VARIABILITY OF GRAIN ARSENIC CONCENTRATION AND SPECIATION IN RICE (*Oryza sativa* L.)

A Dissertation

by

TUSHARA RAGHVAN PILLAI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2009

Major Subject: Molecular and Environmental Plant Sciences

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ABSTRACT

Variability of Grain Arsenic Concentration and Speciation in Rice (*Oryza sativa* L.).

(December 2009)

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Arsenic is not an essential element and can be toxic to both plants and animals in high concentration. There is a demonstrated association between soil arsenic (As) and the occurrence of straighthead (a physiological disorder in rice characterized by panicle sterility and yield loss); however, the relationship between grain-As accumulation and straighthead susceptibility in rice is not yet fully understood. The objective of the current study was to evaluate a set of diverse rice cultivars, including both indica and japonica subspecies, for total grain-As (TGAs) and As-species concentrations in 2004, 2005, and 2007, on a native (moderate As-concentration) paddy soil and an adjacent monosodium monomethylarsonate (MSMA) amended soil. Cultivars were evaluated under both continuously flooded and intermittently flooded (saturated) field conditions.

The genotypic differences in the occurrence of straighthead, total grain-As (TGAs) and As-species concentrations, and their relationships with plant growth parameters, e.g., heading date, plant height, and yield were assessed. The cultivars exhibited a considerable range in both TGAs and grain-As species concentrations.

In 2004 and 2005, twenty-one rice cultivars replicated on native soil under continuous flooding showed significant differences in TGAs and As-species concentrations by genotype and year. In 2005, heading was generally delayed in the rice cultivars, resulting in reduced yields that were likely associated with unusually high temperatures and prolonged exposure to stresses in the field, including prolonged flooding and associated soil-As induced stresses. Lower grain-As concentrations were generally associated with early maturing and high yielding genotypes, but with some exceptions. Total grain-As concentrations were not correlated to straighthead susceptibility suggesting that high As concentration in rice grain might not be a direct cause of the genotype-dependent panicle sterility associated with MSMA in soil.

The rice cultivars grown on the MSMA-flooded treatment could be effectively differentiated for their relative straighthead susceptibility, with scores ranging from 1 to 8 for the most resistant to the most susceptible genotypes, respectively. In general, traits such as low grain-iAs^{III} concentration,early maturity, and high yield were correlated with straighthead resistance. In the MSMA-flooded treatment, very high grain-As accumulation resulted in elevated rice-grain dimethyl-As^{V} (DMAs^{V}) concentration, whereas, the concentration of the more harmful inorganic-As^{III} species was less affected.

The TGAs and As-species concentrations were considerably higher in continuously--flooded soil than the intermittently-flooded soil. The variations in TGAs and grain-DMAs^{V} concentrations were more highly influenced by water regime than by genotype, whereas, grain-iA^{III} concentrations were more highly genotype dependent. In the native soil with intermittent flooding, the concentrations of grain-DMAs^{V} and the less desirable grain-iA^{III} concentrations were lowest. The study concluded that for attaining lower As accumulation in the rice grain both genotype selection and water management are potentially useful approaches.

DEDICATION

To my husband and parents

ACKNOWLEDGMENTS

The author takes this opportunity to acknowledge the excellent academic guidance offered by Dr. Richard Loeppert and Dr. Terry Gentry during my Ph.D. Program at Texas A&M University (TAMU). Working with Dr. Richard Loeppert was a wonderful learning experience in many ways and I will never forget him in my life. I sincerely appreciate the support and excellent guidance provided by Dr. Terry Gentry towards completion of goals in my doctoral research. I am also grateful to Dr. Wengui Yan for expert guidance and organization in rice culture, sample and data collection that enabled my research. The timely guidance and insightful discussions with Dr. Michael Grusak Dr. Wayne Versaw is also appreciated. In general, I am very fortunate to have an extraordinary advisory committee with a wide range of expertise that helped me gain better knowledge during my research from multiple perspectives and shape this dissertation in its current form. I thank Dr. Amir Ibrahim for extending his expertise in statistical challenges and GGE biplot analysis. Also I thank various faculty members in the Department of Soil and Crop Sciences and Dale Bumpers National Rice Research Center (DBNRRC) for providing me with valuable guidance at different stages in my Ph.D. program.

I sincerely acknowledge the USDA for funding the research project through DBNRRC. I appreciate the role of Dr. Anna McClung, Dr. Tony Beaty, Dr. Helen Miller, Dr. Hesham Agrama, and Dr. Bryant Rolfe during the course of my research. I thank

Racheal Joslin, Emiliy Henderson, Tiffany Sookarserm, and Yao from DBNRRC for their assistance in my research.

I offer special thanks to my mother, father and Varun for their continuous support, prayers and encouragement which helped me achieve my Ph.D. goals. I appreciate the support from amma, achan, Sandhya, Rajesh and Devooty and several other family members. I am highly grateful to Vipul for his support and the financial statement, which helped me in keeping my I-20 valid during the later semester of my studies.

Finally, my very special thanks go to my husband, Radhakrishna, for his love, encouragement, and support in achieving this dream.

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1. INTRODUCTION

1.1. RESEARCH MOTIVATION

Rice (*Oryza sativa*) is a staple food of more than half of the world's population. There is increasing interest in rice-grain arsenic (As) concentration and As speciation because of concerns with food quality and interest in minimizing any potential risk from dietary exposure. Wide range of grain-As concentrations have been reported in rice samples in markets around the world (Williams et al., 2005). However the study of grain-As concentrations in cultivars aimed at crop improvement is required. Preliminary work by Yan et al. (2008) showed that As in rice grain is genotype dependent and can vary by a factor of 4 from one genotype to another, regardless of the As concentration in the soil. These findings open possibilities for the development of rice cultivars with low grain-As concentrations. In the past, arsenical herbicides were used on cotton growing areas now replaced by rice culture. The rice yields and grain quality could possibly be impacted by residual effect of arsenical compounds in the soil (Schweizer, 1967, Wells and Gilmour, 1977). Therefore, concerns have been raised about the quality of rice grown in the U.S., especially for the ethnic populations consuming rice as a major source of

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This dissertation follows the format of Crop Science.

carbohydrate (Williams et al., 2005). Studies have shown that the inorganic forms of As such as arsenate [As^V; $H_2AsO_4^{1-}$, $HAsO_4^{2-}$] and arsenite [As^{III}; $H_3AsO_3°$] are more toxic than the organic forms such as mono-methylarsonic acid ($MMAs^V$) and dimethylarsinic acid ($DMAs^V$), because the formers have longer retention times in the human body (Johnson and Farmer, 1990; Cohen et al., 2006). Because at higher grain-As concentrations (i.e., 150 - 400 µg/kg), the proportions of organic As such as $DMAs^V$ is higher than the potentially more harmful inorganic-As species (Williams et al., 2005), the As-speciation studies in rice are encouraging. The study of rice grain-As in wide ranging cultivars is important to identify genotypes with lower concentrations of potentially harmful As species. Study of grain-As concentrations in rice genotypes will be valuable towards the development of cultivars with inherently low grain-As concentrations when grown in soil types with diverse As concentrations.

1.2. DEFINITIONS

The rice cultivars for this study were grown in the field on a Dewitt silt loam soil (fine, smectitic, thermic Typic Albaqualf), at the USDA Dale Bumpers National Rice Research Center near Stuttgart, Arkansas. The four soil treatments including soil-As treatments and irrigation regimes in this document are defined as follows:

- Native-flooded treatment – *Soil*: Native soil used for rice culture. *Water management*: Continuous flooding consisting of flood to a depth of 12 cm at the five-leaf stage of the rice plant and the flood level maintained until one week prior to harvest.

- Native-saturated treatment - *Soil*: Native soil used for rice culture. *Water management*: Intermittent flooding consisting of flood to a depth of 12 cm established at the five-leaf stage of the rice plant. Subsequent irrigation similar to that of the flooded treatment is carried out only when the water layer above the soil surface dissipates and the soil becomes firm. Irrigation (re-flooding) timing was variable but averaged approximately once per week, depending on rainfall.
- MSMA-flooded treatment – *Soil*: A plot situated in an adjacent location to the 'native-soil treatment' plot and used in alternate years for the past 12 years for straighthead-susceptibility screening of rice cultivars. The soil has received an As treatment in alternate years for the past 12 years in the form of monosodium monomethylarsonate (MSMA) at the rate of 6.7 kg ha^{-1} (1.49 kg As ha^{-1}) application. *Water management*: Continuous flooding consisting of flood to a depth of 12 cm at the five-leaf stage of rice plant and the flood level maintained until one week prior to harvest.
- MSMA- saturated treatment – *Soil*: A plot situated in an adjacent location to the 'native-soil treatment' plot and used in past for straighthead susceptibility screening in rice cultivars and received soil-As treatment in alternate years for the past 12 years in the form of MSMA at the rate of 6.7 kg ha^{-1} application. *Water management*: Intermittent flooding consisting of flood to a depth of 12 cm established at the five-leaf stage of rice plant. Subsequent irrigation similar to that of the flooded treatment is carried out only when the water layer above the soil surface dissipates and the soil becomes firm.

1.3. SYMBOLS AND ABBREVIATIONS

Table 1-1. List of symbols and abbreviations used in this dissertation.

Symbol/Abbreviation	Explanation
ADP-As^V	Adenosine diphosphate
As	Arsenic
$As(GS)_3$	Arsenic triglutathione
ATP	Adenosine triphosphate
C	Carbon
C.V.	Coefficient of variance
$DMAs^{III}$	Dimethylarsinous acid (with trivalent As)
$DMAs^V$	Dimethylarsinic acid (with pentavalent As)
G	Genotype
G x I	Genotype by Irrigation interaction
G x Y	Genotype by Year interaction
GGE	Genotype and Genotype by Environment
GGE biplot	Genotype, Genotype-by-Environment biplot
GT-biplot	Genotype by Trait biplot
HPLC/ICP-MS	High performance liquid chromatography/inductively coupled argon-plasma mass spectrometry
I	Irrigation
iAs^{III}	Inorganic As^{III} (arsenite)
iAs^V	Inorganic As^V (arsenate)
$MMAs^{III}$	Monomethylarsonous acid (with trivalent As)
MSMA	Monosodium methyarsonate
O_2	Diatomic oxygen molecule
Pi	Inorganic phosphate
ROS	Reactive Oxygen Species
S	Sulfur
S.S.	Sum of Squares
SVP	Singular value partitioning
TFA	Trifluroacetic acid
TGAs	Total grain-As
USDA	United States Department of Agriculture
Y	Year

2. LITERATURE REVIEW

2.1. EFFECT OF ARSENIC ON RICE CULTIVATION

Rice is a semi-aquatic plant that is predominantly cultivated under flooded conditions. Flooded rice fields are occasionally prone to "straighthead", a physiological disorder characterized by reduced grain yield due to sterility of the florets (Figure 2-1). Since the affected panicles or heads with poor grain fill are not weighted down during maturity and instead remain upright, hence the name "straighthead" was adopted. The symptoms of natural straighthead are similar to the symptoms induced artificially by treating rice fields with As-based chemicals (Schweizer, 1967; Baker et al., 1976; Wells and Gilmour, 1977; Gilmour and Wells, 1980). The As-induced or natural occurrences of straighthead generally exhibit reduced plant growth, partial to complete sterility of the florets and incomplete grain development in the panicles (Atkins, 1974; Bollich et al., 1989; Schweizer, 1967, Wells and Gilmour, 1977).

Figure 2-1. Highly straighthead-susceptible cultivar 'Cocodrie' grown on (A) a native soil with no MSMA treatment in which rice panicles are weighted down by normal grain fill and (B) MSMA-treated soil in which straighthead affected rice has upright panicles due to incomplete grain fill.

Straighthead is usually observed only upon the prolonged flooding of rice fields. In addition, soils with histories of herbicidal MSMA (monosodium monomethylarsonate) applications or high organic matter (Rasamivelona et al., 1995) occasionally exhibit symptoms of straighthead during rice growth. To avoid straighthead, management practices of draining and drying the field during rice growing season are performed widely (Yan et al., 2005). The draining and drying may stress rice plants and limit yield potential, waste water and use extra energy, so increase the overall cost of production; therefore, farmers are advised to use straighthead resistant cultivars (Rasamivelona et al., 1995). Because the symptoms of As injury are similar to rice straighthead, evaluating rice susceptibility to straighthead in breeding programs by incorporation of As into soil form of MSMA has become a common practice (Yan et al., 2008). The straighthead screening of the U.S.A. and worldwide cultivars by Yan et al. (2005) demonstrated that U.S. rice cultivars Cocodrie, Mars, Kaybonnet and Bengal were highly straighthead susceptible whereas several Chinese cultivars such as Zhe-733, Zao-402, Luhongzao and Xiangzaoxian No.1 were straighthead resistant.

2.2. UPTAKE OF SOIL-ARSENIC BY PLANTS

In Bangladesh, high rice-grain As concentrations and associated loss in yields have been attributed to the high soil-As concentrations (Duxbury et al., 2003). Some U.S. and European soils are also known to be As-contaminated because of a history of As pesticide application. Irrespective of the total soil-As concentration, the bioavailability of As to rice depends on soil conditions like pH, redox potential, organic matter, texture, the presence of ligand-exchange sites on iron (Fe) and aluminum (Al) oxide minerals (Ultra et al., 2009) and the As species abundance in the soil (Woolson, 1977). In soil, various abiotic and biotic environmental factors cause oxidation-reduction of the As-species (Masscheleyn et al., 1991). The microorganisms in the soil can convert inorganic-As to $MMAs^V$, $DMAs^V$, gaseous arsines (AsH_3), or conversely to inorganic-

As species (Sohrin et al., 1997; Turpeinen et al., 1999), influencing the form of As available to the plants. In aerobic soil, As^V is immobilized by binding to ferric hydroxide minerals (Lafferty and Loeppert, 2005), however under anaerobic soil conditions favor the formation of reduced, more readily soluble Fe^{2+} and As^{III}, resulting in the release of As^{III} into the soil-pore water (Marin et al., 1993; Masscheleyn et al., 1991) and thus increasing the bioavailability of soil-As.

The prolonged flooding of rice fields is often characterized by anaerobic soil conditions. Under the anaerobic conditions, the rice roots diffuse oxygen into the rhizosphere soil, resulting in the oxidation of dissolved Fe^{2+} and the subsequent formation of iron hydroxide plaque on the root surface (Armstrong, 1967). The iron plaque on rice root serves as a reservoir of nutrients, enhances inorganic phosphate (P_i) availability (Liang et al., 2006), has a very strong affinity for $As^V$and likely impacts As uptake by rice (Meng et al., 2002; Otte et al., 1991). As^V is an analog of P_i (inorganic phosphate) (Dixon, 1997) and both As^V and P_i enter the plant through the root phosphate transporter (Rothstein and Donovan, 1963; Asher and Reay, 1979; Ullrich-Eberius et al., 1989; Meharg and Macnair, 1992). The As^V occurring in aerobic soil is converted to As^{III} under flooded conditions, thus As^{III} is the dominant As-species in the pore water of reduced soils (Marin et al., 1993; Masscheleyn et al., 1991). As^{III} uptake is mediated by the non-selective aquaporin channel in yeast, rice, peas and wheat roots (Wysocki et al., 2001; Meharg and Jardine, 2003). Studies in rice have shown that arsenite is taken up by roots through aquaporin channel, Lsi1 (the aquaporin NIP2;1) and Lsi2 (an efflux carrier) (Ma et al., 2008; Li et al., 2009b). The Lsi1 mutation inhibits uptake of MMAs^{V} and DMAs^{V}, whereas, in the roots of wild-type rice uptake of undissociated methylated especially MMAs^{V} was observed (Li et al., 2009b). The ability of plants to transform As species outside the root to enable uptake is not yet thoroughly understood.

2.3. ARSENIC SPECIATION AND TOLERANCE MECHANISMS

In rice, As exists in the +5 and +3 valence states and often as DMAs^{V}, MMAs^{V}, inorganic As^{V}and phytochelatin-complexed As^{III} (Raab et al., 2007; Smith et al., 2008). In living organisms, oxidation and reduction of As species is believed to take place during uptake, transport and storage processes (Thomas et al., 2004). The first gene identified for As-reduction called ScAcr2p was found in *Saccharomyces cerevisiae* (Mukhopadhyay et al., 2000) and later homologs with similar function were found in *Arabidopsis* and rice. In rice, As^{V} exposure results in expression of two ACR2-like genes, namely OsACR2.1 (expressed in whole plant) and OsACR2.2 (expressed in root) and which are related to As^{V} reduction (Duan et al., 2007). In microorganisms, As detoxification is carried out by proteins encoded by the *ars* operon (Xu et al., 1998) and As respiration is linked to proteins encoded by an *arr* operon (Saltikov and Newman, 2003). The *arrA* gene of the *arr* operon is highly conserved and a reliable marker for detection of As^{V} respiration in environmental samples (Malasarn et al., 2004).

Arsenic is toxic and limits cell function in several ways. For example, inorganic As^{III} reacts with sulfhydryl groups (–SH) of proteins and inhibits enzyme function associated with respiration causing cell death (Ullrich-Eberius et al., 1989). The As^V in cells competes with Pi and replaces the Pi in ATP to form an unstable ADP-As^V complex which disrupts the flow of energy in cells (Terwelle and Slater, 1967). During redox cycling, As and other heavy metals can result in generation of reactive oxygen species (ROS) in plants (Mascher et al., 2002), causing oxidative damage to the cell structure. The antioxidant enzymes and metal chelators present in the cells control ROS generation and metal toxicity. In *Pteris vittata*, an As hyperaccumulator, As induced oxidative damage is prevented by increasing the ascorbate–glutathione pool (Singh et al., 2006) involved in scavenging of ROS and metal sequestration. Other mechanisms of metal detoxification include production of cysteine-rich, metal-binding peptides called metallothioneins and phytochelatins (Hall, 2002). Metallothionein genes are described as encoded polypeptides and phytochelatins are enzymatically synthesized peptides produced in response to metal toxicity. Both compounds can bind with the metal via the -SH group and are transported to the vacuole (Rauser, 1990). In *Saccharomyces cerevisiae*, the ABC-transporter Ycf1p is involved in the uptake of $As(GS)_3$ complex into the vacuole (Wysocki et al., 2001). In plants, ABC-transporters are also known to sequester xenobiotics, pigments, ions and phytochelatin complexes in vacuoles (Rosen, 1999; Goodman et al., 2004). Excessive phytochelatin production has been linked to As tolerance in *Arabidopsis* (Sung et al., 2007), *Helianthus annus* (Raab et al., 2007) and *Silene vulgaris* (Sneller et al., 2000). Phytochelatins reduce the metal toxicity, but do not confer ability to hyperaccumulate metals in the plant (Li et al., 2004; Li et al., 2005).

This behavior was demonstrated by complementation of *Arabidopsis* phytochelatin synthase mutant with over expressed phytochelatin synthase gene *AtPCS1*. The *AtPCS1* gene could overcome As sensitivity in *Arabidopsis* but did not confer excessive As accumulation capacity when compared to the wild type. Similarly, in tobacco, over expression of all three major genes involved in phytochelatin biosynthesis did not result in over accumulation of cadmium (Wawrzynski et al., 2006). These studies indicate that phytochelatins alone do not confer metal tolerance to plants. Heavy metal tolerant plants usually limit influx of toxic metals (Meharg and Macnair, 1992; Catarecha et al., 2007) or are better equipped to prevent disruption of normal cellular function (Ullrich-Eberius et al., 1989). An As^{v}-tolerant *Arabidopsis* mutant with *pht1;1-3*, a mutant allele of the high-affinity Pi transporter PHT1;1, was shown to display a rate of As^{V} uptake that ultimately enables the mutant plant to accumulate double the As found in wild-type plants (Catarecha et al., 2007).

The mode of As transport and storage in plants is also important in tolerance by plants. Pickering et al. (2000) reported that in *Brassica juncea*, oxyanions As^{V} and As^{III} are transported through xylem and the majority of As in root and leaf existed as distinct As^{III}-tris-thiolate complexes. In As hyper-accumulating *P. vittata,* As^{V} is reduced to As^{III} in the frond for storage. Similar to various metal over-accumulating plants, *P. vittata* has a greater concentration of As in fronds than roots (Wang et al., 2002; Webb et al., 2003; Zhao et al., 2003). The As hyper-accumulating *Arabidopsis* mutants contain mostly As^{III} species (Clark et al., 2003; Quaghebeur and Rengel, 2004). Generally,

$MMAs^V$ is found at very low concentrations or below significant detection limits in rice grain (Schoof, 1998; Heitkemper et al., 2001). A similar observation in edible bamboo (Zhao et al., 2006) and rice shoot (Yuan et al., 2005), suggested that $MMAs^v$ is an intermediate compound formed during As metabolism. $DMAs^v$ is found in both shoots and roots of plants, and is believed to be the form transported from one part of the plant to another (Zhao et al., 2006; Raab et al., 2007). In the rice grain sampled from different countries, the grain-As concentrations were variable, and the selected samples from Europe, Bangladesh and India were dominated by inorganic As, whereas some U.S. cultivars predominantly contained DMA (Williams et al., 2005). The variety-dependent variation in rice-grain As speciation reported by Williams et al. (2005) should be further investigated under uniform soil conditions with different rice cultivars to understand whether differences in grain-As speciation are attributable to soil/environmental differences or to rice genetic differences.

3. TOTAL GRAIN-ARSENIC AND ARSENIC-SPECIES CONCENTRATIONS IN DIVERSE RICE CULTIVARS UNDER FLOODED CONDITIONS

3.1. INTRODUCTION

Rice (*Oryza sativa*) is the staple food for more than half of the world's population and in certain cultures accounts for up to 70 % of the total calorie intake (FAO, 2006). In recent years rice-grain As concentrations have received considerable interest, especially in terms of net daily intake by populations that are also ingesting high As levels from drinking water (Meharg and Rahman, 2003; Smith et al., 2006; Saha and Ali, 2007; Heikens et al., 2007; Mondal and Polya, 2008; Rahman et al., 2008; Panaullah et al., 2009). In drinking water, As occurs mainly as inorganic species [arsenate (As^V; $H_2AsO_4^{1-}$, $HAsO_4^{2-}$) or arsenite (As^{III}; $H_3AsO_3^{\circ}$)] (Cullen and Riemer, 1989), whereas in rice grain, organic-As species such as monomethylarsonic acid ($MMAs^V$) and dimethylarsinic acid ($DMAs^V$) as well as the inorganic forms have been reported (Heitkemper et al., 2001; Williams et al., 2005; Zavala et al., 2008). Both inorganic-As^{III} (iAs^{III}) and -As^V (iAs^v) species are toxic and can detrimentally impact metabolism. At cellular levels, iAs^{III} and iAs^V species can be bio-transformed to methylarsenic species (Cohen et al., 2006). In this process intermediate trivalent As species such as iAs^{III}, monomethylarsonous acid ($MMAs^{III}$) and dimethylarsinous acid ($DMAs^{III}$) are produced that can bind to S-containing amino acids and disrupt protein structure and function (Knowles and Benson, 1983; Voet et al., 2005). iAs^V mimics inorganic phosphate due to their structural similarities and can disrupt vital cell processes (Dixon, 1997). In the human body, ingested organic-As species, such as $DMAs^V$ and $MMAs^V$ have shorter retention times, and therefore present less potential hazard than inorganic-As species (Johnson and Farmer, 1990; Cohen et al., 2006). Hence, rice with low inorganic grain-As concentration is a desirable goal.

3.1.1. Causes of Variable Arsenic Availability to Rice

High As concentrations in rice grain have been linked to both soils high in As and the use of As-contaminated irrigation water (Xie and Huang, 1998; Van Geen et al., 2006; Heikens et al., 2007; Panaullah et al., 2009). High soil-As concentrations are associated with a physiological disorder of rice, "straighthead", which results in partial or complete sterility of panicles and eventual yield reduction (Gilmour and Wells, 1980). With prolonged flooding, the O_2 in soil is depleted as a result of soil-microbial activity and organic-matter decomposition processes. In addition, the layer of flood water acts as a physical barrier for O_2 diffusion into the soil. Under these anaerobic soil conditions, poorly crystalline ferric oxides that strongly retain As as a surface adsorbed complex are partially dissolved, resulting in the concurrent release of Fe^{2+} and As to the soil-pore water and the increased bioavailability of iAs^{III} and iAs^V (Masscheleyn et al., 1991). In anaerobic soils, the reduced iAs^{III} species, which is generally considered to be more soluble and more bioavailable than iAs^V, is usually the prevalent As species, compared to oxidized soil systems in which iAs^V is usually the prevalent species. Irrespective of total soil-As concentration, the available As concentration in flooded soil is usually higher than in non-flooded soil due to the differences in specific biochemical processes in these soils (Masscheleyn et al., 1991).

3.1.2. Arsenic Variability in Rice Grain

Wide variations in total grain-As and As-species concentrations have been reported in several market basket surveys of rice (e.g., Robberecht et al., 2002; Roychowdhury et al., 2002; Das et al., 2004; Al Rmalli, 2005; Huq et al., 2006; Van Geen et al., 2006; Heikens et al., 2007; Williams et al., 2007; Zavala and Duxbury, 2008; Zhu et al., 2008; Torres-Escribano et al., 2008). Differences in grain-As concentration might be attributable to differences in rice genetics, soil, or crop management. Genotypic differences in As-concentration in rice grain, seedlings, and roots have been successfully utilized in genetic studies to identify multiple quantitative-trait loci and candidate genes on rice chromosomes (Dasgupta et al., 2004; Zhang et al., 2008; Norton et al, 2008).

Since grain-As concentrations in rice cultivars will vary between soils (Cheng et al, 2006; Panaullah et al., 2009; Marin et al., 1998; Liu et al., 2006; Dasgupta et al., 2004; Zhang et al., 2008) uniform growth conditions are essential to evaluate genotypic differences in rice-grain As accumulation and minimize the complicating influences of soil and environmental variables. The present study was conducted to explore the variability in total grain-As and As-species concentrations among rice cultivars selected from the United States Department of Agriculture (USDA) rice germplasm collection.

The stability of genotypes for grain-As concentrations over multiple years was tested by growing rice on a uniform a native silt-loam soil with moderately high As-bioavailability. The relationship of total grain-As and As-species concentrations with agronomic characteristics such as yield, plant height, and heading date were evaluated.

3.2. METHODS

3.2.1. Rice Culture

Twenty-one rice cultivars originating from China, Nepal, Philippines, Portugal, Russian Federation, and U.S.A. including entries from indica and japonica subspecies, from the USDA rice-germplasm collection were selected for screening during 2004 and 2005 (Table 3-1). Ten cultivars from this study were additionally grown in 2007 (Table 3-1) to further evaluate grain-As species concentrations.

3.2.2. Soil and Environmental Conditions

The rice cultivars were grown on a Dewitt silt loam soil (fine, smectitic, thermic Typic Albaqualf), with a native soil-As concentration of 5.9 ± 1.5 mg As kg^{-1} (Yan et al., 2008), at the USDA Dale Bumpers National Rice Research Center near Stuttgart, Arkansas. The average rainfall during rice growth periods in 2004, 2005, and 2007 was 35, 21, and 20 cm, respectively. The maximum and minimum air temperature averages were correspondingly 30 and 19 °C in 2004, 32 and 19 °C in 2005, and 31 and 19 °C in 2007.

Table 3-1. Rice cultivars selected for studies in the year 2004, 2005, and 2007.

Cultivars studied in 2004 and 2005			Cultivars studied in 2007		
Cultivar Name	**Cultivar ID**	**Sub-species**	**Cultivar Name**	**Cultivar ID**	**Sub-species**
Aijiaonate	**G2**	**I**	**Huri-282**	**G11**	**I**
Danwanbao24	**G8**	**I**	**†IR-9209**	**G13**	**I**
Gui 99	**G10**	**I**	**†Jing-185-7**	**G15**	**J**
IR-44595	**G14**	**I**	**†Medark**	**G18**	**J**
†IR-9209	**G13**	**I**	**†Spalick**	**G24**	**J**
Jinnuo No.6	**G16**	**I**	**Xiangzaoxian**	**G28**	**I**
Luhongzao	**G17**	**I**	**Zanuo No1**	**G30**	**I**
Minkenzao	**G19**	**I**	**†Zao 402**	**G31**	**I**
Tie-90-1	**G25**	**I**	**†Zhe 733**	**G32**	**I**
You-I-B	**G29**	**I**	**Zhenshan 97**	**G33**	**I**
†Zao 402	**G31**	**I**			
†Zhe 733	**G32**	**I**			
Zhong 86-44	**G35**	**I**			
Cocodrie	**G37**	**J**			
†Jing 185-7	**G15**	**J**			
KBNT-1-1	**G38**	**J**			
†Medark	**G18**	**J**			
Ponta Rubra	**G20**	**J**			
Priscilla	**G21**	**J**			
†Spalick	**G24**	**J**			
Wells	**G27**	**J**			

† Indicates that the cultivar was studied in 2004, 2005, and 2007.

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Figure 3-1. Highly susceptible cultivar 'Cocodrie' grown on a typical test plot (1.8 x 1.2 m plot with 6 rows, 1.5 m long and 0.3 m apart) with MSMA-flooded treatment and showing straighthead symptoms of sterile and upright panicles in the center, and a 'border effect' characterized by filled panicles at the edges of the plot.

3.2.3. Field Screening Procedure

The field was prepared using a Northwest tiller (Yakima, WA, USA), and the rice was drill-seeded with a planter (Hege 1000; Hege Equipment Inc., Colwich, KS, USA). The experiment was conducted in a completely randomized block design (RCBD) with four replicates. The dimension of each plot was 1.8 x 1.2 m with 6 rows, 1.5 m long and 0.3 m apart (Figure 3-1). At about the four-leaf stage of rice growth, weeds were controlled by application of 9.3 L ha^{-1} of propanil (3',4'-dichloropropionanilide) mixed with 0.4 kg ha^{-1} of quinclorac (3,7-dichloroquinoline-8-carboxylic acid; Facet, BASF, Florham Park, NJ, USA). At about the five-leaf stage, urea fertilizer was applied at the rate of 134 kg N ha^{-1}, and immediately a permanent flood was established and maintained continuously until one week prior to harvest. The planting and harvesting dates were correspondingly, May 19th and Sep 10th in 2004, April 21st and Aug 27th in 2005, and April 15th and Aug 22nd in 2007. The heading date (defined as the number of days from planting until 50 % panicle emergence from the flag leaf), plant height, and yield were recorded for each plot as described by Yan et al. (2005). The rice grain for As analysis was obtained from the center 0.6 m of the center two rows in each plot to avoid the border effects observed in the rice plots (Figure 3-1).

3.2.4. Analysis of Arsenic Concentrations

The total grain-As (TGAs) concentration in rice samples was determined following open-vessel digestion of powdered milled grain with trace-metal grade HNO_3/H_2O_2. Grain samples (0.5 g) were digested with 0.5 mL HNO_3 in a teflon tube capped with a funnel and heated on a temperature-programmable 48-well graphite-block digestion system (Digi Prep MS, SCP Science, Montreal, Canada). During the HNO_3 digestion step, the digestion block was heated to 50 °C for 240 min, 60 °C for 240 min, and 120 °C for 240 min and allowed to cool. Then two rounds of H_2O_2 digestion, each involving the addition of 3 mL H_2O_2, were followed by heating to 130 °C for evaporation to dryness. For quality control, two samples of a standard reference material (1568a rice flour, NIST, Gaithersburg, MD, USA) with a certified As-concentration of 0.29 ± 0.03 μg As g^{-1}, two blanks, and three sample replicates were included in each digestion batch. The completely digested and dried samples were re-dissolved in 15 mL of 2 % HNO_3 and analyzed by inductively-coupled-plasma mass-spectroscopy (ICP-MS) using a Perkin Elmer ELAN DRC II (Perkin-Elmer Sciex, Concord, ON, Canada) fitted with a Meinhard concentric nebulizer and cyclonic spray chamber. To verify TGAs concentrations, outliers were reanalyzed, but individually verified values were not removed during calculations of mean and standard error, since this variation could have been due to natural genetic variation or localized soil differences or both.

The grain-As species were extracted using a modification of the method described by Heitkemper et al. (2001). Deioinized (DI) water (1690 μL) followed by 310 μL of 99.9 % trifluoroacetic acid (TFA) (a final concentration of 2 M TFA) was added to ~0.5 g of rice flour in a 50 mL polypropylene tube that was then capped and heated at 80 °C for 4 hr using a temperature-programmable graphite-block digestion system (Digi Prep MS, SCP Science, Montreal, Canada). The mixture was then diluted with 20 mL DI water, homogenized by vortexing for 1 min, and centrifuged for 20 min at 3600 rpm. The supernatant solution was then collected, evaporated to near dryness, redissolved in 15 mL DI water, and filtered through a 0.2 μm nylon membrane filter. The As species were separated using a PerkinElmer 200 HPLC system (Waltham, MA, USA) with a guard column (IonPac Dionex AG7, Sunnyvale, CA, USA) and an anion-exchange column (IonPac Dionex AS7), which were attached in-line to the ICP-MS for As analysis. The separation scheme for As speciation consisted of a 1-min elution with 1 mM HNO_3 followed by a 6-min linear-gradient elution from 1 to 50 mM HNO_3. A chromatographic internal standard of 5 μg As L^{-1} As was pulsed at 6.5 min post-injection. The quantification of As species by ICP-MS induces a variable enhancement of the As signal due to the presence of C-containing compounds extracted from rice grain. This potential error was minimized by the addition of 3 % CH_3OH/H_2O into the sample line after the guard and anion-exchange columns (Larsen and Sturup, 1994). A 20-cm coil was added to the ICP-MS sample-input tubing to ensure complete mixing of the CH_3OH with the column eluant (James et al., 2008). The standard-curve was obtained using mixtures of four As species, namely $DMAs^V$ (dimethylarsinic acid; Chem Service, West Chester, PA, USA), $MMAs^V$ (monosodium methylarsonate sesquihydrate; Chem Service), iAs^{III} (As_2O_3; Alfa Aesar, Johnson Matthey Company, Westhill, MA, USA), and iAs^V (SPEX Centriprep, Metuchen, NJ, USA). Perkin Elmer Chromera software was used to control the HPLC and ICP-MS instruments, as well as for data collection and analysis. To evaluate the efficiency of As-species extraction from each rice sample, the TGAs concentrations obtained by the HNO_3/H_2O_2 digestion method were compared with the sum of As species identified by the TFA-extraction method.

3.2.5. Statistical Analysis

Statistical analyses such as analysis of variance (ANOVA), mean comparison, and correlation were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The General Linear Model (GLM) procedure was used for completely randomized design (CRD) variance analysis where main effect genotype (G), year (Y), and their interactions (G x Y) were considered as sources of variance. From the GLM output, the coefficient of variance (*C.V.*) and sum of squares (S.S.) were also obtained. As a part of ANOVA, the percentage variation explained by each source of variation was calculated for the individual traits using the S.S. and the formula $\{[S.S._{(\text{trait x factor})}] / \text{total } S.S.\}*100$, which indicates the percentage sum of squares of each trait x factor combination. The CORR and REG procedures were used to obtain Pearson's correlation coefficients (r) and the corresponding significance levels, and the LSMEAN and MEANS procedures were used to estimate least square mean (mean) and least square difference (LSD), respectively.

