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# International Rice Research Notes

**The etiology of red stripe of rice:  
current status and future directions**



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International Rice Research Notes

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# The etiology of red stripe of rice: current status and future directions

T.W. Mew, N.P. Castilla, F.A. Elazegui, and C.M. Vera Cruz, IRRI  
E-mail: t.mew@cgiar.org

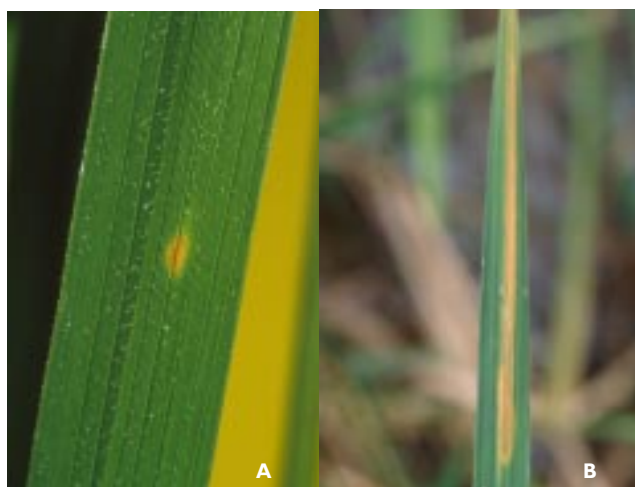


**R**ed stripe is an emerging disease\* of the rice crop that has been observed in recent years in intensive rice production systems of Southeast Asia. Initial lesions are pin-sized spots, often light yellow green to light orange (Fig. 1a). An older lesion appears as an orange spot with a stripe that advances toward the leaf tip (Fig. 1b). Lesions may become necrotic and coalesce, ultimately blighting the leaves. Although more common on leaves, red stripe lesions are also found on sheaths. Typical symptoms are usually observed from flowering to ripening.

Red stripe was first reported in 1988 from Indonesia (Mogi et al 1988). It also occurs in the Philippines (Barroga and Mew 1994), Malaysia (Yazid et al 1996), Thailand (Dhitikiattipong et al 1999), and Vietnam (Du et al 1991a). It has not yet been reported in temperate rice-growing countries. The disease is a potential threat to rice production in Southeast Asia, but a reliable quantification of yield losses has not been done yet.

Since red stripe symptoms do not resemble those caused by any known diseases of rice, various efforts have been exerted to understand its etiology. However, 12 years after red stripe was first reported, the causal organism has yet to be identified and its etiology remains obscure. To understand the etiology of red stripe, the most pressing questions that should be answered are the following: (1) Is red stripe caused by a biotic agent? (2) If so, what is the causal organism? (3) Is red stripe associated with production of toxins? and (4) How is the causal organism transmitted from one plant to another? This article reviews the hypotheses and results

\* In this review, the term “disease” is defined as any disturbance of the plant that interferes with its normal growth and development (e.g., structure and function), economic value, or aesthetic quality and leads to the development of symptoms (Shurtleff and Averre 1997). A disease is caused by biotic or abiotic agents.



**Fig. 1. Lesions of red stripe. A new lesion is a pin-sized spot (A), while an older lesion appears as a spot with a stripe that advances toward the leaf tip (B).**

of studies that have been conducted to answer these questions. It also outlines the research work and other options that should be considered in the future to establish the etiology of red stripe. With this review, we hope to provide baseline information for current and future research on the etiology of red stripe and promote a better exchange of ideas among scientists working on this disease.

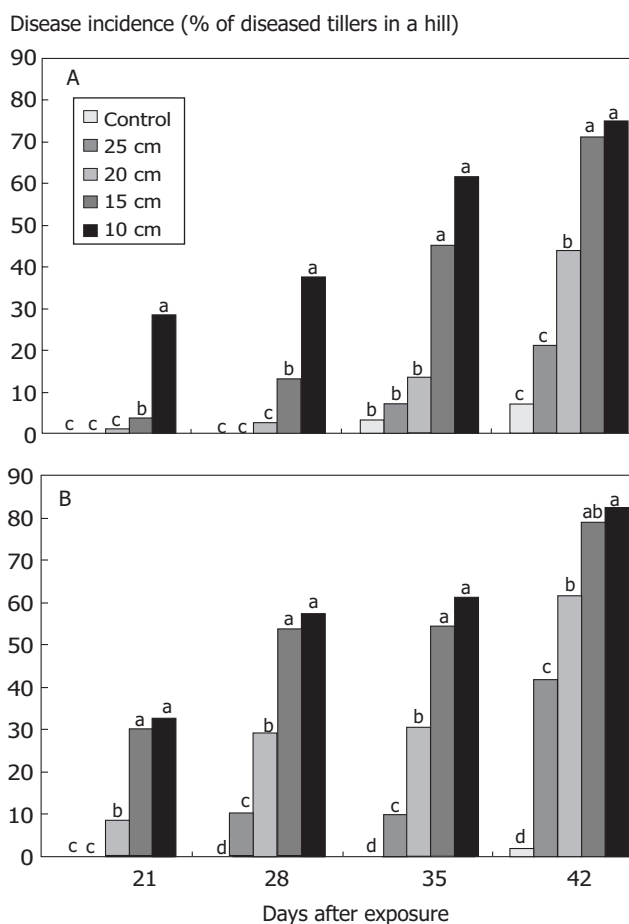
### Is red stripe caused by a biotic agent?

Various observations and experimental results suggest that red stripe is caused by an infectious or biotic agent and not by an abiotic agent. The causal agent of red stripe can be transmitted from diseased to healthy plants placed together inside moist plastic cages. It has been observed that plants produce red stripe lesions when placed beside other plants with red stripe lesions (Suparyono 1999, Vinh et al 1999). Our experiments at IRRI showed that healthy plants that are placed closer to plants with red stripe lesions have more diseased tillers than those placed farther away from diseased plants (Fig. 2). Experiments conducted at IRRI and in Vietnam (Du et al 1991b) also showed that plants sprayed with fungicides have fewer red stripe lesions than the untreated check.

### What is the causal organism?

The above information suggests that the causal agent of red stripe is transmissible from diseased to healthy rice plants. It is then very likely that the causal agent of red stripe is a biotic agent. Scientists have postulated various hypotheses with regard to the causal agent of red stripe. However, testing these hypotheses has been difficult because attempts to isolate the biotic agent using conventional methods in plant pathology have so far been unsuccessful.

To determine with certainty whether a particular biotic agent is the cause of a disease, it is necessary to critically examine



**Fig. 2. Effect of distance from source (diseased) plants on the incidence of red stripe at different days after exposure during two trials (A and B). Each column represents the mean of five replications. In a given day after exposure, bars with the same letter are not significantly different at  $P \leq 0.05$  using the least significant difference test. Analyses were made on arcsine-transformed data. An experimental unit consisted of four healthy plants that were placed around a diseased plant at a given distance (10 to 25 cm). For the control, each of the four healthy plants was placed 10 cm away from another healthy plant.**

its relationship with the host plant. Koch's postulates must be satisfied before a biotic agent can be regarded as a pathogen of red stripe. Koch's postulates state that (1) the suspected pathogen must always be present in the plant when the disease occurs; (2) the organism that is believed to cause the disease must be isolated and grown in pure culture on nutrient media (nonobligate parasites), or it must be grown on a susceptible host plant (obligate parasites); (3) the pure culture of the organism must produce symptoms or signs of the disease when inoculated on a healthy plant; and (4) the suspected causal organism must be reisolated in pure culture from the inoculated plant and must be identical to the organism initially isolated. Until the requirements of Koch's postulates are fulfilled, the causal agent remains obscure.

Below, we compare characteristics of pathogens that are suspected to cause red stripe with the general features of related



plant pathogens. Red stripe symptoms are also compared with typical symptoms of diseases caused by known plant pathogens.

**Bacteria.** Lesions caused by bacterial pathogens on plant leaf blades usually appear as spots, blight, streaks, or stripes in monocotyledonous plants. Pathogenic bacteria characteristically induce water-soaked lesions in infected tissues at initial stages. Bacterial ooze is usually formed from the cut edges of infected tissues.

Some reports indicate that red stripe could be caused by a bacterial pathogen. One of the first steps in determining whether red stripe is induced by a bacterial pathogen is the isolation and culture of bacteria from diseased tissues. Tuat (1999) isolated a bacterium from rice seedlings with typical red stripe symptoms. The bacterium was identified as *Acidovorax avenae* subsp. *avenae* (formerly *Pseudomonas avenae*) based on biochemical tests and fatty acid analysis. *A. avenae* subsp. *avenae* causes a common disease of rice known as bacterial brown stripe. However, the typical symptoms of bacterial brown stripe, which are brown, water-soaked stripes on the leaf blades and leaf sheaths (Webster and Gunnell 1992), differ from the symptoms of red stripe. The stripe extending from the spot of a red stripe lesion on the leaf blades is not water-soaked, and red stripe lesions are rarely observed on the sheath. Although bacterial brown stripe also occurs during the later development stages of the rice crop, it usually occurs during the seedling stage. In contrast, red stripe is normally observed during flowering to ripening stages. Furthermore, *A. avenae* subsp. *avenae* can be easily isolated and characterized (Shakya et al 1986). If it is indeed the causal organism of red stripe, Koch's postulates could have been easily fulfilled for red stripe. Unless red stripe is caused by a nonculturable bacterial species, the isolation and identification of the bacterium suspected to be the causal agent should not have been difficult. Until further evidence is provided, it remains unconfirmed if the isolate of *A. avenae* subsp. *avenae* obtained by Tuat (1999) is the causal agent of red stripe.

Recently, Kaku et al (2000) reported that white, transparent bacterial colonies were consistently isolated from all red stripe lesions of diseased leaf samples collected in Indonesia. Symptoms similar to red stripe developed on leaves of healthy plants that were inoculated with bacterial colonies using either the multineedle pricking method or infiltration method. They further reported that bacterial masses were also observed in the xylem vessels of diseased plants. The 16S rDNA sequence analysis of these isolates shows that the causal bacterium of red stripe is closely related to members of the genus *Microbacterium* (Kaku et al 2000). This study provides new information on the etiology of red stripe. However, lesions resulting from inoculation of rice leaves with the proposed causal organism do not seem to resemble typical red stripe lesions observed in the field. Furthermore, there is a need to determine whether the method described by Kaku et al (2000) will produce the same results in other laboratories and whether the isolate will produce symptoms resembling those that are typically produced by red stripe. At

IRRI, we have isolated several bacterial species from red stripe lesions using the isolation techniques and medium described by Kaku et al (2000). However, in two trials, we observed the predominance of yellow, shiny bacterial colonies in petri dishes. Pathogenicity tests by the pinpricking method showed that the isolated bacterial colonies did not produce red stripe symptoms. Modifying Kaku's method by forgoing surface sterilization of tissues produced white, transparent bacterial colonies. However, these colonies did not also produce red stripe symptoms in pathogenicity tests. At IRRI, we observed sections of red stripe lesions under the light and electron microscope, but we did not find bacteria-like organisms in the xylem. It is therefore necessary that other scientists repeat the isolation techniques described by Kaku et al (2000).

Some species of fastidious vascular bacteria (previously known as rickettsia-like organisms) are also pathogenic, but they cannot be grown on conventional culture media. Red stripe symptoms do not resemble typical symptoms of diseases caused by fastidious phloem-inhabiting bacteria (e.g., leaf stunting and clubbing, shoot proliferation, and greening of floral parts) or by fastidious xylem-inhabiting bacteria (e.g., necrosis of leaves and stunting of infected plants). So far, we have not observed these microorganisms or bacteria-like structures on red stripe lesions using the electron microscope.

**Fungi.** Fungal pathogens may cause local or systemic symptoms, such as general necrosis or death of plant tissues. They may also produce various structures on the surface of the host such as mycelium, sclerotia, and spores. Fungal pathogens are either facultative or obligate. Facultative fungal pathogens can be cultured and isolated using conventional laboratory techniques and their characteristics easily described. In contrast, obligate fungal pathogens cannot be cultured in artificial media, but they can be grown on a susceptible host plant and can produce typical symptoms. Koch's postulates should be satisfied even when dealing with obligate pathogens, although this is not always easy to do.

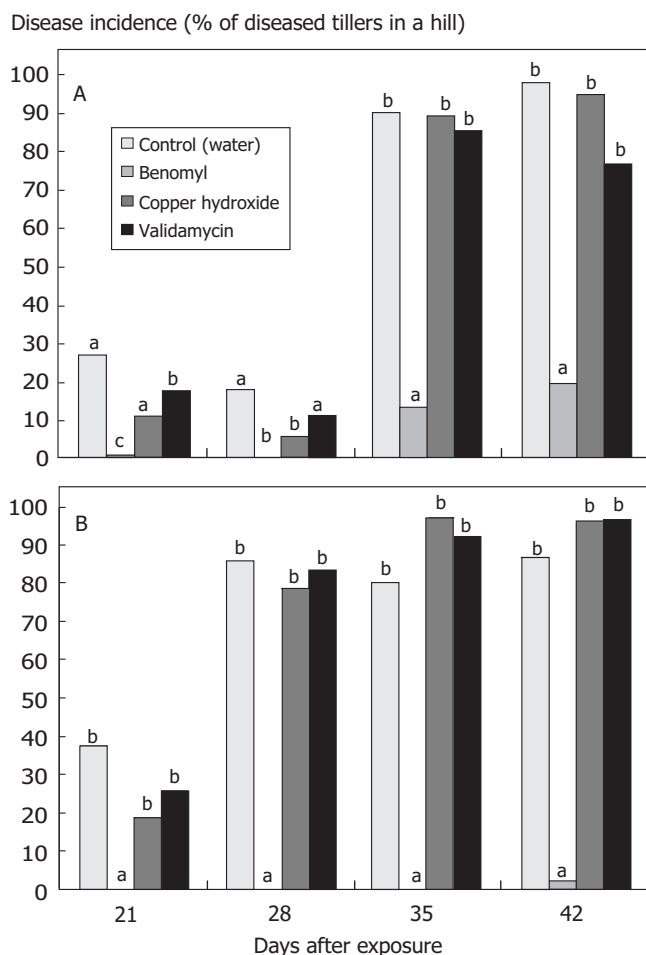
Red stripe lesions are, in many ways, similar to those of foliar diseases caused by some fungal pathogens. However, no fungal structures on infected tissues are visible to the naked eye. Several fungal species have been isolated from leaf tissues with red stripe lesions by plating small pieces of these leaf tissues on nutrient media (Du et al 1991a, Vinh 1997, Wakimoto et al 1998, Vinh et al 1999, Saad 1999). Among the fungi found were *Curvularia lunata*, *Nigrospora oryzae*, *Cercospora* sp., *Alternaria* spp., *Helminthosporium* spp., *Colletotrichum* spp., and *Fusarium* sp. None of these fungal isolates, however, produced typical red stripe lesions when rice leaves were inoculated with pure cultures. Most of these fungal species are saprophytes commonly associated with rice plants. We have also isolated these species from both red stripe lesions and healthy leaves by plating tissues on nonselective and selective media. Considering that the pathogen may be a slow-growing organism, we also attempted to induce sporulation in leaf tissues with red

stripe lesions in a moist chamber and exposed them to continuous light or near ultraviolet light. We then grew the spores that were collected from diseased leaves on nutrient media, but no isolate produced typical red stripe symptoms.

Experiments on the effect of fungicides on red stripe can be useful in establishing its etiology. These experiments can help determine whether red stripe is associated with a fungus even if it cannot be cultured on artificial media. The causal organism of red stripe could be a fungus if the application of certain fungicides is consistently effective against it. Du et al (1991b) reported that the severity of red stripe was lower in plants sprayed with benzimidazole fungicides, specifically benomyl and carbendazim, than in untreated plants. Experiments conducted at IRRI showed that the intensity of red stripe was significantly lower in plants sprayed with benomyl than in plants sprayed with other chemicals (Fig. 3). In another study, we found that the rate of increase

in number of lesions and area under the number of lesions progress curve were significantly lower in diseased plants that were sprayed with benomyl than in those that were sprayed with water (see table). These results suggest that the causal organism of red stripe could be a fungus. Benzimidazole fungicides are systemic and have been widely used to control ascomycete (Keinath and Zitter 1998) and basidiomycete fungal plant pathogens. It is essential to conduct more tests on the efficacy of fungicides including those that specifically control fungal plant pathogens other than ascomycetes and basidiomycetes. Although certain groups of fungicides may be proven to be consistently effective against red stripe, further studies are necessary to confirm whether a fungicide or a group of fungicides has prevented the “causal fungus” from invading plant tissues. In plants that are already diseased, it is necessary to confirm whether the fungicide or the group of fungicides can inhibit the increase in number and size of lesions and the spread of red stripe to other plants.

**Viruses, viroids, and mollicutes.** Viruses and viroids usually cause yellowing and stunting of infected plants. Mollicutes, such as phytoplasmas (formerly known as mycoplasma-like organisms) (Sears and Kirkpatrick 1994, Fletcher et al 1998) and spiroplasmas, induce chlorosis, stunting of plants, and abnormal growth of plant parts. Culture or purification is not yet possible for some viruses and viroids. A few species belonging to the genus *Spiroplasma* can be cultured to comply with Koch’s postulates, but phytoplasmas and spiroplasmas are generally difficult to obtain from their host plants and vectors and culture on nutrient media. Their association with a disease may be confirmed by observations under the electron microscope and by transmission tests. The method of detecting plant viruses by rubbing or injecting healthy plants with sap from an infected plant has been tried but has produced negative results (Dhitikiattipong et al 1999, Vinh et al 1999). Thin sections of red stripe lesion tissues were examined under the transmission electron microscope but no virus-like particles were seen (H. Kaku as cited by Vinh et al 1999). Stunting or any abnormality in plant growth has never been observed even on plants with severe red stripe intensity. No evidence to date links insect vectors to the transmission of the causal agent of red stripe. In an experiment, Vinh et al (unpublished data) allowed the brown planthopper *Nilaparvata lugens* to feed on plants with red stripe lesions and placed the insects in cages with healthy plants. The severity of



**Fig. 3.** Effect of pesticides on the incidence of red stripe at different days after exposure of healthy plants to diseased plants during two trials (A and B). Each bar represents the mean of four replications. In a given day after exposure, columns with the same letter are not significantly different at  $P \leq 0.05$  using the least significant difference test. Analyses were made on arcsine-transformed data. An experimental unit consisted of a diseased plant surrounded by four healthy plants.

**Rate of increase in number of red stripe lesions and area under the number of lesions progress curve (AUNLPC) on plants sprayed with benomyl and water. <sup>a</sup>**

| Treatment       | Rate      | AUNLPC (number-days) <sup>b</sup> |
|-----------------|-----------|-----------------------------------|
| Water (control) | 0.9804 a  | 398 a                             |
| Benomyl         | -0.0031 b | 59                                |

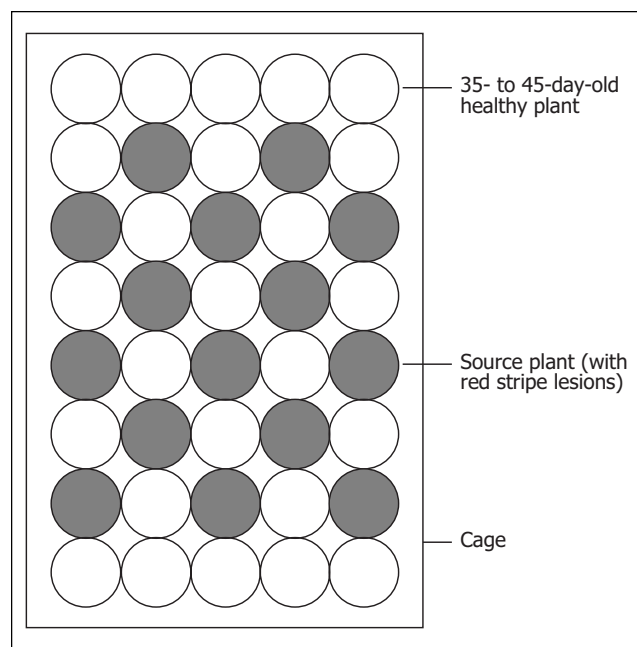
<sup>a</sup>Mean of seven replications. Computations were based on data collected from 0 to 28 d after treatment application. In a column, means followed by different letters are significantly different at  $P = 0.001$  using the least significant difference test. <sup>b</sup>Analysis was based on log-transformed data.



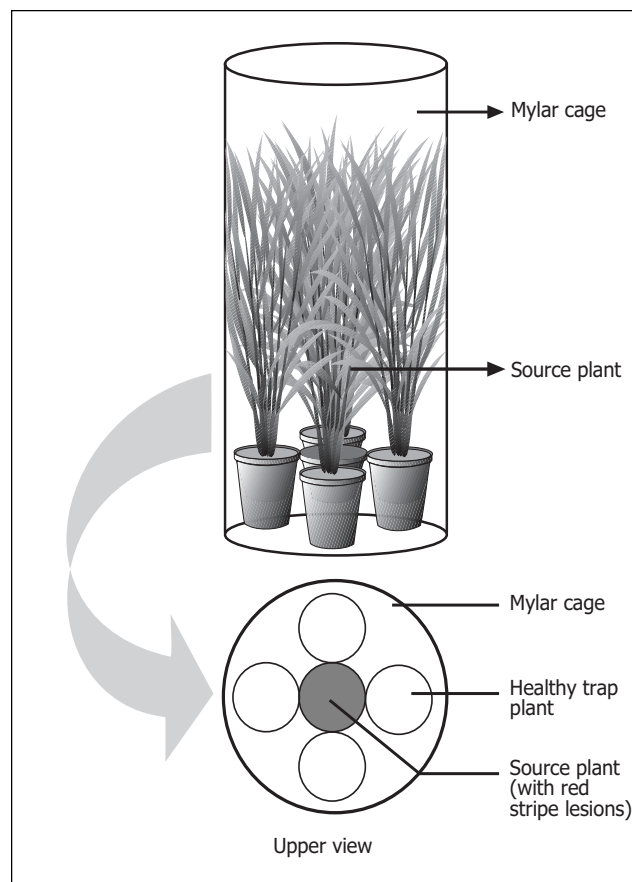
red stripe did not significantly differ between cages with and without the brown planthopper. The involvement of other vectors in the transmission of the causal agent needs to be investigated. More detailed electron microscopy may reveal particles similar to viruses, viroids, or mollicutes. However, even if particles similar to these groups of pathogens are found, it will be necessary to prove that these particles are in fact causing red stripe.

### Is red stripe associated with the production of toxins?

No studies have been conducted to test the hypothesis that a toxin is involved in red stripe development. The stripe emanating from the base of a red stripe lesion on the leaves may be due to a toxin produced by the causal organism. Since the stripe always advances toward the tips of leaves, the toxin is probably water-soluble and mobile within the xylem, and probably has a low molecular weight. To confirm whether the causal organism produces a toxin, filtrates from cultures of the causal agent or lesions should produce the stripe of a red stripe lesion on rice leaves. Future studies should also determine whether the causal organism can be isolated from the base of the lesion or from the stripe that extends toward the tips of leaves. If the causal organism can be isolated from the base of the lesion and not from the stripe, it can be assumed that the pathogen produces the toxin.



**Fig. 4. Arrangement of infected and healthy plants inside a cage for multiplying diseased plants in a screenhouse. Red stripe lesions are observed on healthy plants starting from 2 to 3 wk after exposure to diseased plants. The cage is made of a wooden frame wrapped with polyethylene plastic sheet. The top of the cage has no cover to facilitate spraying of water.**



**Fig. 5. Layout of an experimental unit for studying the effect of plant spacing and chemicals on the intensity of red stripe.**

### What is the mode of transmission of the red stripe causal agent?

At IRRI, diseased plants were multiplied by placing 35- to 45-d-old healthy plants between infected plants (Fig. 4). The basic unit of the experiments, which were conducted at IRRI to determine factors affecting red stripe intensity, consisted of healthy plants placed around a diseased plant (Fig. 5). These plants were enclosed in cages and sprayed with water. After 2 to 3 wk of exposure, red stripe lesions were usually observed on healthy plants surrounding diseased plants. Our experiment showed that plants placed 10 and 15 cm away from plants with red stripe lesions have significantly more diseased tillers than those placed 20 and 25 cm away (Fig. 2). These observations and experiments suggest that tissue contacts facilitate the transmission of the causal agent. We also observed red stripe lesions on plants not in contact with diseased plants, although the level of red stripe incidence was lower than that on plants in contact with diseased plants (Fig. 4). Although these observations have raised several questions on the transmission of the causal agent, they suggest that transmission is possible even without tissue contacts.

Bacterial and fungal pathogens are usually disseminated by tissue contacts, wind, or water. Viruses and viroids are

transmitted by mechanical means through infected sap, insect vectors, or vegetative-propagating materials. Mollicutes are generally transmitted by insect vectors. However, there is no evidence that the vectors involved in the transmission of viruses, such as aphids, mites, planthoppers, and leafhoppers, or even parasitic fungi, are involved in the spread of red stripe. Although planthoppers and leafhoppers commonly occur in rice fields, no reports indicate that rice fields infected with red stripe have more planthoppers and leafhoppers than those without red stripe.

