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Evaluation of herbicide tolerance and interference potential among weedy rice
germplasm

By

Swati Shrestha

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant and Soil Sciences

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2018

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Weedy red rice is conspecific weed of rice, identified as a threat to global rice production. As weedy rice is more tolerant to stresses than cultivated rice and has wide genetic and morphological variation we hypothesized that weedy rice has high herbicide tolerance and weed suppressive potential. Herbicide tolerance and weed suppressive potential of 54 weedy rice accessions were evaluated and the traits were associated with molecular markers. Accessions B2, B20 and S11 showed high tolerance to glyphosate and B49, B51 and S59 had high tolerance to flumioxazin. All the accessions were controlled 100% with 1311g a.i/ha (1.5x) rate of glufosinate. Accession B2 inhibited the growth of barnyardgrass and amazon sprangletop by more than 50% indicating its high weed suppressive potential. Nei's gene diversity and Shannon's information index among the weedy rice accessions were found to be 0.45 and 0.66 respectively indicating high genetic diversity among weedy rice accessions.

DEDICATION

This work is dedicated to my parents. Thank you for all your sacrifices and teaching me the value of education in life. I would never have attempted to gain a higher degree without encouragement and support from my Dad. Also, I would like to dedicate this thesis to the person with whom I shared all the frustrations while researching and writing. Thank you Dr. Pratik Khatiwada for listening and tolerating me when I was difficult. Lastly, I should not forget mentioning Bindu Shrestha and Vishal Shrestha, you guys have always impacted my life and a great deal of whatever I am today is because of your motivation and favors.

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CHAPTER I
LITERATURE REVIEW

1.1. Introduction

Weedy rice is a commonly occurring weed in rice fields, and can be any species of genus *Oryza* that primarily infect crop fields and decreases yield (Delouche et al. 2007). It is among the most damaging and troublesome weed in rice fields (Norsworthy et al. 2013) causing complication in all levels of supply chain, from producers to consumers. It adulterates cultivated rice decreasing its productivity in field, and quality after harvesting, as the crop harvested is contaminated with weedy rice. Genus *Oryza* has twenty-one wild and two cultivated species. The two cultivated species include, *O.sativa* the Asian rice, and *O.glabberima* the African rice, both with diploid AA genome. Among the wild rice, nine are tetraploid (BBCC) (CCDD), and twelve are diploid (Khush 1997). Wild rice found in nature are those derived from *O. sativa* (AA complex), and *O. Officinalis* complex (BB, CC, CCDD) (Singh et al. 2013). *Oryza sativa* complex includes, *O. rufipogon*, *O. barthii*, and *O. longistaminata*; while, *O. officinalis* complex includes, *O. punctata*, *O. latifolia*, and *O. officinalis*. However, weedy rice is distinct from wild rice and is believed to have been evolved through (i) hybridization among and within cultivated and wild rice, (ii) de-domestication of cultivated rice, and, (iii) direct colonization of wild rice in agricultural rice fields (Kanapeckas et al. 2016; Londo and Schaal 2007; De Wet and Harlan 1975).

About 99% of weedy rice found in the USA is red “with red pericarp” thus people use the term weedy rice or red rice interchangeably.

In the USA, there are two major populations of weedy rice: blackhull and strawhull. (Constantin 1960; Shivrain et al. 2010b). However, there is variation among hulltype in weedy rice germplasm, and additionally other hull types, brownhull, greyhull and goldhull, are found in Louisiana and Texas (Noldin 1999). The process of hybridization between cultivated and wild rice, and among wild rice has resulted in enormous diversity of weedy rice germplasm in the US (Londo and Schaal 2007).

Rice production system, both puddled transplanting and direct-seeded, are affected by weeds (Chauhan 2013). However, in puddled rice system, crops have competitive advantage over weeds as they are transplanted into flooded field, while in direct seeded rice system, weeds pose a greater threat (Rao et al. 2007). Weeds compete with crop for light, nutrients, and water, thereby reducing yield of crop. Weeds are a major constraint in increasing rice productivity especially in the face of increasing population and decreasing agricultural land. Rice yield loss due to weeds ranges from 45-95% depending on the planting season, planting density, plant spacing, amount of fertilizers applied, climatic condition, and type and density of weed infestation (Pimentel 1991). Management practices for weed control has been shifting from hand weeding in small farms, and animal power in larger farms, to the use of chemicals because of labor shortage, particularly in developing countries (Mirza et al. 2008). The use of herbicides has further increased due to the economic expansion and development of herbicide tolerant crops (Naylor R 1994). In such a scenario, weedy rice is one of the major, most difficult-to-control, weed in rice fields because of its morphological and physiological similarity with cultivated rice (Chauhan

2013). According to Allston (1846), the first weedy red rice was reported in the US as early as 1800 as seed contaminants from Asia, and since then has been affecting rice crop by limiting its yield. Depending on the amount of infestation, weedy rice can cause yield losses varying from 50-60 % under moderate infestation, to 70-80% under heavy infestation (Chauhan 2013). In Arkansas, the highest rice producing state in the US, economic loss due to red rice has been estimated to be \$274/hectare (Burgos et al. 2008). The threshold for weedy rice infestation is 1-3 plants/m² in USA; plant density higher than this can cause significant yield loss (Smith 1988).

1.2. Competitive nature of weedy rice

Competition for limited resource is the drawing force for natural selection and shaping plant communities. Weeds compete with crop for nutrients, space, and light thus decreasing yield potential of crop. Traits of weedy rice like taller growth habit, higher tillering, and higher nutrient use efficiency makes it dominant and more competitive in the field (Estorninos et al. 2002, Burgos et al. 2006, Shivrain et al. 2010b). These traits which help them establish successfully in field are considered as competitive traits in this thesis. It is difficult to control weedy rice because they mimic cultivated rice morphologically, biochemically, and physiologically (Abraham and Jose 2015). Physical weed management is difficult as weedy rice is morphologically similar to cultivated rice in early stages, and chemical weed management is limited as herbicides controlling weedy rice also kills rice plant (Pantone and Baker 1991). Furthermore, with increasing dry-seeded rice system the problem of weedy rice is increasing worldwide (Chauhan 2013). In many places, farmers have altered their cropping pattern to non-rice system to manage this noxious weed.

There is vast morphological and genetic diversity among and within weedy rice populations (Shivrain et al. 2010a; Shivrain et al. 2010b; Tseng et al. 2013). Genetic variation and high plasticity in weedy rice makes it a highly dominant weed species in rice production system worldwide, with the ability to survive and proliferate in diverse niches.

The success of weedy rice as weeds can be attributed to its high dormancy as they can remain viable in soil for a long period of time, and emerge when conditions are favorable (Goss and Brown 1940). Noldin (2006) conducted experiment to evaluate the dormancy and longevity of various weedy rice biotypes from four states in the US. The study found differential level of dormancy among weedy rice ecotypes buried under soil at different depths and all of them were more viable than commercial rice cultivar. Five weedy ecotypes had viable seeds even after 36 months of burial in soil. The commercial rice seeds were not viable just after 5 months of their burial in soil. Weedy rice has greater viability than cultivated rice under certain environmental conditions and can emerge from deeper soil surface thus developing a robust soil seedbank.

Seed shattering, which distinguishes cultivated rice from its wild forms, is variable in weedy rice. In weedy rice, the abscission layer degrades earlier as compared to cultivated rice, leading to earlier shattering and increasing its fitness for survival in the environment (Thurber et al. 2011). Shattering in weedy rice is controlled by unidentified regulatory genes distinct from wild rice thus suggesting parallel evolution between weedy rice and wild rice (Thurber et al. 2011).

Weedy rice is generally taller than cultivated rice making them more efficient for light and space (Anuwar et al. 2014). According to Burgos et al. (2006), weedy rice has higher nitrogen use efficiency causing greater yield loss in rice fields. Thus, in fields

infested with weedy rice, the application of nitrogen fertilizers may not lead to an eventual increase in rice yields; instead, the weedy rice plants grow bigger and compete more aggressively with rice resulting in rice plants with lower yield. Nitrogen accumulation is higher in weedy rice compared to cultivated rice in “nutrient deficient” conditions, suggesting a more efficient nutrient uptake mechanism in weedy rice than cultivated rice (Sales et al. 2011).

In addition to higher nutrient response, weedy rice also has higher stress tolerance. Unlike commercial rice varieties, weedy rice can perform better in unfavorable environmental conditions like higher carbon dioxide, lower nutrient supply, and higher temperature, indicating they have higher capability of enduring stress than cultivated rice, and therefore, thrive better in stressful environment (Burgos et al. 2006; Ziska et al. 2008). Weedy rice ecotypes have higher leaf area and root weight when grown at carbon dioxide level of 500 $\mu\text{mol/mol}$ which is the projected CO_2 concentration in the middle and end of the 20th century (Ziska et al. 2008).

Growth and germination of most plant species are reduced under saline condition; however, weedy rice accessions have higher germination index and seedling vigor than commercial rice at 16 dSm^{-1} (NaCl) salinity level (Uddin et al. 2015). A weed-crop competition model by Pantone and Baker (1991) showed that weedy rice is dominant than cultivated rice, and that the competitive ability of one weedy rice plant is equivalent to three plants of an old commercial rice variety “Mars”. Ottis et al. (2005) studied the interference potential of red rice on 5 rice cultivars (CL161, Cocodrie, LaGrue, Lemont and XL8). Yield reduction of rice cultivars ranged from 100 to 755 kg/ha for every red rice

plant/m². Owing to higher competitive and stress tolerance ability, weedy rice has the potential to become more problematic in the face of climate change and global warming.

Research discussed above show that weedy rice is one of the most successful weeds in rice due to key weedy traits including high seed dormancy and longevity, high seed shattering, high nutrient uptake and nitrogen use efficiency, more tillers, high panicle number and biomass, and tolerance to stresses including herbicides. For successful implementation of management strategies for weedy rice, the physiological and genetic basis of these competitive traits, persistence, and tolerance to herbicide need to be understood. Further, understanding the mechanism and genetic basis of these competitive traits may provide unique information for rice improvement owing to its close relationship with cultivated rice.

1.3. Allelopathy and Allelopathic potential of rice

Hans Molisch (1856-1937) who is sometimes referred to as father of allelopathy defined allelopathy as interaction between plant and microorganisms which can be both beneficial and detrimental (Willis 2007). The concept has undergone several changes since then and allelopathy in general can be defined as a process where the secondary metabolites produced by one plant species suppresses the growth and development of neighboring species. Allelopathy has been documented in the literature for over 2,000 years and scientists have been trying to explore the mechanism of allelopathy in plants to exploit it in crop improvement programs (Amb and Ahluwalia 2016). The use of allelopathy to control weeds has evolved as a supplemental tool to control weeds in rice culture and has been explored since late 1980. The first experiment on rice allelopathy was conducted in

Arkansas, USA by Dilday et al. (1994) where 10,000 rice accessions were evaluated for their weed suppressive potential against a major aquatic weed of rice, ducksalad (*Heteranthera limosa*). Approximately 347 of them were found to possess allelopathic activity against ducksalad. The weed suppressive potential among cultivated rice is highly variable (Olofsdotter et al. 1998). Dilday et al. (2001) evaluated allelopathic potential of 17,927 rice accessions against ducksalad, barnyardgrass and redstem; 412 of these suppressed the growth of ducksalad (*Heteranthera limosa*), 145 had weed suppressive potential against redstem (*Ammannia coccinea*), and 94 suppressed barnyardgrass (*Echinochloa crus-galli*). Salam et al. (2009) evaluated the allelopathic potential of 102 Bangladeshi rice cultivars and found some rice cultivars that suppressed the growth of cress, lettuce, barnyardgrass and junglerice at different levels. The allelopathic potential of rice differs depending on its source, plant size, developmental stage, plant part used, and hull color (Khanh et al. 2005). Chung et al. (2003) evaluated the allelopathic potential of rice straw, leaves, and hull extract on barnyardgrass growth, and reported the straw extracts caused highest growth inhibition (22%) of barnyardgrass; leaf and hull extracts inhibited barnyardgrass growth by 12 and 8%, respectively. Hassan et al. (1998) evaluated the allelopathic potential of 1,000 rice cultivars against barnyardgrass and rice flatsedge, the most troublesome weeds in Egypt rice fields. They reported that 30 varieties inhibited growth of barnyardgrass by 50-90% and 10 varieties inhibited growth of rice flatsedge by 50-75%. Root exudates of five rice cultivars from Taiwan namely, Zhongzu 14, Zhunliangyou 527, Ganxin 203, Zhongzao 22, and Xiushui 417, reduced germination of lettuce at a concentration of 100 mg/L; inhibition decreased with increasing dilution of root exudate, indicating concentration-dependent effect of compounds present in the extract

(Ma et al. 2014). The above-mentioned studies show that some rice cultivars have innate weed suppressive potential. Rice root exudates, rice straw (left in the field after harvest), and rice leaves, contain allelochemicals, such as momilactone, that suppress some weed species in rice fields (Table 1.1). Since weedy rice demonstrates superior competitive traits than cultivated rice and have vast genetic diversity, weedy rice may also have high potential to suppress weeds in rice culture system. The weed suppressive potential of weedy rice has not been explored. Investigating the potential of weedy rice to suppress weeds in rice fields may help us discover plants with higher weed suppressive potential, which can be used in developing elite rice varieties.

1.4. Herbicide tolerance in weedy rice

Weedy rice has diverse hull color (black, brown, straw and goldhull), variable height (blackhull 75-190 cm tall and strawhull 46-189 cm tall), high number of tillers, diverse phenology (days to flowering ranging from 56-126 days), and variable flag leaf size (Shivrain et al. 2010b; Tseng 2013). The wide variation in morphology of weedy rice can be attributed to its genetic diversity. In the principal component analysis, weedy rice formed multiple clusters each corresponding to either black or strawhull populations thus showing genetic variability within each hull type. Blackhull accessions clustered close to *Oryza sativa indica* whereas strawhull accessions clustered close to *Oryza rufipogon* and *Oryza sativa indica* var *aus* (Londo and Schaal 2007). Wide genetic and morphological variation of weedy rice suggest that these may have differential response to some herbicides. Weeds in general exhibit differential tolerance to herbicides (Brown et al. 1987). Weedy rice accessions from Arkansas have differential tolerance to glyphosate,

being controlled 81- 100% with the commercial dose (Burgos et al. 2011). In the same study, some accessions that were completely necrotic after treatment with glyphosate, recovered and produced viable seeds. In the field, weedy rice accessions surviving glyphosate treatment may evolve higher tolerance in successive generations through iterative selection with a dose that is nonlethal (Burgos et. al 2011). Similarly, these weedy rice populations have differential tolerance to imazethapyr, with 3 of 130 accessions being tolerant to the commercial dose (Kuk et al. 2008). These accessions exhibited 17, 48 and 37-fold more tolerance than the standard susceptible weedy rice accessions. Weedy rice in the US mid-South also responded differently to glufosinate, with blackhull weedy rice from Texas (TX4) being more than twice as tolerant as other weedy rice from Arkansas to glufosinate (Gealy and Black 1998). These studies demonstrate the ability of weedy rice to survive commercial doses of some herbicides. Weedy rice can adapt to abiotic stress such as salinity, increased carbon dioxide, cold, and drought; thus, might harbor genetic mechanisms allowing them to sustain herbicide application and be more aggressive than the rice crop.

