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# A Method to Estimate Field Response to Hydrogen Sulfide Toxicity and Autumn Decline in Rice Cultivars

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## Abstract

In Arkansas where nearly 50 percent of the U.S.A rice is produced, hydrogen sulfide ($H_2S$) toxicity causes the primary symptom that mainly include stunted plants with black root rotting and yellowish foliage in some flooded fields where the disorder prevail. In rice fields where $H_2S$ toxicity is severe, rice root crowns rot rendering an opportunity for the invasion of weak pathogenic fungi causing a phenomenon called autumn decline or akiochi in Japanese. The symptoms in rice plants are irreversible once the fungal invasion established. With the identification of more rice fields displaying $H_2S$ toxicity and autumn decline in the recent five years, we started testing commercial rice cultivars for tolerance to these disorders. In the testing process we faced problem what scale to use. The objective of the study was directed to developing a method that estimates the combined damages from $H_2S$ toxicity and autumn decline on rice root mass and root crown. The scale was developed from percentage values collected separately in 2015 on root mass discoloration and root crown damage. The percentage values were first translated to a 0 to 5 and a 0 to 9 scale for root mass discoloration and root crown damage, respectively. A double weight was assigned to root crown damage due to its greater impact on the survival of a rice plant. To combine the damages from the two disorders, a matrix-addition scale was developed to best reflect our field observations. Correlation coefficients were compared between the prior scales and the transformed matrices in matrix-addition scale. Further verification was carried out in a replicated field experiment in 2016. The matrix-addition scale was verified as reliable and useful to assess rice cultivars grown in fields with a history of $H_2S$ toxicity and autumn decline in the U.S.A and worldwide. The scale can also be used to estimate responses of other wetland vegetation or crops that may have two related phenomena.

### Keywords

Rice Hydrogen sulfide toxicity; Autumn decline; Akiochi; Matrix

## Introduction

Arkansas produces nearly 50 percent of the U.S.A. rice. In some fields, hydrogen sulfide ($H_2S$) toxicity causes black root rot in rice with stunted plants and leaf chlorosis beginning soon after flood establishment as described by Wamishe et al. [1]. Although root blackening is not the direct toxicity effect from $H_2S$, in anaerobic soil, portions of $H_2S$ contribute to form insoluble sulfides such as FeS that accumulate on rice roots turning them black [2-5]. In a situation where $H_2S$ toxicity is severe, rice roots start rotting, creating an opportunity for the invasion of root crowns by weak pathogenic fungi, commonly *Fusarium spp*. [3]. The invasion ultimately obstructs the root crown from water and nutrient translocation [6-15]. Subsequently resulting into reduced content of K, Mg, Ca, Mn and Si in rice plant tissues [5,6].

Although $H_2S$ toxicity predisposes rice plants to autumn decline [3], often both are referred to as the same phenomenon even though they are two forms of disorders. These disorders are known to occur in soils where active iron and cation exchange capacity are low. Soils having excessive insoluble forms of iron have been the primary suspects for root blackening. As a result, $H_2S$ toxicity in rice can sometimes be associated with iron toxicity [4,14]. $H_2S$ is produced through reduction of sulfates under flooded conditions and it becomes harmful to plants when it stays in a soil solution above a certain concentration [5]. $H_2S$ largely accumulates as a result of microbial reduction of sulfate during anaerobiosis [7,11]. However, its level varies depending on the prevailing edaphic conditions. Input levels of sulfur affect severity of $H_2S$ toxicity in soil solution. Discharge from groundwater and run-off from surface waters can make soil richer in sulfate [9,13]. Moreover, increased amounts of organic matter are reported to boost sulfate reduction rates by providing electron donors from the process in decomposition [16]. Overall, $H_2S$ toxicity level in soil solution can be influenced by complex inter-relationships of factors such as soil sulfate content, rate of sulfate reduction, soil temperature, soil redox potential, soil pH, organic matter content, $CO_2$, bicarbonate and sulfide ion immobilization by $Fe^{2+}$ [4]. Other than rice paddies, the risk from $H_2S$ toxicity is an important issue on a global scale, as sulfur concentrations have raised in many fresh waters and wetlands [8,12]. Higher temperatures and salinity are also reported to contribute to the rise of $H_2S$ [7]. However, the causes of $H_2S$ toxicity in Arkansas rice paddies are not yet well studied.