The graphical analyses of relationships between agronomic traits and grain-As concentrations were performed using GGE biplot software (version 6.1) following the methods of Yan and Tinker (2006). The "relationship among testers" view of GGE biplot denoted as "GT-biplot" was produced to assess relationships between traits as an aid in selection of genotypes with multiple desirable traits using a three-way dataset of genotypes, traits, and replicates. This view was obtained by singular value partitioning ("SVP=2"), no data transformation ("transform=0"), scaling by standard deviation ("scaling=1"), and centering by G+GE ("centering=2"). The "mean-trait performance and stability" view of biplot was individually plotted for each trait using two-way data consisting of trait and genotype-by-year columns with singular value partitioning ("SVP=1"), no data transforming ("transform=0"), scaling by standard deviation ("scaling=1"), and centering by G+GE ("centering=2").

3.3. RESULTS AND DISCUSSION

3.3.1. Total Grain-arsenic and Arsenic-species Concentrations

The TGAs concentrations of 21 genotypes evaluated in the 2004 and 2005 tests are summarized in Table 3-2. In 2004, the TGAs concentrations of 21 genotypes ranged from 0.188 mg As kg^{-1} ('Cocodrie') to 0.863 mg As kg^{-1} ('KBNT-1-1'), a ratio of maximum/minimum concentration of 4.6. During 2005, the TGAs concentrations ranged from 0.274 ('Jinnou No.6') to 1.824 mg As kg^{-1} ('KBNT-1-1'), a 6.7 ratio. In individual genotypes, the standard errors (SE) of TGAs concentration varied, ranging from 0.003 to 0.035 mg As kg^{-1} in 2004 and from 0.006 to 0.229 mg As kg^{-1} in 2005.

The large SE of TGAs concentration in some cultivars indicates poor replication due to either genotypic or soil variation (Table 3-2). The only As species identified in the TFA extracts of rice grain were iAs^{III} and $DMAs^{V}$, with 77-100 % total recoveries compared to TGAs concentrations (Table 3-3). The occasional low recovery of As in rice samples by the TFA-extraction method could have resulted from the strong bond between the thiol groups of rice protein and As^{III} (Munoz et al., 1999) or the presence of unknown As-containing compounds. Grain-iAs^{III} concentrations in genotypes grown in 2004 ranged from 0.094 ('IR-9290') to 0.188 mg As kg^{-1} ('Zanou No.1'), a 2-fold ratio and during 2005 ranged from 0.091('Zhenshan 97') to 0.149 mg As kg^{-1} ('Huri-282'), a 1.6-fold ratio (Table 3-3). $DMAs^{V}$ was the dominant As species in the rice samples, constituting 51.4 – 74.6 % of the total TFA extractable As (43.3-64.8 % of the TGAs).

Table 3-2. Mean and standard error of agronomic traits and total grain-As concentrations for 21 rice cultivars grown in 2004 and 2005 under continuously flooded conditions.

			Heading Date (days)				Yield (kg ha^{-1})				†TGAs (mg As kg^{-1})			
Cultivar Name	Cultivar ID	Sub-species	2004	2005	2004-2005	P>|t|	2004	2005	2004-2005	P>|t|	2004	2005	2004-2005	P>|t|
Aijiaonante	G2	I	63	70	-7	**	8686	7310	1376		0.363 ±0.024	0.324 ±0.010	0.039	
Danwanbao24	G8	I	82	82	0		6052	4922	1130	**	0.468 ±0.012	0.544 ±0.010	-0.076	**
Gui 99	G10	I	86	88	-2	*	7537	4072	3465	***	0.537 ±0.008	0.675 ±0.010	-0.138	***
IR-44595	G14	I	72	79	-7	***	9348	6839	2509	**	0.416 ±0.020	0.535 ±0.060	-0.119	
IR-9209	G13	I	70	77	-7	***	7522	6072	1450	***	0.564 ±0.017	0.749 ±0.020	-0.185	**
Jinnuo No.6	G16	I	92	94	-2		9105	6060	3045	**	0.331 ±0.018	0.274 ±0.010	0.057	**
Luhongzao	G17	I	59	69	-10	***	8435	6797	1638	***	0.390 ±0.019	0.467 ±0.010	-0.077	**
Minkenzao	G19	I	61	69	-8	***	8299	6905	1394	**	0.488 ±0.004	0.480 ±0.010	0.008	
Tie-90-1	G25	I	58	66	-8	***	9859	7519	2340	***	0.421 ±0.012	0.368 ±0.060	0.053	
You-I-B	G29	I	60	66	-6	***	7936	6281	1655	**	0.329 ±0.022	0.536 ±0.010	-0.207	***
Zao 402	G31	I	63	72	-9	***	8065	7916	149		0.690 ±0.031	0.679 ±0.040	0.011	
Zhe 733	G32	I	61	62	-1		9464	7800	1664	**	0.268 ±0.031	0.372 ±0.040	-0.104	
Zhong 86-44	G35	I	61	68	-7	***	8478	7839	639		0.447 ±0.012	0.580 ±0.050	-0.133	**
Cocodrie	G37	J	80	81	-1		7511	6492	1019	**	0.188 ±0.003	0.458 ±0.020	-0.27	***
Jing 185-7	G15	J	87	92	-5	**	8333	6015	2318	***	0.402 ±0.008	0.423 ±0.010	-0.021	
KBNT-1-1	G38	J	85	87	-2		6377	5239	1138		0.863 ±0.035	1.824 ±0.230	-0.961	**
Medark	G18	J	78	77	1		7424	6069	1355	**	0.507 ±0.014	0.552 ±0.020	-0.045	
Ponta Rubra	G20	J	60	66	-6	***	6375	4973	1402	***	0.427 ±0.028	0.468 ±0.040	-0.041	
Priscilla	G21	J	83	87	-4		7153	5403	1750	**	0.333 ±0.034	0.380 ±0.010	-0.047	
Spalick	G24	J	57	55	2	**	7430	4693	2737	***	0.535 ±0.013	0.522 ±0.020	0.013	
Wells	G27	J	79	85	-6	**	7630	5513	2117	**	0.418 ±0.018	0.446 ±0.010	-0.028	
Mean			71^a	76^b	-5		7953^a	6225^b	1728		0.447^a	0.555^b	-0.11	
Median			70	77	-6		7936	6072	1638		0.421	0.48	-0.05	
Upper Limit			92	94	2		9859	7916	3465		0.863	1.824	0.057	
Lower Limit			57	55	-10		6052	4072	149		0.188	0.274	-0.96	
LSD (0.05)			3	3			1041	777			0.076	0.161		
C.V. (%)			2	3			9	9			12	21		

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.

†TGAs = mean and standard error total grain-As concentration (mg As kg^{-1}) as determined following HNO_3/H_2O_2 digestion

Means denoted by ^{a} and ^{b} for each trait are significantly different at 0.05probability

Table 3-3. Mean and standard error of grain-As concentrations for ten rice-cultivars grown in 2004 and 2007 under continuously flooded conditions.

Cultivar name	Cultivar ID	Sub-species	†TGAs (mg As kg^{-1})				As species								¶Rec	As^{III}/TGAs
							‡Grain-iAs^{III} (mg As kg^{-1})				§Grain-DMAs^{V} (mg As kg^{-1})					
			2004	2007	2004-2007	$P>\lvert t\rvert$	2004	2007	2004-2007	$P>\lvert t\rvert$	2004	2007	2004-2007	$P>\lvert t\rvert$		
Huri-282	G11	I	0.599±0.730	0.630±0.019	-0.035		0.136±0.003	0.149±0.007	-0.013		0.396±0.000	0.369±0.015	0.027		0.82	0.24
IR-9209	G13	I	0.564±0.800	0.500±0.018	0.061	**	0.094±0.004	0.134±0.013	-0.040	*	0.322±0.020	0.236±0.005	0.086	**	0.73	0.27
Jing-185-7	G15	J	0.402±0.890	0.410±0.040	-0.006		0.121±0.010	0.121±0.007	0.000		0.230±0.010	0.206±0.007	0.024		0.80	0.30
Medark	G18	J	0.507±1.030	0.510±0.017	-0.001		0.146±0.006	0.127±0.006	0.019		0.230±0.060	0.323±0.022	-0.093		0.89	0.25
Spalick	G24	J	0.535±0.920	0.270±0.019	0.268	***	0.152±0.007	0.140±0.009	0.012		0.284±0.010	0.136±0.010	0.148	***	1.03	0.52
Xiangzaoxian	G28	I	0.334±0.830	0.280±0.035	0.052		0.099±0.003	0.114±0.011	-0.015		0.199±0.000	0.146±0.029	0.053		0.92	0.40
Zanuo No1	G30	I	0.409±0.870	0.430±0.011	-0.023		0.188±0.008	0.124±0.007	0.064	***	0.231±0.030	0.234±0.024	-0.003		0.83	0.29
Zao 402	G31	I	0.514±0.860	0.350±0.021	0.165	***	0.106±0.020	0.139±0.005	-0.033		0.250±0.030	0.164±0.005	0.086	*	0.87	0.40
Zhe 733	G32	I	0.268±0.770	0.280±0.015	-0.009		0.103±0.001	0.125±0.002	-0.022	**	0.136±0.020	0.114±0.006	0.022		0.86	0.45
Zhenshan 97	G33	I	0.345±0.850	0.300±0.006	0.049	**	0.109±0.005	0.090±0.003	0.019	*	0.207±0.010	0.138±0.004	0.069	**	0.77	0.31
Mean			0.448^{a}	0.400^{b}	0.052		0.125^{a}	0.126^{a}			0.248^{a}	0.210^{b}	0.042		0.85	0.34
Median			0.458	0.380	0.024		0.115	0.126			0.231	0.190	0.040		0.85	0.30
Upper Limit			0.599	0.630	0.268		0.188	0.149			0.396	0.370	0.148		1.03	0.52
Lower Limit			0.268	0.270	-0.023		0.099	0.090			0.136	0.110	-0.093		0.77	0.24
LSD (0.05)			0.059	0.070			0.020	0.024			0.047	0.050				
C.V. (%)			9	11			12	13			13	15				

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.

†TGAs = total grain-As concentration (mg As kg^{-1}) as determined following HNO_3/H_2O_2 digestion

‡Grain-iAs^{III} = inorganic iAs^{III} concentration (mg As kg^{-1}) in rice grain as determined by TFA extraction

§Grain-DMAs^{V} = dimethylarsinic acid (mg As kg^{-1}) in rice grain as determined by TFA extraction

¶Rec = recovery efficiency of As species by TFA extraction compared to total grain-As concentration following HNO_3/H_2O_2 digestion method

Means denoted by ^{a} and ^{b}for each trait are significantly different at 0.05probability.

3.3.2. Variance Analysis of Total Grain-arsenic Concentration

The ANOVA indicated that both TGAs and grain-$DMAs^V$ concentrations were significantly affected by G, Y, and G x Y interaction effects (Table 3-4 and Table 3-5). The contributions of the G, Y, and G x Y factors towards the total variation in TGAs concentration were 70.1, 3.5, and 17.1 %, respectively. The larger contribution of G indicates that the differences in TGAs concentration were mostly genotype dependent. The average TGAs concentration in 2004 (0.447 mg As kg^{-1}) was significantly lower than that in 2005 (0.555 mg As kg^{-1}). This variation of TGAs concentration by Y was likely impacted by climatic differences. Although adjacent plots were used in 2004 and 2005 the chemical compositions and mineralogies of these soils might not have been identical. The higher temperatures in 2005 compared to 2004, likely contributed to the decreased yields in 2005 (Tao et el., 2007; Feller et al., 1998) and the associated higher TGAs concentrations as discussed below. The significant G x Y interaction of TGAs concentration implied that annual differences in growth condition impacted some individual genotypes disproportionally. As a result, the relative rankings of several cultivars for TGAs concentration were different in 2004 versus 2005.

Table 3-4. Variance analysis of agronomic traits and grain-As concentrations for 21 rice genotypes grown in 2004 and 2005 under continuously flooded conditions.

Source of variation	d.f.	Level of Significance				% of Total Sum of Squares			
		HD	HT	Yield	TGAs	HD	HT	Yield	TGAs
Year (Y)	**1**	***	**	***	***	**3.9**	**0.8**	**35.5**	**3.5**
Genotype (G)	**20**	***	***	***	***	**91.3**	**65.7**	**44.8**	**70.3**
Year x Genotype (GxY)	**20**	***	***	***	***	**2.0**	**18.0**	**3.0**	**17.1**

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.
HD = days to 50 % heading; HT = plant height (cm); Yield = grain yield (kg ha^{-1}); TGAs = total grain-As concentration (mg As kg^{-1}) as determined following HNO_3/H_2O_2 digestion.

Table 3-5. Variance analysis of inorganic iAs^{III} and organic As ($DMAs^{V}$) concentrations in grain of ten genotypes grown in 2004 and 2007 under consistently flooded conditions.

Source of variation	d.f.	Level of Significance		% of Total Sum of Squares	
		iAs^{III}	$DMAs^{V}$	iAs^{III}	$DMAs^{V}$
Year (Y)	**1**		**	**0.00**	**7.1**
Genotype (G)	**9**	***	***	**48.1**	**68.8**
Year x Genotype (GxY)	**9**	***	***	**30.0**	**9.7**

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.
iAs^{III} = inorganic As^{III} (mg As kg^{-1}), $DMAs^{V}$ = dimethylarsinic acid (mg As kg^{-1}) as determined in rice grain following 2 M TFA extraction method.

A field study of rice-grain As and other elemental constituents was conducted by Cheng et al. (2006) using nine genotypes grown over six locations and three years. Cheng et al. (2006) also reported significant differences in TGAs concentration among genotypes, but unlike the current study, found the Y effect to be non-significant. However, they did observe significant differences in TGAs concentration by location (L) and interaction of genotype and location (GxL), which demonstrated that genotype and soil properties had combined effects on TGAs concentration. The specific environmental factors that contributed to variability of TGAs concentration in the current study deserve further investigation.

3.3.3. Relationship between Total Grain-arsenic Concentration and Grain-DMAs^V Concentration

In the GT biplots (e.g., Figure 3-2) the individual traits are connected at the biplot origin by vectors lines. The cosine of the angle between any two vectors approximates to their correlation, with acute and obtuse angles indicating positive and negative associations, respectively (Yan and Tinker, 2006). The GT biplot (Figure 3-2) indicates a strong positive correlation between TGAs and grain-DMAs^V concentrations as represented by the narrow acute angle between their respective vectors. The positive relationship between TGAs and grain-DMAs^V concentrations was confirmed by the highly significant and positive correlation coefficients ($r = 0.934$, $P < 0.001$, in 2004; $r = 0.813$, $P < 0.001$, in 2007). Therefore, higher or lower concentrations of TGAs and grain-DMAs^V occurred simultaneously, similar to the findings in previous studies (Xu et al., 2008; Zavala et al., 2008b). Grain-DMAs^V was the predominant As species in rice grain, contributing 58 to 79 % of TGAs concentration and 52 to 73 % of the sum of As species recovered (Table 3-3). The variance analysis indicated that grain-DMAs^V concentrations were significantly different by G, Y, and G x Y, which explained 68.8, 7.1, and 9.7 %, respectively, of the total variation in TGAs concentration (Table 3-5). This trend of grain-DMAs^V concentration was similar to that of TGAs concentration.

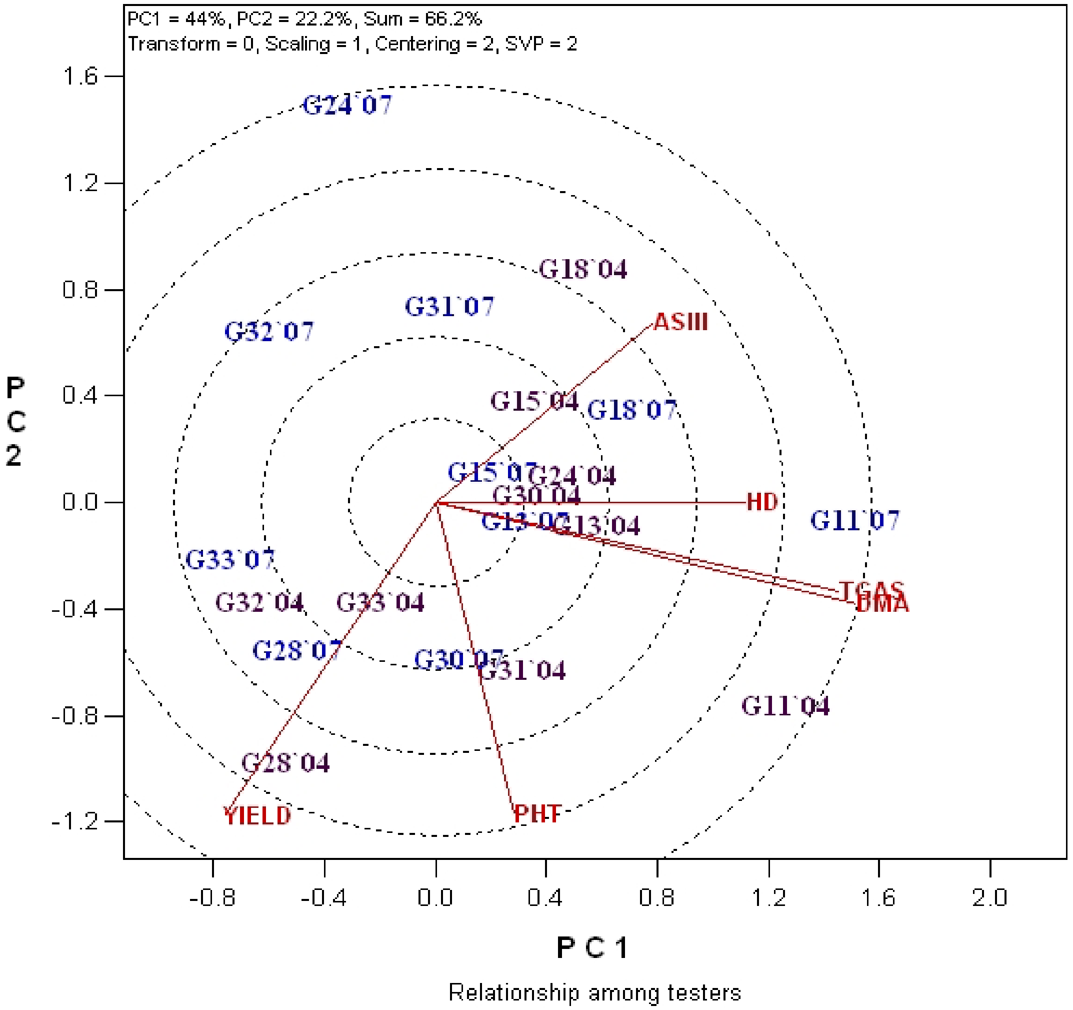

Figure 3-2. GT biplot showing relationships among various agronomic traits with ten genotypes grown in 2004 and 2007under continuous flooded conditions. HD = days to 50 % heading; yield = grain yield (kg ha^{-1}); PHT = Plant Height (cm); TAS = total grain As concentration (mg As kg^{-1}); AsIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}). Genotypes: G11 = Huri 282, G13 = IR-9209, G15 = Jing 185-7, G18 = Medark, G24 = Spalcik, G28 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao 402, G32 = Zhe 733, G33 = Zhenshan 97.

3.3.4. Variance Analysis of Grain-iAs^{III} Concentration and Its Relationship with Total Grain-arsenic Concentration

The proportion of grain-iAs^{III} to TGAs concentration ranged from 0.29 to 0.46 in 2004 and from 0.30 to 0.52 in 2007 (Table 3-3). The grain-iAs^{III} concentrations were significantly different by G and G x Y, explaining 48 and 30 % of the total variation, respectively, indicating the impact of genotype on grain-iAs^{III} concentration. The average grain-iAs^{III} concentrations observed in year 2004 and 2007 were similar; therefore, the Y effect was not significant. However a few cultivars had different grain-iAs^{III} concentrations in the two years resulting in a significant G x Y interaction (Table 3-3)

The GT-biplot (Figure 3-2) indicated poor correlation of grain-iAs^{III} concentration with TGAs and grain-DMAs^{V} concentrations, as represented by the wide acute angle between the grain-iAs^{III} vector and the TGAs and grain-DMAs^{V} vectors. This result was confirmed by the relatively poor correlation of grain-iAs^{III} concentration with grain-DMAs^{V} concentration ($r = 0.210$, $P > 0.226$, in 2004; $r = 0.376$, $P > 0.026$, in 2007). These results also indicate that the control of iAs^{III} accumulation in rice grain might not be directly linked to DMAs^{V} accumulation.

3.3.5. Variance Analysis of Yield and Its Correlation to Grain-arsenic Accumulation

The variations in yield due to G, Y, and G x Y interaction were significant (Table 3-4), explaining 44.8, 35.5, and 3 %, respectively, of the total variation. The G x Y and Y effects reflected the relatively large differences in yield between 2004 and 2005 (Table 3-2), which could be at least partially attributed to the higher temperature in 2005. The significant G x Y interaction for yield indicated that the yearly differences in growth conditions impacted the various genotypes differently. On average, the yield was 22 % higher in 2004 (7953 kg grain ha^{-1}) than in 2005 (6225 kg grain ha^{-1}). On the other hand, the average TGAs concentration was 24 % lower in 2004 (0.447 mg As kg^{-1}) compared to 2005 (0.555 mg As kg^{-1}). The GT biplot (Figure 3-3) indicated an inverse relationship between yield and TGAs concentration as represented by the nearly opposite orientation of the respective vectors. In addition, the negative correlation coefficients between yield and TGAs ($r = -0.417$, $P < 0.001$, in 2004; $r = -0.209$, $P < 0.05$, in 2005) indicate that higher yields were generally associated with a lower grain-As concentrations.

Yield was also negatively associated with grain-iAs^{III} concentration as indicated in the GT biplot (Figure 3-2) by the nearly opposite orientations of vectors of the respective traits and their correlation coefficients (r = -0.229, $P < 0.01$, in 2004; r = -0.364, $P < 0.05$, in 2007). The higher TGAs (Figure 3-3) and iAs^{III} (Figure 3-2) concentrations observed at lower yields might be attributable to the concentration effect that has been reported with various minerals in cereal crops (e.g., Batten, 1986; Graham et al., 1999; Fan et al., 2008). An alternative explanation is that rice-grain yields were reduced in those cultivars with higher TGAs and grain-iAs^{III} concentrations. Previous studies have demonstrated the negative impact of high soil-As concentration on rice-grain yield (Duxbury et al., 2003; Panaullah et al., 2009). Further studies will be required to explain the observed relationship in the current study.

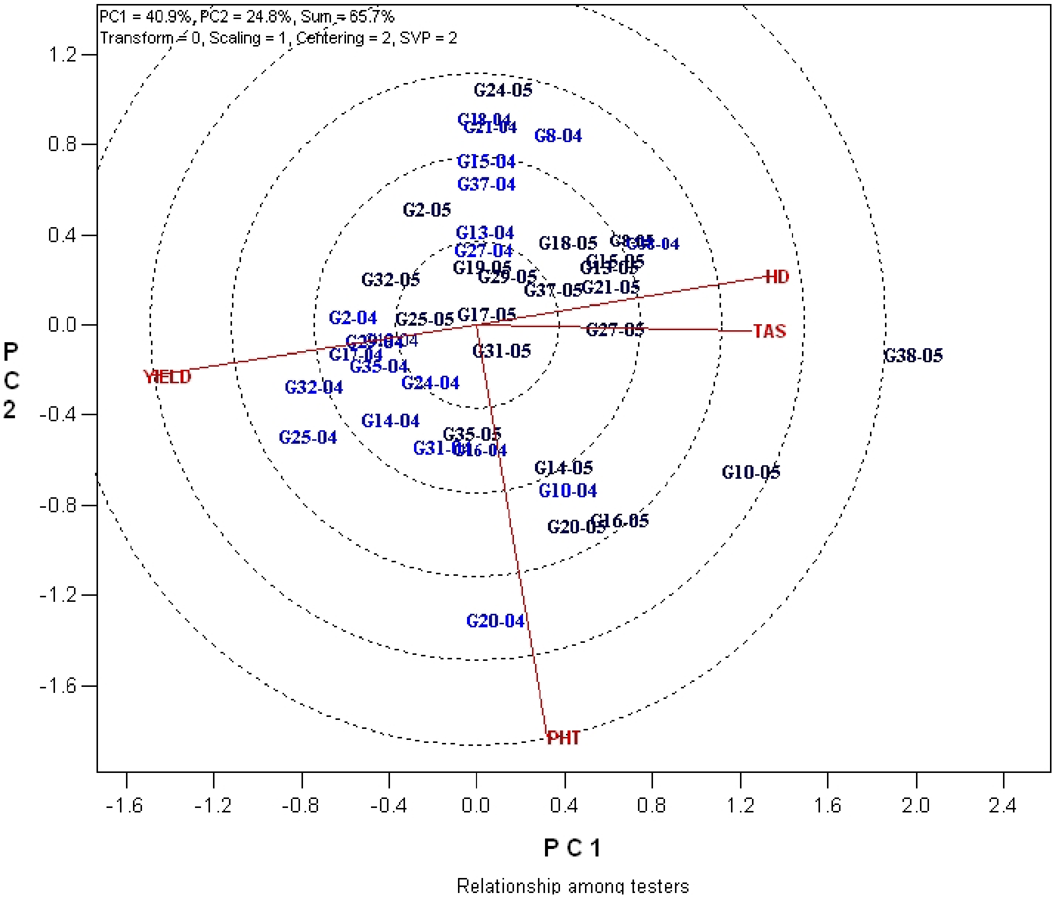

Figure 3-3. GT biplot showing relationships between various agronomic traits with 21 genotypes grown under continuous flooding in years 2004 and 2005. HD = days to 50 % heading; YIELD = grain yield (kg ha^{-1}); PHT = Plant Height (cm); TAS = total grain As concentration (mg As kg^{-1}). Genotypes: G2 = Aijiaonante, G37 = Cocodrie, G8 = Danwanbao 24, G10 = Gui 99, G14 = IR-44595, G13 = IR-9209, G15 = Jing 185-7, G16 = Jinnuo No.6, G38 = KBNT-1-1, G17 = Luhongzao, G18 = Medark, G19 = Minkenzao, G20 = Ponta Rubra, G21 = Priscilla, G24 = Spalick, G25 = Tie-90-1, G27 = Wells, G29 = You-I-B, G31 = Zao 402, G32 = Zhe 733, G35 = Zhong-86-44.

3.3.6. Heading Date and Its Relationship to Grain-arsenic Accumulation

Rice heading date (time from planting to 50 % panicle emergence) was significantly influenced by G, Y, and G x Y, explaining 91.3, 3.9, and 2 % of the total variations, respectively (Table 3-4). Although the proportions of the total variation in heading attributable to Y and G xY were relatively small these, the respective influences were statistically significant and were likely impacted by the delayed heading of cultivars in 2005 (Table 3-4).

Grain-DMAs^{V} concentration was positively correlated with heading in both 2004 ($r = 0.438$, $P = 0.008$) and 2007 ($r = 0.821$; $P < 0.001$). Grain-iAs^{III} concentration was positively correlated with heading in 2007 ($r = 0.318$; $P < 0.05$) but not in 2004 ($r = 0.128$, $P = 0.464$). These results indicate that delay in heading might have contributed to higher As-species concentrations.

Heading date was negatively associated with the yield as indicated in GT biplots (Figure 3-3) and correlation analysis ($r = -0.280$, $P < 0.005$, in 2004; $r = -0.341$, $P < 0.01$, in 2005). Reduced grain-yield has been associated with late heading genotypes that likely experienced additional abiotic-stress from the extended period of flooding (Blom and Voesenek, 1996). Therefore, to reduce grain-As concentrations, both higher yield and early maturity appear to be favorable traits.

3.3.7. Stability Biplots

Genotypic ranking and stability for grain-iAs^{III}, grain-DMAs^{V}, and TGAs concentrations are shown in Figure 3-4, Figure 3-5, and Figure 3-6, respectively. In each of these figures, the single-arrowed line represents the abscissa, which points to the direction of higher trait values. The double-arrowed line points to the direction of larger variability and hence lower stability associated with each genotype (Yan and Tinker, 2006). In the stability-view of grain-iAs^{III} concentration (Figure 3-4), the genotypes 'Xiangzaoxian', 'Jing-185-7', and 'Spalick', which are close to the abssica line, were the most stable genotypes, with correspondingly low, intermediate, and high grain-iAs^{III} concentrations. Genotypes 'Zhe 733' and 'Zhenshan 97' had low grain-DMAs^{V} (Figure 3-5) and TGAs (Figure 3-6) concentrations and were relatively stable for these traits, but lacked grain-iAs^{III} concentration stability. A promising genotype was 'Xiangzaoxian', which exhibited relatively low concentrations and stable values of grain-iAs^{III} (Figure 3-4), grain-DMAs^{V} (Figure 3-5), and TGAs (Figure 3-6) concentration during the two years.

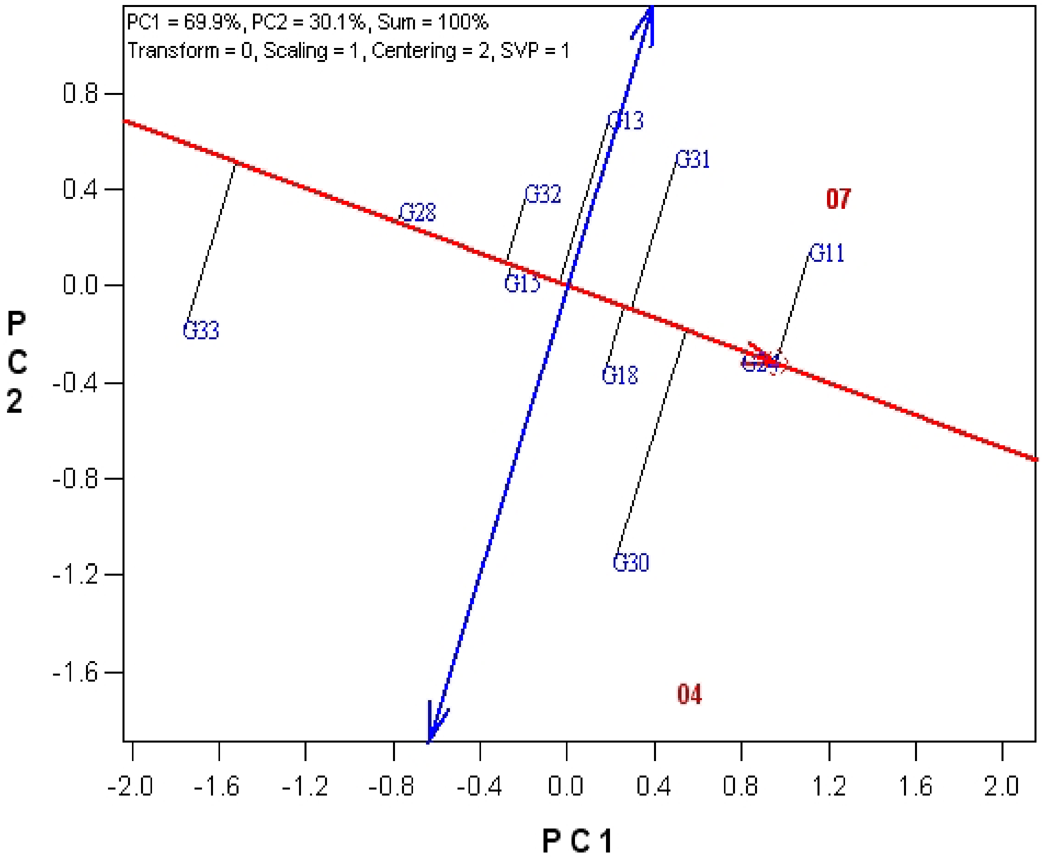

Figure 3-4. Stability-biplot of grain - iAs^{III} concentrations with ten genotypes grown under continuous flooding in years 2004 and 2007. Genotypes: G11 = Huri 282, G13 = IR- 9209, G15 = Jing 185-7, G18 = Medark, G24 = Spalcik, G28 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao 402, G32 = Zhe 733, G33 = Zhenshan 97.

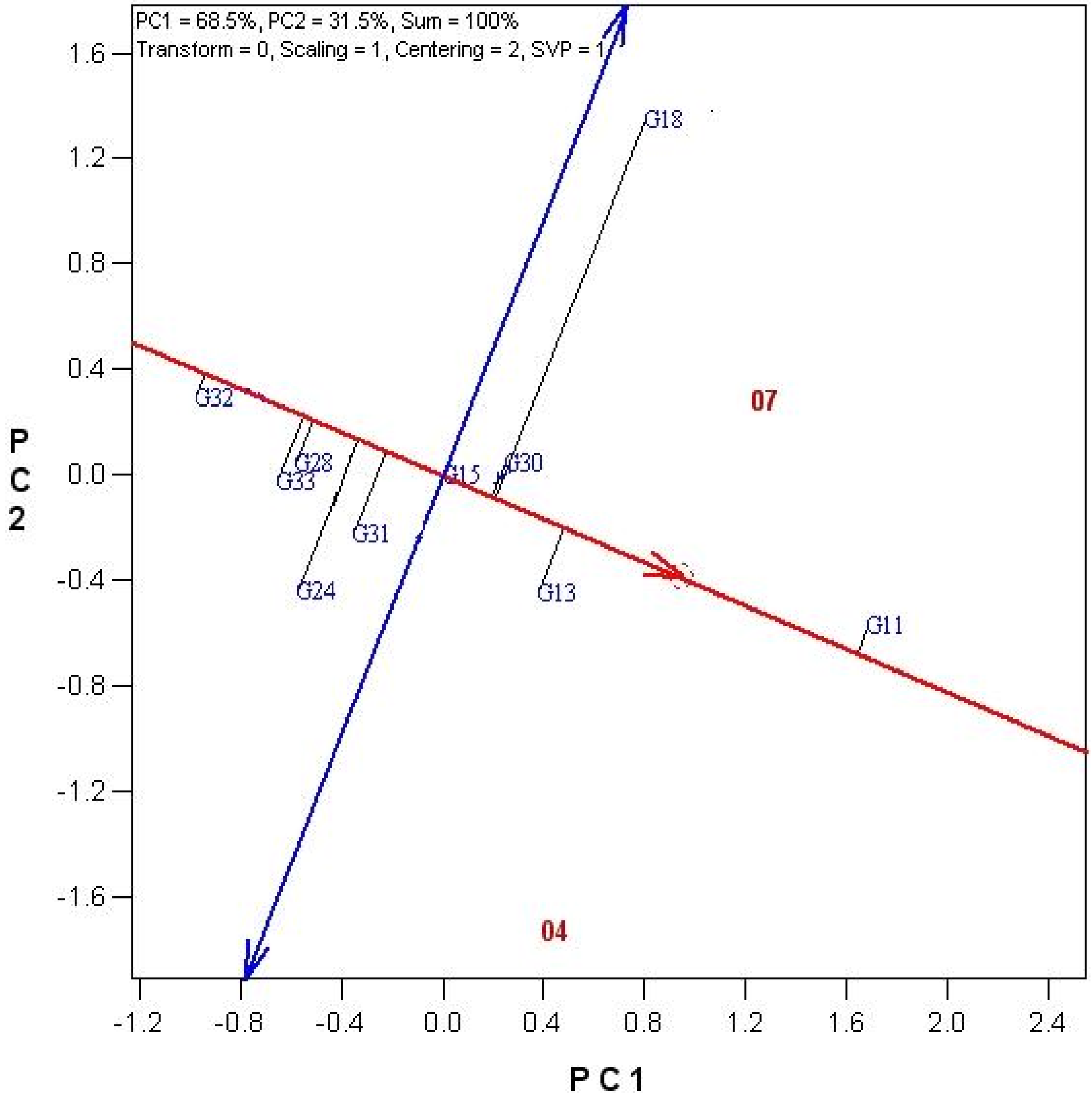

Figure 3-5. Stability biplot of grain -DMAs^{V} concentrations with ten genotypes grown under continuous flooding in years 2004 and 2007. Genotypes: G11 = Huri 282, G13 = IR- 9209, G15 = Jing 185-7, G18 = Medark, G24 = Spalcik, G28 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao 402, G32 = Zhe 733, G33 = Zhenshan 97.

