WILD RICE DISEASE RESEARCH

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INTRODUCTION:

The plant pathology research staff concentrated on the following areas during 1989:

1. Developing a chemically defined hydroponic growth medium for studying the nutrient requirements of wild rice (D. Malvick, J. Percich, R. Zeyen, & P. Bloom, Dept. Soil Science).

2. Filing an application for an emergency exemption (Section 18) for the fungicide propiconazole (Tilt^{R}) (MWRC & J. Percich).

3. Initiation of a disease nursery to screen for fungal brown spot resistance (J. Percich & R. Porter, North Central Exp. Sta. & Dept. of Agronomy and Plant Genetics).

4. Continued investigation of wild rice tissue culture and other <u>in vitro</u> screening methods for fungal brown spot resistance (D. Johnson).

The plant pathology wild rice project is pleased to announce the appointment of Mr. Dean Malvick as Associate Scientist. Dean received his B.S. (Biological Sciences) from Bemidji State Univ., and an M.Sci. (Plant Pathology) from Oregon State Univ. in 1987. Dean was a Research Technician in the Dept. of Plant Pathology at Oklahoma State Univ. before coming to the Univ. of Minnesota.

A CHEMICALLY DEFINED HYDROPONIC GROWTH MEDIUM USEFUL FOR DETERMINING MINERAL NUTRIENT REQUIREMENTS OF WILD RICE (RESEARCH FUNDED BY THE MINNESOTA PADDY WILD RICE RESEARCH AND PROMOTION COUNCIL).

Both commercial field production and experimentation with wild rice suffer from a lack of basic knowledge concerning the plants physiology and mineral nutrient needs. What chemical elements must wild rice absorb to live and grow normally? What are the essential major and minor chemical elements needed for maximum growth and health of wild rice? What are the symptoms of various chemical element deficiencies in wild rice? Field studies have helped define some macronutrient requirements, like nitrogen forms, rates and times of application. However, there is little known about total wild rice nutritional needs.

Experimental procedures like producing wild rice plants form tissue culture are hampered by the lack of information on mineral nutrition requirements. Growing plants from tissue culture could provide a much more rapid means of disease screening, testing for herbicide tolerance, and accelerate future breeding programs. Tissue culture is also necessary to take advantage of advances in molecular biology which may be applied to wild rice improvement.

The only proven and exacting method of determining precise chemical nutritional requirements of plants is to grow them in a soil-free, water-based nutrient system, using ultra-pure chemical elements of known composition and concentration. Plants must be grown in these chemically defined solutions to adult status. This method is also called "hydroponic culture".

Our preliminary experiments indicate that wild rice can be grown in a chemically defined media. However, the chemical composition and concentrations of the media necessary to support normal wild rice growth, pH conditions, and perhaps growth chamber conditions (photoperiod and light quality) are not yet ideal. Wild rice plants produced to hydroponic media to date are small (one-third normal height and mass) and expressed unknown stress symptoms. We need to use our current knowledge to expand these experiments until we can grow normal plants to flowering and seed set. This information will enable us to use various "nutrient plus and minus" experiments to learn the importance of each chemical element wild rice needs.

Objectives Of Hydroponic Research Project:

1. Perfect a chemically defined hydroponic growth medium for wild rice to allow for research into major element nutrition.

2. Determine minor element needs by growing wild rice to seed set in a chemically defined medium. Obtaining viable seed from hydroponic-grown plants is necessary in minor element research, since field grown seed contain enough minor element "carry-over" to permit first generation growth. Therefore, seed from parents grown with known types and concentrations will eliminate "carry-over".

3. Determine the role(s) of silicon for wild rice growth and disease resistance.

4. Use hydroponic methods to assist ("nurse solutions") in the regeneration of wild rice plants from differentiated tissue culture.

References:

Percich, J. A., Zeyen, R. J., Huot, C. M., and Johnson, D. 1987. Wild rice disease research - progress in tissue culture of wild rice. pp. 78-87. In: Minnesota Wild Rice Research 1987. Minnesota Exp. Sta. Misc. Publ. 54-1988. pp. 98.