We investigated whether the causal agent of red stripe can be transmitted through the soil or seeds (Tisalona and Mew, unpublished data). Seeds of plants with red stripe lesions were collected in fields and then grown in pots containing soil that was collected from fields with severe red stripe intensity. The untreated check consisted of soil that was sterilized at about 150 °C for 4 h. We did not observe red stripe lesions on plants that grew on any of the treatments, which led to the suggestion that red stripe may not be associated with a soilborne or seedborne organism. Results also provided some evidence that abiotic factors associated with the soil may not be directly involved in red stripe disease. Our experiments at IRRI, as well as the information obtained from various workers, so far suggest that the causal organism of red stripe is transmitted from diseased to healthy plants. Further tests involving plants grown in soil collected from fields with severe red stripe intensity would provide evidence as to whether a soilborne organism is associated with the spread of the disease.

### What are the next steps?

From all available information, except for the claims of Kaku et al (2000), the requirements of Koch's postulates have not been fulfilled for red stripe. Considering the difficulty in isolating the pathogen in pure culture, it is possible that compliance with Koch's postulates may not be achieved using conventional procedures. The causal agent of red stripe may be a fastidious organism requiring highly specific nutrients, an obligate pathogen, or some other so-called viable but nonculturable organism. It would be difficult to reintroduce such a pathogen as a pure culture into the plant and comply with Koch's postulates.

The tools of molecular plant pathology offer new approaches to identify the causal agent of red stripe. Among the options is the use of nucleic acid-based techniques, such as DNA hybridization and polymerase chain reaction (PCR), which have been useful in detecting and identifying several pathogens that are difficult or impossible to isolate. At IRRI, we are currently using arbitrary PCR primers that, among the multiple amplicons generated, consistently amplify a DNA fragment unique to tissues with red stripe lesions (and absent from healthy tissues). To prove that the unique fragment amplified from diseased tissues is indeed originating from the causal agent, experiments are needed to confirm whether the fragment hybridizes only to extracted DNA from diseased leaves and not to any arbitrary DNA sequence

present in rice leaves. Once sequence homology to DNA from diseased samples has been confirmed, the fragment will be used as a DNA probe to screen the collection maintained at the Entomology and Plant Pathology Division (IRRI) of characterized rice-associated bacteria (Cottyn et al 2001) and fungi to detect similarity to any of the described taxa. Further efforts will be conducted to isolate genomic DNA of the causal agent out of the DNA pool of diseased tissue by using the specific DNA fragment as bait (Henson and French 1993). If this is successful, the DNA of the causal agent can serve as a template to amplify the 16S rDNA gene that will provide phylogenetic information about the causal agent.

A complementary approach is a more detailed investigation of the effect of fungicides, bactericides, and antibiotics on red stripe development. Comparing the efficacy of benzimidazole fungicides with other groups of fungicides, bactericides, and antibiotics can help determine the causal organism of red stripe. Phytoplasmas and spiroplasmas are inhibited by tetracyclines but are resistant to penicillin, whereas fastidious vascular bacteria are sensitive to both tetracyclines and penicillin. Determining the effects of these antibiotics on red stripe development will help establish whether red stripe is caused by mollicutes or fastidious vascular bacteria. A thorough examination of cell sections from lesions under the transmission electron microscope would also be useful. The appearance of particles or masses under the electron microscope can give clues about the identity of the causal agent. It can also determine the occurrence of the causal agent inside the vascular system.

Further improvement of techniques for isolation, culture, and inoculation of the causal agent is necessary. The use of artificial media for fastidious and slow-growing organisms should be continuously explored. A primary consideration is the fact that several species of fungal endophytes are found on apparently healthy rice leaves (Fisher and Petrini 1992, Gonzales et al 2000). These endophytes can colonize internal plant tissues without causing apparent harm to their host and can thus live symptomlessly in the host for some time (Fisher and Petrini 1992). Similarly, several nonpathogenic bacterial species colonize rice plants as epiphytes. Thus, when applicable, isolates or tissues from diseased leaves should always be compared with those obtained from healthy plants of the same variety and age. These healthy plants should be collected from areas where red stripe does not occur because plants collected from areas favorable to red stripe development may appear healthy but may be latently infected by the pathogen. Epiphytes that are collected from leaves with red stripe lesions but not from healthy leaves may provide a clue about the biotic agent of red stripe. It should also be considered that the occurrence of microbial organisms might vary with the stage of lesion development.

Because of difficulties in isolating the causal agent of red stripe, it is possible that the causal agent is very slow-growing and obscured in culture by fast-growing saprophytic isolates. Iso-

lating suspected microorganisms from initial lesions should be considered because initial lesions may have fewer saprophytes and the pathogen may therefore grow better in vitro.

Since the stripe extending from the base of a red stripe lesion resembles the effect of a toxin, the compound that causes this stripe should be extracted, purified, and tested for the induction of red stripe lesions. Should a positive result be obtained, the purified chemical substance should be compared with known toxins of plant pathogens to help identify organisms producing it. Since toxins are mainly produced by either bacterial or fungal pathogens, a better understanding of the toxin may suggest the identity of the pathogen.

Finally, closer interaction among scientists working on red stripe is needed to speed up the research toward understanding the etiology of red stripe. Improved interaction would facilitate sharing of methodologies and approaches, such as formulation of nutrient media, methods for pathogenicity tests, multiplication of inoculum under controlled conditions, and assessment of disease intensity. It would also allow standardization of methods and better evaluation and comparison of results. A workshop held in March 1999, organized by IRRI and the Institute of Agricultural Sciences in South Vietnam, attempted to achieve these objectives. The interaction among scientists who participated in this workshop has resulted in the identification of new approaches and facilitated the exchange of ideas on the etiology of red stripe.

## References

- Barroga JF, Mew TW. 1994. "Red stripe": a new disease of rice in the Philippines. Paper presented at the 25th Anniversary and Annual Scientific Convention of the Pest Management Council of the Philippines, 3-6 May 1994, Cagayan de Oro City, Philippines.
- Cottyn B, Regalado E, Lanoot B, De Cleen M, Mew TW, Swings J. 2001. Bacterial populations associated with rice seed in the tropical environment. *Phytopathology* 91:282-292.
- Hitikiatpong R, Nilpanit N, Surin A, Arunyanart P, Chettanachit D. 1999. Study on rice red stripe in Thailand. Paper presented at the Planning Workshop on Red Stripe, 15-18 March 1999, Ho Chi Minh City, Vietnam.
- Du PV, Lan NTP, Dinh HD. 1991a. Red stripe, a newly discovered disease of rice in Vietnam. *Int. Rice Res. Newsl.* 16(3):25.
- Du PV, Dinh HD, Lan NTP, Sau TT, Ba DX. 1991b. Field evaluation to control 'red stripe', a new rice disease in Vietnam. *Int. Rice Res. Newsl.* 16(5):21-22.
- Fisher PJ, Petrini O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytol.* 120:137-143.
- Fletcher J, Wayadande A, Melcher U, Ye FC. 1998. The phytopathogenic mollicute-insect vector interface: a closer look. *Phytopathology* 88:1351-1358.
- Gonzales AT, Dalisay TU, Telan IF. 2000. Endophytic fungi in rice leaves. Poster presented at the 31st Anniversary and Annual Scientific Convention of the Pest Management Council of the Philippines, 3-6 May 2000, Baguio City, Philippines.
- Henson JM, French R. 1993. The polymerase chain reaction and plant disease diagnosis. *Annu. Rev. Phytopathol.* 31:81-109.
- Kaku H, Subandiyah S, Ochiai H. 2000. Red stripe of rice is caused by a bacterium *Microbacterium* sp. J. *Gen. Plant Pathol.* 66:149-152.
- Keinath AP, Zitter TA. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. *Plant Dis.* 82:479-484.
- Mogi S, Sugandhi Z, Baskoro S. 1988. A new discovered disease (bacterial red stripe) on rice at Indonesia, its symptom and distribution. Abstracts. Fifth International Congress of Plant Pathology, 20-27 August 1988, Kyoto, Japan. p 388.
- Saad A. 1999. The occurrence of red-stripe disease in Malaysia. Paper presented at the Planning Workshop on Red Stripe, 15-18 March 1999, Ho Chi Minh City, Vietnam.
- Sears BB, Kirkpatrick BC. 1994. Unveiling the evolutionary relationships of plant-pathogenic mycoplasma-like organisms. *Am. Soc. Microbiol.* 60:307-312.
- Shakya DD, Chung HS, Vinther F. 1986. Transmission of *Pseudomonas avenae*, the cause of bacterial stripe of rice. *J. Phytopathol.* 116:92-96.
- Shurtleff MC, Averre III CW. 1997. Glossary of plant-pathological terms. St. Paul, Minnesota: American Phytopathological Society. 361 p.
- Suparyono. 1999. An overview on the study of bacterial orange leaf blight of rice in Indonesia. Paper presented at the Planning Workshop on Red Stripe, 15-18 March 1999, Ho Chi Minh City, Vietnam.
- Tuat NV. 1999. A research status of red stripe and associated diseases in Vietnam. Paper presented at the Planning Workshop on Red Stripe, 15-18 March 1999, Ho Chi Minh City, Vietnam.
- Vinh MT. 1997. Etiological studies on the yellow leaf syndrome of rice (*Oryza sativa* L.). MS thesis, University of the Philippines Los Baños, College, Laguna. 77 p.
- Vinh MT, Mew TW, Bien PV. 1999. Etiological studies on the yellow leaf syndrome of rice (*Oryza sativa* L.). Paper presented at the International Workshop on Red Stripe, 15-18 March 1999, Ho Chi Minh City, Vietnam.
- Wakimoto S, Kim PV, Thuy TTT, Tsuno K, Kardin MK, Hartini RH, Surang S, Sunetra P, Nilpanit N, Negishi H, Suyama K. 1998. Micro-fungus closely associated with the lesions of red stripe disease of rice. Abstracts. Vol. 3. 5th International Congress of Plant Pathology, 9-16 August 1998, Edinburgh, Scotland. p 3.6.11.
- Webster RK, Gunnell PS. 1992. Compendium of rice diseases. St. Paul, Minnesota: American Phytopathological Society.
- Yazid ME, Saad A, Jatil AT. 1996. Historical profile and current rice disease management practices in Malaysia. Paper presented at the International Workshop on Rice Disease Management Technology in the Tropics, 11-13 June 1996, Sungai Petani, Kedah, Malaysia.

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## Sterility mechanism in different TGMS and CMS lines of rice

R. Kalaiyarasi and P. Vaidyanathan, Department of Rice, Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India E-mail: kalai\_r@usa.net

Thermosensitive genic male sterility (TGMS) is a new genetic tool used in developing commercial F<sub>1</sub> rice hybrids. This study investigated the stage at which sterility occurs during anther development in TGMS lines. For critical examination, panicles were collected from TGMS lines TS16, TS18, TS29, and cytoplasmic male sterile (CMS) lines IR58025A and IR62829A. In the TGMS lines, the developmental stages of young panicles were examined at stages IV, V, and VI in September 1998 and May 1999 for the expression of fertility and sterility, respectively. Anthers were taken out from the spikelet and fixed in 70% alcohol for 1 h. These were then passed through a series of solutions containing alcohol and methyl salicylate in the proportion of 4:1, 3:2, 2:3, and 1:4, and finally in methyl salicylate for 15 min in each solution with two changes in the last step. Cleared anthers were mounted in methyl salicylate in a cavity microslide and examined using a *Namarski differential interference contrast* in a bright field microscope for optimal viewing and microphotography.

Sterile anthers differed from fertile anthers in size, shape of pollen grain, and anther color in TGMS lines (see figure). In all TGMS lines, sterile anthers were small with empty pollen grains of irregular shape, except in TS16, which showed pollen-free anthers (see figure). This indicated that the sensitive stage of TS16 was at stage IV (stamen and pistil primordia) of panicle development.

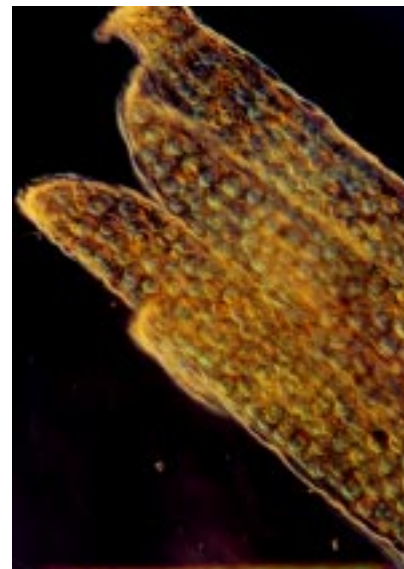
A distinct variation was observed between sterile and fertile expression in anther size, shape, and color besides content and quantity of pollen grains in TS18, TS29, and CMS lines. Pollen grains of sterile anthers were small and irregu-

larly shaped, whereas fertile pollen grains were plump, larger, and yellow. This indicated that the occurrence of sterility in TS18, TS29, and CMS lines is postmeiotic. Results strongly indicate that genes governing premeiotic sterility operate in TS16, leading to the pollen-free nature of the anther, while certain other genes operate

at postmeiotic stages, causing sporophytic sterility in TS18, TS29, and CMS lines. The genetic mechanisms and variations in the developmental pattern of anther and pollen production could be clearly understood by analyzing the expression of sterility/fertility in intermated lines and the segregating progenies of TGMS lines.



**Pollen-free anther in TS16 (May 1999). Sterile phase-sensitive stage—stage IV (stamen and pistil primordia of panicle development).**



**Fertile anthers in TS16 (September 1998). Fertile phase of TGMS line TS16.**



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# High-yielding and good-quality Tianjin 1244 japonica hybrid cultivar series

J. Niu, Y. Li, W. Zhang, Z. Niu, and M. Zhou, Tianjin Rice Research Institute (TRRI), 300112, Tianjin, China

Japonica rice hybrids have been studied and used for 40 years (Yang 1999). For a long time, researchers have always used yield improvement as a priority goal in developing japonica hybrids. Hybrid rice has nearly become synonymous with high yield, leaving quality behind. To breed a japonica hybrid cultivar with high yield and good quality, a japonica restorer line with good quality is needed. The restorer must be genetically different from the sterile line so that good quality and high heterosis can be combined. Tianjin 1244, bred by the TRRI in 1985, is a japonica restorer line with good quality (Niu et al 1988).

Tianjin 1244 was bred from the cross between female parent Hongqi 8 (with good quality and resistance to rice blast) and restorer line C57-10 (with poor quality but strong resistance to drought). The  $F_1$  obtained was crossed with japonica Chusheng, which has good quality and high yield. Tianjin 1244 was the result of several generations of selection. It has good quality, early maturity, flourishing growth, and loose panicles.

Results of a quality analysis done on Tianjin 1244 by the Crop Germplasm Institute (CGI) of the Chinese Academy of Agricultural Sciences (CAAS) showed that the appearance, milling quality, and protein content of Tianjin 1244 are better than those of checks Zhongdan 2 and

Zhonghua 9. Its cooking quality is similar to that of checks and its taste is intermediate. Tianjin 1244 met the Chinese Ministry of Agriculture (CMA) standard for quality (Table 1).

Tianjin 1244 has excellent restoring ability from wide crosses with 15 japonica cytoplasmic male sterile lines from different regions. The six test-cross  $F_1$ s gave five lines with yields higher than that of the check Liyou 57 (6.3–59.6% range).  $F_1$  seed setting ranged from 80.9% to 93.6% (Table 2). Jingyou 6 (Zhongzuo 59A/1244), bred by the Crop Research Institute of the Beijing Academy of Agriculture and Forest

Science, has good quality. The quality analyses made by CGI of CAAS and the China Rice Research Institute showed that mean values of all main characteristics (except for chalkiness, which is 15.3% higher) met the CMA standard. Moreover, Jingyou 6 has stable and high yield (yield higher than that of check Quiguang by 14.5–14.9%) and resistance to blast and green smut. It is being extensively released in Beijing, Tianjin, and Ningxia (Hong et al 1999).

An analysis of the appearance of 63 japonica hybrid combinations in 1999 showed that two parents with good quality

**Table 1. Results of rice quality analysis.**

| Variety      | Appearance       |                | Milling quality |                      |                  | Nutritional quality |            |               |                  |
|--------------|------------------|----------------|-----------------|----------------------|------------------|---------------------|------------|---------------|------------------|
|              | Translucency (%) | Chalkiness (%) | Brown rice (%)  | Partially milled (%) | Fully milled (%) | Protein (%)         | Lysine (%) | Gross fat (%) | Total starch (%) |
| Tianjin 1244 | 1                | 1              | 83.1            | 74.2                 | 73.5             | 9.0                 | 0.3        | 2.5           | 76.5             |
| Zhonghua 9   | 3                | 5              | 81.5            | 62.7                 | 54.6             | 7.9                 | 0.3        | 2.7           | 74.2             |
| Zhongdan 2   | 3                | 5              | 82.9            | 71.2                 | 68.0             | 8.4                 | 0.3        | 2.2           | 73.6             |

| Variety      | Cooking quality |                 |                            | Eating quality <sup>a</sup> |       |        |                    |
|--------------|-----------------|-----------------|----------------------------|-----------------------------|-------|--------|--------------------|
|              | Amylose (%)     | Gel consistency | Gelatinization temperature | Eating                      | Color | Flavor | General evaluation |
| Tianjin 1244 | 16.6            | Soft            | Low                        | 8.4                         | 8.7   | 8.3    | 34.0               |
| Zhonghua 9   | 18.2            | Soft            | Low                        | 8.8                         | 8.8   | 8.7    | 34.8               |
| Zhongdan 2   | 16.2            | Soft            | Low                        | 8.1                         | 8.3   | 8.4    | 33.1               |

<sup>a</sup>Maximum value is 10; the higher the rating, the better.

**Table 2.  $F_1$  generation obtained from cross combinations with Tianjin 1244.**

| Combination            | Plant height (cm) | Panicles plant <sup>-1</sup> (no.) | Panicle length (cm) | Grains panicle <sup>-1</sup> (no.) | Seed setting (%) | 1,000-grain weight (g) | Grain yield (t ha <sup>-1</sup> ) (%) | Increase over |             |
|------------------------|-------------------|------------------------------------|---------------------|------------------------------------|------------------|------------------------|---------------------------------------|---------------|-------------|
|                        |                   |                                    |                     |                                    |                  |                        |                                       | Check 1 (%)   | Check 2 (%) |
| Hong 12A/Tianjin 1244  | 106.5             | 15.6                               | 23.6                | 186.2                              | 84.2             | 26.9                   | 16.4                                  | +68.2         | +59.6       |
| Hong 21A/Tianjin 1244  | 108.1             | 14.6                               | 20.5                | 139.2                              | 88.7             | 25.9                   | 11.7                                  | +19.8         | +13.9       |
| Hong 26A/Tianjin 1244  | 99.0              | 12.2                               | 19.9                | 205.7                              | 85.7             | 23.9                   | 12.8                                  | +31.4         | +24.8       |
| Liao 5A/Tianjin 1244   | 95.5              | 16.0                               | 18.8                | 156.2                              | 80.9             | 26.2                   | 13.2                                  | +35.2         | +28.5       |
| Nong 6A/Tianjin 1244   | 98.0              | 12.0                               | 21.0                | 141.3                              | 93.6             | 27.6                   | 10.9                                  | +12.0         | +6.3        |
| 8436A/Tianjin 1244     | 101.0             | 11.4                               | 19.8                | 115.9                              | 90.3             | 27.0                   | —                                     | -17.7         | -21.8       |
| Tianjin 1244 (check 1) | 101.4             | 11.6                               | 22.4                | 135.4                              | 91.9             | 27.1                   | 7.9                                   |               |             |
| Liyou 57 (check 2)     | 95.2              | 14.1                               | 19.9                | 145.0                              | 87.7             | 23.0                   | 10.2                                  |               |             |

had an  $F_1$  with good quality. A japonica hybrid with good quality can be obtained by combining a sterile line with good quality and a restorer line with good quality. Combining different plant types and different panicle types will make the heterosis of  $F_1$  more powerful.

## References

- Hong LF, Liu BQ, Mao ZW, Li ZG. 1999. Breeding and using japonica hybrid rice Jingyou 6. Hybrid Japonica Rice Breeding Research in Northern China. Beijing (China): Chinese Agricultural Science-Technology Publisher. 6:63–65.
- Niu J, Guo ZJ, Zhang KM, Li YP. 1988. The breeding and studying of the multiple

- functional new variety of Keng Rice Tianjin 1244. Acta Agric. Boreali-Sin. 3(4):61–66.
- Yang Z. 1999. Retrospects and prospects on the development of japonica hybrid rice in northern China. Hybrid Japonica Rice Breeding Research in Northern China. Beijing (China): Chinese Agricultural Science-Technology Publisher. 6:3–11.

## Yield potential of Brazilian upland rice

F. Breseghello and E. Perpétuo Guimarães, Embrapa Arroz e Feijão, Caixa Postal 179, 75375000 Santo Antonio de Goiás, Goiás, Brazil E-mail: eguimara@cnpaf.embrapa.br

Brazil is the only rice-growing country where upland and irrigated lowland rice are equally important. Upland rice area in the 1999-2000 crop season was 2.35 million ha, while lowland rice area was 1.21 million ha. Production from these two ecosystems totaled 4.44 and 6.34 million t, respectively, indicating that average rice yield under irrigated conditions is 177% higher (IBGE 2001).

Nevertheless, EMBRAPA's breeding program has emphasized exploitation of yield potential in upland rice germplasm, as well as improving grain quality and increasing blast resistance. Lately, this effort has paid off. Our objective is to show that the yield potential of upland rice breeding lines is much higher than the national average and higher than that of the most common upland varieties.

Breeding lines generated by the different breeding programs in Brazil are included in a set of common trials (observation, preliminary, and advanced yield) that are evaluated in the Brazilian Network for Rice Germplasm Evaluation (BNRGE). Trials are conducted in the most important upland rice-growing areas in the country. Each trial includes around 16 entries plus 4 checks.

Table 1 compares the mean yields of two elite lines and two widely planted varieties in the 1999-2000 cropping seasons. Under a wide range of environments, the new lines outyielded

the checks by 8–10%. Evaluation trials were conducted under the best soil and weather conditions. The elite breeding lines proved to be even better than the commercial varieties, resulting in 13–16% more yield under the same environment (Table 2). These results reveal not only the responsiveness of elite lines but also their ability to withstand adverse conditions.

The best breeding lines, under good experimental conditions, achieve

yields almost three times higher than the national average ( $1.9 \text{ t ha}^{-1}$ ). The release of new varieties, combined with better agricultural technologies that are already available, will increase the average yield of upland rice in Brazil in the short term.

## Reference

- Instituto Brasileiro de Geografia e Estatística (IBGE). 2000. Levantamento Sistemático da Produção Agrícola. 1980–1999. Rio de Janeiro (Brasil): IBGE. 10 vol.

**Table 1. Average yield ( $\text{t ha}^{-1}$ ) of elite breeding lines (EL) and check varieties (CV) of upland rice evaluated in BNRGE, 1999-2000.**

| Genotype     | Type | 1999 | 2000 | Av  | % increase over check |
|--------------|------|------|------|-----|-----------------------|
| CNA8557      | EL   | 3.6  | 3.9  | 3.8 | 110                   |
| CNA8540      | EL   | 3.7  | 3.8  | 3.8 | 110                   |
| Primavera    | CV   | 3.4  | 3.6  | 3.5 | 102                   |
| Caiapó       | CV   | 3.4  | 3.4  | 3.4 | 100                   |
| Trial av     |      | 3.4  | 3.5  | 3.5 |                       |
| Trials (no.) |      | 51   | 66   | 117 |                       |

**Table 2. Average yield ( $\text{t ha}^{-1}$ ) of elite breeding lines (EL) and check varieties (CV) of upland rice evaluated in high-yield environments, BNRGE, 1999-2000.**

| Genotype     | Type | 1999 | 2000 | Av  | % increase over check |
|--------------|------|------|------|-----|-----------------------|
| CNA8557      | EL   | 5.4  | 5.3  | 5.3 | 116                   |
| CNA8540      | EL   | 4.9  | 5.5  | 5.2 | 114                   |
| Primavera    | CV   | 4.4  | 4.8  | 4.6 | 101                   |
| Caiapó       | CV   | 4.5  | 4.7  | 4.6 | 100                   |
| Trial av     |      | 4.6  | 4.9  | 4.8 |                       |
| Trials (no.) |      | 20   | 22   | 42  |                       |

# ADTRH1, a short-duration promising rice hybrid

A.P.M. Kirubakaran Soundararaj, Hybrid Evaluation Center, Gudalore; R. Vaithilingam, Tamil Nadu Rice Research Institute (TRRI), Aduthurai; S. Giridharan, Sugarcane Research Station, Cuddalore; W. Wilfred Manuel, T.B. Ranganathan, M. Subramanian, Tamil Nadu Agricultural University (TNAU), Coimbatore; P. Babumathi, Agricultural College and Research Institute, Madurai; G. Manimegalai, Department of Food Science and Nutrition, Home Science College and Research Institute, Madurai; and A. Abdul Kareem, TNAU, India

Heterosis breeding in rice is a new and practical approach that aims to break the yield plateau. TRRI Aduthurai started research on heterosis in 1986 and released its first rice hybrid, ADTRH1, in January 1998 for general cultivation.

