status of the soil, temperature, soil pH and presence of organic matter. At 25 C and pH7, the half-life of 2,4-D in water was reported as 39 d. Dissipation of 95% of the initial concentration (DT95) of 2,4-D in paddy rice soil, rain-fed lowland rice, and bareground soil was estimated as 15, 26, and 20 days, respectively (Johnson et al., 1995). The amine salt of 2,4-D was used in our study, which rapidly hydrolyzes in soil and water to form 2,4-D acid (Tomlin, 2006). Microbes further decompose 2,4-D acid to yield CO₂, 2,4-DCP and other non-toxic forms (Ghassemi et al., 1981; Walters, 1999). Therefore, the lower crop injuries caused by fall-applied 2,4-D amine (2.24 kg ha⁻¹) in winter-flood treatments, compared with no flood, could be due to leaching and rapid degradation of this herbicide in water.

Flumioxazin belongs to the N-phenylphthalimide group and is registered as PRE, and POST, treatment for the control of broadleaf weeds and some grasses in cotton, dry beans, field

corn, sweet potato, sugarcane and soybean (Askew et al., 1999; Scott et al., 2001). It is also used as a partner for a nonselective herbicide such as glyphosate, in preplant desiccation of vegetation, to improve the overall efficacy and provide residual activity. In general, flumioxazin is adsorbed by soil colloids but has quick dissipation rate in soil (half-life of 10 to 25 d). Flumioxazin moves down the soil profile with water and is absorbed by plant roots (Ferrell and Vencill, 2003). It has been reported that just by increasing soil water content, flumioxazin can be removed from soil adsorption sites as more water reduces the binding capability of the herbicide molecule to soil (Clay and Koskinen, 1990; Ferrell et al., 2005). This leads to reduced residual effect or short persistence. Therefore, flumioxazin was more effective in controlling volunteer rice when applied to soil without flood and less effective in winter-flooded soil. In dissipation studies, only 24% of flumioxazin was detected 90 d after application in clayey soil (sand =14.4%, silt =35.3%, clay = 50.3%) (Alister et al., 2008). The persistence of flumioxazin in southern US soils depends upon percentage of clay and soil organic matter content with higher adsorption in clay soils (Ferrell et al., 2005). Low organic matter and clay content results in rapid dissipation of flumioxazin. The soils of SEREC (sand =4.35%, silt = 52.6%, clay = 43%) and RREC (sand = 2%, silt = 62%, clay = 36%) have high clay content, which increases adsorption of flumioxazin to soil in no-flood conditions resulting in higher volunteer rice control compared with winter-flooded conditions.

Sulfentrazone belongs to the triazolinone group of herbicides and effectively controls broadleaf and grassy weeds in soybean, sugarcane, sunflower and many vegetables. Its bioactivity is more intense in clayey soils and in soils containing high levels of organic matter (Reddy and Locke, 1998; Ohmes and Mueller, 2007). It can persist in soil for more than 539 d at 1.2 kg ai ha⁻¹ when applied in soybean (Blanco et al., 2012). Szmigielski et al., (2009) observed

negative effects of this herbicide on beetroot plants at 302 days from application, demonstrating the necessity of a longer rotation interval for succeeding crops. Vivian et al., (2006) reported that sulfentrazone persisted up to 467 d at 0-10 cm soil depth at the dosage of 0.9 kg ha⁻¹. Sulfentrazone (0.28 kg ha⁻¹) has very good activity on grasses (Bailey et al., 2009) but its persistence may have negative effect on succeeding crops. In our study, sulfentrazone caused higher injury on the rice crop compared with other herbicides (*S*-metolachlor, flumeturon, thiobencarb, flumioxazin) at 29 WAS. However, lower crop injuries were observed in winter-flooded treatments compared to no-flooded treatments probably due to rapid dissipation in flooded soil.

Pyroxasulfone is a relatively new herbicide which belongs to the pyrazole group of herbicides (Tanetani et al., 2009) and is labeled for corn, cotton and soybean. The half-life (DT₅₀) of pyroxasulfone in soil was estimated to be at 71 d in loam soil, TN (Mueller and Steckel, 2011) and 104-134 d in Nunn fine clay loam soil, CO (Westra et al., 2014). The high variation in half-life of pyroxasulfone was attributed to soil moisture and clay content. In fine clay loam soil with 452 mm rainfall, the half-life (DT₅₀) of pyroxasulfone was estimated at 134 d; with higher rainfall (731mm) DT₅₀ was 104 d (Westra et al. 2014). This indicates that pyroxasulfone would persist in soil longer if there is less soil water such as in non-flooded soil over the winter. Sulfentrazone degradation in soil is enhanced in no-till systems where microbial activity is high, which results in reduced efficacy under such conditions (Locke and Bryson, 1997; Geier et al., 2006; Mahoney et al., 2014). The rice in our test was planted in no-till conditions, which is expected to enhance microbial activity and, therefore, reduce residual activity on rice. Our data showed that where pyroxasulfone (0.12 kg ha⁻¹) was applied into fall, it controlled volunteer rice in spring, and reduced rice crop stand at 29 WAS. However, it did not

affect rice yield because rice recovered from that injury. Rice compensates for low population density by producing more tillers when provided with optimum fertilizer and other crop production inputs (Yoshida, 1981; Zeng and Shannon, 2000).

4.3. Overwintering potential of hybrid and non-hybrid rice seed at different depths

The seed burial experiment showed that winter-flood reduced the number of seed that can survive the winter, averaged over depth of seed placement and rice seed type (Table 9). Baek and Chung, (2012) reported that the viability of a cultivated rice placed on the soil surface without flood over the winter was 5% and was 0.5% when flooded over the winter. It supports the hypothesis that winter-flood reduces germination and viability of rice seed. Overwintering with winter-flood favors the decay of weed seeds including weedy rice (Nelms and Twedt, 1996). In comparison to conventional rice, the viability (germinable + dormant) of hybrid seed was higher (13-53%) than that of non-hybrid rice seed (8-27%). The hybrid rice seed had 2-4 times higher germination than non-hybrid rice seed. Similarly, the proportion of dormant seed was also higher (2 times) in hybrid seed than that of non-hybrid seed. This means that more hybrid rice seeds survived the winter (across flooding treatments) compared with non-hybrid seed. Baek and Chung, (2012) also compared cultivated rice with weedy rice and found higher viability in latter case (90% and 61%) in no-flood and winter-flood respectively. Several other studies have indicated high viability in weedy rice in over-winter studies (Seong et al., 2004; Noldin et al., 2006; Fogliatto et al., 2011). This may have occurred because weedy rice has primary seed dormancy (Cohn and Hughes, 1981) and generally exhibits deeper (secondary) dormancy than cultivated rice (Gu et al., 2003). Our study also showed an interaction effect between seed type and seed-depth on germination capacity and dormancy of hybrid and non-

hybrid rice seeds. Dormant seeds buried in soil under natural conditions would persist longer than non-dormant seeds (Justice and Bass, 1978). The deterioration of seeds is associated with damage to lipid, nucleic acid, and protein owing to oxidation reactions (Yan et al., 1996; Hsua et al., 2003; Baek and Chung, 2012). In plants with freezing tolerance, anti-freeze proteins block the growth of ice crystals in outside spaces of cells in the tissue, preventing cell damage from freezing and thawing (DeVries, 1986; Jeong, 2009). Anti-freeze protein accumulation in the embryo and aleuronic layer of the seed is one of the freeze tolerance mechanisms in weedy rice (Baek and Chung, 2012). The same mechanism could be functioning in hybrid rice. In addition, hybrid seed vigor could contribute to freezing tolerance.

5. Conclusion

Winter-flood alone (without herbicide) can reduce volunteer rice germination by 34% to 40%. The application of 2,4-D pre-plant (1.12 kg ha^{-1}) following pyroxasulfone applied in the fall (0.12 kg ha^{-1}) provided better volunteer rice control (73%) than the other treatments and did not injure the rice crop nor cause yield loss. Therefore, the sequential application of pyroxasulfone and 2,4-D at these rates is a potential herbicide program for controlling volunteer rice. Winter-flood can reduce volunteer rice infestation up to 50% (averaged over depth and seed type). This practice can be integrated with postharvest/pre-plant herbicide use to minimize volunteer rice infestation. Hybrid rice seed has higher capability to survive the winter than non-hybrid rice seed. Therefore, the volunteer rice problem is expected to be higher following a hybrid rice crop than following an inbred cultivar. The volunteer rice population can be reduced significantly simply by keeping the soil saturated over the winter or in conjunction with a

herbicide program such as pyroxasulfone (0.12 kg ha^{-1}) applied in the fall followed by a pre-plant application of 2,4-D (1.12 kg ha^{-1}).

6. Acknowledgements

The authors thank Mariccor S.A.B. Batoy, George Botha, Silvana Spaniol Fin, Meagon Garner, Pamela Carvalho de Lima, Fernando Luis Martini, Linda Martin, Fernando Ramirez, Ana Carolina Roso, Reiofeli Salas, Sirichai Sathuwijarn, and Hussain Tahir for their help in the implementation of the experiment in the field and evaluation of seed germination capacity and dormancy in the laboratory.

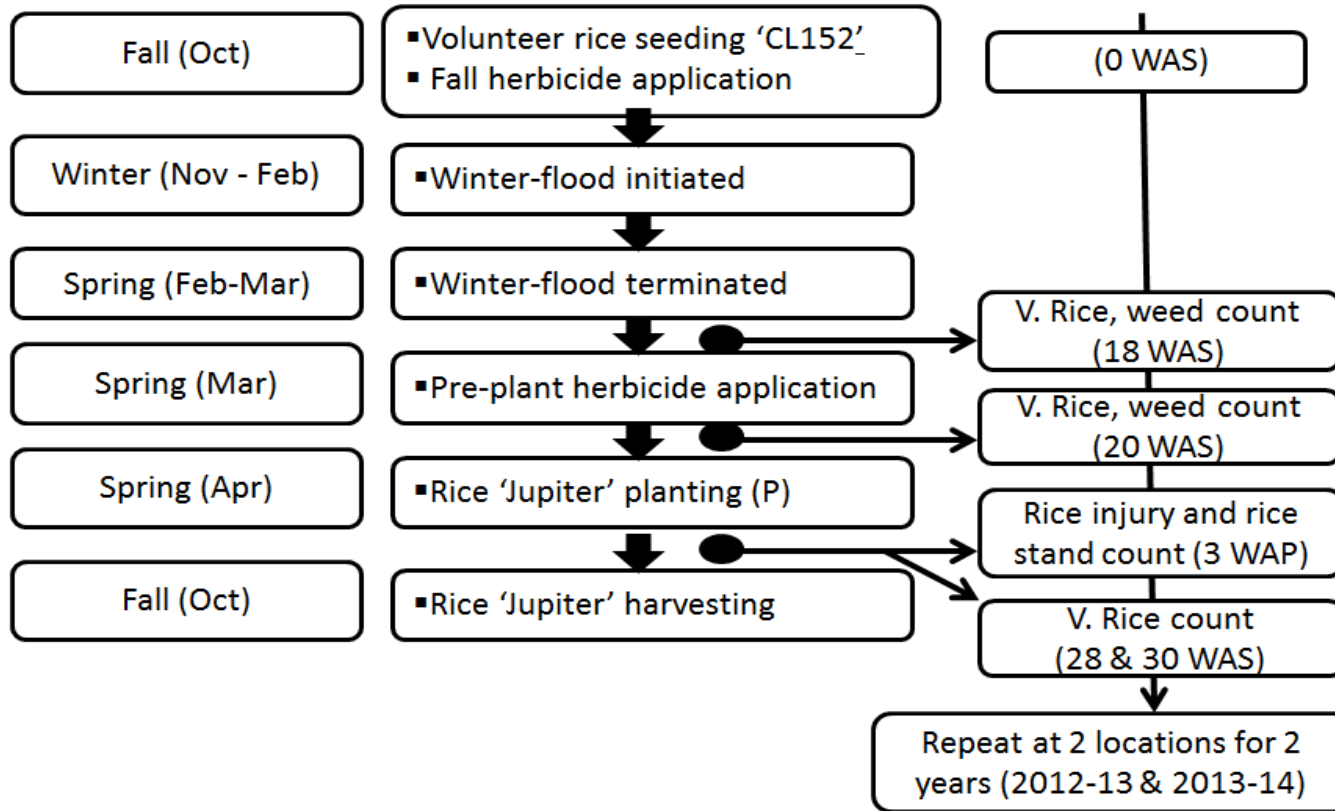


Fig. 1. Schematic diagram of general sequence of field activities for Experiment 1 (2012-13 and 2013-14), at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA. WAS = weeks after volunteer rice seeding (broadcasted); WAP = weeks after rice crop planting (drill seeded); 3 WAP = 29 WAS.

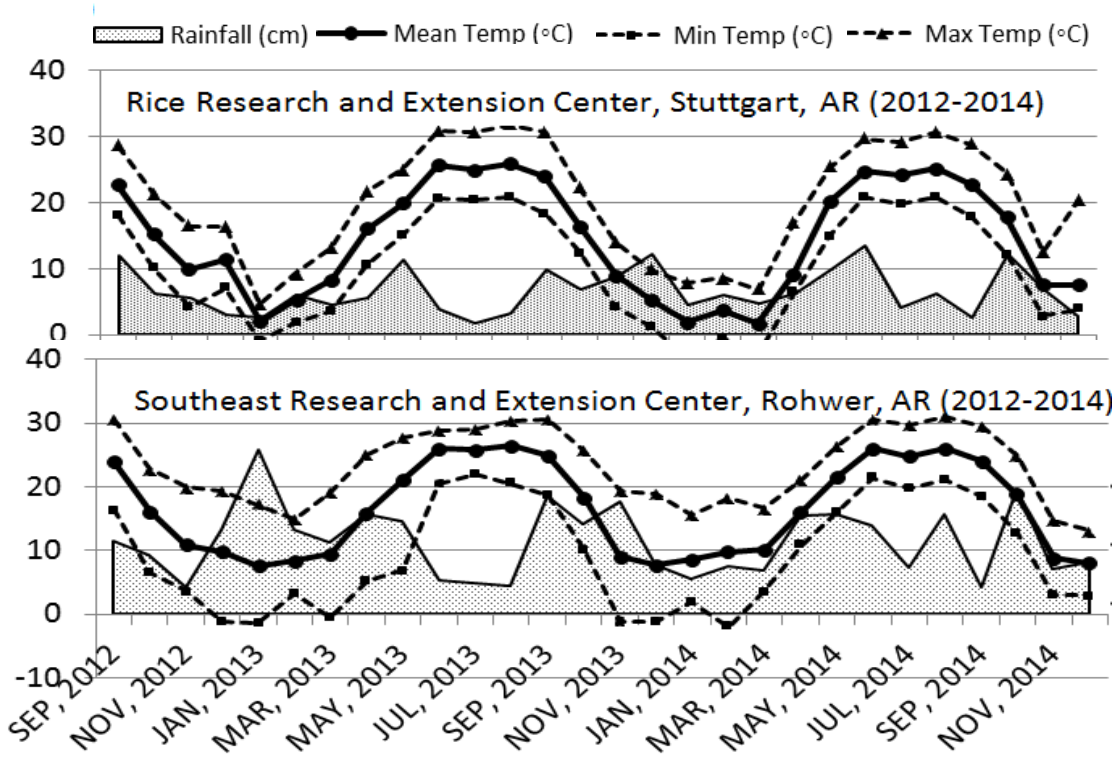


Fig. 2. Weather data during the course of the experiments (Source: United States Department of Agriculture, weather station data archives and Southeast Research and Extension Center weather station data)

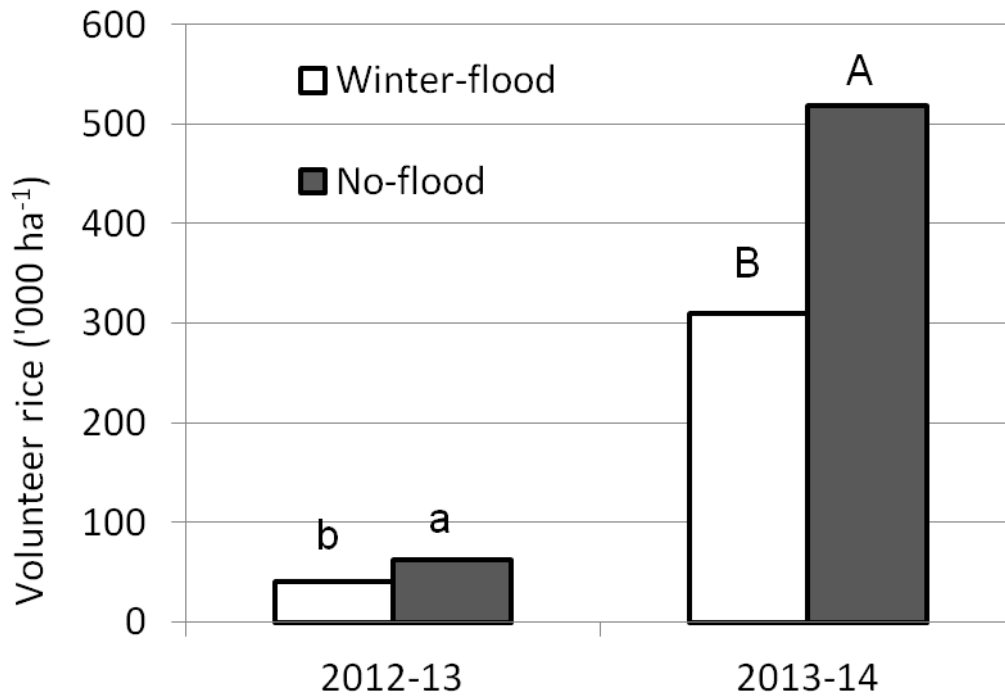


Fig. 3. Influence of winter-flood on volunteer rice germination (averaged over 6 replications across 2 locations) during 2012-13 and 2013-14 (Experiment 1) at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA. For each year, treatments with different letters are significantly different. Uppercase letters are for comparing flood treatments in 2013-14 and lower case letters are for comparing flood treatments in 2012-13.