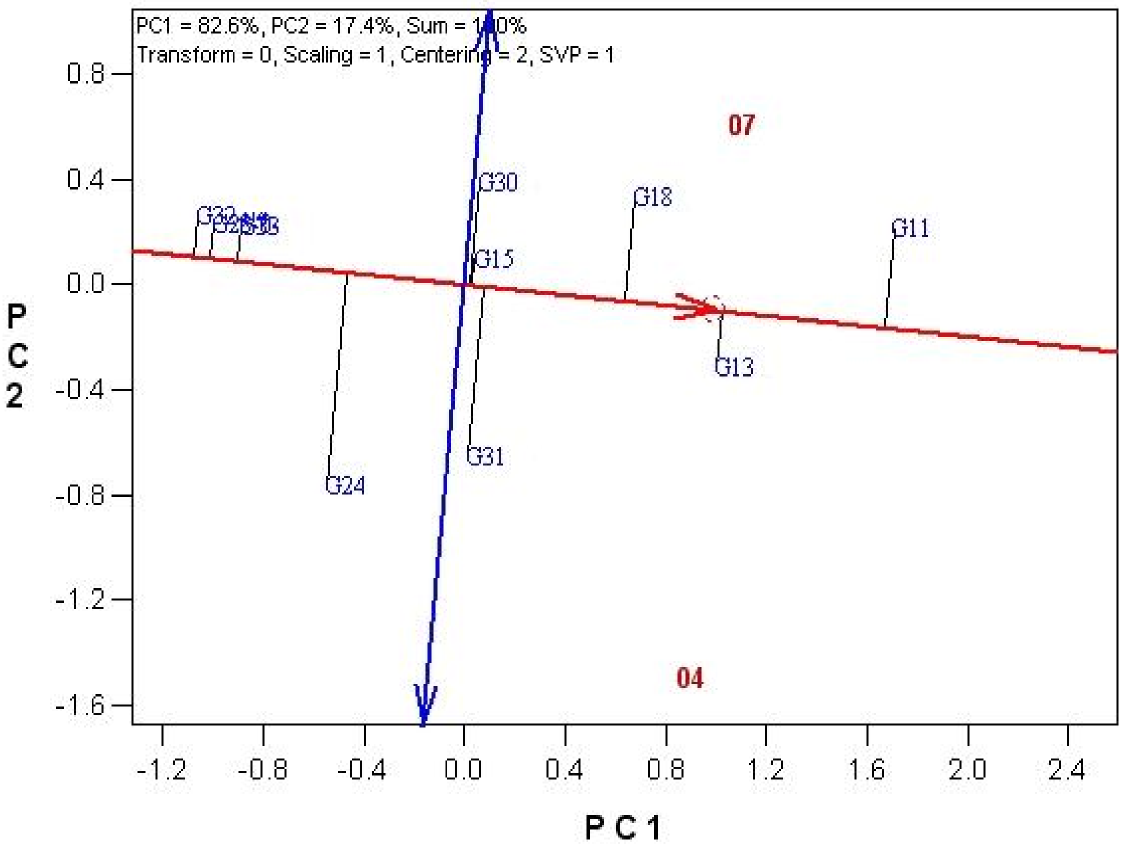

Figure 3-6. Stability biplot of TGAs concentrations with ten genotypes grown under continuous flooding in years 2004 and 2007. Genotypes: G11 = Huri 282, G13 = IR- 9209, G15 = Jing 185-7, G18 = Medark, G24 = Spalcik, G28 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao 402, G32 = Zhe 733, G33 = Zhenshan 97.

3.4. SUMMARY -IMPLICATIONS TO THE IMPROVEMENT OF RICE QUALITY

For improving food quality, development of rice cultivars with low grain-As concentrations is a desirable goal. In the current study, iAs^{V} and $MMAs^{V}$ were not detected in the rice grain, whereas, iAs^{III} and $DMAs^{V}$ were identified and their concentrations differed considerably among genotypes. Since $DMAs^{V}$ was always present in high concentrations in the grain, the TGAs concentration was mostly controlled by grain-$DMAs^{V}$ concentration. The wide genotype-dependent differences in TGAs and As-species concentrations indicated that genotype selection could be used to reduce grain-As concentration, including that of iAs^{III}, which is potentially more harmful to humans than the methyl-As forms. Low TGAs and As^{III} species concentrations were generally associated with higher yields and early heading of rice. The delayed heading of rice plants resulted in both a longer period of vegetative growth and a longer period of exposure to stresses (including possible As-induced stresses) associated with flooded soils. These factors could have contributed to increases in grain-As concentration and concurrently reduced rice-grain yields.

This study also showed a significant G x Y interaction effect on grain-As concentration, which resulted in a non-uniform ranking of some genotypes, especially those exhibiting tendencies towards relatively low TGAs concentrations in 2004 and 2005. Genotypes with very high grain-As concentrations exhibited relatively consistent rankings between years. The cultivars with lower TGAs concentrations exhibited crossover-interactions that were likely impacted by variability in localized soil and especially environmental conditions between years that affected cultivars differently. Due to the G x Y interaction, for the discrimination of relative grain-As concentrations it is always preferable to screen genotypes on a uniform native soil with moderately-high soil-As bioavailability. In the current study, the genotypes were screened on relatively uniform adjacent soils. In spite of this precaution the significant impact of Y and G x Y were evident, indicating the very important role of yearly environmental and crop cultural variables on TGAs and As-species concentration. Due to the importance of G x Y interaction in grain-As accumulation, the impacts of specific environmental and crop management parameters on As acquisition by rice deserve further study.

4. STRAIGHTHEAD SUSCEPTIBILITY IN RICE AND ITS RELATIONSHIP TO GRAIN-ARSENIC CONCENTRATION

4.1. INTRODUCTION

4.1.1. Occurrence of Straighthead in Rice

Straighthead is a physiological disorder of rice observed in lowland cultivation and characterized by reduced grain yield due to sterility of the florets (Takeokea et al., 1990). The exact cause of straighthead is not known, but As is known to be one of several factors that can induce straighthead. In the past, rice was commonly cultivated in rotation with cotton where As-based herbicides, usually monosodium monomethylarsonate (MSMA), were often used for cotton defoliation. In these soils the appearance of straighthead in rice was linked to residual soil As from previous cotton crops, suggesting that straighthead was induced as a result of either high As uptake or As-induced inhibition of healthy rice-root growth (Schweizer, 1967**;** Baker et al., 1976; Marin et al., 1993). However, incidences of natural straighthead have also been observed in other soils without a history of prior As application (Iwamoto et al., 1969; Dunn et al., 2006; Belefant-Miller and Beaty, 2007).

4.1.2. Arsenic-induced Straighthead

The typical symptoms of naturally occurring straighthead and As-induced straighthead are similar (Schweizer, 1967; Baker et al., 1976; Wells and Gilmour, 1977) and include distorted lemma and palea (defined as “parrot-beak”), twisted panicle branches, missing kernels, dark green leaves during the maturity period, abnormally large root systems with few branches, and in extreme cases, totally inhibited panicle emergence. Based on prior observations of MSMA-induced straighthead of rice grown in rotation with cotton, a straighthead susceptibility screening method was developed (Atkins, 1957; Wells and Gilmour, 1977; Yan et al., 2005). The screening method involves growing rice on soil treated with MSMA to evaluate percent grain fill and panicle emergence. The MSMA-based straighthead-screening procedure has become a routine method for cultivar selection and genetic studies of straighthead in rice (Xie, 1993; Rasamivalona et al., 1995; Yan et al., 2008).

4.1.3. Soil and Plant Factors Known to Be Associated with Straighthead

The natural occurrence of straighthead is usually more prevalent in soils with sandy to silty-loam texture (Adair et al., 1973), high organic matter content (Jones et al., 1938; Batten et al., 2006), and low pH (Baba and Harada, 1954). Straighthead can be artificially induced by incorporation of starch (Takeokea et al., 1990), sugar, or crop residue to soil (Williams, 2003), due to the effects of increased microbial activity, O_2 depletion and lower redox potential in the rhizosphere.

Studies of mineral concentrations in soil and rice plants associated with MSMA-induced straighthead (Yan et al., 2008) and naturally-occurring straighthead (Belefant-Miller and Beaty, 2007) indicated that low soil-pH and low Mg concentrations in soil and plant were factors associated with the occurrence of straighthead. The application of nitrogen fertilizer was found to reduce the severity of straighthead (Dilday et al., 2001; Yan et al., 2005; Dunn et al., 2006; Belefant-Miller and Beaty, 2007). The role of As in straighthead could not be established, because in straighthead-affected fields the As concentrations were similar in plants with or without the straighthead symptoms, except in the seeds, (Belefant-Miller and Beaty, 2007) where the As concentration of straighthead-affected plants was higher than that of adjacent healthy rice plants. This difference was attributed to reduced yield and resultant elevated mineral concentrations in the grain of straighthead-affected plants. Rice grown in soils with very high As-concentrations, for example, in regions of Bangladesh, do not always exhibit straighthead symptoms, suggesting that As by itself will not always result in straighthead symptoms (G.M. Panaullah, personal communication, 2009).

4.1.4. Concerns about High Grain-arsenic Concentrations

In general, rice grown in soils with very high As concentrations are known to produce grain with elevated As concentrations (e.g., Duxbury et al., 2003; Williams et al, 2007; Cheng et al., 2006; Hossain et al., 2009; Khan et al, 2009; Panaullah et al., 2009). The TGAs and grain-As species concentrations could vary in rice cultivars due to the strong impact of genotype. Therefore, it is of interest to select rice cultivars with low grain-As concentrations (Cheng et al., 2006; Liu et al., 2006; Pillai et al., 2009).

4.1.5. Objective

It has not yet been established whether the As-induced genotype-dependent susceptibility to straighthead is directly related to the genotype-dependent concentration of As in rice grain. The objective of the current study was to evaluate this relationship.

4.2. METHODS

4.2.1. Soil

The experiments were conducted at the USDA Dale Bumpers National Rice Research Center near Stuttgart, Arkansas, U.S.A. on a Dewitt silt loam soil (fine, smectitic, thermic, Typic Albaqualf). The soil treatments were the native soil with a soil-As concentration of 5.9 ± 1.5 mg As kg^{-1} and an adjacent soil used specifically for straighthead evaluation by application of As in the form of MSMA with a preplant soil-As concentration of 16 mg As kg^{-1} (Yan et al., 2008).

4.2.2. Rice Cultivars

Cultivars for this study were selected from the USDA world rice-germplasm collection, based on previous screening results to evaluate tolerance to straighthead (Yan et al., 2005). The sample set included indica and japonica subspecies with both straighthead-resistant and straighthead-susceptible cultivars. In 2004, 25 rice cultivars were evaluated, and the experiment was repeated with 21 cultivars in 2005. In 2007, ten cultivars tested 2004 and 2005 were selected for repeat testing based on variation in grain-As concentration and susceptibility to straighthead (Table 3-1).

4.2.3. Experimental Design and Data Collection

The experiment was conducted in a split-plot design with soil As as the main plot (i.e., the native-soil and the MSMA-treated soil), and cultivar was a completely randomized sub-plot in four replicates. Each sub-plot with individual cultivar had a dimension of 1.8 x 1.5 m with 6 rows, 1.5 m long and 0.3 m apart. A fresh MSMA application immediately before dry seeding is required each year to successfully differentiate rice cultivars for susceptibility to straighthead (W. Yan, personal communication, 2008). The monosodium monomethylarsonate (MSMA; Monterey Weed-Hoe, Fresno, CA) was sprayed on the MSMA plot (straighthead-test plot) at a rate of 6.7 kg ha^{-1} (1.49 kg As ha^{-1}) to give a final As concentration of 19.5 ± 2.5 kg As ha^{-1}. Then immediately the MSMA-treated soil and the native-soil plots were tilled identically using a Northwest tiller (Yakima, WA, USA) and both treatments were drill- seeded with a planter (Hege 1000; Hege Equipment Inc., Colwich, KS, USA). At about the four-leaf stage of rice growth, weeds were controlled by application of 9.3 L ha^{-1} of propanil (3',4'-dichloropropionanilide) mixed with 0.4 kg ha^{-1} of quinclorac (3,7-dichloroquinoline-8-carboxylic acid; Facet, BASF, Florham Park, NJ, USA). At about the five-leaf stage, urea fertilizer was applied at the rate of 134 kg N ha^{-1}, and immediately a permanent flood was established and maintained continuously until one week prior to harvest. The planting and harvesting dates were correspondingly, May 19th and Sep 10th in 2004, April 21st and Aug 27th in 2005, and April 15th and Aug 22nd in 2007. The heading date (defined as the number of days from planting until 50 % panicle emergence from the flag leaf), plant height, and yield were recorded for each plot from the center 0.6 m of the two center rows in each plot to avoid the border effects (Figure 3-1) in the rice plots (Yan et al., 2005). The straighthead symptoms were visually rated on a scale of 1 (resistant) to 9 (susceptible) based on floret sterility and panicle emergence from the flag leaf sheath (Table 4-1; Yan et al., 2005).

Table 4-1. Straighthead-rating scale for visual ranking of rice cultivars based on panicle emergence and resistance to panicle sterility (Yan et al., 2005).

Straighthead Rating	Symptoms
1	No apparent sterility (more than 80% grains developed) and about 100% of the panicles emerged
2	71 to 80% of the grains developed and 96 to 100% of the panicles emerged
3	61 to 70% of the grains developed and 91 to 95% of the panicles emerged
4	41 to 60% of the grains developed and 61 to 90% of the panicles emerged
5	21 to 40% of the grains developed and 31 to 60% of the panicles emerged (at this stage distorted and parrot-beak grains initially appear)
6	11 to 20% of the grains developed and 10 to 30% of the panicles emerged
7	0 to 10% of the grains developed and most of the panicles emerged but remained totally erect
8	No grains developed and 0 to 10% of the panicles emerged from the flag leaf sheath but erect
9	short stunted plants with no panicle emergence

The rice grain for As analysis was obtained from the center 0.6 m of the center two rows in each plot to avoid the border effects (Figure 3-1) observed in the rice plots. The harvested rice was dehulled using a Kett rice husker (TR-200 Electromotion rice husker, Kett, Tokyo, Japan), milled using a Kett grain polisher (PEARLEST, Kett, Tokyo, Japan) and powdered using a grinder (Foss Cemotec 1090, Slangerupgade, DK-3400 Hilleroed, Denmark). To avoid cross contamination, the grain from the native-soil and MSMA-treated soil were prepared in separate batches and the polisher and grinder were cleaned after each sample processing. Nineteen cultivars representing a range of straighthead susceptibilities and total grain-As concentrations were analyzed for As species [(inorganic arsenite (iAs^{III}); inorganic arsenate (iAs^{V}); monomethylarsonic acid ($MMAs^{V}$), dimethylarsinicacid ($DMAs^{V}$)].

4.2.4. Analysis of Arsenic Concentrations

The total grain-As (TGAs) concentration in rice samples was determined following open-vessel digestion of powdered milled grain with trace-metal grade HNO_3/H_2O_2. Grain samples (0.5 g) were digested with 0.5 mL HNO_3 in a teflon tube capped with a funnel and heated on a temperature-programmable 48-well graphite-block digestion system (Digi Prep MS, SCP Science, Montreal, Canada). During the HNO_3 digestion step, the digestion block was heated to 50 °C for 240 min, 60 °C for 240 min, and 120 °C for 240 min and allowed to cool. Then two rounds of H_2O_2 digestion, each involving the addition of 3 mL H_2O_2, were followed by heating to 130 °C for evaporation to dryness. For quality control, two samples of a standard reference material (1568a rice flour, NIST, Gaithersburg, MD, USA) with a certified As-concentration of

0.29 ± 0.03 μg As g^{-1}, two blanks, and three sample replicates were included in each digestion batch. The completely digested and dried samples were redissolved in 15 mL of 2 % HNO_3 and analyzed by inductively-coupled-plasma mass-spectroscopy (ICP-MS) using a Perkin Elmer ELAN DRC II (Perkin-Elmer Sciex, Concord, ON, Canada) fitted with a Meinhard concentric nebulizer and cyclonic spray chamber. To verify TGAs concentrations, outliers were reanalyzed, but individually verified values were not removed during calculations of mean and standard error, since this variation could have been due to either natural genetic variation or localized soil differences or both.

The grain-As species were extracted using a modification of the method described by Heitkemper et al. (2001). Deioinized (DI) water (1690 μL) followed by 310 μL of 99.9 % trifluoroacetic acid (TFA) (a final concentration of 2 M TFA) was added to ~0.5 g of rice flour in a 50 mL polypropylene tube that was then capped and heated at 80 °C for 4 hr using a temperature-programmable graphite-block digestion system (Digi Prep MS, SCP Science, Montreal, Canada). The mixture was then diluted with 20 mL DI water, homogenized by vortexing for 1 min, and centrifuged for 20 min at 3600 rpm. The supernatant solution was then collected, evaporated to near dryness, redissolved in 15 mL DI water, and filtered through a 0.2 μm nylon membrane filter. The As species were separated using a PerkinElmer 200 HPLC system (Waltham, MA, USA) with a guard column (IonPac Dionex AG7, Sunnyvale, CA, USA) and an anion-exchange column (IonPac Dionex AS7), attached in-line to the ICP-MS for As analysis. The separation scheme for As speciation consisted of a 1-min elution with 1 mM HNO_3

followed by a 6-min linear-gradient elution from 1 to 50 mM HNO_3. A chromatographic internal standard of 5 μg As L^{-1} As was pulsed at 6.5 min post-injection. The quantification of As species by ICP-MS induces a variable enhancement of the As signal due to the presence of C-containing compounds extracted from rice grain. This potential error was minimized by the addition of 3 % CH_3OH/H_2O into the sample line after the guard and anion-exchange columns (Larsen and Sturup, 1994). A 20-cm coil was added to the ICP-MS sample input tubing to ensure complete mixing of the CH_3OH with the column eluant (James et al., 2008). The standard-curve was obtained using mixtures of four As species, namely $DMAs^V$ (dimethylarsinic acid; Chem Service, West Chester, PA, USA), $MMAs^V$ (monosodium methylarsonate sesquihydrate; Chem Service), iAs^{III} (As_2O_3; Alfa Aesar, Johnson Matthey Company, Westhill, MA, USA), and iAs^V (SPEX Centriprep, Metuchen, NJ, USA). Perkin Elmer Chromera software was used to control the HPLC and ICP-MS instruments, as well as for data collection and analysis. To evaluate the efficiency of As-species extraction from each rice sample, the TGAs concentrations obtained by the HNO_3/H_2O_2 digestion method were compared with the sum of As species identified by the TFA extraction method (Pillai et al., 2009a).

4.2.5. Statistical Analysis

Statistical procedures for analysis of variance (ANOVA), mean comparison, and correlation were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Generalized Linear Model (GLM) procedure was used for variance analysis, where the main effects of genotype (G), year (Y), and their interaction (G x Y) were considered as sources of variance. From the GLM output, coefficient of variance (*C.V.*) and sum of squares (S.S.) were also obtained. As a part of ANOVA, the percentage variation explained by each source of variation was calculated for the individual traits using the S.S. and the formula $\{[\text{S.S.}_{(\text{trait x factor})}] / \text{total S.S.}\}*100$, which indicates the percentage sum of squares of each trait x factor combination. The CORR and REG procedures were used to obtain Pearson's correlation coefficients (r) and the corresponding significance levels, and the LSMEAN and MEANS procedures were used to estimate least square mean (mean) and least square difference (LSD), respectively.

The graphical analysis of relationships between agronomic traits and grain-As concentrations was performed using GGE biplot software (version 6.1) following the methods of Yan and Tinker (2006). The biplot is a graphical display of a two-rank matrix as originally described by Gabriel (1971), Kempton (1984), Kroonenberg (1995). Later Yan et al. (2006) developed the principal-components based methodology that is known today as the GGE (Genotype, Genotype-by-Environment) biplot method of cultivar comparison and multi-environment data analysis. The GGE biplot utilizes the first two principal components from the data to display various graphs.

The "Relationship among testers" view biplot was produced to assess relationships between traits using a three-way dataset of cultivars, traits, and replicates. This view was obtained by singular value partitioning ("SVP=2"), no data transformation ("Transform=0"), scaling by standard deviation ("scaling=1"), and centering by G+GE ("centering=2"). In this view, a relatively greater length of a vector represents a relatively better ability of the corresponding trait to discriminate the cultivars (Yan and Kang, 2003). The cosine of the angle between the two vectors approximates the correlation between two traits.

4.3. RESULTS AND DISCUSSION

4.3.1. Grain-arsenic Concentrations and Speciation

The TGAs and grain-DMAs^{V} concentrations of all cultivars from the MSMA-treated soil were significantly higher than those from the native soil (Table 4-2). With the MSMA treatment, the TGAs concentrations in 2004 ranged from 0.690 to 2.697 mg As kg^{-1}, but in the native soil, concentrations ranged from 0.188 to 0.599 mg As kg^{-1} (Table 4-2). In 2004 and 2005, the MSMA treatment resulted in approximately four times (3.6 and 4.5 times, respectively) higher TGAs concentrations than with the native-soil (Appendix A). Soil-As concentrations with the MSMA treatment were about three times (2.7 to 3.3 times, respectively) higher than that those with the native soil treatment. Considerably higher TGAs concentrations were observed with the MSMA treatment in the current study compared to the TGAs concentrations in rice observed in other studies with considerably higher soils-As concentrations (e.g., Panaullah et al., 2009). These results taken together indicate that As bioavailability and accumulation of TGAs in rice grain following MSMA application was much greater than that from naturally occurring inorganic-As species dominant in native soils.

Table 4-2. Comparison of mean As-concentrations from two soil treatments (native soil and MSMA treated soil) for nineteen genotypes grown under consistently flooding conditions in 2004.

Cultivar Name	Sub-species	†TGAs				‡Grain-iAs^{III}				§Grain-DMAs^{V}			
		MSMA-treated soil	Native soil	Percent difference (%)	P<|t|	MSMA-treated soil	Native soil	Percent difference (%)	P<|t|	MSMA-treated soil	Native soil	Percent difference (%)	P<|t|
IR-9209	I	0.990	0.564	76	0	0.077	0.094	-18	*	0.699	0.322	54	***
Minkenzao	I	0.845	0.507	67	***	0.094	0.084	12	*	0.712	0.339	52	***
Luhongzao	I	1.418	0.351	304	***	0.095	0.087	9		1.421	0.247	83	***
IR-31779	I	1.319	0.434	204	***	0.103	0.094	10		1.019	0.267	74	***
Xiangzaoxian	I	1.446	0.229	531	***	0.105	0.099	6		1.549	0.199	87	***
UVSUnblatuzi	I	1.635	0.343	377	***	0.108	0.114	-5		1.242	0.233	81	***
Zhe-733	I	0.810	0.268	202	***	0.112	0.104	8		0.591	0.14	76	***
IR-44595	I	1.112	0.416	167	***	0.113	0.102	12		0.898	0.243	73	***
Jing-185-7	J	0.690	0.402	72	***	0.123	0.121	1		0.489	0.23	53	***
Zao-402	I	2.517	0.514	389	***	0.123	0.107	15		1.574	0.254	84	*
Ponta Rubra	J	2.290	0.519	341	***	0.135	0.143	-6		2.204	0.254	88	***
Zhenshan	I	2.551	0.368	594	***	0.138	0.109	26	*	1.776	0.207	88	***
Spalcik	J	2.467	0.535	361	***	0.139	0.155	-10		1.979	0.283	86	***
Zhongyouzao	I	2.519	0.475	430	***	0.14	0.141	0		1.893	0.278	85	***
Huri-282	I	0.999	0.599	67	*	0.142	0.136	4		0.787	0.396	50	**
CNTLR-800	I	0.794	0.326	144	***	0.143	0.131	9		0.545	0.243	55	*
Medrak	J	2.170	0.507	328	***	0.17	0.147	15		1.931	0.267	86	**
Zanuo No1	I	2.697	0.409	559	***	0.218	0.188	16		2.54	0.231	91	***
Cocodrie	J	-	0.188	-	-	-	0.095	-	-	-	0.093	-	-
Average		1.626	0.418	290		0.126	0.118	6		1.325	0.249	81	
Median		1.432	0.416	316		0.123	0.109	8		1.331	0.247	81	
Lower Range		0.690	0.188	67		0.077	0.084	-18		0.489	0.093		
Upper Range		2.697	0.599	594		0.218	0.188	26		2.54	0.396		
LSD		0.290	0.072			0.029	0.018			0.415	0.04		
C.V. (%)		13	11			15	10			21	11		

†TGAs = total grain-As concentration (mg As kg^{-1}) of determined following HNO_3/H_2O_2 digestion

‡Grain-iAs^{III} = inorganic iAs^{III} concentration (mg As kg^{-1}) in grain as determined by TFA extraction

§Grain-DMAs^{V} = dimethylarsinic acid (mg As kg^{-1}) in rice grain as determined following 2 M TFA extraction method.

In comparison to TGAs, the grain-iAs^{III} concentrations of most cultivars from the MSMA-treated soil were not significantly different from those of the native-soil (Table 4-2). The grain-iAs^{III} concentrations in the current study were not strongly impacted by the differences in soil-As concentration and speciation between the MSMA-amended and native soils. With the MSMA treatment, a relatively narrow range of grain-iAs^{III} concentrations was obtained (0.077 to 0.218 mg As kg^{-1}), compared to the considerably larger ranges in TGAs and grain-DMAs^{V} concentrations (Table 4-2). The proportion of grain-iAs^{III} to TGAs concentration was very low, averaging 28 % with the native soil and 7.7 % with the MSMA treatment. The balance of the total As was principally DMAs^{V}, though there was the small proportion of unaccounted As, varying from 0-33 and 0-30% in samples from the native-soil and MSMA-treatment plots, respectively. The relatively low proportion of grain-iAs^{III} is encouraging in terms of dietary intake of As, since the inorganic species of As are considered to be more harmful than the organic species (Johnson, 1990; Cohen et al., 2006). Grain-DMAs^{V} was the dominant species of As in rice grain from native (59%) and MSMA-amended (81%) soils.

The correlation of grain-DMAs^{V} with TGAs indicates that grain-DMAs^{V} concentration increases with increase in TGAs concentration (Figure 4-1 and Figure 4-2). However the grain-iAs^{III} concentrations were considerably lower and more stable than the grain-DMAs^{V} concentrations.

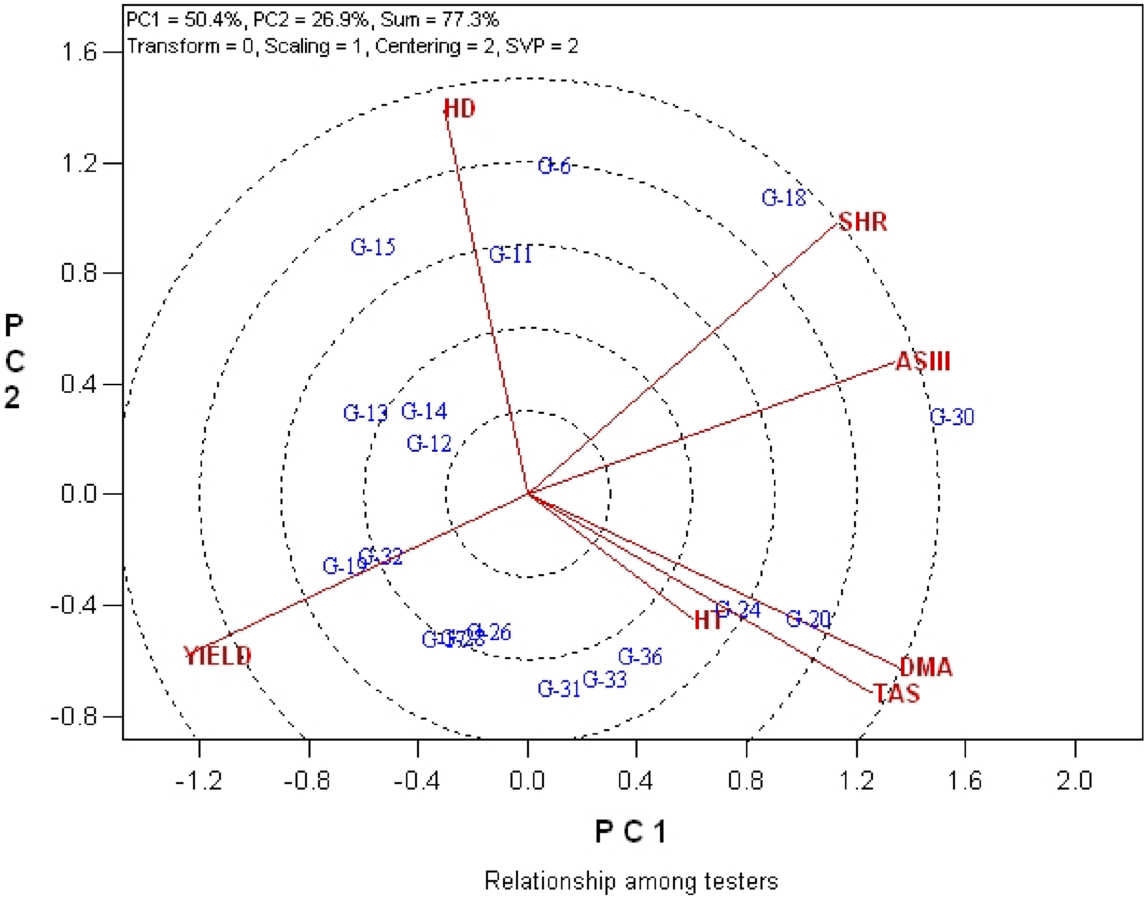

Figure 4-1. GT biplot showing relationships between various agronomic traits of 18 genotypes grown in 2004 under continuous flooding and MSMA-treated soil. HD = days to 50 % heading; yield = grain yield (kg ha-1); PHT = Plant Height (cm); TGAs = total grain As concentration (mg As kg-1); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}). Genotypes: G6 = CNTLR-800, G37 = Cocodrie, G11 = Huri-282, G12 = IR-31779, G14 = IR-44595, G13 = IR-9209, G15 = Jing-185-7, G17 = Luhongzao, G18 = Medrak, G19 = Minkenzao, G20 = Ponta Rubra, G24 = Spalcik, G24 = Spalcik, G27 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao-402, G32 = Zhe-733, G33 = Zhenshan, G36 = Zhongyouzao)

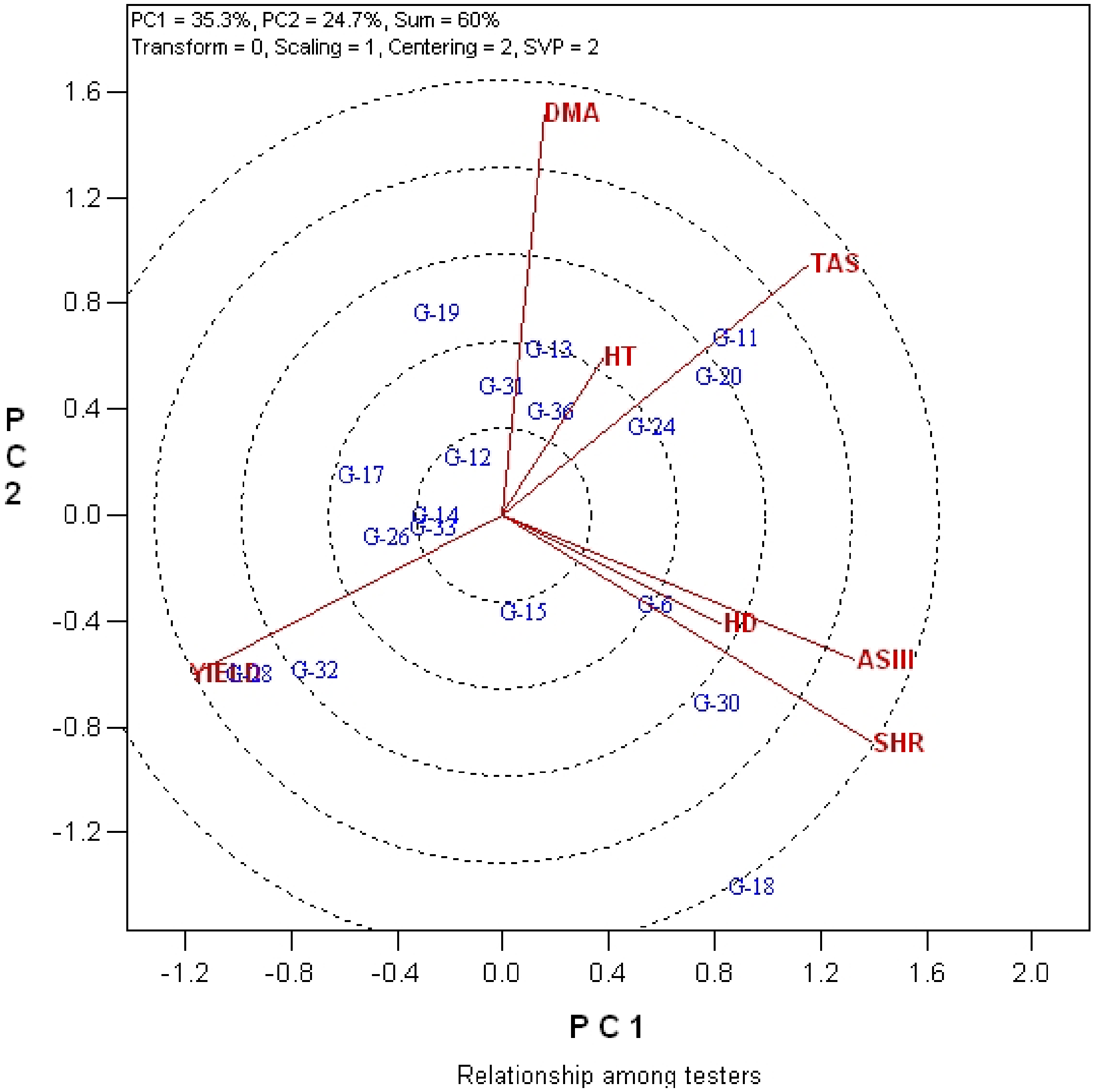

Figure 4-2. GT biplot showing relationships between various agronomic traits of 18 genotypes grown in 2004 under continuous flooding and native soil. HD = days to 50 % heading; yield = grain yield (kg ha-1); PHT = Plant Height (cm); TGAs = total grain As concentration (mg As kg^{-1}); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-$DMAs^{V}$ concentration (mg As kg^{-1}). Genotypes: G6 = CNTLR-800, G37 = Cocodrie, G11 = Huri-282, G12 = IR-31779, G14 = IR-44595, G13 = IR-9209, G15 = Jing-185-7, G17 = Luhongzao, G18 = Medrak, G19 = Minkenzao, G20 = Ponta Rubra, G24 = Spalcik, G24 = Spalcik, G27 = Xiangzaoxian, G30 = Zanuo No1, G31 = Zao-402, G32 = Zhe-733, G33 = Zhenshan, G36 = Zhongyouzao)

In the GGE biplots, the straighthead-rating vectors (representing the MSMA-treatment plots) were nearly perpendicular to the respective TGAs-concentration vectors representing the MSMA-treatment (Figure 4-3) and the native-soil (Figure 4-4), indicating a lack of association between these two traits. This result was confirmed by correlation analyses in which straighthead ratings were not correlated with TGAs concentrations from the MSMA-treated soil ($r = 0.096$, $P = 0.396$, in 2004; $r = 0.071$, $P = 0.519$, in 2005) or native soil ($r = -0.176$, $P = 0.118$, in 2004; $r = -0.063$, $P = 0.576$, in 2005). Therefore, panicle sterility associated with straighthead might not be directly linked to high As-accumulation in the grain. In addition, this result indicates that TGAs concentrations are not necessarily high in straighthead-susceptible cultivars. For example, the TGAs concentration in the straighthead-susceptible cultivar "Cocodrie" was relatively low (0.188 mg As kg^{-1}). On the other hand, the highly straighthead-resistant cultivar "Zhe-733" also exhibited a relatively low TGAs concentration (0.268 mg As kg^{-1}).