Compared to adult rice plants, seedlings were shown to be more sensitive to $H_2S$ [6]. The capability of rice roots to release oxygen to oxidize $H_2S$ in rhizospheres plays role in reducing its accumulation. Therefore, the toxicity level from $H_2S$ can vary depending on the oxidizing power of rice roots [5]. However, in soil solutions where reduction rates of sulfates are high, hydrogen sulfide inhibits oxygen release from rice roots [2,5]. As a result, there can be differences in root systems of rice cultivars in their rates of nutrient uptake [6].

The two disorders were conveyed as $H_2S$ toxicity in Arkansas in a limited number of rice fields in 2004. However, the numbers increased since year 2012. The symptoms in a field can easily be confused with nutrient deficiency particularly nitrogen. The increased flow of reports in recent years is probably due to increased awareness rather than surge in the disorders. Recently, attempts were made to separate the two disorders as $H_2S$ toxicity for the earlier symptoms and autumn decline for the later symptoms associated with the fungal invasion into root crown [3] mainly for management strategy. In rice paddies with levees, symptoms of both disorders are less prominent on levees where soil mostly remains aerobic. Contrarily, symptoms become conspicuous in barrow ditches, water furrows, in field areas deeper in

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flood depth and at well water inlets [1]. Prior to developing this scale, other standard disease rating methods were used in greenhouse and field conditions in our effort to identify tolerant rice cultivars. However, none appeared good enough to estimate the combined effects of root mass discoloration and root crown damage caused by $H_2S$ toxicity and autumn decline, respectively. Thus, the objective of this paper focused on developing a scale to estimate the combined effects of these two phenomena to effectively evaluate rice cultivars growing in soils that have a history of these disorders.

## Experimental Procedure

### Field experiment in 2015

Nineteen commercial rice cultivars (Table 1) were planted in four replications in a production field with a history of $H_2S$ toxicity in Woodruff County, Arkansas, in the spring of 2015. Each plot had 9 rows with 17.8 cm spacing and 4.6 m length. Plots were managed by the producer similar to his adjacent commercial rice. Between heading and anthesis, 20 to 30 rice plants were randomly pulled up from the outer rows of each plot. Roots were washed and percent root mass discoloration/blackening were estimated immediately before roots lose their black color due to exposure to atmospheric oxygen. A subset of 10 randomly chosen rice plants were split vertically down the length of the stem and percentage root crown blockage/browning were recorded in reference to crown length.

### Development of a new scale

Since the percentage values for root mass discoloration and crown damage did not show strong correlation, ranking cultivar responses did not make sense using either of the data set. Based on our observations across years, grain yield were more affected when root crown damage was more prominent. Therefore, based on the economic importance of the disorders, the data were translated to the 0 to 5 and 0 to 9 standard scales. The 0 to 5 scale was assigned to root mass discoloration and the extended 0 to 9 scale to crown damage. In addition, to amplify root crown damage, a double weight was assigned to root crown damage by multiplying the 0 to 9 values by 2. To combine the damages from the two disorders, a matrix was used. Finally, a matrix-addition scale was found to best reflect our field observations. In the matrix-addition table, the percentage values were kept in the corresponding outskirt of the matrix for the purposes of data comparison or exchange (Table 2). To evaluate the viability of the matrices, correlation coefficients were analyzed and compared between the values in the 0 to 5, 0 to 9 and the transformed matrices.

### Validation of the developed matric-addition scale

The matrix-addition scale was validated in 2016 in replicated field plots using 20 rice cultivars (Table 1) in adjacent field to the one in year 2015. The plots were managed by the same farmer similar to the test in year 2015. Again, 20 to 30 rice plants were randomly and carefully pulled up from the outer rows of each plot; roots thoroughly washed and rated immediately using a 0 to 5 scale for root mass discoloration and a 0 to 9 scale for crown damage. In addition, percentage cultivar response data were collected separately for both disorders where higher values represented more root mass discoloration or crown damage.