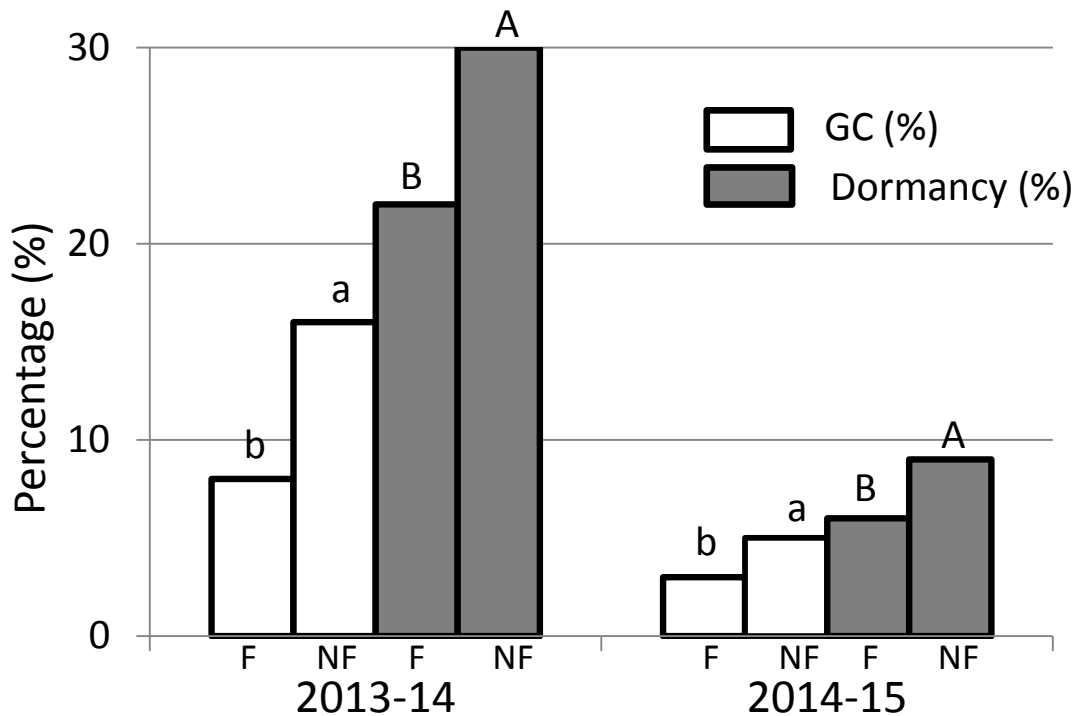


Fig. 4. Influence of winter-flood on germination capacity (GC) and dormancy of rice seed, averaged over seed type and burial depth during 2013-14 and 2014-15 (Experiment 2) at the Rice Research and Extension Center (RREC), Stuttgart, and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA. F = winter-flood, NF = no-winter flood. For each variable within a year, treatments with different letters are significantly different. Uppercase letters are for comparing dormancy means; lower case letters are for comparing germination capacity means.

Table 1. Timing of field operations for off-season control of volunteer rice (Experiment 1) in 2012-13 and 2013-14 at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA.

Field operations	2012-13^a	2013-14
Volunteer Rice 'CL152' Seeding (Fall)	Oct 30	Oct 30
Winter flood duration	Nov 25 - Feb 28	Nov 15 - Mar 5
Herbicide application (Fall)	Nov 10	Oct 30
Herbicide application; pre-plant (Spring)	Apr 2	Mar 27
Rice planting (Jupiter)	May 9	May 2

^aMaximum of three days difference (± 3 d) between two locations within a year

Table 2. Herbicide treatments across two years (2012-13 and 2013-14) at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA.

Herbicide treatments	Dose (kg ai ha ⁻¹)	Time of application	2012-13 ^a	2013-14
no herbicide	NA	NA	√	√
2,4-D	1.12	fall	√	–
2,4-D	2.24	fall	√	√
2,4-D	1.12	35 d pre-plant	√	–
2,4-D	2.24	35 d pre-plant	√	√
flumioxazin	0.014	fall	√	√
flumioxazin	0.014	35 d pre-plant	√	√
flumioxazin fb 2,4-D	0.014 fb 1.12	fall fb 35 d pre-plant	–	√
S-metolachlor	1.68	fall	√	√
sulfentrazone	0.336	fall	√	√
fluometuron	1.121	fall	√	√
pyroxasulfone	0.123	35 d pre-plant	√	–
pyroxasulfone fb 2, 4-D	0.123 fb 1.12	fall fb 35 d pre-plant	–	√
pyroxasulfone	0.123	fall	√	√
thiobencarb	4.48	35 d pre-plant	–	√

^a '√' = treatment was implemented, otherwise '-' for treatments that were implemented only in year 1 or only in year 2.

Table 3. Analysis of variance (*P*-values) for the volunteer rice density, rice crop injury and yield, experiment 1 (2012-13), Arkansas, USA.

Source of variation ^a	df ^b	Total ^c V.Rice	V.Rice ^d reduction	Crop ^e injury (3 WAP)	Crop stand (3WAP)	Yield
----- <i>P</i> -values-----						
Flood (F)	1	0.0655	0.0798	0.0007	0.7302	0.0902
Herbicide (H)	11 (10)	<.0001	<.0001	<.0001	<.0001	<.0001
Flood x Herbicide (F x H)	11 (10)	0.0004	0.0629	0.0386	0.7275	0.7361

^aData were pooled over two locations; RREC, Stuttgart and SEREC, Rohwer, AR (2012-13)

^bParentheses indicate degrees of freedom (df) for volunteer rice (VRice) reduction and crop injury; non-treated weed-free was excluded from analysis

^cSum of volunteer rice (V. rice) recorded at 28 WAS and 30 WAS

^dVolunteer rice reduction was estimated relative to the average plant density in respective non-treated plots with or without winter flood, within a year

^eCrop injury was estimated relative to non-treated weed-free treatment at 21 DAT on a scale of 0 to 100; includes stunting and chlorosis

Table 4. Analysis of variance (*P*- values) for the volunteer rice density, rice crop injury and yield, experiment 1 (2013-14), Arkansas, USA.

Source of variation ^a	df ^b	Total ^c V.Rice	V.Rice ^d reduction	Crop ^e injury (3 WAP)	Crop stand (3WAP)	Yield
----- <i>P</i> -values-----						
Flood (F)	1	0.0136	<.0001	0.522	0.028	0.3955
Herbicide (H)	11 (10)	<.0001	<.0001	<.0001	0.0085	<.0001
Flood x Herbicide (F x H)	11 (10)	0.0200	0.1055	0.0245	0.4037	0.449

^aData pooled over two locations; RREC, Stuttgart and SEREC, Rohwer, AR

^bParentheses indicate degree of freedom (df) for volunteer rice (V. rice) reduction and crop injury

^cSum of volunteer rice (V. rice) recorded at 28 WAS and 30 WAS

^dVolunteer rice reduction was estimated with reference to the average plant density in respective non-treated plots with or without winter flood, within a year

^eCrop visual injury was estimated with reference to non-treated weed-free treatment at 21 DAT on a scale of 0 to 100; includes stunting and chlorosis

Table 5. Herbicide efficacy and rice crop 'Jupiter' performance during 2012-2013 (Experiment 1) at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA^a.

Herbicides	Rate kg ai ha ⁻¹	Timing	Total V. rice ('000/ha)		V. rice reduction (%)	Rice stand, 3 WAP ('000 ha ⁻¹)	Crop injury, 3 WAP (%)		Yield (t ha ⁻¹)
			F	NF			F	NF	
no herbicide	NA	NA	41	62	–	1768	–	–	7.6
2,4-D	1.12	fall	38	53	11	1645	2	4	7.7
2,4-D	2.24	fall	45	19	40	1636	4	8	6.9
2,4-D	1.12	35 d preplant	32	25	44	1703	9	8	7.2
2,4-D	2.24	35 d preplant	20	14	67	1456	15	17	6.4
flumioxazin	0.14	fall	36	15	42	1697	7	12	7.7
flumioxazin	0.14	35 d preplant	29	16	56	1681	12	14	6.8
S-metolachlor	1.68	fall	35	12	56	1583	9	15	6.8
sulfentrazone	0.34	fall	32	8	52	1748	23	35	5.7
fluometuron	1.12	fall	29	12	62	1708	12	18	6.9
pyroxasulfone	0.12	35 d preplant	9	7	85	1181	42	51	5.2
pyroxasulfone	0.12	fall	15	4	81	1708	10	16	7.4
Winter-flood				30	47	1637		13	7.3
No-flood				21	73	1648		18	6.8
LSD_(0.05)^b									
Flood				NS	NS	NS		***	NS
Herbicide				***	15	167		***	0.7
Flood x Herbicide		Within flood		8	NS	NS		5	NS
		Across flood		20	NS	NS		7	NS

^aData pooled over two locations; RREC, Stuttgart and SEREC, Rohwer (2012-13)

^bFisher's protected LSD to compare treatment means

*Indicates significance at ***P ≤ 0.001; only interaction LSD is shown if interaction effect is significant

Table 6. Herbicide efficacy and rice crop 'Jupiter' performance during 2013-2014 (Experiment 1) at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA^a.

Herbicides	Rate kg ai ha ⁻¹	Timing	Total		V. Rice reduction (%)	Rice stand, 3 WAP (⁰⁰⁰ /ha)	Crop injury, 3 WAP (%)		Yield (t ha ⁻¹)
			V. rice (⁰⁰⁰ /ha)				F	NF	
no herbicide	NA	NA	310	518	–	1250	–	–	7.1
2,4-D	2.24	fall	330	366	7	1236	0	0	6.9
2,4-D	2.24	35 d preplant	100	116	69	970	20	25	5.8
flumioxazin	0.14	fall	281	233	33	1098	3	4	7.3
flumioxazin FB	0.14 FB	fall FB 35 d	252	180	53	1153	8	5	7.2
2,4-D	1.12	preplant							
flumioxazin	0.14	35 d preplant	250	234	54	1051	10	10	6.8
S-metolachlor	1.68	fall	360	259	25	1163	3	5	6.9
sulfentrazone	0.34	fall	287	201	44	1190	12	30	6.0
fluometuron	1.12	fall	430	338	12	1039	8	15	6.7
pyroxasulfone	0.12	fall	168	114	64	1090	4	8	6.8
pyroxasulfone	0.12 FB	fall FB 35 d	138	93	73	1121	5	11	6.9
FB 2,4-D	1.12	preplant							
thiobencarb	4.48	35 d preplant	178	198	56	1220	6	4	7.0
Winter-flood			257		38	1027	7		6.6
No-flood			236		52	1242	10		7.0
LSD (0.05)^b									
Flood			*		12	150	NS		NS
Herbicide			***		9	110	***		0.5
Flood x Herbicide		Within flood	40		NS	NS	4		NS
		Across flood	64		NS	NS	5		NS

^aData pooled over two locations; RREC, Stuttgart and SEREC, Rohwer (2013-14)

^bFisher's protected LSD to compare treatment means

*Indicates significance at *P ≤ 0.05, ***P ≤ 0.001; only interaction LSD is shown if interaction effect is significant

Table 7. Analysis of Variance (*P*-values) for germination capacity (GC) and seed dormancy, for the seed overwintering experiment in 2013-14 and 2014-15 (Experiment 2), at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA.

Source of Variation	df	2013-14		2014-15	
		GC	Dormancy	GC	Dormancy
----- <i>P</i> -values-----					
Flood (F)	1	<.0001	<.0001	<.0001	0.0048
Seed type (S)	1	<.0001	<.0001	<.0001	0.0041
Depth of burial (D)	2	<.0001	<.0001	<.0001	<.0001
F × S	1	0.6282	0.4329	0.2186	0.0977
F × D	2	0.4312	0.3612	0.0252	0.0126
S × D	2	0.0286	0.0128	<.0001	0.0244
F × S × D	2	0.5348	0.7657	0.4574	0.4046

Table 8. Interaction effect of winter-flood and seed placement depth on seed dormancy during 2013-14 and 2014-15 (Experiment 2) at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA.

Dormancy (%) ^a						
Depth (cm)	2013-14			2014-15		
	F ^b	NF ^b	Mean	F	NF	Mean
0	16	21	19	4	5	5
7.5	21	31	26	6	9	8
15.0	29	37	33	8	12	10
Mean	22	30	29	6	9	8
LSD _(0.05) ^c ; F x D	6			3		

^aData pooled over two locations within a year (RREC, Stuttgart and SEREC, Rohwer)

^bF = Flooded; NF = Nonflooded over the winter

^cFisher's protected LSD to compare treatment means

Table 9. Germination capacity and dormancy of volunteer rice seeds as influenced by seed type and seeding depth in 2013-14 and 2014-15 (Experiment 2), at the Rice Research and Extension Center (RREC), Stuttgart and Southeast Research and Extension Center (SEREC), Rohwer, Arkansas, USA.

Depth	2013-14 ^a						2014-15 ^a					
	Germination Capacity (%)			Dormancy (%) ^b			Germination Capacity (%)			Dormancy (%)		
	H ^c	NH ^c	Mean	H	NH	Mean	H	NH	Mean	H	NH	Mean
0	27	7	17	27	10	19	8	4	6	6	3	5
7.5	21	6	14	34	19	27	4	3	4	7	5	6
15.0	8	1	5	40	26	33	2	1	2	10	7	9
Mean	19	5	12	34	22	26	5	3	4	8	5	7
LSD _(0.05) ^d												
Seed type (S)	6		7		1		2					
Depth (D)	3		4		1		2					
S x D	4		6		2		3					

^aData pooled over two location within a year (RREC, Stuttgart and SEREC, Rohwer)

^bNon-germinated, viable seeds were considered as dormant. The viability of intact, non-germinated seeds was tested using Tetrazolium

^cSeed type; H = hybrid seed, NH = non-hybrid

^dFisher's protected LSD to compare treatment means

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CHAPTER IV

Effect of Volunteer Rice (*Oryza sativa* L.) Infestation on Rice Grain Quality and Yield

Abstract

Volunteer rice (*Oryza sativa* L.) plants arise from shattered seeds of the previous crop, which could reduce the yield of cultivated rice and the commercial value of harvested grain. Volunteer rice plants from a cultivar other than the current crop produce grains that may differ in physico-chemical traits from the planted cultivar, which reduce the uniformity and milling quality of harvested rice grain. To evaluate the effect of volunteer rice infestation on rice yield and grain quality, 20 commercial fields were surveyed, across six rice growing counties of Arkansas in the fall of 2012. Panicles were collected from 1-m² areas (4-8 sites per field) representing different levels of infestations across the field. Cropping history that included hybrid cultivars over the past two years (2010 and 2011), indicated higher volunteer rice infestation (20%) in 2012 compared with fields planted previously with inbred rice (5.6%). The total grain yield of rice was reduced by 0.4% for every 1% increase in volunteer rice density across all fields, averaged over cultivars. The 1000-kernel weight, kernel length-width ratio, %protein, %amylose, and head rice yield (HRY) were affected by volunteer rice infestation and cultivars grown in the previous two years. The protein content, amylose content, chalk and physical dimensions of harvested grain did not change with repetition of the same cultivar for 3 years. Volunteer rice can potentially have a significant negative impact on rice yield and grain quality and should be managed effectively.

Keywords: amylose, chalkiness, Clearfield™ rice, hybrid rice, milling yield, non-hybrid

1. Introduction

Rice (*Oryza sativa* L.) is a major staple food crop for more than half of the world's population. The volume of production and grain quality are primary considerations in rice production. Grain quality is evaluated by many parameters including milling ratio; head rice recovery; uniformity of grain shape, size, and color; and cooking quality (Septiningsih et al., 2003; Aluko et al., 2004). Rice grain contamination with other grains of various sizes and characteristics results in inferior quality and reduced market value. Volunteer rice plants are the progenies of the previous rice cultivars, arising from shattered seeds or harvest losses. Prior to milling, rough rice is cleaned and dehulled. Milling removes bran layers from the caryopsis, producing the white rice that we consume. The milled rice consists of head rice (the kernels which are at least three-fourth of the original length) and broken kernels. Head rice yield (HRY), is the proportion of head rice to rough rice. Head rice is a major factor that determines rice market value as it is directly related to brown rice yield and milled rice yield, which together constitute the "milling quality" (Webb, 1980; Juliano and Bechtel, 1985; Unnevehr et al., 1992; Aluko et al., 2004). Chalkiness (seen as an opaque portion of the endosperm), percentage of broken kernels, and undesirable grain color can reduce the commercial value of rice. (Bautista et al., 2009). Chalky grains are not as hard as translucent ones and are prone to breakage during milling (Nagato, 1962; Rani et al., 2006). Volunteer rice grains from a different cultivar, or from plant types that mature earlier or later than the planted cultivar, can reduce the overall milling quality if present above threshold levels.

Grain appearance is mostly judged by grain shape and translucence of the endosperm (Tan et al., 2000). However, grain color has a stronger influence on consumer preference or acceptance for end-products (Juliano, 1985; Collado et al., 1997; Bhattacharya et al., 1999).

Protein content can influence the physical and chemical properties of cooked rice (Hamaker and Griffin, 1990; Marshall et al., 1990; Hamaker, 1994). The bran (outer layer of brown rice) contains high amount of protein, most of which is removed during milling. Protein content in general is influenced largely by environmental conditions, the level of available nitrogen (Nanda and Coffman, 1979; Perez et al., 1996), and genotype (Jing et al., 2010). Amylose is one of the major components of starch (Brown and Poon, 2005). Amylose content is responsible for the stickiness and tenderness of cooked rice. Rice starch contains nearly 20% amylose and 75-80% amylopectin (Jane et al., 1999). The amylose content of rice is one of the most important determinants of cooking and eating quality (Bao et al., 2002) and could give rice high or low mark for grain quality.

Besides the potential impact on harvested grain, volunteer rice also has an ecological impact that eventually translates to economic losses. Volunteers from hybrid rice emerge at different times due to variability in germination behavior and difference in depth of seed placement in the soil. In addition, volunteer plants from hybrid rice vary in maturity period as these will be segregating F₂ plants or of a later generation. This non-uniform germination and phenology increase the window for out-crossing and gene flow to weedy rice or the planted cultivar, which is a problem if the hybrid rice is herbicide-resistant. Further, the segregating volunteer plants compete with the crop, but have variable yield potential and grain quality *per se*. The overall crop productivity is therefore reduced by having less productive volunteers in the mix and the grain quality is compromised by contamination of different grain types and grains of different moisture contents due to non-uniform maturity of volunteer plants. The potential economic impact of volunteer rice on the rice industry due to crop yield losses from competition and reduction in grain quality is not known. The objectives of this research were: (1) to evaluate

the effect of hybrid and non-hybrid cropping history on volunteer rice infestation, and (2) to evaluate the effect of volunteer rice on the yield and grain quality of different rice genotypes.

2. Materials and Methods

2.1. Plant Material

In the fall of 2012, rice grain samples were collected from 20 farmers' fields infested with volunteer rice across six rice growing counties of Arkansas, USA (Fig. 1). Pertinent information regarding the field history was obtained from the grower (Table 1) and weedy rice infestation at the time of sampling was recorded. Paddy rice samples were harvested from a 1-m² area in each field, representing different levels of infestation, with two samples collected from non-infested sites per field. The total number of infested samples differed across fields, depending on the levels and patches of weedy/volunteer rice infestation within the field. In the Southern US, the rice crop is generally seeded in rows using a grain drill, on a dry seedbed. All the sampled fields were planted in this manner. The volunteer rice plants were distinguished from cultivated rice based on their location (between the drill rows) and random or patchy distribution. Further, volunteer rice could also be distinguished from the rice crop based on some morphological characteristics (i.e. taller or shorter than the rice crop, different growth habit) and phenology (early or late maturity). The samples were threshed within 30 days of harvest with a mechanical thresher at 18% grain moisture content. The threshed grains were kept at room temperature for further drying to 12% moisture content. The dry grains were then cleaned and 200g composite samples of both volunteer rice and cultivated rice were de-hulled and milled at the Riceland Mill, Stuttgart, Arkansas, USA.

*Composite sample = (200g * % rice yield in sample) + (200g * %Volunteer rice yield in sample)*

2.2. Morphological and physical characterization of grains

The following data were recorded: number of tillers, number of panicles, grain yield at 12% moisture, chalk% using WinSeedle™Pro, Epson Perfection V700 (Epson America Inc., Long Beach, CA, USA), and physical characteristics of grain (color, 1000-grain weight, length-width ratio, and others) using 2312 Graincheck™ Analyzer (FOSS TECATOR, Eden Prairie, MN, USA). All morphological and physical characteristics were recorded for the mixture of seeds (rice + volunteer rice) based on the proportion of volunteer rice seed in respective samples.