Zeyen, R. J., and Percich, J. A. 1988. Silicon enhanced wild rice plant growth, development, yield, and disease resistance in cultivated wild rice. Ext. Abstr. International Wild Rice Conf. Reno, NV.

APPLICATION FOR THE EMERGENCY REGISTRATION (SPECIFIC EXEMPTION UNDER SECTION 18 OF FIFRA) FOR TILT ON CULTIVATED WILD RICE IN MINNESOTA (MINN. PADDY WILD RICE RESEARCH AND PROMOTION COUNCIL & J. PERCICH).

Background:

The manufactures (United States) of fungicides containing the active ingredient ethylene bis dithiocarmate (EBDC) jointly announced a voluntary suspension of numerous registrations involving food crops on September 6, 1989. It is difficult to count exactly how many uses for the EBDC fungicides have been voluntarily withdrawn. An approximation is that 73 registered food uses have been reduced to 13.

The voluntary withdraw affects mancozeb (Dithane M-45, produced by Rohm & Hass) on cultivated wild rice in Minnesota. Also, tank mixes involving Dithane M-45 are affected. Withdraw of Dithane M-45 on wild rice has been in effect since January 2, 1990.

The Minnesota Department of Agriculture (MDA) has indicated the use of Dithane M-45 on cultivated wild rice in Minnesota was approved by the Environmental Protection Agency (EPA) and given a Special EPA Number. However, EPA has withdrawn all such special labels. The MDA cannot re-issue a label for Dithane M-45 on wild rice. Therefore, the cultivated wild rice industry in Minnesota will no longer have Dithane M-45 to control foliar fungal diseases.

Current Status:

The MDA has supported a request by the Minnesota Wild Rice Council and Dr. Jim Percich to obtain a Section 18 for the use of the fungicide propiconazole (Tilt, produced by CIBA-GEIGY) for the 1990 growing season. The documentation is currently (Feb. 12, 1990) under review by the EPA. A decision, hopefully, will be made during the spring of 1990.

Should the EPA reject the wild rice growers' application for Tilt; Dr. Percich will immediately submit supportive documentation for the use of Dithane M-45 on cultivated wild rice in Minnesota.

SCREENING FOR FUNGAL BROWN SPOT (FBS), CAUSED BY BIPOLARIS ORYZAE (D. Malvick, R. Porter and J. Percich).

Introduction:

A cooperative project was initiated with Dr. Ramey Porter to develop a wild rice disease nursery at the North Central Experiment Station at Grand Rapids, Minnesota. Plants were inoculated during early boot and again two weeks later with mixed isolates of B. oryzae. The inoculated plants were misted during the day to provide the necessary moisture (free water) to help facilitate pathogen infection and reproduction. Observations for FBS incidence and severity on the Flag/Flag-1/Flag-2 leaves were recorded two weeks after inoculation and continued at 10 day intervals until the 1/4 grain stage of plant development.

Results and Conclusions:

Fungal brown spot disease incidence and severity was extremely low, observed to be < 5% total leaf area infected per plant by harvest. The resulting low disease pressure did not allow for discriminating evaluation of individual plants which may have been more tolerant to fungal brown spot. The poor disease pressure in our nursery may have been due in avirulent inocula, poor misting of inoculated plants (too much or too little moisture), and/or hot dry weather conditions. Evaluation of FBS tolerance in both the field and greenhouse will be attempted during the 1990 growing season.

SCREENING EXOTIC WILD RICE GERMPLASM FOR RESISTANCE TO FUNGAL BROWN SPOT, CAUSED BY BIPOLARIS ORYZAE (D. R. Johnson).

Introduction:

In cereals, the most common method of improving disease resistance has been the introgression of resistance genes from wild relatives of the crop. Although cultivated wild rice (Zizania palustris L.) varieties exhibit considerable variation for some traits, the genetic base of these varieties is quite narrow. It is possible that greater variation for reaction to Bipolaris oryzae exists in wild populations of Z.palustris and related species. It has been hypothesized that the greatest diversity for genetic traits which confer some adaptive advantage occurs at the plant's center of origin (Wahl et al.,1984) For most cereals, the center of origin is the middle east, but for wild rice it is the lakes and rivers of the U.S.