ADTRH1 is the heterotic combination of a three-line breeding system involving cytoplasmic genic male sterile, maintainer, and restorer lines—A, B, and R. IR58025A and IR66R are the parents of rice hybrid ADTRH1. This hybrid is semidwarf (105 cm) and matures in 115 d. It tillers profusely (12–15 productive tillers hill<sup>-1</sup>) under 20 × 10-cm spacing, with each panicle 27.5 cm long, producing 142 grains, and with more than 90% spikelet fertility. Its 1,000-grain weight is 23.8 g.

In different trials, ADTRH1 showed 26.9% and 24.5% higher yield over CORH1 and ASD18, respectively, with an average yield of 6.6 t ha<sup>-1</sup> (Table 1). The hybrid recorded the highest grain yield of 11.4 t ha<sup>-1</sup> in 1 out of 77 adaptive research trials in farmers' fields in Tamil Nadu. The highest yield of the check in these trials was 9.6 t ha<sup>-1</sup>.

ADTRH1 possesses long slender white kernels with a mild aroma when cooked. Physical, cooking, chemical, and organoleptic traits are all good (Table 2). Its grains contain 25.2% amylose and 6.9% protein. It is moderately resistant to stem borer and leafhopper under field conditions and moderately susceptible to blast and green leafhopper under glasshouse screening (Table 3). It is recommended for cultivation in irrigated transplanted areas during both the wet (April–September) and dry seasons in Tamil Nadu.

**Table 1. Performance of ADTRH1 compared with CORH1 and ASD18.**

| Variety/hybrid | Grain yield (t ha <sup>-1</sup> ) in |                                            |                                             |                                              | Mean (t ha <sup>-1</sup> ) |
|----------------|--------------------------------------|--------------------------------------------|---------------------------------------------|----------------------------------------------|----------------------------|
|                | Station trials, <sup>a</sup> 1994-97 | Multilocation trials, <sup>b</sup> 1996-98 | Adaptive research <sup>c</sup> trials, 1997 | Large-scale demonstration, <sup>d</sup> 1997 |                            |
| ADTRH1         | 6.6 a                                | 6.2 a                                      | 6.5 a                                       | 7.1 a                                        | 6.6                        |
| CORH1          | 5.1 c                                | 4.9 c                                      | 5.7 b                                       | 4.9 b                                        | 5.2                        |
| ASD18          | 5.3 b                                | 5.4 b                                      | 5.7 b                                       | 4.8 b                                        | 5.3                        |

<sup>a</sup>Mean of 4 trials. <sup>b</sup>Mean of 19 trials. <sup>c</sup>Mean of 77 trials. <sup>d</sup>Mean of 6 trials. Means followed by similar letters do not differ significantly from each other by Duncan's multiple range test.

**Table 2. Quality characteristics of hybrid ADTRH1.**

| Character                                         | ADTRH1 | IR58025A | IR58025B | IR66R | CORH1 | ASD18 |
|---------------------------------------------------|--------|----------|----------|-------|-------|-------|
| <i>Physical characteristics (% in rough rice)</i> |        |          |          |       |       |       |
| Hulling                                           | 79.8   | 78.8     | 76.0     | 82.0  | 78.0  | 75.2  |
| Milling                                           | 77.1   | 74.1     | 68.4     | 78.2  | 65.5  | 69.3  |
| Head rice recovery                                | 69.9   | 66.0     | 58.2     | 71.6  | 63.5  | 59.1  |
| <i>Cooking quality characteristics</i>            |        |          |          |       |       |       |
| Weight increase (g)                               | 26.5   | 26.0     | 26.2     | 26.0  | 24.7  | 25.5  |
| Volume increase (mL)                              | 30.2   | 28.0     | 29.0     | 30.0  | 24.8  | 28.0  |
| Water absorption (mL)                             | 28.5   | 28.5     | 28.0     | 28.0  | 24.0  | 28.0  |
| Cooking time (min)                                | 24.0   | 25.0     | 24.0     | 24.0  | 22.0  | 25.0  |
| PS5 BX                                            | 2.3    | 2.5      | 2.8      | 2.5   | —     | 2.9   |
| <i>Organoleptic evaluation (1–9 scale)</i>        |        |          |          |       |       |       |
| Color and appearance                              | 3.9    | 3.8      | 3.7      | 4.0   | 3.0   | 3.7   |
| Flavor                                            | 3.9    | 3.8      | 3.7      | 4.0   | 3.5   | 3.8   |
| Texture                                           | 3.9    | 3.8      | 3.7      | 3.9   | 3.7   | 3.7   |
| Paste                                             | 4.0    | 4.0      | 3.9      | 4.0   | 3.5   | 3.8   |
| Overall acceptability                             | 4.0    | 4.0      | 3.9      | 4.0   | 3.7   | 3.8   |

Note: Scale of 1–9, with 1 = good and 9 = bad.

**Table 3. Reaction of ADTRH1 and other varieties/hybrids against pests and diseases.**

| Pest                | Center <sup>a</sup> | Year    | Score (1–9 scale) |                 |       |     |
|---------------------|---------------------|---------|-------------------|-----------------|-------|-----|
|                     |                     |         | ADTRH1            | CORH1           | ASD18 | TNI |
| <i>Insects</i>      |                     |         |                   |                 |       |     |
| Stem borer          | Aduthurai (F)       | 1995-96 | 3                 | 3               | 3     | 7   |
|                     | Aduthurai (F)       | 1996-97 | 3                 | 1               | 3     | 7   |
| Leaffolder          | Aduthurai (F)       | 1997-98 | 3                 | 1               | 3     | 3   |
| Green<br>leafhopper | Coimbatore (A)      | 1995-96 | 5                 | 5               | 9     | 9   |
| <i>Disease</i>      |                     |         |                   |                 |       |     |
| Blast               | Aduthurai (A)       | 1995-96 | 5                 | 7               | 5     | –   |
|                     | Aduthurai isolate   |         |                   |                 |       |     |
|                     | Coimbatore (A)      | 1996-97 | 5                 | NT <sup>b</sup> | 0     | –   |
|                     | Coimbatore isolate  |         |                   |                 |       |     |

<sup>a</sup>F = field condition, A = artificial condition. <sup>b</sup>NT = not tested. Note: Scale of 1–9, with 1 = resistant and 9 = susceptible.



# Inheritance study of nodal rooting in deepwater rice

P.M. Mohapatra, A.R. Panda, and S.N. Ratho, Division of Genetics and Plant Breeding, Central Rice Research Institute (CRRI), Cuttack 753006, Orissa, India

Deepwater rice varieties are characterized by their ability to elongate under excess water. Besides elongation ability, these varieties possess nodal roots, nodal tillers, and kneeing ability, among others. Nodal roots are needed by deepwater rice because, under conditions of excess water, basal roots rot and plants float on the water surface. Nodal roots keep the plants buoyant and facilitate the uptake of water-soluble nutrients (Khan et al 1982, Boonwite 1979). Boonwite (1979) found an insignificant but positive correlation between elongation ability and nodal rooting. Tripathy and Balakrishna Rao (1985) also studied the inheritance of characters including nodal rooting that are associated with the floating habit.

We studied the inheritance of nodal rooting in eight crosses of rice in the 1995 wet season (WS) at CRRI, Cuttack. Two deepwater rice varieties, Jalamagna and TCA4 (tall, elongating types), capable of producing nodal roots under deepwater conditions, were crossed with three tall, nonelongating varieties, Khao Y Khao, Mtu-6, and Kwan-Fu-1, during the 1994 WS.

Seeds of the parents, their  $F_1$ , and  $F_2$  were sown in big galvanized iron trays (60 × 60 cm). Forty-two-day-old seedlings were placed in 60-cm-deep water in a cemented deepwater tank. After 30 d, the water was drained and plants were observed for nodal rooting. Ten plants of each parent and  $F_1$  and about 200–300 plants of each  $F_2$  were observed for nodal rooting.

All plants of parents Jalamagna and TCA4 had nodal roots, whereas those of Khao Y Khao, Kwan-Fu-1, and Mtu-6 had none (see table). All  $F_1$  plants of crosses involving Jalamagna or TCA4 had nodal roots, indicating dominance of the trait. The  $F_2$  plants of these crosses segregated into nine with nodal rooting to seven without nodal rooting, implying that two dominant complementary genes control nodal rooting. The  $F_1$  and  $F_2$  plants of the crosses between TCA4 and Jalamagna (deepwater varieties) had nodal rooting, whereas those between Khao Y Khao and Mtu-6 (nondeepwater varieties) did not.

The data show the presence of two dominant complementary genes for nodal rooting in rice. Nodal rooting occurs when

both genes are present. The absence of either or both genes results in plants with no nodal roots as in nonelongating rice varieties.

## References

- Boonwite C. 1979. Two methods of scoring nodal rooting ability. In: Proceedings of the 1978 International Deepwater Rice Workshop. Manila (Philippines): International Rice Research Institute. p 145–149.
- Khan MR, Ventura W, Vergara BS. 1982. Uptake through aquatic roots and distribution of  $^{15}\text{N}$ -tagged ammonium in deepwater rice. In: Proceedings of the 1981 International Deepwater Rice Workshop. Manila (Philippines): International Rice Research Institute. p 321–326.
- Tripathy RS, Balakrishna Rao MJ. 1985. Inheritance studies of characters associated with floating habit and their linkage relationship in rice. *Euphytica* 34:875–881.

**Nodal rooting behavior of  $F_1$  and  $F_2$ , 1995 wet season, CRRI, Cuttack.**

| Cross                              | F <sub>1</sub>      |                    |                       | F <sub>2</sub>      |                    |                       | $\chi^2$ (9:7) | P value   |
|------------------------------------|---------------------|--------------------|-----------------------|---------------------|--------------------|-----------------------|----------------|-----------|
|                                    | Plants tested (no.) | Observed frequency |                       | Plants tested (no.) | Observed frequency |                       |                |           |
|                                    |                     | With nodal rooting | Without nodal rooting |                     | With nodal rooting | Without nodal rooting |                |           |
| Khao Y Khao/Jalamagna <sup>a</sup> | 10                  | 10                 | 0                     | 290                 | 150                | 140                   | 2.414 ns       | 0.25–0.10 |
| Khao Y Khao/TCA4 <sup>a</sup>      | 10                  | 10                 | 0                     | 268                 | 146                | 122                   | 0.342 ns       | 0.75–0.50 |
| Kwan-Fu-1/Jalamagna                | 10                  | 10                 | 0                     | 300                 | 175                | 125                   | 0.529 ns       | 0.50–0.25 |
| Kwan-Fu-1/TCA4                     | 10                  | 10                 | 0                     | 300                 | 185                | 115                   | 3.576 ns       | 0.10–0.05 |
| Mtu-6/Jalamagna                    | 10                  | 10                 | 0                     | 287                 | 152                | 135                   | 1.261 ns       | 0.50–0.25 |
| Mut-6/TCA4                         | 10                  | 10                 | 0                     | 185                 | 100                | 85                    | 0.362 ns       | 0.75–0.50 |
| TCA4/Jalamagna                     | 10                  | 10                 | 0                     | 202                 | 202                | 0                     | —              | —         |
| Khao Y Khao/Mtu-6                  | 10                  | 10                 | 10                    | 275                 | 0                  | 275                   | —              | —         |

<sup>a</sup>Both Jalamagna and TCA4 had nodal roots in all 10 plants. Nodal roots were absent in Khao Y Khao, Kwan-Fu-1, and Mtu-6. ns = not significant.

# Japanese cultivar cytoplasm affects seedling vigor and heterosis

J.C. de Leon, T. Abe, and T. Sasahara, Laboratory of Plant Breeding, Yamagata University, Tsuruoka 997, Japan  
E-mail (T. Sasahara): CYN00524@nifty.ne.jp

In higher plants, cytoplasmic genome variation has been associated with male sterility, miniature kernel mutation, chloroplast deficiency, and disease susceptibility and, in rice, with heterosis and combining ability performance for yield and other traits (Young and Virmani 1990). To date, similar studies in relation to early plant growth are scanty. Thus, we report on the phenotypic effect of japonica rice cytoplasm on seedling vigor and heterosis.

Full diallel crosses were developed from IR50 and three japonica cultivars, namely, Akihikari (AK), Koshihikari (KO), and Todorokiwase (TO). Pre- and postgermination characters related to seedling growth and vigor were observed

on a total of 20 materials per treatment divided into two replications of a completely randomized experiment. After 3 d of incubation at 30 °C, seedlings were placed into sectioned wooden frames with a nylon screen bottom support (1 plot was 5 × 5 cm) and grown in standard culture solution (Kasugai 1959) under glasshouse conditions to minimize environmental effects. Seedlings reared for 16 d and 23 d after sowing (DAS) were then evaluated for different traits. Cytoplasmic effect was assessed using linear correlation and the least significant difference (LSD) test (Gomez and Gomez 1984).

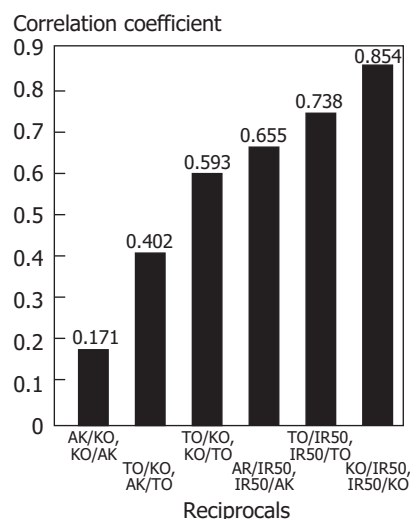
To verify the cytoplasmic effect on seedling phenotypes, correlation analysis using data on overall midparent heterosis

( $H_{mp}$ ) (de Leon et al 1999) was performed. The figure shows a substantial decline in linear association between the reciprocals of AK and KO, and between those of AK and TO. This result confirmed the cytoplasmic effect on seedling vigor traits and on the level of  $H_{mp}$  attained by certain reciprocal pairs. A detailed analysis of this cytoplasmic effect was performed using the LSD test. Results showed that negatively significant effects were associated with  $F_1$  hybrid seedlings with the Japanese cv. Akihikari as the female parent (see table). The consistently poorer performance of these  $F_1$  hybrids, that is, the negative performance persists regardless of the pollen parent used with AK, suggested cytoplasmic effects with

**Cytoplasmic effect on seedling vigor traits in rice.<sup>a</sup>**

| Trait <sup>b</sup> | Cross            |                |                     |                   |                     |                     | LSD<br>(0.05) | LSD<br>(0.01) |
|--------------------|------------------|----------------|---------------------|-------------------|---------------------|---------------------|---------------|---------------|
|                    | AK/TO–<br>TO/AK  | TO/KO<br>KO/TO | TO/IR50–<br>IR50/TO | AK/KO–<br>KO/AK   | AK/IR50–<br>IR50/AK | KO/IR50–<br>IR50/KO |               |               |
| Initial stage      |                  |                |                     |                   |                     |                     |               |               |
| SW                 | <b>-0.1501**</b> | 0.0263**       | 0.0152**            | 0.0114**          | -0.0027 ns          | 0.0519**            | 0.0105        | 0.0145        |
| GI                 | <b>-0.8255**</b> | 1.5230**       | -1.5110**           | <b>-1.1530*</b>   | <b>-0.8000**</b>    | -0.0675 ns          | 0.5368        | 0.7396        |
| SL                 | <b>-0.2975*</b>  | 0.3917**       | -0.0069 ns          | -0.0134**         | -0.1066 ns          | 0.0153 ns           | 0.2713        | 0.3737        |
| RL                 | -0.6990 ns       | 0.9115*        | 0.0190 ns           | <b>-1.4125 ns</b> | <b>-0.8875*</b>     | -0.0035 ns          | 0.7189        | 0.9906        |
| 2nd LEI            | <b>-1.8480**</b> | 0.6395**       | -0.0715 ns          | <b>-1.7090**</b>  | <b>-1.0635**</b>    | 0.0000 ns           | 0.3419        | 0.4712        |
| 16 DAS             |                  |                |                     |                   |                     |                     |               |               |
| LN                 | <b>-0.4125**</b> | 0.0125 ns      | 0.0415 ns           | -0.0540 ns        | -0.1540 ns          | 0.2125 ns           | 0.2786        | 0.3839        |
| SH                 | <b>-4.3500**</b> | 1.9120 ns      | 2.8120 ns           | <b>-4.5500**</b>  | <b>-4.3370**</b>    | 0.6120 ns           | 2.9215        | 4.0250        |
| RTL                | -1.7120 ns       | 1.3500 ns      | 1.6040 ns           | 0.6710 ns         | *1.8000 ns          | -1.1000 ns          | 2.3838        | 3.2847        |
| 2LA                | <b>-0.1510**</b> | 0.0015 ns      | -0.0480 ns          | -0.0035 ns        | 0.0890*             | 0.0385 ns           | 0.0876        | 0.1208        |
| 3LA                | <b>-0.6700**</b> | 0.2575 ns      | -0.1090 ns          | <b>-0.5050*</b>   | <b>-0.5750**</b>    | -0.2080 ns          | 0.3895        | 0.5368        |
| 4LA                | <b>-1.5031**</b> | 0.4485 ns      | 0.2075 ns           | <b>-0.6990*</b>   | <b>-0.8830**</b>    | -0.0435 ns          | 0.6327        | 0.8718        |
| FW                 | <b>-0.1490**</b> | 0.0365 ns      | 0.0640 ns           | <b>-0.0690*</b>   | -0.0405 ns          | 0.0275 ns           | 0.0685        | 0.0942        |
| SDW                | <b>-0.0095**</b> | 0.0035 ns      | 0.0045 ns           | <b>-0.0085**</b>  | <b>-0.0060*</b>     | 0.0010 ns           | 0.0055        | 0.0076        |
| RDW                | -0.0030 ns       | 0.0020 ns      | 0.0020 ns           | -0.0015 ns        | -0.0015 ns          | 0.0015 ns           | 0.0030        | 0.0041        |
| 23 DAS             |                  |                |                     |                   |                     |                     |               |               |
| LN                 | 0.0100 ns        | 0.2500 ns      | 0.0900 ns           | -0.1850 ns        | -0.1170 ns          | 0.2170 ns           | 0.4461        | 0.6147        |
| SH                 | <b>-8.9850**</b> | 1.7910 ns      | 1.4100 ns           | <b>-3.3450**</b>  | <b>-4.5500**</b>    | 0.9380 ns           | 2.3530        | 3.2422        |
| RTL                | <b>-2.6775*</b>  | 0.7575 ns      | 0.5900 ns           | 0.6550 ns         | <b>-2.2675*</b>     | 0.3490 ns           | 2.1421        | 2.9516        |
| 2LA                | -0.0650 ns       | 0.0525 ns      | 0.0310 ns           | 0.0270 ns         | -0.1190 ns          | 0.0575 ns           | 0.1396        | 0.1924        |
| 3LA                | <b>-0.5535*</b>  | 0.1525 ns      | 0.2275 ns           | -0.1575 ns        | <b>-0.7075**</b>    | -0.2125 ns          | 0.4492        | 0.6189        |
| 4LA                | <b>-1.0590**</b> | -0.0490 ns     | 0.5255 ns           | 0.1335 ns         | <b>-0.7805*</b>     | 0.0130 ns           | 0.5934        | 0.8177        |
| 5LA                | <b>-2.4075**</b> | 0.2160 ns      | 0.7240 ns           | -0.3920 ns        | <b>-1.7615**</b>    | -0.0065 ns          | 1.1678        | 1.6091        |
| FW                 | <b>-0.3075**</b> | 0.0950**       | 0.0375*             | <b>-0.0485**</b>  | <b>-0.0915**</b>    | 0.1035**            | 0.0345        | 0.0475        |
| SDW                | <b>-0.0245**</b> | 0.0106 ns      | 0.0095 ns           | -0.0022 ns        | <b>-0.0165**</b>    | 0.0075 ns           | 0.0119        | 0.0164        |
| RDW                | <b>-0.0061**</b> | 0.0029*        | 0.0020 ns           | 0.0002 ns         | <b>0.0027*</b>      | 0.0028*             | 0.0022        | 0.0031        |

<sup>a</sup>Negatively significant cytoplasmic effect in bold. \* = significant at 0.05% level, \*\* = significant at 0.01% level. <sup>b</sup>SW = initial seed weight; GI = germination index; SL = shoot length; RL = radicle length; 2nd LEI = second leaf emergence index; LN = leaf number; SH = seedling height; RTL = root length; 2LA, 3LA, 4LA, 5LA = second, third, fourth, fifth leaf areas, respectively; FW = fresh weight; SDW = shoot dry weight; RDW = root dry weight. GI and 2nd LEI based on transformed data (square root).



**Cytoplasmic or maternal effects on mid-parent heterosis performance of seedling vigor traits revealed by the correlation between reciprocal crosses.**

minimal interaction of the nuclear genome. Slightly similar effects were noted from KO/TO (see table). However, such effects were observed in only a few characters of this cross. In contrast, it can be shown that negatively significant effects of AK as a cytoplasmic parent accounted for about 76% of all such cases observed.

These results led us to consider the possibility of a common maternal ancestor shared by both AK and KO. Terry et al (2000) have elucidated such a possibility by a pedigree analysis of AK, which indicates the following simplified genetic lineage involving only female parents: Aikoku - Ginbozu - Norin 8 - Norin 22 - Hatsunishiki - Sasanishiki - Toyonishiki - AK. This suggests that either Aikoku or Ginbozu is the common ancestral parent of AK and KO. This possibility and its implications for the present findings are under investigation.

## References

- de Leon JC, Abe T, Sasahara T. 1999. Heterosis and combining ability analyses for seedling vigor and related traits in rice (*Oryza sativa* L.). *Breed. Res.* 1 (suppl. 2):265.
- Gomez KA, Gomez AA. 1984. Statistical procedures for agricultural research. 2nd ed. Los Baños (Philippines): International Rice Research Institute.
- Kumazawa K. 1995. Researches on hydroponics (water culture). In: Matsuo T, Kumazawa K, Ishii R, Ishihara K, Hirata H, editors. *Science of the rice plant. Vol. 2. Physiology.* Tokyo (Japan): Food and Agriculture Policy Center. p 274–276.
- Terry RG, Nowak RS, Tausch RJ. 2000. Genetic variation in chloroplast and nuclear ribosomal DNA in Utah juniper (*Juniperus osteosperma*, Cupressaceae). *Am. J. Bot.* 87(2):250–258.
- Young JB, Virmani SS. 1990. Effects of cytoplasm on heterosis and combining ability for agronomic traits in rice (*Oryza sativa* L.). *Euphytica* 48:177–188.

## Digital Literacy for Rice Scientists

To help rice scientists take advantage of new information and communication technologies, the IRRI Training Center has developed the *Digital Literacy Course for Rice Scientists*. The course aims to provide scientists with information about what resources are available on the Internet and how they can go about accessing these resources.

The course is unique in that it focuses on the needs of rice scientists, it provides a forum for rice scientists to share their experiences and Internet resources with other rice scientists online, and it establishes a learner-centered knowledge network in the form of an online community centered on rice research.

The topics covered by the course include

- What is the Internet
- What is the World Wide Web and what makes it work
- Key Internet terminology
- How to use the Internet for communication with other scientists
- How to use Web browsers
- How to search for information efficiently and effectively
- What are some of the good sources of information for rice scientists available on the Internet
- How to cite Internet documents
- What training opportunities are available online

Connection to the Internet offers national scientists a low-cost communication medium with other scientists linked to the Internet, gives them access to the ever-growing body of information available on and through interlinked computers throughout the world, and provides access to formal and informal training offered online from virtually anywhere.



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# Identification of varieties and testing of hybrid purity of rice by ultrathin-layer isoelectric focusing of seed protein

X.F.Wang, College of Biotechnology, South China Agriculture University, Guangzhou 510642, China;  
R. Knoblauch and N. Leist, LUFA Augustenberg, Postfach 430230, 76217 Karlsruhe, Germany  
E-mail: xfwang@scau.edu.cn

Variety characterization and purity are among the most important requirements of a rice seed lot for sale. Therefore, variety identification, especially through examination of seeds, becomes increasingly important in terms of seed quality control and protection of breeders' rights.

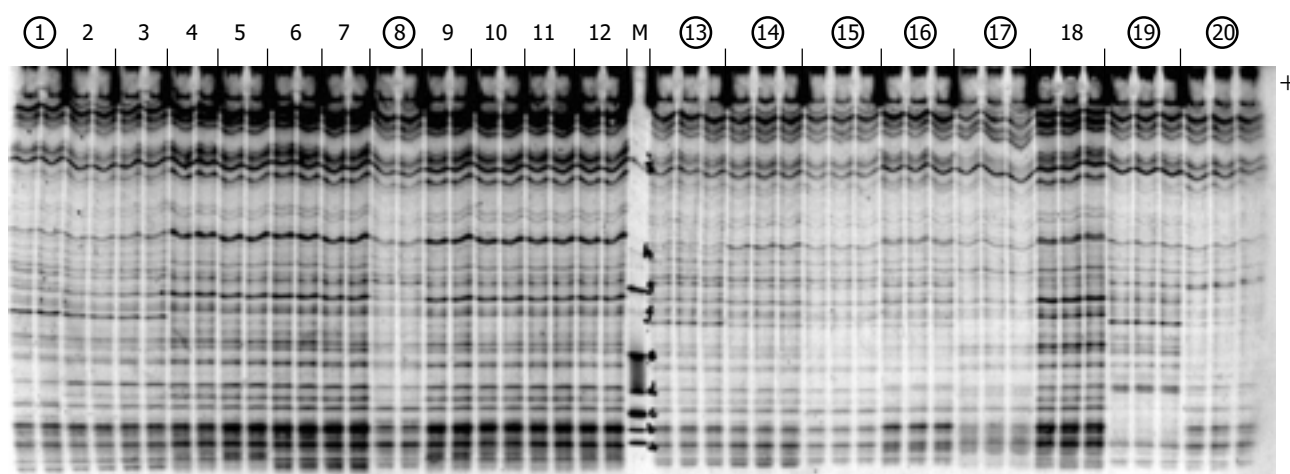
Ultrathin-layer isoelectric focusing (UTLIEF) of seed protein is considered a convenient, quick, cheap, and reliable laboratory method and has been included in the International Seed Testing Association rules for variety verification (ISTA 1999). The cost per kernel of maize purity testing is US\$0.14, \$0.49, and \$1.87 when analyzed by UTLIEF, simple-sequence repeat (SSR) DNA markers, and isozymes, respectively (LUFA Augustenberg, unpubl. data). So far, UTLIEF has not been used in variety testing

of rice. The objective of this study was to identify 20 rice varieties (Fig. 1) collected by the Seed Testing and Applied Botany, State Institute for Agricultural Testing and Research, Augustenberg, Germany, from different countries by UTLIEF.