2.3. Chemical analysis of grains

The threshed rice kernels were dehulled using benchtop de-hulling equipment (Satake Rice Machine, Tokyo, Japan). Dehulled kernels were milled using a benchtop miller (PEARLEST, Kett Electric laboratory, Tokyo, Japan) for 45s to remove the bran. A subsample of the milled kernels (10g) was ground to pass a 0.5-mm screen in a Cyclotech grinder (Foss North American, Eden Prairie, MN, USA). Ground milled rice samples were analyzed for apparent amylose content according to AACC method 61-03.01 (AACC, 2010) using Auto Analyzer 3 (AA3; Seal Analytical, Mequon, WI, USA). Briefly, ground flour (50 mg) was placed in a Fisherbrand 50 ml centrifuge tube and wetted with ethanol (95%, 0.5 ml), followed by the addition of NaOH solution (1M, 4.5 ml) containing Triton X-100 (wetting agent) down the sides of the tube to wash any adhering sample down with the sodium hydroxide. After allowing the samples to sit overnight at 22°C without shaking, the samples were vortexed and 45 ml of deionized water was added to each tube, re-vortexed and allowed to sit overnight at 22°C. Duplicate aliquots were pipetted from each sample and placed on the AA3. Citric acid (0.1N), iodine solution [0.5 ml containing I₂ (3.2 mM) and KI (48.2 mM)], and deionized water were

added by the AA3 to each sample which then measured the absorbance at 620 nm. Amylose content was calculated from a standard curve generated by the AA3.

Crude protein levels were determined using 0.2 g ground brown rice (before milling) using the AACC method 46-30.01 (AACC, 2010) (LECO FP-2000; LECO Corp., St. Joseph, MI, USA). Briefly, rice flour was placed in ceramic weigh boats and then placed in the LECO FP-2000. Nitrogen gas was removed for other volatile combustion products and then measured by the thermal conductivity detector. A conversion factor of 5.95 (Juliano, 1985) was used to convert nitrogen value to crude protein content. Analysis was conducted in duplicates. Weighted averages of protein and amylose content were calculated based on the proportions of rice and volunteer rice seeds to represent protein and amylose content of the volunteer rice-infested sample. This weighted average was used for analysis purposes.

*Weighted average = (PC/AC of rice * % of rice yield in sample) + (PC/AC of V. rice * % of V. rice yield in sample);* where PC= protein content, AC=amylose content, V. rice = volunteer rice

2.4. Cropping systems categorization for data analysis

For objective 1, cultivars grown in previous years were grouped into 5 cropping histories/scenarios according to seed type (Table 1). If Clearfield™ rice was grown repeatedly in all 3 years (2010-2012), it was grouped in cropping history ‘A’ for hybrid Clearfield™ rice or ‘B’ for non-hybrid Clearfield™ rice. Imazethapyr (ALS herbicide) could reduce the volunteer rice density in Clearfield™ rice following a conventional rice cultivar. Therefore, if Clearfield™ rice was grown in at least one year after non-Clearfield™ rice in 2010, this field was grouped into either cropping history ‘C’ for hybrid or ‘D’ for non-hybrid seed type during 2011 and 2012. However, if Clearfield™ rice in 2010 was followed by conventional rice in 2011 and 2012, the field was grouped in cropping history A for hybrid Clearfield™ rice or B for non-hybrid

Clearfield™ rice because HR volunteer rice cannot be controlled in conventional rice.

One field that was planted with soybean in 2010 and fallowed in 2011, but had hybrid rice in 2009 was grouped into cropping history 'E'. Cropping history E, was excluded from data analysis for objective 1. For objective 2, the field with 'CL261' had a small number of samples because the volunteer rice density was similar across the field, hence CL261 was excluded from data analysis for objective 2.

2.5. Statistical analysis

2.5.1. To evaluate the effect of cropping history on volunteer rice infestation, analysis of variance (ANOVA) was conducted and Fisher's protected LSD ($\alpha = 0.05$) was used to compare the treatment means among different cropping histories. Contrast analysis was conducted to differentiate hybrid and inbred rice cropping histories.

2.5.2. To evaluate the effect of volunteer rice on the yield and grain quality, a two-factor linear regression model was used and analysis of covariance was conducted. Volunteer rice density and cultivars were treated as fixed factors and field was treated as random variable. The data were analyzed using Proc Mix in SAS 9.3 and JMP Pro v.11 software. Fisher's protected LSD ($\alpha = 0.05$) was used to compare the slopes of fitted lines when found significant.

3. Results

Volunteer rice infestation in surveyed rice fields differed widely depending upon the cultivar planted during the sampling year, type of cultivars grown in previous years, and farmers' management practices. In general, rice harvested from volunteer rice-infested fields includes a mixture of rice + volunteer rice. The problem becomes severe when volunteer rice matures at

different times. Cropping history that included hybrid cultivars over the past two years (A) had higher volunteer rice infestation (22%) in 2012 compared with all other cropping histories, except C where hybrid rice cultivars in 2010 were followed by Clearfield™ hybrid rice cultivars in 2011 (Fig. 2a). However, cropping history C had similar volunteer rice infestation as cropping history B (inbred cultivars in previous years) and B was similar to D (soybean followed by fallow) in terms of volunteer rice infestation. Contrast analysis of cropping histories A + C versus B, which compares the incidence of volunteer rice in fields planted with hybrid versus inbred cultivars in previous years, revealed that fields that had been planted with hybrid rice are more likely to have higher volunteer rice infestation (20%) than those planted only with inbred rice (5.6%) (Fig. 2b).

3.1. Chemical attributes

3.1.1. Protein content

Volunteer rice density alone could not explain the variation in protein content. Linear regression model with interaction of volunteer rice density and cultivar explained 64% of the variation in the data. The remaining 36% of the variation could be attributed to field and management practices. Protein content ranged from 7.0% in 'Roy J' to 8.8% in 'CL261' not contaminated with volunteer rice seed (Table 2). 'CLXP756' showed 0.26% increase in protein content with every 1% increase in volunteer rice density. The increase in protein content with volunteer rice density was observed only in CLXP756. The protein content of other cultivars declined with increasing density of volunteer rice.

3.1.2. Amylose content

Amylose content was highest in Roy J (22%) and lowest in Jupiter (14%), without

volunteer rice infestation. In general, amylose content differed between cultivars and varied with volunteer rice density. 'Cocodrie' showed 4.4% reduction in amylose content with every 1% increase in volunteer rice density. Cocodrie was planted after hybrid cultivars in fields 'F01' and 'F16' (scenario A) (Table 1). Volunteer plants of these hybrids had lower amylose content than Cocodrie, which in turn lowered the amylose content of the contaminated samples. In the same cropping scenario (A), CLXP756 was preceded by hybrid cultivar CLXL729 in fields F04 and F07, which had similar amylose content as that of CLXP756; in such cases, no change was observed in amylose content even after volunteer rice (CLXL729) contamination. As many cultivars were distributed across scenarios during the sampling year, the averaged effect of cropping scenarios resulted in no change in amylose content with increase in volunteer rice. Similarly, with other cultivars, the change in amylose content was not significant due to averaged effect of diverse volunteer rice with high and low amylose content. During the sampling year, CLXL745 was included in three out of five cropping scenarios. In cropping scenario A, the amylose content of CLXL745 decreased with increase in volunteer rice density and in cropping scenario B, the amylose content of CLXL745 increased with volunteer rice density. These changes were dependent on volunteer rice genotype and led to overall non-significant decline in amylose content of CLXL745 with increase volunteer rice density, (averaged over cropping scenarios).

3.1.3. Chalkiness

Without volunteer rice contamination, the lowest chalk (%) was observed in CL261 (2.1%) and Roy J (2.3%) followed by CLXP756 (3%). Chalk (%) was influenced only by cultivar. Volunteer rice density had no effect on variation in chalkiness of rice kernel. Cocodrie

had the highest chalk (4.8%) and 'Jupiter' had the lowest chalk (4.0%), at 17.7% volunteer rice density (averaged across all fields) (Fig. 5).

3.2. Physical attributes

3.2.1 Head rice yield

Volunteer rice had no effect on head rice yield of CLXL745 and CLXP756 but significantly reduced the head rice yields of Cocodrie, Jupiter and Roy J (Fig 3-D). Cocodrie was contaminated with the volunteer rice seed of 'XL723' and 'XL753' which in general, have lower head rice yield than Cocodrie.

3.2.2. 1000-Kernel Weight

Without volunteer rice contamination, the 1000-kernel weight of CL261 was the lowest (17.0 g) and that of Cocodrie was the highest (21.9 g) followed by that of 'Jupiter' (21.5 g). Kernel weights of Cocodrie and Jupiter sharply declined with increase in volunteer rice density compared with those of other cultivars (Table 4). The 1000-kernel weights of CLXL745 and Roy J were not impacted by increase in volunteer rice density.

3.2.3. Yield reduction

The total yield reduction was independent of cultivars. With every 1% increase in volunteer rice density, the total yield (rice + volunteer rice) was reduced by 0.4% (averaged over all cultivars) (Fig 3-F). Volunteer rice plants, several of which were immature at harvest, generally had less number of panicles and unfilled spikelets compared with cultivated rice. Lesser number of panicles on volunteer rice plants resulted in lower yields. Fields F13 and F14 (Table 1) had the least number of panicles (effective tillers) of volunteer rice (Fig. 4). These fields were planted with a longer-duration cultivar (Taggart) in the previous two years (potential

volunteer rice plants) and planted with shorter-season cultivars CL261 and CLXL745 during the sampling year. This led to many immature volunteer plants with fewer panicles at the time of sampling. A similar trend was observed in other fields planted with a short-season cultivar in the sampling year, but had volunteer rice plants that were offsprings of long- or medium-duration cultivars planted in previous years.

4. Discussion

4.1. Effect of cropping history on volunteer rice infestation

Our study demonstrated how rice cropping history could impact the severity of volunteer rice infestation. A related study demonstrated that 2-4 times more volunteer rice infestation can be expected from hybrid rice than from conventional rice (Singh et al., 2015, unpublished) due to high deterioration of conventional rice seed. Noldin et al., (2006) conducted experiment to study the influence of irrigation, burial depth on seed longevity of red rice and commercial rice (non-hybrid) cultivars. Rice cultivar seeds were not viable after 5 months, regardless of their position in the soil. The decrease in viability of buried non-hybrid rice cultivar seeds was the result of seed decay. Also, the viability of non-hybrid rice placed at soil surface was just 5% in no-flood conditions during winter and decreased to 0.5% in flood conditions (Baek and Chung, 2012). This leads to few volunteer plants from non-hybrid seeds. Our study showed a higher level of volunteer rice infestation in fields planted with hybrid rice in previous years than those planted previously with non-hybrid rice. Also, in a related experiment, we observed that the hybrid rice seed had higher dormancy than the non-hybrid rice (Singh et al., 2015, unpublished). It has been reported that dormancy increases with depth of seed placement (Omami et al., 1999) and the lifespan of dormant seeds buried in the soil under natural conditions may be longer than the

lifespan of non-dormant seeds (Justice and Bass, 1978). Shattered seeds which are buried in soil during field operations are brought back to the soil surface, or fully exposed, with soil disturbance during tillage. These seeds germinate at favourable conditions and increases volunteer rice density. Moreover, if the shattered seeds are of hybrid cultivars then these can germinate even from deeper depths (Singh et. al., 2015, unpublished).

4.2. Effect of volunteer rice on the yield and grain quality of different rice genotypes

Volunteer rice contamination in rice seed has affected many morpho-physiological characteristics of rice grain, except seed color. It has been reported by Basutkar et al., (2014) that milled rice yield, head rice yield, and head rice chalkiness of commingled samples vary according to the weighted average of the head rice yield of each cultivar and head rice chalkiness values, respectively. Chalk in rice kernels has strong negative correlation with head rice yield (Bautista et al., 2009) as higher chalk reduces kernel strength (Nagato, 1962; Rani et al., 2006; Webb, 1991). Physical dimensions (Chen et al., 1999; Chen and Siebenmorgen, 1997; Pomeranz and Webb, 1985; Webb, 1980) and moisture content (Andrews et al., 1992; Reid et al., 1998) have been shown to affect rice milling performance.

4.2.1. Protein content

Protein content, in general, varies by genotype and is further modified by N-fertilizer rate and application timing (Nangju and De Datta, 1970; Tamaki et al., 1989; Ghosh et al., 2004). Cameron and Wang, (2005) reported that two hybrids, 'XL7' and 'XL8', had a slightly higher amylose content, but significantly lower crude protein content than conventional inbred cultivars under the same milling conditions. A similar trend was observed when kernels of different

cultivars were analyzed without volunteer rice contamination (Table 2). Our data shows that inbred cultivars Cocodrie, Jupiter and CL261, except Roy J, had higher protein content than Clearfield™ rice hybrids CLXL745 and CLXP756. Gealy and Bryant (2009) reported that protein content is inversely related with amylose content ($P < 0.0001$). Similarly, our study showed that the cultivars CL261 and Jupiter had higher protein content but lower amylose content than other cultivars. The linear regression model in the current study explained 64% of the variation in protein content. The remaining variation could be attributed to differences between fields and management practices including N-fertilizer application, which can affect the total crude protein content of rice seed. It has been reported that the protein content of rice is influenced by quantity of N-fertilizer application (Perez et al., 1996; Champagne et al. 2009). Nitrogen is a constituent of proteins; higher dose of N-fertilizer results in higher protein content. Moreover, timing of N-fertilizer application also influences the protein content of rice grain. For example, top dressing N-fertilizer at heading increases HRY and protein content of the rice grain (Seetanum and De Datta, 1983). In Arkansas, farmers apply N-fertilizer pre-flood and second split at beginning of internode elongation (BIE). One single optimum pre-flood N application is recommended in fields that can be flooded timely with option of midseason application and split application is recommended in fields (Norman et al., 2013). N application is recommended between late boot and beginning of heading only for hybrids to minimize lodging and to increase milling quality (Norman et. al., 2013). During grain filling stage, grains derive N compounds accumulated in leaves and stem up to flowering stage (Osaki et al., 1991; Zhao et al., 2015). Accumulation of protein in the lateral region of the endosperm provides resilience and protects grain from breakage during milling (Leesawatwong et al., 2005). Therefore, protein content in

grain varied (36%) based on soil type, soil fertility, application and timing of fertilizer and irrigation conditions.

4.2. 2. Amylose content

The relation of amylose content with the grain type has been discussed in many studies. It has been shown that long-grain cultivars have higher amylose content than short-grain cultivars (Williams et al., 1958; Webb, 1985). Long-grain and medium-grain cultivars in the southern US were bred to contain 21-23% and 12-18% amylose, respectively (Moldenhauer et al., 2004). In the current study, Jupiter and CL261, both medium-grains from the University of Arkansas breeding program, had less amylose content (14.0% and 15.9%, respectively) than the long grain varieties (Table 2). Amylose content is governed by waxy (*Wx*) alleles (Shure et al., 1983; Sano, 1984; MacDonald and Preiss, 1985; Ayres et al., 1997) and strong correlation was observed with G-T polymorphism at the *Wx* locus (putative 5'-leader intron splice site) and apparent amylose content. This single nucleotide polymorphism ('T' in short-grain US varieties and 'G' in long grain US varieties) could explain 80.1% of the variation in the apparent amylose content (Ayres et al., 1997). In the current study, apparent amylose was affected by the interaction of volunteer rice density with cultivars planted (Fig. 3-B). This interaction model explained 93% of the variation in apparent amylose content. The remaining variation in amylose content in rice can be attributed to environmental factors, and management practices. The amylose content decreases with high N-fertilization in rice (Alcantara et al., 1996) and also negatively correlated with mean temperature during grain filling (Counce et al., 2005; Jin et al., 2005). N-fertilizer prolongs rice growing period which influences temperature of seed setting that leads to low amylose content (Hao et al., 2007). Sato, (1979) reported that activity of starch-synthesizing enzymes decreases under temperature stress and also reduces starch transport to developing kernels. The amylose

content of the harvested grain is expected to decline if contaminated with volunteer rice grains containing low amylose. The increase in volunteer rice density would also increase the grain contamination level; however, the overall impact of volunteer rice grain contamination on the amylose content of harvested grain depends upon both the level of contamination and the amylose content of the planted cultivar.

4.2.3. Chalkiness

Chalk is one of the major issues with rice quality which reduces its market value. Chalky endosperm decreases the kernel strength and results in lower head rice yield (Webb, 1991; Siebenmorgen and Qin, 2005; Ambardekar et al., 2011). Chalk increases when night air temperature is high at the time of grain filling (Counce et al., 2005; Ambardekar et al., 2011). The response of cultivars to high night air temperature is variable (Mackill et al., 1996; Yamakawa et al., 2007; Cooper et al., 2008; Lanning et al., 2011). The differential response of cultivars to night time air temperatures results in varying levels of chalkiness (Linscombe et al., 1991; Cooper et al., 2008). The current study indicated that chalkiness differs between cultivar, but is independent of volunteer rice density. However, increase in volunteer rice density can contaminate the harvested grain with seeds from different cultivars or plant types with lower or higher chalkiness. As the difference in chalkiness between crop seed and volunteer rice seed increases, the impact of seed contamination is also expected to increase. The exclusion of volunteer rice density from significant effects on chalkiness only indicates that not all volunteer seeds have different chalkiness than the planted cultivar and that chalkiness among volunteer rice seed is highly variable.

4.2.4. Milling quality

Whole grain milling yields are impacted by both genotype and environmental factors (Jodari and Linscombe, 1996). Calderwood et al., (1980) delayed rice harvest to reduce drying costs and found that head rice yield peaked shortly after maturity and declined sharply thereafter. The delay in rice harvest correlated with increased broken kernels, which reduced rice milling quality. On the other hand, harvesting the grains too early also reduces milling quality because immature kernels have high moisture content and soft endosperm (Reid et al., 1998; Siebenmorgen et al., 2006). Late-germinated volunteer rice plants were not mature at the time of sampling in all cropping scenarios. Fields F14, F13 and F12 in cropping history D and field F20 in cropping history B where long- or mid-season cultivar Taggart and Jupiter was grown in 2010 and 2011 (Table 1), had many immature volunteer rice plants. The seeds with high MC% generally break during milling thereby reducing head rice recovery. Head rice recovery is also low when grains are dry (< 14% MC) and undergo alternate drying and wetting. Volunteer rice seed from a short-season rice cultivar would mature earlier and, therefore, would have less moisture content than the long-season rice crop at the time of harvest. Early-maturing volunteer rice usually leads to higher chalkiness and fissuring in mature rice seed, as these seeds remain in the field until harvest of the planted cultivar. In cropping scenario B, Jupiter and CLXL745 had lower HRY, where these were preceded by Francis (F06) and CL151, CL131 (F02), respectively in previous years. Similarly, CLXL745 in cropping scenario C and Cocodrie in cropping scenario A had lower HRY where these were preceded by XL723 due to more breakage of the over-dried, contaminating shorter-season volunteer rice seed. Because genotype affects head rice yield, the genotype of volunteer rice affects the milling quality of contaminated, harvested rice grain. In general, CLXL745 and CLXP756 have lower HRY than other cultivars (Wilson et al.,

2013), which can reduce the head rice recovery of a rice crop contaminated by volunteer plants from such hybrid lines. As observed by Basutkar et al., (2014), head rice recovery decreased from 66.7% to 62.5% as the share of CLXL745 increased from 0% to 100% in original CL151 sample. They further observed that Clearfield™ hybrids require less time for milling because the kernels have thinner bran layer than those of conventional rice. Some of the kernels therefore will be overmilled, and break, when commingled with conventional rice.