1.5. Weedy rice as source of desirable crop protection traits

Competitive ability and stress tolerance in weedy rice are often elite in comparison with cultivated rice because of higher genetic variability as it has gained characters from both wild and cultivated varieties (Vaughan et al. 2001). Twenty-eight novel blast resistance QTLs have been identified in two ecotypes of weedy rice with broad resistance spectrum, suggesting US weedy rice can be a source of blast resistance R gene for future rice breeding program (Liu et al. 2015). The aggressive and persistent nature of weedy rice

can be a good source for superior traits like herbicide tolerance and weed suppression. Once the QTL responsible for these traits are identified, the information can be utilized in rice improvement program as both weedy rice and cultivated rice are generally the same species. Additionally, understanding differential tolerance of weedy rice to herbicides is important for improving weed management program, and herbicide resistance management.

1.6. Molecular markers and population structure study

Genetic diversity is the basis of variability among plant species, and crop improvement program relies heavily on the genetic diversity of plants. The success of weeds in the agroecosystem can be attributed to their genetic variability and plasticity (Green et al. 2001). This allows weeds to survive and proliferate in diverse habitat, adapt according to environmental conditions, and at times mimic the crop, making their control difficult. Diverse variation in weedy rice morphology and phenology is observed which implies wide genetic diversity among weedy rice population.

The study of genetic diversity in weedy rice is essential for successful management of this weed and can also serve as a good resource for diversifying rice gene pool due to the close relationship of weedy rice with cultivated rice. Molecular markers are a convenient resource for accessing the genetic diversity and population structure of plants (Parker et al. 1998). These are small fragments of DNA having a particular location in the genome and is able to distinguish among and within species at the genomic level. An ideal molecular marker should be polymorphic, distributed evenly throughout the genome, affordable, have distinct linkage to specific phenotype, reliable, and need little amount of

DNA (Powell 1996). Different types of molecular markers used in rice breeding and marker assisted selection program are Restriction fragment length polymorphism (RFLP), Random amplification of polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP) and Simple sequence repeats (SSR) markers (Wang et al. 1994; Welsh and McClelland 1990; Yu et al. 2005). Among these molecular markers RFLP is non-PCR based marker and is not very popular due to the requirement of large amount of high quality genomic DNA and involvement of expensive radioactive materials (Agarwal et al. 2008). RAPD is PCR based marker but not very reliable as the profiling is dependent on reaction conditions and might differ within the laboratories (Bardakci 2001). AFLP and SSR markers are robust, reliable and reproducible, but SSR markers are cost effective and readily available hence more commonly used in rice marker assisted selection (Tanksley et al. 1989; Powell 1996).

The genetic diversity of weedy rice has been estimated using various markers. Cao et al. (2006) studied the genetic diversity of weedy rice populations from Liaoning province of North Eastern China using SSR markers. The genetic diversity among populations was found to be relatively high with H_e value of 0.313, and 35% of genetic variation was among regions. Yu et al. (2005) used both RAPD and SSR markers to access genetic diversity of weedy rice population from Liaoning China and reported that, overall, SSR markers are superior to RAPD in detecting genetic diversity among weedy rice populations. Gealy and Sneller (2002) could distinguish among weedy rice, weedy rice-cultivated rice hybrids, and rice cultivars, using microsatellite SSR markers. In contrast to the negative influence of weedy rice in rice production system, the genetic diversity of weedy rice could be used to

enhance the performance of cultivated rice by transferring favorable traits of weedy rice into the cultivated rice.

Understanding the molecular mechanism for herbicide tolerance and weed suppression using SSR markers will help us understand why some accessions of weedy red rice are tolerant to herbicides, more aggressive, or weed suppressive while others are not. Knowing molecular basis of these traits will help develop potential novel tools for weed management in rice. These tools might include markers for herbicide tolerance and weed suppression, which can be selected for increased expression in cultivated rice to provide competitive advantage to the crop.

Table 1.1 Allelochemicals identified in rice.

Plant source	Analysis method	Potential allelochemicals	Reference year
Rice Straw	Gas Chromatography	p-hydroxybenzoic, vanillic, p-coumaric, ferulic acid, benzoic, salicylic, syringic, protocatechuic, β -resorcylic, caffeic, sinapic, galic and gentisic acid	Kuwatsuka and Shindo (1973)
Rice Husk	Silica gel column chromatography, Thin layer chromatography, High performance liquid chromatography	Ineketone, S(-)+dehydrovomifoliol, momilactone A and B, momilactone C, p-coumaric acid	Kato et al. 1977
Rice residue	Paper Chromatography	p-hydroxybenzoic, p-coumaric, vanillic, ferulic, and o-hydroxyphenylacetic acid	Chou and Lin 1976
Rice seed	High performance liquid chromatography	Momilactone A and B	Cartwright et al. 1981
Rice leaves	Gas chromatography-mass spectrometry-selected ion monitoring	Oryzalexins A, B, C and D, momilactone A	Kodama et al. 1988

Rice straw	High performance liquid chromatography-mass spectrometry	Momilactone A and B	Lee et al. 1999
Rice seedling	High resolution mass spectrometry	3-Isopropyl-5-acetoxycyclohexene-2-one-1, momilactone B, and 5,7,4'-trihydroxy-3',5'-dimethoxyflavon	Kong et al. 2004
Table 1.1 (continued)			
Rice root exudate	Chromatographic fractionation	Momilactone B	Kato-Noguchi, H., 2004
Rice residue	High performance liquid chromatography	momilactone B, p-hydroxybenzoic, p-coumaric, ferulic, syringic and vanillic acids	Kong et al. 2006
Rice hull	Co-chromatography (TLC)	stigmastanol-3 β -p-glyceroyldihydrocoumaroate (1), stigmastanol-3 β -p-butanoyldihydrocoumaroate (2), lanast-7,9(11)-dien-3 α ,15 α -diol-3 α -d-glucopyranoside (3) and 1-phenyl-2-hydroxy-3,7-dimethyl-11-aldehydic-tetradecane-2- β -d-glucopyranoside	Chung et al. 2006
root exudates	High performance liquid chromatography	gallic, protocatechuic, chlorogenic, vanillic, syringic, ferulic, benzoic, ellagic, cinnamic acids and vanillin	Khang et al. 2016
Blended root, stem and leaf extract	Column Chromatography	syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), (-) loliolide, 3 β -hydroxy-5 α ,6 α -epoxy-7-megastigmen-9-one and 3-hydroxy- β -ionone	Masum et al. 2018

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CHAPTER II
SCREENING FOR HERBICIDE TOLERANCE AMONG WEEDY RICE
GERMPLASM

2.1. Abstract

Weedy rice is one of the most competitive weeds in rice production system. Traits such as rapid growth, high tillering, enhanced ability to uptake fertilizers, asynchronous maturation, seed shattering, and long dormancy periods, make weedy rice more competitive than cultivated rice. Although weedy rice is a major limitation to rice crop yield it may have traits that could facilitate crop improvement. Greenhouse studies were conducted to evaluate the response of 54 weedy rice accessions collected between 2008-2009 from Arkansas, USA, to glyphosate, glufosinate, and flumioxazin applied at field rates. Rice cultivars, CL163 and REX were included for comparison. Accessions B20, B2 and S11 were highly tolerant to glyphosate and B49, B51 and S59 had high tolerance to flumioxazin with injury of less than 40%, 5 WAT (weeks after treatment). All the weedy rice accessions and cultivated rice were 100% controlled at 1.5x rate of glufosinate. Rice cultivars (CL163 and REX) were sensitive to both glyphosate and flumioxazin controlled 100% 5 WAT to these herbicides. On an average, blackhull accessions were more tolerant to herbicides than strawhull accessions. Potential “herbicide tolerant” accessions were identified, which can be used as a source of genetic material for crop improvement. Weedy

rice accessions have differential tolerance to herbicides and this information should be kept in mind while developing improved weedy rice management strategies.

Keywords: crop improvement; herbicide tolerance;

Flumioxazin, 2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propionyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione; glufosinate, glufosinate-ammonium, glyphosate, N-(phosphomethyl) glycine

2.2. Introduction

Rice is the staple food crop for more than half of the world's population at present and is an important crop in achieving global food security (Subudhi et al. 2006). In the US, rice is predominantly produced in Arkansas, Mississippi, Missouri, Louisiana, Texas and California (USDA ERS 2016). Weeds are among the major causes of rice yield reductions worldwide, and are becoming a bigger threat as growers shift from transplanted to direct-seeded rice due to water and labor limitations (Chauhan 2012; Rao et al. 2007). The most problematic weeds in Arkansas and Mississippi rice fields are, barnyardgrass (*Echinochloa crus-galli*), sprangletops (*Leptochloa* spp.), weedy rice (*Oryza sativa* L), northern jointvetch (*Aeschynomene virginica*), and palmer amaranth (*Amaranthus palmeri*) (Norsworthy et al. 2013). In Arkansas, season-long weed interference can reduce rice yield from 10% with eclipta (*Eclipta prostrata*), to 82% with weedy rice (*Oryza sativa* L). Yield reductions vary depending on weed species and density (Smith et al. 1977; Smith 1988).

Weedy rice is a conspecific, highly competitive and aggressive weed of cultivated rice (Vaughan et al. 2001). According to Allston (1846), the first weedy rice infestation was reported in the USA in 1840 as seed contaminant from Asia, and since then has been

affecting rice fields reducing crop yield and quality. Its vegetative and morphological similarity with cultivated types in early stages makes it difficult to identify and selectively handweed, while its genetic similarity to cultivated rice limits herbicide use (Delouche et al. 2007). Weedy rice is believed to have evolved through hybridization among and within cultivated and wild rice; hence it has diverse genetic, physiological, and morphological characteristics compared to cultivated rice (Londo and Schaal 2007). Weedy rice varies widely in terms of hull type (blackhull, strawhull, brownhull and goldhull), awn color (black, straw, pink), awn length (long awn, short awn, awnless), plant height (76-190 cm), number of tillers, panicle length, and seed production potential (Shivrain et. al 2010b). Characteristic traits in weedy rice such as vigorous vegetative growth and large biomass production, greater response to nutrients, variable time of seed germination and maturity, high seed shattering, high tillering, differential dormancy, and seed longevity makes weedy rice more competitive than cultivated rice (Constantin 1960; Burgos et al. 2006; Tseng et al. 2013; Burgos et al. 2011; Diarra et al. 1985; Fogliatto et al. 2011). Pantone and Baker (1991), through weed-crop competition modeling, demonstrated the dominant nature of weedy rice, and showed that competitive ability of one weedy rice was equivalent to that of four cultivated rice plants. The aggressive and persistent nature of weedy rice makes it one of the most troublesome weeds in rice fields. However, ability of weedy rice to tolerate various biotic and abiotic stress conditions makes it an important resource for plant breeders. Liu et al. (2015) identified 28 novel blast resistant QTL in two US weedy rice indicating robust nature of weedy rice to fight against disease and stress. Weedy rice produces higher biomass and leaf area under elevated carbon dioxide condition suggesting

their potential to thrive in the face of climate change and global warming (Ziska and McClung 2008).

Generally, weeds have variable tolerance to herbicides (Brown et al. 1987). Likewise, weedy rice has differential tolerance to imazethapyr and glyphosate (Burgos et al. 2011; Kuk et al. 2008). Weedy rice accessions that have escaped or survived herbicide applications may be able to tolerate a higher herbicide rates in successive generation through natural selection (Burgos et al. 2011). Weedy rice has adapted to flourish in man-made environment and outcompete crops. Owing to the ability of weedy rice to thrive under unfavorable environmental conditions where crops do not perform well, we hypothesized that weedy rice has differential tolerance to glyphosate, glufosinate and flumioxazin. The three herbicides were selected based on their wide use in numerous crops, non-selective nature and their approval as pre-plant burn down in numerous crops such as, cotton, corn, and rice. Previous research revealed that weedy rice ecotypes collected in the year 2002-2003 from Arkansas had differential tolerance to glyphosate (Burgos et al. 2011). The exposure of weedy rice to glyphosate has increased tremendously with the popularity of Roundup Ready soybean and use of glyphosate as burn down before planting rice. Thus, tolerance level of weedy rice to glyphosate may have increased with time. Estimating tolerance level of weedy rice to these herbicides is important for development of successful weed management strategies. The potential tolerant accessions, if any, would also provide natural source of herbicide tolerance that could be used by plant breeders to increase herbicide tolerance in crops, to protect them from herbicide drift or development of herbicide resistant crops. In this current study we aimed to (i) screen a diverse germplasm of weedy rice collected between 2008 and 2009 for tolerance to glyphosate, glufosinate

and flumioxazin (ii) To associate morphological trait of weedy rice (hullcolor) with herbicide tolerance.

2.3. Materials and Methods

Seed Collection

Seeds of 200 weedy rice accessions were collected from all major rice growing counties in Arkansas in 2008-2009. All accessions were grown at the USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, and characterized in terms of plant height, tillering, days to flower, hull color, culm length, panicle length, grain shattering and grain yield (Tseng 2013). Using these morphological data, 54 accessions deemed to be most competitive were selected based on key weedy traits such as high tillering capacity, high seed shattering, and tall culm length; these traits being generally linked to aggressiveness and persistence of plants (Delouche et al. 2007). These 54 accessions were tested for herbicide tolerance.