UTLIEF gels were prepared on polyester support films as described by Radola (1980). The polymerization solution for 10 gels contained 16 g urea, 1.6 g taurine, 50 mL acrylamide ( $T = 6.8\%$ ,  $C = 2.5\%$ ), 4.4 mL ampholytes (Servalyt pH 2–9, 40  $\mu\text{L}$  N N N'N'-tetramethylethylenediamine, and 300  $\mu\text{L}$  of 20% (w/v) ammonium peroxydisulfate. One hundred twenty microliters of a  $\text{PO}_4$  buffer solution (1,000 mL of such solution contained 0.194 g  $\text{K}_2\text{HPO}_4$ , 0.528 g  $\text{KH}_2\text{PO}_4$ , 25.0 mL glycerine, 1.0 g dithioerythritol, and 0.38 g ethylenediaminetetracetic acid) was used to extract proteins from

individual seed crushed by Kataskapt III (LUFA Augustenberg). About 20  $\mu\text{L}$  of the supernatant was pipetted onto the 0.15-mm-thick gels and electrophoresis was carried out on Desaga horizontal electrophoresis units (Desaphor, Heidelberg) connected to a cooling apparatus (Haake R) at a temperature of 10 °C for 70 min. After focusing, gels were fixed in 12% (w/v) trichloroacetic acid and stained in Coomassie brilliant blue solution.

Based on the morphological seed characters of the 20 varieties, Egyptian Jasmin (basic seed) and Giza 181 (basic seed) from Egypt; Jinhuaazhan, Luhuangzhan, Yuexiangzhan, and Peiza 67 from China; and RD6 (glutinous) and KDML 105 (nonglutinous) from Thailand were classified as indica type with long seed. The remaining 12 varieties were



**Fig. 1.** Electrophoregram of seed proteins extracted by  $\text{PO}_4$  buffer from 20 rice varieties on the gel with Servalyt pH 2–9. M = standard protein markers (isoelectric focusing calibration kit, range pH 3–10, Pharmacia). The pIs of the marker protein bands, from bottom to top, are 8.65, 8.45, 8.15, 7.35, 6.55, 5.85, 5.20, 4.55, and 3.50. Numbers 1 to 12 are Egyptian Jasmin (basic seed), Giza 178 (basic seed), Giza 178 (Gharbia), Giza 171 (Gharbia), Giza 176 (basic seed), Giza 177 (basic seed), Giza 177 (Gharbia), Giza 181 (basic seed), Sakha 101 (basic seed), Sakha 101 (Gharbia), Sakha 102 (basic seed), and Sakha 102 (Gharbia) from Egypt; 13 to 16 are Jinhuaazhan, Luhuangzhan, Yuexiangzhan, and Peiza 67 from China; 17 and 18 are A and B varieties from the Philippines; 19 and 20 are RD6 (glutinous) and KDML 105 (nonglutinous) from Thailand. ○ = indica.



classified as japonica type with short seed. In the electrophoregram (Fig.1), 34–40 protein bands were identified for each variety. Among the many protein bands, 10 distinct bands were polymorphic among the 20 varieties tested, with pI (isoelectric point) values of 4.40, 5.06, 6.01, 6.38, 6.49, 6.75, 7.00, 7.24, 7.58, and 8.38. With these polymorphic protein bands and morphological characters of seeds, eight indica and three japonica [Giza 178 (basic seed), Giza 178 (Gharbia), and variety A from the Philippines] varieties could be distinguished from each other. The other nine japonica varieties could be in two subgroups; the electrophoregram was similar in each subgroup. Other protocols of the UTLEIF such as different protein extraction solutions, ampholytes of different pH ranges, different focusing distances between anode and cathode, and other staining methods (silver staining) could be used to further discriminate the varieties in the two japonica subgroups. This result shows that UTLEIF can be used to discriminate among rice varieties, even among genetically close varieties such as Giza 178 (basic seed), Giza 178 (Gharbia), and Giza 181 (basic seed).

A two-line breeding technique using photothermosensitive genic male sterile (PTGMS) indica rice (Peiai 64S) as the male sterile line and maintainer line is being developed in China and some other countries. However, the purity of the  $F_1$  seeds of two-line hybrid rice is usually low because the PTGMS line will self-pollinate when light and temperature conditions are not met. Therefore, testing the hybrid purity of  $F_1$  seeds of two-line hybrid rice is

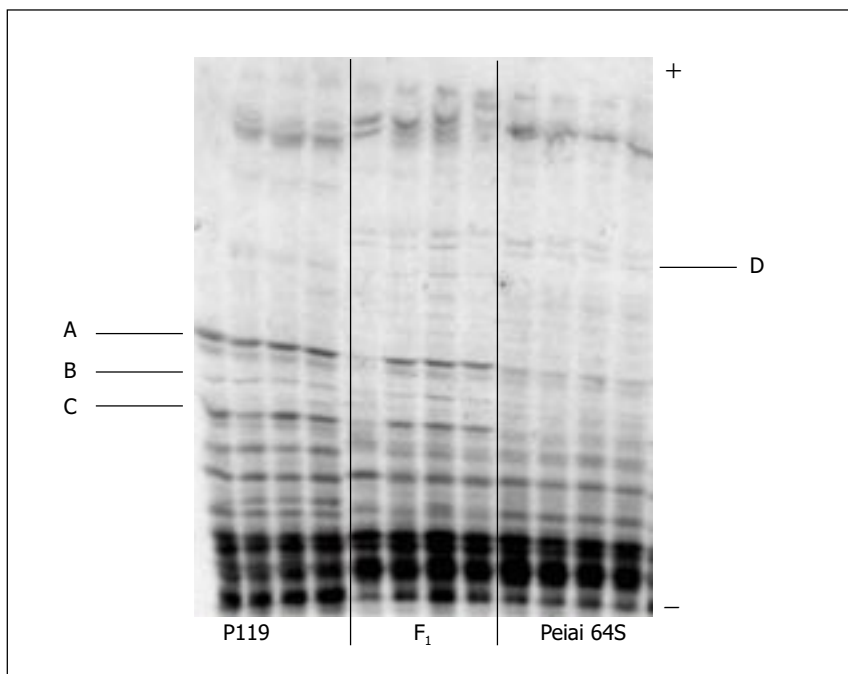
becoming increasingly important. Figure 2 shows the electrophoregram of seed proteins from a two-line hybrid combination: Peiai 64S/P119. One protein marker band (D) was found to exist in the PTGMS line Peiai 64S in the pH range used, whereas the restorer line P119 had three marker bands (A, B, and C). Therefore, the real hybrid  $F_1$  seeds of Peiai 64S/P119 should contain bands A, B, and C from the restorer line and band D from the male sterile line. Figure 2 shows that, of the four individual  $F_1$  seeds tested, one seed had only the marker band from Peiai 64S. This  $F_1$  seed was inbred. The other three  $F_1$

seeds had four marker bands (A, B, C, and D) from both P119 and Peiai 64S, and thus were considered real hybrids.

A field test is being carried out to confirm the UTLEIF results.

## References

- ISTA (International Seed Testing Association). 1999. International rules for seed testing. Annexes 1999. Seed Sci. Technol. 27 (supplement).
- Radola BJ. 1980. Ultrathin-layer isoelectric focusing in 50–100 mm polyacrylamide gels on silanized glass plate or polyester films. Electrophoresis 1:43–56.



**Fig. 2. Electrophoregram of proteins extracted by 30% 2-chloroethanol of parent and  $F_1$  seeds of two-line hybrid combination (Peiai 64S/P119) rice on the gel with Servalyt pH 4–9.**



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IRRISTAT is a computer program for data management and basic statistical analysis of experimental data. It can be run in any 32-bit Windows operating system.

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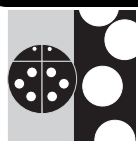
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# Basal application of fertilizer reduces golden apple snail population

M.S. de la Cruz, R.C. Joshi, and A.R. Martin, Crop Protection Division, Philippine Rice Research Institute (PhilRice), Maligaya, Muñoz, Nueva Ecija, 3119 Philippines E-mail: joshiravi@hotmail.com

Commercial molluscicides are widely used by rice farmers to control the golden apple snail (GAS) *Pomacea canaliculata* (Lamarck). Chemical control is an additional expense and a burden to rice farmers. In addition, misuse of these synthetic pesticides not only pollutes the environment but also poses hazards to applicators, farm workers, work animals, and other nontarget organisms such as fish, frogs, and beneficial arthropods.

In the experimental fields of PhilRice in Maligaya, Muñoz, Nueva Ecija, Philippines, we observed dead GAS after transplanting in fields with no pesticide. Farm interviews did not provide conclusive answers on whether farm implements (i.e., hand tractor) caused the death of GAS. Neighboring farmers claimed that, when applied before planting (soil incorporation), inorganic fertilizers reduced the GAS population and minimized damage, such as missing hills. We searched various literature databases and contacted snail researchers in the country and abroad but

did not find published or unpublished information on the effects of inorganic fertilizers on snails.

Thus, a series of trials on the effect of different inorganic fertilizers were conducted at PhilRice. A basal rate recommendation of complete fertilizer (14-14-14), urea (40-0-0), and complete fertilizer + urea was used in trial 1 during the 1999 wet season (WS). Handpicking, molluscicide, and no fertilizer served as the untreated control. In trials 2 and 3, during the 2000 dry season (DS) and WS, respectively, the recommended rate of ammonium phosphate (16-20-0) + urea was added as a treatment in place of handpicking. In trial 4, single-element fertilizers (urea, solophos, muriate of potash, and commercial organic fertilizer) were compared with molluscicide (niclosamide 250 EC) to pinpoint the fertilizer element that caused GAS mortality.

Each microplot measured 4 m<sup>2</sup>, replicated four times. Treatments were

arranged in a randomized complete block design in all trials. IR64 seedlings (18-d-old) were transplanted at one seedling hill<sup>-1</sup>, spaced 20 × 20 cm. Levees of 30 × 30 cm were constructed to separate microplots and to avoid GAS transfer and fertilizer seepage. Fertilizer treatments were applied before final leveling and transplanting was done immediately after leveling. Freshly collected adult GAS were sorted according to size and acclimatized for 48 h. Shell length measured 20–35 mm. Twelve GAS of assorted sizes (4 each of small, medium, and large, with 1 female:1 male) were randomly distributed in each microplot. After several days, the number of dead GAS was counted. Dead GAS had the following characteristics: they floated on the water surface, they had partially opened opercula, they did not respond to mechanical stimuli, and they emitted an unpleasant odor.

Minimal damage (missing hills) was observed on different fertilizer treatments during the first 2 d (Tables 1 and 2). GAS

**Table 1. Missing hills (%) caused by GAS in various basal fertilizer treatments, PhilRice, 1999 WS and 2000 DS and WS. <sup>a</sup>**

| Treatment                                          | Rate<br>(kg ha <sup>-1</sup> ) | 1999 WS |         |        | 2000 DS |        | 2000 WS |          |         |         |         |
|----------------------------------------------------|--------------------------------|---------|---------|--------|---------|--------|---------|----------|---------|---------|---------|
|                                                    |                                | 1 DAT   | 3 DAT   | 5 DAT  | 1 DAT   | 3 DAT  | 1 DAT   | 2 DAT    | 3 DAT   | 4 DAT   | 7 DAT   |
| 14-14-14<br>(complete fertilizer)                  | 40-40-40                       | 5.5 ab  | 16.2 ab | 32.8 a | 5.5 c   | 17.7 b | 11.2 ab | 30.2 bc  | 31.7 b  | 54.0 b  | 58.0 bc |
| Urea                                               | 40-0-0                         | 8.7 ab  | 22.0 ab | 40.0 a | 17.2 bc | 69.2 a | 18.7 a  | 43.2 b   | 44.2 b  | 64.0 ab | 73.5 ab |
| 14-14-14 + urea<br>(complete fertilizer +<br>urea) | 60-40-40                       | 6.0 b   | 12.2 b  | 32.7 a | 3.7 c   | 25.7 b | 3.5 b   | 20.0 cb  | 23.0 bc | 44.7 b  | 53.2 cb |
| 16-20-0 + urea<br>(ammonium phosphate<br>+ urea)   | 60-40-0                        | —       | —       | —      | 23.5 ab | 84.0 a | 7.0 ab  | 28.0 bcd | 30.0 b  | 42.2 b  | 44.0 c  |
| Molluscicide<br>(niclosamide 250 EC)               | 1.0 L ha <sup>-1</sup>         | 0.3 c   | 1.5 c   | 7.3 b  | 2.0 c   | 7.2 c  | 1.5 b   | 6.7 d    | 6.7 c   | 6.7 c   | 7.7 d   |
| Untreated<br>(no fertilizer, no molluscicide)      | —                              | 10.5 a  | 19.7 ab | 33.5 a | 35.0 a  | 83.2 a | 12.0 ab | 69.2 a   | 72.5 a  | 84.5 a  | 92.0 a  |

<sup>a</sup>Values followed by a common letter in a column are not significantly different at 5% level by DMRT. — = no data available, DAT = days after treatment, WS = wet season, DS = dry season. Values were arcsine-square root-transformed before analysis. Untransformed values are reported.

became inactive and immobile in plots treated with inorganic fertilizer and may have died because of visibly intensified mucus secretion. The major organ in GAS responsible for mucus production and release is the skin, in which various types of mucus cells can be found. As the GAS became immobilized, they may have additionally been exposed to unfavorable conditions. Desiccation by exposure to sunlight may have accelerated the lethal effects of inorganic fertilizers, but this appeared to be a side effect rather than a direct cause of death. Dead GAS in

fertilizer-treated plots had opened opercula but in niclosamide-treated plots the opercula were closed. GAS mortality (48.0–95.9%) was highest on molluscicide-treated microplots (Table 3). Fertilizer treatment reduced the GAS population to a maximum of 54.1% 7 d after treatment using a basal recommendation of 60-40-40 kg ha<sup>-1</sup> of complete fertilizer combined with urea. However, in the single-element and commercial organic fertilizer trial, no GAS mortality was observed (Table 2). Therefore, we conclude that the

combination of three elements (N, P, and K) caused the death of GAS.

Basal application of complete fertilizer (soil incorporation) in combination with urea at the recommended rate reduced the GAS population by 54%, thus preventing GAS damage to the crop during the first 2 d. This reduces the need to apply molluscicide. In addition, handpicking of snails becomes more efficient as the rice field water clears after application of inorganic fertilizers.

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**Table 2. Effect of single-element fertilizer on GAS mortality (%) and missing hills (%), PhilRice, 2000 WS.<sup>a</sup>**

| Treatment                                                | Rate<br>(kg ha <sup>-1</sup> ) | 6 h              |                    | 1 DAT            |        | 3 DAT            |        | 5 DAT            |         | 7 DAT            |          |
|----------------------------------------------------------|--------------------------------|------------------|--------------------|------------------|--------|------------------|--------|------------------|---------|------------------|----------|
|                                                          |                                | GAS<br>mortality | MH                 | GAS<br>mortality | MH     | GAS<br>mortality | MH     | GAS<br>mortality | MH      | GAS<br>mortality | MH       |
| Urea                                                     | 40-0-0                         | 0.0 b            | 1.2 a <sup>a</sup> | 0.0 b            | 3.0 dc | 0.0 b            | 11.5 c | 0.0 b            | 17.2 d  | 0.0 b            | 21.7 d   |
| Solophos<br>(0-8-10)                                     | 0-40-0                         | 2.0 b            | 2.2 a              | 2.0 b            | 7.7 b  | 2.0 b            | 15.5 c | 2.0 b            | 21.2 cd | 2.0 b            | 28.0 cd  |
| Muriate of potash<br>(0-18-0)                            | 0-0-40                         | 0.0 b            | 1.2 a              | 0.0 b            | 8.2 ab | 0.0 b            | 24.0 b | 0.0 b            | 31.2 b  | 0.0 b            | 37.0 b   |
| Organic fertilizer<br>(commercial organic<br>fertilizer) | 8 bags ha <sup>-1</sup>        | 0.0 b            | 2.7 a              | 0.0 b            | 12.2 a | 0.0 b            | 36.0 a | 0.0 b            | 51.2 a  | 0.0 b            | 60.0 a   |
| Molluscicide<br>(niclosamide 250 EC)                     | 1.0 L ha <sup>-1</sup>         | 31.2 a           | 1.0 a              | 93.7 a           | 1.2 d  | 93.7 a           | 1.2 d  | 93.7 a           | 1.2 e   | 93.7 a           | 1.2 e    |
| Untreated<br>(no fertilizer, no molluscicide)            | –                              | 0.0 b            | 1.5 a              | 0.0 b            | 6.0 c  | 0.0 b            | 13.7 c | 0.0 b            | 25.2 bc | 0.0 b            | 32.0 bcd |

<sup>a</sup>Values followed by a common letter in a column are not significantly different at 5% level by DMRT. – = no data available, MH = missing hills, DAT = days after treatment, WS = wet season, DS = dry season. Values were arcsine-square root-transformed before analysis. Untransformed values are reported.

**Table 3. GAS mortality (%) as affected by basal application of fertilizers, PhilRice, 1999 WS and 2000 DS and WS.<sup>a</sup>**

| Treatment                                          | Rate<br>(kg ha <sup>-1</sup> ) | 1999 WS |        |         | 2000 DS |        | 2000 WS |        |         |         |         |
|----------------------------------------------------|--------------------------------|---------|--------|---------|---------|--------|---------|--------|---------|---------|---------|
|                                                    |                                | 1 DAT   | 3 DAT  | 5 DAT   | 1 DAT   | 3 DAT  | 1 DAT   | 2 DAT  | 3 DAT   | 4 DAT   | 7 DAT   |
| 14-14-14<br>(complete fertilizer)                  | 40-40-40                       | 18.7 c  | 18.7 c | 23.0 bc | 12.5 b  | 20.8 b | 37.3 b  | 37.3 b | 41.5 bc | 41.5 bc | 43.5 bc |
| Urea                                               | 40-0-0                         | 2.1 b   | 2.1 b  | 6.2 b   | 2.0 b   | 8.3 b  | 25.0 b  | 25.0 b | 27.0 b  | 27.0 b  | 33.3 b  |
| 14-14-14 + urea<br>(complete fertilizer +<br>urea) | 60-40-40                       | 25.0 c  | 25.0 c | 37.0 c  | 12.5 b  | 22.9 b | 33.3 b  | 33.3 b | 52.0 bc | 52.0 bc | 54.1 bc |
| 16-20-0 + urea<br>(ammonium phosphate<br>+ urea)   | 60-40-0                        | –       | –      | –       | 4.1 b   | 8.3 b  | 18.8 b  | 18.8 b | 27.2 bc | 29.3 b  | 31.3 b  |
| Molluscicide<br>(niclosamide 250 EC)               | 1.0 L ha <sup>-1</sup>         | 48.0 d  | 75.0 d | 77.1 d  | 83.3 c  | 95.9 c | 70.8 c  | 70.8 c | 75.0 c  | 75.0 c  | 75.0 c  |
| Untreated<br>(no fertilizer, no molluscicide)      | –                              | 0.0 a   | 0.0 a  | 0.0 a   | 0.0 a   | 0.0 a  | 0.0 a   | 0.0 a  | 0.0 a   | 0.0 a   | 0.0 a   |

<sup>a</sup>Values followed by a common letter in a column are not significantly different at 5% level by DMRT. – = no data available, DAT = days after treatment, WS = wet season, DS = dry season. Values were arcsine-square root-transformed before analysis. Untransformed values are reported.

# Meloidogyne graminicola and Sclerotium rolfii interaction in rice

B.P. Hazarika, Department of Plant Pathology, Indian Council for Agricultural Research, (RC) Manipur Center, Imphal 795004, India

Rice root-knot nematode *Meloidogyne graminicola* causes yield losses of up to 18% in rainfed upland rice in eastern India, and seedling blight disease of rice, caused by *Sclerotium rolfii*, is seen to be associated with it (Mathur 1973). This observation led to the investigation of the nature and type of association among rice cultivar IR8, *M. graminicola*, and *S. rolfii*.

Samples of IR8 plants with an intact root system and rhizosphere soil collected from the 0–5-cm depth of a rainfed upland rice field served as a source of inoculum for the greenhouse experiments.

Earthen pots (15 × 15 cm) were used after rinsing the samples in 2% formaldehyde and air-drying for potting soil. Upland rice field soil (79 sand:16 silt:5 clay), with a pH of 5.0 from the top 16 cm, was steam-sterilized at 15 kg cm<sup>-2</sup> pressure and placed in pots at 1,000 g pot<sup>-1</sup>. Seeds of IR8 were dehusked, soaked in 0.52% sodium hypochlorite for 1 h (Keoboonreung 1971), and rinsed in distilled water thrice before sowing in 2% agar in 15 × 15-cm jars in an all-glass seed germination chamber. When seedlings reached the 3–4-leaf stage 18–23 d after sowing, they were planted at one seedling pot<sup>-1</sup>. Water was always adjusted to

approximately 32% of soil moisture content.

Interaction was studied by introducing six treatments in six replications (see table). Based on earlier research (Rao and Israel 1972) on the rate of invasion and behavior of nematodes in roots, *M. graminicola* was inoculated at 100 larvae sprout<sup>-1</sup> at 3 d and at 500 larvae seedling<sup>-1</sup> at 15 d. An equal amount of soil and water or PDA without fungus was added with the nematode to simulate fungus inoculation. Ten grams of substrate containing spore and mycelium was diluted with 90 mL of distilled water to provide inocula at 1 mL 3-d-old seedling<sup>-1</sup> and 2.5 mL 15-d-old seedling<sup>-1</sup>.

The prevalence of nematode and fungus was estimated from all treatments 30 d after the second inoculation, and plant growth characters were compared. The number of galls decreased significantly in the presence of the fungus, indicating the negative effect of the fungus on nematode development. The negative effect of fungal invasion was demonstrated by the reduced number of endoparasites (all stages) in treatments in which the fungus was inoculated first and the nematode a week later. In these treatments, the percentage

of males was highest and fungal development was best. Fresh weight of roots and tillers, shoot height, and dry weight decreased in nematode and fungus associations. Similar observations were reported by other researchers working with *M. javanica* and *Fusarium moniliforme* or maize variety Hybrid 17 A with *F. solani* or *Rhizoctonia solani*—the association decreased plant growth more significantly than did either the nematode or the fungus alone (Ibrahim and Rezk 1978).

All these factors indeed show an interaction between *M. graminicola* and *S. rolfii* in rice. Roots invaded by *S. rolfii* were not suitable for the development of *M. graminicola*.

## References

- Ibrahim IKA, Rezk MA. 1978. Pathogenicity of *M. javanica* and certain fungi on corn. *Alex. J. Agric. Res.* 26(2):441–446.
- Keoboonreung S. 1971. Effect of rice-root nematode *Hirschmanniella* (Van Breda de Hann 1902) Luc and Goodey 1963 on rice seedlings. PhD thesis, Louisiana State University, USA.
- Mathur SC. 1973. Studies on seedling blight of rice caused by *Sclerotium rolfii*. *Riso* 22(3):231–237.
- Rao YS, Israel P. 1972. Influence of inoculum density on the final population of the rice root-knot nematode. *Indian J. Nematol.* 2:72–76.

**Meloidogyne graminicola and Sclerotium rolfii interaction in rice cv. IR8.**

| Treatment                       | Galls (no.) | Total endoparasites (all stages) (log 10 value) | % of males | Fungal development <sup>a</sup> (no.) | Tillers plant <sup>-1</sup> (no.) | Shoot       |             | Fresh root wt (g) |
|---------------------------------|-------------|-------------------------------------------------|------------|---------------------------------------|-----------------------------------|-------------|-------------|-------------------|
|                                 |             |                                                 |            |                                       |                                   | Height (cm) | Dry wt (mg) |                   |
| T1 <i>M. graminicola</i> alone  | 263.4       | 3.68                                            | 38.8       | –                                     | 1.8                               | 39.4        | 617.0       | 4.81              |
| T2 <i>S. rolfii</i> alone       | –           | –                                               | –          | +                                     | 1.6                               | 38.6        | 608.6       | 4.61              |
| T3 T1 + T2 together             | 194.8       | 3.74                                            | 41.5       | ++                                    | 1.4                               | 34.6        | 549.0       | 4.14              |
| T4 T1 first and T2 a week later | 189.7       | 3.41                                            | 40.5       | +                                     | 1.3                               | 30.2        | 399.2       | 4.14              |
| T5 T2 first and T1 a week later | 211.0       | 3.30                                            | 51.2       | ++                                    | 1.6                               | 31.2        | 578.2       | 3.82              |
| T6 Uninoculated                 | –           | –                                               | –          | –                                     | 2.1                               | 43.9        | 764.0       | 5.81              |
| CD 0.0537.3                     | 0.13        |                                                 |            | 0.27                                  | 2.5                               | 78.7        | 0.43        |                   |

<sup>a</sup>+ = less, ++ = more, – = none.