Screening of diverse collections of wild rice for FBS resistance has not been done. The purpose of this study was to determine if FBS resistance exists in non-cultivated wild rice.

Materials and Methods:

Seed of Zizania spp. and interspecific hybrids were screened by one of two methods: Rare seed, such as from interspecific matings, was tested using the in vitro embryo rescue method of Johnson and Percich (1988). Seed which was plentiful was screened in greenhouse seedling tests. Seeds were first germinated in water, then transplanted to greenhouse pots. The tests were conducted in 16.5 x 23.5 x 3 cm trays each containing six 7 x 7 x 7 cm pots. The pots

were filled with moistened vermiculite and planted with four wild rice seedlings each. The trays were filled with water and kept in a greenhouse at 20-24 C. Two grams of fertilizer, 21-7-7 "acid special" (Peters Fertilizer Products, Allentown, Pa.) was added to the water in each tray. Supplemental fluorescent light (130 uE m-1 s-2) was supplied at 12-hour intervals. Seedlings were inoculated when the floating leaves appeared (usually 14 days after germination). An aqueous suspension of B. oryzae conidia (ca. 10^5 conidia /ml) was sprayed on the plants with an air brush. Inoculated seedlings were placed in a dew chamber at 20 C and 100% relative humidity for 40 hr, then returned to the greenhouse.

Results and Discussion:

Fungal brown spot infection was visible on inoculated leaves after two or three days. Disease reactions were observed and recorded after 10 days to allow for maximum lesion expansion. Disease reactions were divided into three classes:

Resistant	=	Circular, dark brown necrotic spots < 3 mm diameter.
Intermediate	=	Oblong, light brown necrotic spots with dark brown margins 3-5 mm diameter.
Susceptible	=	Diffuse areas of light and dark brown necrotic tissue > 5 mm diameter.

The reactions of Zizania spp.,interspecific hybrids, and Zizaniopsis milaceae , are presented in Table 1.

Resistance was observed in several Zizania species, including the cultivated variety Meter. The resistance was less distinct when young aerial leaves are inoculated, and could not be distinguished in adult plants. Perhaps resistance is expressed only in seedlings, or perhaps the pathogen is better adapted to the aerial leaves. It is possible that a subtle type of resistance, such as a slower rate of lesion expansion, persists in adult plants. This would be obscured by heavy inoculum loads.

There appears to be no exceptional resistance to FBS in the collection screened in this study. It should be noted that the size of this collection was exceedingly small when compared with the size and number of wild Zizania populations. Additional collections, especially from regions where the pathogen is prevalent, should be obtained and evaluated. Zizaniopsis milaceae, a perennial relative of Zizania, was immune to B. oryzae in these tests. Attempts to cross cultivated wild rice to Z. milaceae were unsuccessful. The immunity of Z. milaceae may represent a non-host reaction which cannot be transferred to Zizania species.

Table 1. Reactions of Zizania species and hybrids to infection with Bipolaris oryzae.

Name	Number and reaction class[a]
Z. aquatica subbrevis	4S,2I
Z. aquatica aquatica	11S
Z. aquatica aquatica (Wading River Ecotype)	1S
Z. palustris palustris	1R, 11S
Z. palustris interior	1R, 2I, 9S
Z. aquatica aquatica x Z. plaustris interior	1I
Z. texana x Z. palustris interior	1R, 1I
Z. texana x Z. aquatica aquatica	1S
Z. palustris interior 'Meter'	4R, 12I, 230S
Z. palustris interior 'Voyager'	3I, 145S
Zizaniopsis milacea	10 immune[b]

[a] R= resistant, I= intermediate, S= susceptible
[b] No evidence of infection

Literature Cited:

Johnson, D. R. and Percich, J. A. 1988. A laboratory method for evaluation of wild rice (Zizania palustris) germplasm for resistance to fungal brown spot caused by Bipolaris oryzae. Phytopathology 78: 1616.

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INFLUENCE OF (+/-) ABSCISIC ACID ON SOMATIC EMBRYOGENESIS IN TISSUE CULTURES OF WILD RICE (Zizania palustris) (D. R. Johnson).