## Results and Discussion

From the replicated field plots of 19 commercial rice cultivars, data on root mass discoloration and crown damage were collected between heading and anthesis developmental stage but before the farmer lowered the flood depth in preparation to harvest. The percentages of root mass discolored and root crown damaged were also collected. While variation in root mass discoloration and crown damage were clearly perceived in this field test, the intensity of root mass discoloration did not appear to consistently correlate with root crown damage across cultivars. In some instances, roots intensely discolored had little or no crown damage and the vice versa. Since an assessment method should hypothetically serve as a good predictor of the overall impact of the disorders on grain yield or quality, the percentage values were first translated to a 0 to 5 and 0 to 9 scale. The extended standard scale, the 0 to 9 was assigned to root crown damage and the 0 to 5 scale to root mass discoloration (Table 2). From years of our field observations, we knew that root mass blackening is a reversible phenomenon since it slowly disappears once the flood level is lowered allowing oxygen to enter the soil surface. However, crown damage is irreversible and can severely affect crop yield by inhibiting nutrient translocation from soil up. Therefore, similar scales to assess damages by $H_2S$ toxicity and autumn decline were not believed to be adequate to reflect the impact levels of each phenomenon.

The 0 to 9 values were arranged vertically parallel to the percentages they represent and the 0 to 5 values horizontally likewise to their respective percentage values (Table 2). To additionally amplify the impact of crown damage, the 0 to 9 values of root crown damage were assigned double weight (Table 2). Finally, when matrix was applied to combine the vertical and horizontal rating values for root crown damage and root mass discoloration, matrices from the matrix-addition appeared to reflect our field observations rendering distinct five tolerance categories. The categories were termed as T (tolerant), MT (moderately tolerant), ST (slightly tolerant), IT (intolerant), and VIT (very intolerant). These phenomena were considered to relate to disorders in soils and the oxidation power of rice root systems. The matrices within the matrix-addition ranged from 0 to 23 where 0 to 2 represented T, 3 and 4 MT, 5 and 6 ST, 7 and 8 IT and 9 to 23 VIT. The matrix-addition scale indicated that a rice cultivar with up to 25 percent root mass discoloration can still be tolerant; up to 75 percent moderately tolerant as long as there are no root crown damages. With up to 10 percent root crown damage, a cultivar can move down to MT as long as the root mass blackening remained below 25 percent. 20 percent root crown damage, however, can take a cultivar down to intolerant (IT) when accompanied with 50 percent or more root mass discoloration. As shown in Table 2, most rice cultivars fell in the category of VIT. These categories were in agreement with our multiple field observations across years where a larger proportion of Arkansas commercial rice appeared intolerant. We were not able to show yield data in these tests due to difficulty in setting up control plots with different water management. In our field tests, our plots were fully managed by the rice farmer similar to his adjacent field.

Incorporating the percentage data values in the outskirt of the matrix was considered important to show how data were translated to the 0-5 or 0 to 9 scales. In developing the matrix-addition scale, Pearson's correlation coefficients from the translated data of 2015 between the 0 to 5 and 0 to 9 scale was 0.63 at 0.05 prob>0.0033; between the 0 to 5 scale and the transformed matrices moved to 0.79 at 0.05 prob>0.0001. It went further up to 0.97 at 0.05 prob>0.0001 between the matrices and the 0 to 9 scale before or after doubling the weight. Doubling the weight the 0 to 9 scale used to estimate root crown damage appeared useful of to distinctly separate the categories for rice cultivar tolerance. Without the double value the categories were not that distinct (Table 2).

Through years, we have seen up to 50 percent grain yield loss in the

**Table 1:** Rice cultivars tested for $H_2S$ toxicity and autumn decline tolerance in a producer's fields at Woodruff County, Arkansas in year 2015 and 2016.

| **Year 2015** | | **Year 2016** | |
|---|---|---|---|
| **Cultivar** | **Cultivar** | **Cultivar** | **Cultivar** |
| CL111 | Mermentau | CL111 | LaKast |
| CL151 | Roy J | CL151 | Mermentau |
| CL153 | Taggart | CL153 | G214CL |
| CL163 | Thad | CL163 | Roy J |
| CL172 | Titan | CL172 | Thad |
| CL271 | RT CLXL745 | CL272 | Titan |
| CL272 | RTCLXL729 | RTGemini214CL | RTCLXL745 |
| Diamond | RTXL753 | RT7311 | RTXL753 |
| Jupiter | RTXL760 | Diamond | RTXL760 |
| LaKast | | Jupiter | RTCLXP766 |

**Table 2:** Matrix-addition scale to combine and estimate the damages of hydrogen sulfide toxicity and autumn decline in rice cultivars.