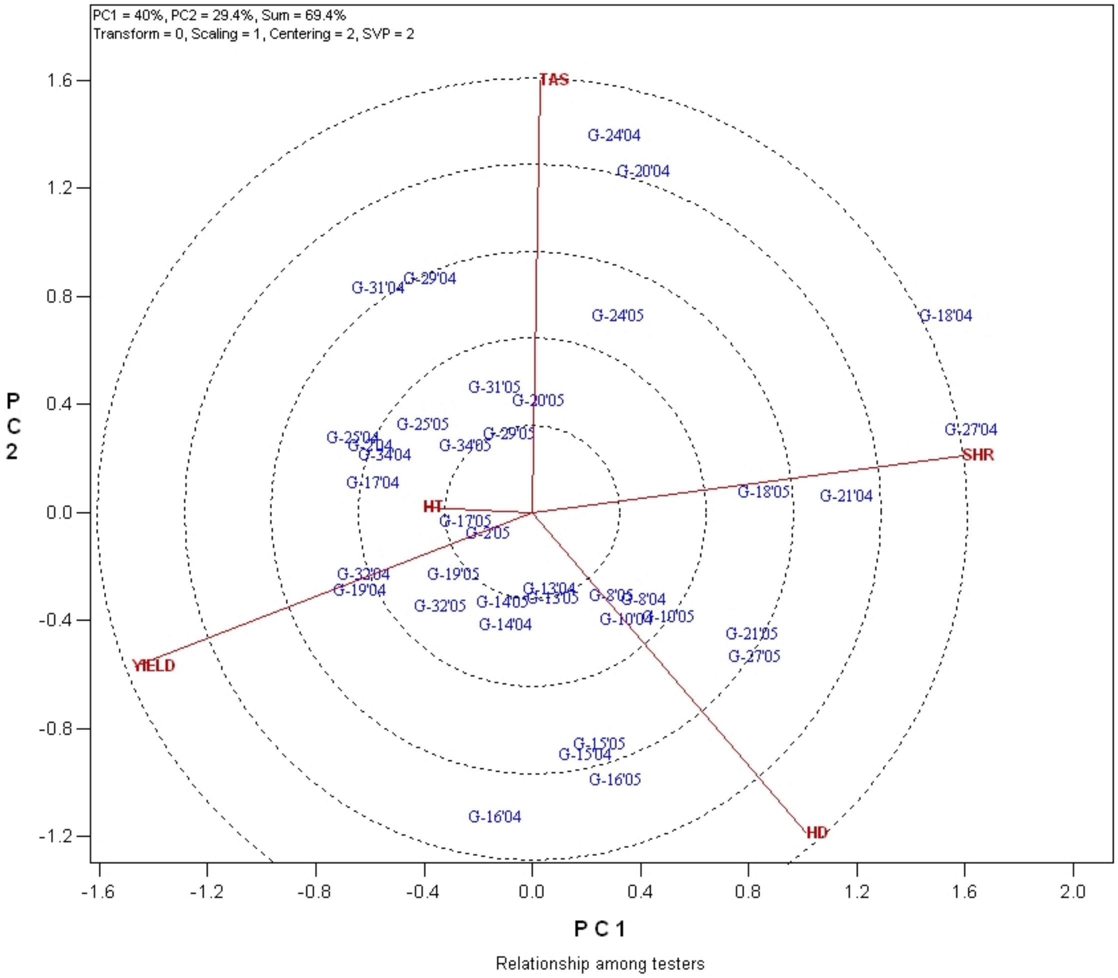

Figure 4-3. GT biplot showing relationships between various agronomic traits of 21 genotypes grown in years 2004 and 2005 under continuous flooding and MSMA treated soil. HD = days to 50 % heading; yield = grain yield (kg ha^{-1}); PHT = Plant Height (cm); TGAs = total grain As concentration (mg As kg^{-1}) Genotypes: G2 = Aijiaonante, G37 = Cocodrie, G8 = Danwanbao, G10 = Gui 99, G14 = IR-44595, G13 = IR-9209, G15 = Jing-185-7, G16 = Jinnuo No.6, G38 = KBNT-1-1, G17 = Luhongzao, G18 = Medark, G19 = Minkenzao, G20 = Ponta Rubra, G21 = Priscilla, G24 = Spalick, G25 = Tie-90-1, G27 = Wells, G29 = You-I-B, G31 = Zao 402, G32 = Zhe 733, G34 = Zhong-86-44)

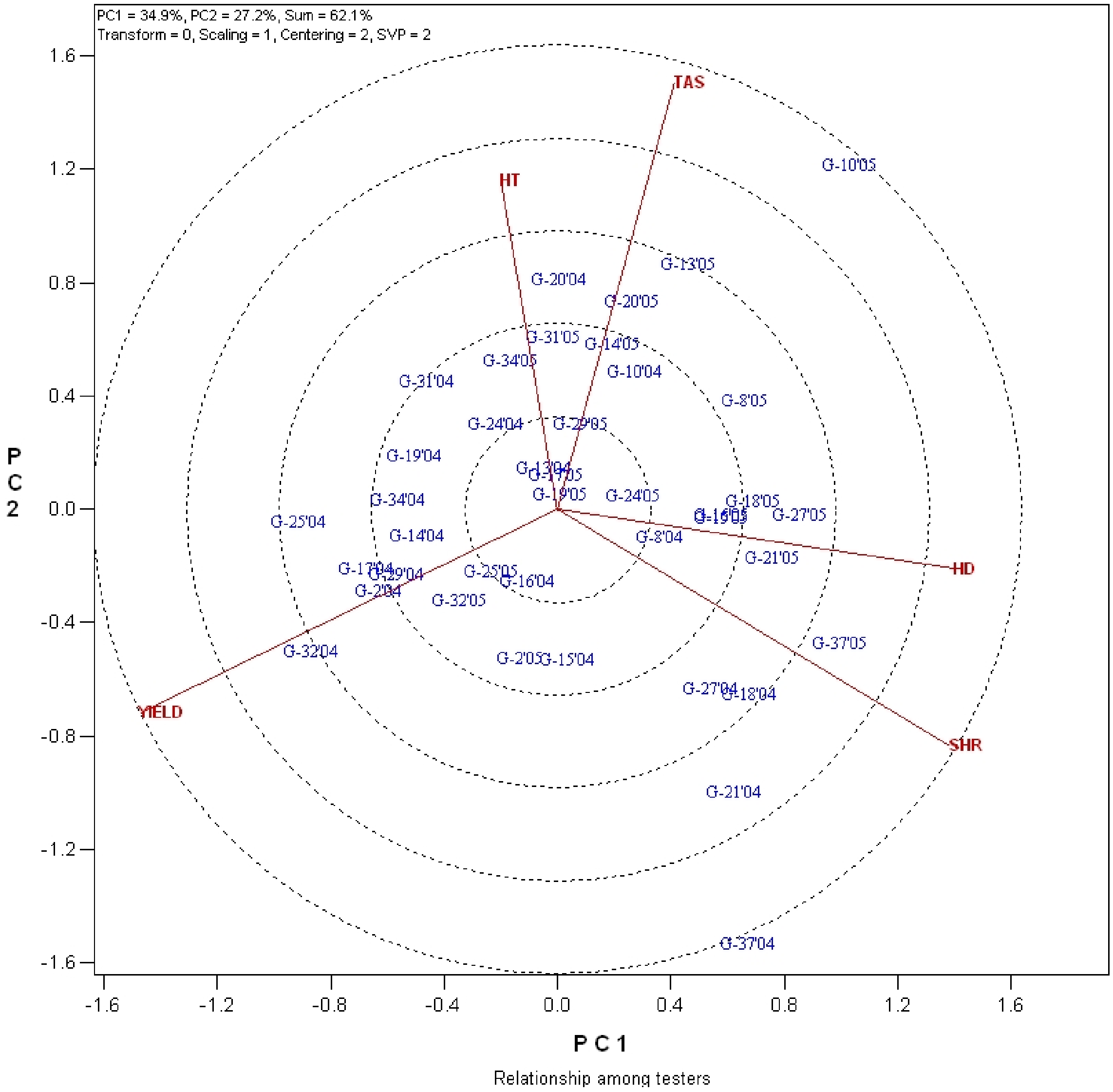

Figure 4-4. GT biplot showing relationships between various agronomic traits of 21 genotypes grown in years 2004 and 2005 under continuous flooding and native soil. HD = days to 50 % heading; yield = grain yield (kg ha^{-1}); PHT = Plant Height (cm); TGAs = total grain As concentration (mg As kg^{-1}) Genotypes: G2 = Aijiaonante, G37 = Cocodrie, G8 = Danwanbao, G10 = Gui 99, G14 = IR-44595, G13 = IR-9209, G15 = Jing-185-7, G16 = Jinnuo No.6, G38 = KBNT-1-1, G17 = Luhongzao, G18 = Medark, G19 = Minkenzao, G20 = Ponta Rubra, G21 = Priscilla, G24 = Spalick, G25 = Tie-90-1, G27 = Wells, G29 = You-I-B, G31 = Zao 402, G32 = Zhe 733, G34 = Zhong-86-44)

4.3.2. Influence of Genotype on Grain-arsenic Concentration and Speciation with MSMA Treatment

Analysis of variance (ANOVA) indicated that the TGAs concentrations from the MSMA-treated and continuously flooded soil were significantly affected by genotype (G), year (Y) and G x Y interaction effects (Table 4-3). The influence of the G was dominant, with 73% of the total variance explained by genotype. The percent of the total variance in TGAs concentration due to Y and G x Y interaction effects were considerably lower (9 and 11%, respectively) than that due to G. Similarly, the G effect explained 76% of the total variance in grain-$DMAs^{V}$ concentration, with a smaller percent of the total variation attributable to G x Y interaction (8%) and Y (4%) (Table 4-4). The iAs^{III} concentration was relatively stable across years, and was mostly impacted by genotype by G (33%) and G x Y interaction (45%). From these results, it can be concluded that with the MSMA-treated soil, grain-As concentration in rice is strongly genotype-dependent and that selection and breeding for low-As cultivars would be an important component of strategies to minimize As hazard in rice.

Table 4-3. Variance analysis of agronomic traits and grain-As concentrations for nineteen rice genotypes grown in 2004 and 2005 on MSMA-treated soil under consistently flooded conditions.

Source of variation	d.f.	Level of significance					% of total Sum of Squares				
		HD	HT	Yield	TGAs	SHR	HD	HT	Yield	TGAs	SHR
Year (Y)	1	**			***	***	1	0	0	9	1
Genotype (G)	18	***	***	***	***	***	97	71	75	73	78
Year x Genotype (G x Y)	18	***	***	***	***	***	1	18	13	11	10

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.
HD = days to 50 % heading; HT = plant height (cm); yield = grain yield (kg ha^{-1}); TGAs = total As concentration (mg As kg^{-1}) following HNO_3/H_2O_2 digestion

Table 4-4. Variance analysis of As species concentrations for twenty-one rice genotypes grown in 2004 and 2005 on MSMA-treated soil under consistently flooded conditions.

Source of variation	d.f.	Level of significance		% of total Sum of squares	
		iAs^{III}	$DMAs^{V}$	iAs^{III}	$DMAs^{V}$
Year (Y)	1		**	0	4
Genotype (G)	9	***	***	33	76
Year x Genotype (G x Y)	9	***	***	45	8

iAs^{III} = inorganic As^{III}(mg As kg^{-1}), $DMAs^{V}$ = dimethylarsinic acid (mg As kg^{-1}), as determined following 2 M TFA extraction method.
*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.

4.3.3. Influence of Genotype on Grain-arsenic Concentration and Speciation with Native-soil

In the native soil under continuous flooding, both TGAs and grain-$DMAs^{V}$ concentrations were significantly affected by G, Y, and G x Y interaction effects (Table 3-4 and Table 3-5). The contributions of the G, Y, and G x Y factors towards the total variation in TGAs concentration were 70.1, 3.5, and 17.1 %, respectively. The larger contribution of G indicates that the differences in TGAs concentration were mostly genotype dependent. The average TGAs concentration in 2004 (0.447 mg As kg^{-1}) was significantly lower than that in 2005 (0.555 mg As kg^{-1}). This variation of TGAs concentration by Y was likely impacted by climatic differences. Although adjacent plots were used in 2004 and 2005, the chemical compositions and mineralogies of these soils might not be identical. The higher temperatures in 2005 compared to 2004, likely contributed to the decreased yields in 2005 (Satake et al., 1982; Feller et al., 1998; Tao et el., 2007) and the associated higher TGAs concentrations as discussed below. The significant G x Y interaction of TGAs concentration implies that annual differences in growth condition impacted some individual genotypes disproportionally. As a result, the relative rankings of several cultivars for TGAs concentration were different in 2004 versus 2005. The variance analysis indicated that grain-DMAs^{V} concentrations were significantly different by G, Y, and G x Y, which explained 68.8, 7.1, and 9.7 %, respectively, of the total variation in TGAs concentration (Table 3-5). This trend of grain-DMAs^{V} concentration was similar to that of TGAs concentration. The average grain-iAs^{III} concentrations observed in year 2004 and 2007 were similar; therefore, the Y effect was not significant. However, a few cultivars had different for grain-iAs^{III} concentrations in the two years resulting in a significant G x Y interaction (Table 3-3).

A field study of rice-grain As and other elemental constituents was conducted by Cheng et al. (2006) using nine genotypes grown over six locations (agricultural soil) and three years. Cheng et al. (2006) also reported significant differences in TGAs concentration among genotypes, but unlike the current study, found the Y effect to be non-significant. However, they did observe significant differences in TGAs concentration by location (L) and interaction of genotype and location (GxL), which demonstrated that genotype and soil properties had combined effects on TGAs concentration. The specific environmental factors that contributed to variability of TGAs concentration in the current study deserve further investigation.

4.3.4. Association of Straighthead with Grain Arsenic and Agronomic Traits

In the native soil, rice grew normally without straighthead symptoms. The MSMA treatment effectively differentiated cultivars for straighthead performance ranging from resistant (rated 1) to highly susceptible (rated 8) in both 2004 and 2005 (Table 4-5). The ANOVA indicated that G, Y, and G x Y effects explained 78, 1, and 10 %, respectively, of the total variation in MSMA-induced straighthead rating. These results indicate that with the MSMA treatment, straighthead ratings were mostly genotype dependent in the MSMA-amended soil.

Table 4-5. Comparison of mean agronomic-traits from two soil treatments (native soil and MSMA treated soil) for nineteen genotypes grown under consistently flooding conditions in 2004.

Cultivar	Sub-species	Heading (days)				Yield (kg/ha)				Straighthead Rating			
		MSMA-treated soil	Native soil	Difference (days)	P<|t|	MSMA-treated soil	Native soil	Percent difference (%)	P<|t|	MSMA-treated soil	Native soil	Absolute difference	P<|t|
IR-9209	**I**	**76**	**70**	**6**	*	**5861**	**7522**	**-22**	***	**1.75**	**1**	**1**	*
Minkenzao	**I**	**64**	**61**	**3**	**	**8260**	**8299**	**0**		**1**	**1**	**0**	
Luhongzao	**I**	**65**	**59**	**7**	***	**7776**	**8435**	**-8**	**	**1**	**1**	**0**	
IR-31779	**I**	**75**	**67**	**8**	**	**5996**	**7694**	**-22**	**	**1.5**	**1**	**1**	
Xiangzaoxian	**I**	**66**	**63**	**3**	***	**8396**	**10993**	**-24**	***	**1.25**	**1**	**0**	
UVSUnblatuzi	**I**	**57**	**54**	**3**	*	**5740**	**8134**	**-29**	*	**1**	**1**	**0**	
Zhe-733	**I**	**62**	**61**	**2**		**7696**	**9464**	**-19**		**1**	**1**	**0**	
IR-44595	**I**	**82**	**72**	**10**	***	**6873**	**9348**	**-26**	***	**1.5**	**1**	**1**	
Jing-185-7	**J**	**92**	**87**	**5**	*	**6199**	**8333**	**-26**	**	**1.5**	**1**	**1**	
Zao-402	**I**	**66**	**63**	**4**	**	**7627**	**8065**	**-5**		**1**	**1**	**0**	
Ponta Rubra	**J**	**63**	**60**	**3**	**	**1874**	**6375**	**-71**	***	**3.25**	**1**	**2**	*
Zhenshan	**I**	**66**	**66**	**0**		**7096**	**8432**	**-16**	*	**1**	**1**	**0**	
Spalcik	**J**	**58**	**57**	**1**		**2794**	**7430**	**-62**	***	**2.75**	**1**	**2**	*
Zhongyouzao	**I**	**66**	**62**	**4**	**	**5529**	**7815**	**-29**		**1**	**1**	**0**	
Huri-282	**I**	**91**	**87**	**4**	**	**6147**	**8164**	**-25**		**3.25**	**2**	**1**	
CNTLR-800	**I**	**89**	**86**	**3**	*	**3055**	**7374**	**-59**	***	**4**	**1.25**	**3**	***
Medrak	**J**	**83**	**78**	**5**	*	**2509**	**7424**	**-66**	***	**6.25**	**2**	**4**	***
Zanuo No1	**I**	**66**	**69**	**-3**		**2306**	**8912**	**-74**	***	**5.5**	**2**	**4**	***
Cocodrie	**J**	**87**	**80**	**8**	**	**76**	**7511**	**-99**	***	**8**	**2**	**6**	***
Average		**72**	**68**	**4**		**5358**	**8196**	**-36**		**2.5**	**1.22**	**1**	
Median		**66**	**66**	**4**		**5996**	**8134**	**-26**		**1.5**	**1**	**1**	
Lower Range		**57**	**54**	**-3**		**76**	**6375**	**-99**		**1**	**1**	**0**	
Upper Range		**92**	**87**	**10**		**8396**	**10993**	**0**		**8**	**2**	**6**	
LSD		**2.7**	**2.1**			**1365**	**1045**			**1**	**0.16**		
***C.V.* (%)**		**3**	**2**			**18**	**9**			**28**	**9**		

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.

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4.3.5. Association of Straighthead with Grain Arsenic and Agronomic Traits

The grain-iAs^{III} concentration was positively correlated with straighthead rating as indicated in the GT biplots (Figure 4-1 and Figure 4-2) by the acute angles between the respective vectors and confirmed by the correlation analysis ($r = 0.569$, $P < 0.001$, in the MSMA-treated soil; $r = 0.657$, $P < 0.001$, in the native soil). Therefore, in general, the cultivars with high grain-iAs^{III} concentrations in native soil or MSMA-treated plots were also highly susceptible to straighthead. High soil-As concentrations have been linked to the occurrence of straighthead (Schweizer, 1967; Atkins, 1957; Wells and Gilmour, 1977). The current study indicates that the panicle sterility observed in straighthead incidences are not directly related to grain-As concentration.

4.3.6. Relationship of Straighthead Ratings to Heading Date

The GGE biplot indicates a positive correlation between straighthead rating and heading with the MSMA-treated and the native soils, as represented by the acute angles between the respective vectors (Figure 4-3 and Figure 4-4Figure 4-4). The straighthead rating (from the MSMA treatment) was significantly correlated to heading in rice cultivars from the native soil ($r = 0.446$, $P > 0.001$, in 2004; $r = 0.462$, $P > 0.001$, in 2005) and the MSMA-treated soil ($r = 0.442$, $P > 0.001$, in 2004; $r = 0.303$, $P > 0.006$, in 2005). These results indicate that early maturity of rice cultivars was beneficial in reducing straighthead severity, possibly by reducing the impact of potential abiotic stress factors (including As availability) resulting from prolonged flooding of soil (Blom and Voesenek, 1996).

4.3.7. Relationship of Straighthead Rating to Yield

The grain yields in both the MSMA-treated (Figure 4-3) and native (Figure 4-4) soils were negatively correlated with straighthead rating from the MSMA-treated plot as indicated by the wide obtuse angles between the respective vectors. These results were confirmed by correlation analyses of straighthead rating with grain yields in the native soil ($r = -0.810$, $P< 0.001$, in 2004; $r = -0.775$, $P< 0.001$, in 2005) and the MSMA-treated soil ($r= -0.364$, $P<0.001$, in 2004; $r = -0.261$, $P< 0.02$, in 2005). The relatively high correlation coefficients obtained with the native soil indicates that in general the cultivars with high susceptibility to straighthead also exhibited relatively poor grain yields in the native soil, possibly from a lower tolerance to abiotic-stress factors (including soil As) in the flooded-rice fields. The high and negative correlation coefficients with the MSMA-treatment indicated a large reduction of grain yield caused by MSMA-induced straighthead, which has been reported by Yan et al. (2005; 2008).

4.4. SUMMARY - IMPLICATIONS OF STRAIGHTHEAD TO GRAIN-ARSENIC ACCUMULATION

The natural incidence of straighthead in rice fields can result in partial to almost complete yield loss. High soil-As concentrations have been linked to the occurrence of straighthead (Schweizer, 1967; Atkins, 1957; Wells and Gilmour, 1977). The current study indicates that the panicle sterility observed in straighthead incidences might not be directly related to grain-As concentration. Cultivars with lower TGAs concentrations were found in both straighthead-susceptible and straighthead-resistant cultivar groupings.

In rice cultivars, the TGAs concentrations with MSMA treatment were higher than the native soil and mostly increased grain-DMAs^{V} concentration. The grain-DMAs^{V} concentrations within native soil and MSMA treatment varied considerably, which was likely impacted by genotype, soil-As speciation and its related bioavailability and plant response to localized soil conditions (*C.V.*% = 21; Table 4-2). The poor correlation between grain-DMAs^{V} and grain-iAs^{III} concentrations indicates that different predominant processes control the relative concentrations of these species in grain.

The grain-iAs^{III} concentrations were significantly different by genotype. Unlike grain-DMAs^{V}, the concentrations of the less desirable iAs^{III} were not substantially different with native versus MSMA-treated soil (Table 4-2), indicating the possibility of a genotype-dependent concentration limit in grain-iAs^{III} concentration.

The straighthead susceptible cultivars from native soil generally had higher grain-iAs^{III} concentrations indicating the possible role of iAs^{III} in straighthead susceptibility (Figure 4-2). This relationship deserves further investigation. Straighthead resistance was generally associated with early maturing cultivars, indicating that shorter exposure to abiotic stress prior to and during grain fill of flooded rice resulted in lower susceptibility to straighthead.

5. RICE GRAIN ARSENIC CONCENTRATIONS AND PLANT GROWTH PARAMETERS AS IMPACTED BY WATER MANAGEMENT IN THE FIELD

5.1. INTRODUCTION

5.1.1. Arsenic Species in Rice

Rice is a major food source of ingested arsenic (As) in populations relying on rice as a staple food (Mondal and Polya, 2008). In rice, inorganic-As species such as arsenate (iAs^{V}) and arsenite (iA^{III}) and organic-As species such as dimethylarsinic acid ($DMAs^{V}$) and monomethylarsonic acid ($MMAs^{V}$) have been reported (Heitkemper, 2001; Williams et al., 2005; Pillai et al., 2009a). The inorganic As species are considered to be less desirable in food materials since these have longer retention times in the body than the organic-As species (Johnson and Farmer, 1990; Cohen et al., 2006).

5.1.2. Arsenic Bioavailability in Rice Culture

The bioavailability of As to rice depends on the soil characteristics such as As concentration and speciation, pH, redox potential, organic-matter content, texture, mineralogy of soil Fe oxides, and the availability of adsorption sites for immobilization of As (e.g., Woolson, 1977; Williams et al., 2005; Ultra et al., 2009; Hossain et al., 2009). In aerobic soils, As^{V} is usually the dominant As species and is immobilized by bonding to Fe^{3+}-hydroxide minerals (Lafferty and Loeppert, 2005; Zhang et al., 2007), and hence is poorly bioavailable to most plants. As uptake by rice is usually higher than with other grain crops because of the flooded soil conditions of paddy fields, in which soil As is generally more soluble and more bioavailable.

The continuously flooded rice field is characterized by anaerobic soil conditions, under which Fe oxides can be dissolved by biologically mediated reductive dissolution. This process usually results in the release of adsorbed As species. Also, iAs^{V} is converted to the more readily solubilized iAs^{III}, thus increasing the bioavailability of soil-As to rice (Marin et al., 1993; Masscheleyn et al., 1991). Oxidized soil-conditions might also promote a microbially induced transformation of inorganic-As species to their methyl-substituted analogs such as MMAs, DMAs, and gaseous arsines (e.g., AsH_3) (Sohrin et al., 1997; Turpeinen et al., 1999).

iAs^{V} is an analog of inorganic phosphate (P_i) (Dixon, 1997), and both As^{V} and P_i enter the plant through the root plasma membrane phosphate transporter (Rothstein and Donovan, 1963; Ullrich-Eberius et al., 1989; Meharg and Macnair, 1992). The uptake of iAs^{III} is mediated by the non-selective aquaporin channel in rice (Ma el al., 2008). Evidence is mounting that the methyl As species are especially bioavailable to wetland rice (Kertulis et al., 2005; Raab et al., 2007; Pillai et al, 2009b; Li et al., 2009b). A recent study in rice has shown that arsenite is taken up by roots through aquaporin channel, Lsi1 (the aquaporin NIP2;1) and Lsi2 (an efflux carrier) (Ma et al., 2008; Li et al., 2009b). The Lsi1 mutation inhibits uptake of $MMAs^{V}$ and $DMAs^{V}$, whereas, in the roots of wild-type rice uptake of undissociated methylated especially $MMAs^{V}$ was observed (Li et al., 2009b). The ability of plants to transform As species outside the root to enable uptake is not yet thoroughly understood.

In rice grain, the TGAs and As-species concentrations vary by genotype and are strongly impacted by soil-As concentration and speciation (Williams et al., 2005; Zavala et al., 2008; Pillai et al., 2009a; Pillai et al., 2009b). There is yet little known about the impact of paddy-water management practices on grain-As speciation.

Rice cultivars exhibit substantial differences in relative grain-As accumulation at when grown on high soil-As concentrations (Abedin et al. 2002; Duxbury and Panaullah, 2007; Pillai et al., 2009b). Pillai et al. (2009a) reported negative correlation between TGAs concentration and yield as well as a genotype-dependent impact of TGAs concentration in rice. High-As availability to the rice plant has been linked to a physiological disorder called ‘straighthead’ which results in panicle sterility and reduction in grain yield. To prevent yield loss due to straighthead incidence, the use of resistant cultivars and mid-season draining of flooded fields is a common practice (Yan et al., 2005). A recent study has indicated that the relative susceptibility of rice cultivars to straighthead and the relative grain-As concentrations among cultivars are not correlated, indicating that these two characteristics are not controlled by directly linked processes (Pillai et al., 2009b).

In Bangladesh, paddy fields are often irrigated with As-contaminated water, resulting in higher soil-As concentrations and consequent elevation in rice-grain As concentrations (Meharg and Rahman, 2003; Williams et al., 2006; Panaullah et al., 2009). When rice is grown on high As soil, a relatively lower grain-As concentrations can be obtained if, aerobic, non-flooded soil conditions are maintained as opposed to rice grown under continuous flooding (Duxbury and Panaullah, 2007; Xu et al., 2008; Ma et al., 2008). Under these conditions, As solubility in the soil and its subsequent availability to the plant is decreased (Duxbury and Panaullah, 2007; Xu et al., 2008; Ma et al., 2008). Management strategies to reduce water use in rice fields are gaining interest as approaches to reduce grain-As concentration (Ma et al,. 2008).

5.1.3. Objective

The objective of this study was to determine the impacts of water management, soil-As species and genotype on total grain-As and As-species concentrations. Rice cultivars selected from the USDA rice germplasm collection were grown on a native soil and an adjacent soil with monosodium monomethylarsonate (MSMA) treatment utilized for screening of cultivars for straighthead susceptibility. Both soils included the water-management treatment of continuously- and intermittently flooded conditions.

5.2. METHOD

5.2.1. Soil

The experiments were conducted on a Dewitt silt loam soil (fine, smectitic, thermic Typic Albaqualf) at the USDA Dale Bumpers National Rice Research Center near Stuttgart, Arkansas. A native soil and an adjacent straighthead testing soil with a history of MSMA application were selected for growing rice under two irrigation methods. The soil-As concentration in the native-saturated, native-flooded, MSMA-saturated, and MSMA-flooded treatments were 5.6 ± 0.5, 6.2 ± 0.4, 22.2 ± 1.5, and 21.5 ± 1.5, respectively (Somenahally et al., 2008).

5.2.2. Rice Genotypes

In 2007, twenty-one rice cultivars, including both japonica and indica subspecies, from the USDA world-germplasm collection were selected for evaluation. These cultivars were selected based on previous screening results to represent wide ranges of tolerance to straighthead and grain-As accumulation (Yan et al., 2005; Pillai et al., 2009b).

5.2.3. Field Screening Procedure

Before field preparation, the straighthead-testing plots were freshly sprayed with MSMA (Monterey Weed-Hoe, Fresno, CA) at the rate of 6.7 kg ha^{-1} (1.49 kg As ha^{-1}). Soils in both the native and MSMA-treated plots were immediately mixed thoroughly with a Hedge 80 planter to a depth of 15 cm, and followed immediately by dry seeding. The experiment was conducted as a randomized split-spilt plot design. The main plot consisted of two soil-As concentrations, namely a “native-soil” and a “MSMA-treatment soil”. The MSMA-treatment soil has received 6.7 kg MSMA ha^{-1} application in alternate years for the past 12 years). The sub-plot consisted of two type of water management: continuous flood and intermittently flooded. The continuously-flooded treatment consisted of a flood establishment to a depth of 12 cm from the five-leaf stage of rice plant until one week prior to harvest. This is a common management practice in dry-seeded lowland-rice cultivation. Herein, this type of water management in the native and the MSMA-treated soils will be denoted as ‘native-flooded’ and ‘MSMA-flooded’ treatments, respectively. The second type of water management also begins with flooding at the five-leaf stage of rice; however, subsequent irrigation similar to that of the flooded treatment is carried out only when the water layer above the soil surface dissipates and the soil becomes firm. Herein, this alternate method of flooding and drying will be denoted in the native and the MSMA-treated soils as ‘native-saturated’ and ‘MSMA-saturated’ treatments, respectively. The sub-sub-plots consisted of cultivars in four replicates. Each sub-sub-plot was 1.8 x 1.2 m in dimension, with six rows, 0.3 m apart (Figure 3-1).

At about the four-leaf stage of rice growth, weeds were controlled by the application of 9.3 L ha^{-1} of propanil (3',4'-dichloropropionanilide) mixed with 0.4 kg ha^{-1} of quinclorac (3,7-dichloroquinoline-8-carboxylic acid; Facet, BASF, Florham Park, NJ, USA). At about the five-leaf stage, urea was applied at the rate of 134 kg N ha^{-1}, immediately after which the respective water-management treatments were established and maintained uninterrupted until one week prior to harvest. The planting and harvesting dates were April 15th and Aug 22nd in 2007. From each sub-sub-plot, heading date (number of days from planting to 50 % panicle emergence from the flag leaf), plant height, grain yield and straighthead rating were recorded as described by Yan et al., (2005). The rice grain for As analysis was obtained only from the center 0.6 m of the center two rows of each plot to avoid the edge effects found in the rice plots (Figure 3-1). The harvested rice was dehulled using a Kett rice husker (TR-200 Electromotion rice husker, Kett, Tokyo, Japan), milled using a Kett grain polisher (PEARLEST, Kett, Tokyo, Japan) and powdered using a grinder (Foss Cemotec 1090, Slangerupgade, DK-3400 Hilleroed, Denmark). To avoid cross contamination, the grain from the native-soil and MSMA-treated soil were prepared in separate batches and the polisher and grinder were cleaned after each sample processing.

5.2.4. Analyses of Arsenic

The total grain-As (TGAs) concentrations in rice samples were determined following open-vessel digestion of powdered milled grain with trace-metal grade HNO_3/H_2O_2. Grain samples (0.5 g) were digested with HNO_3 (0.5 mL) in a teflon tube capped with a funnel using a temperature-programmable 48-well graphite block-digestion system (Digi Prep MS, SCP Science, Montreal, Canada). During the HNO_3 digestion step, the digestion block was heated to 50, 60, and 120 °C for successive 240 min heating steps and allowed to cool. Then two rounds of H_2O_2 digestion, each involving the addition of 3 mL H_2O_2, were followed by heating to 130 °C for evaporation to dryness. For quality control, two standard reference material (1568a rice flour, NIST, Gaithersburg, MD, USA) with a certified As-concentration of 0.29 ± 0.03 μg As g^{-1}, two blank samples, and three sample replicates were included in each digestion batch. The completely digested and dried samples were re-dissolved in 15 mL of 2 % HNO_3 and then analyzed for TGAs concentration by inductively-coupled-plasma mass-spectroscopy (ICP-MS) using a

Perkin Elmer ELAN DRC II (Perkin-Elmer Sciex, Concord, ON, Canada) which was fitted with a Meinhard concentric nebulizer and cyclonic spray chamber. To verify TGAs concentrations, outliers were re-analyzed, but individually verified values were not removed during the calculations of mean and standard error, since this variation could have been due to either natural genetic variation or localized soil variation or both.

The speciation of As was performed using a modification of the method described by Heitkemper et al. (2001). For extracting As species, deioinized (DI) water (1690 µL) followed by 310 µL of 99.9 % trifluoroacetic acid (TFA) was added (a final concentration of 2 M TFA) to ~0.5 g rice flour in a 50 mL polypropylene tube that was then capped and heated at 80 °C for 4 h using a temperature-programmable graphite block-digestion system. The mixture was diluted with 20 mL DI water, homogenized by vortexing for 1 min, and centrifuged for 20 min at 3600 rpm. The supernatant solution was collected, evaporated to near dryness, re-dissolved in 15 mL DI water, and filtered through a 0.2 µm nylon membrane filter. The As species were separated using by HPLC (PerkinElmer Series 200, Waltham, MA. USA) with the guard column (Dionex AG7, Sunnyvale, CA, USA), and anion-exchange column (Dionex AS7), that were attached in-line to the ICP-MS for As analysis. The separation scheme for As speciation consisted of a 1 min elution with 1 mM HNO_3 followed by a 6-min linear-gradient elution from 1 to 50 mM HNO_3. A chromatographic internal standard of 5 µg As kg^{-1} As was pulsed at 6.5 min post-injection. The quantification of As species by ICP-MS induces a variable enhancement of the As signal due to the variable presence of C-containing compounds extracted from rice grain. This potential error was minimized by the addition of 3 % CH_3OH/H_2O into the sample line (Larsen and Sturup, 1994). A 20-cm coil was added to the ICP-MS sample input tubing to ensure complete mixing of the CH_3OH with the column eluant (James et al., 2008). The standard-curve was obtained using mixtures of four As-species, namely $DMAs^V$ (dimethylarsinic acid; Chem Service, West Chester, PA, USA), $MMAs^V$ (monosodium monomethylarsonate sesquihydrate; Chem Service), iAs^{III} (As_2O_3; Alfa Aesar, Johnson Matthey Company, Westhill, MA, USA), and iAs^V (SPEX Centriprep, Metuchen, NJ, USA). Perkin Elmer Chromera software was used to control the HPLC and ICP-MS instruments, as well as for data collection and analysis. To evaluate the efficiency of As-species extraction from each rice sample, the TGAs concentrations obtained by the HNO_3/H_2O_2 digestion method were compared with the sum of As species identified by the TFA extraction method.

