Distribution of Arsenic and Other Minerals in Rice Plants Affected by Natural Straighthead

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ABSTRACT

Because of the inconsistency and unpredictability of naturally occurring straighthead, arsenical herbicides are sometimes used to induce straighthead-like symptoms to study this sterility disorder. In 2005, an outbreak of naturally occurring (nonchemically induced) straighthead in rice (Oryza sativa L.) study fields in Stuttgart, AR provided an opportunity to examine the role of minerals in this generally unpredictable disorder. The outbreak affected areas of yield and N rate tests thus permitting examination of the effect of N levels on straighthead. It was found that at the higher N levels, straighthead symptoms were reduced. Since several minerals, including As, have been associated with straighthead, samples of the soil and plants from three of the affected cultivars were analyzed for their levels of several minerals. Straighthead-affected and nonstraighthead-affected plants of each cultivar were separated into roots, stems, leaves, and seeds. Each plant part was analyzed for its level of macro- and micronutrients plus As, from which a relative (straighthead/nonstraighthead) mineral level for each cultivar was calculated. Relative levels of As did not show a consistent pattern among the plant parts. Magnesium may play a role in natural straighthead; only its relative concentrations were consistent across the three cultivars in the soil, stems (and its subsection, stem internodes), leaves, seeds (and its subsection, seed hulls); though not in the roots, brown rice, or stem nodes. The data provide a description of nutrient levels in the rice plant from a rare occurrence and so may provide comparisons for other studies of natural and induced straighthead.

Straighthead is a physiological disorder of rice causing sterility. The panicles remain upright because of the light weight of the unfilled kernels, and the empty hulls are distorted into a crescent or parrot-beak. The plants show few other symptoms. Straighthead differs from sterility derived from other causes such as drought, disease, alkali injury, etc. (Tisdale and Jenkins, 1921; Padwick, 1950). Straighthead occurs early in flower development while many other causes of sterility can occur after heading in an otherwise healthy flower. In fact, plants affected by straighthead appear healthy, even being darker green, as opposed to stunted or dried-up plants that have sterile panicles. Straighthead-like symptoms can be induced with As-containing herbicides such as monosodium methanarsenate (MSMA) (Wells and Gilmour, 1977; Yan et al., 2005), which was used on cotton (Gilmour and Wells, 1980).

Straighthead occurs in the United States, South America, Japan, and elsewhere. Naturally occurring (without any apparent chemical intervention) straighthead is difficult to study because it is unpredictable in occurrence. Also, growers commonly drain and dry their rice fields (Wilson et al., 2001) to avoid straighthead occurrences. As a result, very little is known about the physiological changes that occur during straighthead. To our knowledge, only two sources in the scientific literature (Iwamoto, 1969; Dunn et al., 2006) investigated natural straighthead since the 1960s. Numerous descriptions of the disorder and field treatments are made in agriculture extension bulletins, progress reports, handbooks, etc. (e.g., Hewitt, 1912; Cheaney, 1955; Atkins, 1974).

Natural straighthead has been associated with a number of minerals: N, P, or K (Baba et al., 1965); sulfates, Fe, and thiols (Iwamoto, 1969); potassium permanganate, boric acid, ferric sulfate, copper sulfate, zinc oxide, and iron and aluminum sulfates (Evatt and Atkins, 1957); Cu (Ou, 1985); ammonium sulfate (Padwick, 1950); but not with Zn (Bollich et al., 1988) or potassium persulfate (Ricardo and Cunha, 1968). Straighthead has also been associated with higher nutrient absorption (Joshi et al., 1975). It must also be considered that any associated change in nutrient levels may be a result, rather than a cause of the straighthead sterility.

An alteration in soil minerals leading to straighthead may act by changing the mineral status of the plant, in which case the levels of minerals in several parts of the straighthead plant would consistently be high or low relative to the nonstraighthead plant. Since straighthead is known to be induced early, during panicle formation (Todd and Beachell, 1954), minerals that are known to affect flower viability and, hence, seed production in rice would be of special interest. Potassium deficiency will result in a decrease in the number of rachis branches per panicle (Hirata, 1995). Panicles do not emerge from rice that suffers from B deficiency at the panicle formation stage (Obata, 1995) while application of B increases pollen vitality in rice (Garg et al., 1979). Relative amounts of N and P alter the morphology of the panicle during spikelet differentiation (Takeoka et al., 1993).