Introduction: The ability to initiate callus from organized plant tissue and regenerate plants from callus is essential for use of somaclonal variation and in vitro techniques in plant improvement. In wild rice (Zizania palustris L.) the initiation and maintainence of callus cultures has been achieved (Percich et al., 1988) but regeneration of plants has been difficult. Plant regeneration from tissue cultures of all monocotyledonous plant, especially those in the family Gramineae, has only been moderately successful. Regeneration can occur by organogenesis or embryogenesis, but the latter process is most desirable because it is more likely to result in the production of complete plantlets. Reports of plant regeneration via embryogenesis have been numerous since it was first reported by Green and Phillips (1975), but some cereal cultures, including those of wild rice, rarely or never produce somatic embryos.

There are several reports of successful induction of embryogenesis in cereal tissue cultures using abscisic acid (ABA)(Maddock et al., 1983, Reddy and Reddy, 1987, Vasil and Vasil, 1981). Exogenous ABA also prevented precocious germination of somatic embryoids (Qureshi et al., 1989), suggesting that ABA promotes embryo maturation. Fluridone inhibition of ABA synthesis in plants and callus tissues has been demonstrated, (Henson 1984, Moore and Smith 1984 Raikhel et al., 1985) but its effect on embryogenesis have not been reported. This study was undertaken to examine the effects

of exogenous ABA on embryogenesis in wild rice callus cultures and to learn if embryogenesis can be induced in non-morphogenic cultures.

Materials and Methods:

Callus cultures. Callus was initiated from mature embryos of wild rice varieties K-2 and Meter using the methods of Percich et al. 1987. Breifly, embryos were extracted aseptically, surface disinfested, rinsed and placed on a modified Murishige-Skoog (MS)(1962) solid culture medium ammended with 1.0 mg 2,4-dichlorophenoxyacetic acid (2,4-D). The embryos were incubated for 30 days at 25 C in darkness. Necrotic areas and remnants of the original embryo were trimmed from the calli before transfer to the next medium.

Medium. Abscisic acid (+/-)-cis, trans-ABA 99% was obtained from Sigma. Fluridone (1-methyl-3-phenyl5-([trifluoro-methyl]phenyl)-4-[1H]pyridinone) was obtained from Ciba-Geigy. Stock solutions were prepared by dissolving the technical material in 95% ethanol. One liter of the base culture medium contained the following: MS macro- and micro-nutrients, MS vitamins, 15 g maltose, 15 g sucrose, 10 ml antibiotic/antimycotic solution (Gibco),1 mg naphthalene acetic acid (NAA), 100 mg casein hydrolysate, and 2 g gelrite (Scott). The test media contained 0.1, 0.2, 0.5 or 1.0 mg ABA or 1 mg Fluridone. The base medium was used as a control.

Incubation. The established callus cultures were transferred to 100 x 25 mm petri dishs containing 50 ml of test medium. Twenty-five plates, each containing three calli (ca. 2 cm dia) were prepared for each treatment. The plates were sealed with parafilm and incubated under grow lamps (20 $uEm^{-2}s^{-1}$) in a growth cabinet at 25 C. After 30 days, the number of somatic embryos/callus were recorded. Healthy cultures were transferred to regeneration medium.

Results and Discussion: Embryogenesis was significantly higher in medium containing 0.1 or 0.2 mg ABA as compared with the control (Table 2). Optimal ABA concentration for embryogenesis was 0.2, while 1.0 mg was associated with a high degree of necrosis. No embryogenesis was observed in the fluridone treatments. Although callus growth appeared to be stimulated by fluridone, the tissue was mushy, white, and amorphous.

The results of this study support the hypothesis that ABA has a significant positive effect on the production of somatic

embryos in callus cultures of wild rice. There is some evidence that ABA can restore lost embryogenic capacity of some cultures.

Table 2. Average number of somatic embryos/callus in 75 cultures of Zizania palustris 'Meter' after 30 day incubation on abscisic acid, fluridone, or control medium.

Treatment	Average no. embryos
ABA(mg)	
0.1	2.9b
0.2	4.2a
0.5	0.6c
1.0	0.2c
Fluridone	0.0
Control	0.4c

Means followed by the same letter are not significantly different at the p = 0.05 level according to Duncan's New Multiple Range Test.

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