4.2.5. Yield attributes

Genotype is the main source of variation in kernel weight, density, number of panicles and overall yield (Rao, 2005). Delayed maturation, immature panicles and contamination of seed with other types of different sizes and weights were some of the consequences of volunteer rice infestation. In the fields with volunteer rice, the panicles were fewer on volunteer rice plants compared with the planted cultivar. Many volunteer rice plants in each field germinated late, and showed reduced growth. Rice kernels that are set at later stages have poor grain quality and reduced dry matter (Mohapatra et al., 1993). Fields in cropping scenario D (Table 1) had many immature volunteer rice plants without panicles (Fig. 4) where CLXL745 was preceded by Taggart, Roy J or Jupiter in 2010 and 2011 (F14, F13). This was also the case with fields in cropping scenario B where Jupiter was preceded by Taggart (2010-2011) (F20). These volunteer plants competed with planted rice for resources, but did not contribute to the total yield. Similar cases were observed with other cultivars, where volunteer rice that germinated late, or had later maturity period, produced less panicles per unit area, resulting in reduced harvested grain per unit area. Thus, yield loss increased with increasing volunteer rice infestation. Moreover, the 1000-kernel weight of CLXL729, XL723, and XL753 (grown in 2010 and 2011) were lower than

that of Cocodrie, CLXP756 and CLXL745 (grown in the sampling year) (Wilson et. al. 2013). Thus, when planted rice is infested with volunteer rice from cultivars with lesser kernel weight, the overall productivity of the field will be reduced.

Planting the same cultivar across several years would not affect protein content, amylose content, 1000-grain weight and head rice yield (at 15% volunteer rice density of the same genotype). However, yield reduction may be observed due to lesser panicles on volunteer rice plants and immature plants without panicles at harvest (Table 5). It is expected that if permanent-flood delays or rice planted in dry direct-seeded conditions, volunteer rice plants may germinate late which will mature later than crop plants. The delay in harvest or difference in maturity of grains at harvest can lead to differences in chalk, crude protein content and apparent amylose content and HRY of the rice even for plants of the same genotype, depending upon the volunteer rice density.

5. Conclusion

The volunteer rice infestation was four times higher in fields with the cropping history of hybrid rice cultivars over the past two years (2010 and 2011), compared with fields planted previously with inbred rice. Volunteer rice affected the total yield (rice + volunteer rice) and head rice yield of the rice crop. The total grain yield of rice was reduced by 0.4% for every 1% increase in volunteer rice density across all fields, averaged over cultivars. The 1000-kernel weight, kernel length-width ratio, %protein, %amylose, and head rice yield (HRV) were affected by volunteer rice density and genotypes grown in the previous two years. The impact of volunteer rice on the overall productivity of the rice field stems from the reduced productivity of

volunteer plants while these plants also compete with the planted cultivar. Volunteer rice seed can potentially have a significant negative impact on rice grain quality.

6. Acknowledgment

The authors thank Riceland, Stuttgart, AR, USA for processing the samples to generate data on milling quality and Heather Box for her assistance in protein and amylose content analysis at the Dale Bumpers National Rice Research Center, Stuttgart, AR. We would also like to thank Terry Gray (Gray Farms), Dustin Engler, Brad Koen, Kevin Norton (Consultants), Randy Chlapecka, Dave Freeze and Lance Schmidt (Extension Agents) for providing field history information. This research was funded by NSF (National Science Foundation) Grant # IOS -1032023.

Table 1.

Summary of cropping scenarios with cultivars grown in 20 farmers' fields from 2010-2012 across six counties in Arkansas, USA.

-----Cropping scenarios-----							
Coding	Sampling year (2012)	Cropping history (Previous years) (2011-2010)	Sampling year 2012	Previous years 2011 2010		Fields (ID)	County
A	Inbred /Hybrid	Hybrid-Hybrid	Cocodrie	XL723	XL753	F01	Ashley
			CLXP756	CLXL729	CLXL729	F07	Greene
			Cocodrie	XL723	XL723	F16	Greene
			CLXP756	CLXL729	CLXL729	F04	Jackson
			CL261	CLXL745	CLXL729	F05	Jackson
			CLXL745	CLXL745	CLXL745	F17	Mississippi
			CLXL745	CLXL745	CLXL745	F18	Mississippi
B	Inbred /Hybrid	Inbred- Inbred	Jupiter	Jupiter	CL152	F15	Mississippi
			CLXL745	CL151	CL131	F02	Arkansas
			Jupiter	Jupiter	Francis	F06	Jackson
			Roy J	Jupiter	Jupiter	F11	Jackson
			Jupiter	Jupiter	Jupiter	F19	Jackson
			Jupiter	Taggart	Taggart	F20	Jackson
C	Hybrid	CL Hybrid- Hybrid	CLXL745	CLXL745	XL723	F09	Mississippi
			CLXL745	CLXL745	XL753	F10	Mississippi
			CLXL745	CLXL729	XL723	F08	Greene
D	CL Inbred/CL Hybrid	Inbred- Inbred	CL261	Taggart	Taggart	F12	Jackson
			CLXL745	Roy J	Jupiter	F13	Jackson
			CLXL745	Taggart	Taggart	F14	Mississippi
E	Inbred	Fallow- Soybean	Roy J	Fallow	Soybean	F03	Craighead

Table 2.

Summary of chemical and physical attributes of rice grain harvested (without volunteer rice contamination) across six counties (2012), Arkansas, USA.

Cultivar	Protein ^a	Amylose ^a	Chalk ^b	Length / Width ^b	Head rice ^b	1000- Kernel Weight ^b	Yield
	(%)	(%)	(%)	Ratio	(%)	(g)	(t/ha)
CL261	8.8	15.9	2.1	2.4	66	17.0	7.8
CLXL745	7.6	19.6	4.2	3.3	64	21.7	9.3
CLXP756	7.5	20.6	3.0	3.3	64	21.7	9.5
Cocodrie	8.1	20.5	5.5	3.4	68	21.3	8.5
Jupiter	8.4	14.0	3.8	2.2	67	21.3	8.4
Roy J	7.9	22.0	2.3	3.4	67	20.0	8.5
LSD _(0.05) ^c	0.3	0.6	1.1	0.2	1.9	0.5	0.7

^aProtein and amylose were measured in duplicates; brown rice was used for protein content analysis and milled rice was used for amylose content analysis

^bChalk, length-width ratio, head rice and 1000-kernel weight were measured using brown rice

^cFisher's protected LSD_(0.05) was used to compare the treatment means

Table 3.

Analysis of variance (ANOVA) *P*-values for chemical and physical attributes of milled and brown rice from cultivars produced in fields infested with volunteer rice (2012), Arkansas, USA.

Source of variation	df	Protein ^a	Amylose ^a	Chalk ^b	Length/ Width ^b	Head rice ^b	1000- Kernel weight ^b	Total yield reduction	V. rice panicle reduction ^c
----- <i>P</i> -value-----									
Volunteer rice (V)	1	0.9744	0.0249	0.9162	0.0761	<.0001	<.0001	<.0001	0.0670
Cultivar (C)	4	0.004	<.0001	0.0124	<.0001	0.0606	0.0009	0.7079	0.5006
V x C	4	<.0001	<.0001	0.0624	<.0001	<.0001	<.0001	0.2411	0.7074

^aProtein and amylose content were analysed in duplicates, milled rice was used for amylose and brown rice was used for protein content analysis

^bChalk, Length/width, head rice, kernel weight were analyzed using brown rice

^cV. rice panicle reduction= Volunteer rice tiller without panicles

Table 4.

Rate of change (slopes) in variables affected by cultivar and volunteer rice interaction (2012), Arkansas, USA.

Cultivar	Protein ^a	Amylose ^a	Length / Width ^b	Head rice ^b	1000- kernel weight ^b	Total yield reduction ^c
CLXL745	-0.006 b	-0.017 c	-0.002 b	-0.058 c	-0.019 b	
CLXP756	0.003 a	-0.006 bc	-0.003 b	-0.128 bc	-0.026 ab	
Cocodrie	-0.009 b	-0.044 d	-0.005 b	-0.396 a	-0.029 ab	0.42
Jupiter	-0.018 c	0.030 a	0.012 a	-0.175 b	-0.037 a	
Roy J	-0.012 c	0.006 b	-0.005 b	-0.258 ab	-0.006 c	

^aProtein and amylose content were analysed in duplicates separately for rice crop and volunteer rice; weighted average were used for analysis; milled rice was used for amylose and brown rice was used for protein content analysis

^bChalk, Length/width, head rice, kernel weight were analyzed using brown rice

^cTotal yield (TY)= rice yield + volunteer rice; TYR was affected by interaction of volunteer rice infestation and cultivar

Table 5.

Analysis of variance (ANOVA) *P*-values for chemical and physical attributes of milled and brown rice from cultivars produced in fields infested with volunteer rice of the same variety (2012), Arkansas, USA^a.

Cultivar ^b	Source of Variation	df	Protein	Amylose	Chalk	Length/ Width	Head rice	1000- Kernel weight	V. rice panicle reduction ^d	Total yield reduction
----- <i>P</i> -value-----										
CLXL745 ^c	Volunteer rice density	2	0.1901	0.0526	0.0193	0.7222	0.581	0.6314	<0.0001	0.0108
Jupiter ^c		1	0.1166	0.0683	0.8121	0.8916	0.2394	0.0276	0.0014	0.0183

^aEach cultivar was compared at 15% volunteer rice density (same genotype) and without volunteer rice infestation

^bCultivars grown consecutively for three years (2010-2012)

^cCLXL745 averaged over eighteen volunteer infested samples and four control samples of two fields, field was treated as random variable; Jupiter rice cultivar averaged over three volunteer infested samples and two control samples of same field

^dV. rice panicle reduction = Tillers without panicle

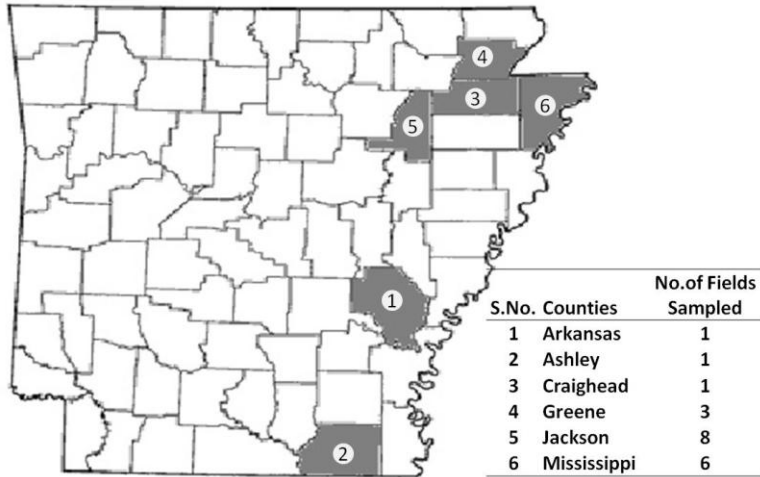


Fig. 1. Distribution of fields surveyed (2012) across six counties in Arkansas, USA.

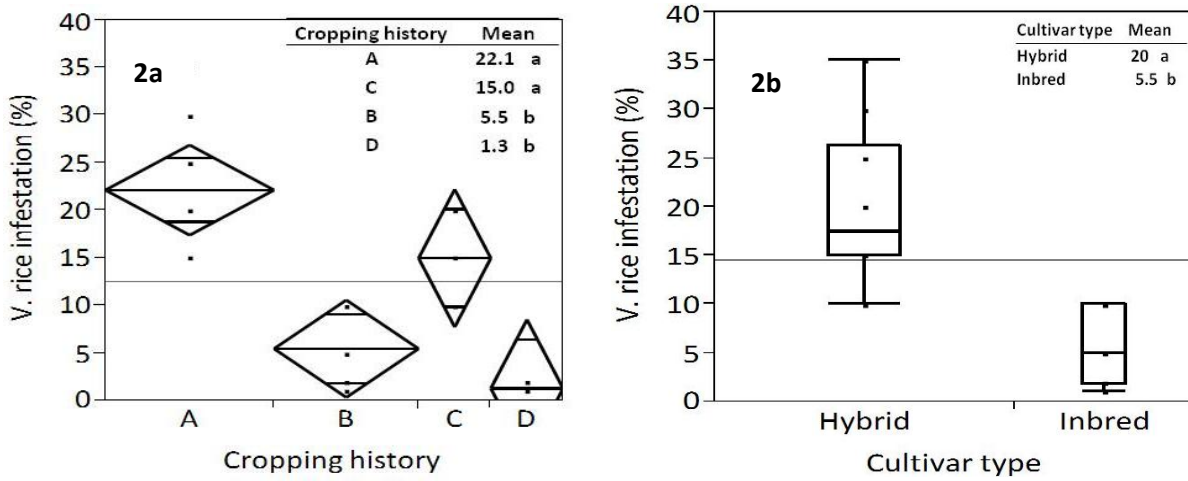


Fig. 2. Effect of cropping history (2a) and cultivar type (2b) on volunteer rice infestation; A= Hybrid-Hybrid, B= Inbred-Inbred, C= ClearfieldTM Hybrid-Hybrid, D= Inbred-Inbred with ClearfieldTM rice variety in sampling year 2012.

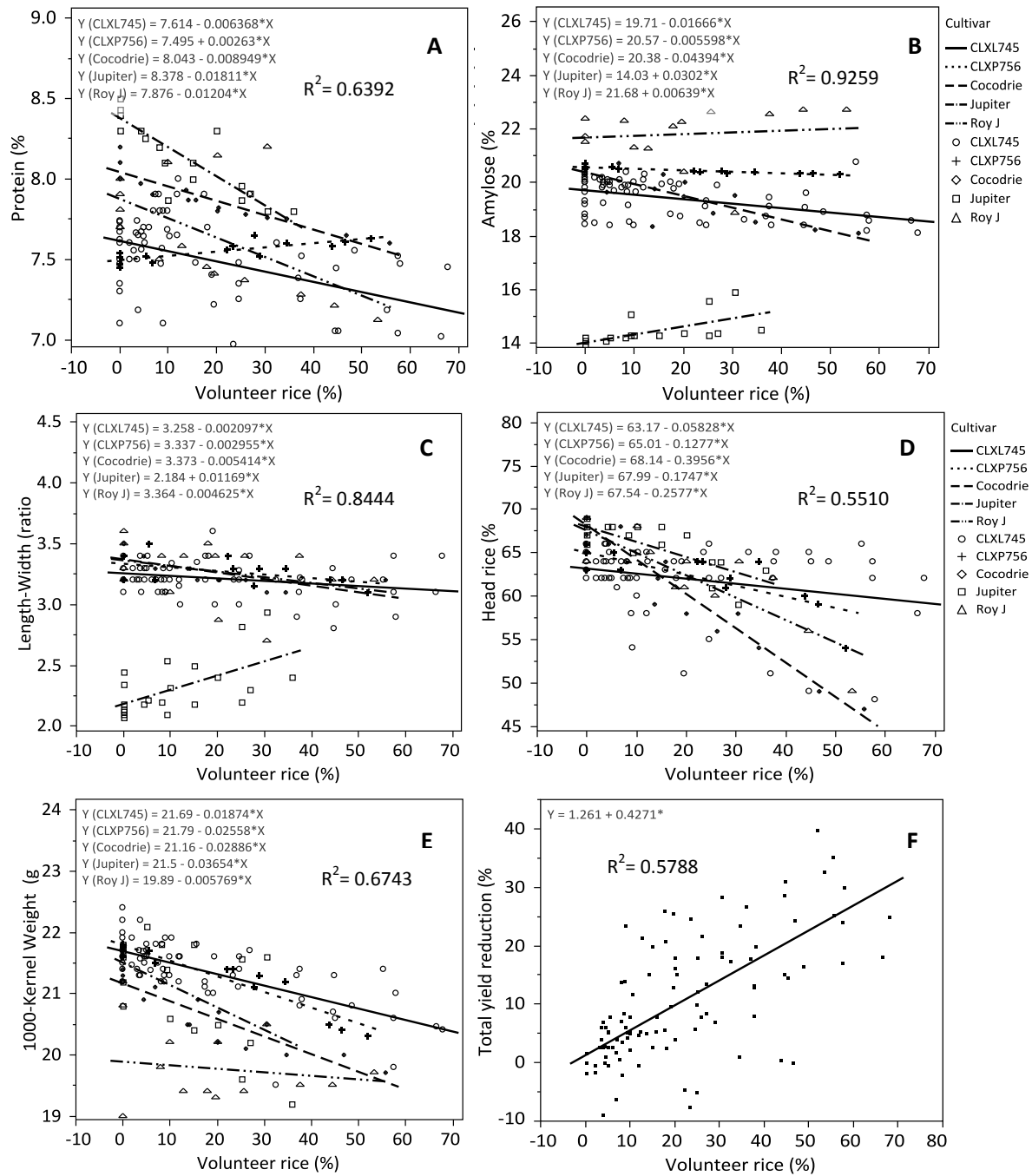


Fig. 3. Linear regression representation for A= Protein%, B= Amylose%, C= Length-Width (ratio), D= Head rice%, E= 1000-Kernel Weight, F= Total yield reduction%.

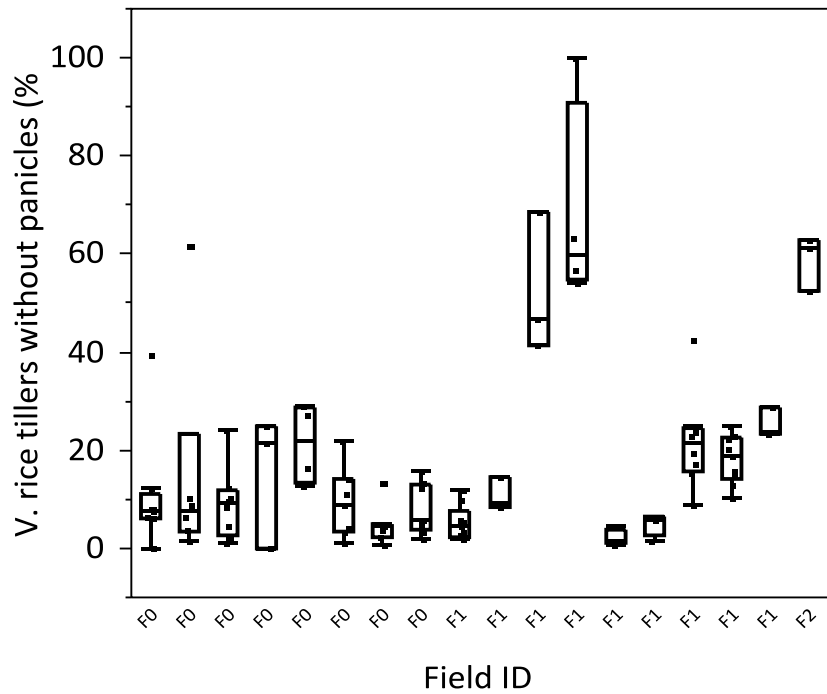


Fig. 4. Field to field variations on volunteer rice maturity and panicle development, volunteer rice infested fields surveyed across six counties in 2012, Arkansas, USA.