Bioassay in the Greenhouse

Experiments were conducted in 2016 and 2017 in two runs, with completely randomized design, in greenhouse at the RR Foil Plant Science Research Center, Mississippi State University. Maximum and minimum greenhouse temperature were set up at 25°C and 35°C, respectively, and humidity was maintained at 70%. Two rice cultivars CL163 and REX were included as reference. Seeds were pre-germinated in 0.3% agar medium. After one-week, pre-germinated seeds were transferred to 50-cell trays filled with Sunshine #1 (Sun Gro Horticulture Canada Ltd, Vancouver, Canada), an all-purpose

potting medium with sphagnum peat moss, perlite, dolomitic limestone, long lasting wetting agent, and RESiLIENCE[®]. Twenty-five plants per accession (1 tray = 2 Accession), with two replications, were treated with glyphosate, glufosinate, or flumioxazin, in separate experiments. Information on the product and doses used are presented in Table 2.1. Treatments were applied at 3-4 leaf stage in a spray chamber equipped with TP8002VS VisFlo Flat Spray Tip (TeeJet[®], Spraying Systems Co. World Headquarters, P.O. Box 7900, Wheaton, IL 60187) calibrated to deliver 187 L ha⁻¹ at 6895 Pa. Since smaller plants are more sensitive to herbicides than larger plants (Shrestha et al. 2007; Norsworthy et al. 2008), glyphosate and glufosinate were applied to young seedlings (3-4 leaf) to observe more differentiation in response among accessions. Three doses of glufosinate were used as the initial dose (1x) did not injure plants and all weedy rice accessions recovered from the lowest dose used. Flumioxazin was applied as pre-emergence. Trays were immediately transferred to the greenhouse after herbicide application, and watering was resumed after 24 hours. Plants were watered everyday using a micro sprinkler. Plant injury from glyphosate and glufosinate were evaluated 3 and 5 weeks after treatment (WAT) on a scale of 0 to 100%, where 0 meant no injury, and 100% meant complete plant mortality with no green tissue (Frans et al. 1986). Visible injury symptoms were chlorosis followed by necrosis of leaf tissue, and stunting. For flumioxazin, germination was evaluated 1 and 2 weeks after planting (WAP). Height and injury were recorded 2, 3, 4 and 5 WAT. Stunting of plants relative to non-treated checks was calculated from plant height data using the formula:

$$\text{Stunting (\%)} = \frac{\text{Height of control plant (cm)} - \text{Height of treated plant (cm)}}{\text{Height of control plant (cm)}} \times 100$$

Plants showing less than 40% injury 5 WAT were transferred into bigger pots to obtain grain yield. Control plants were also grown until maturity to record yield.

Statistical Analysis

Weedy rice accessions and herbicide treatment were considered as fixed effects, while replications and runs as random effects. The experimental design was a completely randomized and data were pooled across runs. Data were subjected to analysis of variance using PROC GLM method (version 9.4, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA), and mean values were separated using Fisher's protected LSD at an alpha level of less than or equal to 0.05. The ANOVA model used in the experiment was,

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

Where Y_{ijk} is the response variable which includes injury and height of weedy rice accessions, μ is mean of response variable, α_i is the accessions effect, β_j is the effect of run on the responses, $(\alpha\beta)_{ij}$ is the errors associated with run*accession and, ϵ_{ijk} is the error associated with the model.

$$\beta_j \sim \text{NN}(0, \sigma^2_{\beta}), \text{ iid}$$

$$(\alpha\beta)_{ij} \sim \text{NN}(0, \sigma^2_{\alpha\beta}), \text{ iid}$$

$$\epsilon_{ijk} \sim \text{NN}(0, \sigma^2), \text{ iid are independent}$$

Hierarchical clustering method of JMP 13 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA) statistical software was used to group accessions into different clusters based on their injury 5 WAT. In this method, mean injury of each accession is considered

as a single point initially and the two closest points are combined, the process continuing until all points are combined forming a single cluster. Ward's minimum variance was used to group accessions into different clusters where the ANOVA sum of squares between two nearest clusters added up over all variables is the distance between two variables.

2.4. Results and Discussion

Response of accessions to glyphosate, glufosinate, and flumioxazin

Owing to their genetic and morphological variation we expected differential herbicide sensitivity of numerous weedy rice accessions. Data from the experiment supported the above assumption as 54 weedy rice accessions and 2 rice cultivars CL163 and Rex showed differential tolerance to glyphosate and flumioxazin ($p < 0.0001$). However, the accessions did not vary in terms of their glufosinate response. Glufosinate applied at 874 g ai/ha (1x) and (1.25x) did not cause any significant injury to plants; whereas, glufosinate applied at 1.5x rate killed all plants. Similar results were obtained by Pearson (2005) where glufosinate was found to be the weakest herbicide among glyphosate, glufosinate and imazethapyr, providing only 49% control of weedy rice when applied at full rate. Glufosinate applied alone for weedy rice control is not very effective and 92% control is obtained only when a high rate of 1100 kg/ha (1.8x) is applied (Sankula 1997). Glufosinate is primarily effective on broadleaf weeds, however, efficacy of this herbicide on grasses can be enhanced by mixing with other herbicides such as acifluorfen (Sankula 1997).

Weedy rice injury from glyphosate ranged from 30 (B20) to 97% (S118), 5 WAT (Fig 2.1); with B20 being most tolerant to glyphosate, and S118 being most sensitive.

Cultivated rice, CL163 and REX, were most sensitive to glyphosate with injury of 97 and 98% respectively, 5 WAT. Of the 54 weedy rice accessions, 3 exhibited higher tolerance to glyphosate than others with less than 40% injury. We considered these 3 as accessions potentially tolerant to glyphosate as they recovered from glyphosate injury with new flush of green leaves, 5 WAT. Sensitive accessions and rice cultivars, on the other hand, showed gradual increase in injury from 3 to 5 WAT. Injury of the three most tolerant accessions B20, B2 and S11, decreased from 36, 39 and 49%, 3 WAT, to 30, 34 and 38%, respectively, 5 WAT (Fig 2.1), reflecting a high rate of recovery. On the other hand, injury of the three most sensitive accessions, namely ALR-1, B44, and S118, increased from 86, 89, and 95%, to 94, 94 and 97%, respectively, from 3 to 5 WAT.

In the study conducted by Burgos et al. (2011), weedy rice accessions from Arkansas were controlled 81-100% by commercial glyphosate rate. Similarly, in our study most accessions were controlled 70-100% by glyphosate, which reveals effectiveness of glyphosate in controlling weedy rice. In contrast, B2, B20 and S11 were not sensitive to glyphosate. High glyphosate tolerance in these weedy rice accessions may be due to cropping practices and herbicide use pattern in rice production system. Most farmers in the southern US rotate rice with soybean to control infestation of major weeds in rice (Smith et al. 1977; Griffin et al. 1986). In soybean, glyphosate was initially used as a burndown herbicide to control weedy rice and other grass weeds, but with the commercialization of roundup ready soybean in 1996, over-the-top application of glyphosate in soybean has become very common. Weedy rice, has variable germination and dormancy (Tseng et al. 2013) and some weedy rice germinate later in the season. This has possibly led to a tremendous increase in exposure of weedy rice populations to glyphosate applied over-the-

top in soybeans. Weedy rice accessions may have therefore undergone high selection pressure and developed increased tolerance to glyphosate with time.

Flumioxazin is used as preplant burndown for weed control in rice, cotton and soybean. Like glyphosate response, most weedy rice accessions were susceptible to flumioxazin, indicating its effectiveness in controlling this weed. With flumioxazin, most accessions were injured more than 80%, 5 WAT; however, B49, B51 and S59, showed lower injury of 22, 30 and 39%, respectively, and were considered tolerant to flumioxazin (Fig 2.2). Among these 3 flumioxazin tolerant accessions, S59 showed a high rate of recovery from herbicide injury, while, B49 and B51 showed slight increase in injury at 5WAT, when compared to 3WAT. Flumioxazin did not affect weedy rice germination and germination rate of all accessions were greater than 80%. Herbicides for controlling weedy rice in rice fields are limited and flumioxazin has 60 days waiting period for rice. This infers rice is highly sensitive to flumioxazin. As rice and weedy rice are closely related flumioxazin can be a good herbicide for weedy rice control, if we could develop flumioxazin tolerant rice cultivars. Three weedy rice accessions showed high tolerance to flumioxazin and these can be a source of raw genetic material for rice breeder to develop flumioxazin tolerant rice which could broaden herbicide option for rice. However, it is important to understand how frequently these tolerant accessions occur in rice and soybean fields. Repeated exposure of tolerant accessions to herbicides will eventually select for these accessions and soon we might develop weedy rice resistant to these herbicides making weedy rice management difficult in rice and soybean fields. Thus, the development of strategic and integrated weed management practices to control weedy rice is important before they become resistant to glyphosate and other herbicides, and possess a bigger threat

in the future. The tolerant accessions identified in this study can be used in genetic analysis to identify QTLs or genomic regions associated with glyphosate and flumioxazin tolerance.

Effect of herbicide treatment on yield and height of accessions

There was no difference in grain yield of tolerant accessions because of glyphosate and flumioxazin injury when compared with the control plants. Grain yield of accessions B20 and B2 with high glyphosate tolerance produced 130 g/plant each, and S11 produced 149 g/plant. B49, B51 and S59, with high flumioxazin tolerance had grain yield of 71, 94 and 64 g/plant, respectively (Table 2.2). This indicates their potential to quickly produce numerous weedy rice plants with high herbicide tolerance in subsequent generation. Seeds from these tolerant accessions may also contribute to soil seedbank, and as weedy rice seeds have long dormancy periods and variable emergence, these may germinate after several years causing sudden outbreak of weedy rice in the fields outcompeting the crop (Tseng et al. 2013; Goss and Brown 1939).

Reduction in plant height, following glyphosate application, ranged from 4 to 40% 5 WAT, as compared to control plants. The highest stunting of 40% was observed in ALR-1, which was higher than stunting in all other accessions. The three most tolerant accessions namely B20, B2 and S11, showed minimal stunting of 4, 5 and 8%, respectively. Glyphosate injury and stunting on plants were positively correlated but with low Pearson correlation coefficient of 0.16. Due to high sensitivity of most accessions to flumioxazin, stunting was computed only for B49, B51 and S59, which was 19, 22 and 38%, respectively.

Response of weedy rice accessions to herbicide treatment based on hulltypes

Three hull types of weedy rice accessions were used in this study namely, blackhull, strawhull, and brownhull. Cultivated rice, CL163 and REX, were grouped separately for comparison. Even though there was variable response to herbicide within each hull type, among the hull types, blackhull were least injured by herbicides than other hull types, both 3 and 5 WAT (Table 2.3). Injury due to glyphosate and flumioxazin for blackhull types was 76 and 89%, respectively, 5 WAT. Brownhull and strawhull biotypes were controlled 85-100 % by both herbicides. Rice cultivars were most sensitive with 100% injury by both herbicides. In a similar study by Gealy et al. (1999), blackhull accessions were more tolerant to imazethapyr (Pursuit) than strawhull accessions. Weedy rice accession TX4 showed high tolerance to glufosinate and was not completely controlled even by 2x (1.12 kg/ha) rate of the herbicide (Noldin et al. 1999). TX4 with low susceptibility to glufosinate, was a blackhull biotype with high dormancy, obtained from Texas. Higher herbicide tolerance in blackhull biotypes may be due to the presence of diverse genes. The genetic diversity within blackhull is higher (0.76) than strawhull populations (Shivrain et al. 2010b), suggesting higher genetic diversity in blackhull and thus well adapted to stresses. Blackhull and strawhull strains evolved independently and their genetic basis for weediness varies (Li et al. 2017). Tseng et al. (2013) reported blackhull biotypes have higher dormancy linked loci than strawhulled biotypes, again, indicating higher variation in genetic makeup of blackhull biotypes. Thus, infestation of blackhull weedy rice in rice fields may pose a bigger threat to rice growers owing to their differential selectivity with higher tolerance to herbicides and highly diverse genetic makeup. Identification and control of blackhull accessions in early stages of rice culture can therefore minimize yield

reduction in rice. However, it is not practically possible to distinguish blackhull strains from other strains in the early stage; thus, integrated weed management practices such as fallow tillage, stale seed bed, and crop rotation, should be used along with chemicals to suppress weedy rice. Care should be taken to ensure weedy rice biotypes do not escape management tactics and farmers should be encouraged not to use sub-lethal dose of herbicides that would promote selection among tolerant weedy rice accessions. However, as blackhull showed higher tolerance to glyphosate and flumioxazin than strawhull, it provides a unique opportunity to further examine the basis of differential tolerance among these accessions. Genetic diversity has always been the basis for crop improvement and diversity in weedy rice can be harnessed to improve the biotic and abiotic stress tolerance in rice cultivars.

Hierarchical clustering of weedy rice based on injury 5WAT with glyphosate

Weedy rice accessions were divided into four distinct clusters on the basis of their differential sensitivity to glyphosate, 5 WAT (Fig 2.3). Glyphosate injury was chosen for cluster analysis as the weedy rice accessions showed greatest variation in injury with this herbicide. The hierarchical clustering grouped accessions in response to their mean injury. Accessions B20, B2 and S11, which were considered more tolerant than others were grouped together in cluster 1 and had mean injury of 36%. Cluster 2 consisted mostly of blackhull accessions with mean injury of 73%. Cluster 3 consisted of a mixture of both blackhull and strawhull accessions with mean injury of 88%. Cluster 4 represented highest injury of 94% and primarily consisted of strawhull accessions. Accessions in clusters 1 showed signs of recovery and regrowth from the glyphosate injury at 5 WAT when

compared at 3 WAT. On the other hand, cluster 3 and 4 showed no recovery signs from glyphosate injury 5 WAT. Both rice cultivars, CL163 and REX, fell in cluster 4 indicating that commercial rice cultivars are highly susceptible to glyphosate and show high injury symptoms with no recovery when exposed to this rate of glyphosate. Based on the result of cluster analysis, accessions in cluster 1 were designated as glyphosate tolerant. The recovered accession in this category were able to produce viable seeds at maturity. Accessions in cluster 2 were sensitive to glyphosate and although showed some signs of recovery from glyphosate injury, remained stunted over time. Information from the cluster analysis should be considered while developing weedy rice management strategies. Further, with commercial rice being highly sensitive to glyphosate, steps should be taken to prevent drift of glyphosate into rice fields.

2.5 Conclusion

Differential susceptibility of weedy rice accessions to herbicides, as evident from this study, suggests genetic variability among weedy rice accessions; a factor that promotes their successful adaptation to varying cropping patterns. This should be kept in mind while using herbicides to control weedy rice, as the usage of herbicides will increase in the future with the development and commercialization of herbicide resistant rice cultivars. Additionally, the potential tolerant accessions can be explored by plant breeders for development of herbicide tolerant crops.

Table 2.1 Herbicide information and rates used.

Treatment	Rate (g ai or ae/ha)	Product	Concentration	Manufacturer
Glyphosate	1120	Roundup PowerMAX®	540 g acid/l	Monsanto Company St Louis, Missouri
Flumioxazin	72	Valor® SX	51% a.i (w/w)	Valent USA Corp., Walnut Creek CA
Glufosinate	874	Liberty® 280 SL	280 g a.i/l	Bayer CropScience LP, NC
Glufosinate (1.25x)	1093	Liberty® 280 SL	280 g a.i/l	Bayer CropScience LP, NC
Glufosinate (1.5x)	1311	Liberty® 280 SL	280 g a.i/l	Bayer CropScience LP, NC

Table 2.2 Grain yield of accessions tolerant to glyphosate and flumioxazin.

SN	Accession	Grain yield (g/plant)
1	B2 ^a	130
2	B20 ^a	130
3	S11 ^a	149
4	B49 ^b	71
5	B51 ^b	94
6	S59 ^b	64

a- Potential accessions tolerant to glyphosate

b- Potential accessions tolerant to flumioxazin

Table 2.3 Injury of different accessions based on hull type 3 and 5 weeks after treatment with glyphosate and flumioxazin.