# Effects of antibiotics on biological control agents and their efficacy on rice sheath blight (*R. solani* AG-I.1)

W. Tan and T.W. Mew, IRRI E-mail: wanzhong.tan@bbsrc.ac.uk and t.mew@cgiar.org

The biological control of rice diseases with antagonistic microbial organisms has been studied extensively and is considered a promising technology (Mew et al 1993). Many factors have been shown to affect the efficacy of biocontrol agents (BCAs), but there have been few studies on effects of pesticides on disease biocontrol (Zhao et al 1992). Some antibiotics have been reported to be effective against bacterial diseases of crops. It is important to determine whether antibiotic applications in the field affect disease suppression by BCAs. In biocontrol studies, mutation with antibiotics has been exploited to obtain bacterial mutants with acquired or increased antagonism to pathogens (Georgakopoulos et al 1994). Little information has been available on bacterial BCA mutations in relation to biocontrol of diseases in rice systems. The objectives of this study were to (1) analyze the direct effects of different antibiotics on the growth of BCAs, (2) obtain BCA mutants with enhanced biocontrol activity, and (3)

examine the effects of antibiotics on rice sheath blight and BCA function.

To determine the effect of antibiotics on the growth of different bacterial BCAs, four BCA strains, B-916 (*Bacillus subtilis*), P7-14 (*Pseudomonas fluorescens*), P9409 (*P. resinovorans*), and P10353 (*P. malculicola*), were grown on peptone potassium nitrate medium (PPM) containing 0.5–1,000  $\mu\text{g mL}^{-1}$  of four antibiotics: ampicillin, hygromycin, kanamycin, and rifampicin. The four antibiotics affected growth of the four BCAs quite differently (Table 1). Kanamycin had a profound effect on B-916, P7-14, and P9409, which could not grow at 3  $\mu\text{g mL}^{-1}$ , but it did not affect the growth of P10353 at concentrations of more than 250  $\mu\text{g mL}^{-1}$ . Rifampicin at the highest concentration used in the experiment (1,000  $\mu\text{g mL}^{-1}$ ) did not influence the growth of P7-14, but the other three bacteria stopped growing at 3  $\mu\text{g mL}^{-1}$ . However, apart from that of B-916, the growth of the other three bacteria

was not affected by ampicillin at rather high concentrations ( $>100 \mu\text{g mL}^{-1}$ ).

Some BCAs mutated easily, such as P7-14 and P9409 on hygromycin, P10353 and P9409 on rifampicin, and B-916 on ampicillin. Mutants of these BCAs resistant to 1,000  $\mu\text{g mL}^{-1}$  of the antibiotics were obtained. In other cases, BCAs were very stable, such as B-916 and P9409 on kanamycin and B-916 on rifampicin.

Cell growth (Table 1), pathogenicity, and antagonistic activity of the mutants were not significantly changed compared with parent bacteria, but the growth of  $\text{amp}^R$ -916 decreased markedly and antagonistic activity of  $\text{hyg}^R$ -714 and  $\text{hyg}^R$ -9409 increased markedly ( $P < 0.05$ ).

The three antibiotics (50  $\mu\text{g mL}^{-1}$ ) reduced sheath blight by 60–80% as measured by area under the disease progress curve (AUDPC). B-916 and P9409 [ $10^7$  colony-forming units (CFU)  $\text{mL}^{-1}$ ] controlled the disease by more than 75%, whereas P7-14 and P10353 were less effective. When an antibiotic (25  $\mu\text{g mL}^{-1}$ )

**Table 1. Effect of antibiotics on growth of BCAs and mutants.**

| Antibiotic | BCA     | Highest growth <sup>a</sup><br>concentration ( $\mu\text{g mL}^{-1}$ ) |        | Mutant                | Mutant growth on <sup>b</sup> |                  |                  |
|------------|---------|------------------------------------------------------------------------|--------|-----------------------|-------------------------------|------------------|------------------|
|            |         | BCA                                                                    | Mutant |                       | PPM                           | PPM + antibiotic | Wild type on PPM |
| Ampicillin | B-916   | 1                                                                      | 1,000  | $\text{amp}^R$ -B916  | 341 b                         | 243 c            | 735 a            |
|            | P7-14   | 500                                                                    | —      |                       |                               |                  |                  |
|            | P 10353 | 500                                                                    | —      |                       |                               |                  |                  |
|            | P 9409  | 250                                                                    | —      |                       |                               |                  |                  |
| Hygromycin | B-916   | 10                                                                     | 100    | $\text{hyg}^R$ -714   | 752 a                         | 755 a            | 767 a            |
|            | P 7-14  | 50                                                                     | 1,000  |                       |                               |                  |                  |
|            | P 10353 | 250                                                                    | —      |                       |                               |                  |                  |
|            | P 9409  | 50                                                                     | 1,000  |                       |                               |                  |                  |
| Kanamycin  | B-916   | 1                                                                      | 10     | $\text{hyg}^R$ -9409  | 596 a                         | 582 a            | 581 a            |
|            | P 7-14  | 1                                                                      | 250    |                       |                               |                  |                  |
|            | P 10353 | 500                                                                    | —      |                       |                               |                  |                  |
|            | P 9409  | 1                                                                      | 10     |                       |                               |                  |                  |
| Rifampicin | B-916   | 0.5                                                                    | 3      | $\text{rif}^R$ -10353 | 654 a                         | 633 a            | 639 a            |
|            | P 7-14  | 500                                                                    | —      |                       |                               |                  |                  |
|            | P 10353 | 10                                                                     | 1,000  |                       |                               |                  |                  |
|            | P 9409  | 1                                                                      | 1,000  |                       |                               |                  |                  |
|            |         |                                                                        |        | $\text{rif}^R$ -9409  | 611 a                         | 573 a            | 581 a            |

<sup>a</sup>BCA growth was considered not affected and mutation was not performed when it could grow at 250  $\text{mg mL}^{-1}$ . <sup>b</sup>Each value is the mean of six replicates. Means (number of colonies per plate) in a row with the same letter are not significantly different ( $P > 0.05$ ) by DMRT.



was mixed with a BCA ( $0.5 \times 10^7$  CFU mL<sup>-1</sup>) sensitive to it, disease suppression was consistently weakened, for example, B-916 + ampicillin, P10353 + rifampicin, and P9409 + rifampicin. When an antibiotic and a tolerant BCA were combined, their efficacy was unchanged or enhanced, as shown by P7-14 + hygromycin and P9409 + hygromycin.

Compared with the parent BCA, biocontrol efficacy of hyg<sup>R</sup>-714 and hyg<sup>R</sup>-9409 was significantly enhanced ( $P < 0.05$ ) or unchanged in other cases ( $P > 0.05$ ). The combined spray of mutant with its inducer antibiotic markedly improved biocontrol efficiency in some cases, but it was not altered in other cases based on the control effect of the mutant.

In conclusion, antibiotics affected the growth of some BCAs but not others. Some BCAs tolerated high concentrations of antibiotics and thus their growth was not affected. Of the five mutants obtained from the BCAs, two had enhanced antagonistic activity and one had lowered growth capability. No mutant developed pathogenicity to rice plants. The antibiotics alone controlled sheath blight, and BCAs and mutants also controlled the disease effectively. When a BCA or mutant was combined with an antibiotic at half the dosage ( $0.5 \times 10^7$  CFU mL<sup>-1</sup> and 25 µg mL<sup>-1</sup>, respectively), the antibiotic enhanced, weakened, or had no effect on sheath blight control, depending on the compatibility of the BAC and antibiotic.

## References

Georgakopoulos DG, Henderson M, Panopoulos NJ, Schroth MN. 1994. Analysis of expression of a phenazine biosynthesis locus of *Pseudomonas aureofaciens* PGS12 on

**Table 2. Comparison of sheath blight severity (lesion area in mm<sup>2</sup>) difference between BCA and antibiotic treatments.<sup>a</sup>**

| Treatment                     | Days after treatment |         |         |          |          | AUDPC |
|-------------------------------|----------------------|---------|---------|----------|----------|-------|
|                               | 3                    | 4       | 5       | 6        | 7        |       |
| B-916                         | 5.4 c                | 12.3 c  | 31.2 c  | 98.7 c   | 163.3 c  | 226.6 |
| Amp <sup>R</sup> -B916        | 1.0 c                | 8.0 c   | 24.7 cd | 72.7 c   | 124.0 c  | 167.9 |
| Amp. 50                       | 4.3 c                | 12.3 b  | 29.7 c  | 73.7 c   | 115.3 c  | 175.5 |
| B-916 + A25                   | 15.3 b               | 33.4 b  | 86.7 ab | 172.0 b  | 248.5 b  | 423.8 |
| Amp <sup>R</sup> -B916 + A25  | 3.0 c                | 7.7 c   | 19.0 d  | 40.0 d   | 75.3 d   | 105.9 |
| Check                         | 22.0 a               | 72.7 a  | 145.0 a | 262.3 a  | 363.3 a  | 672.7 |
| 7-14                          | 16.3 a               | 45.7 b  | 99.0 ab | 124.3 bc | 201.7 b  | 383.3 |
| Hyg <sup>R</sup> -714         | 14.7 ab              | 33.7 b  | 55.3 c  | 96.7 c   | 146.5 c  | 266.3 |
| Hyg. 50                       | 5.3 b                | 15.7 c  | 45.7 c  | 86.7 c   | 127.7 c  | 214.6 |
| 7-14 + H25                    | 22.0 a               | 58.3 ab | 75.3 bc | 135.0 b  | 191.3 b  | 375.3 |
| Hyg <sup>R</sup> -714 + H25   | 8.0 b                | 21.7 c  | 53.0 c  | 89.3 c   | 130.3 c  | 227.2 |
| Check                         | 22.0 a               | 72.7 a  | 145.0 a | 262.3 a  | 363.3 a  | 672.7 |
| 10353                         | 9.7 b                | 34.0 b  | 62.7 b  | 104.3 b  | 191.0 c  | 301.4 |
| Rif <sup>R</sup> -10353       | 10.3 b               | 22.7 c  | 53.0 b  | 94.3 b   | 150.0 c  | 250.2 |
| Rif. 50                       | 5.0 b                | 17.3 c  | 55.3 b  | 89.7 b   | 158.3 c  | 244.0 |
| 10353 + R25                   | 8.0 b                | 30.7 b  | 74.7 b  | 124.3 b  | 253.3 b  | 360.4 |
| Rif <sup>R</sup> -10353 + R25 | 7.7 b                | 18.5 c  | 35.3 c  | 61.4 c   | 98.7 d   | 168.4 |
| Check                         | 22.0 a               | 72.7 a  | 145.0 a | 262.3 a  | 363.3 a  | 672.7 |
| 9409                          | 6.5 b                | 15.7 b  | 35.3 c  | 84.7 b   | 173.5 b  | 225.7 |
| Hyg <sup>R</sup> -9409        | 0 c                  | 5.3 c   | 10.7 d  | 39.7 c   | 90.3 c   | 100.9 |
| 0.Hyg. 50                     | 5.3 b                | 15.7 b  | 45.7 b  | 86.7 b   | 127.7 c  | 214.6 |
| 9409 + H25                    | 1.7 b                | 8.3 c   | 23.7 d  | 35.3 c   | 68.7 d   | 102.5 |
| Hyg <sup>R</sup> -9409 + H25  | 1.3 b                | 6.4 c   | 21.3 d  | 33.3 c   | 45.7 d   | 89.5  |
| Check                         | 22.0 a               | 72.7 a  | 145.0 a | 262.3 a  | 363.3 a  | 672.7 |
| 9409                          | 6.5 c                | 15.7 c  | 35.3 c  | 84.7 c   | 173.5 c  | 225.7 |
| Rif <sup>R</sup> -9409        | 8.3 bc               | 20.3 bc | 44.7 c  | 95.0 c   | 203.7 bc | 266.0 |
| Rif. 50                       | 5.0 c                | 17.3 c  | 55.3 bc | 89.7 c   | 158.3 c  | 243.9 |
| 9409 + R25                    | 11.7 b               | 31.3 b  | 66.0 c  | 156.3 b  | 251.0 b  | 389.9 |
| Rif <sup>R</sup> -9409 + R25  | 1.3 c                | 22.4 c  | 51.3 bc | 93.3 c   | 135.7 c  | 240.5 |
| Check                         | 22.0 a               | 72.7 a  | 145.0 a | 262.3 a  | 363.3 a  | 672.7 |

<sup>a</sup>Each value is the mean of nine replicates. Means in a column with the same letter are not significantly different ( $P > 0.05$ ) by DMRT. AUDPC values were calculated from the means in each treatment.

seeds with a mutant carrying a phenazine biosynthesis locus-ice nucleation reporter gene fusion. Appl. Environ. Microbiol. 60:4573-4579.

Mew TW, Rosales AM, Maningas GV. 1993. Biological control of *Rhizoctonia* sheath blight and blast of rice. In: Ryder MH, Stephens PM, Brown GD, editors. Improving plant productivity with rhizosphere bacteria. Australia:

Commonwealth Science and Industrial Research Organisation. p 9-13.

Zhao ZM, Chen Y, Li HR, Liu ZJ, Yan SQ. 1992. Studies on the resistance of *Bacillus cereus* R2 to commonly used insecticides, fungicides, and herbicides. In: Yan SQ, editor. Rice growth-promoting rhizobacterium. Beijing (China): Scientific and Technical Document Publishing House. p 67-72.

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# First report of palea browning in China and characterization of the causal organism by phenotypic tests and Biolog

Xie Guanlin, Institute of Biocontrol of Plant Diseases, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, China  
E-mail: glxie@zju.edu.cn

In recent years, a bacterial disease of rice was observed in the field in Jiangxi and Yuhang, Zhejiang Province, China. The disease usually occurs at early flowering of late rice (japonica varieties). Initially, light, rusty, water-soaked lesions appear on the lemma or palea. The lesions then turn brown. The discoloration occurs most frequently on the palea. More immature and lighter grains are observed on panicles at harvest. The disease was reported in Japan, Korea, and the Philippines but not previously in China (Azegami et al 1983, Kim et al 1989, Xie 1996). Therefore, the author determined the causal organism and compared it with *Pantoea agglomerans* (formerly *Erwinia herbicola*) (Gavini et al 1989), the causal organism of palea browning in rice from Japan.

Three and seven seed samples of rice (4–5 g sample<sup>-1</sup>) with palea browning were collected from Jiangxi and Yuhang, respectively, in 1998–99. Isolations were done by plating serial dilution of seed washes (10<sup>-3</sup>–10<sup>-5</sup> g<sup>-1</sup>) on nutrient agar medium. Colony morphology was described and phenotypic and pathogenicity tests followed (Mew and Misra 1994, Azegami et al 1983, Xie 1996). Four standard reference strains (*Erwinia herbicola* J301742, *E. herbicola* J301752, *E. ananas* J301714, and *E. ananas* J301722) were provided by the National Institute of Agrobiological Resources, Japan. Additionally, Biolog (Biolog Inc., Investment Blvd. 3447, Suite 3, Hayward, Calif. 94545, USA) with software version 3.5 was used to further characterize the isolates.

Twelve bacterial isolates from 10 rice samples with palea browning showed characteristics similar to those of the reference strains of *P. agglomerans* in the phenotypic, pathogenicity, and Biolog

tests. The bacterium was Gram-negative and facultative fermentative. It had 2–6 peritrichous flagella, and it produced a

yellow water-soluble pigment. Table 1 shows 39 phenotypic characteristics of the isolates similar to those of *P. agglomerans*

**Table 1. Phenotypic characterization of 12 isolates isolated from rice grains showing palea browning collected from Jiangxi and Yuhang of Zhejiang Province, China.**

| Phenotypic test                | Isolates tested             |                            | Reference strains <sup>a</sup>                    |                                              |
|--------------------------------|-----------------------------|----------------------------|---------------------------------------------------|----------------------------------------------|
|                                | 6<br>(Jiangxi) <sup>b</sup> | 6<br>(Yuhang) <sup>c</sup> | <i>P. agglomerans</i><br>(J301742 and<br>J301752) | <i>P. ananas</i><br>(J301714 and<br>J301722) |
| Gram staining                  | –                           | –                          | –                                                 | –                                            |
| Flagellation                   | +                           | +                          | +                                                 | +                                            |
| O-F test <sup>d</sup>          | +                           | +                          | +                                                 | +                                            |
| Yellow pigment                 | +                           | +                          | +                                                 | +                                            |
| Fluorescent pigment            | –                           | –                          | –                                                 | –                                            |
| Pink diffusible pigment        | –                           | –                          | –                                                 | –                                            |
| Growth factors required        | –                           | –                          | –                                                 | –                                            |
| Growth at 40 °C                | +                           | +                          | +                                                 | +                                            |
| H <sub>2</sub> S from cysteine | +                           | +                          | +                                                 | –                                            |
| Reducing sugar                 | –                           | v                          | –                                                 | +                                            |
| Indole test                    | +                           | +                          | +                                                 | +                                            |
| Gelatin liquefaction           | +                           | +                          | +                                                 | +                                            |
| Acetoin                        | +                           | +                          | +                                                 | +                                            |
| Nitrate reduction              | –                           | v                          | –                                                 | –                                            |
| Urease                         | –                           | –                          | –                                                 | –                                            |
| Phenylalanine deaminase        | v                           | –                          | –                                                 | –                                            |
| DNase                          | –                           | –                          | –                                                 | –                                            |
| Lecithinase                    | –                           | –                          | –                                                 | –                                            |
| Gas from glucose               | –                           | –                          | –                                                 | –                                            |
| Casein hydrolysis              | –                           | –                          | –                                                 | –                                            |
| Cotton seed oil hydrolysis     | –                           | –                          | –                                                 | –                                            |
| Growth in 5% NaCl              | +                           | +                          | +                                                 | +                                            |
| Acid production from           |                             |                            |                                                   |                                              |
| Melibiose                      | +                           | v                          | +                                                 | +                                            |
| Arabinose                      | +                           | +                          | +                                                 | +                                            |
| Mannitol                       | +                           | +                          | +                                                 | +                                            |
| Salicin                        | v                           | +                          | +                                                 | +                                            |
| Raffinose                      | v                           | +                          | +                                                 | +                                            |
| Lactose                        | v                           | +                          | +                                                 | +                                            |
| Maltose                        | +                           | +                          | +                                                 | +                                            |
| Cellobiose                     | v                           | +                          | +                                                 | +                                            |
| Dextrin                        | –                           | –                          | –                                                 | –                                            |
| Glycerol                       | v                           | +                          | +                                                 | +                                            |
| Mannose                        | +                           | +                          | +                                                 | +                                            |
| Rhamnose                       | +                           | +                          | +                                                 | –                                            |
| Ribose                         | +                           | +                          | +                                                 | +                                            |
| Sorbitol                       | +                           | v                          | +                                                 | +                                            |
| Sucrose                        | +                           | +                          | +                                                 | +                                            |
| Trehalose                      | +                           | +                          | +                                                 | +                                            |
| Starch                         | v                           | –                          | –                                                 | +                                            |
| Isolates tested (no.)          | 6                           | 6                          | 2                                                 | 2                                            |

<sup>a</sup>+ = 83–100% isolates positive; v = 33–82% isolated variable, – = 83–100% isolates negative; four reference strains isolated from rice grains in Japan. <sup>b</sup>Isolated from rice grains in Jiangxi. <sup>c</sup>Isolated from rice grains in Yuhang. <sup>d</sup>Oxidation-fermentation test.

reference strains. Production of H<sub>2</sub>S from cysteine, reducing sugar, and production of acid from starch and rhamnose differentiated the 12 isolates from *Pantoea ananas* (*E. ananas*). Six isolates induced discoloration of palea when inoculated at flowering stage by spraying. The rate of discolored grains on a panicle was 2.1–12.6%, which varied based on isolate, age of the rice plant, and environmental conditions (mainly temperature and moisture) for inoculation (Azegami et al 1983). The six selected strains isolated from discolored grains were identified as *P. agglomerans* by Biolog, which confirmed the two reference strains as *P. agglomerans* with similarity indices of 0.611 and 0.930 (Table 2).

The causal organism of palea browning in Zhejiang Province has been phenotypically identified as *P.*

**Table 2. Identity of causal bacterium of palea browning of rice by Biolog.**

| Code of isolate | Biolog identification      | Similarity index |
|-----------------|----------------------------|------------------|
| CB98301         | <i>Pantoea agglomerans</i> | 0.971            |
| CB97311         | <i>P. agglomerans</i>      | 0.930            |
| CB98370         | <i>P. agglomerans</i>      | 0.911            |
| CB97303         | <i>P. agglomerans</i>      | 0.894            |
| CB97318         | <i>P. agglomerans</i>      | 0.863            |
| CB98369         | <i>P. agglomerans</i>      | 0.827            |

*agglomerans*. It can be concluded that it is the same causal organism reported in Japan in 1983.

## References

- Azegami K, Ozaki K, Matsuda A. 1983. Bacterial palea browning, a new disease of rice caused by *Erwinia herbicola*. Bull. Natl. Inst. Agric. Sci. Ser. C 39:1–12.
- Gavini F, Mergaert J, Beji A, Mielcarek C, Izard D, Kersters K, Ley J de. 1989. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Int. J. Syst. Bacteriol. 39(3):337–345.
- Kim YC, Kim KC, Cho BH. 1989. Palea browning disease of rice caused by *Erwinia herbicola* and ice nucleation activity of the pathogenic bacteria. Korean J. Plant Pathol. 5(1):72–79.
- Mew TW, Misra JK. 1994. A manual of rice seed health testing. Manila (Philippines): International Rice Research Institute. 220 p.
- Xie GL. 1996. Characterization of *Pseudomonas* spp. and other bacterial species associated with rice seeds. PhD thesis, University of the Philippines Los Baños, Philippines. 170 p.

# Expression of *Bt* genes under control of different promoters in rice at vegetative and flowering stages

R.M. Aguda, K. Datta, J. Tu, S.K. Datta, and M.B. Cohen, IRRI E-mail: m.cohen@cgiar.org

Toxin-encoding genes from the bacterium *Bacillus thuringiensis* (*Bt*) have been widely used in plant transformation to confer resistance to insect pests. These genes have been placed under the control of various promoters. Some, such as the cauliflower mosaic virus (CaMV) 35S and rice actin promoters, are constitutive promoters, that is, they drive gene expression in most tissues at most stages of plant development. Other promoters are tissue-specific, such as the maize phosphoenolpyruvate carboxylase (PEPC) promoter, which is active only in tissues that are photosynthetically active, and the maize tryptophan synthase alpha subunit promoter, which is active in pith. To achieve adequate and sustainable insect control with *Bt* genes, the associated promoter should provide strong gene expression in tissues attacked by target pests during all stages of plant development that are attacked by the pests (Cohen et al 2000). In some cases, it may

also be useful to avoid toxin gene expression in tissues not attacked by target pests, such as roots or grain.

In rice, the principal target pests for control by *Bt* genes are the yellow stem borer (YSB) *Scirpophaga incertulas* (Walker), striped stem borer (SSB) *Chilo suppressalis* (Walker), and leafhoppers such as *Marasmia patnalis* Guenée. Alinia et al (2000) found that a rice line transformed with *Bt cryIAb* toxin gene under control of the PEPC promoter was highly resistant to stem borers and leaf-feeding pests at the vegetative stage, but that toxin titer and insect resistance dropped substantially at the flowering stage. In this study, we examined the resistance at the vegetative and flowering stages of *cryIAb*-transformed rice lines with the CaMV, actin, and pith-specific promoters. These lines were previously found to be resistant to YSB at the vegetative stage (Datta et al 1998, Tu et al 1998).