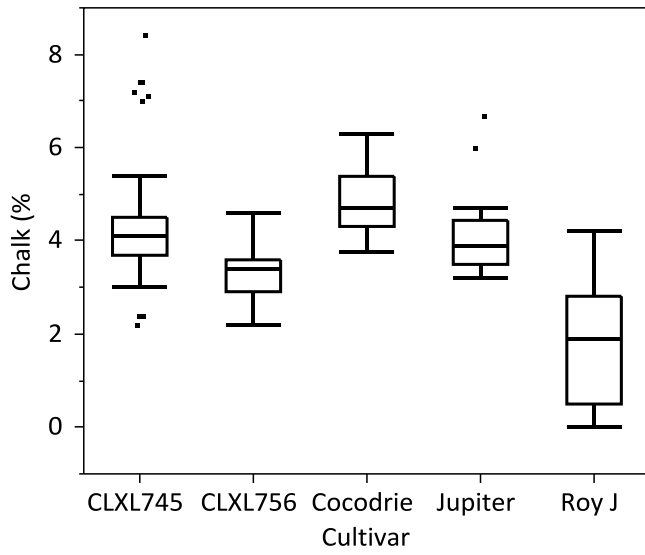


Fig. 5. Chalk percentage affected by genotypes at 17.7% volunteer rice density (averaged across all fields), volunteer rice infested fields surveyed across six counties in 2012, Arkansas, USA.

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CHAPTER V

**Introgression of Clearfield™ Rice Crop Alleles into Weedy Rice Populations in Arkansas,
USA**

Abstract

Studies were conducted to determine the impact of introgression of crop alleles into weedy rice populations. Eighty nine weedy rice accessions collected from fields in 11 counties with cropping history of Clearfield™ rice cultivars, were treated with imazethapyr (0.071 kg ai ha⁻¹) in 2011, Stuttgart, AR, USA. Survivor weedy rice plants were genotyped using the allele-specific PCR assay to detect resistance-endowing *als* mutations, S₆₅₃N and G₆₅₄E. Hybridization between Clearfield™ rice and weedy rice was verified using SSR markers (RM 215, RM 220, RM 234 and RID12). Two-to five herbicide-resistant (HR) plants per accession per replication (727 plants) representing different plant types were characterized for 14 morphological and biological traits. Seed dormancy was evaluated 75 and 270 days after harvest. Seventy-nine percent of accessions were resistant to imazethapyr. However, only S₆₅₃N mutation was detected in HR weedy red rice, which is also present in popularly grown Clearfield™ rice cultivars. These weedy rice accessions were outcrosses of Clearfield™ rice cultivars and weedy red rice as confirmed by SSR markers. Ten weedy rice accessions hybridized with more than one cultivar or hybrid weedy rice previously as these carry multiple alleles same as that of many cultivars. Nearly 70% of the HR weedy rice flowered at the same time as that of Clearfield™ rice. Weedy rice grouped into 3 distinct clusters based on germination but showed no variation in dormancy over time. Kernels of 20% of the parent accessions had both white and red bran color, irrespective of hull color. Two of the parent weedy rice accessions (goldhull) were homozygous for white bran and passed this trait to their offsprings. Overall, 31% of the characterized offsprings had white bran color. Plant height, stem angle, flag leaf length; width, bran color, seed shattering and dormancy of majority of weedy rice accessions were similar to cultivated rice. Proliferation of HR weedy populations with crop-like traits, in rice fields could result in

evolution of new hybrid weedy rice genotypes. On the other hand, reduced seed shattering and dormancy would make it easy to control weedy rice in integrated systems involving crop rotation and alternative HR technology.

1. Introduction

Hybridization is a common and important component of plant evolution (Rieseberg and Ellstrand, 1993). More than 70% of the plant species might have descended from hybrids (Grant, 1981). Gene flow from crop species to wild or weedy species leads to introgression of alleles of one plant population into the gene pool of another (Anderson, 1949; Futuyma, 1998).

Hybridization between crop species and weedy relatives results into evolutionary genotypes with intermediate characteristics (Burgos et al., 2014; Futuyma, 1998; Suh et al., 1997) and in some cases more weedy in nature (Langevin et al., 1990; Shivrain et al., 2006). Segregating hybrids exhibit diverse phenotypes and may enhance fitness of weedy hybrid lineages (Arnold and Hodges, 1995; Lexer et al., 2003). Introgression of crop alleles becomes common due to fitness advantage (Barton, 1993; Haygood et al., 2004). The introgression of single-gene transgenic traits, such as herbicide-resistance may lead to even greater fitness advantages in out-crossed populations compared with conventional crop traits (Davis et al., 1999; Snow et al., 2003).

Over the last decade many researchers have worked on gene flow from rice crop to weedy rice populations (Gealy et al., 2003, Shivrain et al., 2006, 2009, 2010; Zhang et al., 2006). Owing to genetic and morphological similarities between weedy rice and rice, weedy rice could not be controlled selectively in rice fields (Burgos et al., 2006; Smith et al., 1997). Non-transgenic, herbicide-resistant (HR) ClearfieldTM rice (Croughan et al. 1996; Tan et al., 2005) was introduced in 2002 which provided 95 to 100% weedy rice control selectively in rice fields with application of imazethapyr, IMI (Imidazolinone) herbicide (Avila et al., 2005; Shivrain et al., 2007; Steele et al., 2002). Despite Clearfield rice technology, it is hard to achieve 100% weedy rice control. Red rice escapes are often observed due to issues in herbicide-application, environmental and biological factors (Burgos et al., 2008). These escaped weedy rice plants

hybridize with Clearfield™ rice cultivars due to flowering synchronization (Shivrain et al., 2007). The resulting HR red rice or F1 outcross is difficult to control as it segregates into several weedy types which competes with rice and has questionable grain yield and quality.

To avoid pollen-mediated gene flow from adjacent fields in commercial rice seed production, an isolation distance is generally maintained (Khush, 1993) but in weedy rice infested-field, hybridization can easily happen (Shivrain et al., 2007). Several studies have reported hybridization between weedy rice and rice cultivars ranged from 0.1% to 3.2%, (Langevin et al., 1990; Shivrain et al., 2007; Zhang et al., 2006). However, most of studies have reported $\leq 1\%$ outcrossing between weedy rice and rice cultivars (Cao et al., 2006; Gealy et al., 2003; Shivrain et al. 2007). Once outcrossed, the crop alleles introgress in weedy populations indefinitely within a few generations (Gealy et al., 2003). The low outcrossing rate and introgression plays an important role in long-term evolution of weedy rice populations (Cao et al., 2006; Chen et al., 2004; Gealy et al., 2003; Slatkin, 1987). It has been reported that hybrids between red rice and cultivated rice were taller, had more flag leaf area, and more tillers than their parents (Langevin et al., 1990; Shivrain et al., 2006). The introgression of varietal traits in weedy populations has the potential to change population dynamics, morphological characteristics and genetic structure of weedy populations (Burgos et al., 2014). There is vast diversity among inter-and intra-weedy rice populations and between weedy rice and cultivated rice (Dodson, 1898; Knapp, 1899; Londo and Schaal, 2007; Shivrain et al., 2009; Tseng et al., 2013). Insight into the consequences of hybridization between weedy rice and Clearfield™ rice cultivars on evolution of HR weedy populations would be important for the management of this troublesome weed. Sustainable weedy rice management necessitates a thorough understanding of red rice diversity and evolutionary changes in morphological characteristics and genetic structure

of the HR weedy populations. Therefore, the objective of this study was to evaluate the impact of introgression of crop alleles into weedy populations.

2. Materials and Methods

2.1. Screening for herbicide-resistance

Weedy rice collected from 11 counties in Arkansas (Fig.1) were tested for resistance to IMI herbicide in a field experiment at Rice Research and Extension Center (RREC) Stuttgart, AR (2011). The soil was a DeWitt silt loam (fine, smectitic, thermic Typic Albaqualfs) with 0.9% organic matter and pH 6.2. The red rice (89) accessions and 3 ClearfieldTM rice cultivars were planted in 6.1 m row per accession; 50 seeds/ row on 20th May, 2011. Imazethapyr at 1x rate (70 g ha⁻¹) and 0.5x rate (35 g ha⁻¹) was applied at two-to three- leaf stage and second application 7 d later. Injury and mortality were recorded at 21 days after treatment (DAT). Mortality (%) was calculated based on the number of plants alive out of total plant sprayed.

2.2. Molecular marker analysis

2.2.1 DNA extraction

Green leaf tissues were harvested from 89 HR plants representing the various accessions. Total genomic DNA was extracted using a modified hexadecyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1990). Briefly, 0.05 g of leaf tissue was placed in 2 ml microtubes (Qiagen, Germantown, MD, USA) containing two stainless steel beads (Qiagen, Germantown, MD, USA). To each microtube, 500 µl of CTAB extraction buffer (containing 100 mM Tris-HCl, 20 mM EDTA, 2 M NaCl, 2% CTAB, 2% polyvinylpyrrolidone-40, 1 mM phenanthroline, and 0.3% β- mercaptoethanol) was added. The sample was then homogenized

using a Retsch Mixer Mill MM400 (Verder Scientific Inc., Newtown, PA, USA) at 30 Hz for 2 min. After adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to each tube, the mixture was incubated at 55 °C for 60 min, followed by centrifugation at 12,000 rpm for 10 min. The supernatant was transferred to a new 1.5 ml centrifuge tube (Eppendorf North America, Hauppauge, NY, USA) containing an equal volume of absolute isopropanol, mixed by inverting, and incubated overnight at -80 °C. DNA was then pelleted by centrifugation at 12,000 rpm for 10 min. The DNA pellet was washed with absolute ethanol, air dried, and re-suspended in 30 µl of 1x TE (containing 10 mM Tris-HCl, and 1 mM EDTA). The genomic DNA was quantified using a NanoDrop 2000c V. 1.0 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), diluted to 100 ng/µl with deionized water.

2.2.2 *Allele-Specific PCR (AS-PCR)*

Polymerase chain reaction (PCR) reactions were carried out for ALS gene SNPs (Ser₆₅₃Asn; S₆₅₃N and Gly₆₅₄Glu; G₆₅₄E) using a set of allele specific forward primer and a common reverse primer as described by Kadaru et al., (2008) (Table 1). For each sample, a 20 µl PCR mix consisting of 3 µl of genomic DNA (20ng µl⁻¹) mixed with 10 µl of 2x Taq Mastermix; EmeraldAMP[®] MAX HS PCR Master Mix (Takara Bio, Madison, WI, USA), 1 µl each of 10 µM forward and reverse primers and 5 µl deionized water. DNA amplification reactions were carried out using thermo cycle conditions of 95°C—2 min; 95°C—20 s, 60°C—20 s, 72°C—20 s for 28 cycles and 72°C—5 min. After amplification, 7 µl of each PCR product was loaded onto a 2% OmniPur[®] agarose gel (EMD Chemicals Inc., Gibbstown, NJ, USA), ran for 60 min in 1x TBE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and images were taken after staining with GelRed[™] Nucleic Acid Stain (Biotium, Hayward, CA, USA).

2.2.3. Microsatellite DNA amplification (Hybridization test)

Verification of hybridization between red rice and Clearfield™ rice cultivars was done with 10 cultivars (8 Clearfield™ rice cultivars including hybrids and one conventional inbred 'Jupiter') and 4 micro-satellite (RID12, RM 215, RM 220, and RM 234) markers. For PCR amplification, 2 µl of Zymo purified template DNA (80 ng/µl) were used in a reaction containing HotStarTaq DNA Polymerase, Qiagen Multiplex PCR Buffer with 6 mM MgCl₂, ultrapure quality dNTP Mix, and 0.25 µM of each primer (Table 1). The PCR was performed using Qiagen Multiplex PCR Kit, (Qiagen, Valencia, CA, USA) using the cycling protocol of a 15 minute hot start at 95 °C followed by 35 cycles of traditional 3-step thermal cycling starting with denaturation at 94 °C for 30 seconds, primer annealing at 55 °C for 90 seconds, and elongation at 72 °C for 60 seconds. The reactions were then brought to 60 °C and incubated for 30 minutes before storing the completed reactions at 8 °C. Two microliters of each diluted PCR product were added to the Genescan 400HD [ROX] Size Standard (Applied Biosystems, Foster City, CA, USA). The PCR products were resolved on an Applied Biosystems 3730 DNA Analyzer using POP-7 polymer on a 36 cm 48-capillary array. The fluorescent signal of the resolved amplicons was analyzed using GeneMapper v4.0 genetic analysis software.

2.3. Morphological characterization

Two-to five- plants per row; representing different phenotypes of each segregating accession were characterized for sixteen morpho-physiological traits in field according to guidelines of International Board for Plant Genetic Resources (IBPGR-IRRI, 1980). The traits evaluated were stem angle, stem color, leaf color; texture, flag leaf length; width; angle, leaf exertion, days to flower, panicle length, hull color, awn color, awn length, grain yield per plant,

seed shattering and seed dormancy (75 DAH and 270 DAH). Culm angle was determined relative to the horizontal plane (small value = open canopy), flag leaf angle was determined with reference to the culm (low value = erect flag leaf). Length and width of flag leaves were measured from five tillers per plant; leaf width was measured at the widest point of the leaf. Leaf texture was evaluated by rubbing the finger along the leaf blade. Plant height was measured from the base of the plant to tip of the flag leaf on the main culm. Panicle exertion was determined based on the portion of panicle still enclosed by the flag leaf. Flower initiation of characterized plants was recorded weekly. A plant was considered flowering when four panicles had been exerted. The length of 5 panicles was measured for each plant. Awn length was measured for 5 seeds of the same panicle. Awn color and hull color were evaluated visually. After characterization; plants were enclosed in Delnet[®] bags (Delstar Technologies, Middletown, DE, USA) to catch shattered seeds. Plants were harvested 75 days after flowering, which spanned a period of 10 weeks because of the diversity of flowering dates of the weedy red rice. Each plant was cut at maturity at 10 cm height from the ground and air-dried in shade for 2 weeks.

2.4. Seed shattering

All of the characterized plants in a field were enclosed in Delnet[®] bags (Delstar Technologies, Middletown, DE, USA). All plants were subjected to the same force (50 kgf) to determine the level of seed shattering. Different sets of weights were attached to each bundle of harvested plants to obtain the same weight of 2.5 kg. The bundle was then dropped from a height of 1 meter into a metal container so that each plant was subjected to same force of 50 kgf. The shattered seeds were then recorded. The panicles with remaining seeds were separated from the

rest of the shoot biomass, threshed and total yield of plants were recorded (shattered seeds + non-shattered seeds). Seed shattering (%) was calculated as:

$$\text{Shattering (\%)} = [\text{shattered seed weight (g)} / \text{total yield (g)}] * 100$$

2.5. Dormancy

Seed dormancy was evaluated twice, at 75 days after harvest (DAH) and at 9 months (270 DAH) after harvest. Seeds of harvested plants were germinated in batches according to harvest dates of plants. Thirty seeds from each accession with 3 replications, in a Petri-dish (9 cm diameter), were moistened with 6 ml of deionized water and were dark-incubated in a growth chamber at 30° C. Petri-dishes were placed in trays and arranged in randomized complete block design. Trays were covered with plastic sheet and Petri-plates were re-watered every 4th day after each germination evaluation. Germinated seeds were removed every 4th day, up-to 12 days. Seeds were considered germinated when the radical protrudes from the caryopsis. The Petri-plates were re-randomized after each evaluation period. Firm, non-germinated seeds were counted as viable, dormant seeds.

2.6. Kernel characteristics

Size and pericarp color of seeds harvested from growers' fields and seeds of offsprings characterized at the RREC were evaluated using Graincheck (2312 FOSS TECATOR). Samples were de-hulled and bran color was recorded in accordance with guidelines of International Board for Plant Genetic Resources (IBPGR-IRRI, 1980). There were seven categories for bran color in this system – white, light brown, speckled brown, brown, red, variable purple, and purple. Length (L), width (W), L/W ratio and 1000-kernel weight were recorded.

3. Statistical analyses

All statistical analyses were performed using JMP Pro (version 11.0; SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was conducted for all the quantitative plant traits using a completely randomized design. Differences among and within ecotypes in plant traits were determined using one-way ANOVA and means were separated by Fisher's Protected LSD at the 5% probability level. Principal component analyses (PCA) for morphological traits was carried out based on 6 prominent weedy traits selected in three steps; removed non-significant variables in each step based on their contribution and 'Eigen' values. *K*-means clustering (JMP Pro v. 11) was performed to group the weedy rice accessions based on the 6 selected traits and separate cluster analysis based on germination and seed shattering. *K*-means cluster analysis was carried out with dataset where columns were scaled individually (all distances were scaled by an overall estimate of the standard deviation of each variable) by default in JMP Pro (v.11). Numbers of clusters in each analysis were selected based on fit statistic, with largest CCC (Cubic Clustering Criterion). PCA and *K*-means cluster analysis were carried out based on all kernel characteristics separately to distinguish weedy population based on their kernel characteristics

4. Results and Discussion

4.1. Frequency of herbicide-resistant red rice

Red rice infested up to 60% of the total rice area in Arkansas (Burgos et al., 2008). In recent years weedy red rice infestation has been reduced to roughly 20% due to stewardship program to minimize weedy rice seed production (R.C Scott, 2015, personal communication). However, weedy rice seed can remain dormant in the soil for up to 10 years (Goss and Brown, 1939) which increases the risk of out-crossing between weedy rice and HR rice cultivars if not

eradicated. Occurrence of resistant offsprings from parent accessions sampled across all 26 counties (Fig. 2) indicates that HR weedy rice is a common problem in rice fields in Arkansas. Clearfield™ rice has been planted in these counties in the last decade. Seventy-nine percent of the accessions were resistant (0-79% injury) to imazethapyr; of these, 98% were highly resistant (0-20% injury) (Fig. 3). Only S₆₅₃N (Serine to Asparagine) mutation was detected in tested red rice accessions responsible for herbicide-resistance (Fig. 4). In 2004, 19% of the area in Arkansas was planted with Clearfield™ inbred rice ('CL121', 'CL141' and 'CL161') and Clearfield™ hybrid rice ('CLXL8') (Shivrain et al. 2007; Wilson and Branson, 2004). Cultivars harboring G₆₅₄E (CL121 and CL141) were less resistant to imazethapyr (Avila et al. 2005; Wenefrida et al., 2007) compared with cultivars harboring S₆₅₃N mutation released in 2003 (McClain, 2003) and thereafter. With the introduction of Clearfield™ inbred rice cultivars (eg. 'CL161') and hybrid cultivars (eg. 'CLXL8', 'CLXL745' and 'CLXL729') harboring S₆₅₃N mutation, adoption of this technology increased many folds owing to their higher yield potential and resistance to IMI herbicides. The trend resulted in 45% acreage under Clearfield™ rice in 2009 in Arkansas, USA with majority of the area under Clearfield™ hybrid rice (Wilson et al., 2010) where all cultivars carried S₆₅₃N mutation. The rice acreage under Clearfield™ rice in Arkansas increased to 54% in 2013 (Hardke, 2014) with 36% under Clearfield™ hybrid cultivars. Hybrid rice cultivars owing to longer duration of flowering (2 to 3 d), and high competitive ability showed higher out-crossing with weedy red rice (Shivrain et al., 2007; 2008; 2009b). The out-crossing rate of 'CLXL8' (Clearfield™ hybrid) with weedy rice was higher (0.23%) compared with that of 'CL161' (0.07%) (Clearfield™ inbred) (Shivrain et al., 2009a). This indicates that the frequency of HR weedy rice in rice fields would increase with the increasing trend of Clearfield™ hybrid rice in Arkansas.