Hull Color ^a	Herbicide	Injury (%) 3WAT ^b	Injury (%) 5WAT
CR	Glyphosate	95a	98a
BR	Glyphosate	85b	93a
SH	Glyphosate	84b	85b
BH	Glyphosate	74c	76c
CR	Flumioxazin	84a	100a
BR	Flumioxazin	50d	100a
SH	Flumioxazin	66b	94b
BH	Flumioxazin	61c	88c

^bWAT = weeks after treatment. Means within a column followed by the same letter are not different based on Student's t-test at $\alpha=0.05$

^aCR = cultivated rice; BR = brownhull; SH = strawhull; BH = blackhull

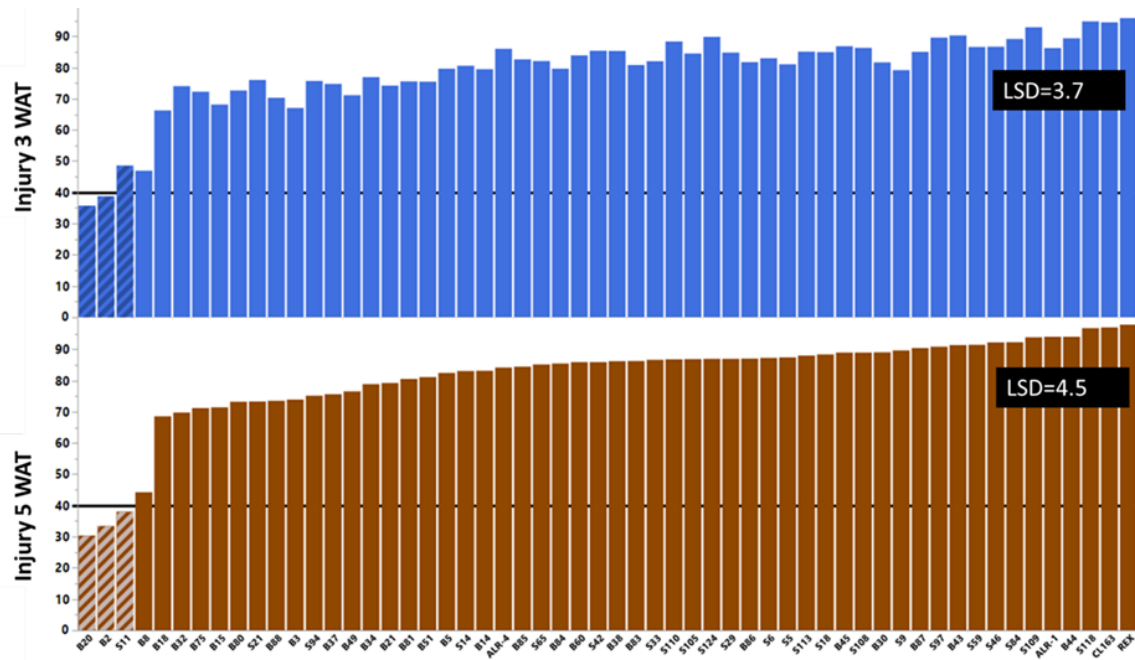


Figure 2.1 Response of weedy rice accessions to glyphosate at 3 and 5 WAT.

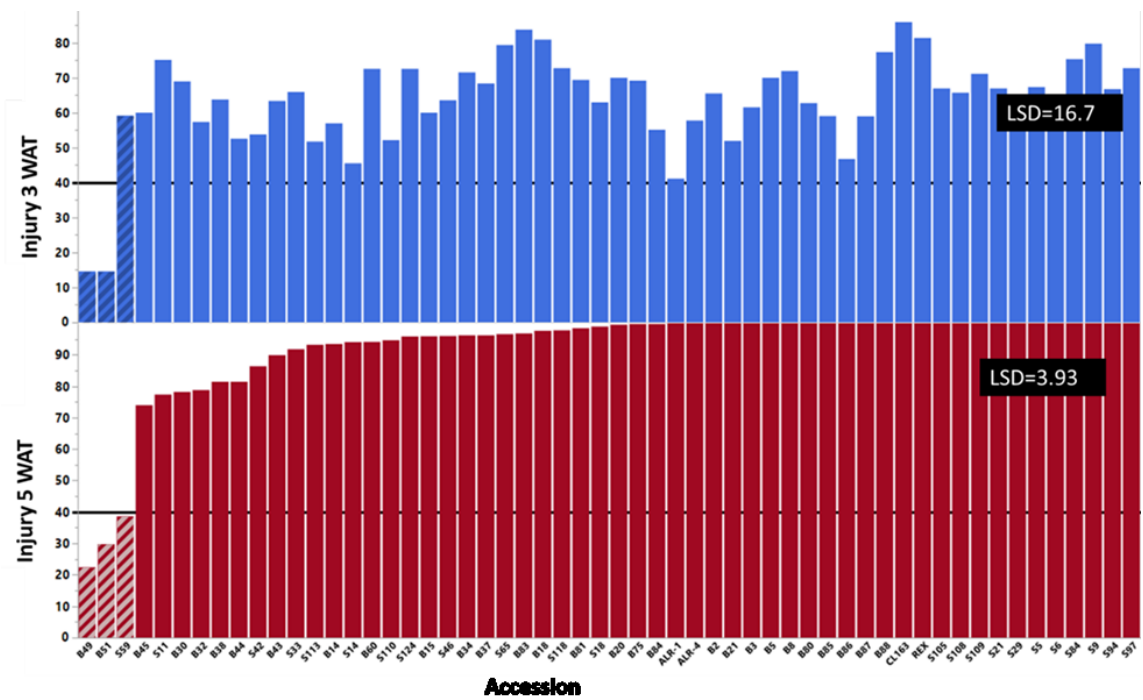


Figure 2.2 Injury among weedy rice accessions with flumioxazin herbicide 3 and 5 WAT.

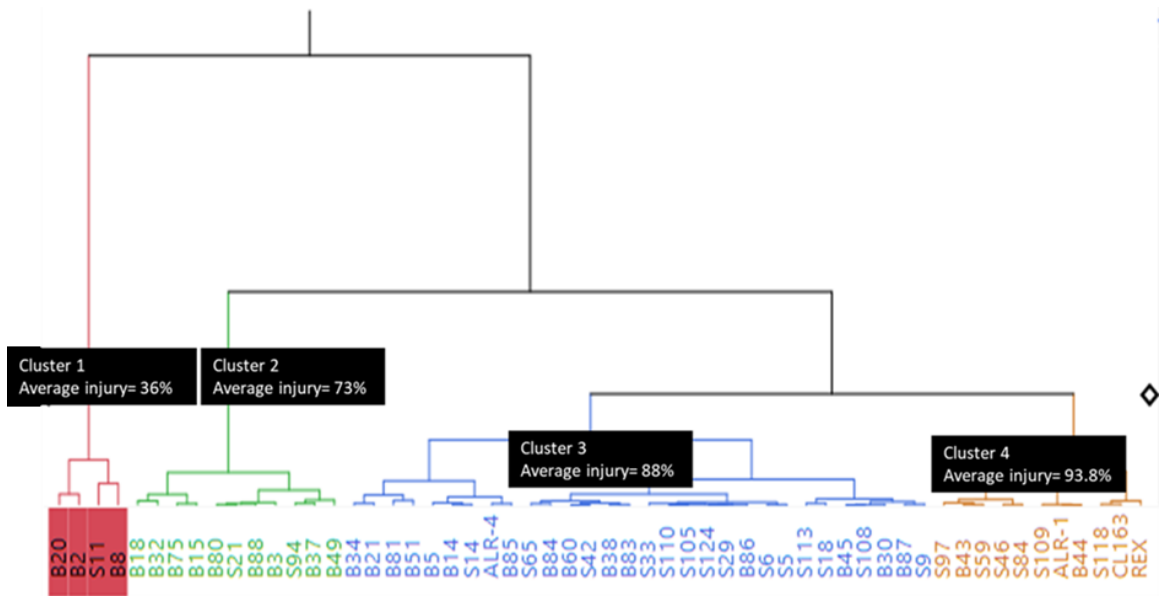


Figure 2.3 Hierarchical clustering of weedy rice accessions and rice cultivars based on plant injury ratings 5 WAT with glyphosate.

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CHAPTER III
EVALUATING INTERFERENCE POTENTIAL OF WEEDY RICE: STEP
TOWARDS BREEDING WEED-SUPPRESSIVE RICE CULTIVARS

3.1. Abstract

Rice is a vital crop to attain global food security. With increasing shortage of resources like labor and water, there has been a shift in rice establishment method from Puddled Transplanting (PTR) to Direct Seeded Rice (DSR) system. Even though DSR uses less resource, weeds are a major yield constraining factor. Herbicide use for controlling weeds has become limited with the development of herbicide resistant weeds in rice production. Development of allelopathic rice can be a good option for sustainable weed management in DSR. As wild relative is often explored by plant breeders for crop improvement program, 54 weedy rice accessions- weedy relatives of cultivated rice were evaluated for their interference or weed suppressive potential against barnyardgrass and amazon sprangletop. Three allelopathic rice cultivars (PI312777, PI338046, and RONDO) and two non-allelopathic rice cultivars (CL163, REX) were included as positive and negative control. Accession B2 (61%) had higher interference potential against *E. crus-galli* and accession B81 (52%) had greatest interference potential against *L. panicoides*. Accession B81 had more than 50% inhibition on the growth of both *E. crus-galli* and *L. panicoides*, two major weeds of rice. Morphologically, there was difference in weed suppressive potential of weedy rice with respect to hull type, blackhull being more

competitive than straw and brownhull. Potential allelopathic weedy rice accessions were identified and can be used as a source of raw genetic materials in rice breeding programs for developing allelopathic rice cultivars.

Keywords: allelopathy, amazon sprangletop (*Leptochloa panicoides*), barnyardgrass (*Echinochola crus-galli*), crop improvement, weed suppression,

3.2. Introduction

Rice (*Oryza sativa*) is the staple food crop for more than half of the world's population and is considered a major food crop to ensure future global food security (Fairhurst & Dobermann, 2002). In USA, rice is mainly cultivated in Arkansas, Mississippi, Texas, Louisiana and California, covering approximately 1274760 ha of land with an average yield of 3471.3 kg/ha (USDA NASS, 2017). United States is the major exporter of high quality rice contributing more than 10% to the global rice trade annually (USDA-ERS, 2017). Conventionally, in most of the developing countries rice is transplanted into standing water thus providing competitive advantage to the crop and enhancing crop productivity by suppressing weeds. However, with the scarcity of labor and water there has been a significant shift from puddled transplanting (PTR) to direct seeded rice (DSR) system in Asian countries in the past two decades (Pandey & Velasco, 2002). In USA, all rice fields are cultivated through highly mechanized DSR system. DSR involves rice stand establishment directly by sowing seeds in the fields and uses less water, labor, and emits less methane than the PTR system (Chauhan, 2012). Even though DSR has numerous advantages over the PTR, sustainability of DSR with reference to weed control is questionable. Weeds are a major yield reducing factor in DSR and can cause yield reduction up to 100% under cases of severe infestation (Figure 1- Rao et al., 2007).

Wide adoption of DSR has also raised concerns on weed adaptation and ecology, with higher weed diversity observed in DSR than PTR system (Tomita et al., 2003). Furthermore, there has been an increase in difficult to control weeds such as grasses and sedges under DSR. For example, in Malaysia, broadleaf weeds were most prevalent in rice fields during 1970, when DSR was recently introduced, but by 1990 grass weeds like barnyardgrass (*Echinochloa crus-galli*) and red sprangletop (*Leptochloa chinensis*) turned out to be dominant (Azmi et al., 2003).

Use of herbicides to control weeds in DSR is cost effective and uses less labor, however with the evolution of herbicide resistant weeds the effectiveness of herbicides alone for weed management has become ineffective. In rice, the first case of herbicide resistant weed was reported in *Sphenoclea zeylanica* (gooseweed) in Philippines, in 1983 (Heap, 2018). In the US, *Echinochloa crus-galli var crusgalli* (barnyardgrass) was the first weed reported to be herbicide resistant in 1990, with 50 different weed species documented to have developed resistance to numerous herbicide modes of action in rice currently (Heap, 2018). In such a case, alternative weed control need to be considered for successful, sustainable, and cost effective DSR production. One promising weed control option is to use the weed-suppressive ability already present in crop varieties, known as allelopathy. The word allelopathy was coined by Austrian plant Physiologist, Hans Molisch, who is sometimes referred to as father of allelopathy (Willis, 2007). Allelopathy, in general is defined as the harmful or beneficial effect of chemical(s) secreted by one organism on organisms present in the surrounding environment. Putnam and Duke (1974), came up with the concept of integrating allelopathic crop to increase their competitive ability against weeds in the agroecosystem. They evaluated the allelopathic potential of cucumber species

from 41 nations and reported that one accession of cucumber suppressed weed growth by 85%, while 25 accessions inhibited growth of indicator species by 50%. Numerous field and greenhouse screenings has proven that rice plant and their residues can produce allelochemicals having the potential to reduce weeds in rice fields (Amb, 2016). Rice root exudates, straw, and hull, are shown to have significant allelopathic potential and can potentially be used as sustainable weed management strategy in rice (Dilday et al., 2001; Ma et al., 2014). Use of allelopathy has evolved as an alternative option to control weeds in rice culture system and has gained popularity since the late 1980's (Olofsdotter, 1998). Even though allelopathy in rice has been evaluated, allelopathic rice cultivars are not popular among farmers. This is primarily because less information is available among farmers about these cultivars and/or they have inconsistent/lower yields compared to other commercial rice cultivars. Thus, developing rice cultivars with high allelopathic potential and stable yield and creating awareness among the farmers about these cultivars will be a real asset to rice production system worldwide.

Scrutinizing wild relative of crops for genetic diversity is a common strategy in crop improvement programs (Bessey C. E, 1906). Weedy rice, belonging to the same genus and species as the cultivated rice has wide genetic and morphological diversity (Shivrain et al., 2010a; Shivrain et al., 2010b). Studies have shown that weedy rice is more competitive than cultivated rice. For instance, weedy rice grows taller than cultivated rice, has high levels of shattering, and variable dormancy (Noldin et al., 1999). Patone & Baker (1991), through the weed- competition model, suggested that weedy rice was more dominant than cultivated rice in the field and that the competitive ability of one weedy rice plant was equivalent to that of three plants of commercial rice variety "Mars". Weedy rice

also has higher nitrogen use efficiency than cultivated rice (Burgos et al., 2006). Nitrogen applied at the rate of 20 g m⁻² produced more culm biomass in weedy rice than the rice cultivar “Mars”. Since weedy rice is conspecific but more competitive and aggressive than cultivated rice it is possible that weedy rice may have higher weed suppressive potential than cultivated rice. Finding out potential germplasm of weedy rice having high weed suppressive potential will be helpful in diversifying the rice gene pool and rice breeding program as both (rice and weedy rice) are closely related. Thus, the objective of the study was, (i) To evaluate the weed suppressive potential of diverse weedy rice germplasm, and (ii) Characterize weedy rice accessions morphologically based on their allelopathic potential.