A fortuitous occurrence of straighthead in test plots provided a unique opportunity of collecting straighthead and nonstraighthead affected plant and soil material within single rice plots. The straighthead in this study occurred in an experimental field plot designed as a yield trial for some developing lines as well as in a yield test of OL 5 at the other end of the same block of bays. Since the straighthead in this study occurred in a several cultivars in the same area and in the same growing season, mineral levels in the plants and soil could be compared to gain insight into the role, if any, that minerals play in the occurrence of straighthead. These mineral

Abbreviations: ICP, inductively coupled argon plasma; MSMA, monosodium methanarsenate.
levels may serve to provide a benchmark for comparison in other studies of natural and herbicide-induced straighthead. It has not been determined if natural and chemically-induced straighthead are the same, related, or unrelated phenomena. Future study may provide information on the relationship as well as indications for its control.

**MATERIALS AND METHODS**

In 2005, yield trials were conducted at the Rice Research and Extension Center near Stuttgart, AR on a Crowley silt loam (fine, smectite, thermic Typic Albaqualf). Soil in this area has not been treated with arsenical pesticides, nor has straighthead been observed at or near these plots, for at least 13 yr; nor was it observed the year after these observations, 2006.

In one yield trial, six genotypes were grown within one bay in a four replication, split-plot design with three fertilizer rates ($56, 112, 168$ kg ha$^{-1}$, equivalent to 50, 100, and 150 lb acre$^{-1}$) being the main plots and the six genotypes being the subplots. Two genotypes were U.S. commercial cultivars (Francis and Wells) and four were M8 generation indica selections designated Indica 10, Indica 11, Indica 12, and Indica 13. The planting date was 19 April. Plots were nine row plots on 19-cm row centers and were 5.2 m long. The seeding rate was 430 seeds m$^{-2}$.

In another yield trial, 24 genotypes were grown within one bay in a two replication, randomized complete block design. Two genotypes were U.S. commercial cultivars (Francis and Wells), one was the parent check Oryzica Llanos 5 (OL 5), and 21 were the M10 generation of induced mutations of OL 5. The planting date was 3 May. Nitrogen was applied as urea (112 kg ha$^{-1}$) at only one time, when the plants were at the five leaf stage. The plots were six row plots on 30-cm row centers and were 5.2 m long. The seeding rate was 270 seeds m$^{-2}$.

All plots were flooded from between the fourth and fifth leaf stages until maturity when visual ratings of straighthead were taken. Ratings were on a scale from 1 to 9 (Yan et al., 2005) with 1 being no symptoms and 9 being severely affected. Soil and plant samples were taken at harvest from both yield trials. To focus on any plant-wide (across cultivar) changes that occurred during straighthead, mineral analyses were done on three of the highly affected rice cultivars. Samples of plant and soil were taken from the three cultivars and from corresponding plant and soil within the same plot that were not affected. Approximately 10 contiguous plants within a control or affected area were obtained for each sample. Approximately 300 g of soil was obtained for each sample from within the area penetrated by the roots of the sampled plants. In the N rate test, the two most severely affected lines (Indica 11 and Indica 12 in the 56 kg ha$^{-1}$ test) were sampled along with their corresponding, non-affected tests (from the adjacent 168 kg ha$^{-1}$ plots). In the yield test (all one N rate) of OL 5 in a nearby bay, another incidence of straighthead occurred within the bay from which straighthead and nonstraighthead samples were taken. To allow easier comparisons among the cultivars, mineral levels in the soil from the straightheaded were divided by the mineral levels from the nonstraighthead material for each cultivar to give a straighthead to control ratio of mineral levels.

An initial set of plant samples used bulked material, divided into seeds, stems, leaves, and roots, from each straighthead and nonstraighthead test plot. A second set of analyses from the same plots used stems and seeds that had been subdivided. Stems were divided into nodes and internodes, which were numbered from the ground up. For example, node 1 was the node adjacent to the main roots. Internode 1 was the internode between nodes 1 and 2. Seeds were divided into hulls and brown rice. Not enough plant material was available to carry out As analyses on the subdivided stem or seed parts.