Lines with the pith-specific and CaMV 35S promoter were in the background of variety CBII, and the line with the actin promoter was variety IR72. All lines were homozygous-positive for the *cryIAb* gene. Nontransgenic plants of CBII and IR72 were used as controls. Bioassays were conducted in petri dishes using cut stems for YSB and SSB and cut leaves for *M. patnalis*, with one stem or leaf per petri dish. Six first-instar stem borer larvae or second-instar *M. patnalis* larvae were placed in each petri dish, and the number of dead, live, and unrecovered larvae was recorded after 4 d. One stem and one leaf were tested from each of 75 plants of each *cryIAb* line at the vegetative stage. Another stem and leaf from 50 of these plants were tested at the booting stage, and another stem and leaf from 25 plants at the flowering stage. Three stems or leaves were tested from one CBII and IR72 control plant at each growth stage. Each petri dish

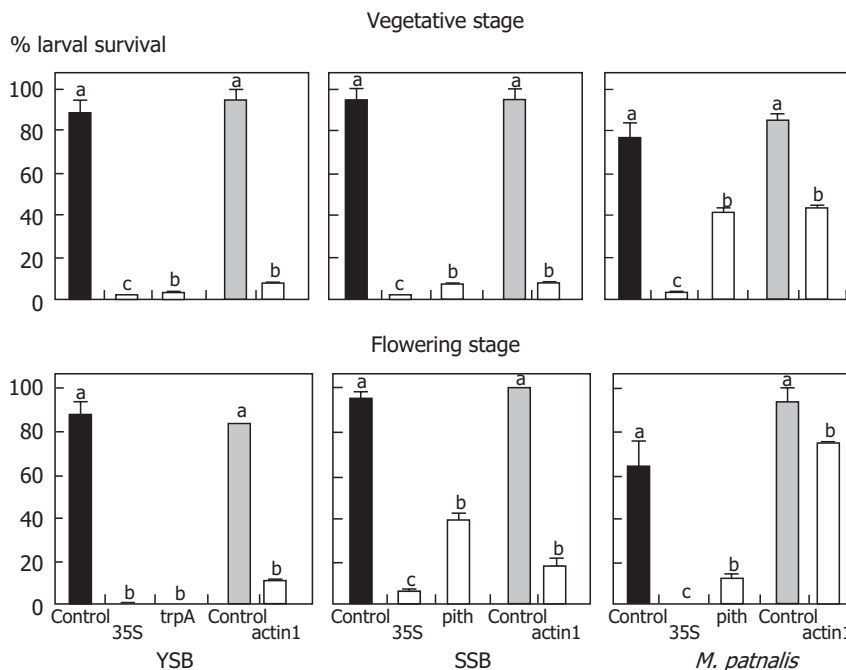
was considered a replicate. Petri dishes were arranged in a completely randomized design. Whole-plant bioassays of plants at the vegetative, booting, and flowering stages were conducted with YSB. At each growth stage, eight plants of each *cry1Ab* line and eight control plants of CBII and IR72 were infested with 25 first-instar YSB larvae. Four plants were dissected after 4 d and four plants after 25 d. All data were analyzed by analysis of variance, and arcsine-square root-transformed means were compared by the LSD test.

In the petri dish assays, larval survival on all three *cry1Ab* lines was significantly lower than on control lines at the vegetative and flowering stages (Fig. 1). For both SSB and *M. patnalis*, survival was significantly lower on the line with the CaMV 35S promoter than on that with the pith-specific promoter. In the whole-plant assays, YSB larval survival was significantly lower on all three *cry1Ab* lines than on control plants at both growth stages (Fig. 2). Similar results were also obtained at the booting stage in both the petri dish and whole-plant assays (data not shown).

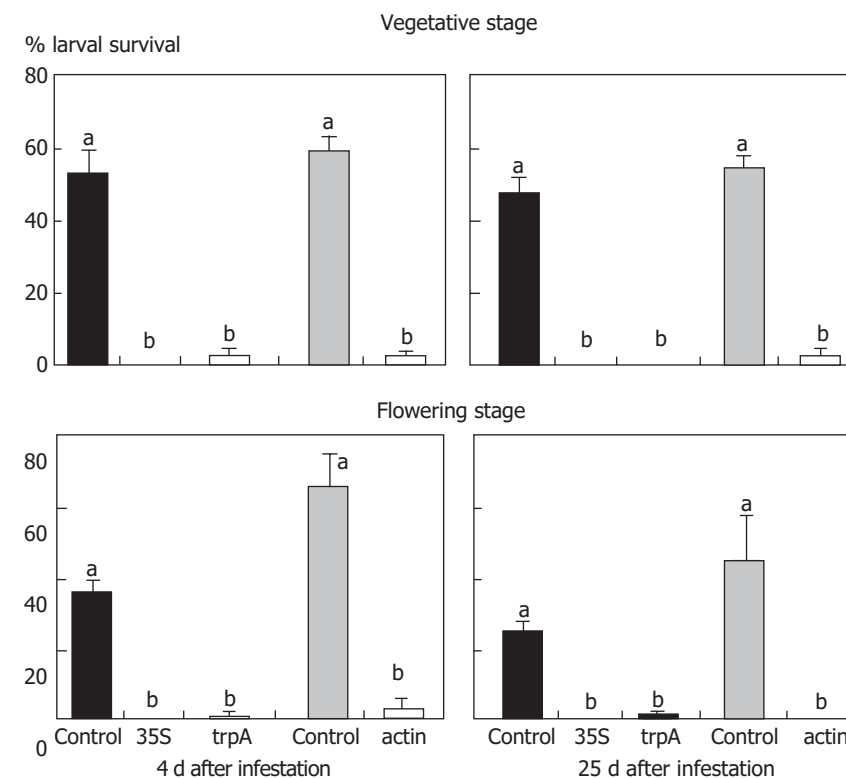
Our results demonstrate that *cry1Ab* lines with the actin, CaMV 35S, and pith-specific promoters retain resistance to stem borers and leaffolders at the flowering stage. Quantification of toxin titer by immunological methods would provide a more sensitive test of whether there is a decline in toxin titer at the flowering stage. However, it is apparent that any toxin decline in these lines is much less substantial than that observed by Alinia et al (2000) in a *cry1Ab* rice line with the PEPC promoter. Because toxin expression can vary greatly among transgenic lines containing the same construct, additional lines must be tested before general conclusions can be drawn.

## References

- Alinia F, Ghareyazie B, Rubia LG, Bennett J, Cohen MB. 2000. Effect of plant age, larval age, and fertilizer treatment on resistance of a *cry1Ab*-transformed aromatic rice to lepidopterous stem borers and foliage feeders. *J. Econ. Entomol.* 93:484–493.
- Cohen MB, Gould F, Bentur JS. 2000. *Bt* rice: practical steps to sustainable use. *Int. Rice Res. Notes* 25(2):4–10.
- Datta K, Vasquez A, Tu J, Torrizo L, Alam MF, Oliva N, Abrigo E, Khush GS, Datta SK. 1998. Constitutive and tissue-specific differential expression of the *cry1Ab* gene in transgenic



**Fig. 1. Survival (mean  $\pm$  SE) of stem borer and leaffolder larvae after 4 d on cut stems and leaves of *cry1Ab* and control lines. Means within a growth stage, species, and variety (CBII or IR72) sharing the same letters are not significantly different ( $P > 0.05$ , LSD test).**



**Fig. 2. Survival (mean  $\pm$  SE) of YSB larvae in *cry1Ab* and control lines in whole-plant bioassays. Means within a growth stage and variety (CBII or IR72) sharing the same letters are not significantly different ( $P > 0.05$ , LSD test).**

rice plants conferring resistance to rice insect pest. *Theor. Appl. Genet.* 97:20–30.

Tu J, Datta K, Alam MF, Fan Y, Khush GS, Datta SK. 1998. Expression and function of hybrid

*Bt* toxin gene in transgenic rice conferring resistance to insect pest. *Plant Biotechnol.* 15:195–203.

# Testing a yield loss simulation model for rice in Chinese rice-wheat system production environments

D. Zhu, China National Rice Research Institute (CNRRI), Hangzhou 310006, Zhejiang, China; L. Willocquet, CBGP, Campus International de Baillarguet, CS 30 016, 34988 Montpellier/Lez cedex, France; Q. Tang, S. Huang, X. Lin, CNRRI; L. Fernandez, F.A. Elazegui, IRRI, Philippines; and S. Savary, CBGP

A yield loss simulation model for rice pests was developed (Willocquet et al 1998, 2000) as a tool for setting research priorities and improving pest management. This model is production situation (PS)-specific and addresses yield loss caused by several rice pests. The model has been tested in several PSs in the Philippines, Vietnam, and northern India, using field experiments done at different sites. In most cases, the model adequately simulated the attainable growth of a rice crop, as well as losses from various pests (diseases, insects, and weeds) (Willocquet et al 1998, 1999a,b, 2000). The work reported here was aimed at further testing this model under PSs and injuries prevailing in the Chinese rice-wheat system. The PSs and injuries addressed here were defined based on the characterization of rice injury profiles and PSs recently done across tropical Asia (Savary et al 2000). The first PS, PS2, is a reference used in all field-test experiments. Two other PSs (PS6 and PS7) represent PSs prevailing in Zhejiang Province. Whiteheads caused by stem borers, sheath blight, and weeds are considered as common rice pests in the province.

An experiment was done at CNRRI during the 1998 rainy season using "early rice." Crop establishment in the field was done on 27 April. In each PS, injury-free plots and rice plots injured by pests (alone or in combination) were established. Crop growth, environmental factors, and pest injuries were monitored throughout the growing season. In a given PS, data from injury-free plots were used to calibrate parameters to simulate attainable yield. Data from injured plots were used to test simulations of yield losses.

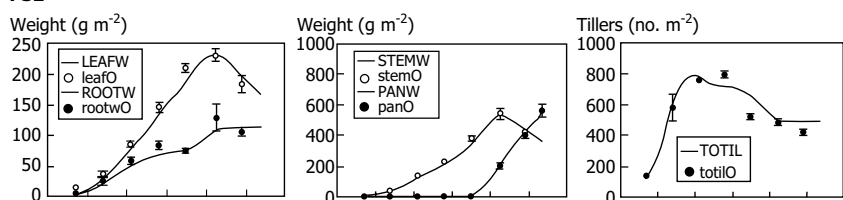
Three PSs were addressed: PS2 (IR72 transplanted with young seedlings,

110 kg N ha<sup>-1</sup>, irrigated rice), PS6 (Jian Yu 293, direct-seeded at a density of 170 germinated seeds m<sup>-2</sup>, 180 kg N ha<sup>-1</sup> in three splits, 15 kg P ha<sup>-1</sup> at basal, 75 kg K ha<sup>-1</sup> in two splits), and PS7 (Fan 97 transplanted with 28-d-old seedlings, 100 kg N ha<sup>-1</sup> in seedbed, same fertilization as PS6). Three injury treatments were established in each PS: whiteheads (WH), sheath blight (ShB), weeds (WEED), a combination of the three injuries (COMBI), and a noninjured (CTRL) treatment. The injury treatments were randomized with three replications in each PS. Individual plots were 2.8 × 2.8 m and included four zones from the outer to the inner part of the plot: a border row, 20 cm wide; a sampling zone, 20 cm wide, to monitor crop growth (destructive sam-

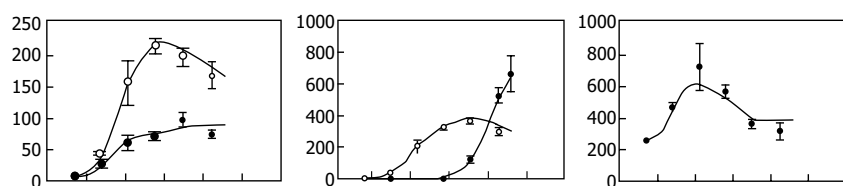
plings) and injuries; a second row, 20 cm wide; and a central harvest area.

The simulations of attainable growth (leaf, stem, root, and panicle dry weight, and tiller population) and final yield were close to observed values (see figure) in the three PSs considered. In all PSs, the same patterns were observed: increase in leaf weight until flowering, then a decline (leaf senescence); increase in root weight until flowering, remaining stable afterward; increase in stem weight until flowering, then a decline (translocation of stored starch from the stems to the panicles); and an increase in panicle weight from flowering to maturity. Tillering occurred until 30–40 d after crop establishment (DACE). The number of

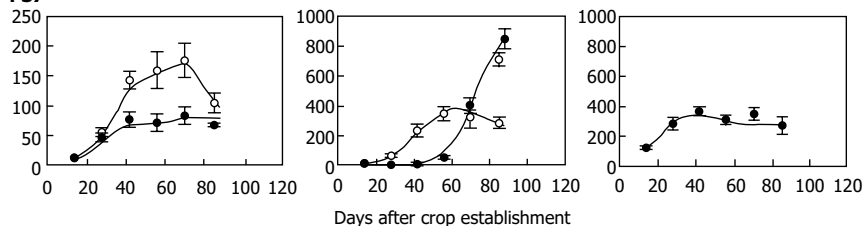
## PS2



## PS6



## PS7



**Attainable rice growth in three PSs (A: PS2, B: PS6, C: PS7):** observed (dots) ± SEM and simulated (plain line) dry weight (g m<sup>-2</sup>) of roots (ROOTW), dry weight of leaves (LEAFW), dry weight of stems (STEMW), dry weight of panicles (PANW), and number (m<sup>-2</sup>) of tillers (TOTIL). Observed data were collected from the experiment done at CNRRI in 1998 in control plots (see text for details).



tillers declined afterward because of tiller death and remained constant after flowering. The crop cycle was shorter in PS6 and PS7 than in PS2. The maximum leaf dry weight and the maximum number of tillers were much smaller in PS7 than in PS2 and PS6. This is probably because of the crop establishment method in PS7 (using old transplanted seedlings), which favored the growth of a small number of big tillers. The maximum dry weight of stems was observed in PS2 and was attributed to the longer crop cycle in this PS, which allowed the stem to grow for a long time. The final yield was largest in PS7, followed by PS6 and PS2. The lower fertilization in PS2 than in the two other PSs explains the poorer performance of this PS.

The table gives simulated and observed grain yields for the 15 (PS×injury) combinations addressed in the experiment. Yield loss ranged from 0.3% to 24%. An acceptance interval of ± 10% of the observed grain yield was defined to assess simulated yield outputs. The model simulated yields within this acceptance interval in all cases. It can thus be assumed that the model accounted well for the effects of different injuries manipulated in this experiment.

Results show that the simulation model adequately describes PSs and re-

**Simulated and observed grain yield (g m<sup>-2</sup>) in the experiment done at CNRRI in 1998.**

| Treatment <sup>a</sup> | PS2 <sup>b</sup> |           | PS6 <sup>b</sup> |           | PS7 <sup>b</sup> |           |
|------------------------|------------------|-----------|------------------|-----------|------------------|-----------|
|                        | Observed         | Simulated | Observed         | Simulated | Observed         | Simulated |
| CTRL                   | 422              | 418       | 579              | 568       | 787              | 787       |
| WH                     | 420              | 408       | -                | -         | -                | -         |
| SHB                    | 393              | 402       | 519              | 517       | 689              | 711       |
| WEED                   | 319              | 331       | 523              | 550       | 669              | 686       |
| COMBI                  | 361              | 343       | 509              | 493       | 611              | 670       |

<sup>a</sup>CTRL = control, WH = white heads, SHB = sheath blight, WEED = weeds, COMBI = combination of the three injuries.

<sup>b</sup>PS2 = reference production situation, PS6 and PS7 = production situations that prevail in Zhejiang Province.

flects fairly well the damage caused by sheath blight, weeds, and whiteheads, alone or in combination. Previous evaluations of the model yielded similar conclusions under different sets of PSs (Willocquet et al 1998, 1999a,b, 2000). This study shows that the model behaves fairly well under other rice production environments, such as those found in Zhejiang Province.

The simple model structure and the approach used to calibrate and test it can be used by different research teams to address specific (PS×injuries) combinations. The model is flexible enough to address other injuries, such as those caused by brown planthopper, bacterial leaf blight, or defoliators. It can thus be used for scenario analyses that may help in prioritizing research for rice pest management.

## References

- Savary S, Willocquet L, Elazegui FA, Teng PS, Du PV, Zhu D, Tang Q, Huang S, Lin X, Singh HM, Srivastava RK. 2000. Rice pest constraints in tropical Asia: characterization of injury profiles in relation to production situations. *Plant Dis*. 84:341–356.
- Willocquet L, Savary S, Fernandez L, Elazegui FA, Teng PS. 1998. Simulation of yield losses caused by rice diseases, insects and weeds in tropical Asia. *IRRI Disc. Pap. Ser.* 34. 62 p.
- Willocquet L, Fernandez L, Singh HM, Srivastava RK, Rizvi SMA, Savary S. 1999a. Further testing of a yield loss simulation model for rice in different production situations. I. Focus on rice-wheat system environments. *Int. Rice Res. Notes* 24(2):26–27.
- Willocquet L, Fernandez L, Savary S. 1999b. Further testing of a yield loss simulation model for rice in different production situations. II. Focus on water-stressed environments. *Int. Rice Res. Notes* 24(2):28–29.
- Willocquet L, Savary S, Fernandez L, Elazegui FA, Teng PS. 2000. Development and evaluation of a multiple-pest, production situation-specific simulation model of rice yield losses in tropical Asia. *Ecol. Model.* 131:133–159.

## Analysis of spatial structure and distribution of brown planthopper at a macro-scale level

R. Zhang, Q. Zhou, and D. Gu, Institute of Entomology/State Key Laboratory for Biocontrol, Zhongshan University, Guangzhou 510275, China

Brown planthopper (BPH) is a migrant insect pest and is observed to complete about seven to eight generations in Guangdong Province of China (Bao et al 1996). Its spatial structure and distribution are complex and difficult to predict at a macro-scale level. Early studies tried to

describe distribution using classical statistics, neglecting the spatial location of samples and their interdependence. To capture these spatial relations, we have adapted a geostatistical modeling approach to predict the density distribution of BPH.

In this study, BPH density was considered as a typical regionalized variable, and the semivariogram—a function describing the relationship between distance and sample values—was used as a tool to analyze its spatial structure (Journel 1984). The Kriging interpolation technique,

which estimates spatial values by taking a weighted linear average of available samples (Kriging and Magri 1982), was applied to estimate BPH density distribution. Data on BPH density in 82 plots in the province in 1997 were used for analysis. The geographic information system (GIS) software, IDRISI, was employed to assemble and collate data. Analytical procedures were as follows: (1) the region was divided into grid cells ( $2 \times 2$  km) and digitized, (2) the semivariogram was developed, (3) spatial structure and anisotropy (directional semivariogram) were analyzed, (4) Kriging interpolation was used to estimate BPH density for each unknown grid cell, and (5) a scenario of BPH spatial structure and distribution was generated with GIS.

Results indicated that the BPH population aggregated at a macro-scale level and clump diameter ranged from 70 to 400 km in immigration generations and from 198 to 205 km in other generations. The spatial structure of BPH in the early rice-growing season was more stable than

in the late rice season, and a significant anisotropy appeared from east to west.

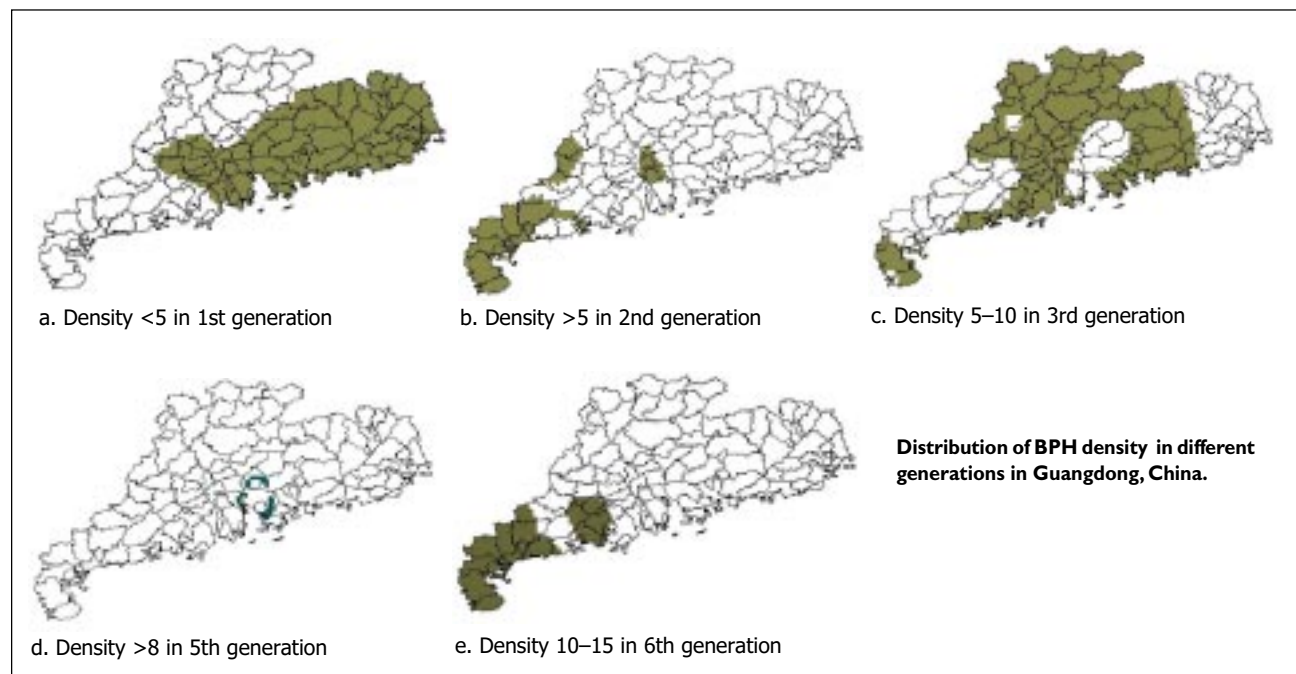
BPH density and its distribution areas increased with generation. In the first generation of the early rice-growing season, the density in most areas was less than 1 BPH hill<sup>-1</sup>, but in the second generation, the population increased to 5 BPH hill<sup>-1</sup> and was distributed over 3 million ha in the Leizhou Peninsula and Pearl River Delta. In the third generation, areas with a density of 5–10 BPH hill<sup>-1</sup> expanded to 11 million ha, mainly located in the central and northern parts of the province (see figure, a-c).

A similar situation occurred in the late rice-growing season, when BPH immigrated back to the province. Densities of 5–8 BPH hill<sup>-1</sup> were observed in the Pearl Delta over 180,000 ha. As the population developed into the sixth generation, however, areas with more than 20 BPH hill<sup>-1</sup> reached 3 million ha, covering the southwest part of the province (see figure, d-e).

The technique of regionalized variable, semivariogram, and Kriging interpolation performed well in predicting the spatial distribution of BPH density, as shown by comparisons with data collected in fields different from those sampled to obtain input data for the GIS analysis. The technique appeared to be useful for macro-scale-level forecasting of insect density. A spatio-temporal model may be one type of modification of this technique to further improve the prediction level.

## References

- Bao H, Chao Z, Yang L, Chen H. 1996. Regionalization of planthoppers in Guangdong Province and their control strategy. *Guangdong Agric. Sci.* 4:8–11.
- Journel AG. 1984. The place of non-parametric geostatistics. In: Verly G, David M, Marechal A, Journel A, editors. *Geostatistics for natural resources characterization. Part 1*. Dordrecht (The Netherlands): D. Reidel. p 307–335.
- Krige DG, Magri EJ. 1982. Studies of the effects of outliers and data transformation on variogram estimates for a base metal and a gold ore body. *Math. Geol.* 14:557–564.





## Polyolefin-coated urea as a fertilizer for rice on soil with high nitrogen leaching loss

O.O. Fashola and T. Wakatsuki, Shimane University, Faculty of Life and Environmental Science, Matsue, Shimane 6908504, Japan E-mail: d97a2011@matsu.shimane-u.ac.jp or oofashola@hotmail.com

Rice farmers in the inland valleys (IVs) of sub-Saharan Africa cultivate under rainfed conditions with little or no bunding. Their fields often alternate between being in flooded and droughty conditions, thus subjecting added inputs, especially N, to leaching and surface runoff. Unfortunately, soil nutrient status in most IVs is very poor and soil is sandy, invariably lowering the efficiency of commonly available N fertilizers, such as urea, and consequently decreasing the yields of improved varieties. Attaining reasonable yields of about 3 t ha<sup>-1</sup> requires two to three split applications at 90 kg N ha<sup>-1</sup>. Farmers do not often attain these yields. Our trial was aimed at finding out the efficiency of polyolefin-coated urea as an alternate N source, with one basal application at 60 kg N ha<sup>-1</sup> in sandy soil under poor water management.

A greenhouse study with 54 Wagner pots (500 cm<sup>3</sup>) was conducted from June to October 1997 and June to October 1998 in Shimane, western Japan. The soil was an Entisol—sandy (91%) and nutritionally poor. Total N was 0.43 g kg<sup>-1</sup>, total C was 7.46 g kg<sup>-1</sup>, CEC was 5.35 cmol (+) kg<sup>-1</sup>, and pH was 5.6. The trial was a randomized complete block design with two factors (fertilizer and water management) and three replications. Fertilizer treatments consisted of (1) no N (N<sub>0</sub>), (2) prilled urea at 60 kg N ha<sup>-1</sup> applied in two splits—basal and 35 d after transplanting (DAT) (N<sub>x</sub>), and (3) polyolefin-coated urea (POCU) at 60 kg N ha<sup>-1</sup> applied once basally. Water management was either (1) good water management (GWM)—i.e., submergence throughout the growing season, with periodic water sampling made fortnightly, or (2) poor water management (PWM), with periodic flooding to induce leaching in 1997 and leaching plus surface runoff in 1998. Pots with PWM had their bottom

drainage hole unplugged at 33 DAT in 1997; in 1998, four additional side holes (10-mm diameter) were made. Side holes were unplugged at 14 DAT and the bottom drain hole was unplugged at 25 DAT. GWM pots were not leached, except during water sampling and for mid-season drainage for 24 h. In 1997, 4,500 mm of irrigation water was used in 123 d per pot for PWM; in 1998, 4,200 mm was used in 121 d. The N<sub>0</sub> treatment with GWM had 320 mm and 400 mm of irrigation in 1997 and 1998, respectively. POCU and urea, on the other hand, had 600 mm and 760 mm of irrigation in 1997 and 1998, respectively, to maintain submerged conditions during the growing season.