3.3. Materials and Methods

Bioassay in the Greenhouse

Greenhouse experiments were conducted in 2016-17 at RR Foil Plant Science Research Center at Mississippi State University to evaluate the allelopathic potential of 54 weedy rice accessions against *E. crus-galli* and *L. panicoides*. From our previous weedy rice morphological characterization study using 208 weedy rice accessions (Tseng, 2013), a total of 54 competitive accessions were selected based on 10 traits, namely, ALS-inhibiting herbicide tolerance (<10% injury), cold tolerance (<10% injury), early flowering (<85 days after seeding), culm height (120-140 cm), high grain yield (140-160 g/plant), high leaf area (50-75 cm²), high tillering capacity (culm number 110-130), lodging resistance (culm strength moderately-strong to strong), high panicle shattering (>50%), and high spikelet fertility (>75% well-developed spikelets in proportion to total number of spikelets on 5 panicles). Two rice cultivars (CL 163 and Rex), and 3 allelopathic rice

cultivars/lines (Rondo, PI 338046, and PI 312777) (Gealy & Yan, 2012) were included in bioassay screenings together with weedy rice accessions to serve a comparison.

Seeds of weedy rice, allelopathic and non-allelopathic rice cultivars, were pre-germinated in 0.3 % agar media to obtain uniform plant stand. Pots of 8.5-inch diameter were filled with Sunshine #1 (Sun Gro Horticulture Canada Ltd, Vancouver, Canada), an all-purpose growing mix with sphagnum peat moss, perlite, dolomitic limestone, long lasting wetting agent and RESiLIENCE®. Weed suppressive potential was evaluated using the method described by Li et al., (2015) with slight modification. Briefly, rice plants were planted at equal spacing (~2.5 cm) from each other in corner of each pot. When rice plants reached 5-leaf stage, 3 pre-germinated seedling of *E. crus-galli* were planted in circle at the center of the pot to evaluate interference effects of weedy rice on *E. crus-galli* (Fig 3.1). Height of *E. crus-galli* was recorded at 7, 14 and 21 days after planting (DAP). In preliminary experiment it was observed that the roots of weedy rice and test species (*E. crus-galli*, *L. panicoides*) in separate pots, do not overlap with each other until 3 weeks after growing together in 8.5 in pots when arranged in the same manner as indicated above, which is why data were recorded only up to 21 DAP. This will also eliminate the possibility of any interaction between allelopathy and competition. Optimum light and water conditions were maintained to minimize competition as much as possible. Greenhouse temperature and humidity were maintained at 25°C and 70% respectively. As the activity of allelochemicals is concentration dependent (Khang et al., 2016), 1000 ml of water was added to each tray every alternate day in which pots were placed. *E. crus-galli* grown without rice were considered as control. Each treatment was replicated three times and experiment was conducted twice (Run 1 and 2). *E. crus-galli* was uprooted carefully at 21

DAP keeping the roots intact. Roots and shoots were separated using a sharp scissor, soil particles were removed from the roots gently using a brush, and dry mass was recorded after drying in oven for 78 hours. The same experimental setup was used for *L. panicoides*. Inhibition on the growth of weeds was calculated as inhibition in height, biomass and rootlength.

$$\text{Inhibition (\%)} = \frac{\text{Value of control plant} - \text{value of treated plant}}{\text{Value of control plant}} \times 100$$

Statistical Analysis

In all experiments, accessions were considered as fixed effect while replications and runs as random effects. The experimental design was completely randomized with 3 replications and 2 runs. Data were analyzed using general linear model PROC GLM of SAS 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA) and mean values were separated using Fisher's protected LSD at or below 0.05 probability level. Principle component analysis of JMP 13 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA) was used to visualize correlation among the original variables and between the variables and components.

3.4. Results

Interference potential of weedy rice was measured in terms of ability of plants to reduce height, above ground biomass and root length of weed species when grown in proximity. Average interference potential was calculated taking means of percent reduction of height, biomass and root length. No interaction was observed between two runs so data from both runs were pooled for analysis.

Weed suppressive potential of weedy rice accessions against *E. crus-galli*

There was difference among the weedy rice accessions in terms of weed suppressive potential ($p < 0.0001$) against *E. crus-galli*. The interference potential of weedy rice accession B2 (61%) against *E. crus-galli* was higher than all 3 weed suppressive rice cultivars (PI312777, PI338046 and RONDO). The average height of *E. crus-galli* grown in monoculture, was 26 cm. The average height of *E. crus-galli* planted with test accessions ranged from 20.7 to 10.3 cm. Reduction in height of *E. crus-galli* by known allelopathic rice cultivars was, ranging from 50 to 52%. The weedy rice accession B81 had similar weed suppressive potential as the allelopathic rice cultivars. Rice cultivars CL163 and REX were least inhibitory on *E. crus-galli*, causing height reductions of only 21% and 25%, respectively. Among weedy rice, S33, a strawhull accession, effected the least height reduction (22%) of *E. crus-galli*, like CL163 and REX (Table 3.1).

The average shoot biomass of monoculture *E. crus-galli* in this study was 0.29 g whereas that of weedy rice accessions ranged from 0.10 to 0.24 g. The weedy rice accession B2 reduced *E. crus-galli* biomass by 65%, and caused higher biomass reduction than all the accessions. The allelopathic rice cultivars and weedy rice accession B81 reduced the biomass of *E. crus-galli* by 57, 56, 54 and 59% respectively. Weedy rice accession S33, caused the least *E. crus-galli* biomass reduction (17%), while CL163 and REX reduced *E. crus-galli* biomass by 21 and 23%, respectively (Table 3.1).

Average root length of *E. crus-galli* planted as control was 22 cm, and that of *E. crus-galli* planted with test accession ranged from 19 to 8 cm. In terms of percent root length reduction of *E. crus-galli*, the allelopathic rice cultivar and 2 weedy rice accessions B2 and B81, were found to be similar with reductions of 63, 53, 52, 58 and 52%,

respectively. Rice cultivars CL163 and REX had root length reductions of 14 and 17% respectively, which was lower than most of weedy rice accessions. Among weedy rice accessions, S113 reduced *E. crus-galli* root length least by 12% (Table 3.1).

Unlike the three weed-suppressive rice cultivars, the commercial rice cultivars, CL163 and REX, had lower weed-suppressive potential than weedy rice. Two weedy rice accessions (B2 and B81) caused more than 50% inhibition of growth of *E. crus-galli*. The yield of weedy rice accessions ranged from 51 g/plant for B14, B15 and B18 to 158 g/plant for B8, B83, B84, B85, B86, B87, B88, B80 and B81. The yield of most suppressive weedy rice (B2) was 130 gm/plant, which was higher than 51% of the weedy rice accessions tested.

Principal component analysis based on height, biomass and root length inhibition revealed 72% of variation in weed suppressive potential occurred due to component 1 and 16% of the variation was due to component 2. All parameters used for measuring allelopathy (height, shoot biomass and root length reduction) were found to be positively correlated to component 1. Most of the allelopathic rice and some of blackhull weedy rice accessions clustered together in the PCA indicating high weed suppressive ability of accessions in this cluster (Fig 3.2).

Interference potential against *E. crus-galli* based on hull type

Weedy rice accessions used in this study represented 3 hull types namely, brownhull, blackhull and strawhull. Average inhibition of *E. crus-galli* by brownhull, blackhull and strawhull accessions were 41, 36 and 32%, respectively. Each hull type had different weed-suppressive potential on average. Percentage inhibition in height 3WAT by blackhull, brownhull and strawhull accessions were 36, 41 and 34% respectively.

Reduction in biomass of *E. crus-galli* was 39, 41 and 34%, respectively by brownhull, blackhull and strawhull accessions. *E. crus-galli* root length was inhibited 43, 33 and 28% by brownhull, blackhull and strawhull accessions, respectively (Table 3.3).

Interference potential of the accessions against *L. panicoides*

Among the weedy rice, B81 caused the highest overall inhibition (52%) of *L. panicoides* (Table 3.2). Height reduction of *L. panicoides* by B81 was 59%. The allelopathic rice cultivars PI312777, PI338046 and RONDO reduced the height by 56, 57 and 47 % respectively. Non-allelopathic rice cultivars caused the least height reduction of *L. panicoides* by 16 and 13%, respectively. Among weedy rice, S18 inhibited shoot growth of *L. panicoides* the least (13%).

Average biomass of *L. panicoides* grown with test accessions ranged from 0.33 to 0.22 g. Weedy rice accession B81 reduced height of *L. panicoides* the most (42%), and was similar to height reduction shown by allelopathic rice cultivar PI338046 (38%). PI31277 and RONDO reduced the biomass by 33 and 28%, respectively; while rice cultivars, CL163 and REX, reduced the biomass by 13 and 14%, respectively. S124, showed least reduction of *L. panicoides* biomass (12%) (Table 3.2).

Average root length of *L. panicoides* grown as control was 30 cm, while root length grown with test accessions ranged from 8 to 19 cm. Highest reduction in root length of *L. panicoides* was caused by PI338046 (63%) and was similar to root length reduction by B2, PI312777, B81 and RONDO. Rice cultivars, CL163 and REX, caused least reduction in root length (14 and 17%, respectively). It was worth noting that B81 had highest inhibition in all three factors, thus indicating its consistent weed suppressive potential and superior nature than other accessions used in the experiment. B81 was the only accession showing

an average weed suppressive potential of more than 50%; majority of accessions (57%) showed inhibition of less than 30% while the rest of the accessions (41%) had inhibition ranging 30-40%. Weedy rice accession B81 with high allelopathic potential was also among the weedy rice with highest yield (Table 3.2).

Principal component analysis based on percentage height, biomass and root length inhibition was similar to that of the PCA for *E. crus-galli* growth inhibition. Component 1 represented 85% of the variation while component 2 represented 9% of variation. The three factors were positively correlated with the component 1. All allelopathic rice and most blackhull accessions clustered together suggesting high allelopathic potential of blackhull weedy rice accessions (Fig 3.3).

Weed suppressive potential against *L. panicoides* based on hull type

The average inhibition of *L. panicoides* by blackhull, brownhull and strawhull accessions were 33, 20 and 22%, respectively. Blackhull accessions had higher weed suppressive potential against *L. panicoides* than brownhull and strawhull accessions. Suppression in height, biomass and root length by blackhull accessions were 38, 24 and 38%, respectively. Brownhull accessions suppressed *L. panicoides* height 21%, shoot biomass 16% and root length 22%. Strawhull accessions suppressed height, biomass and root length by 20, 16 and 28% respectively (Table 3.4).

3.5. Discussion

In agriculture, the concept of utilizing crops for weed suppression was first investigated by Putnam and Duke in 1974. Numerous crops are found to have allelopathic effect on weeds and these can be utilized as natural herbicides (Khanh et al., 2005). Since

the outset of rice allelopathic research by Dilday in 1994, allelopathy in rice has gained high popularity as a means of sustainable weed control option in rice. At present, PI312777, PI330846 and RONDO are the proven weed suppressive rice cultivars (Gealy et al. 2003; Gealy & Yan 2012), however weed suppressive potential of weedy rice has not been evaluated. Allelopathy in rice cultivars varies depending on cultivar and origin (Khanh et al., 2005; Chung et al., 2003). Our data showed this variability in terms of suppressing the growth of major rice weeds *E. crus-galli* and *L. panicoides*. Thus, interference potential in weedy rice is accession dependent as in cultivated rice. The genetic characteristic of rice varieties affects the allelopathic potential and comparatively *indica* rice has lower allelopathic potential than the *japonica* rice (Khanh et al., 2007). As weedy rice in the USA has high genetic variability (Shivrain et al., 2010b), it is possible that some of the potential weed suppressive accessions identified in the study, such as B2 and B81, have novel genes than the cultivated rice coding for production of allelochemicals to suppress weed species. Some rice cultivars can suppress growth of multiple weed species. For example, in the study by Dilday et al., (1994), rice accessions B850/Cros 1-7-18-3-2, Johna 349, and Mahlar 346, were found to suppress growth of both ducksalad and redstem by 70 to 85%. Similarly, weedy rice accession B81, suppressed height, biomass and root length of *E. crus-galli* and *L. panicoides* at a higher level than most weedy rice accessions and the allelopathic rice. To the best of our knowledge, no rice cultivar has been identified that can suppress the growth of both *E. crus-galli* and *L. panicoides*, the two most problematic weeds in rice production system in Arkansas and Mississippi (Norsworthy et al., 2013). Thus, B81 with the ability to suppress both weeds can serve as an important resource for crop improvement program. It is a common practice in crop breeding programs to select

for biotic and abiotic stress tolerance from wild lines. Wild relatives of wheat have been used as an important resource for developing disease resistant wheat lines (Fedak, 1999). Thus, this unique ability of B81 to suppress *E. crus-galli* and *L. panicoides* can be bred into cultivated rice to provide competitive advantage to crops. Furthermore, with the advancement of molecular biology, genetic modification of crops conferring tolerance to various biotic and abiotic stress has become common practice. Genes responsible for production of allelochemicals in rice have been identified. For example, 4-copalyl-diphosphate synthetase and kaurene synthase-like 4 are responsible for synthesis of momilactone-B which has high activity against weeds (Xu et al., 2012). In such a scenario, exploring the potential weed suppressive accessions identified in the study at the molecular level to identify genes associated with allelopathy in these accessions would provide new insight to molecular assisted breeding programs. These accessions, even though can be a good resource for crop improvement, may also have the potential to suppress cultivated rice through release of secondary metabolites, thus leading to significant yield loss in rice fields through both competition for resources and allelopathic activity of. It may thus be essential to evaluate the allelopathic activity of weedy rice against cultivated rice in the future.

Most weedy rice accessions were not highly inhibitory. For instance, only 2 out of 54 accessions inhibited growth of *E. crus-galli* by more than 50%, and only 1 out of 54 accessions inhibited growth of *L. panicoides* by more than 50%. These accessions might have developed high allelopathic potential through selection pressure to outcompete surrounding plants. The sturdy genes in these unique accessions needs to be studied and identified to get an insight on their competitive nature. Rice cultivars, CL163 and REX,

were found to have lower weed suppressive potential than most weedy rice accessions thus affirming strong nature of weedy rice and their usefulness in rice improvement study. In the present study, phenotypic characteristics of weedy rice, hull type, was found to be associated with interference potential. Weedy rice accessions differed significantly in allelopathic potential with respect to hull type (Table 3.3, 3.4). Weed suppressive potential of rice cultivars are influenced by genetic and phenotypic characters (flowering, maturity, plant parts- leaves, straw, hull) (Chung et al., 2003; Ahn et al., 2005), thus indicating that a similar mechanism(s) may be responsible for allelopathy/weed suppressive potential in rice and weedy rice.

Rice cultivars with high allelopathy will help in natural control of weeds thus reducing the use of herbicides, enhancing sustainability, and at the same time provide effective control of herbicide resistant weeds. Because of the close relationship between weedy and cultivated rice, the weed suppressive potential of accessions such as B2 and B81 can be transferred into cultivated rice without much genetic barrier. Furthermore, the morphological characteristics like hull type can be used as phenotypic markers for choosing allelopathic weedy rice accessions.