Weedy rice plants can be distinguished and classified by hull colors (prominently, strawhull and blackhull) as these are phenotypically and genetically different (Gealy et al., 2002; Noldin et al., 1999; Reagon et al., 2010; Vaughan et al., 2001). Generally, strawhull weedy rice has longer flowering overlap with rice cultivars compared with blackhull ecotype but flowering overlap could not be directly related to outcrossing rates (Shivrain et al., 2008). Similarly, the frequency of resistant offsprings was independent of hull color of mother plants in our study (Fig. 5). In general, gene flow from cultivated rice to weedy rice depends upon several factors like flowering synchronization, floral morphology, pollen load, genetic compatibility and environmental conditions (Shivrain et al., 2009b). Another reason for same frequency of resistant offsprings among hull types in current study is the possibility of multiple outcrossing events over the years within weedy rice populations and between weedy rice and cultivated rice.

Molecular markers (Fig. 6); detected 42 accessions which carried alleles same as that of cultivars. Twenty five percent of these 42 accessions carried multiple alleles same to more than one cultivar. This happens if weedy rice either outcross with different cultivars or with hybrid weedy rice over a period of time. Remaining 28 HR weedy rice accessions might have hybridized with other ClearfieldTM rice cultivars that were not included in the test. Forty eight percent of weedy rice accessions were homozygous resistant and 31% were heterozygotes carrying both mutant and wild allele (Fig. 4). Homozygous alleles in weedy rice indicate outcrosses that have taken place few years back and now introgressed in weedy populations.

4.2. Differentiation of morpho-physiological traits

Seventy six percent of the plants were taller than cultivated rice (130 cm) (Fig. 7-A). Shivrain et al., (2006) reported that outcrosses of weedy rice and cultivated rice were taller than parents and ranged from 40 cm to 180 cm in plant height. Weedy rice was reported to be 15-

65% taller than cultivated rice (Do Lago, 1982; Shivrain et al., 2006). However, differences in plant height were observed among two prominent hull types - strawhull and blackhull ecotypes. Strawhull weedy rice ecotypes were shorter than blackhull weedy rice which was also reported by Shivrain et al., (2010). Cluster analysis based on 6 morpho-physiological characteristics (selected based on PCA) (Table 2) indicated that the two clusters (1 and 2) representing 55% of the characterized plants were with mean height similar to that of cultivated rice. This suggests that only 45% of the weedy rice plant types were more competitive in terms of shading and access to sunlight and nutrient consumption. Plant height was expected to contribute in gene flow. Studies have indicated that weedy rice plants with the same height as that of cultivated rice tends to be the pollen receiver from cultivated rice whereas taller plants facilitates the transfer of pollen (donor) to cultivated rice (Shivrain et al., 2007; Zhang et al., 2003). However, recent studies have reported that differences in plant height did not influence the gene flow rate from cultivated rice to red rice or vice versa (Shivrain et al., 2009; 2010).

The range of flowering initiation in weedy rice was 70 to 135 d after planting (DAP) and 71% of them flowered 96-99 DAP (Table 2). Similarly, 90% of the weedy rice reported to flower 98 DAP with flowering range of 70 to 130 DAP (Shivrain et al., 2006). Blackhull and brownhull weedy rice ecotypes flowered later (101 DAP) than strawhull ecotype (96 DAP) which was also reported in previous studies (Do Lago, 1982; Shivrain et al., 2004). Clearfield™ rice cultivars flowered at 97 DAP (Table 2) which indicated the potential overlap of flowering between strawhull weedy rice outcrosses and rice cultivars. Hybrid cultivars like 'CLXL8' even has longer flowering overlap (+ 2 to 3 d) with weedy rice versus conventional cultivars (eg. 'CL161') (Shivrain et al., 2009b). Longer flowering overlap results in higher chances of hybridization between weedy rice and rice cultivars. The area planted with hybrid

rice and Clearfield™ hybrid rice is increasing in Arkansas owing to their higher yield potential (Hardke, 2014). The increasing area under Clearfield™ hybrid rice would alleviate the HR weedy rice issues and the frequency of diverse weedy populations as hybrid rice cultivars has higher outcrossing rate.

Based on PCA, 6 significant variables were identified for cluster analysis (plant height, flowering, panicle length, hull color, seed shattering and grain yield) (Fig. 8). *K*-means cluster analysis grouped 698 weedy rice offsprings into 3 clusters (Table 2). The biggest cluster '3' consists of 314 very tall individuals (166 cm) which flowered 99 DAP with longer awns, intermediate seed shattering (56%) and lower yield potential compared with other clusters. Seventy eight percent of the weedy rice plants in cluster 3 had dark hull color (45% blackhull and 33% brownhull). Cluster 1 individuals (26%) were similar to cultivated rice in morpho-physiological characteristics. Cluster 2 consisted of individuals (29%) with same height as that of Clearfield™ rice cultivars, upright leaves and closed canopy but delayed flowering (+7 DAP), higher seed shattering (+35%), shorter panicles (-2.3 cm) and lower yield potential (-20.1 g) compared with Clearfield™ rice cultivars. Majority of weedy rice offsprings (71%) in this study flowered at same time (96-99 DAP) as that of cultivated rice cultivars (97 DAP). Cluster 1 and 2 with crop-like characteristics both had 57 and 20% strawhull ecotypes. Similar characterization study in 2010 (Shivrain et al., 2010) with 215 weedy rice plants grouped them into 5 clusters. Weedy rice accessions of largest clusters (64%) flowered at 90-92 DAP and had medium height (128-134 cm). The flowering time was same as that of rice cultivars in Arkansas. Majority of these accessions in clusters 1 and 2 (80-92%) were strawhull types. The numbers of blackhull and brownhull ecotypes in 2010 study were low (47 and 15, respectively) (Shivrain et al, 2010) whereas in our current study characterized population consisted of 260

blackhull, 210 brownhull and 30 goldhull ecotypes apart from 200 strawhull ecotypes. Our study has given more extensive analysis of morho-physiological and phenological traits across all hulltypes. Crop-like characteristics were observed in all ecotypes irrespective of hull color.

The majority of weedy rice accessions were with upright leaves similar to cultivated rice and all clusters were similar in terms of stem angle. Similarly, one of the cluster in 2010 study (Shivrain et al., 2010) with accessions (9%) of medium height (122 cm), had almost upright leaves (flag leaf angle 31° , relative to perpendicular axis) but flowered very early (57 DAP). Several offsprings of outcrosses between weedy rice and rice had upright leaves (Do Lago, 1982; Shivrain et al., 2007; 2010). However, historical weedy rice populations in general have droopy leaves and wide canopy (Burgos et al., 2006). The variation in flag leaf angle or culm angle between weedy rice accessions may be the consequence of hybridization with cultivated rice (Shivrain et al., 2010).

The strawhull and blackhull weedy rice types are two major hull types reported for historical weedy rice populations (Gealy et al., 2002; Reagon et al., 2010) but rare types may also exist (Shivrain et al., 2010). In this current study, several intermediate hull colors were observed (yellowish straw, gray, and different shades of brown), potentially due to weedy rice evolution over the years under natural environmental conditions. Strawhull and blackhull ecotypes are genetically different (Gealy et al., 2002; Vaughan et al, 2001) but sometimes represent similar traits (Gealy et al., 2003; Shivrain et al, 2010). Many of the morho-physiological characteristics of weedy rice like stem angle, flag leaf length; width (Table 3), and dormancy (Table 4) were similar to cultivated rice irrespective of the hull color of weedy populations. Cultivated rice plants were of intermediate height (123 cm), upright with mean stem angle of 73° , 31 cm flag leaf length and GC of 90-91% at 75 and 270 DAH.

K-means cluster analysis of germination capacity at 75 and 270 DAH for offsprings (698) produced 3 distinct clusters (Table 4). Cluster 1, comprised of 69% of individuals had GC (94 and 97%) similar to cultivated rice (90 and 91%). Only 31% of the individuals had lower GC than cultivated rice which were represented by cluster 2 and 3. It was reported that blackhull weedy rice had three times lower germination than strawhull weedy rice (Do Lago, 1982). Higher intra-population variation was also observed among blackhull populations in terms of dormancy when compared with strawhull weedy rice populations (Do Lago, 1982; Tseng et al., 2013). However, no variation was observed in GC among strawhull and blackhull populations in current study. The potential gene flow from similar genotype parent (cultivated rice) to both prominent ecotypes (strawhull and blackhull) of weedy rice could be one of the factors that can result in homogeneity or similar characteristics.

Apart from dormancy, seed shattering is one of the major characteristics of historical weedy rice which enables rice plants to dehisce the seeds in the field that helps in seed distribution for its survival (Cao et al., 2006; Delouche et al., 2007; Thurber et al., 2010). The shattered seed remain in the soil and germinates intermittently over many years. Blackhull weedy rice plants had highest mean seed shattering (60%) and goldhull weedy rice were with lowest seed shattering (39%) (Table 3). Forty two percent of weedy rice offsprings in our study showed similar or less seed shattering compared with cultivars (Table 5), Therefore, many of these weedy rice outcrosses have shown minimal dormancy, (Table 4) and low seed shattering (Table 5) compared to historical weedy populations, which would help in decreasing soil seed bank and efficient control of this weed through use of broad-spectrum herbicide or tillage to kill the already germinated seeds (stale seed bed technique).

4.3. Differentiation of kernel characteristics

In general, weedy red rice has been identified based on its red bran/pericarp (Smith, 1981; Gross et al., 2010). Red bran is a dominant trait of weedy red rice (Gealy et al., 2003). No study has investigated the evolutionary changes in bran (pericarp) color of weedy red rice owing to natural hybridization of weedy red rice with ClearfieldTM rice in commercial rice fields. Weedy rice with white kernel have been studied in Southern America and Asian countries but all of the weedy rice in North American region were characterized with red bran (Delouche et al., 2007). However, white-kernel weedy rice was once reported in Mississippi, USA (Do Lago, 1982). Weedy rice plants with white kernels were similar to cultivated rice in characteristics (Delouche et al., 2007; Do Lago, 1982). PCA analysis based on kernel characteristics revealed that grain width, length, and 1000-kernel weight were major contributors to component 1 and bran color was the main contributor to the variation in component 2 (Fig. 9). Together they explained 81% of the variation in grain characteristics of the offsprings. *K*-means cluster analysis on the basis of bran color, kernel length, width and 1000-kernel weight grouped weedy rice offsprings into 3 distinct clusters (Table 7). Clusters 1, 2 and 3 had 40, 26 and 13% of kernels with white bran color. Kernels in cluster 2 and 3 were longer with higher 1000-grain weight. As the percentage of red bran increased, data showed increase in length, width and 1000-kernel weight. This indicates that HR weedy rice kernels with red bran are bigger and characteristics associated with red bran positively affect yield parameters. However, grains with light brown bran were the largest (Table 8). The proportion of kernels with light brown bran was low (<1%) and did not influence the average kernel size and weight.

Of the parents with mixed-color kernels (19), 66% of the offsprings were with red bran and 33% of the offsprings were with white bran (Fig. 10). Offsprings (477) of 60 parent accessions with red bran segregated into both red bran (69%) and white bran (30%). Thirty five percent of these segregating offsprings with white bran were blackhull ecotypes and 28% strawhull ecotypes. Two of the parent weedy rice accessions (goldhull types) with white bran passed this trait to all of their offsprings (Fig. 10). Kernels with white bran were observed in 31% of the offsprings, overall (Fig. 10; Table 6). Only 6 parent weedy rice accessions with red bran were homozygous for red bran trait as no segregation was recorded among their offsprings. White bran was observed in weedy populations irrespective of hull color. Molecular analysis of 89 individuals (offsprings), each representing one parent accession, showed that 51% and 20% of individuals were homozygous for red and white bran, respectively. Twelve percent were heterozygous, containing alleles for both red and white bran color. White bran (*rc* allele) is the mutant (nonfunctional) version of the ancestral *O. rufipogon* (*Rc*) red allele (dominant allele) and RID12 micro-satellite marker can detect the presence of red allele (Sweeney et al., 2006). Whereas *Rc-g* is another dominant wild type allele which resulted into red pericarp of 'Wells' through natural mutation within *rc* allele but that could not be detected through RID12 micro-satellite marker (Brooks et al., 2008). In our study, detection of red (*Rc*) and white bran alleles (*rc*) with RID12 marker and allele similarity with other SSR markers confirmed that white bran is not the result of natural mutation in weedy rice, rather it is related to gene flow from cultivated rice. Appearance of white-bran in weedy rice populations (Fig. 10, Table 6) suggests that these populations resulted from out-crossing of cultivated rice to weedy rice that segregated over few generations and produced both red and white pericarp. Gealy et al., (2003) have demonstrated the effect of single outcross and further segregation of pericarp color along with HR trait over the

subsequent years (Fig. 11). Assuming that red seed coat (RR) and herbicide resistance (NN) are dominant traits, the population resulting from a single hybrid (single hybridization between HR cultivated white rice; rrNN and herbicide-susceptible weedy red rice; RRnn) becomes more than 95% resistant (half with white seed coats and the other half with red seed coats) to the herbicide within six generations. This indicates that the present HR weedy rice populations that segregated into both red and white kernels were F3 or later generations based on proportion of red and white kernels in their offsprings. All the weedy rice accessions carrying only white bran/pericarp allele are homozygous for that allele (*rc* being a recessive trait) which suggests that some of the genes have already introgressed in these populations. However, some of the outcrosses were recent which were heterozygous for red bran allele; red allele being the dominant, and segregated into several weedy type plants with diverse characteristics. The presence of only S₆₅₃N polymorphism in resistant weedy rice populations indicate that these populations could not be beyond F7 as Clearfield™ cultivars harboring S₆₅₃N were commercialized in 2003. Crop genes have the potential to introgress in weedy populations within few generations (Gealy et al., 2003) and these weedy rice outcrosses in our study have become similar to cultivated rice in terms of many morpho-physiological characteristics, especially the strawhull types. Four accessions with more than 60% of their progenies with white bran; were selected (Table 9). Two of these parent accessions had white bran and passed this trait to their progenies. Characteristics were averaged over all progenies with white bran for respective accession. Progenies of white bran parents were upright with erect leaves and had similar stem angle as that of reference cultivars. White kernel progenies of red kernel parents were more diverse and had wider range in terms of stem angle, 1000-kernel weight and GC. These type of outcrosses showed 100% flowering overlap with reference cultivars. High similarity in characteristics of weedy rice and cultivated rice would

increase the chance of contamination in rice seed and continued infestation in rice fields. These crop-like HR weedy populations could not be controlled through Clearfield™ rice technology due to their herbicide resistance trait. This emphasizes the need for alternative HR technology with different mode of action. 'Provisia® rice' is a non-transgenic HR technology (developed by BASF) based on ACCase group. This technology would provide a potential solution to this problem (Webster et al., 2015) by selectively controlling IMI resistant weedy rice with the application of quizalofop-p-ethyl herbicide. Rotating rice with soybean and use of alternate HR technologies would provide near-full control of HR weedy rice owing to their reduced weedy characteristics.

5. Conclusion

The majority of weedy rice remaining in rice fields, after some years with herbicide-resistant rice, carry the resistant allele of *ALS* with the S₆₅₃N mutation. This was introgressed into the weedy population via gene flow. The HR weedy rice populations exhibit more crop-like traits and reduced weediness such as reduced seed shattering and reduced seed dormancy. This is favorable for weedy rice management in general and weedy red rice in particular in Arkansas. However, some of these plants have white kernels and will no longer be detectable as contaminant of the harvested rice grain; thus, it is important to prevent seed production of HR weedy rice. Also, weedy rice plants with crop-like characteristics would be difficult to detect in field. An alternative HR technology based on a different mode of action would help curtail the evolution of HR weedy rice if integrated with cultural practices stipulated in the best management practices for resistance management.

6. Acknowledgement

The authors thank Te Ming Tseng, Leopoldo Estorninos, Mariccor S.A.B. Batoy, George Botha, and Reiofeli Salas for their help in the implementation of the experiment in the field and evaluation of seed germination capacity and dormancy in the laboratory. The study was funded and supported by the National Science Foundation Grant # IOS -1032023.

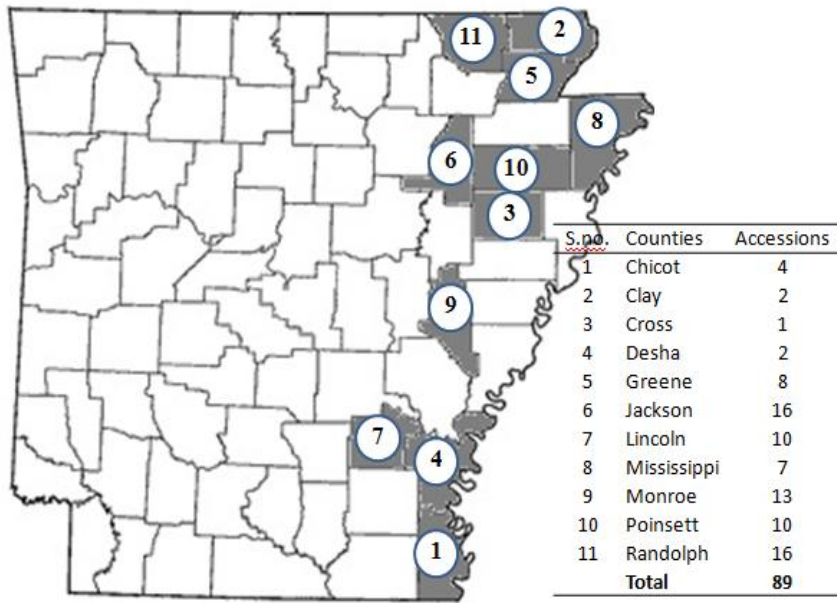


Fig. 1. Geographic distribution of red rice accessions collected from eleven counties in 2012, Arkansas, USA.

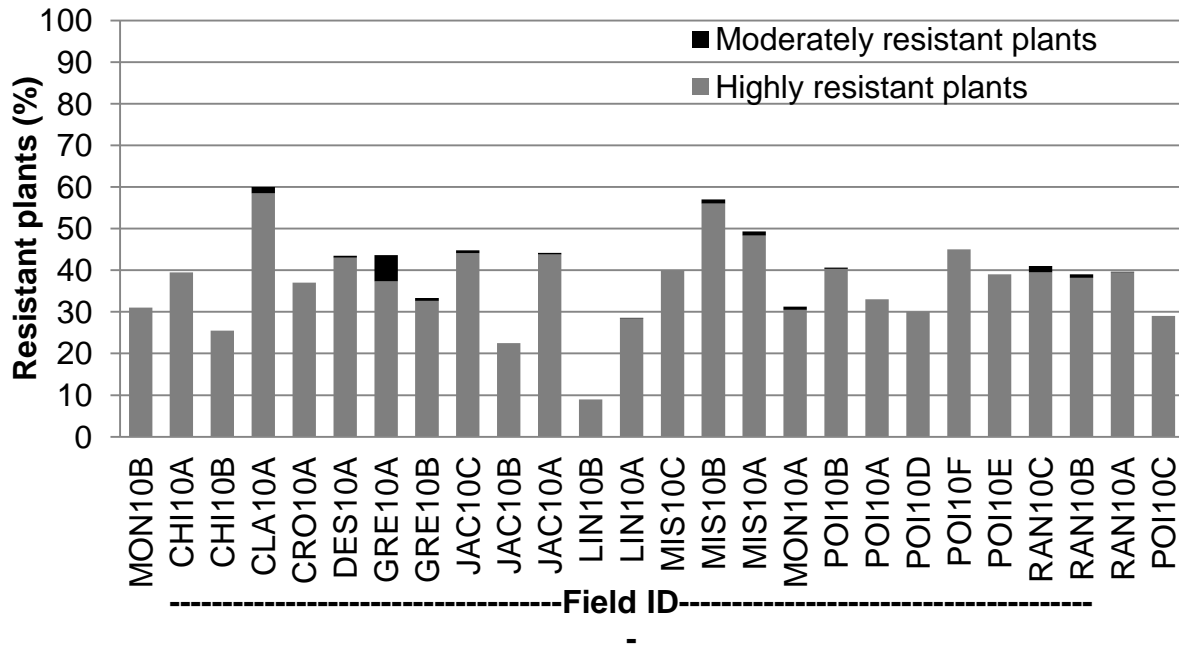


Fig. 2. Proportion of HR offsprings of weedy rice (*Oryza sativa* L.) accessions (89) collected from 26 fields across Arkansas, USA.