5.2.5. Statistical Analysis Procedures

Statistical analyses such as ANOVA, mean comparison, and correlation were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The GLM procedure was used for ANOVA. Genotype (G), irrigation treatment (I), and their interaction (G xI) were considered as the sources of variance for plant growth traits within the native and MSMA-treated soils. For each trait, percent variation explained by each factor was calculated using the sum of squares (S.S.) and the formula $\{[S.S._{(\text{trait x factor})}] / \text{total } S.S.\} * 100$, where $S.S._{(\text{trait x factor})}$ indicates the S.S.for each trait x factor combination. The MEANS and LSMEAN procedure was used to estimate least square mean (mean) and least square difference (LSD). The CORR procedure was used to obtain correlation coefficients (r), and the corresponding significance-levels.

5.2.6. GGE Biplot

The graphical analysis of relationships between agronomic traits and grain-As concentrations were obtained using GGE biplot software (version 6.1) following the methods of Yan and Tinker (2006). The biplot is a graphical display of a rank-two matrix as originally introduced by Gabriel (1971), Kempton (1984), and Kroonenberg (1995) and later Yan et al. (2000) who developed the principal components based methodology that is known today as the GGE [(genotype (G), genotype-by-environment (GE)] biplot method for genotype comparison and multi-environment data analysis. The GGE biplot utilizes the first two principal components from the data to display various graphs. The "relationship among testers" view of GGE biplot denoted as "GT-biplot" was produced to assess the relationship between traits as an aid in selection of genotypes with multiple desirable traits using a three way dataset of genotypes, traits, and replicates. This view was obtained by singular value partitioning ("SVP=2"), no data transformation ("transform=0"), scaling by standard deviation ("scaling=1"), and centering by G+GE ("centering=2"). In this view, a relatively greater length of a vector represents a relatively better ability of the corresponding trait to discriminate the genotypes (Yan and Kang, 2003). The cosine of the angle between two vectors approximates the correlation between two traits. The results of trait associations in GT-biplot were confirmed by bivariate-correlation analysis obtained using SAS software. The "mean-trait performance and stability" view of biplot was individually plotted for each trait using two-way data consisting of trait and genotype-by-year columns with singular value partitioning ("SVP=1"), no data transforming ("transform=0"), scaling by standard deviation ("scaling=1"), and centering by G+GE ("centering=2").

5.3. RESULTS AND DISCUSSION

5.3.1. Analysis of Variance

The MSMA-treated soil, with artificially high methyl-As species and total-As concentrations, was distinct from the native soil that was dominated by inorganic As species (Somenahally, 2008). The resulting grain-As concentrations from the MSMA-treated soil were also considerably higher than concentrations from the native soil, as also reported by Pillai et al. (2009b). With the two water-management treatments and the two soil-treatments, the TGAs, grain-DMAs^{V}, and grain-iAs^{III} concentrations generally decreased in the order: MSMA-flooded > MSMA-saturated, native-flooded > native-saturated.

Since the primary objective of the current study was to evaluate the influence of irrigation treatment on grain-As concentration and speciation rather than to evaluate the effect of As treatment, the analysis of variance for traits in the MSMA and native-soil treatments were conducted separately (Table 5-1and Table 5-2). The proportions of the total variation explained by G, I, and G x I are depicted graphically in (Figure 5-1 and Figure 5-2). The total variation explained by these factors was less than 100% for some traits because of additional variation likely introduced by unknown factors such as soil variability. With the native soil as well as the MSMA-treated soil, the ANOVA indicated that TGAs, grain-DMAs^{V} and grain-iAs^{III} concentrations were significantly affected by genotype (G), irrigation (I), their interaction (GxI) (Table 5-1and Table 5-2). The percent of total variation explained by G, I, G x I for each of these traits (TGAs, grain-DMAs^{V} and grain-iAs^{III} concentrations) was different with native and MSMA-treated soil (Table 5-1and Table 5-2).

Table 5-1. Variance analysis of agronomic traits and grain-As concentrations for twenty-one rice genotypes grown in 2007 under continuously flooded and intermittently flooded conditions.

Soil Type	**Source of Variation**	**d.f.**	**Level of Significance**				
			HD	**PHT**	**Yield**	**TGAs**	**SHR**
Native soil	Genotype (G)	1	***	***	***	***	***
	Irrigation (I)	20	*		*	***	***
	Genotype x Irrigation (G x I)	20	*			***	***
MSMA-treated soil	Genotype (G)	1	***	***	***	***	***
	Irrigation (I)	20	***	***	***	***	***
	Genotype x Irrigation (G x I)	20	*	*	***	***	***

HD = days to 50 % heading; HT = plant height (cm); yield = grain yield (kg ha^{-1}); TGAs = total grain-As concentration (mg kg^{-1}) as determined following HNO_3/H_2O_2 digestion;
*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.

Table 5-2. Variance analysis of grain-As species concentrations for twelve rice genotypes grown in 2007 under continuously flooded and intermittently flooded treatments.

Soil Type	Source of Variation	d.f.	Level of Significance	
			iAs^{III}	DMAs^{V}
Native soil	Genotype (G)	1	***	***
	Irrigation (I)	11	***	***
	Genotype x Irrigation (GxI)	11	*	***
MSMA-treated soil	Genotype (G)	1	***	***
	Irrigation (I)	11	***	***
	Genotype x Irrigation (GxI)	11	*	***

*, **, and *** represent significance at 0.05, 0.01, and 0.001 probability, respectively.
iAs^{III} = inorganic As^{III} (mg kg^{-1}) in rice grain, DMAs^{V} = dimethylarsinic acid (mg As kg^{-1}) in rice grain as determined following 2 M TFA extraction method.

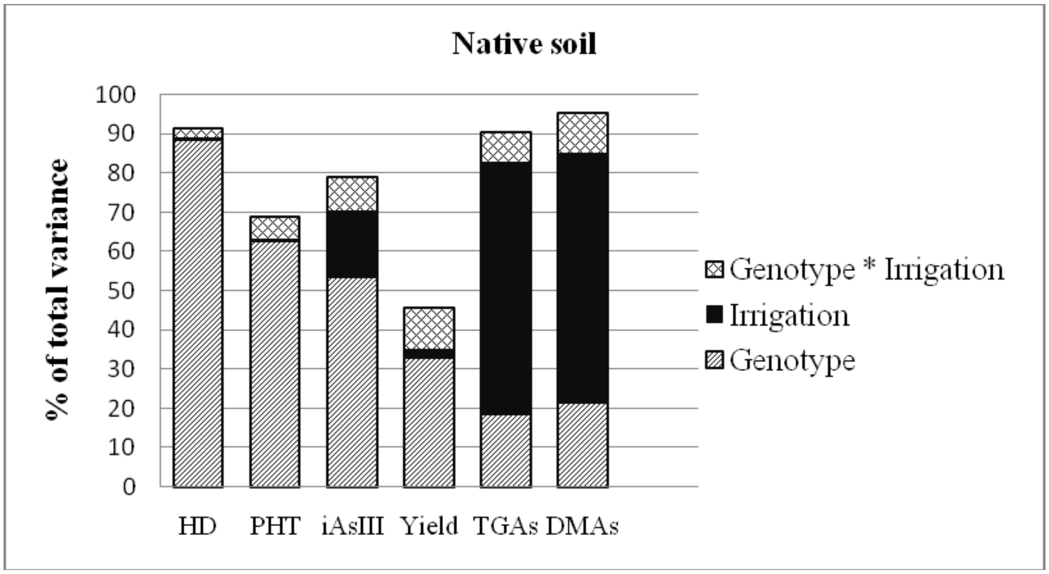

Figure 5-1. Percent of the total variance of agronomic traits and grain-As concentrations for cultivars in the native soil under the two irrigation treatments (continuous flood and intermittent flood) explained by genotype, irrigation, genotype by irrigation. Traits - HD = days to 50 % heading; PHT = Plant Height (cm); iAsIII = grain-iAs^{III} concentration (mg As kg^{-1}); yield = grain yield (kg ha^{-1}); TGAs = total grain As concentration (mg As kg^{-1}); DMAs = grain-DMAs^{V} concentration (mg As kg^{-1}).

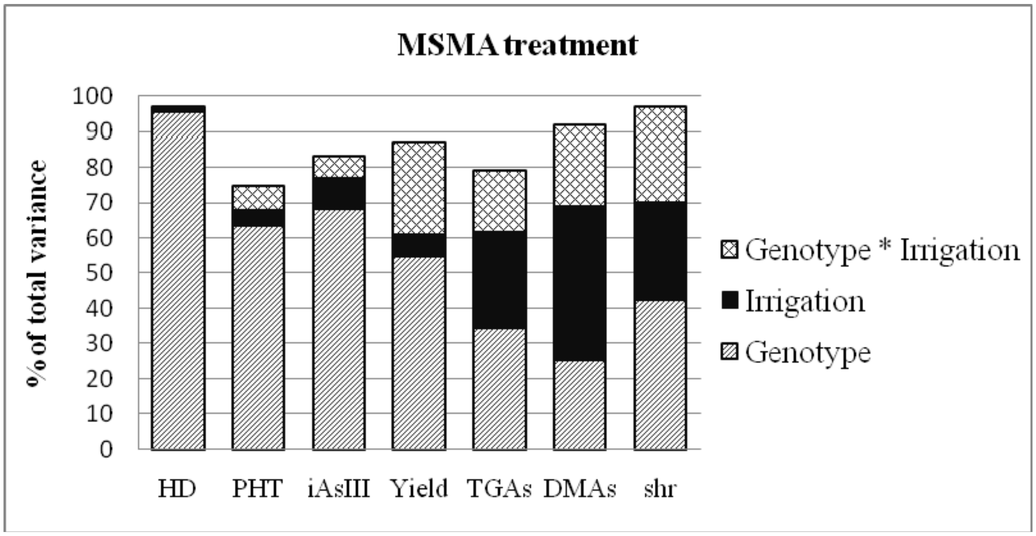

Figure 5-2. Percent of the total variance of agronomic traits and grain-As concentrations for cultivars in the MSMA-treated soil under the two irrigation treatments (continuous flood and intermittent flood) explained by genotype, irrigation, genotype by irrigation. Traits - HD = days to 50 % heading; PHT = Plant Height (cm); iAsIII = grain-iAs^{III} concentration (mg As kg^{-1}); yield = grain yield (kg ha-1); TGAs = total grain As concentration (mg As kg^{-1}); DMAs = grain-DMAs^{V} concentration (mg As kg^{-1}); SHR = straighthead rating.

5.3.2. Grain Arsenic Concentration - Native Soil

The lowest TGAs concentrations (0.095 to 0.253 mg As kg^{-1}) were generally obtained in the native-saturated treatment, whereas, with the native-flooded treatment the concentrations averaged approximately 2.5 times higher, ranging from 0.247 to 0.634 mg As kg^{-1} (Figure 5-3). In the native-flooded treatment, the concentrations of grain-DMAs^{V} ranged from 0.111 to 0.369 mg As kg^{-1} and were on average 5.5 times higher than those from the native-saturated treatment (0.000 to 0.079 mg As kg^{-1}) (Figure 5-4). The near zero to very low grain-DMAs^{V} concentrations with the native-saturated treatment, indicate that the intermittent aerobic condition were likely prevalent in the soil, reducing grain-DMAs^{V} concentrations (Xu et al., 2008; Li et al., 2009a). With the native soil, the irrigation treatment (I) accounted for 64 and 63 % of the total variation in TGAs and grain-DMAs^{V} concentration, respectively, compared to correspondingly 19 and 20 % of the total variation that was attributable to genotype (G) (Figure 5-1). This result indicates the dominant impact of water management on TGAs and grain-DMAs^{V} concentrations, with a somewhat smaller relative impact of G. In contrast to TGAs and grain-DMAs^{V} concentrations, the percent of total variance in grain-iAs^{III} concentration that were impacted by I was 18 %, with a comparatively larger impact of G (53 %). A differential relative impact of genotype and water-management on grain-As speciation is evident in these results. The grain-iAs^{III} concentrations with the native-saturated treatment ranged from 0.070 to 0.162 mg As kg^{-1} and were on average 18 % lower than those with the native-flooded treatment (0.090 to 0.196 mg As kg^{-1}) (Figure 5-5). A low range of genotype-dependent grain-iAs^{III} concentrations obtained with the native-saturated treatment indicate that cultivar selection can reduce the concentrations of potentially harmful inorganic As^{III} species. The lower TGAs, grain-DMAs^{V}, and grain-iAs^{III} concentrations obtained in the native-saturated treatment unlike the (native-flooded treatment) are likely at least partially attributable to a relatively higher redox potential and a lower solubility of soil As under intermittently flooded conditions (Masscheleyn, 1991; Xu et al, 2008; Ma et al., 2008). These current results indicate a substantial impact of genotype and irrigation regimes on rice-grain As concentration and speciation.

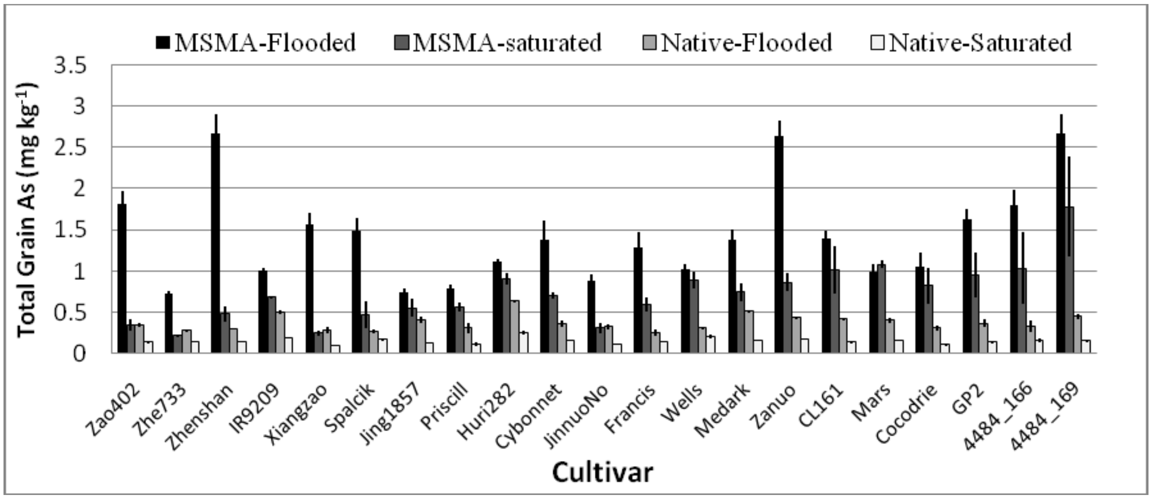

Figure 5-3. Mean and standard error of TGAs concentrations with 21 rice cultivars grown under four soil treatments (consisting of two soil-As concentrations and two water regimes) in 2007.

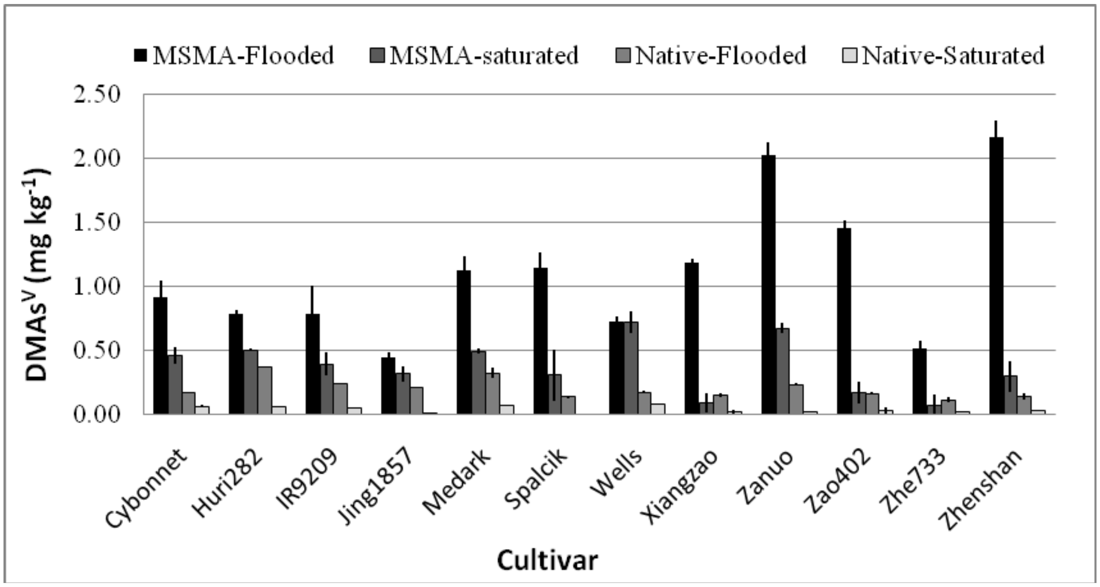

Figure 5-4. Mean and standard error of grain-DMAs^V concentrations with 21 rice cultivars grown under four soil treatments (consisting of two soil-As concentrations and two water regimes) in 2007.

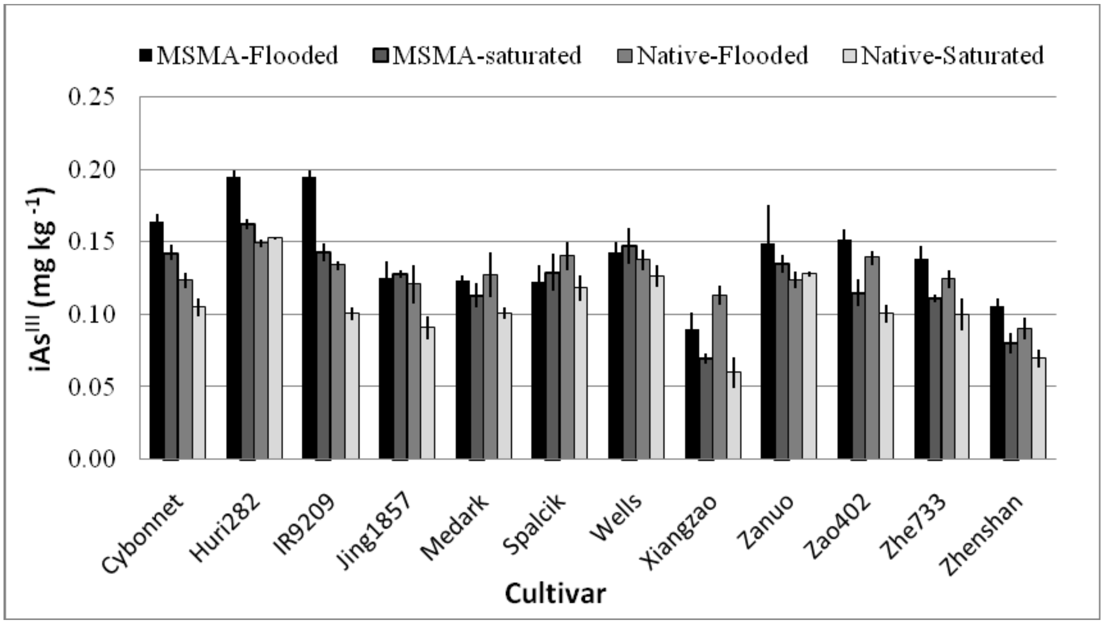

Figure 5-5. Mean and standard error of grain-iAs^{III} concentrations with 21 rice cultivars grown under four soil treatments (consisting of two soil-As concentrations and two water regimes) in 2007.

5.3.3. Analysis of Variance: MSMA-treated Soil

The soil-As concentration in the MSMA-treatment plot was ~3 times higher than that of the native-soil (21.4 ± 1.5 and 6.2 ± 0.4 mg kg^{-1}, respectively; Somenahally et al., 2008). In MSMA-treatment with already high-As soil, the additional 1.49 kg ha^{-1} As (MSMA) was added before planting. Rice cultivars in the MSMA-flooded treatment produced the highest TGAs concentrations, ranging from 0.548 to 2.656 mg As kg^{-1}(Figure 5-3). Compared with the MSMA-flooded treatment, the TGAs concentrations in the MSMA-saturated treatment were lower (ranging from 0.218 to 1.778 mg As kg^{-1}) indicating that grain-As accumulation was reduced, though still considerably higher than the range observed with the native-soil treatments. The grain-As concentrations with the

MSMA-flooded and MSMA-saturated treatments in the current study were considerably higher than those normally observed with even a higher concentrations of inorganic As in soil (e.g., Das et al., 2004; Panaullah et al., 2009) and indicates the likely higher uptake of methylated-As species by rice cultivars. The accumulation of methyl-As species by various plants is often higher than the inorganic-As species (Raab et al., 2007; Kertulis et al., 2005) and rice aquaporin channels are known to uptake methylated-As species (Li et al., 2004). In aerobic soils, the solubility of As species such as iAs^{III}, iAs^{V} and $MMAs^{V}$ is usually relatively low due to their strong adsorption characteristics, whereas, $DMAs^{V}$ is less strongly adsorbed and more soluble (Lafferty and Loeppert, 2005; Zhang et al., 2007). Therefore the role of rhizosphere microorganisms in methylation (Sohrin et al., 1997; Turpeinen et al., 1999) and the possible increased bioavailability of As (Li et al., 2009b) deserve further attention.

With the MSMA-treated soil, G, I, and G x I each accounted for substantial proportions of the total variation in both TGAs and grain-$DMAs^{V}$ concentrations (Figure 5-2). The G x I interaction of TGAs and grain-$DMAs^{V}$ concentrations effected large proportions of the total variation and resulted from different relative rankings of the genotypes in MSMA-flooded versus MSMA-saturated treatment. For example, the TGAs concentrations of cultivar “Zhe 733” was relatively low with both irrigation treatments, whereas, the cultivar “Zhenshan” had a relatively high TGAs concentration with the MSMA-flooded treatment and a relatively low concentration with the MSMA-saturated treatment (Figure 5-1). The different trait performance of cultivars with the different water-management suggests a likely genotype-dependent preference for As species existing in these treatments. With the MSMA treatment, the variation in grain-iAs^{III} concentrations were mostly impacted by G (68 %), whereas the variation in both grain-DMAs^{V} and TGAs concentrations were strongly impacted by both genotype and water management. The genotypic differences in As uptake have been attributed to transport of iAs^{III}, DMAs^{V} and MMAs^{V} through aquaporin channel variants expressed in rice roots (Ma et al., 2008; Li et al., 2009b).

5.3.4. Correlation: TGAs and Arsenic Species Concentration

The GT-biplots indicates that TGAs and grain-DMAs^{V} concentrations were positively correlated in each of the four treatments, as indicated by the acute angles between the respective vectors (Figure 5-6, Figure 5-7, Figure 5-8, and Figure 5-9). The correlation coefficients of TGAs and grain-DMAs^{V} concentration were highly significant ($r > 0.900$, $P \leq 0.001$; Table 5-3) with three treatments, namely MSMA-flooded (Figure 5-6), MSMA-saturated (Figure 5-7) and native-flooded (Figure 5-8) treatments. The grain_DMAs^{V} proportions relative to the TGAs concentrations in these three soil treatments were 74 (MSMA-flooded), 59 (MSMA-saturated) and 51% (native-flooded). In these three treatments DMAs^{V} represented major proportion of the TGAs concentration. On the contrary, the grain-DMAs^{V} and TGAs concentrations were considerably lower with the native-saturated treatment and represented an average of only 22 % of the TGAs concentration. Consequently, the correlation between TGAs and grain-DMAs^{V} concentration was relatively lower in the native-saturated treatment (r = 0.637, P = 0.001; Table 5-3) as also indicated in the GT-biplot (Figure 5-9) by a larger acute angle and a lower correlation coefficient. The grain-iAs^{III} constituted 65 % of the TGAs concentration in the native-saturated treatment and therefore better represented the variation in TGAs concentration. In the GT-biplot (Figure 5-9), the vectors of grain-iAs^{III} and TGAs concentration formed a small acute angle and exhibited a high correlation coefficient (r = 0.774; P < 0.001; Table 5-3).

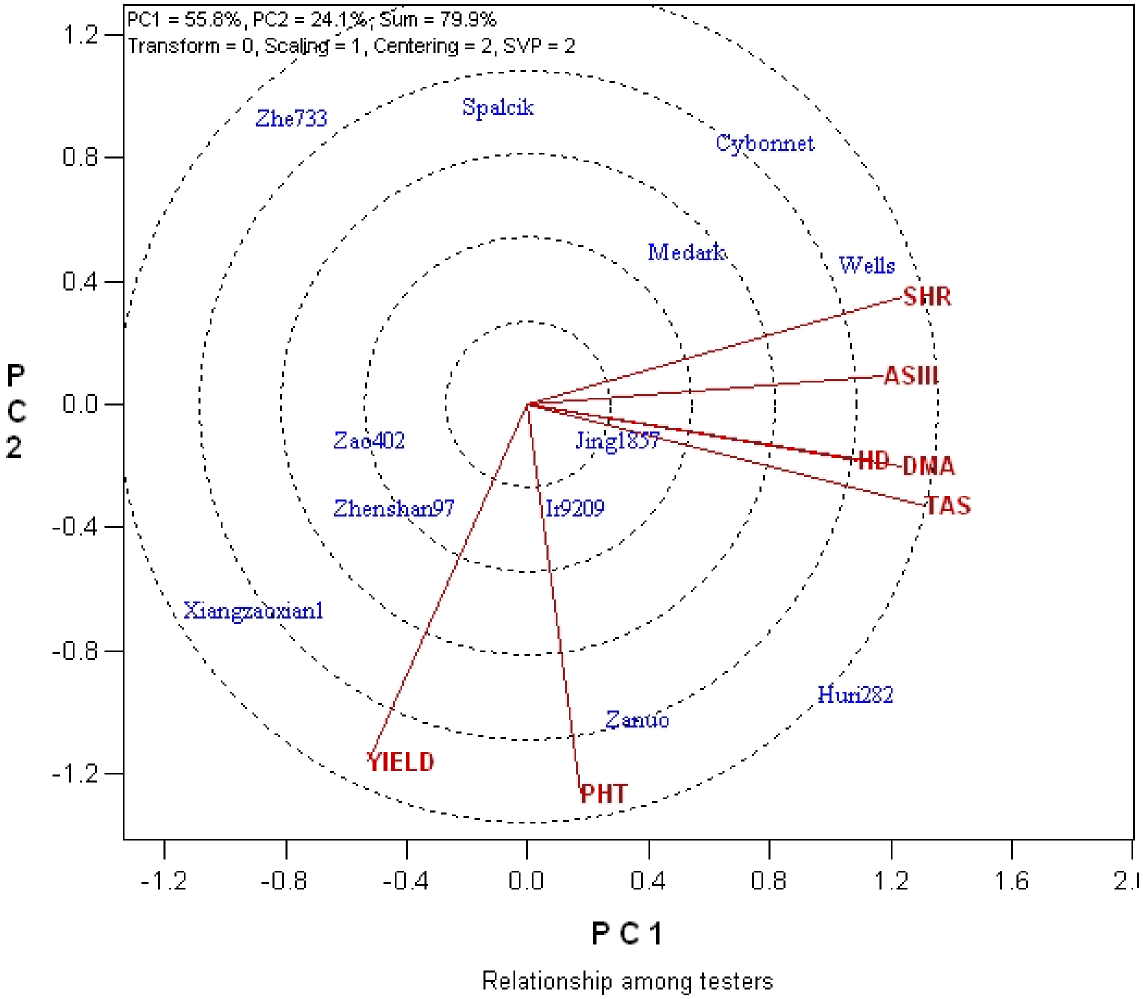

Figure 5-6. GT biplot showing relationships among agronomic traits in twelve genotypes grown in 2007 under MSMA-saturated treatment. Traits: HD = days to 50 % heading; Yield = grain yield (kg ha^{-1}); PHT = Plant height (cm); TGAs = total grain As concentration (mg As kg^{-1}); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}).

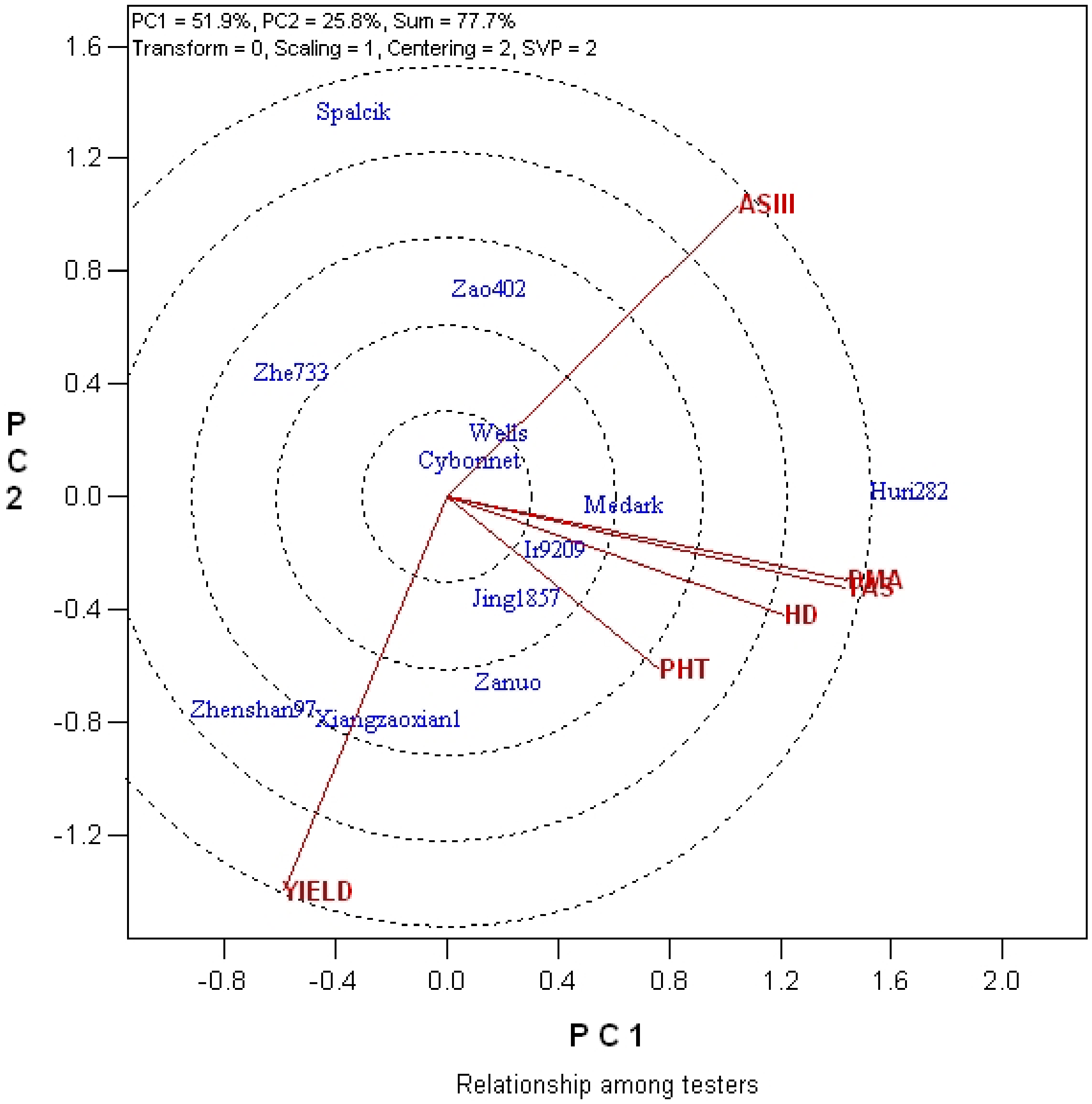

Figure 5-7. GT biplot showing relationships among agronomic traits with twelve genotypes grown on Native-flood treatment in 2007. Traits: HD = days to 50 % heading; Yield = grain yield (kg ha^{-1}); PHT = Plant height (cm); TGAs = total grain As concentration (mg As kg^{-1}); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}).

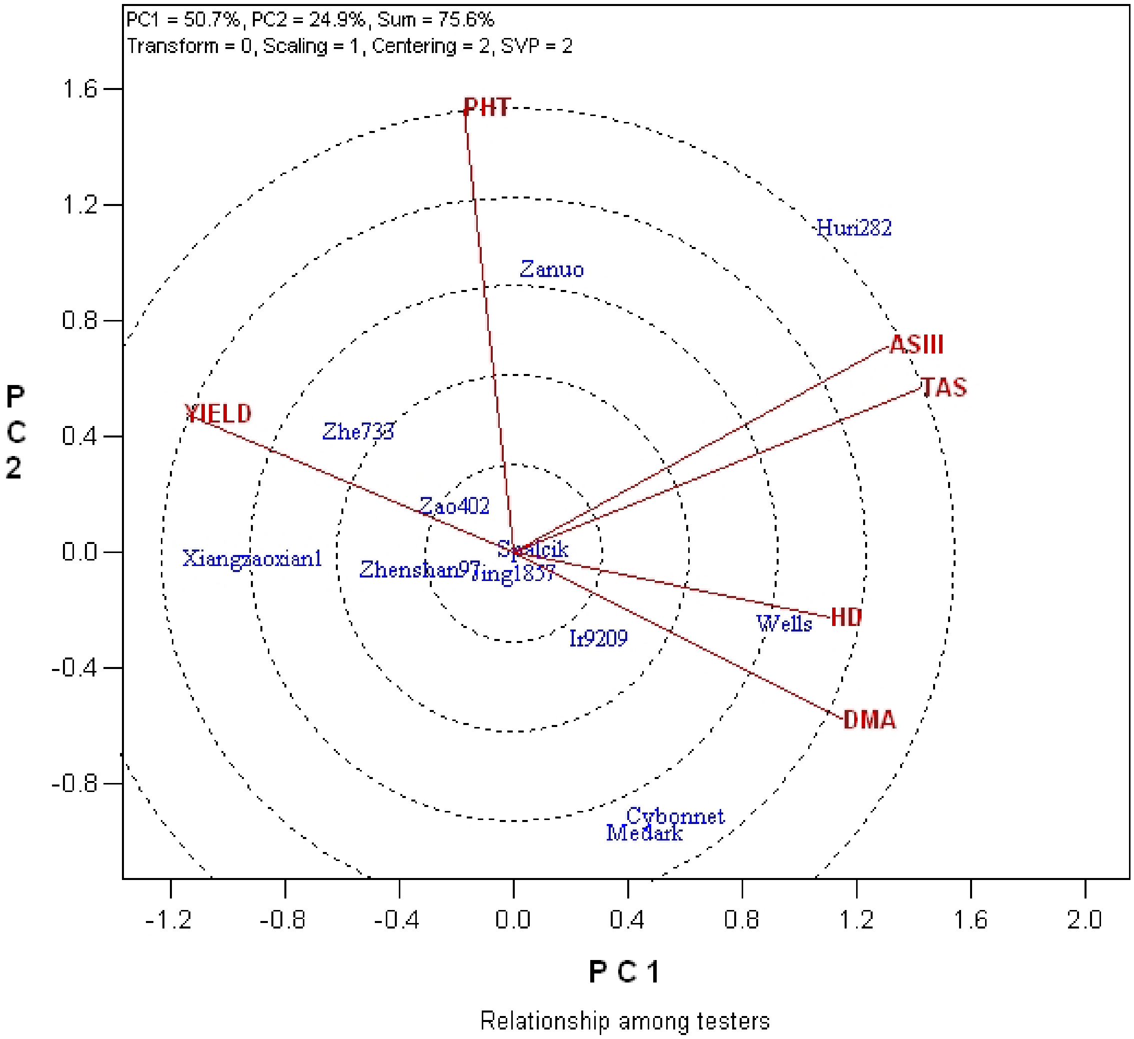

Figure 5-8. GT biplot showing relationships among agronomic traits in twelve genotypes grown in 2007 under native-saturated treatment. Traits: HD = days to 50 % heading; Yield = grain yield (kg ha^{-1}); PHT = Plant height (cm); TGAs = total grain As concentration (mg As kg^{-1}); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}).