| | | | **% Root blackening aligned with a 0 to 5 scale** | | | | | |
|---|---|---|---|---|---|---|---|---|
| **% Crown** | **0 to 9** | **2X** | **0** | **10** | **25** | **50** | **75** | **>75** |
| **Infection** | **Scale** | **(0-9)*** | **0** | **1** | **2** | **3** | **4** | **5** |
| 0 | 0 | 0 | 0=T | 1=T | 2=T | 3=MT | 4=MT | 5=ST |
| 10 | 1 | 2 | 2=T | 3=MT | 4=MT | 5=ST | 6=ST | 7=IT |
| 20 | 2 | 4 | 4=MT | 5=ST | 6=ST | 7=IT | 8=IT | 9=VIT |
| 30 | 3 | 6 | 6=ST | 7=IT | 8=IT | 9=VIT | 10=VIT | 11=VIT |
| 40 | 4 | 8 | 8=IT | 9=VIT | 10=VIT | 11=VIT | 12=VIT | 13=VIT |
| 50 | 5 | 10 | 10=VIT | 11=VIT | 12=VIT | 13=VIT | 15=VIT | 15=VIT |
| 60 | 6 | 12 | 12=VIT | 13=VIT | 14=VIT | 15=VIT | 16=VIT | 17=VIT |
| 70 | 7 | 14 | 14=VIT | 15=VIT | 16=VIT | 17=VIT | 18=VIT | 19=VIT |
| 80 | 8 | 16 | 16=VIT | 17=VIT | 18=VIT | 19=VIT | 20=VIT | 21=VIT |
| 90 | 9 | 18 | 18=VIT | 19=VIT | 20=VIT | 21=VIT | 22=VIT | 23=VIT |

[**Note:** The 0-9 scale was multiplied by 2 for a double weight to crown damage since it is irreversible and debilitates the crop than the root mass discoloration. Plant root crown refers to the base of the stem right above the root system.

T=Tolerant; MT=moderately tolerant; ST= slightly tolerant; IT=intolerant; VIT=very intolerant]

**Table 3:** Tolerance levels* of 20 commercial rice cultivars evaluated for hydrogen sulfide toxicity and autumn decline using the new matrix-addition scale transforming mean percentage data into a 0 to 5 and a 0 to 9 scale and double weight based on Economic importance of the disorders in Arkansas rice paddies.

| **Rice Cultivar** | **Black Root Mass Mean %** | **Black Root Mass 0-5 scale** | **Root Crown Damage 0-9 Scale** | **Root Crown Damage Double Weight** | **Matrix Addition Value** | **Tolerance Level*** |
|---|---|---|---|---|---|---|
| CLX1024 | 45 | 3 | 0 | 0 | 3 | MT |
| LaKast | 48 | 3 | 0 | 0 | 3 | MT |
| CLXP766 | 55 | 4 | 0 | 0 | 4 | MT |
| XL753 | 58 | 4 | 0 | 0 | 4 | MT |
| XL760 | 50 | 3 | 2 | 4 | 7 | IT |
| Jupiter | 45 | 3 | 2 | 4 | 7 | IT |
| Roy J | 55 | 4 | 2 | 4 | 8 | IT |
| CL163 | 48 | 3 | 3 | 6 | 9 | VIT |
| Titan | 50 | 3 | 4 | 8 | 11 | VIT |
| Thad | 43 | 3 | 4 | 8 | 11 | VIT |
| CLXL745 | 48 | 3 | 4 | 8 | 11 | VIT |
| CL272 | 53 | 4 | 5 | 10 | 14 | VIT |
| CL172 | 48 | 3 | 8 | 16 | 19 | VIT |
| CL111 | 48 | 3 | 8 | 16 | 19 | VIT |
| RtGemini214 CL | 60 | 4 | 8 | 16 | 20 | VIT |
| RT7311 CL | 60 | 4 | 8 | 16 | 20 | VIT |
| CL151 | 60 | 4 | 8 | 16 | 20 | VIT |
| Diamond | 58 | 4 | 9 | 18 | 22 | VIT |
| Mermentau | 55 | 4 | 9 | 18 | 22 | VIT |
| CL153 | 58 | 4 | 9 | 18 | 22 | VIT |