3.6. Conclusion

Certain weedy rice accessions with higher weed suppressive potential than proven allelopathic rice cultivar were identified. However, the mechanism behind their high allelopathic potential is unknown. In the future, it is essential to study the chemicals responsible for allelopathy in these accessions. Furthermore, investigating the physiology and mechanism of allelopathy in these accessions will help generate tools for rice breeding program.

Table 3.1 Inhibition in height, biomass and root length of barnyardgrass by weedy rice, cultivated rice and allelopathic rice accessions.

SN	Accession	Percent Inhibition			Average
		Height	Biomass	Root Length	
1	ALR-1	37.2	33.0	31.8	34.0
2	ALR-4	40.6	40.6	42.6	41.3
3	B14	40.6	43.7	36.9	40.4
4	B15	30.0	37.9	38.5	35.5
5	B18	32.1	34.1	27.2	31.1
6	B2	60.6	64.8	58.5	61.3
7	B20	33.0	38.7	28.7	33.5
8	B21	37.2	37.9	22.1	32.4
9	B3	41.9	41.0	30.8	37.9
10	B30	32.6	46.0	35.4	38.0
11	B32	40.6	42.1	20.5	34.4
12	B34	30.0	28.7	23.1	27.3
13	B37	30.0	31.0	31.8	31.0
14	B38	27.5	38.3	35.4	33.7
15	B43	41.9	39.8	40.5	40.8
16	B44	34.3	24.9	32.8	30.7
17	B45	28.3	37.9	30.3	32.2
18	B49	36.4	34.1	34.4	35.0
19	B5	40.2	36.4	21.0	32.5
20	B51	32.6	32.2	30.8	31.8
21	B60	35.5	37.9	37.4	37.0
22	B75	34.7	37.2	39.5	37.1
23	B8	27.1	32.2	25.1	28.1
24	B80	33.0	36.8	39.0	36.3
25	B81	51.7	58.6	52.3	54.2
26	B83	47.0	45.6	38.5	43.7
27	B84	38.5	47.9	41.0	42.5
28	B85	33.8	32.2	31.3	32.4
29	B86	29.2	35.6	24.1	29.6
30	B87	32.6	35.2	30.3	32.7
31	B88	29.6	35.6	25.7	30.3
32	CL163	20.7	20.7	14.4	18.6
33	PI312777	52.1	57.1	52.8	54.0
34	PI338046	51.2	55.9	62.6	56.6
35	REX	24.9	23.0	16.9	21.6

Table 3.1 (continued)

36	RONDO	50.4	53.9	52.1	52.1
37	S105	29.6	31.4	29.2	30.1
38	S108	41.1	38.3	35.9	38.4
39	S109	36.8	33.3	29.8	33.3
40	S11	30.9	34.5	26.2	30.5
41	S110	40.2	36.0	28.2	34.8
42	S113	25.4	29.5	11.8	22.2
43	S118	36.8	42.1	33.3	37.4
44	S124	33.0	33.7	31.8	32.8
45	S14	34.3	38.3	32.8	35.1
46	S18	40.6	40.6	34.9	38.7
47	S21	29.6	38.3	31.3	33.1
48	S29	40.6	44.1	27.2	37.3
49	S33	22.4	17.2	15.4	18.3
50	S42	41.1	42.5	31.3	38.3
51	S46	31.7	40.6	37.4	36.6
52	S5	39.8	29.9	19.0	29.6
53	S59	36.0	42.5	36.4	38.3
54	S6	29.2	25.3	28.2	27.6
55	S65	36.0	39.1	31.8	35.6
56	S84	37.7	30.7	22.6	30.3
57	S9	35.1	29.5	21.0	28.6
58	S94	27.5	23.4	16.9	22.6
59	S97	30.9	33.7	17.4	27.3

Table 3.2 Inhibition in height, shoot biomass and root length of amazon sprangletop by weedy rice, cultivated rice and allelopathic rice accessions.

SN	Accessions	Height	Percent Inhibition		Average
			Biomass	Root length	
1	ALR-1	17.0	14.4	20.1	17.2
2	ALR-4	20.6	16.4	22.3	19.8
3	B14	44.7	26.4	37.9	36.3
4	B15	36.2	22.0	39.0	32.4
5	B18	34.8	21.4	37.2	31.1
6	B2	41.9	24.7	33.1	33.2
7	B20	43.3	23.8	33.5	33.5
8	B21	44.7	26.1	40.9	37.2
9	B3	40.4	23.2	37.2	33.6
10	B30	34.1	21.1	34.2	29.8
11	B32	43.3	25.2	40.2	36.2
12	B34	39.7	25.6	43.5	36.3
13	B37	39.0	25.5	39.8	34.8
14	B38	43.3	27.3	42.0	37.5
15	B43	31.9	24.9	32.7	29.9
16	B44	37.6	22.9	37.2	32.6
17	B45	27.7	21.1	32.7	27.2
18	B49	24.8	18.2	27.5	23.5
19	B5	38.3	24.4	37.5	33.4
20	B51	44.7	25.2	37.2	35.7
21	B60	19.9	16.2	30.1	22.0
22	B75	45.4	25.8	40.5	37.3
23	B8	39.0	24.9	40.2	34.7
24	B80	46.8	26.7	41.6	38.4
25	B81	58.9	42.0	55.8	52.2
26	B83	44.7	27.3	44.2	38.7
27	B84	33.3	20.8	41.6	31.9
28	B85	33.3	20.3	34.2	29.3
29	B86	40.4	24.4	36.8	33.9
30	B87	29.1	22.6	32.7	28.1
31	B88	34.8	21.4	35.7	30.6
32	CL163	16.3	13.2	26.0	18.5
33	PI312777	56.7	33.2	49.8	46.6
34	PI338046	56.0	38.1	50.2	48.1
35	REX	13.5	14.4	24.2	17.4

Table 3.2 (continued)

36	RONDO	47.4	28.4	43.1	39.6
37	S105	18.5	16.2	27.1	20.6
38	S108	24.1	15.9	29.0	23.0
39	S109	20.6	15.9	27.5	21.3
40	S11	22.0	17.3	27.5	22.3
41	S110	20.6	13.5	27.9	20.7
42	S113	19.9	15.9	28.3	21.3
43	S118	18.5	14.1	27.9	20.1
44	S124	18.5	11.8	26.8	19.0
45	S14	18.5	17.9	30.1	22.2
46	S18	13.5	13.8	20.4	15.9
47	S21	21.3	17.6	31.2	23.4
48	S29	22.7	16.7	27.9	22.4
49	S33	18.5	16.7	27.5	20.9
50	S42	24.1	18.2	28.6	23.7
51	S46	19.2	16.7	27.9	21.3
52	S5	17.0	12.3	26.8	18.7
53	S59	25.5	20.0	32.3	26.0
54	S6	19.9	17.6	29.0	22.2
55	S65	24.8	18.2	29.7	24.3
56	S84	35.5	21.4	35.3	30.7
57	S9	14.9	15.0	27.9	19.3
58	S94	15.6	14.1	28.6	19.4
59	S97	21.3	17.9	27.5	22.2

Table 3.3 Inhibition of barnyardgrass by weedy rice accessions based on morphological character, hull color.

SN	Character	Height	Percent Inhibition		
			Biomass	Root length	Average
1	Hull color				
	Blackhull	36a	39a	33b	36b
	Brownhull	41a	41ab	42.5a	41a
	Strawhull	34b	34b	27.5c	32c

Table 3.4 Inhibition of amazon sprangletop by weedy rice accessions based on morphological characters, hull color.

SN	Character	Height	Percent Inhibition		Average
			Biomass	Rootlength	
1	Hull color				
	Blackhull	38a	24a	38a	33a
	Brownhull	21b	16b	22c	20b
	Strawhull	20b	16b	28b	22b

Means in the column followed by the same letters are not significantly different from each other based on the means separated by student's t test at 0.05 α

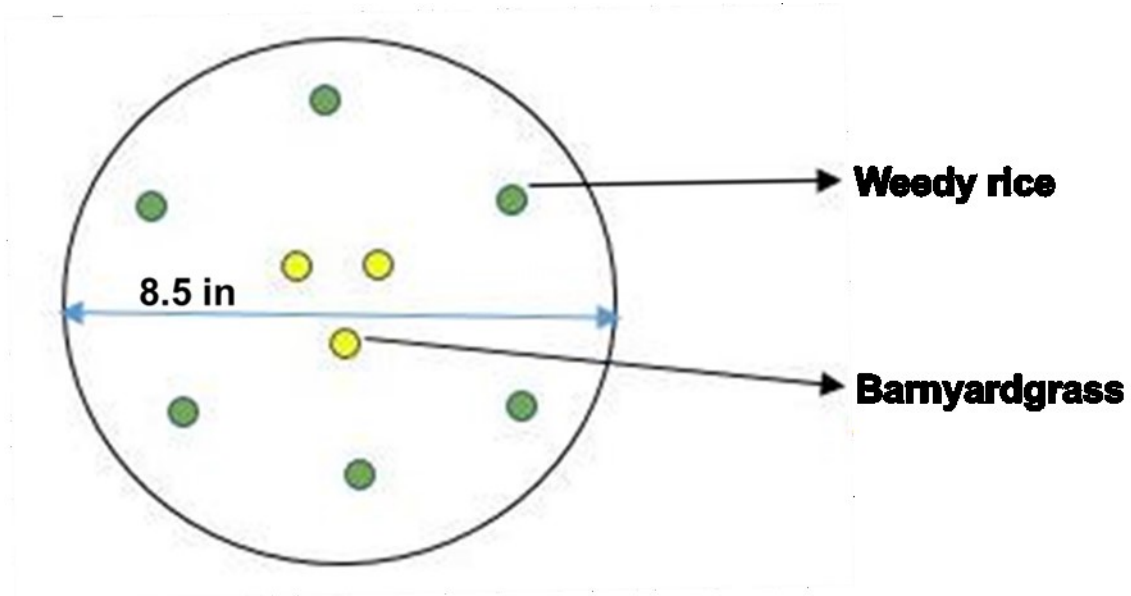


Figure 3.1 Diagrammatic representation of experimental setup for evaluating weed suppressive potential of weedy rice against barnyardgrass.

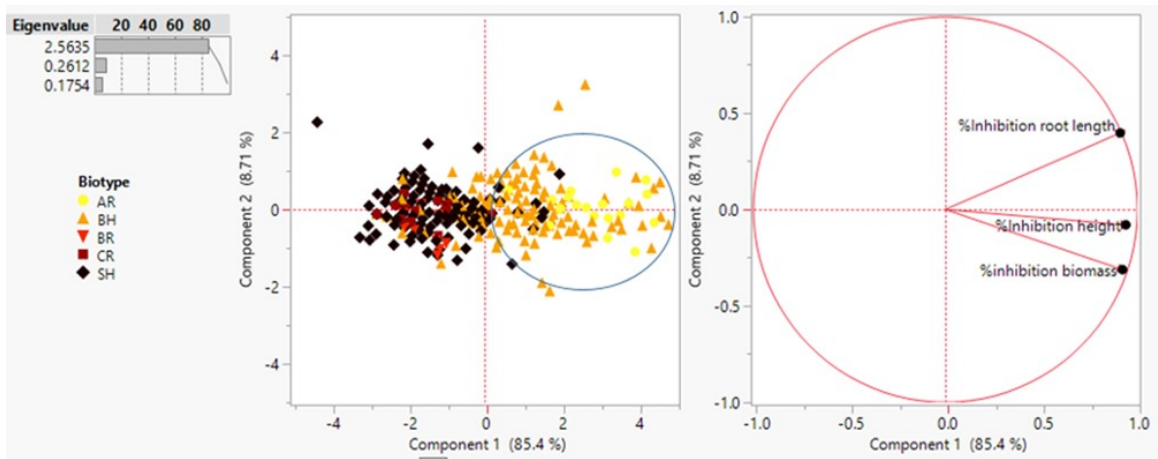


Figure 3.2 Principle component analysis with inhibition in height, shoot biomass and root length, 3 weeks after transplanting of *E. crus-galli* by allelopathic rice (AR), blackhull weedy rice (BH), brownhull weedy rice (BR), strawhull weedy rice (SH) and cultivated rice (CR).

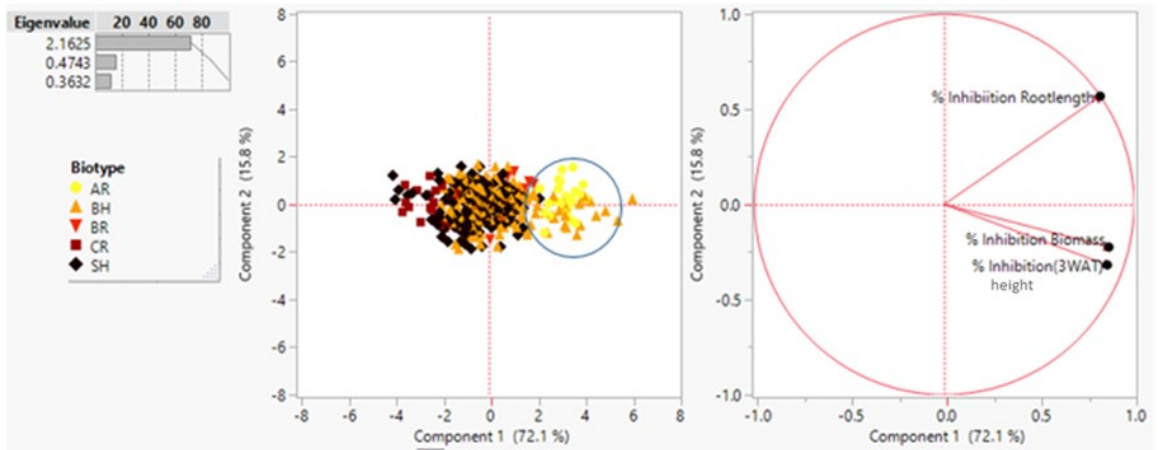


Figure 3.3 Principal component analysis of three different parameters used for evaluating allelopathic potential of allelopathic rice (AR), blackhull weedy rice (BH), brownhull weedy rice (BR), strawhull weedy rice (SH) and cultivated rice (CR) against *L. panicoides*.