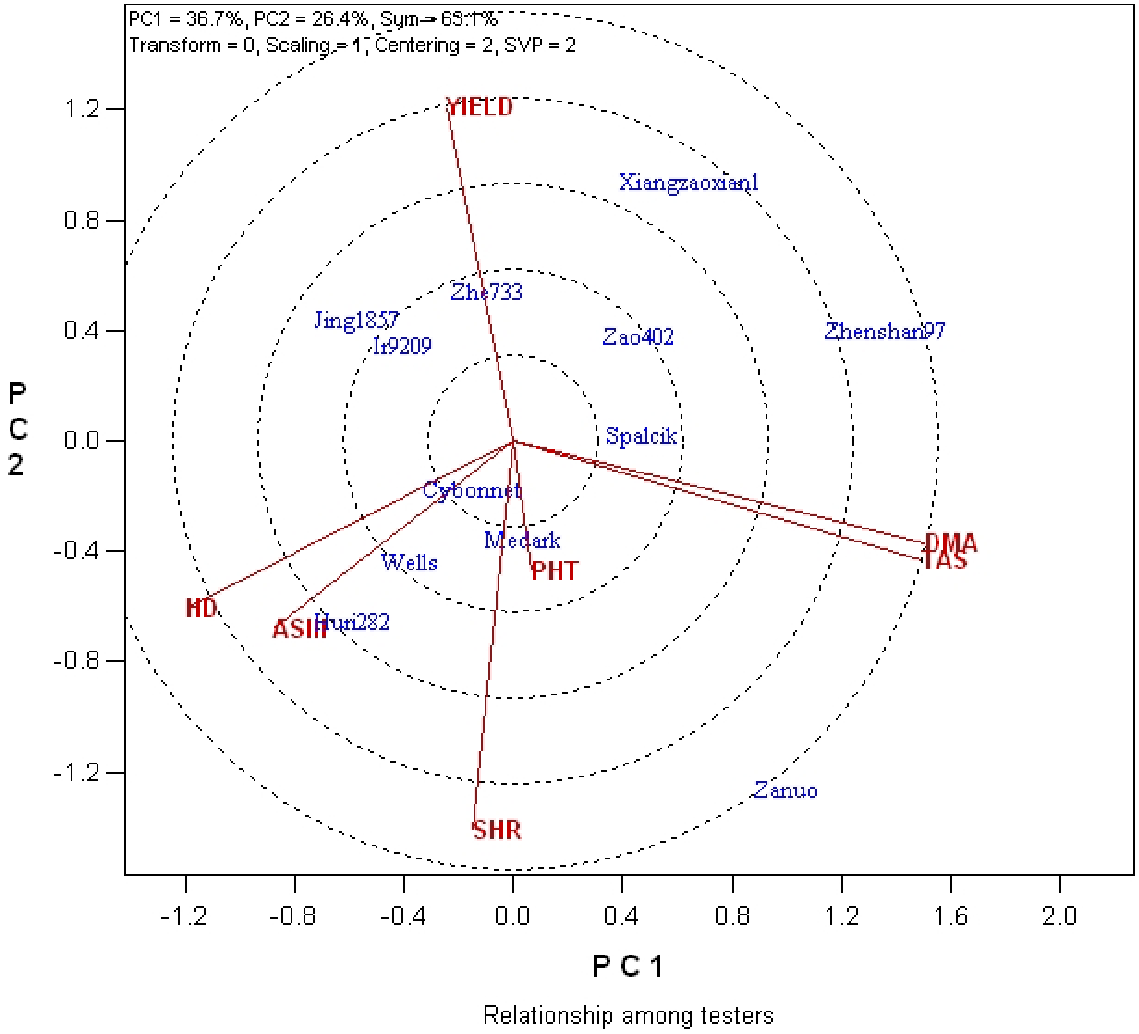

Figure 5-9. GT biplot showing relationships among agronomic traits in twelve genotypes grown in 2007 under MSMA-flood. Traits: HD = days to 50 % heading; Yield = grain yield (kg ha^{-1}); PHT = Plant height (cm); TGAs = total grain As concentration (mg As kg^{-1}); ASIII = grain-iAs^{III} concentration (mg As kg^{-1}); DMA = grain-DMAs^{V} concentration (mg As kg^{-1}).

5.3.5. Heading Date

The heading was significantly different by G, I, and GxI interaction in the MSMA treated soil and the native soil and the percent of total variation in heading dates were mostly explained by G (Figure 5-1 and Figure 5-2) with a small percent of variation explained by I (0.6 %) and G x I (0.9 %). The heading dates with MSMA-saturated treatment (82 days) were delayed by an average of 2 days when compared to MSMA-flooded treatment (80 days). Similar trend of delayed heading between native-saturated treatment (81 days) and native-flooded treatment (79 days) suggested possible abiotic stress conditions associated with the intermittent flooding (Blom, and Voesenek, 1996).

5.3.6. Correlation between Heading and Grain-arsenic Concentration

The heading date was positively associated with grain-iAs^{III} and grain-DMAs^{V} concentrations in MSMA-saturated (Figure 5-6), and native-saturated (Figure 5-8) treatments as indicated in the GT-biplots by acute angles between their respective vectors. Similar observation of early heading and lower grain-As species concentrations was previously reported by Pillai et al. (2009b). Unlike this trend in the MSMA-flooded treatment, GT-biplot (Figure 5-9) shows that heading date was negatively associated with grain-iAs^{III} concentration and this relationship was confirmed by significant correlation coefficients (Table 5-3). In the MSMA-flooded treatment, the heading date was negatively associated with grain-DMAs^{V} concentration (Table 5-3) as indicated by large obtuse angle between their vectors and highly significant correlation coefficients (r = -0.504, P < 0.001); Table 5-3). These result indicate that MSMA was taken up in greater quantities by some early heading cultivars which indicates high As availability to rice in early rice developmental stages likely in the form of methylated As.

Table 5-3. Correlation analysis of agronomic traits and grain As concentrations in cultivars grown in 2007.

Treatment		
	TGAs and As^{III}	As^{III} and Yield
1	(r = -0.282; P = 0.058)	(r = 0.039; P = 0.797)
3	(r = 0.694; P = 0.001)	(r = 0.327; P = 0.033)
2	(r = 0.341; P = 0.020)	(r = 0.331; P = 0.025)
4	(r = 0.774; P = 0.001)	(r = -0.415; P = 0.006)
	TGAs and DMAs^{V}	TGAs and Yield
1	(r = 0.988; P = 0.001)	(r = 0.267; P = 0.050)
3	(r = 0.925; P = 0.001)	(r = -0.021; P = 0.849)
2	(r = 0.917; P = 0.001)	(r = -0.114; P = 0.301)
4	(r = 0.637; P = 0.001)	(r = -0.063; P = 0.571)
	DMAs^{V} and As^{III}	DMAs^{V} and Yield
1	(r = -0.271; P = 0.068)	(r = -0.261; P = 0.079)
3	(r = 0.618; P <0.001)	(r = -0.188; P = 0.226)
2	(r = 0.345; P = 0.019)	(r = -0.084; P = 0.577)
4	(r = 0.295; P = 0.061)	(r =-0.137; P = 0.394)
	As^{III} and HD	DMAs^{V} and HD
1	(r = 0.382; P = 0.009)	(r = -0.504; P = 0.001)
3	(r = 0.515; P ≤ 0.001)	(r = 0.390; P = 0.010)
2	(r = 0.321; P = 0.030)	(r = 0.676; P = 0.001)
4	(r = 0.359; P = 0.020)	(r = 0.400; P = 0.010)
	SHR and HD	TGAs and HD
1	(r = 0.672; P = 0.001)	(r = -0.156; P = 0.156)
3	(r = 0.484 P = 0.001)	(r = 0.306; P = 0.005)
2	-	(r = 0.376; P = 0.001)
4	-	(r = 0.046; P = 0.676)
	SHR and As^{III}	SHR and DMAs^{V}
1	(r = 0.116; P = 0.441)	(r = 0.088; P = 0.561)
3	(r = 0.581; P ≤ 0.001)	(r = 0.559; P = 0.001)
	SHR and TGAs	SHR and Yield
1	(r = 0.192; P = 0.079)	(r = -0.802; P = 0.001)
3	(r = 0.226; P = 0.039)	(r = -0.514; P = 0.001)

Treatments: 1= MSMA-flooded; 2 = Native-flooded, 3 = MSMA-saturated; 4 = Native-saturated

5.3.7. Yield

In spite of possible abiotic stress associated with the saturated system of irrigation (suggested by delayed heading in cultivars), the yield was not significantly different between native-saturated treatment (8345 kg ha^{-1}) and native-flooded treatment (7891 kg ha^{-1}). In the native-soil, ANOVA indicated that yield was significantly affected by G, I contributing to 33.1 and 1.8 % of total variation, respectively. This result indicated larger impact of genotype on yield and lower impact of irrigation (Figure 5-1). In the native soil, the significant variation introduced by I for yields and the slightly higher yield with saturated treatment indicated that intermittent flooding may not be detrimental to yield and will also be useful in conserving water (Figure 5-10).

The MSMA-flooded treatment represents a typical straighthead screening system where continuous flooding is essential to produce maximum genotype specific straighthead symptoms associated with yield loss (personal communication, Dr. Wengui Yan). Therefore, the yield was on an average lowest with the MSMA-flooded treatment (4421 kg ha^{-1}) followed by higher yield in the MSMA-saturated treatment (5750 kg ha^{-1}).

5.4. CONCLUSION

In lowland rice cultivation, prolonged flooding of soil can result in increased As solubility and bioavailability to the rice plant and corresponding increase in grain-As concentration (Duxbury and Panaullah, 2007; Xu et al., 2008; Li et al., 2009a) . In some cases As-induced reductions in grain yield in also observed (Smith et al., 2008; Dilday et al., 2001; Yan et al., 2005; Panaullah et al., 2009). Therefore, water management practices with reduced water use are gaining interest as strategies to improve food quality and ensure better yields of rice grain (Xu et al., 2008; Ma et al., 2008; Li et al., 2009a). In the present study all cultivars from the native-saturated treatment exhibited the lowest TGAs, grain-DMAs^{V}, and iAs^{III} concentrations. The cultivars in the native-saturated treatment exhibited wide concentration ranges of both grain-iAs^{III} (0.070 to 0.162 mg As kg^{-1}) and grain-DMAs^{V} (0.000 to 0.079 mg As kg^{-1}). The wide ranges of TGAs, grain-DMAs^{V}, and grain-iAs^{III} concentration indicate that cultivar selection in addition to water management would be useful in decreasing total grain-As and As-species concentrations in rice grain. Exceptionally high TGAs concentrations were obtained with the MSMA-flooded and the MSMA-saturated treatments. The soil-As concentration of the MSMA-treatment (17.3 ± 0.9 mg As kg^{-1}) was similar or lower than other studies (e.g., 24 mg As kg^{-1}, Meharg and Rahman, 2002; 27 mg As kg^{-1}, Das et al. 2004; 67 mg As kg^{-1}, Panaullah et al., 2009) observing similar or a lower grain-As concentrations. The higher grain-As concentrations obtained with the MSMA treatments likely indicates high bioavailability of the methylated As species. In the current study, grain-DMAs^{V} concentrations were highly variable and especially high in flooded and MSMA-treated soil. On the other hand, grain-As^{III} concentrations were much less impacted by the treatment variables. As a result, DMAs^{V} was the dominant form of As species in rice grain with higher TGAs concentrations from the native-flooded, MSMA-flooded, and MSMA-saturated treatments; whereas, iAs^{III} was the dominant grain-As species in the native-saturated treatment having lower TGAs concentrations. The grain-

iAs^{III} concentration was positively correlated with heading and indicates the possible role of high iAs^{III} concentrations in inducing delayed heading by means of abiotic stress (Kato et al., 2008). This relationship deserves further investigation. In the current study heading was delayed by an average of 2 days with intermittent flooding. In rice, abiotic stress conditions such as both high soil-As toxicity (Yan et al., 2005) and water deficit (Kato et al., 2008) are linked to delayed heading, sterility of flowers and associated yield reduction. In the present study, the straighthead symptoms were more severe with the MSMA-flooded treatment than the MSMA-saturated treatment indicating greater role of As toxicity in delayed heading and yield reduction than the impact of lower water use in the saturated system. In the current study, the average of yield with the native-saturated treatment was higher than with the native-flooded treatment. Therefore no yield loss was associated with the lower water-use in this study. The intermittent flooding of native soil was successful in substantially lowering of grain-As concentrations. This method requires further yield-trial studies to confirm the practical utility.

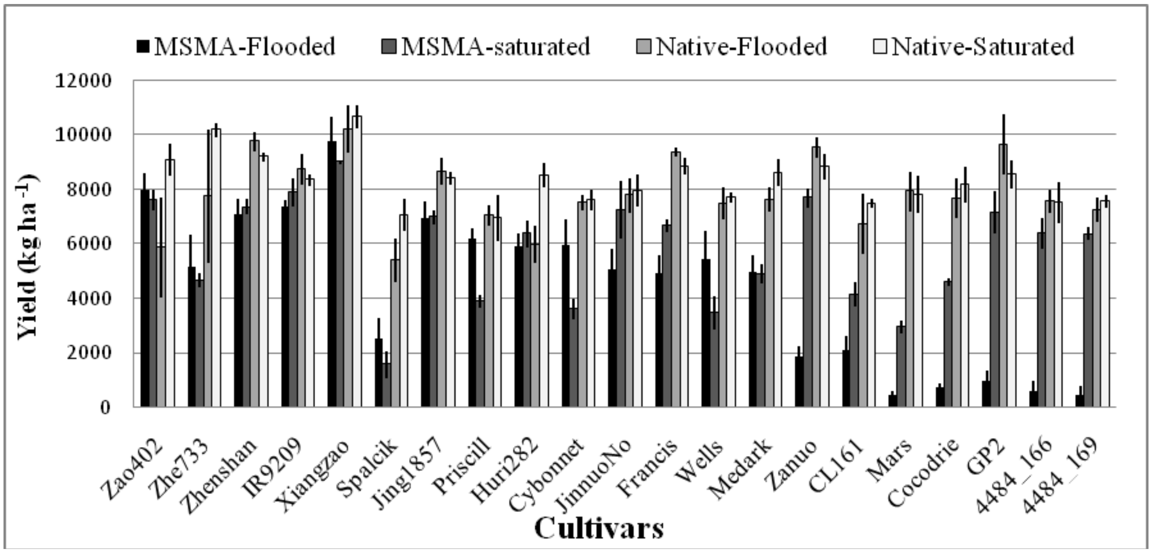

Figure 5-10. Mean yield (kg ha^{-1}) traits in 21 cultivars grown under four soil treatments in 2007. (Treatments, 1= MSMA & continuous flooded, 2 = MSMA & saturated, 1= native soil & continuous flooded, 2 = native soil & saturated).

6. SUMMARY, CONCLUSIONS, AND FUTURE RECOMMENDATIONS

6.1. SUMMARY AND CONCLUSIONS

The As species identified in the rice grain by the TFA-extraction method were iAs^{III} and $DMAs^{V}$. Wide ranges of genotype-dependent TGAs, grain-$DMAs^{V}$, and grain-iAs^{III} concentrations were obtained with the four soil treatments in 2004, 2005 (native-flooded and MSMA-flooded) and 2007 (MSMA-flooded, MSMA-saturated, native-flooded, and native-saturated).

TGAs, grain-$DMAs^{V}$, and grain-iAs^{III} concentrations generally decreased in the order: MSMA-flooded > MSMA-saturated, native-flooded > native-saturated. The grain-As concentrations with the MSMA-flooded and MSMA-saturated treatments in the current study are considerably higher than those normally observed with even higher concentrations of inorganic As in soil and indicates the likely preference of rice cultivars for uptake of methyl-As species.

Although the TGAs and grain-$DMAs^{V}$ concentrations of all cultivars from the MSMA-flooded treatment were significantly higher than those from the native-flooded soil, the concentrations of grain-iAs^{III} were not substantially different in these soil treatments, indicating a genotype-dependent upper concentration limit of grain-iAs^{III} concentration. However, the grain-iAs^{III} concentrations were significantly lower with the native-saturated treatment compared to the native-flooded treatment, likely due to lower As bioavailability from the intermittently flooded soil.

With the native-flooded, MSMA-flooded, and MSMA-saturated treatments, higher TGAs concentrations resulted in the predominance of DMAs^{V}, whereas in the native-saturated treatment with low TGAs concentrations, grain-As^{III} was the dominant As species. In each of the soil treatments, the strong positive-correlation of grain-DMAs^{V} with TGAs indicates that grain-DMAs^{V} concentration increased with increase in TGAs concentration. With the low TGAs concentrations in the native-saturated treatment the variation in TGAs concentration was more highly correlated with grain-As^{III} than with grain-DMAs^{V} concentration. The variation in grain-iAs^{III} concentration was mostly impacted by genotype, whereas the variation in both grain-DMAs^{V} and TGAs concentrations were strongly impacted by both water management and genotype. Therefore in general, the grain-iAs^{III} concentrations were more stable than the grain-DMAs^{V} concentrations across irrigation treatment. The often poor correlation observed between grain-DMAs^{V} and grain-iAs^{III} concentrations indicates that different predominant processes likely control the relative concentrations of these species in grain.

The cultivars that were replicated during 2004 and 2005 exhibited significant G x Y (genotype x year) crossover-interaction in TGAs concentration. Therefore, the relative rankings of some cultivars with low TGAs concentrations were different between years, which were likely impacted by variable annual environmental conditions, e.g., temperature, that affected the cultivars differently. Cultivars tested on similar soils with the different water-management treatments (flooded and saturated) exhibited significant G x I (genotype x irrigation) interactions in TGAs concentration. Therefore, with some cultivars, TGAs concentrations under different water-management regimes were not proportional indicating that cultivars might have exhibited a differential genotype-dependent preference for uptake of different soil-As species predominant with continuously flooded versus intermittently-flooded treatments.

In general, TGAs, $DMAs^{V}$, and grain-iAs^{III} concentrations were inversely correlated to yield, indicating either a concentration effect of grain-As as a result of decreased yield or a yield reduction due to high As-accumulation in rice. $DMAs^{V}$ and grain-iAs^{III} concentrations were, in general, positively correlated with heading date, indicating that early heading of cultivars might favor lower concentrations of As-species in grain. Therefore, to reduce total grain-As and As-species concentrations, both higher yield and early maturity appear to be favorable traits.

The MSMA-flood treatment successfully differentiated straighthead susceptibility in cultivars. Straighthead related panicle sterility and yield reduction were not correlated with grain-$DMAs^{V}$ and TGAs concentrations, which indicates that TGAs concentrations are not necessarily high in straighthead-susceptible cultivars. However, grain-iAs^{III} concentrations were positively correlated with straighthead susceptibility in native-flooded and MSMA-flooded treatments, indicating a possible As induced abiotic stress involved in straighthead incidence.

Straighthead rating (obtained from the MSMA-flood treatment) was negatively correlated with grain yields in both native-flooded and MSMA-flooded treatments. In the native-flooded treatment, a negative correlation of straighthead rating (obtained from

MSMA-flood treatment) with grain yield indicates that in general the cultivars with high susceptibility to straighthead also exhibited relatively poor grain yields in the native soil, possibly from a lower tolerance to abiotic-stress factors (including soil As) in the flooded-rice fields.

The most effective soil treatment for obtaining low TGAs, grain-DMAs^{V}, and grain-iAs^{III} concentrations was the native-saturated treatment, likely due to a lower As bioavailability to the rice plant during intermittent-flooding. The average of yield of with the native-saturated treatment was higher than with the native-flooded treatment, indicating that, in the current study, no yield loss was associated with the lower water-use treatment unlike previous observation (Li et al., 2009a). The wide ranges of TGAs, DMAs^{V}, and iAs^{III} concentrations indicate that cultivar selection in addition to water management would be useful in decreasing total grain-As and As-species concentrations in rice grain.

The genotype 'Xiangzaoxian' was high yielding and early heading and exhibited relatively low concentrations and stable values of grain-iAs^{III}, grain-DMAs^{V}, and TGAs during the 2004 and 2007. The cultivars 'Xiangzaoxian' and 'Zhe 733' were consistently high yielding, straighthead resistant, and exhibited relatively low total grain-As concentrations over the three years with different soil-As concentrations and water management regimes.

6.2. FUTURE RECOMMENDATIONS

The recovery of As species in rice samples by the TFA extraction method were usually lower than 100%. The incomplete recovery of As species could result from the presence of unknown As-containing compounds. Future studies should be directed towards identifying the As-binding compounds in rice and improving As species recovery from the rice grain.

In the current study, the TGAs concentrations of rice grown on MSMA-amended soil were higher than those of rice grown on native soil. However, the grain-iAs^{III} concentrations were not significantly different in the MSMA-amended and native soils. A study of shoot iAs^{III} and organic-As species concentrations relative to those of the grain would be useful in explaining the high stability and genotype-dependent grain-iAs^{III} concentration.

Generally cultivars with higher grain-iAs^{III} concentrations were straighthead susceptible. The further investigation of this relationship in rice plant is needed for confirmation of the role of As in straighthead susceptibility.

Early heading and high yielding cultivars usually exhibited low total grain-As concentrations. However not all cultivars followed this general trend, indicating that further studies will be required to understand genetic and biochemical mechanisms controlling low grain-As characteristics.

Due to the strong dependence of TGAs and $DMAs^{V}$ concentrations on water management and the genotype-dependent iAs^{III} concentrations on genotype, it is recommended to incorporate both cultivar selection and water management to reduce grain-As concentration.

In aerobic soils, the solubility of As species such as iAs^{III}, iAs^{V} and $MMAs^{V}$ is usually relatively low due to their strong adsorption characteristics, whereas, $DMAs^{V}$ is less strongly adsorbed and more soluble. Rhizosphere microorganisms can methylate As and possibly increased the bioavailability of As. Soil-As solubility and availability to the rice plant as influenced by microbial activity deserve further study.

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APPENDIX A

Agronomic data and grain-As concentrations in cultivar grown in 2004, 2005, and 2007 under four soil treatments.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Aijiaonante	G-2	China	I	2004	MSMA	Flooded	1	66	92	8024	1	1.681	.	.
Aijiaonante	G-2	China	I	2004	MSMA	Flooded	2	65	100	9099	1	1.465	.	.
Aijiaonante	G-2	China	I	2004	MSMA	Flooded	3	65	94	7191	1	1.739	.	.
Aijiaonante	G-2	China	I	2004	MSMA	Flooded	4	67	98	7358	1	1.770	.	.
Aijiaonante	G-2	China	I	2004	Native	Flooded	1	63	92	7412	1	0.398	.	.
Aijiaonante	G-2	China	I	2004	Native	Flooded	2	61	102	8854	1	0.322	.	.
Aijiaonante	G-2	China	I	2004	Native	Flooded	3	64	96	8816	1	0.409	.	.
Aijiaonante	G-2	China	I	2004	Native	Flooded	4	63	96	9661	1	0.322	.	.
CL161	G-5	U.S.A.	J	2004	MSMA	Flooded	1	84	80	774	8	1.876	.	.
CL161	G-5	U.S.A.	J	2004	MSMA	Flooded	2	87	82	566	7	1.758	.	.
CL161	G-5	U.S.A.	J	2004	MSMA	Flooded	3	85	86	1036	6	1.518	.	.
CL161	G-5	U.S.A.	J	2004	MSMA	Flooded	4	87	84	1036	6	1.802	.	.
CL161	G-5	U.S.A.	J	2004	Native	Flooded	1	82	84	6835	2	0.439	.	.
CL161	G-5	U.S.A.	J	2004	Native	Flooded	2	84	84	7170	2	0.515	.	.
CL161	G-5	U.S.A.	J	2004	Native	Flooded	3	86	96	7192	2	0.408	.	.
CL161	G-5	U.S.A.	J	2004	Native	Flooded	4	86	96	7843	2	0.378	.	.
Cocodrie	G-37	U.S.A.	J	2004	MSMA	Flooded	1	84	84	180	8	.	.	.
Cocodrie	G-37	U.S.A.	J	2004	MSMA	Flooded	2	89	78	41	8	.	.	.
Cocodrie	G-37	U.S.A.	J	2004	MSMA	Flooded	3	87	78	28	8	.	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
Cocodrie	G-37	U.S.A.	J	2004	MSMA	Flooded	4	89	78	55	8	.	.	.
Cocodrie	G-37	U.S.A.	J	2004	Native	Flooded	1	78	84	7026	2	0.188	0.099	0.089
Cocodrie	G-37	U.S.A.	J	2004	Native	Flooded	2	78	88	7854	2	0.184	0.090	0.097
Cocodrie	G-37	U.S.A.	J	2004	Native	Flooded	3	79	80	7572	2	0.192	0.095	0.093
Cocodrie	G-37	U.S.A.	J	2004	Native	Flooded	4	83	100	7592	2	0.326	.	.
Cybonnet	G-7	U.S.A.	J	2004	MSMA	Flooded	1	83	82	2633	4	1.350	.	.
Cybonnet	G-7	U.S.A.	J	2004	MSMA	Flooded	2	89	78	3531	6	1.452	.	.
Cybonnet	G-7	U.S.A.	J	2004	MSMA	Flooded	3	89	88	2257	5	2.114	.	.
Cybonnet	G-7	U.S.A.	J	2004	MSMA	Flooded	4	88	80	3170	4	1.760	.	.
Cybonnet	G-7	U.S.A.	J	2004	Native	Flooded	1	76	80	6332	1	0.432	.	.
Cybonnet	G-7	U.S.A.	J	2004	Native	Flooded	2	81	82	8202	2	0.437	.	.
Cybonnet	G-7	U.S.A.	J	2004	Native	Flooded	3	86	110	8046	2	0.367	.	.
Cybonnet	G-7	U.S.A.	J	2004	Native	Flooded	4	79	86	7156	2	0.450	.	.
Danwanbao24	G-8	China	I	2004	MSMA	Flooded	1	84	86	2715	1	0.992	.	.
Danwanbao24	G-8	China	I	2004	MSMA	Flooded	2	87	92	2946	1	1.069	.	.
Danwanbao24	G-8	China	I	2004	MSMA	Flooded	3	84	82	3979	2	1.251	.	.
Danwanbao24	G-8	China	I	2004	MSMA	Flooded	4	85	96	4661	2	0.813	.	.
Danwanbao24	G-8	China	I	2004	Native	Flooded	1	76	82	5818	1	0.472	.	.
Danwanbao24	G-8	China	I	2004	Native	Flooded	2	86	84	6038	1	0.445	.	.
Danwanbao24	G-8	China	I	2004	Native	Flooded	3	83	86	5748	1	0.499	.	.
Danwanbao24	G-8	China	I	2004	Native	Flooded	4	83	90	6602	1	0.454	.	.
Francis	G-9	U.S.A.	J	2004	MSMA	Flooded	1	87	94	4185	5	1.019	.	.
Francis	G-9	U.S.A.	J	2004	MSMA	Flooded	2	87	84	1589	6	2.183	.	.
Francis	G-9	U.S.A.	J	2004	MSMA	Flooded	3	87	92	4501	5	1.382	.	.
Francis	G-9	U.S.A.	J	2004	MSMA	Flooded	4	81	94	4067	6	1.338	.	.
Francis	G-9	U.S.A.	J	2004	Native	Flooded	1	76	90	7085	2	0.400	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Francis	G-9	U.S.A.	J	2004	Native	Flooded	2	76	92	7347	2	0.402	.	.
Francis	G-9	U.S.A.	J	2004	Native	Flooded	3	78	92	8306	2	0.387	.	.
Francis	G-9	U.S.A.	J	2004	Native	Flooded	4	76	96	7635	2	0.335	.	.
Gui99	G-10	China	I	2004	MSMA	Flooded	1	89	108	6120	1	1.101	.	.
Gui99	G-10	China	I	2004	MSMA	Flooded	2	90	104	3122	4	1.392	.	.
Gui99	G-10	China	I	2004	MSMA	Flooded	3	88	110	6251	3	1.053	.	.
Gui99	G-10	China	I	2004	MSMA	Flooded	4	89	102	5457	2	1.176	.	.
Gui99	G-10	China	I	2004	Native	Flooded	1	86	110	7089	1	0.554	.	.
Gui99	G-10	China	I	2004	Native	Flooded	2	86	114	8110	1	0.520	.	.
Gui99	G-10	China	I	2004	Native	Flooded	3	86	112	7157	1	0.529	.	.
Gui99	G-10	China	I	2004	Native	Flooded	4	86	122	7793	1	0.545	.	.
Huri282	G-11	Combodia	I	2004	MSMA	Flooded	1	93	110	6188	1	1.236	0.137	0.940
Huri282	G-11	Combodia	I	2004	MSMA	Flooded	2	91	108	3522	5	1.049	0.143	0.920
Huri282	G-11	Combodia	I	2004	MSMA	Flooded	3	90	106	8266	4	0.846	0.146	0.655
Huri282	G-11	Combodia	I	2004	MSMA	Flooded	4	91	102	6610	3	0.864	0.142	0.633
Huri282	G-11	Combodia	I	2004	Native	Flooded	1	89	114	7897	2	0.661	0.135	0.399
Huri282	G-11	Combodia	I	2004	Native	Flooded	2	87	108	8710	2	0.592	0.127	0.385
Huri282	G-11	Combodia	I	2004	Native	Flooded	3	86	108	6873	2	0.547	0.139	0.398
Huri282	G-11	Combodia	I	2004	Native	Flooded	4	86	116	9177	2	0.594	0.143	0.401
IR44595	G-14	Nepal	I	2004	MSMA	Flooded	1	81	90	6506	1	1.060	0.110	1.018
IR44595	G-14	Nepal	I	2004	MSMA	Flooded	2	83	94	6715	2	1.045	0.113	0.776
IR44595	G-14	Nepal	I	2004	MSMA	Flooded	3	81	92	7382	1	1.059	0.114	0.821
IR44595	G-14	Nepal	I	2004	MSMA	Flooded	4	81	100	6890	2	1.282	0.116	0.978
IR44595	G-14	Nepal	I	2004	Native	Flooded	1	72	100	9263	1	0.436	0.104	0.241
IR44595	G-14	Nepal	I	2004	Native	Flooded	2	72	106	10157	1	0.378	0.102	0.224
IR44595	G-14	Nepal	I	2004	Native	Flooded	3	72	104	8597	1	0.388	0.101	0.223

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
IR44595	G-14	Nepal	I	2004	Native	Flooded	4	70	112	9376	1	0.462	0.099	0.283
IR9209	G-13	Philippines	I	2004	MSMA	Flooded	1	74	82	5419	2	0.991	0.072	0.748
IR9209	G-13	Philippines	I	2004	MSMA	Flooded	2	74	84	6188	2	1.107	0.079	0.704
IR9209	G-13	Philippines	I	2004	MSMA	Flooded	3	74	80	5709	1	0.989	0.082	0.737
IR9209	G-13	Philippines	I	2004	MSMA	Flooded	4	81	86	6127	2	0.873	0.076	0.607
IR9209	G-13	Philippines	I	2004	Native	Flooded	1	70	88	7992	1	0.559	0.100	0.363
IR9209	G-13	Philippines	I	2004	Native	Flooded	2	70	94	7328	1	0.595	0.098	0.344
IR9209	G-13	Philippines	I	2004	Native	Flooded	3	70	94	7503	1	0.543	0.095	0.296
IR9209	G-13	Philippines	I	2004	Native	Flooded	4	70	88	7263	1	0.557	0.084	0.285
Jing1857	G-15	China	J	2004	MSMA	Flooded	1	90	82	6587	1	0.702	0.121	0.463
Jing1857	G-15	China	J	2004	MSMA	Flooded	2	89	84	6872	1	0.623	0.121	0.443
Jing1857	G-15	China	J	2004	MSMA	Flooded	3	90	88	4764	2	0.701	0.118	0.529
Jing1857	G-15	China	J	2004	MSMA	Flooded	4	97	86	6573	2	0.734	0.130	0.521
Jing1857	G-15	China	J	2004	Native	Flooded	1	86	84	8366	1	0.316	0.130	0.246
Jing1857	G-15	China	J	2004	Native	Flooded	2	89	82	8183	1	0.438	0.115	0.220
Jing1857	G-15	China	J	2004	Native	Flooded	3	86	90	8211	1	0.451	0.142	0.244
Jing1857	G-15	China	J	2004	Native	Flooded	4	86	88	8571	1	0.401	0.098	0.209
JinnuoNo6	G-16	China	I	2004	MSMA	Flooded	1	93	112	8812	1	0.592	.	.
JinnuoNo6	G-16	China	I	2004	MSMA	Flooded	2	97	110	7598	2	0.569	.	.
JinnuoNo6	G-16	China	I	2004	MSMA	Flooded	3	87	116	8319	1	0.522	.	.
JinnuoNo6	G-16	China	I	2004	MSMA	Flooded	4	97	112	5655	2	0.502	.	.
JinnuoNo6	G-16	China	I	2004	Native	Flooded	1	95	116	7981	1	0.343	.	.
JinnuoNo6	G-16	China	I	2004	Native	Flooded	2	91	100	10890	1	0.350	.	.
JinnuoNo6	G-16	China	I	2004	Native	Flooded	3	91	110	8630	1	0.352	.	.
JinnuoNo6	G-16	China	I	2004	Native	Flooded	4	91	116	8917	1	0.278	.	.
KBNT11	G-38	U.S.A.	J	2004	MSMA	Flooded	1	87	98	221	7	.	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
KBNT11	G-38	U.S.A.	J	2004	MSMA	Flooded	2	88	96	235	7	.	.	.
KBNT11	G-38	U.S.A.	J	2004	MSMA	Flooded	3	89	88	193	8	.	.	.
KBNT11	G-38	U.S.A.	J	2004	MSMA	Flooded	4	87	94	304	7	.	.	.
KBNT11	G-38	U.S.A.	J	2004	Native	Flooded	1	86	88	5532	2	0.803	.	.
KBNT11	G-38	U.S.A.	J	2004	Native	Flooded	2	86	100	6911	2	0.944	.	.
KBNT11	G-38	U.S.A.	J	2004	Native	Flooded	3	86	98	6747	2	0.804	.	.
KBNT11	G-38	U.S.A.	J	2004	Native	Flooded	4	83	92	6318	2	0.901	.	.
Luhongzao	G-17	China	I	2004	MSMA	Flooded	1	65	94	8109	1	1.370	0.094	1.410
Luhongzao	G-17	China	I	2004	MSMA	Flooded	2	66	102	7958	1	1.375	0.101	1.419
Luhongzao	G-17	China	I	2004	MSMA	Flooded	3	63	100	7605	1	1.374	0.092	1.446
Luhongzao	G-17	China	I	2004	MSMA	Flooded	4	66	94	7432	1	1.554	0.092	1.407
Luhongzao	G-17	China	I	2004	Native	Flooded	1	58	96	8351	1	0.370	0.090	0.249
Luhongzao	G-17	China	I	2004	Native	Flooded	2	58	102	8564	1	0.324	0.081	0.234
Luhongzao	G-17	China	I	2004	Native	Flooded	3	60	104	8375	1	0.297	0.089	0.251
Luhongzao	G-17	China	I	2004	Native	Flooded	4	58	96	8449	1	0.412	0.087	0.255
Mars	G-45	U.S.A.	J	2004	Native	Flooded	1	78	102	6189	2	0.509	.	.
Mars	G-45	U.S.A.	J	2004	Native	Flooded	2	82	108	6737	2	0.510	.	.
Mars	G-45	U.S.A.	J	2004	Native	Flooded	3	81	108	6093	2	0.486	.	.
Mars	G-45	U.S.A.	J	2004	Native	Flooded	4	79	102	6723	2	0.443	.	.
Medark	G-18	U.S.A.	J	2004	MSMA	Flooded	1	83	78	2455	8	1.621	0.116	1.364
Medark	G-18	U.S.A.	J	2004	MSMA	Flooded	2	81	78	2408	6	2.473	0.261	2.578
Medark	G-18	U.S.A.	J	2004	MSMA	Flooded	3	87	76	2439	6	2.217	0.132	1.852
Medark	G-18	U.S.A.	J	2004	MSMA	Flooded	4	81	84	2734	5	2.370	.	.
Medark	G-18	U.S.A.	J	2004	Native	Flooded	1	78	78	6715	2	0.510	0.142	0.032
Medark	G-18	U.S.A.	J	2004	Native	Flooded	2	78	84	7704	2	0.502	0.140	0.014
Medark	G-18	U.S.A.	J	2004	Native	Flooded	3	78	84	7509	2	0.546	0.160	0.034