Adapted from: Burgos, N.R., Singh, V., Tseng, T.M., Black, H., Young, N.D., Huang, Z., Hyma, K.E., Gealy, D.R., Caicedo, A., 2014. The Impact of Herbicide-Resistant Rice (*Oryza sativa* L.) Technology on Phenotypic Diversity and Population Structure of US Weedy Rice. *Plant Physiol.* 166 (3), 1208-1220. DOI:10.1104/pp.114.242719

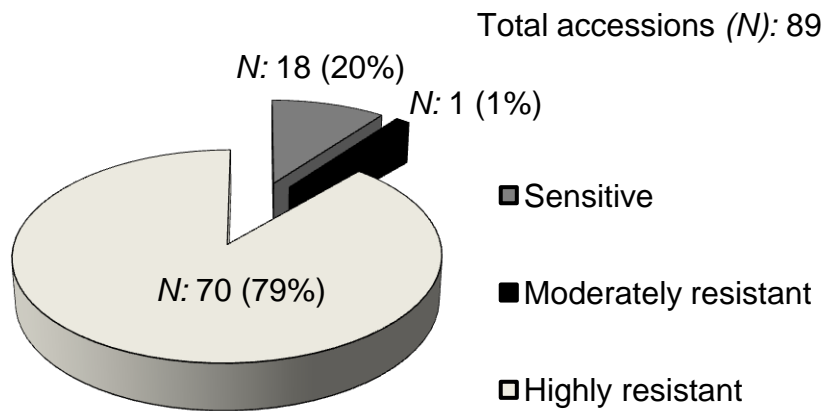


Fig. 3. Resistance profile of offspring of weedy rice (*Oryza sativa* L.) plants collected from Arkansas, USA rice fields with history of Clearfield rice. Three- to four-leaf seedlings were treated with imazethapyr (two applications @ 70 g ha⁻¹). Sensitive plants= 80 to 100% injury, moderately resistant plants= 21 to 79% injury, highly resistant plants= 0 to 20% injury.

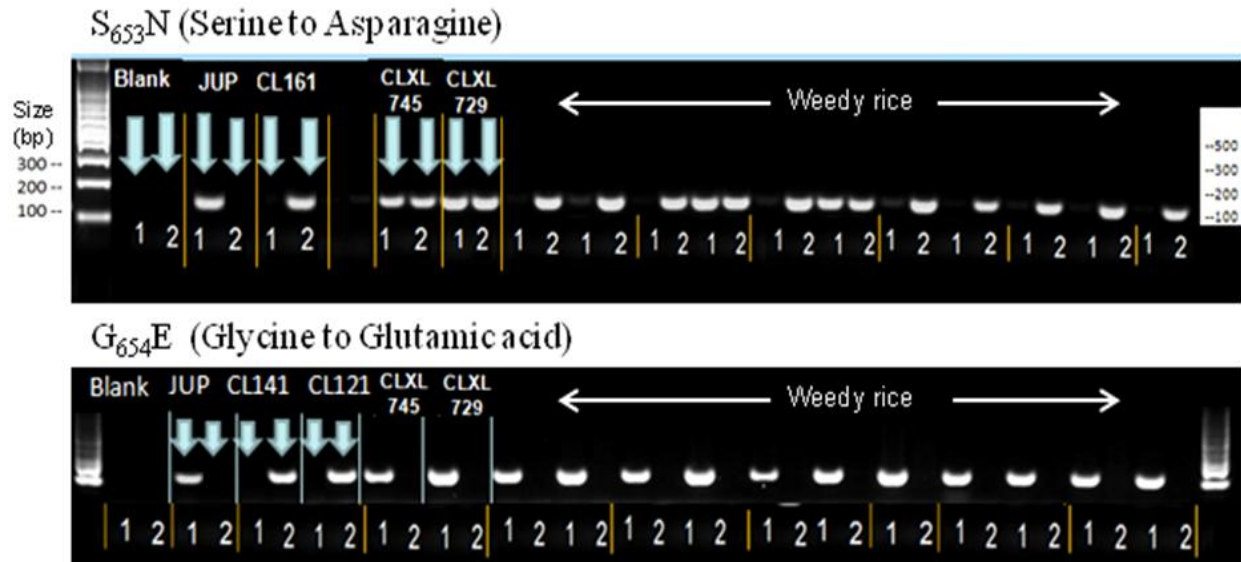


Fig.4.

Representative electrophoresis gel image of S₆₅₃N and G₆₅₄E *ALS* mutation assays of 89 weedy rice accessions. Allele-Specific PCR products (1=wild allele, 2= mutant allele; 134 bp band size) for the S₆₅₃N mutation are shown on the top panel; products for the G₆₅₄E mutation assay (1=wild allele, 2 = mutant allele; 131 bp band size) are on the bottom panel. Position of S₆₅₃N mutation in *ALS* gene is at 1880 bp and G₆₅₄E at 1883 bp. Cultivar JUP='Jupiter' (susceptible standard); CL=ClearfieldTM cultivars and CLXL=ClearfieldTM hybrids (resistant standards).

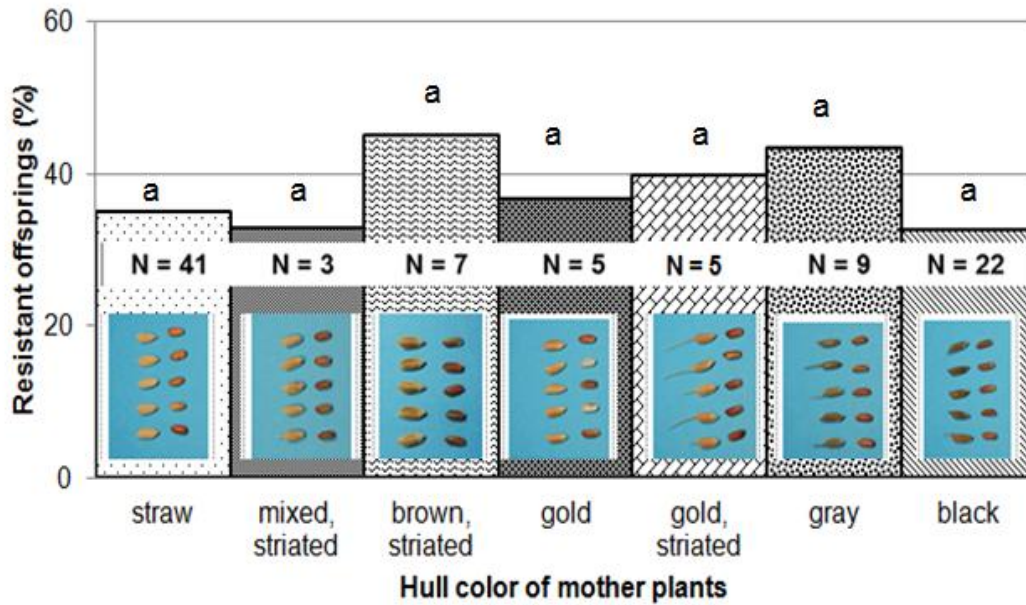


Fig. 5. Frequency of resistant weedy red rice (*Oryza sativa* L.) offspring with respect to hull color of mother plants. Bars followed by the same letters are not different.

Adapted from: Burgos, N.R., Singh, V., Tseng, T.M., Black, H., Young, N.D., Huang, Z., Hyma, K.E., Gealy, D.R., Caicedo, A., 2014. The Impact of Herbicide-Resistant Rice (*Oryza sativa* L.) Technology on Phenotypic Diversity and Population Structure of US Weedy Rice. *Plant Physiol.* 166 (3), 1208-1220. DOI:10.1104/pp.114.242719

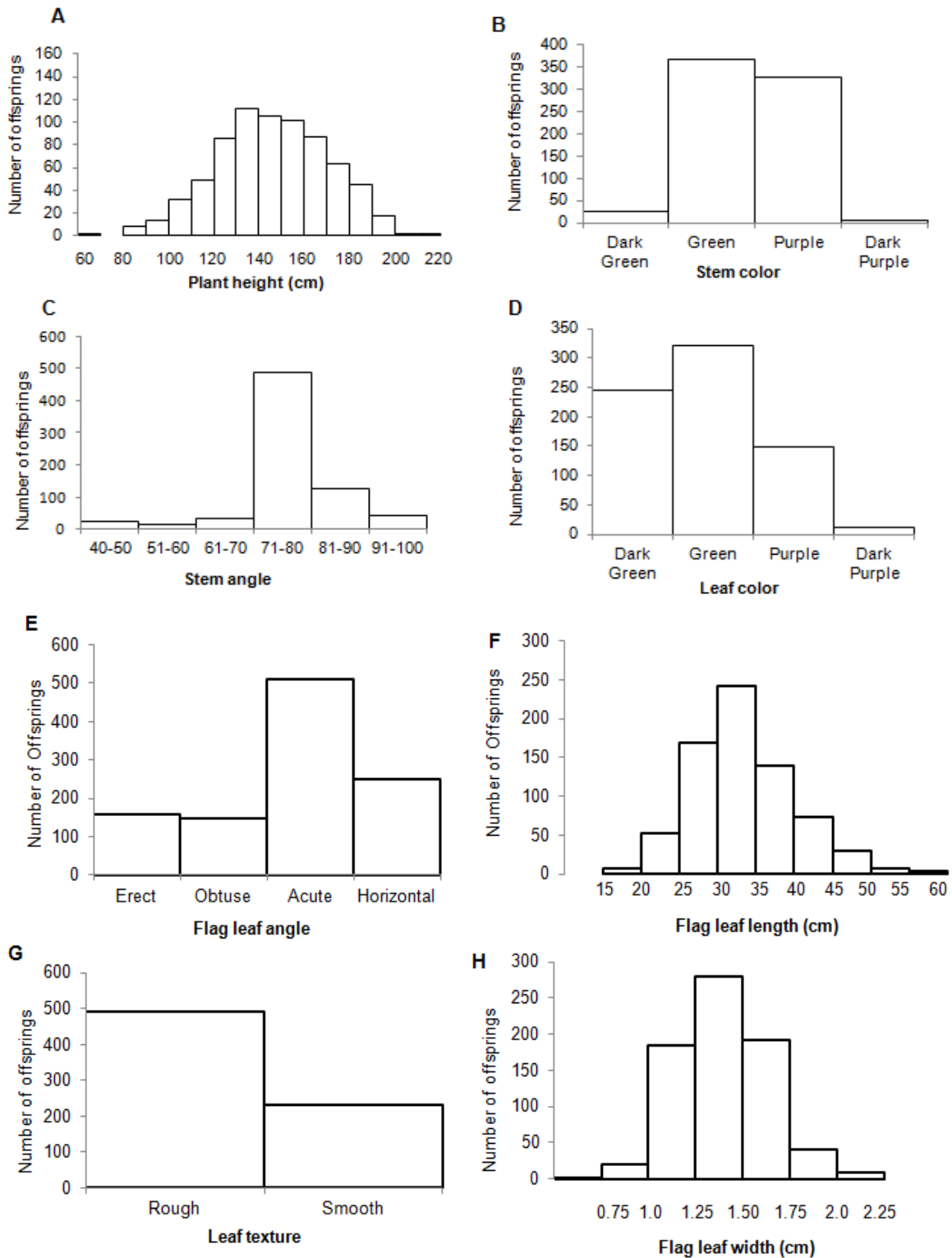


Fig. 7. Frequency distribution of weedy rice (698), resistant to imazethapyr (70 g ha^{-1} , two applications), Arkansas Rice Research and Extension Center, USA. (Cont.).

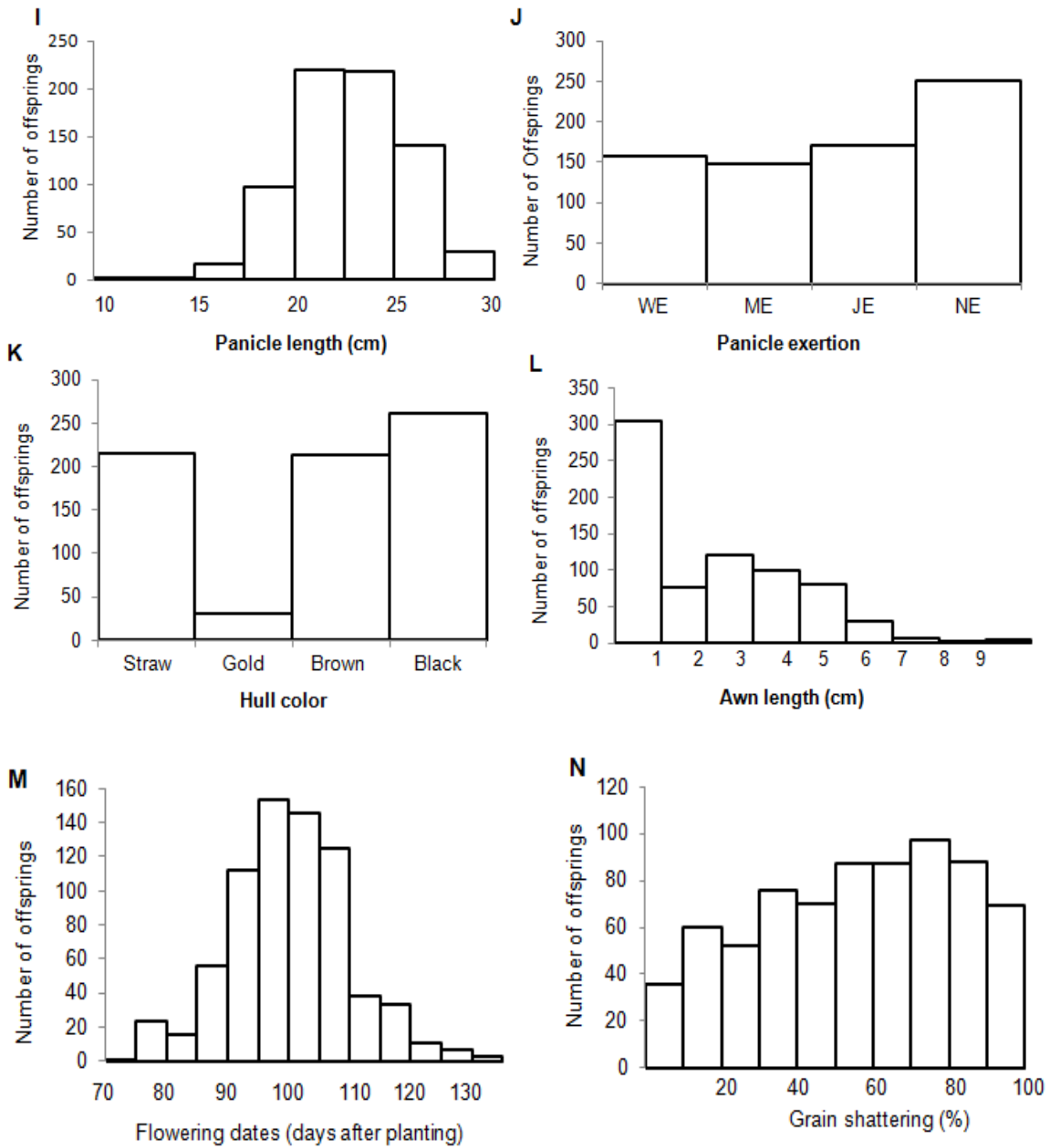


Fig. 7. Frequency distribution of weedy rice offsprings (698), resistant to imazethapyr (70 g ha⁻¹, two applications), Arkansas Rice Research and Extension Center, USA. Panicle exertion 'J': WE = well exerted, ME = moderately exerted, JE = just exerted, NE = not exerted.

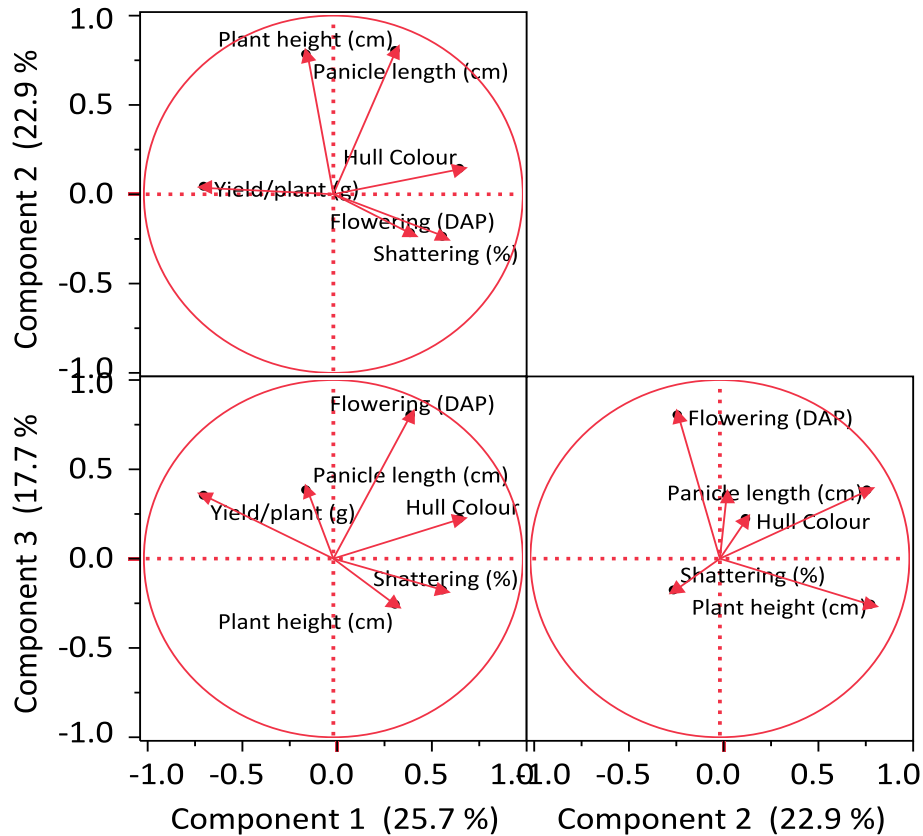


Fig. 8.

Graphical representation of principal component analysis based on plant height, flowering, panicle length, hull color, seed shattering and yield/plant. Traits are represented by vectors extending from the plot origin; vectors of traits that point to the same direction are positively correlated. Components 1, 2, and 3 are indicated with their respective contribution to the total variation in the dataset.