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CHAPTER IV
EVALUATING GENETIC DIVERSITY AMONG WEEDY RICE ACCESSIONS
USING SSR MARKERS

4.1. Abstract

Increasing agricultural productivity is indispensable to meet future food demand. Crop improvement programs rely heavily on genetic diversity. Success of weeds in the ecosystem can be attributed to genetic diversity and plasticity. Diverse variation in weedy rice morphology and phenology is present which implies wide genetic diversity. Studies were conducted to evaluate the genetic diversity among weedy rice, cultivated rice (CL163 and REX) and allelopathic rice (RONDO, PI312777, PI338047) using 30 SSR markers. Nei's genetic diversity among weedy rice (0.45) was found to be higher than cultivated rice (0.24) but less than allelopathic rice (0.56). Genetic relationship and population structure based on herbicide tolerance and allelopathic potential were evaluated. Herbicide tolerant and susceptible accessions formed distinct clusters in the dendrogram indicating their genetic variation whereas no distinction was observed between allelopathic and non-allelopathic weedy rice accessions. Accession B2, which had high weed suppressive potential; and herbicide tolerance was found to be genetically distinct than other weedy rice accessions. This information will be helpful for marker assisted breeding in the future,
Key words: crop-improvement; genetic diversity

4.2. Introduction

Commercial rice production in the US started in 1650's and extended towards South America in the eighteenth century (Singh et al. 2017). According to Allston (1846), weedy rice was introduced as a contaminant from Asia in 1846 and since then has been affecting US rice production. Weedy rice belongs to the same genus and species as cultivated rice (Hoagland and Paul 1978) thus limiting chemical control, as both rice and weedy rice are susceptible to same herbicides. Weedy rice can be controlled in fields by rotation with soybean, sorghum, maize, and other cultural practices such as winter flooding and fallow tillage (Burgos et al. 2008; Burgos et al. 2011). However, because of the popularity of rice monoculture among farmers in rice growing areas, infestation of weedy rice is becoming more severe year after year. Further, with widespread adoption of Clearfield™ rice, gene flow among weedy rice and herbicide tolerant Clearfield rice has been reported thus complicating weedy rice management (Shivrain et al. 2009; Gealy et al. 2005). Weedy rice has evolved as one of the most difficult to control weeds in the countries where rice is established through DSR system (Ziska et al. 2015).

Weedy rice infesting rice fields are genetically and morphologically diverse (Shivrain et al. 2010a; Shivrain et al. 2010b); plants of diverse hullcolor (strawhull, blackhull, brownhull, goldhull,grayhull), awn length, variable flag leaf length and different maturity period are present (Constantin 1960; Noldin et al. 1999; Do Lago 1983). Height of weedy rice found in Arkansas varies from 46-190 cm, with blackhull being taller than strawhull (Shivrain et al. 2010b). Weedy rice germination varies from 34-84% among accessions, and possess high shattering and variable dormancy (Burgos et al. 2006, Cohn and Hughes 1981). These morphological variations in weedy rice can be attributed to

genetic variation. For example, Shivrain et al. (2010a) reported that blackhull, brownhull and strawhull weedy rice accessions are genetically diverse from each other with Nei's genetic diversity of 0.7 and this genetic variation is higher than in cultivated rice. Cao et al. (2006) reported that weedy rice from China has high genetic diversity with a heterozygosity of 0.313, and Shannon's diversity index of 0.572. Mechanism behind the origin of weedy rice, which are (i) hybridization between cultivated rice and wild rice (*O. rufipogon*) (ii) natural hybridization among weedy rice and cultivated rice, and (iii) hybridization within weedy rice populations, may be the cause of the diverse genetic makeup of weedy rice (Londo and Schaal 2007).

High genetic diversity in weedy rice can be used in favor of rice in crop improvement programs by selecting weedy accessions with favorable traits such as herbicide tolerance, allelopathic potential, and disease resistance. Twenty-eight blast resistant quantitative trait loci (QTL) were identified in two weedy rice accessions by Liu et al. (2015), which can be used in rice breeding programs for development of blast tolerant rice cultivars. Ziska and Mc. Clung (2008) reported higher biomass (55%) and leaf area (62%) in weedy rice compared to cultivated rice under elevated carbon dioxide condition indicating the ability of weedy rice to survive efficiently in response to climate change and global warming. Weedy rice also has higher response to nitrogen fertilizer than the cultivated rice, where a continuous increase in weedy rice biomass was observed with addition of nitrogen fertilizer 2 weeks after heading; however, cultivated rice did not respond as efficiently (Burgos et al. 2006). Thus, exploring weedy rice at the molecular level to identify QTL's associated with competitive traits will be vital for rice breeding programs by increasing competitive potential of the crop.

Shrestha et al. (2017) found that 3 of the fifty-four weedy rice accessions collected from Arkansas, USA had higher tolerance to herbicides (< 40% injury) than cultivated rice (97-100% injury). Likewise, 2 weedy rice accessions had greater than 50% allelopathic potential against barnyardgrass and amazon sprangletop, which was substantially higher than the allelopathic rice cultivars RONDO, PI312777 and PI338046. Understanding the genetic basis of herbicide tolerance and allelopathic potential in weedy rice accessions would provide insight on the QTL and genes associated with these competitive traits. Associating the competitive traits with molecular markers will aid in traditional breeding programs through marker assisted selection for the development of competitive rice cultivars.

Numerous markers can be used for evaluating genetic diversity and population structure within a plant species (Parker et al. 1998). First markers used for analyzing genetic polymorphism were Restriction Fragment Length Polymorphism (RFLP) using restriction enzymes for cutting DNA at specific sites creating fragments of varying length. The fragments are then separated and identified by gel electrophoresis or southern blot hybridization (Parker et al. 1998). Random Amplified Polymorphic DNA (RAPD) uses numerous primers about 10 bp long to identify variation in genomes. Primers are chosen at random, hence no prior information of genomic DNA is required for carrying out RAPD. Polymerase Chain Reaction (PCR) is carried out on genes of interest using these short primers and amplified fragments are loaded in agarose gel for separation (Williams et al. 1995). Amplified Fragment Length Polymorphism (AFLP) uses RFLP and PCR method to identify the polymorphic DNA with higher reliability. Restriction enzymes are used to cleave the DNA, which are then ligated with specific adapter before PCR. The PCR

products are then separated in agarose gel for DNA finger printing (Chial H 2008). Inter-Simple Sequence Repeats (ISSR) is a PCR based technique, where nucleotide sequences between two Simple Sequence Repeats (SSR) are amplified using specific primers. Genome sequence information is not required for ISSR markers (Abdel-Mawgood AL 2012). Simple-Sequence Repeats (SSR) are short nucleotide sequences 1 to 6 bp long which repeat themselves at specific interval throughout the genome. These regions are highly polymorphic and effectively represent the genetic diversity among individuals. Simple Sequence Repeat (SSR) markers have numerous benefits over other markers because they are commercial and easy to use, analysis can be performed easily without radioactive materials, are codominant, and can be easily assayed by PCR (Powell et al. 1996; Guilford et al. 1997). These markers are commonly used in rice and weedy rice to determine genetic diversity (Lu et al. 2005; Liakat Ali et al. 2011; Shivrain et al. 2010b). Because of the ability of weedy rice to cross-pollinate among themselves and cultivated rice, there is high probability of genetic variability among different weedy rice accessions. Thus, objectives of this study were, (i) Evaluate genetic diversity among and within weedy rice, cultivated rice, and allelopathic rice populations. (ii) Associate herbicide tolerance and allelopathic potential in weedy rice with molecular markers.

4.3. Materials and Methods

Plant Material and Genomic DNA Extraction

Seeds of 54 different weedy rice accessions were obtained from Weed Physiology Lab at University of Arkansas, Fayetteville, AR. These were the 54 most competitive weedy rice accessions selected from a larger germplasm of 200 accessions collected in 2008-2009, from major rice producing states in Arkansas. Competitiveness was determined

based on morphological traits like high seed shattering, tall culm length, and high dormancy, which are the primary traits associated with aggressiveness of weedy rice in rice fields. Seeds of rice cultivars CL163 and REX were obtained from Rice Breeding Lab at Delta Research and Extension Centre, Stoneville, MS, and seeds of allelopathic rice was provided by Dr. David Gealy at the Dale Bumpers National Rice Research Centre in Stuttgart, AR.

For DNA extraction, fresh tissues were collected from 5-week old plants and stored at -80°C. Tissues were used for DNA extraction after 24 hours, using CTAB method with slight modifications (J. J Doyle 1987). Quality and quantity of DNA was determined using a Nanodrop 2000 spectrophotometer (Wilmington, US). Extracted DNA was stored at -20°C until PCR amplification.

Polymerase chain reaction (PCR) using simple sequence repeat (SSR) markers

DNA samples were diluted to 100 ng/μL before PCR. A total of 30 SSR primers from the standard panel of 50 developed by McCouch et al (2002) available publically in Gramene database (http://archive.gramene.org/markers/microsat/50_ssr.html) were used for analyze genetic diversity of the weedy rice accessions (Table 4.1). These markers can be used to evaluate genetic similarity and differences among *oryza* species with AA genome (Yang et al. 1994). PCR reactions were carried out in 96 well plates with 25 μl reaction volume. Reaction mixture consisted of 12.5 μl of PCR master mix (Taq polymerase, dATP, dGTP, dCTP, dTTP, MgCl₂), 1μl of forward primer, 2 μl of reverse primer, 1 μl of DNA, and 8.5 μl of nuclease free water. PCR profile used for DNA amplification was: denaturation at 94°C for 5 min, 35 cycles of 94°C for 1 min followed by annealing temperature from 55°C to 67°C which was marker dependent (Table 4.1), and

a final extension at 72°C for 5 min. PCR products were separated in 6% polyacrylamide gels for 45 Min at 180 volts, and stained with ethidium bromide. Stained gels were visualized under UV trans-illuminator and photographed.

Data Analysis

Individual bands were considered as co-dominant markers and scored using Cross Checker 2.91 (Buntjier 1999). Bands were scored as binary characters with 1 for presence of bands and 0 for absence of bands. POPGENE version 1.32 was used to obtain number of alleles per locus (A), percentage of polymorphic loci (P), Nei's gene diversity (h) and Shannon's index (I) using data matrix from Cross Checker. Nei's genetic distance was used to develop dendrogram with UPGMA algorithm to evaluate genetic relationship among accessions. STRUCTURE 2.3.4 was used to determine the population structure of the accessions using the genetic data generated by the microsatellite SSR markers (Pritchard et al. 2000). Data was run in STRUCTURE from K=1 to K=8 with three iterations for each K value, and burn-in period of 100,000 and 500,000 replications. Best fit value of K was obtained using Structure Harvester and Distruct (Rosenberg 2004) was used to generate a graphical display of population structure.

4.4. Results and Discussion

Genetic diversity among weedy rice, cultivated rice, and allelopathic rice cultivars used in the study

All 30 markers were polymorphic for all accessions used and number of alleles per locus ranged from 2-3 with an average of 2.9. Overall Nei's gene diversity (h) among weedy rice, cultivated rice, and allelopathic rice was 0.45, with lowest h (0.14) observed with M15, and M18 markers, and highest h (0.65) detected with M8 marker (Table 4.2).

Weedy rice accessions, cultivated rice, and allelopathic rice had h of 0.4, 0.24, and 0.56, respectively. Nei's gene diversity measures the heterozygosity within and between populations/individuals and its value ranges from 0 to 1. Higher the value greater the genetic diversity (Nei 1973). Comparable genetic diversity of 0.31 was observed in weedy rice population from China. High diversity among weedy rice accessions may be responsible for their extensive morphological variation and adaptation in wide range of environments (Cao et al. 2006). Ability of weedy rice to hybridize among themselves and with cultivated rice (Londo and Schaal 2007) may have resulted in diverse genetic characteristic among weedy rice. Cultivated rice (CL 163 and REX) showed low h (0.24) and was similar ($h=0.26$) to the 37 rice cultivars commonly grown in Arkansas, USA (Shivrain et al. 2010a). Both, REX and CL 163, are semi-dwarf rice cultivars released in 2014/15 and 2010, respectively, primarily for cultivating in the Southern United States (Ed Redona 2015; Solomon et al. 2012). As both cultivars are developed for cultivation in similar climatic condition, and are morphologically alike, lower genetic diversity among them was expected and results from our study supported the hypothesis. Nei's gene diversity among allelopathic rice was relatively high (0.56). Allelopathic rice used in this study, PI312777 and PI338046, originated from Phillipines, while Rondo is rice cultivar with Chinese origin (Yan and Mc. Clung 2010; Dilday et al. 1994). Rice cultivars from Asia have high genetic diversity (Lapitan et al. 2007) which might be the reason for high GD among allelopathic rice used in this study.

Mean Shannon Information Index (I) for the entire population was 0.74 and ranged from 1.08 to 0.02. Shannon's index is the uncertainty of predicting an individual, to belong to a population when randomly picked. Higher the Shannon's Index, greater the genetic

diversity in the population. Among the three groups, allelopathic rice had highest I of 0.85, and cultivated rice had the lowest I, with value of 0.38. Shannon's Information index for weedy rice was 0.66, indicating higher genetic diversity than southern rice cultivars, CL163 and REX (Table 4.2). Dendrogram based on Nei's genetic distance clustered weedy rice and rice cultivars (CL163 and REX) in one group, while allelopathic rice clustered separately (Fig 4.1). The genetic distance between weedy rice and rice cultivar was 0.13, and between weedy rice and allelopathic rice was 0.26. Weedy rice are well adapted to flourish in cultivated rice fields under human disturbances (Chauhan, 2013). Since weedy rice and cultivated rice are conspecific, gene flow from cultivated rice to weedy rice is common (Xia et al. 2011; Chen et al. 2004). The frequency of gene flow from rice cultivar (Minghui-63) to weedy rice accessions ranged from 0.011 to 0.046% (Chen et al. 2004). Although, weedy rice are self-pollinated, out crossing among weedy rice ranged from 0.4 to 11.7% (Xia et al. 2011). Rate of gene flow from crop to weed and vice versa is affected by morphological characters of the plant and environmental conditions (Levin and Kerster 1974). As weedy rice has high morphological variation the chances of gene flow between cultivated rice and weedy rice flowering at the same time is quite high. In such a case, genes from rice cultivars can be incorporated in weedy rice gene pool resulting in shorter genetic distance between weedy rice and cultivated rice.