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Medark	G-18	U.S.A.	J	2004	Native	Flooded	4	79	84	7766	2	0.469	.	.
MinkezaoNo22	G-19	China	I	2004	MSMA	Flooded	1	64	90	7290	1	0.817	0.100	0.692
MinkezaoNo22	G-19	China	I	2004	MSMA	Flooded	2	63	92	8891	1	0.807	0.099	0.709
MinkezaoNo22	G-19	China	I	2004	MSMA	Flooded	3	63	94	8808	1	0.869	0.084	0.709
MinkezaoNo22	G-19	China	I	2004	MSMA	Flooded	4	65	90	8051	1	0.887	0.092	0.738
MinkezaoNo22	G-19	China	I	2004	Native	Flooded	1	61	88	7455	1	0.487	0.083	0.341
MinkezaoNo22	G-19	China	I	2004	Native	Flooded	2	61	106	8868	1	0.550	0.085	0.335
MinkezaoNo22	G-19	China	I	2004	Native	Flooded	3	62	102	7982	1	0.493	0.087	0.339
MinkezaoNo22	G-19	China	I	2004	Native	Flooded	4	61	98	8890	1	0.497	0.079	0.341
PontaRubra	G-20	Portugal	J	2004	MSMA	Flooded	1	63	118	1409	2	2.709	0.138	2.025
PontaRubra	G-20	Portugal	J	2004	MSMA	Flooded	2	63	134	3088	2	2.114	0.147	2.525
PontaRubra	G-20	Portugal	J	2004	MSMA	Flooded	3	63	130	1547	5	2.046	0.119	2.062
PontaRubra	G-20	Portugal	J	2004	MSMA	Flooded	4	61	132	1450	4	2.290	.	.
PontaRubra	G-20	Portugal	J	2004	Native	Flooded	1	59	112	6105	1	0.522	.	.
PontaRubra	G-20	Portugal	J	2004	Native	Flooded	2	60	128	6294	1	0.530	0.161	0.279
PontaRubra	G-20	Portugal	J	2004	Native	Flooded	3	60	134	6275	1	0.524	0.125	0.229
PontaRubra	G-20	Portugal	J	2004	Native	Flooded	4	61	122	6824	1	0.500	0.143	0.254
Priscilla	G-21	U.S.A.	J	2004	MSMA	Flooded	1	85	86	4342	6	1.530	.	.
Priscilla	G-21	U.S.A.	J	2004	MSMA	Flooded	2	87	80	3646	6	1.733	.	.
Priscilla	G-21	U.S.A.	J	2004	MSMA	Flooded	3	89	90	3961	5	1.726	.	.
Priscilla	G-21	U.S.A.	J	2004	MSMA	Flooded	4	85	86	4957	5	1.080	.	.
Priscilla	G-21	U.S.A.	J	2004	Native	Flooded	1	79	78	7191	2	0.378	.	.
Priscilla	G-21	U.S.A.	J	2004	Native	Flooded	2	83	84	6518	2	0.382	.	.
Priscilla	G-21	U.S.A.	J	2004	Native	Flooded	3	86	90	6690	2	0.237	.	.
Priscilla	G-21	U.S.A.	J	2004	Native	Flooded	4	84	84	8211	2	0.333	.	.
Spalcik	G-24	Russia	J	2004	MSMA	Flooded	1	58	102	2720	2	2.068	0.139	1.958

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Spalcik	G-24	Russia	J	2004	MSMA	Flooded	2	59	98	4807	2	2.427	0.148	1.969
Spalcik	G-24	Russia	J	2004	MSMA	Flooded	3	57	98	2307	3	2.797	0.141	1.877
Spalcik	G-24	Russia	J	2004	MSMA	Flooded	4	58	102	1340	4	2.576	0.127	2.112
Spalcik	G-24	Russia	J	2004	Native	Flooded	1	57	100	7459	1	0.518	.	.
Spalcik	G-24	Russia	J	2004	Native	Flooded	2	58	104	7194	1	0.530	0.155	0.283
Spalcik	G-24	Russia	J	2004	Native	Flooded	3	56	106	7751	1	0.559	0.166	0.275
Spalcik	G-24	Russia	J	2004	Native	Flooded	4	58	100	7317	1	0.532	0.143	0.291
Tie901	G-25	China	I	2004	MSMA	Flooded	1	63	94	7609	1	1.702	.	.
Tie901	G-25	China	I	2004	MSMA	Flooded	2	66	104	8643	1	1.590	.	.
Tie901	G-25	China	I	2004	MSMA	Flooded	3	66	100	8108	1	1.831	.	.
Tie901	G-25	China	I	2004	MSMA	Flooded	4	66	106	8478	1	1.752	.	.
Tie901	G-25	China	I	2004	Native	Flooded	1	58	100	9845	1	0.438	.	.
Tie901	G-25	China	I	2004	Native	Flooded	2	58	108	9711	1	0.387	.	.
Tie901	G-25	China	I	2004	Native	Flooded	3	59	104	9703	1	0.429	.	.
Tie901	G-25	China	I	2004	Native	Flooded	4	56	108	10178	1	0.430	.	.
Wells	G-27	U.S.A.	J	2004	MSMA	Flooded	1	89	92	1589	6	1.305	.	.
Wells	G-27	U.S.A.	J	2004	MSMA	Flooded	2	89	86	1036	6	1.506	.	.
Wells	G-27	U.S.A.	J	2004	MSMA	Flooded	3	89	92	1989	6	1.812	.	.
Wells	G-27	U.S.A.	J	2004	MSMA	Flooded	4	88	94	1340	6	1.968	.	.
Wells	G-27	U.S.A.	J	2004	Native	Flooded	1	76	86	6944	2	0.418	.	.
Wells	G-27	U.S.A.	J	2004	Native	Flooded	2	78	96	8011	2	0.470	.	.
Wells	G-27	U.S.A.	J	2004	Native	Flooded	3	83	100	7390	2	0.389	.	.
Wells	G-27	U.S.A.	J	2004	Native	Flooded	4	79	92	8174	2	0.394	.	.
Xiangzaoxian1	G-28	China	I	2004	MSMA	Flooded	1	65	98	8132	2	1.376	0.094	1.576
Xiangzaoxian1	G-28	China	I	2004	MSMA	Flooded	2	66	98	8826	1	1.398	0.115	1.522
Xiangzaoxian1	G-28	China	I	2004	MSMA	Flooded	3	66	92	9109	1	1.563	0.105	1.549

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Xiangzaoxian1	G-28	China	I	2004	MSMA	Flooded	4	66	106	7518	1	1.446	.	.
Xiangzaoxian1	G-28	China	I	2004	Native	Flooded	1	64	102	10500	1	0.221	0.097	0.196
Xiangzaoxian1	G-28	China	I	2004	Native	Flooded	2	62	104	11675	1	0.286	0.106	0.202
Xiangzaoxian1	G-28	China	I	2004	Native	Flooded	3	62	106	11107	1	0.165	0.091	0.194
Xiangzaoxian1	G-28	China	I	2004	Native	Flooded	4	63	106	10688	1	0.244	0.102	0.202
YouIB	G-29	China	I	2004	MSMA	Flooded	1	62	92	6510	1	2.258	.	.
YouIB	G-29	China	I	2004	MSMA	Flooded	2	62	104	7458	1	2.484	.	.
YouIB	G-29	China	I	2004	MSMA	Flooded	3	63	90	5599	1	2.175	.	.
YouIB	G-29	China	I	2004	MSMA	Flooded	4	63	92	4998	1	2.222	.	.
YouIB	G-29	China	I	2004	Native	Flooded	1	60	96	7352	1	0.320	.	.
YouIB	G-29	China	I	2004	Native	Flooded	2	60	100	8966	1	0.293	.	.
YouIB	G-29	China	I	2004	Native	Flooded	3	61	88	7776	1	0.311	.	.
YouIB	G-29	China	I	2004	Native	Flooded	4	60	112	7650	1	0.393	.	.
Zanuo	G-30	China	I	2004	MSMA	Flooded	1	67	100	3240	5	2.750	0.199	2.119
Zanuo	G-30	China	I	2004	MSMA	Flooded	2	68	94	2114	6	2.344	0.234	2.866
Zanuo	G-30	China	I	2004	MSMA	Flooded	3	64	98	1602	6	2.697	.	.
Zanuo	G-30	China	I	2004	MSMA	Flooded	4	64	98	2266	5	2.996	0.222	2.636
Zanuo	G-30	China	I	2004	Native	Flooded	1	68	106	8626	2	0.446	0.164	0.241
Zanuo	G-30	China	I	2004	Native	Flooded	2	65	110	7859	2	0.450	0.199	0.304
Zanuo	G-30	China	I	2004	Native	Flooded	3	70	106	9670	2	0.395	0.193	0.186
Zanuo	G-30	China	I	2004	Native	Flooded	4	72	106	9491	2	0.345	0.194	0.191
Zao402	G-31	China	I	2004	MSMA	Flooded	1	65	100	7044	1	2.747	0.137	2.122
Zao402	G-31	China	I	2004	MSMA	Flooded	2	66	110	8329	1	2.476	0.118	1.999
Zao402	G-31	China	I	2004	MSMA	Flooded	3	67	96	7352	1	2.623	0.113	0.601
Zao402	G-31	China	I	2004	MSMA	Flooded	4	67	102	7782	1	2.221	.	.
Zao402	G-31	China	I	2004	Native	Flooded	1	63	106	8790	1	0.447	0.068	0.197

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Zao402	G-31	China	I	2004	Native	Flooded	2	63	108	7807	1	0.520	0.120	0.287
Zao402	G-31	China	I	2004	Native	Flooded	3	61	104	7710	1	0.514	0.133	0.278
Zao402	G-31	China	I	2004	Native	Flooded	4	64	114	7952	1	0.576	.	.
Zhe733	G-32	China	I	2004	MSMA	Flooded	1	63	90	7690	1	0.780	0.108	0.533
Zhe733	G-32	China	I	2004	MSMA	Flooded	2	63	100	9763	1	0.844	0.102	0.562
Zhe733	G-32	China	I	2004	MSMA	Flooded	3	60	100	6326	1	0.855	0.127	0.678
Zhe733	G-32	China	I	2004	MSMA	Flooded	4	63	100	7006	1	0.761	.	.
Zhe733	G-32	China	I	2004	Native	Flooded	1	60	104	10420	1	0.296	0.105	0.158
Zhe733	G-32	China	I	2004	Native	Flooded	2	59	102	8403	1	0.279	0.102	0.109
Zhe733	G-32	China	I	2004	Native	Flooded	3	62	96	9967	1	0.216	0.105	0.152
Zhe733	G-32	China	I	2004	Native	Flooded	4	61	104	9067	1	0.280	.	.
Zhenshan97	G-33	China	I	2004	MSMA	Flooded	1	66	102	7451	1	2.561	0.141	1.767
Zhenshan97	G-33	China	I	2004	MSMA	Flooded	2	66	100	6134	1	2.324	0.128	1.472
Zhenshan97	G-33	China	I	2004	MSMA	Flooded	3	66	102	7212	1	2.500	0.136	1.785
Zhenshan97	G-33	China	I	2004	MSMA	Flooded	4	67	102	7585	1	2.817	0.146	2.078
Zhenshan97	G-33	China	I	2004	Native	Flooded	1	66	106	8870	1	0.370	0.100	0.184
Zhenshan97	G-33	China	I	2004	Native	Flooded	2	66	108	7862	1	0.378	.	.
Zhenshan97	G-33	China	I	2004	Native	Flooded	3	65	100	7574	1	0.369	0.117	0.224
Zhenshan97	G-33	China	I	2004	Native	Flooded	4	67	110	9421	1	0.353	0.110	0.212
Zhong8644	G-35	China	I	2004	MSMA	Flooded	1	67	104	7648	1	1.382	.	.
Zhong8644	G-35	China	I	2004	MSMA	Flooded	2	66	106	8063	1	1.497	.	.
Zhong8644	G-35	China	I	2004	MSMA	Flooded	3	66	110	7462	1	1.567	.	.
Zhong8644	G-35	China	I	2004	MSMA	Flooded	4	66	112	6683	2	1.822	.	.
Zhong8644	G-35	China	I	2004	Native	Flooded	1	60	104	8900	1	0.462	.	.
Zhong8644	G-35	China	I	2004	Native	Flooded	2	60	102	9208	1	0.436	.	.
Zhong8644	G-35	China	I	2004	Native	Flooded	3	61	92	7738	1	0.418	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
Zhong8644	G-35	China	I	2004	Native	Flooded	4	61	104	8065	1	0.470	.	.
Aijiaonante	G-2	China	I	2005	MSMA	Flooded	1	68	82	7009	2	1.160	.	.
Aijiaonante	G-2	China	I	2005	MSMA	Flooded	2	68	90	6304	2	1.131	.	.
Aijiaonante	G-2	China	I	2005	MSMA	Flooded	3	68	86	5755	2	1.037	.	.
Aijiaonante	G-2	China	I	2005	MSMA	Flooded	4	68	86	8107	2	1.093	.	.
Aijiaonante	G-2	China	I	2005	Native	Flooded	1	72	88	7570	2	0.347	.	.
Aijiaonante	G-2	China	I	2005	Native	Flooded	2	68	90	8083	2	0.323	.	.
Aijiaonante	G-2	China	I	2005	Native	Flooded	3	72	90	7809	2	0.315	.	.
Aijiaonante	G-2	China	I	2005	Native	Flooded	4	68	90	5779	2	0.310	.	.
Cocodrie	G-37	U.S.A.	J	2005	MSMA	Flooded	1	83	82	764	8	0.507	.	.
Cocodrie	G-37	U.S.A.	J	2005	MSMA	Flooded	2	86	92	442	8	0.486	.	.
Cocodrie	G-37	U.S.A.	J	2005	MSMA	Flooded	3	86	86	1791	8	1.084	.	.
Cocodrie	G-37	U.S.A.	J	2005	MSMA	Flooded	4	86	88	490	7	1.036	.	.
Cocodrie	G-37	U.S.A.	J	2005	Native	Flooded	1	81	96	6925	2	0.447	.	.
Cocodrie	G-37	U.S.A.	J	2005	Native	Flooded	2	80	102	6304	2	0.421	.	.
Cocodrie	G-37	U.S.A.	J	2005	Native	Flooded	3	81	98	6579	2	0.499	.	.
Cocodrie	G-37	U.S.A.	J	2005	Native	Flooded	4	81	96	6161	2	0.464	.	.
Danwanbao24	G-8	China	I	2005	MSMA	Flooded	1	80	94	5027	2	0.935	.	.
Danwanbao24	G-8	China	I	2005	MSMA	Flooded	2	80	94	5158	2	0.951	.	.
Danwanbao24	G-8	China	I	2005	MSMA	Flooded	3	80	88	4955	2	0.922	.	.
Danwanbao24	G-8	China	I	2005	MSMA	Flooded	4	80	86	4466	3	1.109	.	.
Danwanbao24	G-8	China	I	2005	Native	Flooded	1	86	98	5397	2	0.504	.	.
Danwanbao24	G-8	China	I	2005	Native	Flooded	2	81	90	5170	2	0.544	.	.
Danwanbao24	G-8	China	I	2005	Native	Flooded	3	80	96	4286	2	0.556	.	.
Danwanbao24	G-8	China	I	2005	Native	Flooded	4	81	98	4836	2	0.571	.	.
Gui99	G-10	China	I	2005	MSMA	Flooded	1	88	104	4131	2	1.159	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Gui99	G-10	China	I	2005	MSMA	Flooded	2	88	110	4788	4	1.166	.	.
Gui99	G-10	China	I	2005	MSMA	Flooded	3	88	114	4645	3	0.932	.	.
Gui99	G-10	China	I	2005	MSMA	Flooded	4	88	102	4919	3	1.023	.	.
Gui99	G-10	China	I	2005	Native	Flooded	1	88	118	.	2	0.697	.	.
Gui99	G-10	China	I	2005	Native	Flooded	2	88	118	4167	2	0.686	.	.
Gui99	G-10	China	I	2005	Native	Flooded	3	88	115	3594	2	0.653	.	.
Gui99	G-10	China	I	2005	Native	Flooded	4	86	114	4454	2	0.665	.	.
IR44595	G-14	Nepal	I	2005	MSMA	Flooded	1	80	108	6364	2	1.068	.	.
IR44595	G-14	Nepal	I	2005	MSMA	Flooded	2	80	114	6842	2	1.090	.	.
IR44595	G-14	Nepal	I	2005	MSMA	Flooded	3	80	108	6746	2	1.213	.	.
IR44595	G-14	Nepal	I	2005	MSMA	Flooded	4	80	106	7116	2	1.183	.	.
IR44595	G-14	Nepal	I	2005	Native	Flooded	1	80	106	7152	2	0.431	.	.
IR44595	G-14	Nepal	I	2005	Native	Flooded	2	80	112	7570	2	0.452	.	.
IR44595	G-14	Nepal	I	2005	Native	Flooded	3	76	112	6686	2	0.693	.	.
IR44595	G-14	Nepal	I	2005	Native	Flooded	4	80	120	5946	2	0.563	.	.
IR9209	G-13	Philippines	I	2005	MSMA	Flooded	1	80	86	5755	2	1.121	.	.
IR9209	G-13	Philippines	I	2005	MSMA	Flooded	2	80	94	5922	2	1.029	.	.
IR9209	G-13	Philippines	I	2005	MSMA	Flooded	3	80	94	6245	2	1.090	.	.
IR9209	G-13	Philippines	I	2005	MSMA	Flooded	4	80	96	6674	2	1.166	.	.
IR9209	G-13	Philippines	I	2005	Native	Flooded	1	76	94	5779	2	0.759	.	.
IR9209	G-13	Philippines	I	2005	Native	Flooded	2	76	100	6316	2	0.797	.	.
IR9209	G-13	Philippines	I	2005	Native	Flooded	3	80	92	5982	2	0.696	.	.
IR9209	G-13	Philippines	I	2005	Native	Flooded	4	76	98	6209	2	0.745	.	.
Jing1857	G-15	China	J	2005	MSMA	Flooded	1	88	90	6054	2	0.593	.	.
Jing1857	G-15	China	J	2005	MSMA	Flooded	2	88	94	6627	2	0.705	.	.
Jing1857	G-15	China	J	2005	MSMA	Flooded	3	94	98	5743	2	0.731	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
Jing1857	G-15	China	J	2005	MSMA	Flooded	4	94	92	5839	2	0.708	.	.
Jing1857	G-15	China	J	2005	Native	Flooded	1	94	98	5385	2	0.398	.	.
Jing1857	G-15	China	J	2005	Native	Flooded	2	88	98	6269	2	0.406	.	.
Jing1857	G-15	China	J	2005	Native	Flooded	3	93	98	6101	2	0.471	.	.
Jing1857	G-15	China	J	2005	Native	Flooded	4	93	95	6304	2	0.416	.	.
JinnuoNo6	G-16	China	I	2005	MSMA	Flooded	1	94	124	5946	3	0.499	.	.
JinnuoNo6	G-16	China	I	2005	MSMA	Flooded	2	95	116	6006	3	0.541	.	.
JinnuoNo6	G-16	China	I	2005	MSMA	Flooded	3	94	124	5755	3	0.506	.	.
JinnuoNo6	G-16	China	I	2005	MSMA	Flooded	4	94	122	6615	3	0.597	.	.
JinnuoNo6	G-16	China	I	2005	Native	Flooded	1	95	120	6292	2	0.286	.	.
JinnuoNo6	G-16	China	I	2005	Native	Flooded	2	94	118	5588	2	0.238	.	.
JinnuoNo6	G-16	China	I	2005	Native	Flooded	3	93	122	6125	2	0.284	.	.
JinnuoNo6	G-16	China	I	2005	Native	Flooded	4	94	118	6233	2	0.287	.	.
KBNT11	G-38	U.S.A.	J	2005	MSMA	Flooded	1	87	110	2269	6	2.681	.	.
KBNT11	G-38	U.S.A.	J	2005	MSMA	Flooded	2	86	104	4525	5	2.812	.	.
KBNT11	G-38	U.S.A.	J	2005	MSMA	Flooded	3	86	98	2495	5	3.473	.	.
KBNT11	G-38	U.S.A.	J	2005	MSMA	Flooded	4	86	98	2281	6	2.507	.	.
KBNT11	G-38	U.S.A.	J	2005	Native	Flooded	1	87	100	5337	2	2.494	.	.
KBNT11	G-38	U.S.A.	J	2005	Native	Flooded	2	87	103	4716	2	1.453	.	.
KBNT11	G-38	U.S.A.	J	2005	Native	Flooded	3	86	110	5373	2	1.658	.	.
KBNT11	G-38	U.S.A.	J	2005	Native	Flooded	4	86	105	5528	2	1.692	.	.
Luhongzao	G-17	China	I	2005	MSMA	Flooded	1	68	94	6794	2	1.375	.	.
Luhongzao	G-17	China	I	2005	MSMA	Flooded	2	68	102	7534	2	1.188	.	.
Luhongzao	G-17	China	I	2005	MSMA	Flooded	3	68	96	6686	2	1.107	.	.
Luhongzao	G-17	China	I	2005	MSMA	Flooded	4	68	88	7403	2	1.127	.	.
Luhongzao	G-17	China	I	2005	Native	Flooded	1	72	102	6925	2	0.471	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Luhongzao	G-17	China	I	2005	Native	Flooded	2	66	94	6758	2	0.450	.	.
Luhongzao	G-17	China	I	2005	Native	Flooded	3	68	100	6949	2	0.467	.	.
Luhongzao	G-17	China	I	2005	Native	Flooded	4	68	98	6555	2	0.479	.	.
Medark	G-18	U.S.A.	J	2005	MSMA	Flooded	1	80	84	4454	5	1.753	.	.
Medark	G-18	U.S.A.	J	2005	MSMA	Flooded	2	82	88	4907	5	1.238	.	.
Medark	G-18	U.S.A.	J	2005	MSMA	Flooded	3	85	86	5528	4	1.479	.	.
Medark	G-18	U.S.A.	J	2005	MSMA	Flooded	4	82	82	4872	5	1.532	.	.
Medark	G-18	U.S.A.	J	2005	Native	Flooded	1	81	88	5839	2	0.662	.	.
Medark	G-18	U.S.A.	J	2005	Native	Flooded	2	81	96	5349	2	0.507	.	.
Medark	G-18	U.S.A.	J	2005	Native	Flooded	3	66	104	7427	2	0.487	.	.
Medark	G-18	U.S.A.	J	2005	Native	Flooded	4	80	88	5660	2	0.551	.	.
MinkezaoNo22	G-19	China	I	2005	MSMA	Flooded	1	66	86	7892	2	0.861	.	.
MinkezaoNo22	G-19	China	I	2005	MSMA	Flooded	2	66	90	7773	2	0.833	.	.
MinkezaoNo22	G-19	China	I	2005	MSMA	Flooded	3	66	90	6483	2	0.797	.	.
MinkezaoNo22	G-19	China	I	2005	MSMA	Flooded	4	66	86	7928	2	1.049	.	.
MinkezaoNo22	G-19	China	I	2005	Native	Flooded	1	72	96	7080	2	0.476	.	.
MinkezaoNo22	G-19	China	I	2005	Native	Flooded	2	68	100	7642	2	0.492	.	.
MinkezaoNo22	G-19	China	I	2005	Native	Flooded	3	68	90	6030	2	0.465	.	.
MinkezaoNo22	G-19	China	I	2005	Native	Flooded	4	68	92	6866	2	0.485	.	.
PontaRubra	G-20	Portugal	J	2005	MSMA	Flooded	1	66	108	5910	2	1.629	.	.
PontaRubra	G-20	Portugal	J	2005	MSMA	Flooded	2	66	110	5015	2	1.506	.	.
PontaRubra	G-20	Portugal	J	2005	MSMA	Flooded	3	66	110	4692	3	1.463	.	.
PontaRubra	G-20	Portugal	J	2005	MSMA	Flooded	4	66	104	4334	3	1.531	.	.
PontaRubra	G-20	Portugal	J	2005	Native	Flooded	1	66	112	5051	2	0.421	.	.
PontaRubra	G-20	Portugal	J	2005	Native	Flooded	2	66	114	5266	2	0.393	.	.
PontaRubra	G-20	Portugal	J	2005	Native	Flooded	3	66	122	4633	2	0.557	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
PontaRubra	G-20	Portugal	J	2005	Native	Flooded	4	66	124	4943	2	0.499	.	.
Priscilla	G-21	U.S.A.	J	2005	MSMA	Flooded	1	83	92	2304	4	0.710	.	.
Priscilla	G-21	U.S.A.	J	2005	MSMA	Flooded	2	86	94	4800	3	0.755	.	.
Priscilla	G-21	U.S.A.	J	2005	MSMA	Flooded	3	86	94	3188	4	0.727	.	.
Priscilla	G-21	U.S.A.	J	2005	MSMA	Flooded	4	86	100	4298	4	0.781	.	.
Priscilla	G-21	U.S.A.	J	2005	Native	Flooded	1	86	102	5851	2	0.378	.	.
Priscilla	G-21	U.S.A.	J	2005	Native	Flooded	2	88	104	4919	2	0.394	.	.
Priscilla	G-21	U.S.A.	J	2005	Native	Flooded	3	86	96	5098	2	0.382	.	.
Priscilla	G-21	U.S.A.	J	2005	Native	Flooded	4	86	96	5743	2	0.364	.	.
Spalcik	G-24	Russia	J	2005	MSMA	Flooded	1	55	82	2949	2	1.319	.	.
Spalcik	G-24	Russia	J	2005	MSMA	Flooded	2	55	82	3809	3	1.336	.	.
Spalcik	G-24	Russia	J	2005	MSMA	Flooded	3	55	80	2639	3	1.453	.	.
Spalcik	G-24	Russia	J	2005	MSMA	Flooded	4	55	82	3307	3	1.591	.	.
Spalcik	G-24	Russia	J	2005	Native	Flooded	1	55	78	4394	2	0.474	.	.
Spalcik	G-24	Russia	J	2005	Native	Flooded	2	54	78	5385	2	0.550	.	.
Spalcik	G-24	Russia	J	2005	Native	Flooded	3	55	84	4860	2	0.534	.	.
Spalcik	G-24	Russia	J	2005	Native	Flooded	4	55	80	4131	2	0.528	.	.
Tie901	G-25	China	I	2005	MSMA	Flooded	1	66	100	7188	2	1.768	.	.
Tie901	G-25	China	I	2005	MSMA	Flooded	2	66	100	9015	2	1.715	.	.
Tie901	G-25	China	I	2005	MSMA	Flooded	3	66	92	7737	2	1.813	.	.
Tie901	G-25	China	I	2005	MSMA	Flooded	4	66	96	8012	2	1.698	.	.
Tie901	G-25	China	I	2005	Native	Flooded	1	66	94	7916	2	0.490	.	.
Tie901	G-25	China	I	2005	Native	Flooded	2	66	106	7845	2	0.459	.	.
Tie901	G-25	China	I	2005	Native	Flooded	3	66	98	7295	2	0.264	.	.
Tie901	G-25	China	I	2005	Native	Flooded	4	66	94	7021	2	0.258	.	.
Wells	G-27	U.S.A.	J	2005	MSMA	Flooded	1	86	98	4633	4	0.622	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	$DMAs^{V}$
Wells	G-27	U.S.A.	J	2005	MSMA	Flooded	2	86	96	5469	5	0.758	.	.
Wells	G-27	U.S.A.	J	2005	MSMA	Flooded	3	86	92	5469	5	0.820	.	.
Wells	G-27	U.S.A.	J	2005	MSMA	Flooded	4	86	98	4692	5	0.779	.	.
Wells	G-27	U.S.A.	J	2005	Native	Flooded	1	86	100	6054	2	0.424	.	.
Wells	G-27	U.S.A.	J	2005	Native	Flooded	2	81	100	5576	2	0.445	.	.
Wells	G-27	U.S.A.	J	2005	Native	Flooded	3	86	106	4322	2	0.473	.	.
Wells	G-27	U.S.A.	J	2005	Native	Flooded	4	86	105	6101	2	0.441	.	.
YouIB	G-29	China	I	2005	MSMA	Flooded	1	65	98	5039	2	1.362	.	.
YouIB	G-29	China	I	2005	MSMA	Flooded	2	66	88	6639	2	1.396	.	.
YouIB	G-29	China	I	2005	MSMA	Flooded	3	65	86	5528	2	1.565	.	.
YouIB	G-29	China	I	2005	MSMA	Flooded	4	66	86	6340	2	1.536	.	.
YouIB	G-29	China	I	2005	Native	Flooded	1	66	94	6424	2	0.537	.	.
YouIB	G-29	China	I	2005	Native	Flooded	2	66	98	6006	2	0.516	.	.
YouIB	G-29	China	I	2005	Native	Flooded	3	66	94	6889	2	0.576	.	.
YouIB	G-29	China	I	2005	Native	Flooded	4	66	96	5803	2	0.515	.	.
Zao402	G-31	China	I	2005	MSMA	Flooded	1	72	96	6209	2	1.895	.	.
Zao402	G-31	China	I	2005	MSMA	Flooded	2	72	102	7474	2	2.050	.	.
Zao402	G-31	China	I	2005	MSMA	Flooded	3	72	100	6018	2	2.024	.	.
Zao402	G-31	China	I	2005	MSMA	Flooded	4	72	98	6794	2	2.114	.	.
Zao402	G-31	China	I	2005	Native	Flooded	1	72	98	7558	2	0.757	.	.
Zao402	G-31	China	I	2005	Native	Flooded	2	72	101	8107	2	0.640	.	.
Zao402	G-31	China	I	2005	Native	Flooded	3	72	100	8262	2	0.615	.	.
Zao402	G-31	China	I	2005	Native	Flooded	4	72	104	7737	2	0.703	.	.
Zhe733	G-32	China	I	2005	MSMA	Flooded	3	65	90	8418	2	0.581	.	.
Zhe733	G-32	China	I	2005	MSMA	Flooded	4	65	94	6340	2	0.626	.	.
Zhe733	G-32	China	I	2005	Native	Flooded	1	61	88	8286	2	0.387	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Zhe733	G-32	China	I	2005	Native	Flooded	2	61	96	7403	2	0.393	.	.
Zhe733	G-32	China	I	2005	Native	Flooded	3	60	96	8382	2	0.345	.	.
Zhe733	G-32	China	I	2005	Native	Flooded	4	66	96	7128	2	0.361	.	.
Zhe733	G-32	China	I	2005	MSMA	Flooded	1	65	98	6221	2	0.737	.	.
Zhe733	G-32	China	I	2005	MSMA	Flooded	2	65	108	8322	2	0.654	.	.
Zhong8644	G-35	China	I	2005	MSMA	Flooded	1	68	102	6866	2	1.659	.	.
Zhong8644	G-35	China	I	2005	MSMA	Flooded	2	68	100	6770	2	1.735	.	.
Zhong8644	G-35	China	I	2005	MSMA	Flooded	3	68	94	7379	2	1.433	.	.
Zhong8644	G-35	China	I	2005	MSMA	Flooded	4	68	100	6806	2	1.548	.	.
Zhong8644	G-35	China	I	2005	Native	Flooded	1	68	104	7857	2	0.705	.	.
Zhong8644	G-35	China	I	2005	Native	Flooded	2	66	110	8155	2	0.587	.	.
Zhong8644	G-35	China	I	2005	Native	Flooded	3	68	106	7928	2	0.548	.	.
Zhong8644	G-35	China	I	2005	Native	Flooded	4	68	110	7415	2	0.480	.	.
4484_1665	G-47	China	I	2007	MSMA	Flooded	1	97	92	136	9	2.090	.	.
4484_1665	G-47	China	I	2007	MSMA	Flooded	2	93	102	676	9	1.777	.	.
4484_1665	G-47	China	I	2007	MSMA	Flooded	3	91	102	1630	8	1.268	.	.
4484_1665	G-47	China	I	2007	MSMA	Flooded	4	93	82	95	9	2.027	.	.
4484_1665	G-47	China	I	2007	Native	Flooded	1	90	108	8806	2	0.335	.	.
4484_1665	G-47	China	I	2007	Native	Flooded	2	91	118	6979	2	0.150	.	.
4484_1665	G-47	China	I	2007	Native	Flooded	3	90	122	7085	2	0.428	.	.
4484_1665	G-47	China	I	2007	Native	Flooded	4	91	104	7347	2	0.409	.	.
4484_1665	G-47	China	I	2007	MSMA	Saturated	1	99	78	7032	2	0.477	.	.
4484_1665	G-47	China	I	2007	MSMA	Saturated	2	93	88	5212	2	0.534	.	.
4484_1665	G-47	China	I	2007	MSMA	Saturated	3	93	96	7588	1	2.318	.	.
4484_1665	G-47	China	I	2007	MSMA	Saturated	4	93	90	5739	2	0.808	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
4484_1665	G-47	China	I	2007	Native	Saturated	1	90	110	7984	2	0.108	.	.
4484_1665	G-47	China	I	2007	Native	Saturated	2	90	110	8596	2	0.177	.	.
4484_1665	G-47	China	I	2007	Native	Saturated	3	88	110	5281	2	0.162	.	.
4484_1665	G-47	China	I	2007	Native	Saturated	4	91	104	8222	2	0.186	.	.
4484_1693	G-48	China	I	2007	MSMA	Flooded	1	97	70	0	9	3.025	.	.
4484_1693	G-48	China	I	2007	MSMA	Flooded	2	91	82	1359	9	2.375	.	.
4484_1693	G-48	China	I	2007	MSMA	Flooded	3	92	80	449	9	2.124	.	.
4484_1693	G-48	China	I	2007	MSMA	Flooded	4	93	72	0	9	3.101	.	.
4484_1693	G-48	China	I	2007	Native	Flooded	1	90	98	8566	2	0.466	.	.
4484_1693	G-48	China	I	2007	Native	Flooded	2	90	104	6849	2	0.350	.	.
4484_1693	G-48	China	I	2007	Native	Flooded	3	88	100	6836	2	0.516	.	.
4484_1693	G-48	China	I	2007	Native	Flooded	4	90	102	6745	2	0.459	.	.
4484_1693	G-48	China	I	2007	MSMA	Saturated	1	93	86	5984	2	0.678	.	.
4484_1693	G-48	China	I	2007	MSMA	Saturated	2	91	78	6162	2	1.208	.	.
4484_1693	G-48	China	I	2007	MSMA	Saturated	3	92	88	6283	3	1.748	.	.
4484_1693	G-48	China	I	2007	MSMA	Saturated	4	91	92	7055	2	3.474	.	.
4484_1693	G-48	China	I	2007	Native	Saturated	1	90	100	7466	2	0.134	.	.
4484_1693	G-48	China	I	2007	Native	Saturated	2	90	100	8159	2	0.180	.	.
4484_1693	G-48	China	I	2007	Native	Saturated	3	88	110	7063	2	0.143	.	.
4484_1693	G-48	China	I	2007	Native	Saturated	4	97	110	7618	2	0.156	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Flooded	1	85	76	1221	8	1.451	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Flooded	2	85	82	2603	8	1.336	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Flooded	3	85	86	3341	6	1.146	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Flooded	4	85	86	1303	8	1.613	.	.
CL161	G-5	U.S.A.	J	2007	Native	Flooded	1	82	84	3462	2	0.417	.	.
CL161	G-5	U.S.A.	J	2007	Native	Flooded	2	82	94	7931	2	0.438	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
CL161	G-5	U.S.A.	J	2007	Native	Flooded	3	85	98	7505	2	0.437	.	.
CL161	G-5	U.S.A.	J	2007	Native	Flooded	4	85	98	8043	2	0.367	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Saturated	1	90	74	3640	3	0.658	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Saturated	2	90	76	3261	3	0.498	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Saturated	3	85	72	4501	3	1.803	.	.
CL161	G-5	U.S.A.	J	2007	MSMA	Saturated	4	85	84	5227	2	1.087	.	.
CL161	G-5	U.S.A.	J	2007	Native	Saturated	1	82	92	7224	2	0.136	.	.
CL161	G-5	U.S.A.	J	2007	Native	Saturated	2	85	104	7634	2	0.124	.	.
CL161	G-5	U.S.A.	J	2007	Native	Saturated	3	85	90	7236	2	0.122	.	.
CL161	G-5	U.S.A.	J	2007	Native	Saturated	4	82	100	7885	2	0.148	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Flooded	1	85	84	477	8	1.457	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Flooded	2	82	76	750	8	1.176	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Flooded	3	81	92	729	9	0.642	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Flooded	4	83	86	1082	8	0.930	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Flooded	1	80	94	9524	2	0.318	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Flooded	2	80	96	7215	2	0.362	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Flooded	3	78	80	7881	2	0.326	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Flooded	4	80	102	6112	2	0.208	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Saturated	1	82	78	4748	3	0.697	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Saturated	2	80	78	4524	3	0.620	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Saturated	3	82	88	4839	3	1.456	.	.
Cocodrie	G-37	U.S.A.	J	2007	MSMA	Saturated	4	87	76	4281	3	0.502	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Saturated	1	82	98	7836	2	0.097	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Saturated	2	81	106	8182	2	0.152	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Saturated	3	82	86	6723	2	0.085	.	.
Cocodrie	G-37	U.S.A.	J	2007	Native	Saturated	4	82	94	9984	2	0.104	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Flooded	1	82	86	3418	4	2.046	.	.
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Flooded	2	82	80	7615	4	1.069	0.138	0.845
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Flooded	3	80	92	5918	4	1.011	0.139	0.792
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Flooded	4	82	76	6947	4	1.385	0.216	1.108
Cybonnet	G-7	U.S.A.	J	2007	Native	Flooded	1	82	88	7756	2	0.347	0.121	0.173
Cybonnet	G-7	U.S.A.	J	2007	Native	Flooded	2	82	92	8034	2	0.288	0.141	0.164
Cybonnet	G-7	U.S.A.	J	2007	Native	Flooded	3	81	94	7665	2	0.366	0.117	0.176
Cybonnet	G-7	U.S.A.	J	2007	Native	Flooded	4	80	90	6651	2	0.447	0.115	0.151
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Saturated	1	85	72	3130	4	0.691	0.135	0.506
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Saturated	2	85	72	3114	3	0.797	.	.
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Saturated	3	88	82	3596	3	0.601	0.138	0.380
Cybonnet	G-7	U.S.A.	J	2007	MSMA	Saturated	4	82	74	4713	3	0.707	0.155	0.490
Cybonnet	G-7	U.S.A.	J	2007	Native	Saturated	1	82	86	6665	2	0.148	0.104	0.058
Cybonnet	G-7	U.S.A.	J	2007	Native	Saturated	2	82	82	8326	2	0.169	0.110	0.081
Cybonnet	G-7	U.S.A.	J	2007	Native	Saturated	3	82	90	7350	2	0.146	0.101	0.064
Cybonnet	G-7	U.S.A.	J	2007	Native	Saturated	4	82	92	8070	2	0.150	0.105	0.047
Francis	G-9	U.S.A.	J	2007	MSMA	Flooded	1	81	88	4254	6	1.754	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Flooded	2	82	82	3477	5	1.268	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Flooded	3	82	98	6291	4	0.824	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Flooded	4	82	94	5728	6	1.275	.	.
Francis	G-9	U.S.A.	J	2007	Native	Flooded	1	81	94	8889	2	0.306	.	.
Francis	G-9	U.S.A.	J	2007	Native	Flooded	2	81	98	9547	2	0.143	.	.
Francis	G-9	U.S.A.	J	2007	Native	Flooded	3	81	96	9686	2	0.247	.	.
Francis	G-9	U.S.A.	J	2007	Native	Flooded	4	81	108	9401	2	0.293	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Saturated	1	82	86	7251	2	0.485	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Saturated	2	85	90	6943	2	0.404	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Francis	G-9	U.S.A.	J	2007	MSMA	Saturated	3	82	86	6254	2	0.763	.	.
Francis	G-9	U.S.A.	J	2007	MSMA	Saturated	4	82	94	6278	2	0.703	.	.
Francis	G-9	U.S.A.	J	2007	Native	Saturated	1	82	94	9455	2	0.154	.	.
Francis	G-9	U.S.A.	J	2007	Native	Saturated	2	82	94	9027	2	0.124	.	.
Francis	G-9	U.S.A.	J	2007	Native	Saturated	3	82	98	8897	2	0.137	.	.
Francis	G-9	U.S.A.	J	2007	Native	Saturated	4	82	102	7994	2	0.171	.	.
GP2	G-46	China	I	2007	MSMA	Flooded	1	97	92	1967	9	1.371	.	.
GP2	G-46	China	I	2007	MSMA	Flooded	2	97	110	1099	9	1.985	.	.
GP2	G-46	China	I	2007	MSMA	Flooded	3	97	104	629	8	1.592	.	.
GP2	G-46	China	I	2007	MSMA	Flooded	4	97	104	177	9	1.558	.	.
GP2	G-46	China	I	2007	Native	Flooded	1	65	102	12879	2	0.234	.	.
GP2	G-46	China	I	2007	Native	Flooded	2	93	108	8201	2	0.383	.	.
GP2	G-46	China	I	2007	Native	Flooded	3	93	110	8467	2	0.381	.	.
GP2	G-46	China	I	2007	Native	Flooded	4	93	104	9119	2	0.462	.	.
GP2	G-46	China	I	2007	MSMA	Saturated	1	100	94	5615	2	0.214	.	.
GP2	G-46	China	I	2007	MSMA	Saturated	2	93	108	6351	1	1.374	.	.
GP2	G-46	China	I	2007	MSMA	Saturated	3	97	100	7585	1	0.830	.	.
GP2	G-46	China	I	2007	MSMA	Saturated	4	97	102	9109	1	1.386	.	.
GP2	G-46	China	I	2007	Native	Saturated	1	97	116	9869	2	0.153	.	.
GP2	G-46	China	I	2007	Native	Saturated	2	97	112	7409	2	0.120	.	.
GP2	G-46	China	I	2007	Native	Saturated	3	97	112	8263	2	0.142	.	.
GP2	G-46	China	I	2007	Native	Saturated	4	97	116	8734	2	0.122	.	.
Huri282	G-11	Combodia	I	2007	MSMA	Flooded	1	91	104	6113	3	1.163	0.230	0.851
Huri282	G-11	Combodia	I	2007	MSMA	Flooded	2	91	110	5904	4	1.065	0.188	0.751
Huri282	G-11	Combodia	I	2007	MSMA	Flooded	3	91	98	6967	4	1.015	0.182	0.688
Huri282	G-11	Combodia	I	2007	MSMA	Flooded	4	91	94	4712	5	1.192	0.182	0.848