Thirty-day-old seedlings (cv. IR36) were transplanted. Each pot had two hills with two seedlings per hill. All pots received a basal application of 13.2 kg P ha<sup>-1</sup> as calcium phosphate and 24.9 kg K ha<sup>-1</sup> as muriate of potash. Two slow-release POCU fertilizers, LPS40 and LPS100, were used in both years. In 1997, LPS100 was used alone; in 1998, a 1:1 combination of LP40 and LPS100 was used. LPS100 belongs to the delayed-release group and has a

sigmoid-shaped release pattern. It is released very slowly in the first 40 d of application. In water at 25 °C, 80% of the N is released in 100 d. LP40, on the other hand, belongs to the ordinary-release group with a linear release pattern. Yield data were corrected to 14% moisture content.

Results showed that mean grain yield and number of grains per panicle were higher in 1997 than in 1998, whereas productive tiller number and 1,000-grain weight were similar in both years (Tables 1 and 2). In 1997, the urea treatment produced yield not significantly different from the POCU treatment under GWM, but the POCU treatment was significantly higher (378 g m<sup>-2</sup>) than the urea treatment (316 g m<sup>-2</sup>) under PWM. Under GWM, the POCU treatment had significantly more grains. In 1998, the grain yields of both the POCU and urea treatments were comparable under GWM. However, the POCU treatment under PWM yielded significantly higher (343 g m<sup>-2</sup>) than the urea treatment (261 g m<sup>-2</sup>). The number of grains per panicle of the POCU treatment under both water management regimes was significantly higher than that

**Table 1. Mean grain yield, yield component, PFP, and ANUE for 1997.**

| Treatment                    | Tillers pot <sup>-1</sup><br>(no.) | 1,000-grain<br>wt (g) | Grain yield<br>(g m <sup>-2</sup> ) | Grains<br>panicle <sup>-1</sup><br>(no.) | PFP <sup>a</sup><br>(kg kg <sup>-1</sup> N) | ANUE <sup>b</sup><br>(kg kg <sup>-1</sup> N) |
|------------------------------|------------------------------------|-----------------------|-------------------------------------|------------------------------------------|---------------------------------------------|----------------------------------------------|
| <i>Good water management</i> |                                    |                       |                                     |                                          |                                             |                                              |
| No N                         | 4.7                                | 22                    | 103                                 | 74.2                                     | —                                           | —                                            |
| POCU <sup>c</sup>            | 10.3                               | 22                    | 401                                 | 115.6                                    | 66.9                                        | 49.7                                         |
| Urea                         | 13.7                               | 23                    | 411                                 | 71.8                                     | 68.5                                        | 51.3                                         |
| 5% LSD <sup>d</sup>          | 1.6 <sup>e</sup>                   |                       | 52 ns                               | 29.2 <sup>e</sup>                        | 7.3 ns                                      | 11.7 ns                                      |
| <i>Poor water management</i> |                                    |                       |                                     |                                          |                                             |                                              |
| No N                         | 7.4                                | 22                    | 156                                 | 68.3                                     | —                                           | —                                            |
| POCU <sup>c</sup>            | 12.9                               | 21                    | 378                                 | 100.1                                    | 62.9                                        | 36.9                                         |
| Urea                         | 12.5                               | 22                    | 316                                 | 71.2                                     | 52.8                                        | 26.8                                         |
| 5% LSD <sup>d</sup>          | 1.6 ns                             |                       | 52 <sup>e</sup>                     | 29.2 ns                                  | 7.3 <sup>e</sup>                            | 11.7 ns                                      |

<sup>a</sup>PFP (partial factor productivity) = Yield/N<sub>x</sub>. <sup>b</sup>ANUE (agronomic nitrogen-use efficiency) = (yield at N<sub>x</sub> - yield at N<sub>0</sub>)/applied N at N<sub>x</sub>. <sup>c</sup>POCU is a controlled-release fertilizer; in 1997, LPS100 was used. <sup>d</sup>LSD is a comparison between POCU and prilled urea. In 1997, POCU was LPS100. <sup>e</sup> = significant at P = 0.05. ns = not significant.

**Table 2. Mean grain yield, yield components, PFP, and ANUE for 1998.**

| Treatment                    | Tillers pot <sup>-1</sup><br>(no.) | 1,000-grain<br>wt (g) | Grain yield<br>(g m <sup>-2</sup> ) | Grains<br>panicle <sup>-1</sup><br>(no.) | PFP <sup>a</sup><br>(kg kg <sup>-1</sup> N) | ANUE <sup>b</sup><br>(kg kg <sup>-1</sup> N) |
|------------------------------|------------------------------------|-----------------------|-------------------------------------|------------------------------------------|---------------------------------------------|----------------------------------------------|
| <i>Good water management</i> |                                    |                       |                                     |                                          |                                             |                                              |
| No N                         | 5.2                                | 21                    | 111                                 | 60.0                                     | —                                           | —                                            |
| POCU <sup>c</sup>            | 11.8                               | 23                    | 354                                 | 77.5                                     | 59.0                                        | 40.5                                         |
| Urea                         | 14.3                               | 23                    | 330                                 | 57.7                                     | 54.9                                        | 36.5                                         |
| 5% LSD <sup>d</sup>          | 2.1 <sup>e</sup>                   |                       | 44 ns                               | 14.1 <sup>e</sup>                        | 4.6 ns                                      | 12.8 ns                                      |
| <i>Poor water management</i> |                                    |                       |                                     |                                          |                                             |                                              |
| No N                         | 7.8                                | 21                    | 133                                 | 47.1                                     | —                                           | —                                            |
| POCU <sup>c</sup>            | 13.3                               | 21                    | 343                                 | 71.6                                     | 57.2                                        | 35.0                                         |
| Urea                         | 12.8                               | 21                    | 261                                 | 57.1                                     | 43.4                                        | 21.2                                         |
| 5% LSD <sup>d</sup>          | 2.1 ns                             |                       | 44 <sup>e</sup>                     | 14.1 <sup>e</sup>                        | 4.6 <sup>e</sup>                            | 12.8 <sup>e</sup>                            |

<sup>a</sup>PFP (partial factor productivity) = Yield/N<sub>x</sub>. <sup>b</sup>ANUE (agronomic nitrogen-use efficiency) = (Yield at N<sub>x</sub> - yield at N<sub>0</sub>)/applied N at N<sub>x</sub>. <sup>c</sup>POCU is a controlled-release fertilizer; in 1998, the combination of LP40 and LPS100 was 1:1. <sup>d</sup>LSD is a comparison between POCU and prilled urea. <sup>e</sup> = significant at P = 0.05. ns = not significant.

under the urea treatment (Table 2). The POCU treatment performed significantly better than prilled urea, either with leaching alone or with a combination of

leaching and surface runoff. This can be attributed to the slow-release pattern of N, which matches the need of the plant,

consequently giving POCU more grains per panicle.

In 1997, partial factor productivity (PFP)—but not agronomic nitrogen-use efficiency (ANUE)—of the POCU treatment under PWM was significantly higher than that of the urea treatment. However, in 1998, both PFP and ANUE of the POCU treatment were significantly higher than those of the urea treatment. This implies that the slow-release mechanism of polyolefin-coated urea enhances N-use efficiency of the plant. This better performance of POCU under PWM indicates a potential for increased rice yield if POCU is basally applied at 60 kg N ha<sup>-1</sup> to nutritionally poor soil with high N leaching loss.

## Nitrogen source and application time before flooding affect rice yield in Spain

R. Carreres, J. Sendra, R. Ballesteros, Departamento del Arroz, Instituto Valenciano de Investigaciones Agrarias, Sueca 46410, Valencia, Spain; E. Fernández-Valiente, A. Quesada, D. Carrasco, M. Crespo, and F. Leganés, Departamento de Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain E-mail: r.carreres@master.ivia.es

Valencian rice growers usually apply excess N, which results in loss of soil quality because of its negative effects on the soil N<sub>2</sub>-fixing cyanobacteria (Carreres et al 1996). In Valencia, constraints in the irrigation system have led to lack of control in water management, with difficulties in irrigating after incorporating N fertilizer. The low N efficiency reflects substantial N losses (Fernández Valiente et al 2000). Improving N efficiency and soil use by preserving and developing the ability of N<sub>2</sub>-fixing organisms for biofertilization would reduce N fertilizer use and result in ecological, economic, and environmental benefits. N efficiency cannot be improved by split application in Valencia (Carreres et al 1998) but it can be enhanced by slow-release fertilizers (SRF) in Europe (Moletti et al 1989). The relationships between N<sub>2</sub> fixation, physical and chemical characteristics of water and sediments, and rates and

timing of inorganic N fertilizers were described in previous papers (Quesada et al 1997, Carreres et al 1998). Studies dealing with the effect of SRF on N<sub>2</sub> fixation are lacking. This work evaluates the use of different SRF and nitrification inhibitors (NI) in rice fields of Valencia, Spain.

Experiments were conducted during 1998-99 at the Rice Department in Valencia, Spain. Eight N sources (ammonium sulfate [AS], urea, polymer-coated urea [PCU, 32% and 40% N], sulfur-coated urea [MSCU], isobutylidene diurea [IBDU], N ammoniacal form [AF] + dicyandiamide [DCD, 11:1], and AF + dimethyl pyrazolo phosphate [DMPP, 11:1], including a control [no N] were applied at 120 kg N ha<sup>-1</sup> and at 2 or 15 d before flooding (DBF) during 1998. Four N sources (urea blended with PCU [40% N] at four ratios: 1:0, 3:1, 1:1, 1:3) were applied at four rates (70, 95, 120, and 145

kg N ha<sup>-1</sup>) and at 15 DBF during 1999.

Treatment plots, each replicated four times, were laid out in a split-plot design (delay in flooding after N application as main plot and N source as subplot) during 1998 and in a randomized block design in 1999. N fertilizer was given as a single broadcast application. After flooding, seeds were broadcast at 180 kg ha<sup>-1</sup>.

In June and July 1998, *in situ* acetylene-reducing activity (ARA) was measured only in plots where fertilizer was applied 2 DBF (see table). At crop maturity, grain and straw yields and N content in plants were determined (Carreres et al 1996). In 1998, traits were subjected to a split-plot analysis of variance (N source and timing) and in 1999 to a one-way (no N and 16 treatments: four N sources at four N rates) and a split-plot (N rates as main plot and N source as subplot) analysis of variance.

**Effect of N source and delay in flooding after N application on N<sub>2</sub> fixation, grain yield, and N content, N uptake, and N efficiencies.<sup>a</sup>**

| N source            | ARA <sup>b</sup>  | Grain yield (t ha <sup>-1</sup> ) |          | Grain <sup>c</sup><br>N content<br>(%) | N uptake <sup>c</sup><br>(kg ha <sup>-1</sup> ) | AE                |           | RE (%) <sup>c</sup><br>Δ plant N/<br>applied N |
|---------------------|-------------------|-----------------------------------|----------|----------------------------------------|-------------------------------------------------|-------------------|-----------|------------------------------------------------|
|                     |                   | Δ grain yield/applied N           |          |                                        |                                                 |                   |           |                                                |
|                     |                   | 15 ddf                            | 2 ddf    |                                        |                                                 | 15 ddf            | 2 ddf     |                                                |
| 1998                |                   |                                   |          |                                        |                                                 |                   |           |                                                |
| No N                | 24.0 a            | 6.13 d                            | 6.59 d   | 1.00 c                                 | 85.9 c                                          | —                 | —         | —                                              |
| AS                  | 3.0 b             | 7.78 c                            | 8.90 abc | 1.14 a                                 | 137.2 ab                                        | 13.7 cd           | 19.2 abcd | 42.7 ab                                        |
| CU                  | 7.7 b             | 8.19 abc                          | 8.69 abc | 1.11 ab                                | 135.2 ab                                        | 17.1 abcd         | 17.4 abcd | 41.0 ab                                        |
| PCU (32% N)         | 13.3 a            | 8.26 abc                          | 8.46 abc | 1.12 a                                 | 132.4 b                                         | 17.7 abcd         | 15.6 bcd  | 38.7 b                                         |
| MSCU                | 6.3 b             | 8.22 abc                          | 8.77 abc | 1.12 a                                 | 138.2 ab                                        | 17.4 abcd         | 18.2 abcd | 43.5 ab                                        |
| PCU (40% N)         | 21.3 a            | 9.37 a                            | 9.15 ab  | 1.13 a                                 | 147.8 a                                         | 26.9 a            | 21.3 abcd | 51.5 a                                         |
| IBDU                | 21.7 a            | 8.95 abc                          | 8.74 abc | 1.08 b                                 | 134.6 ab                                        | 23.5 abc          | 17.9 abcd | 40.5 ab                                        |
| DCD                 | 10.3 ab           | 8.86 abc                          | 8.69 abc | 1.12 a                                 | 142.6 ab                                        | 22.7 abcd         | 17.4 abcd | 47.2 ab                                        |
| DMPP                | 9.4 ab            | 9.04 ab                           | 8.14 bc  | 1.11 ab                                | 138.1 ab                                        | 24.4 ab           | 12.9 d    | 43.5 ab                                        |
| SE N source (48 df) | 6.84 <sup>d</sup> | 0.347                             |          | 0.012                                  | 4.25                                            | 2.94 <sup>e</sup> |           | 3.68 <sup>e</sup>                              |
| 1999                |                   |                                   |          |                                        |                                                 |                   |           |                                                |
| No N                | —                 | 4.08 <sup>f</sup>                 |          | 1.06 <sup>f</sup>                      | 57.0 <sup>f</sup>                               | —                 | —         |                                                |
| 100% CU             | —                 | 7.46 b                            |          | 1.10 a                                 | 105.2 b                                         | 30.7 b            | 43.5 b    |                                                |
| 75% CU + 25% PCU    | —                 | 7.18 b                            |          | 1.08 a                                 | 108.2 b                                         | 29.0 b            | 47.2 b    |                                                |
| 50% CU + 50% PCU    | —                 | 7.48 b                            |          | 1.10 a                                 | 110.6 b                                         | 32.6 b            | 50.8 b    |                                                |
| 25% CU + 75% PCU    | —                 | 8.03 a                            |          | 1.11 a                                 | 120.1 a                                         | 38.6 a            | 61.2 a    |                                                |
| SE N source (45 df) | —                 | 0.183                             |          | 0.012                                  | 3.34                                            | 1.90              | 3.24      |                                                |

<sup>a</sup>In 1998, split-plot design (four replications); in 1999, randomized complete block design (four replications), means of four N rates. In each year, means in a column followed by the same letter are not significantly different ( $P = 0.05$ ) by Duncan's multiple range test (DMRT); AS = ammonium sulfate; CU = conventional urea; PCU = polymer-coated urea; MSCU = sulfur-coated urea; IBDU = isobutylidene diurea; DCD = N inhibitor; DMPP = N inhibitor; ARA = acetylene-reducing activity (mmol ethylene h<sup>-1</sup> m<sup>-2</sup>); AE = agronomic efficiency; RE = recovery efficiency; df = degrees of freedom; ddf = days delay in flooding. <sup>b</sup>Mean of two sampling periods, analysis of variance conducted on data transformed to log (1 + x). The table gives backtransformed values. <sup>c</sup>In 1998, means of two delays in flooding (interaction "delay × N source" was not significant). <sup>d</sup>24 df. <sup>e</sup>42 df. <sup>f</sup>Control treatment (no N) was significantly different (grain yield and N uptake) or not (grain N content) from all other treatments (four rates × four N sources; data not shown).

Duncan's multiple range test (DMRT) and orthogonal polynomial contrasts were used to test differences.

In June–July, N from SRF (PCU and IBDU) had been totally released and soil inorganic N level was lower in plots fertilized with SRF than with conventional fertilizers (CF), with or without NI. Therefore, N<sub>2</sub> fixation during months with maximum ARA values (Carreres et al 1996) was not affected by SRF (PCU and IBDU) application compared with when N fertilizer was absent (see table). This trend was similar to that reported by Carreres et al (1998) for split N application.

SRF, particularly PCU 40% N applied basally before flooding, was the best N source for grain yield and improved agronomic efficiency (AE) and recovery efficiency (RE) (see table). However, it was only significant when applied 15 DBF (significant interaction between N source and DBF) and compared with AS. These results agree with the findings of Moletti et al (1989) and the orthogonal contrasts confirm the results ( $P < 0.05$ ). Accordingly, in 1999 and as shown by orthogonal polynomial contrasts, grain yield ( $P < 0.01$ ),

N uptake, and N efficiency ( $P < 0.05$ ) increased linearly as the coated-conventional urea ratio increased. This indicates that the slow N release effectively reduced substantial N losses resulting from the delay in flooding after N application using CF, alone or blended with NI. N losses can be attributed more to ammonia volatilization than to nitrification-denitrification processes, thus confirming the findings of Fernández Valiente et al (2000).

## References

- Carreres R, González Tomé R, Sendra J, Ballesteros R, Fernández Valiente E, Quesada A, Nieva M, Leganés F. 1996. Effect of nitrogen rates on rice growth and biological nitrogen fixation. *J. Agric. Sci.* 127:295–302.
- Carreres R, Sendra J, Ballesteros R, Fernández Valiente E, Quesada A, Leganés F. 1998. Effect of different rates and application times of nitrogen fertilizers in a rice cropping system. In: Chataigner J, editor. *Rice quality: a pluridisciplinary approach*. Paris: Centre Internationale de Hautes Etudes Agronomiques Méditerranéennes / FAO.
- Fernández Valiente E, Ucha A, Quesada A, Leganés F, Carreres R. 2000. Contribution of N<sub>2</sub>-fixing

cyanobacteria to rice production: availability of nitrogen from <sup>15</sup>N-labeled cyanobacteria and ammonium sulphate to rice. *Plant Soil* 221:109–114.

Moletti M, Fiore G, Nipoti E, Villa B. 1989.

Valutazione dei fertilizzanti azotati a lenta cessione e degli inhibitory della nitrificazione in risaia. *L'Inf. Agric.* 9:141–148.

Quesada A, Leganés F, Fernández Valiente E.

1997. Environmental factors controlling N<sub>2</sub>-fixation in Mediterranean rice fields. *Microb. Ecol.* 34:39–48.

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# A simple method of producing green manure *Sesbania rostrata* to achieve N synchrony in lowland rice

G. Seneviratne and E.M.H.G.S. Ekanayake, Biological Nitrogen Fixation Project, IFS, Kandy, Sri Lanka  
E-mail: gaminis@ifs.ac.lk

There is concern, particularly in the tropics, that some high-quality leguminous green manures (LGM) and crop residues may release nitrogen (N) into the soil so rapidly that losses of N may occur through denitrification, ammonia volatilization, and leaching. In Sri Lanka, for example, it is reported that as much as 75% of *Sesbania rostrata*-N is lost in dry-zone lowland soils (Seneviratne and Kulasoorya 1994). The concept of synchrony of N supply with crop demand has thus resulted. An organic material of the right quality may release N at approximately the same time and rate required by the crop, thereby reducing N losses. This has been tested by mixing low- and high-quality plant materials in different proportions (Myers et al 1994). However, this method adds an additional cost to green manuring because of the labor involved in transporting and applying plant materials with a high C:N ratio. This study investigated a simple cultural method to produce low- and high-quality green manures *in situ* with *S. rostrata*.

The study was conducted in two farmers' fields (sites 1 and 2) of the low-country intermediate zone of Sri Lanka during the fallow period before the 1998

dry season. Soil in the area was a Tropaqualf with pH 6.4, organic carbon (C) 1.2%, total N 0.06%, and available phosphorus (P) 9.5  $\mu\text{g g}^{-1}$  soil. Fifteen plots of 6  $\times$  3 m were prepared in three rows, with five plots per row at each site. They were surrounded by 45-cm-wide bands. Irrigation canals were constructed between rows. Five seeding rates of *S. rostrata* ranging from 100 to 300 kg ha<sup>-1</sup> were applied in a completely randomized design with three replications (see table). Plots were puddled, leveled, and pretreated with concentrated H<sub>2</sub>SO<sub>4</sub> for 7 min and allowed to stay this way overnight, and inoculated (with *Azorbizobium caulinodans* ORS 571) seeds were broadcast evenly onto moist soil. Basal dressings of K and P were applied at 16.6 kg K<sub>2</sub>O ha<sup>-1</sup> and 11 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively. After a week, plots were flooded to about 3-cm depth. Stem inoculation with the rhizobial strain was carried out 2, 4, and 6 wk after broadcast by sprinkling broth onto the shoot. When plants were 9 wk old, three samples of whole plants were carefully uprooted from 1  $\times$  1-m squares from each plot. Root systems were washed and plant number was

counted. Samples were then oven-dried (65 °C for 72 h), weighed, and ground. Ground subsamples were analyzed for total N by the Kjeldahl method. C content was calculated as 45% of the plant dry weight.

Because of the lower coefficient of variation (CV) at site 1 compared with site 2, different seed rates at the first site produced significantly different plant densities and biomass (see table). The higher the plant density, the higher the biomass and the lower the plant dry weight at both sites. N concentration also declined under higher plant densities. N accumulation was highest at plant densities of 44–46  $\times 10^5$  ha<sup>-1</sup> at the two sites. C:N ranged from 16 to 19 at plant densities below 6  $\times 10^5$  ha<sup>-1</sup> and increased sharply up to about 40 at plant densities of 44–53  $\times 10^5$  ha<sup>-1</sup>. The C:N achieved here for low and high plant densities is ideal for attaining synchrony because it was shown recently that tropical litter with C:N below and above 27 has contrasting N dynamics, that is, net mineralization and immobilization, respectively (Seneviratne 2000).

This study showed that green manure quality of *S. rostrata* can easily be

**Growth parameters and green manure quality of *Sesbania rostrata* under different seeding rates in two farmers' field<sup>a</sup> sites.**

| Seeding rate<br>(kg ha <sup>-1</sup> ) | Plant density<br>( $\times 10^5$ ha <sup>-1</sup> ) |        | Biomass<br>production<br>(t dry matter ha <sup>-1</sup> ) |        | Plant dry<br>weight<br>(g) |      | N (%) |      | N<br>accumulation<br>(kg ha <sup>-1</sup> ) |     | C:N  |      |
|----------------------------------------|-----------------------------------------------------|--------|-----------------------------------------------------------|--------|----------------------------|------|-------|------|---------------------------------------------|-----|------|------|
|                                        | S1                                                  | S2     | S1                                                        | S2     | S1                         | S2   | S1    | S2   | S1                                          | S2  | S1   | S2   |
|                                        |                                                     |        |                                                           |        |                            |      |       |      |                                             |     |      |      |
| 100                                    | 2.8 e                                               | 2.9 b  | 2.4 e                                                     | 2.3 d  | 8.57                       | 7.93 | 2.71  | 2.47 | 65                                          | 57  | 16.6 | 18.2 |
| 140                                    | 4.0 d                                               | 3.7 b  | 2.8 d                                                     | 2.5 cd | 7.00                       | 6.76 | 2.75  | 2.32 | 77                                          | 58  | 16.4 | 19.4 |
| 210                                    | 6.0 c                                               | 5.6 b  | 3.8 c                                                     | 3.1 c  | 6.33                       | 5.54 | 2.76  | 2.61 | 105                                         | 81  | 16.3 | 17.2 |
| 250                                    | 44.4 b                                              | 46.2 a | 10.5 b                                                    | 9.8 b  | 2.36                       | 2.12 | 1.76  | 1.41 | 185                                         | 138 | 25.6 | 31.9 |
| 300                                    | 52.5 a                                              | 49.6 a | 11.3 a                                                    | 10.7 a | 2.15                       | 2.16 | 1.28  | 1.13 | 145                                         | 121 | 35.2 | 39.8 |
| LSD (0.05)                             | 0.97                                                | 3.50   | 0.29                                                      | 0.73   | —                          | —    | —     | —    | —                                           | —   | —    | —    |
| CV (%)                                 | 2.36                                                | 8.64   | 2.46                                                      | 6.86   | —                          | —    | —     | —    | —                                           | —   | —    | —    |

<sup>a</sup>Values in the same column followed by the same letter are not significantly different at 5% probability level. LSD = least significant difference, CV = coefficient of variation, S1 = site 1, S2 = site 2.

changed by varying its plant density. This has an important implication for achieving synchrony with *in situ* green manure crops. In practice, linear alternate plots of low and high plant densities in the field can produce green manure with contrasting quality. Green manure produced in this manner can be mixed and incorporated during land preparation to achieve better

synchrony between N release and demand in lowland rice.

## References

Myers RJK, Palm CA, Cuevas E, Gunatilleke IUN, Brossard M. 1994. The synchronization of nutrient mineralization and plant nutrient demand. In: Woomer PL, Swift MJ, editors. *The biological management of tropical soil fertility*. Chichester (UK): Wiley. p 81–116.

Seneviratne G. 2000. Litter quality and nitrogen release in tropical agriculture: a synthesis. *Biol. Fert. Soils* 31:60–64.

Seneviratne G, Kulasooriya SA. 1994. Fate of applied N in traditional, modern, and conservation farming systems of lowland rice in Sri Lanka. *Int. Rice Res. Notes* 19(1):18–19.