Genetic relationship and differentiation based on herbicide tolerance

Based on the response of 54 weedy rice accessions to glyphosate and flumioxazin (Shrestha et al., 2018), herbicide tolerant and susceptible accessions were selected. Accessions that showed signs of recovery and with injury of less than 50% were considered herbicide tolerant, while accessions with more than 90% injury were considered as

herbicide susceptible. Cultivated rice (CL163 and REX) were found to be highly susceptible to both glyphosate and flumioxazin with almost 100% control 5 WAT, and were therefore considered as separate populations (susceptible rice cultivars). Overall Nei's gene diversity among all three populations was 0.47, varying from 0.12 to 0.66, and Shannon's information index was 0.78, varying from 0.2 to 1.09. Dendrogram based on Nei's genetic distance divided the population into 4 major clusters (Figure 4.2). Weedy rice accession ALR4, which was susceptible to both glyphosate and flumioxazin, clustered together with rice cultivars CL163 and REX, which were also highly susceptible to both herbicides indicating higher level of genetic similarity among these accessions. ALR-4 is a brownhull accession and studies by Shivrain et al. (2010a) showed that brownhull accessions share closer genetic background with cultivated rice, which may be the reason they clustered together in the dendrogram. Accession B2 with high tolerance to glyphosate did not grouped together with other herbicide tolerant accessions but instead formed a distinct cluster. Blackhull accessions have higher genetic diversity than strawhull accessions (Shivrain et al. 2010a; Tseng et al., 2013). Accession B2 was collected from Grand Prairie rice production zone in Arkansas. Weedy rice accessions from Grand Prairie are considered more dormant than from other locations such as White River, and Delta (Tseng et al. 2013); thus, due to higher level of seed dormancy it may have escaped herbicide treatments and persisted in rice fields. This may have allowed gene flow between cultivated rice and B2 accession thus leading to genetic changes in B2 with time. Late emerging weedy rice seedlings that grow simultaneously with cultivated rice have high potential of cross pollination and introgression, leading to genetic changes in the accession isolating it from other weedy rice population (Sun et al. 2013). B2, however, was most

closely related to cluster 3 which consisted of all glyphosate and flumioxazin tolerant accessions, thus suggesting accessions with herbicide tolerance trait being closely related to each other. Cluster 2 consisted of all herbicide susceptible accessions indicating the genetic similarity of these population. Individuals belonging to the same clusters were more genetically related and with lesser genetic distance, than individuals belonging to different clusters. Grouping of accessions was primarily associated with herbicide tolerance and all herbicide tolerant and susceptible accessions grouped separately indicating diverse genetic background of tolerant and susceptible accessions. Results from the STRUCTURE analysis correlated with the PopGene data and divided the herbicide tolerant and susceptible accessions into K=4 clusters, again inferring distinct clustering of accessions based on herbicide tolerance. Both, blackhull and strawhull herbicide tolerant accessions, showed similar coloring pattern in the figure obtained from Distruct (Figure 4.4) indicating close genetic relationship among weedy rice accessions in terms of herbicide tolerance irrespective of hull color. Likewise, both herbicide susceptible blackhull and strawhull accessions showed similarity in their genetic makeup (Figure 4.2 and 4.4) indicating differential tolerance to herbicide not being associated with hull color genetically. Herbicide resistant weeds that originated after the commercialization of Clearfield rice are not separated genetically based on hull color, however historical weedy rice forms distinct clusters for blackhull and strawhull populations (Burgos et al. 2014). Results from our study supported this finding as both herbicide tolerant hull types did not distinctly group into separate clusters representing their similar genetic background.

Genetic diversity among the accessions with respect to allelopathic potential

From the previous greenhouse screening weedy rice accessions with high allelopathic potential (more than 50% weed inhibition), non-allelopathic (less than 20% weed inhibition), cultivated rice (CL163 and REX), and allelopathic rice cultivars (PI312777, PI338046 and RONDO), were included in the study. All 30 microsatellite (SSR) markers were polymorphic for all accessions. Overall, the observed number of alleles (n_a) and effective number of alleles (n_e) were 2.9 and 2.2, respectively. Nei's gene diversity (h) and Shannon's information index (I) for the entire population was 0.51 and 0.86, respectively. High value of h and I indicates high level of genetic diversity among populations. Clustering based on Nei's genetic distance divided the accessions into three different clusters (Figure 4.3). Cluster 1 consisted of cultivated rice (CL163 and REX) and 2 allelopathic weedy rice accessions, ALR-1 and ALR-4, all with very low allelopathic potential. Both rice cultivars clustered together indicating the genetic similarity between these. Cluster 2 consisted of both allelopathic and non-allelopathic accessions suggesting the weak association of the markers with weed suppressive potential or allelopathy, and thus not able to distinguish between allelopathic and non-allelopathic accessions. The markers used in this study are primarily for accessing genetic diversity among the *Oryza spp.* and are not specific to allelopathy. Thus, the markers grouped allelopathic and non-allelopathic weedy rice together. Cluster 3 consisted of allelopathic rice cultivars and one of the most allelopathic weedy rice accession B2, thus indicating their genetic similarity. Weedy rice accession B2 which had high weed suppressive potential against barnyardgrass clustered with allelopathic rice; however, other allelopathic weedy rice did not fall in this cluster indicating lack of strong association among the genes controlling allelopathy in

weedy rice or weak association of the markers used in the study with allelopathy. Population structure of the allelopathic and non-allelopathic weedy and cultivated rice showed that allelopathic rice and weedy rice had close genetic background; however, some of the allelopathic weedy rice also shared genetic similarity with non-allelopathic weedy rice (Figure 4.5). Allelopathy is a complex mechanism and weed suppression is possible through several different allelochemicals. Momilactone B has been identified as one of the most important allelochemicals for suppression of barnyardgrass in rice (Xu et al. 2012), however there may be additional unknown allelochemicals in the weedy rice involved in weed suppression. In the future, it is important to identify novel allelochemicals and the precise mechanisms involved in weed suppression. This effort will help provide an insight on the mode of action of allelochemicals.

4.5. Conclusion

Weedy rice, which belongs to the same genus and species as cultivated rice is more competitive and can flourish in extreme environmental conditions where the cultivated rice do not perform well. In our previous greenhouse screening of 54 weedy rice accessions (Shrestha et al. 2018), potential herbicide tolerant and allelopathic weedy rice accessions were identified. In the present study, we used 30 microsatellite (SSR) markers to assess the genetic diversity among these accessions and behind these competitive traits. Herbicide tolerance was associated with markers irrespective of their hull color, and allelopathic trait did not show any strong association with markers used in this study. There, may be some distinct allelochemicals and allelopathic mechanism responsible for allelopathy in weedy rice than the ones already identified in cultivated rice. Knowing the basis of allelopathy in

weedy rice at the molecular level through techniques such as whole genome sequencing to identify single nucleotide polymorphisms (SNPs), and genotyping by sequencing (GBS), will be helpful in generating tools for improved rice breeding programs.

Table 4.1 List of markers used with nucleotide sequence, annealing temperature and PCR cycles.

Chromosome	Forward Primer	Reverse Primer	Annealing temperature	PCR Cycles
1	aatccaaggcagagatgg	caacgatgacgaacacaacc	55	30
1	gtctacatgtaccctgttggg	cggcatgagagtctgtgatg	61	30
1	caaatcccgactgctgtcc	tgggaagagagcactacagc	55	30
1	tcctgcgaactgaagagttg	agagcaaacctggttcac	55	30
2	accctctccgctcgcctctc	ctcctcctctgcgaccgctcc	61	30
2	ctgatcgagagcgtaaggg	gggatcaaaccacgtttctg	61	30
3	cattgtgcgtcacggagta	agccacagcgcctctctc	53	40
3	cacaggagcaggagaagagc	ggcaaacgatactcagtc	55	40
3	agattgatctcccattccc	cacgagcatattactagtgg	55	30
4	atcgtctgcgttgcggctgctg	catggatcaccgagctcccc	67	30
5	ctaagctccagccgaaatg	ctcaccctcatcatcgcc	55	30
5	ggcgattcttgatgaagag	tccccaccaatctgtcttc	53	30
5	tgcagatgagaagcggcgcctc	tgtgtcatcagacggcgtccg	61	30
6	ttggattgtttgctggctcgc	ggaacacggggtcggaaagcagc	63	30
6	gccagcaaacaggatccgg	caaggcttgtgcggcttgcgg	61	30
7	atcagcagccatggcagcgacc	aggggatcatgtgccgaaggcc	63	30
7	aacaaccaccactgtctc	agaaggaaaaggctcgatc	57	30
7	ccaatcggagccaccggagagc	cacatcctccagcgaccggag	67	30
8	caacgagctaactccgtcc	actgctactgggtagctgacc	55	30
8	gaaaccaccacactcaccg	ccgtagaccttctgaagtag	53	40
8	acgggcaatccgaacaacc	tcgggaaaacctaccctacc	53	30
8	atctctgatactccatccatcc	cctgtacgftgatccgaagc	55	30
8	tgcgctgaactaaacacagc	agacaaacctggccattcac	53	40
8	Cccttgctgtctctctc	acgggcttctctctctc	55	30
9	ctagttgggcatac gatggc	acgcttatatgttacgtcaac	55	30
9	caaaatggagcagcaagagc	tgagcacctcctctctgtag	55	30
10	tcagatctacaattccatcc	tcggtgagacctagagagcc	55	30
10	Tctcctctcaccattgtc	tgctgcctctctctctc	55	30
11	Tctctcctcttgttgctc	acacaccaacacgaccacac	55	30
12	cggcaaatcatcacctgac	caaggcttcaaggaag	55	30

Table 4.2 Genetic variation among the population indicated through allele's number, Nei's gene diversity and Shannon's Index.

Marker no.	Observed alleles	Nei's gene diversity	Shannon's Index
1	3	0.31	0.58
2	3	0.64	1.06
3	3	0.45	0.76
4	3	0.65	1.067
5	2	0.49	0.69
6	2	0.19	0.34
7	2	0.19	0.34
8	3	0.66	1.08
9	3	0.27	0.48
10	3	0.51	0.75
11	3	0.64	1.05
12	3	0.17	0.37
13	3	0.45	0.76
14	3	0.64	1.05
15	3	0.147	0.33
16	3	0.44	0.76
17	3	0.64	1.06
18	3	0.147	0.33
19	3	0.63	1.03
20	3	0.46	0.78
21	3	0.31	0.58
22	3	0.35	0.64
23	3	0.64	1.05
24	3	0.325	0.60
25	3	0.524	0.88
26	3	0.64	1.06
27	3	0.34	0.63
28	3	0.524	0.88
29	3	0.64	1.06
30	3	0.34	0.63
Mean	2.9	0.44	0.75

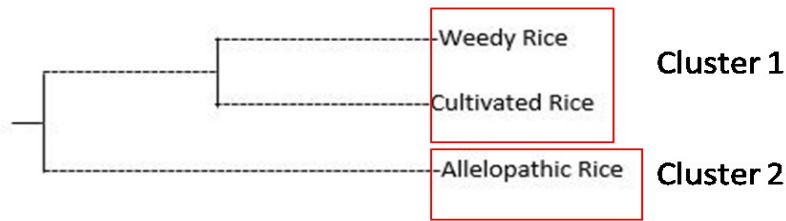


Figure 4.1 Dendrogram based on Nei's genetic distance indicating genetic relationship between weedy rice, cultivated rice, and allelopathic rice.

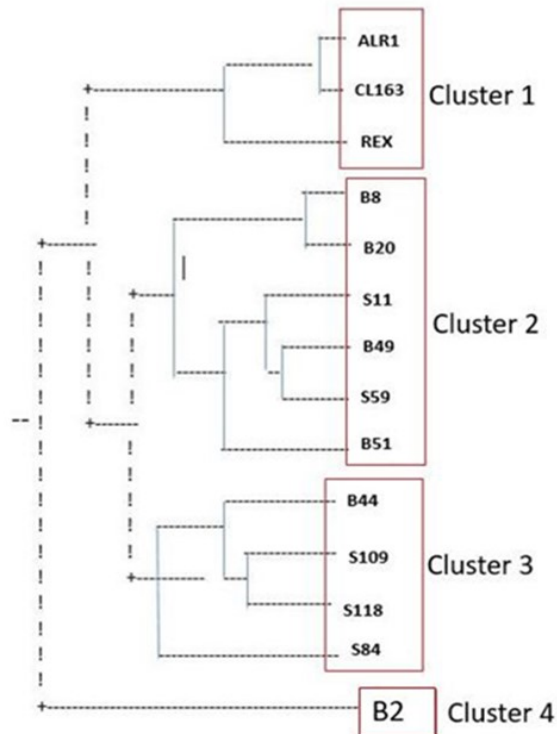


Figure 4.2 Dendrogram obtained from Nei's genetic distance calculated using 30 SSR markers representing relationship among accessions with respect to herbicide tolerance.

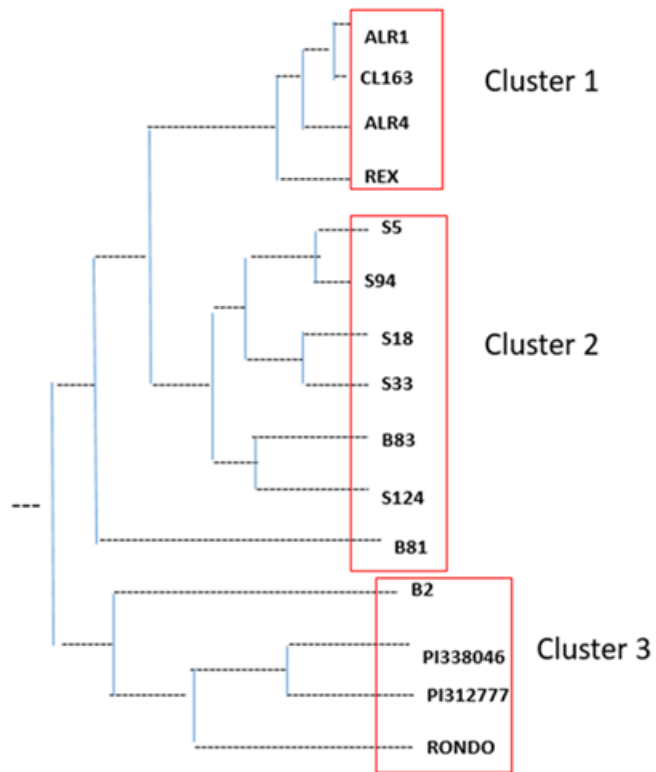


Figure 4.3 Dendrogram exhibiting genetic relationship among the accessions in terms of interference potential based on Nei's genetic distance.

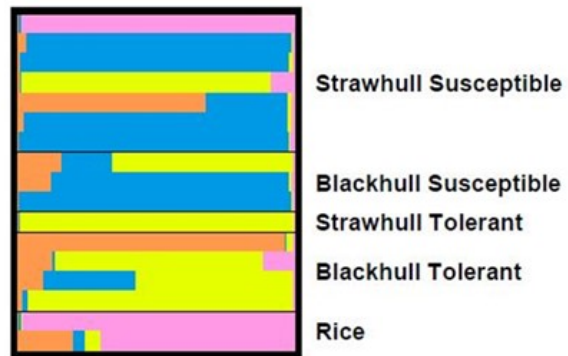


Figure 4.4 Population structure of accessions based on herbicide tolerance.

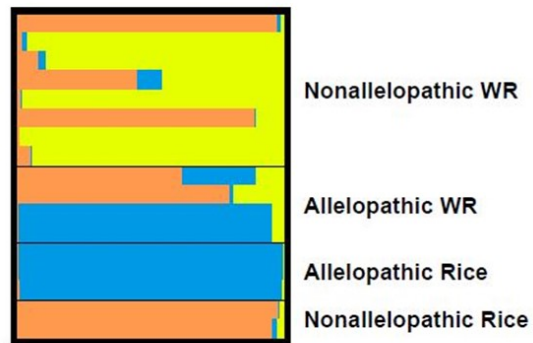


Figure 4.5 Population structure of accessions based on allelopathic potential.

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