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain-As	Inorganic As^{III}	DMAs^{V}
Huri282	G-11	Combodia	I	2007	Native	Flooded	1	90	114	5173	2	0.670	0.149	0.395
Huri282	G-11	Combodia	I	2007	Native	Flooded	2	90	104	6053	2	0.659	0.167	0.379
Huri282	G-11	Combodia	I	2007	Native	Flooded	3	88	116	7854	2	0.589	0.136	0.328
Huri282	G-11	Combodia	I	2007	Native	Flooded	4	91	104	4878	2	0.618	0.144	0.375
Huri282	G-11	Combodia	I	2007	MSMA	Saturated	1	93	90	6161	3	0.975	0.157	0.604
Huri282	G-11	Combodia	I	2007	MSMA	Saturated	2	93	102	5669	3	0.924	0.170	0.280
Huri282	G-11	Combodia	I	2007	MSMA	Saturated	3	93	104	7836	3	1.017	0.155	0.537
Huri282	G-11	Combodia	I	2007	MSMA	Saturated	4	93	108	5877	3	0.713	0.167	0.588
Huri282	G-11	Combodia	I	2007	Native	Saturated	1	93	100	7417	2	0.227	0.181	0.000
Huri282	G-11	Combodia	I	2007	Native	Saturated	2	91	108	8762	2	0.291	0.142	0.088
Huri282	G-11	Combodia	I	2007	Native	Saturated	3	88	108	8344	2	0.216	0.131	0.051
Huri282	G-11	Combodia	I	2007	Native	Saturated	4	91	114	9597	2	0.278	0.157	0.087
IR9209	G-13	Philippines	I	2007	MSMA	Flooded	1	75	86	6880	1	1.027	0.182	0.848
IR9209	G-13	Philippines	I	2007	MSMA	Flooded	2	75	88	7832	1	1.056	0.182	0.688
IR9209	G-13	Philippines	I	2007	MSMA	Flooded	3	75	86	7737	1	0.960	0.188	0.751
IR9209	G-13	Philippines	I	2007	MSMA	Flooded	4	75	86	7139	1	1.013	0.230	0.851
IR9209	G-13	Philippines	I	2007	Native	Flooded	1	75	90	8333	2	0.534	0.132	0.246
IR9209	G-13	Philippines	I	2007	Native	Flooded	2	75	90	8088	2	0.464	0.172	0.235
IR9209	G-13	Philippines	I	2007	Native	Flooded	3	69	108	10472	2	0.479	0.113	0.224
IR9209	G-13	Philippines	I	2007	Native	Flooded	4	75	100	8111	2	0.534	0.119	0.237
IR9209	G-13	Philippines	I	2007	MSMA	Saturated	1	77	78	8709	1	0.682	0.146	0.317
IR9209	G-13	Philippines	I	2007	MSMA	Saturated	2	77	86	8357	2	0.653	0.145	0.334
IR9209	G-13	Philippines	I	2007	MSMA	Saturated	3	77	82	6444	1	0.641	0.135	0.360
IR9209	G-13	Philippines	I	2007	MSMA	Saturated	4	77	88	8092	2	0.740	0.147	0.566
IR9209	G-13	Philippines	I	2007	Native	Saturated	1	76	98	8527	2	0.194	0.091	0.050
IR9209	G-13	Philippines	I	2007	Native	Saturated	2	75	84	8239	2	0.176	0.095	0.048

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
IR9209	G-13	Philippines	I	2007	Native	Saturated	3	75	100	8850	2	0.178	0.116	0.046
IR9209	G-13	Philippines	I	2007	Native	Saturated	4	77	90	7840	2	0.174	.	.
Jing1857	G-15	China	J	2007	MSMA	Flooded	1	91	74	5998	2	0.851	0.131	0.574
Jing1857	G-15	China	J	2007	MSMA	Flooded	2	90	82	6080	2	0.661	0.123	0.339
Jing1857	G-15	China	J	2007	MSMA	Flooded	3	91	84	7504	2	0.678	0.108	0.407
Jing1857	G-15	China	J	2007	MSMA	Flooded	4	90	90	8336	2	0.771	0.140	0.455
Jing1857	G-15	China	J	2007	Native	Flooded	1	82	103	8620	2	0.394	0.103	0.226
Jing1857	G-15	China	J	2007	Native	Flooded	2	91	96	9368	2	0.475	0.137	0.201
Jing1857	G-15	China	J	2007	Native	Flooded	3	82	84	7316	2	0.301	0.117	0.197
Jing1857	G-15	China	J	2007	Native	Flooded	4	90	88	9393	2	0.463	0.126	0.201
Jing1857	G-15	China	J	2007	MSMA	Saturated	1	97	80	6934	2	0.744	0.143	0.421
Jing1857	G-15	China	J	2007	MSMA	Saturated	2	97	86	6278	2	0.307	0.104	0.146
Jing1857	G-15	China	J	2007	MSMA	Saturated	3	93	80	7279	2	0.709	0.136	0.379
Jing1857	G-15	China	J	2007	MSMA	Saturated	4	93	84	7468	2	0.448	.	.
Jing1857	G-15	China	J	2007	Native	Saturated	1	92	88	8641	2	0.162	0.098	0.033
Jing1857	G-15	China	J	2007	Native	Saturated	2	93	116	8766	2	0.106	0.077	0.000
Jing1857	G-15	China	J	2007	Native	Saturated	3	90	100	7706	2	0.125	0.081	0.000
Jing1857	G-15	China	J	2007	Native	Saturated	4	91	90	8575	2	0.125	0.108	0.000
JinnuoNo6	G-16	China	I	2007	MSMA	Flooded	1	93	122	3963	5	1.080	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Flooded	2	93	118	6943	4	0.757	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Flooded	3	93	116	5573	4	0.803	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Flooded	4	93	116	3944	5	0.913	.	.
JinnuoNo6	G-16	China	I	2007	Native	Flooded	1	93	118	7804	2	0.410	.	.
JinnuoNo6	G-16	China	I	2007	Native	Flooded	2	93	120	6267	2	0.286	.	.
JinnuoNo6	G-16	China	I	2007	Native	Flooded	3	92	126	9400	2	0.265	.	.
JinnuoNo6	G-16	China	I	2007	Native	Flooded	4	93	112	7720	2	0.321	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
JinnuoNo6	G-16	China	I	2007	MSMA	Saturated	1	99	104	8061	2	0.267	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Saturated	2	101	98	4691	2	0.153	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Saturated	3	97	108	6688	2	0.345	.	.
JinnuoNo6	G-16	China	I	2007	MSMA	Saturated	4	97	108	9642	2	0.453	.	.
JinnuoNo6	G-16	China	I	2007	Native	Saturated	1	97	116	7770	2	0.110	.	.
JinnuoNo6	G-16	China	I	2007	Native	Saturated	2	97	112	6804	2	0.127	.	.
JinnuoNo6	G-16	China	I	2007	Native	Saturated	3	97	122	7729	2	0.097	.	.
JinnuoNo6	G-16	China	I	2007	Native	Saturated	4	97	108	9558	2	0.095	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Flooded	1	85	96	286	7	0.989	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Flooded	2	82	94	816	8	1.233	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Flooded	3	85	94	488	8	0.971	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Flooded	4	85	92	285	8	0.762	.	.
Mars	G-45	U.S.A.	J	2007	Native	Flooded	1	82	108	10036	2	0.371	.	.
Mars	G-45	U.S.A.	J	2007	Native	Flooded	2	82	114	7868	2	0.389	.	.
Mars	G-45	U.S.A.	J	2007	Native	Flooded	3	82	122	6775	2	0.365	.	.
Mars	G-45	U.S.A.	J	2007	Native	Flooded	4	90	98	7039	2	0.474	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Saturated	1	85	86	2397	2	1.157	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Saturated	2	85	82	3084	2	1.157	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Saturated	3	82	84	2868	3	0.999	.	.
Mars	G-45	U.S.A.	J	2007	MSMA	Saturated	4	85	92	3527	3	0.999	.	.
Mars	G-45	U.S.A.	J	2007	Native	Saturated	1	85	98	7140	2	0.150	.	.
Mars	G-45	U.S.A.	J	2007	Native	Saturated	2	85	108	6790	2	0.151	.	.
Mars	G-45	U.S.A.	J	2007	Native	Saturated	3	83	112	7517	2	0.199	.	.
Mars	G-45	U.S.A.	J	2007	Native	Saturated	4	82	104	9836	2	0.146	.	.
Medark	G-18	U.S.A.	J	2007	MSMA	Flooded	1	82	72	3266	6	1.661	0.099	1.251
Medark	G-18	U.S.A.	J	2007	MSMA	Flooded	2	82	74	5501	7	1.282	0.124	0.981

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Medark	G-18	U.S.A.	J	2007	MSMA	Flooded	3	83	82	5009	6	1.071	0.137	1.047
Medark	G-18	U.S.A.	J	2007	MSMA	Flooded	4	82	84	6162	6	1.484	0.133	1.215
Medark	G-18	U.S.A.	J	2007	Native	Flooded	1	82	88	7768	2	0.467	0.145	0.281
Medark	G-18	U.S.A.	J	2007	Native	Flooded	2	81	84	8803	2	0.549	0.119	0.386
Medark	G-18	U.S.A.	J	2007	Native	Flooded	3	80	90	6842	2	0.508	0.120	0.313
Medark	G-18	U.S.A.	J	2007	Native	Flooded	4	82	90	7139	2	0.508	0.125	0.313
Medark	G-18	U.S.A.	J	2007	MSMA	Saturated	1	85	74	4185	3	0.440	0.106	0.257
Medark	G-18	U.S.A.	J	2007	MSMA	Saturated	2	88	78	4908	3	0.786	0.115	0.540
Medark	G-18	U.S.A.	J	2007	MSMA	Saturated	3	69	78	4674	3	0.887	0.119	0.602
Medark	G-18	U.S.A.	J	2007	MSMA	Saturated	4	85	78	5823	3	0.858	0.113	0.569
Medark	G-18	U.S.A.	J	2007	Native	Saturated	1	82	82	8829	2	0.176	0.122	0.076
Medark	G-18	U.S.A.	J	2007	Native	Saturated	2	82	84	7209	2	0.158	0.091	0.082
Medark	G-18	U.S.A.	J	2007	Native	Saturated	3	82	84	8998	2	0.159	.	.
Medark	G-18	U.S.A.	J	2007	Native	Saturated	4	82	92	9433	2	0.140	0.089	0.060
Priscilla	G-21	U.S.A.	J	2007	MSMA	Flooded	1	85	74	5124	3	0.905	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Flooded	2	82	74	6809	4	0.710	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Flooded	3	82	90	6079	4	0.714	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Flooded	4	83	76	6767	3	0.808	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Flooded	1	82	84	7250	2	0.280	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Flooded	2	90	104	8039	2	0.309	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Flooded	3	82	110	6335	2	0.469	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Flooded	4	82	92	6644	2	0.177	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Saturated	1	88	78	4217	2	0.574	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Saturated	2	85	66	3161	3	0.585	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Saturated	3	85	70	4253	2	0.415	.	.
Priscilla	G-21	U.S.A.	J	2007	MSMA	Saturated	4	85	74	3950	3	0.678	.	.

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Priscilla	G-21	U.S.A.	J	2007	Native	Saturated	1	83	90	6964	2	0.167	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Saturated	2	82	96	8322	2	0.128	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Saturated	3	85	94	4559	2	0.073	.	.
Priscilla	G-21	U.S.A.	J	2007	Native	Saturated	4	90	96	7980	2	0.085	.	.
Spalcik	G-24	Russia	J	2007	MSMA	Flooded	1	64	88	245	2	1.942	0.090	1.473
Spalcik	G-24	Russia	J	2007	MSMA	Flooded	2	64	82	2885	2	1.196	0.126	0.906
Spalcik	G-24	Russia	J	2007	MSMA	Flooded	3	64	88	3586	1	1.368	0.143	1.007
Spalcik	G-24	Russia	J	2007	MSMA	Flooded	4	64	90	3410	2	1.448	0.133	1.164
Spalcik	G-24	Russia	J	2007	Native	Flooded	1	61	92	6356	2	0.315	0.166	0.153
Spalcik	G-24	Russia	J	2007	Native	Flooded	2	61	86	3198	2	0.223	0.122	0.106
Spalcik	G-24	Russia	J	2007	Native	Flooded	3	61	82	5325	2	0.257	0.139	0.142
Spalcik	G-24	Russia	J	2007	Native	Flooded	4	61	86	6744	2	0.274	0.134	0.141
Spalcik	G-24	Russia	J	2007	MSMA	Saturated	1	64	72	2498	1	0.201	0.104	0.068
Spalcik	G-24	Russia	J	2007	MSMA	Saturated	2	64	80	1884	1	0.460	.	.
Spalcik	G-24	Russia	J	2007	MSMA	Saturated	3	64	72	1745	2	0.296	0.138	0.149
Spalcik	G-24	Russia	J	2007	MSMA	Saturated	4	64	98	181	2	0.923	0.145	0.701
Spalcik	G-24	Russia	J	2007	Native	Saturated	1	61	88	7239	2	0.175	0.126	0.000
Spalcik	G-24	Russia	J	2007	Native	Saturated	2	61	88	6630	2	0.186	.	.
Spalcik	G-24	Russia	J	2007	Native	Saturated	3	61	100	5780	2	0.166	0.128	0.000
Spalcik	G-24	Russia	J	2007	Native	Saturated	4	61	98	8601	2	0.128	0.100	0.000
Wells	G-27	U.S.A.	J	2007	MSMA	Flooded	1	82	88	2624	6	1.110	0.135	0.827
Wells	G-27	U.S.A.	J	2007	MSMA	Flooded	2	85	94	7546	4	0.910	0.128	0.696
Wells	G-27	U.S.A.	J	2007	MSMA	Flooded	3	85	96	5651	6	0.948	0.150	0.545
Wells	G-27	U.S.A.	J	2007	MSMA	Flooded	4	85	92	5945	6	1.137	0.160	0.799
Wells	G-27	U.S.A.	J	2007	Native	Flooded	1	82	98	8331	2	0.329	0.142	0.187
Wells	G-27	U.S.A.	J	2007	Native	Flooded	2	82	102	8201	2	0.305	0.148	0.195

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Wells	G-27	U.S.A.	J	2007	Native	Flooded	3	82	100	7648	2	0.296	0.125	0.166
Wells	G-27	U.S.A.	J	2007	Native	Flooded	4	82	98	5763	2	0.288	0.136	0.143
Wells	G-27	U.S.A.	J	2007	MSMA	Saturated	1	85	80	3063	3	1.000	0.152	0.552
Wells	G-27	U.S.A.	J	2007	MSMA	Saturated	2	91	80	2835	4	0.813	0.160	0.799
Wells	G-27	U.S.A.	J	2007	MSMA	Saturated	3	90	78	2767	3	1.110	0.130	0.797
Wells	G-27	U.S.A.	J	2007	MSMA	Saturated	4	82	84	5293	3	0.639	.	.
Wells	G-27	U.S.A.	J	2007	Native	Saturated	1	83	92	7563	2	0.170	0.135	0.041
Wells	G-27	U.S.A.	J	2007	Native	Saturated	2	82	98	7206	2	0.179	0.115	0.060
Wells	G-27	U.S.A.	J	2007	Native	Saturated	3	82	92	8062	2	0.271	0.130	0.136
Wells	G-27	U.S.A.	J	2007	Native	Saturated	4	82	94	7998	2	0.207	.	.
Xiangzaoxian1	G-28	China	I	2007	MSMA	Flooded	1	65	82	7231	2	1.840	0.091	1.457
Xiangzaoxian1	G-28	China	I	2007	MSMA	Flooded	2	65	92	11304	1	1.317	0.099	0.955
Xiangzaoxian1	G-28	China	I	2007	MSMA	Flooded	3	65	100	10547	1	1.295	0.082	1.045
Xiangzaoxian1	G-28	China	I	2007	MSMA	Flooded	4	65	94	10013	1	1.785	0.089	1.250
Xiangzaoxian1	G-28	China	I	2007	Native	Flooded	1	65	96	10893	2	0.257	.	.
Xiangzaoxian1	G-28	China	I	2007	Native	Flooded	2	65	100	12241	2	0.244	0.091	0.094
Xiangzaoxian1	G-28	China	I	2007	Native	Flooded	3	65	106	9511	2	0.241	0.105	0.119
Xiangzaoxian1	G-28	China	I	2007	Native	Flooded	4	93	114	8278	2	0.387	0.143	0.226
Xiangzaoxian1	G-28	China	I	2007	MSMA	Saturated	1	68	90	9100	1	0.177	0.057	0.051
Xiangzaoxian1	G-28	China	I	2007	MSMA	Saturated	2	65	98	8913	1	0.308	0.092	0.144
Xiangzaoxian1	G-28	China	I	2007	MSMA	Saturated	3	65	88	9144	1	0.291	0.056	0.100
Xiangzaoxian1	G-28	China	I	2007	MSMA	Saturated	4	65	90	8896	1	0.203	0.073	0.048
Xiangzaoxian1	G-28	China	I	2007	Native	Saturated	1	65	90	10129	2	0.108	0.068	0.020
Xiangzaoxian1	G-28	China	I	2007	Native	Saturated	2	65	108	9932	2	0.092	0.063	0.013
Xiangzaoxian1	G-28	China	I	2007	Native	Saturated	3	65	98	11835	2	0.097	0.059	0.015

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Xiangzaoxian1	G-28	China	I	2007	Native	Saturated	4	65	100	10762	2	0.083	0.049	0.015
Zanuo	G-30	China	I	2007	MSMA	Flooded	1	69	90	2010	6	3.005	0.155	2.250
Zanuo	G-30	China	I	2007	MSMA	Flooded	2	69	98	2896	6	2.148	0.156	1.650
Zanuo	G-30	China	I	2007	MSMA	Flooded	3	69	102	1423	6	2.684	0.154	2.028
Zanuo	G-30	China	I	2007	MSMA	Flooded	4	69	90	1288	8	2.719	0.133	2.156
Zanuo	G-30	China	I	2007	Native	Flooded	1	69	106	10170	2	0.430	0.129	0.212
Zanuo	G-30	China	I	2007	Native	Flooded	2	77	102	8634	2	0.456	0.113	0.305
Zanuo	G-30	China	I	2007	Native	Flooded	3	69	110	9223	2	0.402	0.142	0.200
Zanuo	G-30	China	I	2007	Native	Flooded	4	69	102	10209	2	0.440	0.111	0.217
Zanuo	G-30	China	I	2007	MSMA	Saturated	1	69	96	6744	1	0.539	0.118	0.313
Zanuo	G-30	China	I	2007	MSMA	Saturated	2	69	96	7679	2	1.006	0.145	0.831
Zanuo	G-30	China	I	2007	MSMA	Saturated	3	69	96	8481	1	0.895	0.146	0.740
Zanuo	G-30	China	I	2007	MSMA	Saturated	4	69	94	7893	1	1.013	0.130	0.806
Zanuo	G-30	China	I	2007	Native	Saturated	1	69	106	10281	2	0.193	0.137	0.028
Zanuo	G-30	China	I	2007	Native	Saturated	2	69	110	8275	2	0.176	0.113	0.028
Zanuo	G-30	China	I	2007	Native	Saturated	3	69	110	8170	2	0.188	0.122	0.027
Zanuo	G-30	China	I	2007	Native	Saturated	4	69	104	8629	2	0.140	0.140	0.000
Zao402	G-31	China	I	2007	MSMA	Flooded	1	65	82	6952	1	1.780	0.154	1.601
Zao402	G-31	China	I	2007	MSMA	Flooded	2	69	102	8211	1	1.597	0.152	1.234
Zao402	G-31	China	I	2007	MSMA	Flooded	3	68	100	9587	1	1.627	0.164	1.248
Zao402	G-31	China	I	2007	MSMA	Flooded	4	69	88	7266	1	2.259	0.138	1.726
Zao402	G-31	China	I	2007	Native	Flooded	1	69	104	979	2	0.338	0.132	0.158
Zao402	G-31	China	I	2007	Native	Flooded	2	69	106	9316	2	0.399	0.152	0.176
Zao402	G-31	China	I	2007	Native	Flooded	3	68	94	7867	2	0.362	0.145	0.169
Zao402	G-31	China	I	2007	Native	Flooded	4	69	98	5317	2	0.297	0.128	0.152
Zao402	G-31	China	I	2007	MSMA	Saturated	1	71	80	7372	1	0.349	0.122	0.169

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Zao402	G-31	China	I	2007	MSMA	Saturated	2	75	94	8291	1	0.561	0.126	0.353
Zao402	G-31	China	I	2007	MSMA	Saturated	3	71	70	8134	1	0.232	0.104	0.076
Zao402	G-31	China	I	2007	MSMA	Saturated	4	71	88	6701	1	0.248	0.109	0.080
Zao402	G-31	China	I	2007	Native	Saturated	1	69	90	9871	2	0.180	0.112	0.045
Zao402	G-31	China	I	2007	Native	Saturated	2	69	106	9773	2	0.113	.	.
Zao402	G-31	China	I	2007	Native	Saturated	3	75	98	9425	2	0.132	0.093	0.010
Zao402	G-31	China	I	2007	Native	Saturated	4	71	102	7347	2	0.119	0.097	0.018
Zhe733	G-32	China	I	2007	MSMA	Flooded	1	61	72	6222	1	0.793	0.144	0.545
Zhe733	G-32	China	I	2007	MSMA	Flooded	2	61	100	7953	1	0.684	0.131	0.453
Zhe733	G-32	China	I	2007	MSMA	Flooded	3	61	92	2746	1	0.731	.	.
Zhe733	G-32	China	I	2007	MSMA	Flooded	4	61	94	3841	1	0.716	0.142	0.534
Zhe733	G-32	China	I	2007	Native	Flooded	1	61	89	11712	2	0.271	0.129	0.122
Zhe733	G-32	China	I	2007	Native	Flooded	2	61	96	7741	2	0.312	.	.
Zhe733	G-32	China	I	2007	Native	Flooded	3	61	92	838	2	0.241	0.119	0.095
Zhe733	G-32	China	I	2007	Native	Flooded	4	61	90	10688	2	0.282	0.126	0.116
Zhe733	G-32	China	I	2007	MSMA	Saturated	1	61	76	5293	1	0.204	.	.
Zhe733	G-32	China	I	2007	MSMA	Saturated	2	61	64	4873	1	0.197	0.112	0.062
Zhe733	G-32	China	I	2007	MSMA	Saturated	3	64	74	4044	1	0.200	0.105	0.063
Zhe733	G-32	China	I	2007	MSMA	Saturated	4	61	80	4443	1	0.269	0.117	0.084
Zhe733	G-32	China	I	2007	Native	Saturated	1	61	96	10460	2	0.151	0.099	0.021
Zhe733	G-32	China	I	2007	Native	Saturated	2	61	98	10734	2	0.124	.	.
Zhe733	G-32	China	I	2007	Native	Saturated	3	61	96	10091	2	0.140	0.102	0.017
Zhe733	G-32	China	I	2007	Native	Saturated	4	61	108	9492	2	0.130	0.099	0.015
Zhenshan97	G-33	China	I	2007	MSMA	Flooded	1	65	82	6722	1	3.025	0.096	2.433
Zhenshan97	G-33	China	I	2007	MSMA	Flooded	2	65	90	8353	1	2.375	0.114	2.056
Zhenshan97	G-33	China	I	2007	MSMA	Flooded	3	65	90	7566	1	2.124	0.109	1.597

Cultivar	Cultivar ID	Origin	Sub-species	Year	MSMA	Flood	Replicate	Days to heading	Plant height (cm)	Yield (kg ha^{-1})	Straighthead Rate	Total grain -As	Inorganic As^{III}	DMAs^{V}
Zhenshan97	G-33	China	I	2007	MSMA	Flooded	4	65	82	5711	1	3.101	0.105	2.542
Zhenshan97	G-33	China	I	2007	Native	Flooded	1	65	98	9813	2	0.291	0.087	0.128
Zhenshan97	G-33	China	I	2007	Native	Flooded	2	65	90	10276	2	0.286	0.084	0.139
Zhenshan97	G-33	China	I	2007	Native	Flooded	3	65	90	8776	2	0.313	0.094	0.148
Zhenshan97	G-33	China	I	2007	Native	Flooded	4	65	94	10217	2	0.295	0.097	0.135
Zhenshan97	G-33	China	I	2007	MSMA	Saturated	1	71	76	6911	1	0.326	0.064	0.161
Zhenshan97	G-33	China	I	2007	MSMA	Saturated	2	75	86	6888	1	0.302	0.078	0.113
Zhenshan97	G-33	China	I	2007	MSMA	Saturated	3	71	94	8034	1	0.646	0.087	0.430
Zhenshan97	G-33	China	I	2007	MSMA	Saturated	4	69	88	7622	1	0.646	0.092	0.478
Zhenshan97	G-33	China	I	2007	Native	Saturated	1	69	98	9482	2	0.157	0.079	0.031
Zhenshan97	G-33	China	I	2007	Native	Saturated	2	69	98	8995	2	0.152	0.070	0.039
Zhenshan97	G-33	China	I	2007	Native	Saturated	3	69	104	8872	2	0.121	0.058	0.022
Zhenshan97	G-33	China	I	2007	Native	Saturated	4	69	96	9491	2	0.132	0.072	0.026

VITA

Tushara Raghvan Pillai was born in Kannur, India and was brought up in Delhi, India. In August 2000, she received her Bachelor of Science degree in Medical (general) from Government Post Graduate College, Kurukshetra University, Hisar, India. In Dec 2002 she earned a degree in Master of Science (M.S.) Ecology and Environment from Indian Institute of Ecology and Environment, Sikkim Manipal University of Health, Medical & Technological Sciences, New Delhi, India. In August 2004, she joined Texas A&M University, College Station, Texas and as a graduate student of Molecular and Environmental Plant Sciences program obtained her doctoral degree in December 2009. During her doctoral studies, she worked as a graduate research assistant and a graduate teaching assistant with Soil and Crop Sciences Department, Texas A&M University.

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