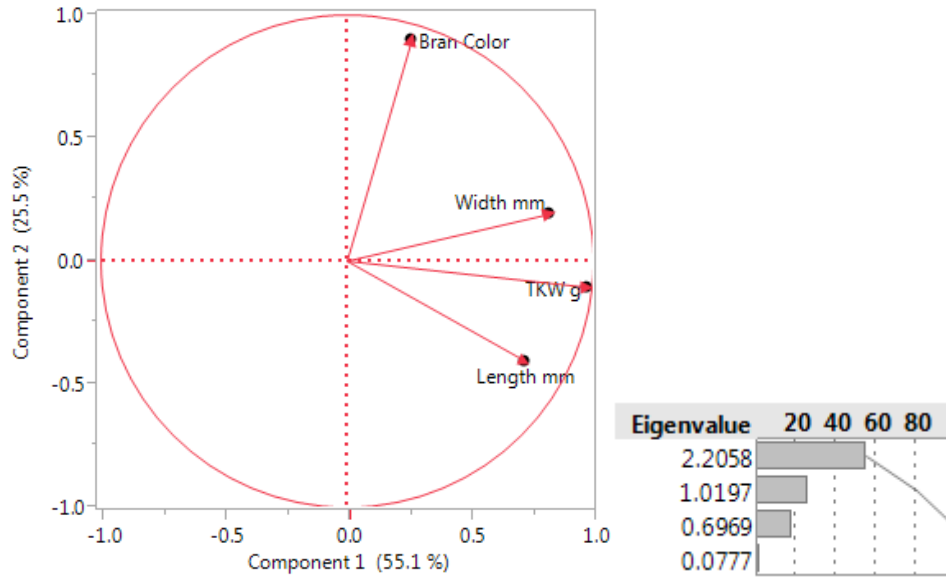


Fig. 9. Graphical representation of principal component analysis based on bran color, kernel length, width and 1000-kernel weight. Traits are represented by vectors extending from the plot origin; and traits that point to the same direction are positively correlated. Components 1 and 2 indicated with their respective contribution to the total variation in the dataset.

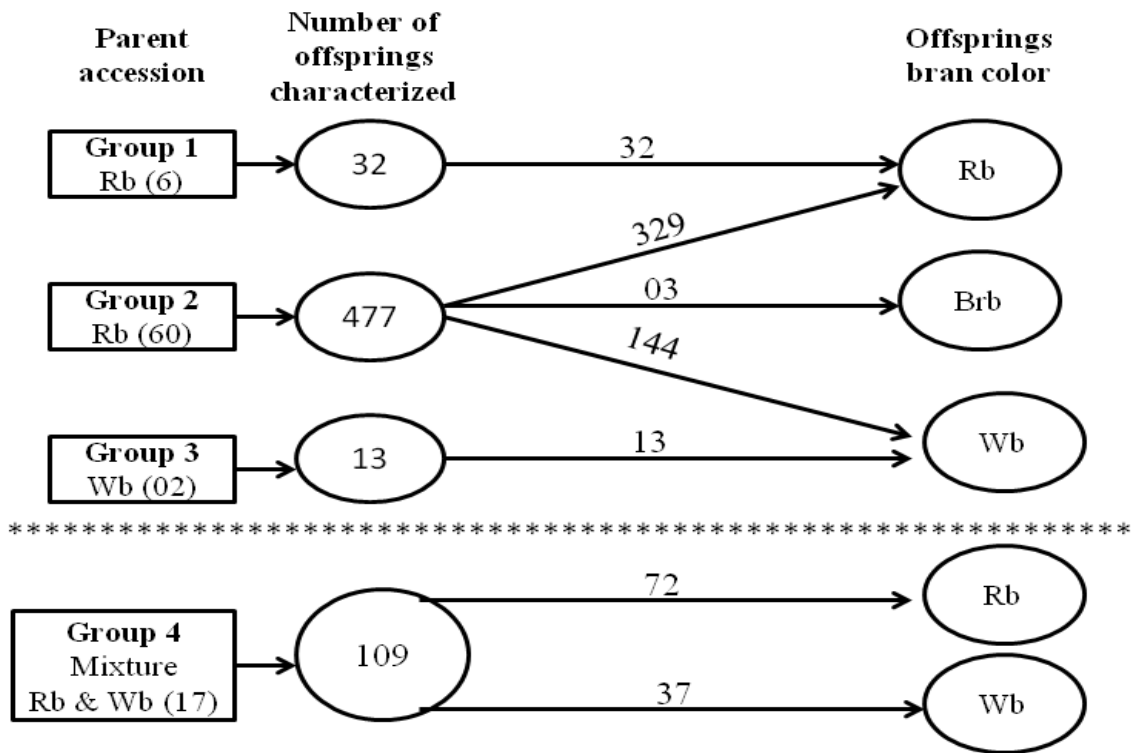


Fig.10. Graphical representation of bran color segregation in HR weedy red rice (2010-2011), Arkansas, USA. Parenthesis represents number of parent accessions. Rb = red bran, Wb = white bran, Brb = brown bran. Group 4, with a mixture of white and red kernels were not considered in segregation analysis. Parental data were not available for 3 accessions.

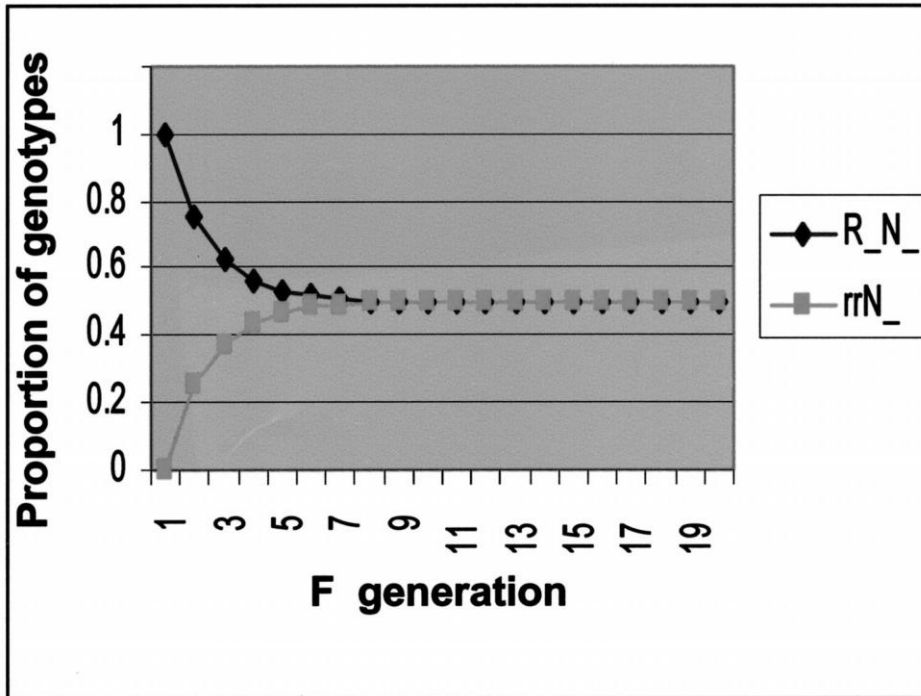


Fig. 11.

Idealized population distribution of HR red rice types expected after several generations of herbicide application (assuming 100% control) following a single hybridization between HR cultivated white rice (rrNN) and herbicide-susceptible red rice (RRnn). Assuming that red seed coat (RR) and herbicide resistance (NN) are dominant traits, the population resulting from a single hybrid becomes more than 95% resistant (half with white seed coats and the other half with red seed coats) to the herbicide within six generations.

Used with permission from 'allen press', publishing servicesSource file: Gealy D.R., Mitten D.H., Rutger J.N., 2003. Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): Implications for weed management. Weed Technol. 17 (3), 627-645. doi: <http://dx.doi.org/10.1614/WT02-100>

Table 1.

Primers and micro-satellite markers used for DNA fingerprinting of HR weedy red rice accessions from Arkansas, USA.

Mutation		Primers	Size (bp)	References
G ₆₅₄ E (Susceptible)	Forward	5'-CTGCCTATGATCCCAAGGGG-3'	131	Kadaru et al., (2008)
	Reverse	5'-TGGGTCATTCAGGTCAAACA-3'		
G ₆₅₄ E (Resistant)	Forward	5'-CTGCCTATGATCCCAAGGGA-3'	131	
	Reverse	5'-TGGGTCATTCAGGTCAAACA-3'		
S ₆₅₃ N (Susceptible)	Forward	5'-GTGCTGCCTATGATCCTAAG-3'	134	
	Reverse	5'-TGGGTCATTCAGGTCAAACA-3'		
S ₆₅₃ N (Resistant)	Forward	5'-GTGCTGCCTATGATCCTAAA-3'	134	
	Reverse	5'-TGGGTCATTCAGGTCAAACA-3'		
Micro-satellite markers			Size (nt)	
RM215	Forward	5'-AAAATGGAGCAGCAAGAGC-3'	143-154	Gealy et al., (2002)
	Reverse	5'-TGAGCACCTCCTTCTCTGTA-3'		
RM220	Forward	5'-GGAAGGTAAGTGTTCCTCAAC-3'	102-121	Akagi et al., (1996)
	Reverse	5'-GAAATGCTTCCCACATGTCT-3'		
RM234	Forward	5'-ACAGTATCCAAGGCCCTGG-3'	135-153	Zhang et al., (2006)
	Reverse	5'-CACGTGAGACAAAGACGGAG-3'		
RID12	Forward	5'-GCCTTGTCACCTCTTGGCATT-3'	151, 165	Sweeney et al., (2006)
	Reverse	5'-GGTTGGCACTGAAATCACCT-3'		

Table 2.

K-means cluster analysis of herbicide-resistant weedy rice (*Oryza sativa* L.) based on 6 morpho-physiological characteristics.

Clusters ^a	N	Plant height	Stem angle	Leaf texture ^b		Flag leaf length	Flowering	Panicle length	-----Hull color ^c -----				Awn length	Shattering	Yield/plant
		(cm)	(°)	S	R	(cm)	(DAP)	(cm)	S	G	Br	B	(cm)	(%)	(g)
1	182	131	73	144	38	34	96	23.5	103	11	33	35	1.7	40	95.7
2	202	127	74	137	65	32	104	20.8	40	7	74	81	1.8	67	32.7
3	314	166	76	188	125	34	99	24.5	57	11	102	144	2.3	56	40.3

^aClusters based on 6 morpho-physiological characteristics: plant height, flowering, panicle length, hull color, seed shattering, and yield/plant

^bLeaf texture: S= smooth, R = rough

^cHull color; S=strawhull, G=goldhull, Br=Brownhull, B=Blackhull

Table 3.

Morpho-physiological characteristics based on hull color of weedy red rice collected from Arkansas, USA.

Hull Color ^a	N	Plant height	Stem angle	Flag leaf color ^b			Flag leaf length	Flowering	Panicle length	Awn length	Shattering	Yield/plant
		(cm)	(°)	G	PM	P	(cm)	(DAP)	(cm)	(cm)	(%)	(g)
straw	200	137 b	74.6 a	144	27	29	33.1a	96 c	23.2 a	1.6 b	52.7 b	64.1 a
gold	30	147 a	74.8 a	19	6	5	32.8 a	97 bc	23.8 a	1.7 b	39.3 c	65.7 a
brown	210	148 a	74.6 a	151	33	26	33.7 a	102 a	23.1 a	2.2 a	53.6 b	47.5 b
black	260	149 a	75.2 a	179	36	45	33.4 a	101 ab	23.0 a	2.1 a	60.0 a	46.1 b

^aMeans followed by different letters are significantly different^bFlag leaf color; G=green, PM=Purple margin, P=purple

Table 4.

Cluster analysis (*K*-means) of germination capacity of weedy rice based on two stages; 75 and 270 days after harvest (DAH).

Clusters ^b	N	Germination capacity (%); 75 DAH				Cluster ^d mean	Germination capacity (%); 270 DAH				Cluster ^d mean
		S ^a	G ^a	Br ^a	B ^a		S	G	Br	B	
		Mean (Std dev) ^c	Mean (Std dev)	Mean (Std dev)	Mean (Std dev)		Mean (Std dev)	Mean (Std dev)	Mean (Std dev)	Mean (Std dev)	
1	478	95 (5)	94 (6)	94 (5)	93 (5)	94	96 (5)	99 (3)	97 (4)	97 (5)	97
2	101	67 (12)	64 (11)	63 (13)	66 (14)	66	84 (10)	88 (7)	86 (10)	86 (10)	85
3	65	70 (22)	56 (39)	76 (13)	82 (13)	77	54 (12)	49 (13)	50 (14)	52 (14)	52

^aHull colors: S=Strawhull; G=Goldhull; Br=Brownhull; B=Blackhull

^bAverage germination of three reference cultivars (CL 151, CLXL 729, CLXL 745) at 75 DAH = 90%; and 270 DAH = 91%

^cParentheses () denotes standard deviation









Table 5.
Cluster analysis of HR weedy red rice (*Oryza sativa* L.) based on seed-shattering.

Cluster ^a	N	Proportion (%)	Hull colors				Mean	Std Dev	Range
			Straw	Gold	Brown	Black			
1	175	25	46	4	46	79	87	6	77-100
2	221	31	55	3	69	94	65	7	53-76
3	174	24	49	13	54	48	40	7	28-52
4	130	18	40	10	41	39	15	8	0-27

^aAverage seed shattering of three ClearfieldTM Rice reference cultivars was 31%

Table 6.

Frequency of bran color from prominent hull color seeds of herbicide-resistant weedy rice offsprings (*Oryza sativa* L.).

		Bran color				Total offsprings ^a
		White	Light Brown	Brown	Red	
Hull color						
		(%)	(%)	(%)	(%)	
Straw		43	1	0	56	215
Gold		47	0	0	53	31
Brown		22	1	1	77	213
Black		26	0	1	74	263

^aNumber of plants based on hull color

Table 7.

K-means cluster analysis for kernel characteristics based on bran color, kernel length; width and 1000-kernel weight.

Clusters	Bran color distribution				Total N	Length (mm)		Width (mm)		TKW ^a (g)	
	White	Light brown	Brown	Red		M ^b	R ^b	M	R	M	R
1	113	0	0	167	280	6.2	5.0 - 6.9	2.4	1.8 - 2.9	17.8	12.5 - 24.7
2	81	4	3	218	306	6.9	6.2 - 8.5	2.3	2.0 - 2.7	20.0	15.4 - 25.4
3	9	0	0	71	80	7.2	6.4 - 8.1	2.8	2.5 - 3.3	26.6	22.7 - 35.4

^aTKW = 1000-kernel weight in grams;

^bM=Means; R=Range

Table 8.

Kernel characteristics of herbicide-resistant weedy red rice (*Oryza sativa* L.) offsprings by bran color category.

Bran color ^a	Length (mm)	Width (mm)	Length-Width ratio	TKW ^a (g)
<i>P</i> -values	0.1969	<0.0001	<0.0001	0.0021
White	6.6 a	2.34 b	2.83 bc	19.2 c
Light brown	7.2 a	2.32 b	3.08 a	21.7 a
Brown	6.7 a	2.30 b	2.90 b	18.6 d
Red	6.8 a	2.45 a	2.78 c	20.3 b

^cMeans followed by different letters are significantly different

^aTKW = 1000-kernel weight in grams

Table 9.

Morpho-physiological and kernel characteristics of weedy rice (*Oryza sativa* L.) offsprings with white bran.

Accession ID ^a	N ^b	Parent bran color	Hull color ^c				Plant height (cm)		Stem angle (°) ^f		Flag leaf length (cm)		Flag leaf width (cm)	
			S	G	Br	Bl	M ^d	R ^e	M	R	M	R	M	R
			AR10-03	5	white	3	1	0	1	141	119-170	79	75-90	30
AR10-100	5	Red	3	1	0	1	136	126-141	68	45-80	35	30-47	1.6	1.5-1.9
AR10-102	4	Red	2	0	2	0	147	112-169	68	45-75	35	28-41	1.5	1.2-1.7
AR10-32	8	white	3	3	1	1	136	105-157	74	70-75	36	28-59	1.4	1.2-1.5
CL151	3	white					119	97-130	75	75	30	22-35	1.4	1.1-1.4
CLXL729	3	white		S			126	116-131	82	75-90	30	28-34	1.2	1.1-1.3
CLXL745	3	white					126	113-134	77	75-80	33	32-34	1.3	1.2-1.3

	Flowering (DAP)		Kernel length (mm)		Kernel width (mm)		1000-kernel weight		Shattering (%)		Germination capacity (%)	
	M	R	M	R	M	R	M	R	M	R	M	R
	AR10-03	91	85-98	6.4	6.0-7.3	2.2	2.0-2.4	16.6	14.9-18.9	55	30-86	84
AR10-100	97	88-105	6.1	5.9-6.4	2.4	2.1-2.5	17.4	15.6-18.4	64	53-80	61	31-94
AR10-102	102	90-109	6.6	6.0-7.4	2.5	2.4-2.6	20.2	15.6-23.4	56	24-75	98	97-99
AR10-32	90	78-108	6.6	6.0-7.5	2.3	2.1-2.3	17.7	15.3-21.3	50	20-92	97	95-99
CL151	96	93-97	7.0	6.5-7.5	2.4	2.0-2.6	19.1	18.6-22.9	9	10-44	95	89-97
CLXL729	96	93-97	6.9	6.6-7.2	2.2	2.1-2.4	18.6	17.3-20.9	31	10-34	96	94-98
CLXL745	91	87-96	6.9	6.7-7.5	2.3	2.1-2.5	20.5	19.7-21.7	33	22-45	91	82-97

^aCL151=ClearfieldTM inbred rice; CLXL729 and CLXL745= ClearfieldTM hybrid rice; AR10-03, 100, 102, 32 = Weedy rice^bN= averaged over number of offsprings^cHull color: S=Strawhull; G=Goldhull; Br=Brownhull; Bl=Blackhull^dM=Mean; ^eR=Range^fStem angle relative to ground (horizontal axis); 75-90° = upright, erect

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CHAPTER VI

Conclusions

Conclusions

Volunteer rice can potentially have a significant negative impact on rice yield and grain quality. Hybrid rice seed that has higher capability to survive the winter than non-hybrid rice seed leads to a higher problem of volunteer rice in field with the cropping history of hybrid rice compared with that of non-hybrid rice. Volunteers from hybrid rice amplify the problem as these segregate into several weedy type plants of variable productivity, competing with cultivated rice, resulting in yield loss. The studies on volunteer rice provided insight into severity of the volunteer rice problem and indicated potential herbicide options for effective control. The application of 2,4-D pre-plant (1.12 kg ha^{-1}) following pyroxasulfone applied in the fall (0.12 kg ha^{-1}) provided better volunteer rice control (73%) than the other treatments and neither injured the rice crop nor caused yield loss. Winter-flood alone can reduce 34-40% of volunteer rice in field and should be part of an integrated management system for weedy or volunteer rice.

The transfer of HR rice genes via pollen flow to diverse weedy red rice populations has the potential to change population dynamics, and morpho-physiological characteristics of weedy rice. The hybridization of weedy red rice and ClearfieldTM cultivars has resulted into many crop-like plants due to introgression of crop gene into these weedy populations. Seed shattering, seed dormancy, plant height, stem angle, flag leaf length; width and bran color of majority of hybrid red rice accessions were similar to that of cultivated rice. These crop-like characteristics would make it hard to detect weedy rice in field and cannot be controlled by ClearfieldTM rice technology due to their resistance to IMI herbicides. Proliferation of these kinds of HR weedy populations in crop fields could result in evolution of new hybrid weedy red rice genotypes. However, reduced weediness in terms of seed shattering and dormancy would make it easy to control weedy red rice if coupled with soybean in rotation and use of alternate HR technology based on different modes of action.