Mineral analyses were performed by A&L Laboratories (Memphis, TN). The soil samples (150 g) were analyzed for pH (1:1, soil/H$_2$O); for metals by inductively coupled argon plasma (ICP) spectroscopy of Mehlich III extractable Ca, Mg, K, Na, Fe, Cu, Mn, and Zn; and for organic matter by a modified Walkley-Black procedure (Walkley and Black, 1934). Dried plant tissue (10 g) was ground and digested using a nitric/peroxide/HCl wet ash digestion. Concentrations of Al, Ca, Mg, Na, K, Fe, Cu, Mn, Cu and Zn were obtained by ICP. Total N was measured using a FP 528 combustion analyzer (LECO Corp., St. Joseph, MI).

Paired $t$ tests were used to determine if differences between each pair (straighthead plants and nonstraighthead plants) of mineral measurements were significant ($P < 0.1$) across all three cultivars.

**RESULTS**

**Straighthead Ratings and Nitrogen**

Straighthead symptoms were observed in several rice cultivars growing in a N rate test plot designed for yield studies. The typical symptoms were observed (Fig. 1): upright panicles from sterile seeds, parrot beak shape of the kernels from distorted lemma and palea, twisted panicle stems, and missing kernels (Yan et al., 2005).

Very little to no straighthead was observed at the higher N rates while moderate to high levels of straighthead were observed in the indica genotypes at the lowest N rates of 56 kg ha$^{-1}$ (Fig. 2). Significant effects of N on reduction of straighthead ratings have been noted previously on natural straighthead (Dunn et al., 2006) and on MSMA-induced straighthead (Dilday et al., 2001; Yan et al., 2005).

Wells was found to be susceptible to straighthead by Yan et al. (2005). Wells and Francis are both rated moderately resistant to MSMA-induced straighthead in Louisiana but moderately susceptible in Arkansas (Katz, 2003). Straighthead ratings are unknown for Indica 10, 11, 12, and 13 or for OL 5 but, based on our results, they appear to be susceptible to straighthead.

**Soil Minerals**

The mineral levels in the soil from the straightheaded plants of the three cultivars tested were nearly equivalent to those from the nonstraightheaded plants, as can be seen by the relative proportion of minerals rarely straying from 1.0 (Fig. 3). Only for Zn, Cu, Ca, Mg, and Mn do the proportion of minerals vary in the same direction in all three cultivars, with less mineral in the straighthead soil than in the corresponding nonstraighthead soil. Though this could make it appear that an absolute decrease of these four minerals in soil may lead to straighthead, a comparison of the actual mineral concentrations shows that for each mineral, the “low” level in a straighthead soil from one cultivar is more than or the same as the level of that mineral in a nonstraighthead soil from a different cultivar (data not shown; for a complete listing of the soil mineral values, see Miller (2007).
Other studies have found relationships between MSMA-induced straighthead and soil-amendments containing inorganic S (Wells and Gilmour, 1977), sulfides (Joshi et al., 1975), thiols, organic matter, Fe, and Ca (Iwamoto, 1969), and N (Yan et al., 2005). However, none of these minerals appear in similar relative ratios in the soils from all three cultivars (Fig. 3).

Pesticides containing As are able to produce straighthead-like symptoms in rice. The total As levels of the soils in this test were 4.5 mg kg$^{-1}$ or less, which is lower than As levels of control soils in tests used to measure the effects of As in soils, e.g., 31.3 mg kg$^{-1}$ (Abedin et al., 2002a), 15.1 mg kg$^{-1}$ (Tlustoš et al., 2002), and 19.2 mg kg$^{-1}$ (Bhattacharyya et al., 2003), as well as the 7 mg kg$^{-1}$ found in soil from natural straighthead (Iwamoto, 1969). Organic matter is also considered to be a factor in the induction of straighthead (Groth and Lee, 2003; Kataoka et al., 1983) but the percent organic matter of each of the three straighthead soils was lower than two of the control soils (Fig. 3). The pH, however,
was consistently lower in the straighthead soils than in the nonstraighthead soil (Table 1), which has also been shown to be related to the disorder (Baba and Harada, 1954; Iwamoto, 1969; Tisdale and Jenkins 1921).

### Plant Minerals

A number of minerals—N, S, K, Mg, Na, Zn, Fe, and Cu—were lower in straighthead plant stems than in control stems of each cultivar (Fig. 4). Another set of stems obtained from the same sites were further subdivided into nodes and internodes and analyzed as well for their mineral contents (Fig. 5 and 6). No mineral showed the same relationship in the stem, node, and internode, though some were similar in two of the parts. In the internodes, as in the whole stem, the amounts of N, K, and Mg were less in the straighthead relative to the nonstraighthead plants (Fig. 6). In the nodes (Fig. 5), as in the whole stems, S and Na were in the same relative proportions. However, the low relative amounts of Zn, Cu, and Fe in the whole stem were not observed in the nodes or internodes (data not shown).