**Note:** The matrices obtained from matrix-addition of values of the 0 to 5 scale and double weighted 0 to 9 values gave three categories: MT=moderately tolerant; IT=Intolerant; VIT=Very intolerant. None of the rice cultivars fell in the categories of T (tolerant) or ST (slightly tolerant) showing the importance of root crown damage compared to root mass discoloration/blackness.]

state of Arkansas due to these two disorders. Cultivars with no black root discoloration are unlikely to show root crown damage associated with $H_2S$ toxicity or autumn decline. We have seen cases of "false akiochi/autumn decline" in a separate greenhouse study and in a few rice fields due to drought stress. False akiochi can also be caused by metallic toxicity in an area where metals were buried. In such instances, rice root crowns can get the damage similar to autumn decline without root mass discoloration. Rotten-egg smell of $H_2S$, field history and soil iron and sulfur amount can distinguish the true autumn decline from the false.

In 2016, the new matrix-addition scale was used to evaluate 20 cultivars planted in a field with a history of $H_2S$ toxicity and autumn decline. Data were collected using the 0 to 9 scale for root crown damage and the 0 to 5 for root mass discoloration. At the same time percentage estimates were also collected for both but separately. The data collected from the 2016 from replicated field plots verified the matrix-addition scale for the evaluation of $H_2S$ toxicity and autumn decline as a useful and feasible predictor of field grown cultivar responses. Accordingly, the matrices of the 20 rice cultivars fell into three categories (Table 3).

Although root crown damage was caused by the invasion of opportunistic fungi, these phenomena were considered as soil related disorders rather than 'proper' diseases. However, since grain yield is affected, assessment methods were needed. Field assessment methods, however, can vary depending on the nature of the problem and the crop. Hence, the matrix-addition method of estimating cultivar responses to hydrogen sulfide toxicity and autumn decline was found to be adequate.

The management strategy currently available in Arkansas is to carefully drain and dry for a few days to allow oxygen into rhizospheres [3]. The drain and dry practice has proven to be effective as a preventative in rice paddies with either one or both disorders. Nevertheless, autumn decline is not reversible and if late it may be difficult to fully rescue the crop. Moreover, draining and drying may not always be an option with large field sizes and low water resource or pumping capacity that limit the ability to drain and re-flood within a few days [17]. It takes a judgement call as to how long and how dry the soil should be dried before re-flooded. It is possible that drought stress can also cause substantial damage to the crop at reproductive developmental stages and it may also predispose the rice crop to blast disease.

Often, rice roots release oxygen in the rhizosphere and $H_2S$ oxidized. Consequently, the toxicity level may vary depending on the oxidizing power of rice cultivars [5]. When flood is lowered to dry the soil, atmospheric oxygen that enters the rhizosphere temporarily enhances the oxidation of $H_2S$ and new roots start to grow from upper parts of root crown as an immediate survival response. Greenhouse research and field observations have shown growth of new roots in 3 to 5 days [1].

It is anticipated that exposure time length under anaerobic/flooded condition may have differences in the level of root mass discoloration and crown damage. In our field experiments, the cultivars included in the tests, do not have similar heading dates. Nevertheless, they were planted at the same time but samples were pulled up and evaluated all on the same day. For instance, rice cultivar CL111 is relatively fast maturing compared to Roy J, which means the latter needs longer flood exposure until drainage for harvest in a normal situation. As a result, rice cultivar Roy J may suffer more from $H_2S$ toxicity and autumn decline than CL111. Yet, for an equal flood exposure time, CL111 appeared more prone to the disorders than Roy J (Table 3). In our test, regardless of cultivar maturity, data were collected after all the cultivars headed but before the fields lost their flood level or drained in preparation for harvest.

The levels of root mass discoloration and root crown damage may also vary from field to field. This study, however, did not explore variations related to soil types or flood exposure timing in relation to cultivar maturity and tolerance variability. Profound study is required to explain the differential effects of $H_2S$ and autumn decline in these areas. This matrix-addition scale has been verified as beneficial to compare rice cultivars grown in similar conditions and submerged for equal period of time. This matrix-addition scale is the first to evaluate rice cultivars grown in rice fields with a history of $H_2S$ toxicity and autumn decline. It can also be used in other crops or vegetation to combine and evaluate the damages of inseparable phenomena similar to $H_2S$ toxicity and autumn decline.

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