Perhaps of some note, the levels of two other minerals showed distinctive patterns within the stem. The relative levels of S showed a general downward trend in the nodes from the soil to the panicle (Fig. 5). Phosphorus, on the other hand, showed a general upward trend in the internodes going from the soil to the panicle (Fig. 6). For a complete listing of the plant mineral values, see Miller (2007).

Only Na and Mg were consistent (below 1.0) for all three cultivars in relative levels in leaves (Fig. 7). A study of minerals in straighthead rice also showed a slight but consistent decrease in Na and Mg in flag leaves of straighthead plants (W. Yan, personal communication, 2006).

None of the minerals in the whole seed (Fig. 8) or seed parts (Fig. 9) had a consistent ratio of less than one in all three cultivars. However, six minerals appeared in ratios greater than one in all three cultivars for the whole seed (Fig. 8). None of these six was also greater in the brown rice and only Mg was also greater in the hull. Nitrogen is the only mineral that appeared in ratios greater than one in both hull and brown rice (Fig. 9).

Six other minerals are found in higher concentrations in the hulls of straighthead seed. Rice hulls contain a large percentage of hydrated silicon dioxide. The high levels of the minerals in the straighthead hulls may

### Table 1. The percent organic matter and pH of soil from around straighthead and nonstraighthead plants.

<table>
<thead>
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<th>Variety</th>
<th>Condition</th>
<th>Organic matter</th>
<th>pH</th>
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</thead>
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<td>OL 5</td>
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<td>5.4</td>
</tr>
<tr>
<td>OL 5</td>
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<td>straighthead</td>
<td>7</td>
<td>5.4</td>
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<td>nonstraighthead</td>
<td>9</td>
<td>5.9</td>
</tr>
<tr>
<td>Indica 12</td>
<td>straighthead</td>
<td>7</td>
<td>5.4</td>
</tr>
<tr>
<td>Indica 12</td>
<td>nonstraighthead</td>
<td>6</td>
<td>5.6</td>
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Fig. 4. Levels of minerals in stems of plants exhibiting straighthead relative to stems of plants not exhibiting straighthead. A value of one (line) indicates no difference in the amount of that mineral between the two sets of stems. An asterisk indicates a mineral for which the data for the straighthead plants is significantly ($P < 0.1$) different than the nonstraighthead plants.

Fig. 5. Relative levels for selected minerals in the stem nodes for straighthead and nonstraighthead plants. The relative levels are graphed as a function of the position of that node on the stem, with node 1 being the node between the root and stem. A value of one (line) indicates no difference in the amount of that mineral between the two nodes.

Fig. 6. Relative levels for selected minerals in the stem internodes for straighthead and nonstraighthead plants. The relative levels are graphed as a function of the position of that internode on the stem, with internode 1 being the closest to the roots and P being the panicle stem (including the rachis, branches, and pedicels). A value of one (line) indicates no difference in the amount of that mineral between the two internodes.
somehow be related to the ability of the silicon dioxide in rice hulls to bind and sequester compounds (Khalid et al., 1998). Baker et al. (1976) found almost twice as much As in the rice kernels harvested from plants grown in MSMA-treated soil than in untreated soil, similar to the proportions seen here (Fig. 8), though the straighthead soil here was not higher in As.

In the root, relative levels for P, Zn, and Cu were consistent among the three cultivars, in this case higher in straighthead plants (Fig. 10). No attempt was made to get rid of the Fe plaque surrounding rice roots, which is known to also bind As (Liu et al., 2004), so it cannot be discerned which minerals were actually taken up into the root or just bound to the outside.

**DISCUSSION**

Rice growers practice “draining and drying” to avoid the occurrence of straighthead. Draining and reflooding a field costs approximately $10 to $15 acre\(^{-1}\) (C.E. Wilson, Jr., personal communication, 2006), in the same range as the cost for N fertilizer. Normal recommendations for N application for rice in Arkansas are 84 kg ha\(^{-1}\) pre-flood, then 67 kg ha\(^{-1}\) more in either one or split in two applications for a combined recommended rate of 151 kg ha\(^{-1}\). At the nearest test rate, 168 kg ha\(^{-2}\), no variety displayed straighthead (Fig. 2). Slightly higher N applications in the field may serve to alleviate straighthead and eliminate the need for draining and reflooding since a high N application reduced the incidence of straighthead, as seen here and as reported by Dunn et al. (2006). Other amendments may also alleviate straighthead. Iwamoto (1969) concluded that the application of Ca, Fe, and nonsulfate fertilizers could be more effective than draining, while McDonald et al. (1972) reduced straighthead by applying copper sulfate, lime with trace elements, or sulfuric acid.

If high levels of As had been available in the soil, correspondingly high levels would be observed in the
plant (Abedin et al., 2002a,b). However, levels of As were not higher in the straighthead plant parts, except in the whole seed. So it is unlikely that an unusually high level of localized soil As was responsible for the straighthead observed here. Williams et al. (2005) compiled reports of As levels in rice from West Bengal and Bangladesh, and elsewhere. Although As, through arsenical herbicides, is associated with straighthead in the United States, straighthead has not been seen in Bangladesh where As-containing water and soil are resulting in As-contaminated rice for consumption.

Williams et al. (2005), in a pot experiment, measured As uptake into grain and shoots and Frans et al. (1988), using hydroponically grown rice, measured As uptake into stem, leaves, flag leaves, and panicles of straight- head susceptible and tolerant rice plants. The tolerant plants did not take up As differently than susceptible plants. The As levels in the straighthead plants were greater only in the whole seed (Fig. 8) of all three cultivars, but showed no consistent relationship in the stems, leaves, or roots (Fig. 4, 7, 10).

Many factors are involved in the uptake, transport, and activity of minerals in the soil, including the presence or absence of other minerals such as N. While soil pH appears to be of some potential significance in this and another (Iwamoto, 1969) natural straighthead occurrence, Tisdale and Jenkins (1921) were not able to affect straighthead by altering the pH of irrigation water.

If unusually high or low mineral levels in the soil result in straighthead, that mineral’s level would be expected to be consistently elevated or reduced in the soil of straighthead plants relative to nonstraighthead. The most likely minerals for this role appear to be Zn, Cu, Ca, Mg, and Mn as they were consistently lower in soil from straighthead plants. But, in the straighthead rice plants, these five minerals that were low in the soil had different relationships in the plant, being higher in the root for Zn and Cu and higher in the whole seed for Zn, Mn, and Cu, but of no particular relationship in hulls or brown rice.

If a mineral is involved in the appearance of straight- head, it is likely that the abundance or deficiency of the minerals would be reflected in the mineral levels in one or several plant parts of the straighthead affected plants of each cultivar. The appearance of straighthead could then be related to uptake into the roots, transport through the stems, and/or biochemical alterations within the flowers. However, in examining the levels of each mineral in each part of the straighthead plants (the root, leaf, stem, or seed), only Mg seems to show a consistent discernable pattern. Iwamoto (1969) measured leaves and stems of straighthead and normal plants for their levels of N, P, K, Ca, Mg, Fe, and Mn. Mg is the only mineral with similar relative levels in this study and that of Iwamoto.

To confirm and identify more specifically the location of mineral differences of the stem and seed, these plant parts were further subdivided and analyzed. The hull and brown rice mineral levels, however, bore no relationship to that of the whole seed, perhaps because straighthead is induced early, during panicle formation (Todd and Beachell, 1954) and no longer bore a relation to the initial cause. Mineral relationships in the stem carried through for N, K, and Mg in the internode, indicating some potential alteration of mineral transport during straighthead. In comparing the mineral levels of the soil with the stem, of the minerals low in straighthead soil, Zn and Cu were also low in whole stems, Mg was similarly low in internodes, while only Mn was low in nodes.

These data are presented as being descriptive of a natural phenomenon that occurs only sporadically and then almost never with multiple cultivars involved. Our results indicate that straighthead symptoms do not occur directly by changes in mineral levels, although there is some indication of an involvement of soil pH, N application, and/or Mg levels in the stem and leaves, which deserve further study. It is to be hoped that further study will reveal explanations for the mineral levels observed here. Straighthead may involve a change in other, non-mineral components, for example, hormones, enzymes, transcription, that may have been altered by specific but as yet unknown environmental conditions such as fungal infection or temperature changes.

REFERENCES