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# Low-cost management of iron toxicity in farmers' fields in Sierra Leone

I. Baggie and A.R. Bah, Rice Research Station, PMB 736, Freetown, Sierra Leone

Iron (Fe) toxicity affects up to 60% of the total cultivable inland valley swamps (IVS) in West Africa and reduces grain yield by up to 80%. Resource-poor farmers cannot afford to use high-cost technologies such as chemical amendments to reduce Fe toxicity. Over the years, on-station experiments have shown the great potential of organic amendments and toxicity-tolerant rice genotypes to improve Fe-toxic soils in the IVS. However, their adaptability in farmers' fields with varying soil types, water regimes, and extent and severity of Fe toxicity needs to be evaluated.

Two types of trials (to determine the effects of variety and organic amendment) were conducted on Fe-toxic IVS at 12 sites in Sierra Leone in the 1995–96 and 1996–97 seasons. Farmer groups from four villages participated. The soil characteristics varied and were in these ranges: 3.8–5.3 for pH in water, 3.6–4.1 for pH in KCl, 0.07–0.35% N, 0.4–3.5 mg Bray-1 P kg<sup>-1</sup> soil, 7.2–13.2% organic C, 3.1–6.9% Fe<sub>2</sub>O<sub>3</sub>, 31–75% sand, 12–31% silt, and 11–38% clay.

The first-season trials were managed by both farmers and researchers, whereas the second-season trials were farmer-managed. In the first trials, two rice genotypes, one tolerant (ROK24) and one

susceptible (ROK5), were grown unreplicated with the farmers' variety in 120-m<sup>2</sup> plots. Four-week-old seedlings were transplanted at 0.15 × 0.2-m spacing. Only N as urea was applied uniformly at 10 kg ha<sup>-1</sup>. Iron toxicity ratings were scored 7 wk after transplanting using the *Standard evaluation system for rice* (SES) developed at IRRI. Harvesting was done at physiological maturity; materials were sun-dried and weighed and the grain yield adjusted to 14% moisture content. The second trials involved three treatments with organic amendment (5t *Calopogonium mucunoides* ha<sup>-1</sup>, 5 t partially decomposed rice husk ha<sup>-1</sup>, and the control [no amendment]) using the farmers' variety as the test variety. Urea was also applied at 10 kg N ha<sup>-1</sup> and data were collected with the same method used in the first trials.

The selected sites were acidic and had low available P and high Fe. Tolerant genotype ROK24 significantly outyielded susceptible genotype ROK5 (Table 1). The mean grain yield increase in ROK24 over that of susceptible ROK5 and the local check was 90.4% and 57.6%, respectively. Overall, yields were higher in the first season than in the second season, presumably because of differences in management.

Incorporation of 5 t ha<sup>-1</sup> partially decomposed rice husk or *C. mucunoides* increased grain yield over the control by 76% and 77%, respectively (Table 2). This proves the ameliorating power of organic residues to correct Fe toxicity. Higher grain yield corresponds to lower Fe toxicity scores and vice versa, indicating a strong negative association between severity of Fe

**Table 1. Varietal differences in response to iron toxicity in farmers' fields over two seasons.<sup>a</sup>**

| Variety            | Effective tillers m <sup>-2</sup> (no.) | Grain yield (t ha <sup>-1</sup> ) |
|--------------------|-----------------------------------------|-----------------------------------|
| ROK24 (tolerant)   | 95                                      | 1.5 (3)                           |
| ROK5 (susceptible) | 53                                      | 0.8 (7)                           |
| Local              | 59                                      | 0.9 (5)                           |
| CV (%)             | 15.4                                    | 24                                |
| Variety            | 11                                      | 0.2                               |
| Site (replication) | ***                                     | ***                               |
| Season (year)      | **                                      | **                                |

<sup>a</sup>Numbers in parentheses indicate Fe toxicity score by the *Standard evaluation system for rice* (SES). \*\* and \*\*\* = statistically significant at 1% and 0.1%, respectively.

toxicity and grain yield. Tiller production showed a trend similar to that of grain yield. Differences between cropping seasons were not significant.

In conclusion, tolerant genotype ROK24 manifested a great potential for use in iron-toxic IVS. Some local rice genotypes showed a moderate degree of tolerance for Fe toxicity. Application of 5 t ha<sup>-1</sup> of partially decomposed rice husk or *C. mucunoides* alleviates Fe toxicity and increases rice yield in farmers' fields with varying water and toxicity regimes.

**Table 2. Effect of organic residue in alleviating grain yield reduction caused by iron toxicity in farmers' fields over two seasons.<sup>a</sup>**

| Organic amendment    | Effective tillers m <sup>-2</sup> (no.) | Grain yield (t ha <sup>-1</sup> ) |
|----------------------|-----------------------------------------|-----------------------------------|
| Decomposed rice husk | 74                                      | 1.4 (3)                           |
| <i>C. mucunoides</i> | 77                                      | 1.4 (3)                           |
| Control              | 55                                      | 0.8 (5)                           |
| CV (%)               | 11.2                                    | 23.1                              |
| Organic amendment    | 9                                       | 0.3                               |
| Site (replication)   | ***                                     | ***                               |
| Season (year)        | ns                                      | ns                                |

<sup>a</sup>Numbers in parentheses indicate Fe toxicity score by SES. ns = not statistically significant. \*\*\* = significant at 0.1%.

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# Growth of rice in modified microclimates of agroforestry

A. Kohli, Department of Soils, Punjab Agricultural University, Ludhiana; and B.C. Saini, Department of Soil Science, G.B. Pant University of Agriculture and Technology, Pantnagar, India  
E-mail: anshuman\_kohli@hotmail.com

In an agroforestry system, radiation received at the ground level is highly variable and consists of alternate patches of light and shade. The level of incident and diffuse radiation affects radiation-use efficiency in crops. Differences in air movement and radiation may also result in spatial variation in temperature and humidity. The importance of soil and floodwater temperatures in the rice crop has been emphasized by Collinson et al (1995). Modifying a crop's microclimate in agroforestry systems is therefore likely to affect crop photosynthesis and evapotranspiration. This study was carried out to measure some components of the microclimate in rice under trees and observe the growth parameters of rice in such modified microclimates.

Cultivar Saket 4 was transplanted at two seedlings hill<sup>-1</sup> on 8 Jul 1997 as a lowland puddled rice in an agroforestry system containing 8-y-old *Eucalyptus tereticornis* trees planted in a Nelder fan design (Nelder 1962), and in a control (sole crop) at Pantnagar (India). There were 15 tree-row orientations with each 24° from

the adjacent row (like a wheel with 15 spikes, each making a 24° angle with the adjacent spike), with 10 trees in each row enclosing a total of 15 plots between tree rows. Plots were serially numbered from 1 to 15 in a counterclockwise direction starting from a row oriented to 0° corresponding to the north direction. Trees in each row were at a distance of 2.0, 5.4, 13.5, 17.8, 21.5, 24.6, 27.4, 29.9, 32.2, and 34.4 m, respectively, from the center of the Nelder design, resulting in a constant tree stand of 333 trees ha<sup>-1</sup>. The trees had an average height of 15.3 m and an average diameter at breast height (DBH) of 16.6 cm. Canopy height, number of panicles and tillers, and grain yields were observed at a selected 1-m row in each plot and in the control. Observations on net radiation (R<sub>n</sub>), soil heat flux (G), air temperature (T<sub>a</sub>), and floodwater temperature (T<sub>w</sub>) were recorded at regular intervals from 0800 to 1700 on 30 Aug, when the crop was at the maximum tillering stage. The instantaneous values of R<sub>n</sub> and G were measured with net radiometers and soil heat flux plates (Middleton Instruments,

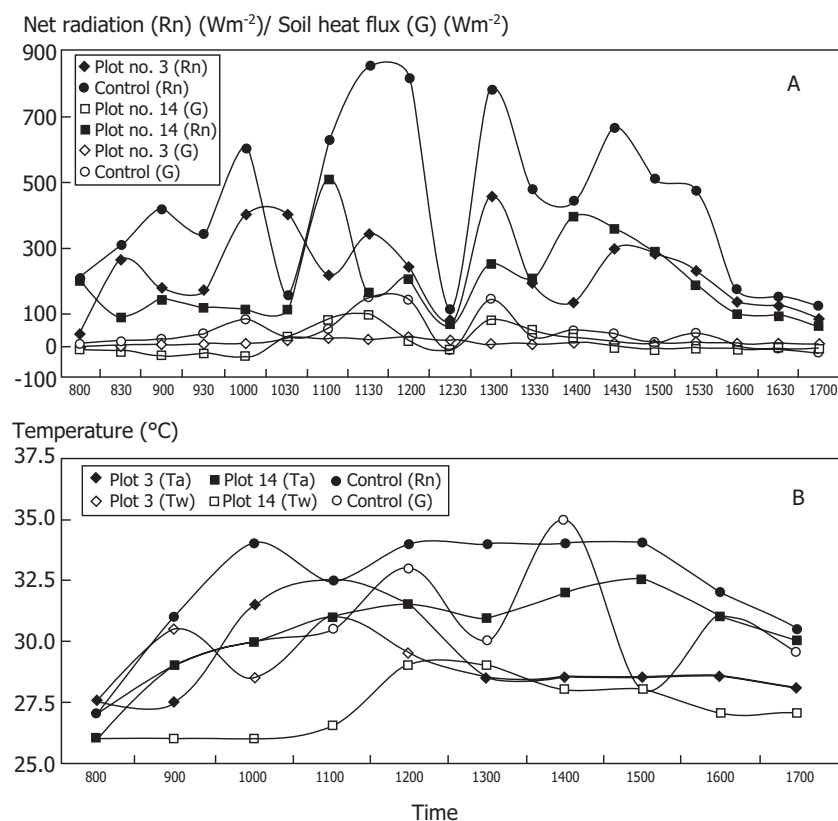
Australia), and read on portable battery-operated micro-voltmeters (Century Instruments, Chandigarh, India).

The observed growth parameters show a high spatial variation within the experimental area. The percentage of effective panicle-bearing tillers was 96%, 57%, 78%, and 89% in plots 3 (48–72°), 9 (192–216°), and 14 (312–336°) and the control, respectively. These show that the microclimatic conditions were better in plot 3 and poorer in plots 9 and 14 than in the control (see table). Canopy height was greater in plots 3 and 9 than in the control during part of the vegetative growth period (see table), indicating poor light availability at these sites. Grain yields were 6.0, 4.8, 5.4, and 5.1 t ha<sup>-1</sup> in plots 3, 9, 14, and the control, respectively.

The R<sub>n</sub> and G were greater in the control than in the agroforestry plots (see figure). In addition to variations in cloudiness and changes in the angle of the sun's rays during the day, in agroforestry, tree canopy shading and wind movement through the trees produce fluctuations in radiation received by the rice crop. The

**Number of tillers and panicles and canopy height at selected agroforestry sites and control, 1997.**

| Date of observation | Tillers 1-m row <sup>-1</sup> (no.) |        |         |         | Canopy height (cm) |        |         |         | Panicles 1-m row <sup>-1</sup> (no.) |        |         |         |
|---------------------|-------------------------------------|--------|---------|---------|--------------------|--------|---------|---------|--------------------------------------|--------|---------|---------|
|                     | Plot 3                              | Plot 9 | Plot 14 | Control | Plot 3             | Plot 9 | Plot 14 | Control | Plot 3                               | Plot 9 | Plot 14 | Control |
| 9 Aug               | 78                                  | 57     | 59      | 89      | 70                 | 50     | 38      | 57      | —                                    | —      | —       | —       |
| 16 Aug              | 72                                  | 64     | 61      | 89      | 88                 | 65     | 44      | 64      | —                                    | —      | —       | —       |
| 23 Aug              | 70                                  | 66     | 70      | 78      | 105                | 81     | 64      | 74      | —                                    | —      | —       | —       |
| 30 Aug              | 71                                  | 64     | 77      | 83      | 105                | 96     | 75      | 76      | —                                    | —      | —       | —       |
| 6 Sep               | 72                                  | 65     | 72      | 74      | 112                | 101    | 89      | 90      | —                                    | —      | —       | —       |
| 13 Sep              | 73                                  | 65     | 73      | 69      | 112                | 107    | 92      | 98      | —                                    | —      | —       | —       |
| 18 Sep              | —                                   | —      | —       | —       | —                  | —      | —       | —       | 18                                   | 4      | 0       | 7       |
| 20 Sep              | 73                                  | 68     | 73      | 66      | 112                | 107    | 98      | 112     | 51                                   | 6      | 3       | 10      |
| 22 Sep              | —                                   | —      | —       | —       | —                  | —      | —       | —       | 60                                   | 17     | 8       | 30      |
| 25 Sep              | —                                   | —      | —       | —       | —                  | —      | —       | —       | 68                                   | 27     | 17      | 45      |
| 27 Sep              | 71                                  | 72     | 65      | 56      | 112                | 105    | 102     | 120     | —                                    | —      | —       | —       |
| 29 Sep              | —                                   | —      | —       | —       | —                  | —      | —       | —       | 67                                   | 41     | 34      | 50      |
| 2 Oct               | —                                   | —      | —       | —       | —                  | —      | —       | —       | 67                                   | 41     | 49      | 50      |
| 9 Oct               | 71                                  | 72     | 65      | 56      | 112                | 105    | 102     | 120     | 67                                   | 41     | 51      | 50      |



**Diurnal pattern of net radiation (Rn) and soil heat flux (G) (A), and air temperature (Ta) and floodwater temperature (Tw) (B) in rice at selected agroforestry sites and control, 30 Aug 1997.**

magnitude of these fluctuations in Rn, observed on a short-term (second) basis, has a profound effect on crop growth (Knapp and Smith 1990). The development rate of rice is highest at

24–26 °C (Summerfield et al 1992). In this study, air and floodwater temperatures were far greater than this optimum range in the sole crop than in agroforestry (see figure).

Modified microclimates and differences in crop growth parameters can have profound effects on rice yields. The data set presented here is limited because it only indicates possible responses that could be expected under agroforestry. The data, however, suggest options for modifying the crop microclimate by using tree row spacing, orientation, and pruning as management tools in agroforestry. Hence, apart from modifying the crop microclimate by manipulating the canopy architecture through breeding programs, obtaining favorable growth parameters at the same levels of input use under agroforestry conditions can also be a viable management tool.

## References

- Collinson ST, Ellis RH, Summerfield RJ, Roberts EH. 1995. Relative importance of air and floodwater temperatures on the development of rice (*Oryza sativa*). Exp. Agric. 31:151–160.
- Knapp AK, Smith WK. 1990. Stomatal and photosynthetic responses to variable sunlight. Physiol. Plant 78:160–165.
- Nelder JA. 1962. New kinds of systematic designs for spacing experiments. Biometrics 18:283–301.
- Summerfield RJ, Collinson ST, Ellis RH, Roberts EH, Penning De Vries FWT. 1992. Photothermal responses of flowering in rice (*Oryza sativa*). Ann. Bot. 69:101–112.

# Seeding density of rice and its effects on ratoon crop

D.Alfonso-Morel, D.A.Althoff, and R.C. Dittrich, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A.-EPAGRI; E.E. Urussanga, Cx P. 49, Urussanga-SC, CEP 88.840-000, Brazil E-mail: dario@epagri.rct-sc.br

We studied the effect of seeding density on the main crop and on a ratoon crop in Santa Catarina State, southern Brazil, located at 28° 56' S in the summer of 1994, 1995, and 1996. The field experiments were conducted in a randomized complete block design with five treatments in 1994 and six in the other 2 years, in four replications at the same site in the 3 years. The soil at the experimental site was Cambicisol Gley. Fertilizer applications for the main crop were 90-40-60 kg NPK ha<sup>-1</sup> as urea,

triple superphosphate, and potassium chloride.

An early maturing cultivar (115 d), EPAGRI 106, was seeded with pregerminated seeds in an irrigated lowland rice field during the recommended period (15 October–15 November) at the following treatment rates (kg ha<sup>-1</sup>): 130 (recommended); 130 + 25% of recommended rate (rr); 130 + 50% rr; 130 + 75% rr; and 130 + 100% rr (260 kg ha<sup>-1</sup>). In the last 2 years, one more treatment with 100

kg ha<sup>-1</sup> was added. The cycle of the main crop (115 d) plus the ratoon (60 d) was 175 d. All data were subjected to analysis of variance (ANOVA) with the F test and linear regression, estimating the response for linear or quadratic effect whenever these effects were significant (F test,  $P < 0.05$ ). The effect of density on yield of the main crop was not significant for any of the 3 years. For the last 2 years, the density × year interaction was not significant (see table).

**Grain yield (t ha<sup>-1</sup>) of main crop and ratoon crop.**

| Treatment                                        | Year |      |      | Av of<br>1995-96 |
|--------------------------------------------------|------|------|------|------------------|
|                                                  | 1994 | 1995 | 1996 |                  |
| <i>Main crop</i>                                 |      |      |      |                  |
| 100 kg ha <sup>-1</sup>                          | —    | 6.7  | 8.3  | 7.5              |
| 130 kg ha <sup>-1</sup>                          | 5.8  | 6.8  | 8.2  | 7.5              |
| 130 kg ha <sup>-1</sup> + 25% of rr <sup>a</sup> | 5.3  | 7.1  | 8.3  | 7.7              |
| 130 kg ha <sup>-1</sup> + 50% of rr              | 5.3  | 7.0  | 8.3  | 7.7              |
| 130 kg ha <sup>-1</sup> + 75% of rr              | 7.5  | 7.3  | 8.1  | 7.7              |
| 130 kg ha <sup>-1</sup> + 100% of rr             | 5.5  | 6.9  | 8.3  | 7.6              |
| Av                                               | 5.9  | 7.0  | 8.2  | 7.6              |
| CV (%)                                           | 19.2 | 4.8  | 3.5  | 4.1              |
| <i>Ratoon crop</i>                               |      |      |      |                  |
| 100 kg ha <sup>-1</sup>                          | —    | 2.2  | 2.6  | 2.4              |
| 130 kg ha <sup>-1</sup>                          | 0.9  | 2.4  | 2.6  | 2.5              |
| 130 kg ha <sup>-1</sup> + 25% of rr              | 0.8  | 2.6  | 2.6  | 2.6              |
| 130 kg ha <sup>-1</sup> + 50% of rr              | 0.6  | 2.4  | 2.5  | 2.5              |
| 130 kg ha <sup>-1</sup> + 75% of rr              | 0.6  | 2.4  | 2.6  | 2.5              |
| 130 kg ha <sup>-1</sup> + 100% of rr             | 0.6  | 2.3  | 2.5  | 2.4              |
| Av                                               | 0.7  | 2.4  | 2.6  | 2.5              |
| CV (%)                                           | 11.6 | 9.1  | 6.4  | 7.8              |

<sup>a</sup>rr = recommended rates.

In all treatments, the main crop lodged because of heavy rain during grain filling, thus reducing crop yields, particularly ratoon crop yields. Thus, the 1994 ratoon crop data were not analyzed. For 1995 and 1996, neither the density × year interaction nor the main effect of density was significant for the ratoon crop (see table).

Since an increase in seeding density did not have a significant effect on ratoon grain yield or on main crop yield, it is recommended that the normal seeding density (130 kg ha<sup>-1</sup>) be used. During years when lodging is a problem in the main crop, ratoon production is not recommended.

## Effect of seeding time and fertilizer rate on rice ratooning

D.Alfonso-Morel, D.Althoff, and R.C. Dittrich, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A.-EPAGRI. E.E. de Urussanga, Cx. P. 49, Urussanga-SC, CEP 88.840-000, Brazil E-mail: epagri@epagri.rct-sc.br

Experiments were conducted to study the effect of (1) seeding time of the main crop on the ratoon crop, planted in observation plots, and (2) fertilizer rate on the ratoon crop in Santa Catarina State, southern Brazil, in the summer of 1994, 1995, and 1996. The soil at the experimental site was a Cambicisol Gley.

An early maturing cultivar (115 d), EPAGRI 106, was seeded in both experiments using pregerminated seeds in an irrigated lowland rice field at the recommended rate of 130 kg ha<sup>-1</sup>. Observation plots (experiment 1) were seeded at three different times: early (20 September–10 October), normal (recommended, 15 October–15 November), and late (20 November–20 December), with three replications in each trial.

In experiment 2, seeding was done at the recommended time in a randomized complete block design with four replications and six fertilizer treatments.

The soil used in this experiment had a pH of 4.7; organic matter, 2.1%; P, 2.5 ppm; and K, 60 ppm. The complete cycle of the main crop (115 d) and ratoon crop (60 d) was 175 d. The six fertilizer treatments applied on the ratoon crop were fractions of that applied on the main crop, which was the recommended rate according to the soil analysis (90-40-60 of NPK). The treatments were (1) 1/2 of N, (2) 1/4 of N, (3) 1/2 of NPK, (4) 1/2 of NK, (5) 1/2 of NP, and (6) no fertilizer. All treatments were applied right after harvesting the main crop and then flooding.

In 1994, the main and ratoon crops lodged in all treatments because of heavy rain, resulting in a large yield reduction in all experiments.

For seeding time, only the early seeding date produced a good main crop and ratoon crop together (Table 1). For fertilizer rate, treatment 1 had the same results as the untreated plot (no. 6). The other treatments had statistically higher yields according to Dunnett's one-tailed T-test with  $P < 0.05$  (Table 2).

**Table 1. Grain yield (t ha<sup>-1</sup>) of main crop and ratoon seeded at three different times.**

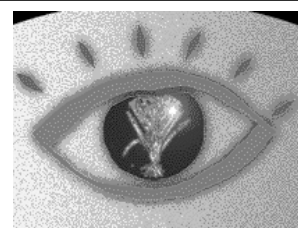
| Treatment | 1994      |             | 1995 |             | 1996 |        | Main crop + ratoon |      |      |
|-----------|-----------|-------------|------|-------------|------|--------|--------------------|------|------|
|           | Main crop | Ratoon crop | Main | Ratoon crop | Main | Ratoon | 1994               | 1995 | 1996 |
| Early     | 6.4       | 1.5         | 4.9  | 2.1         | 6.3  | 2.0    | 7.9                | 7.0  | 8.3  |
| Normal    | 6.3       | 2.1         | 5.7  | 1.5         | 4.1  | 0      | 8.4                | 7.2  | 4.1  |
| Late      | 4.6       | 0           | 5.3  | 0           | 4.5  | 0      | 4.6                | 5.3  | 4.5  |



**Table 2. Grain yield (t ha<sup>-1</sup>) of main crop and ratoon under various fertilizer treatments.**

| Treatment     | 1994      |        | 1995      |        | 1996      |              | Av        |              |
|---------------|-----------|--------|-----------|--------|-----------|--------------|-----------|--------------|
|               | Main crop | Ratoon | Main crop | Ratoon | Main crop | Ratoon       | Main crop | Ratoon       |
| 1/2 of N      | 5.4       | 1.2    | 6.2       | 2.3    | 4.9       | 2.2          | 5.5       | 1.9          |
| 1/4 of N      | 5.1       | 1.2    | 6.5       | 2.4    | 4.7       | 2.3          | 5.4       | 2.0          |
| 1/2 of NPK    | 5.2       | 1.3    | 6.2       | 2.4    | 6.0       | 2.5          | 5.8       | 2.1          |
| 1/2 of NK     | 5.4       | 1.4    | 6.4       | 2.7    | 6.2       | 2.1          | 6.0       | 2.0          |
| 1/2 of NP     | 5.6       | 1.4    | 6.4       | 2.5    | 6.1       | 2.2          | 6.0       | 2.0          |
| No fertilizer | 5.2       | 1.2    | 6.5       | 2.3    | 6.0       | 1.8          | 5.9       | 1.8          |
| CV (%)        | —         | 17.1   | —         | 7.2    | —         | 8.7          | —         | 9.9          |
| F test        | —         | ns     | —         | ns     | —         | <sup>a</sup> | —         | <sup>a</sup> |

<sup>a</sup>Significant at  $P < 0.05$ . ns = not significant..



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## Rooting inhibitors in soil solution under an intensive rice-cropping system

S. Kubota, IRRI, Philippines; Jong-Ho Seo, National Crop Experiment Station, Rural Development Administration, Suwon, Korea; E. Laureles, J. Padilla, and O. Ito, IRRI E-mail: s.kubota@cgiar.org

The productivity of double- and triple-rice cropping conducted at IRRI over the last 30 years has been shown to be declining (Cassman et al 1995). Although extensive studies on various aspects such as soil nutrient supply, deficiency and/or toxicity of nutrients, and climate change over time have been carried out to identify the causes, the phenomenon has not been fully understood. Some toxic substances, accumulated under anaerobic conditions after amendment of plant residues, have been reported to inhibit root elongation (Tanaka et al 1990). Appropriate development and function of root systems are essential for satisfactory yield performance of the crop. This study was conducted to determine the presence of a rooting inhibitor in a soil solution collected from IRRI fields subjected to intensive rice cropping.

Soil samples were collected from 0- to 30-cm depths in fertilized plots under the long-term continuous cropping experiment (LTCCE) and long-term fertility experiment (LTFE) at IRRI (see table). A soil solution (500 mL) was extracted using a centrifuge (560 g for 10 min at 20 °C) and its pH adjusted to 3.5. The soil solu-

tion was loaded in 5 g of Amberlite XAD-8. The column was eluted with 50% ethanol, 100% ethanol, and chloroform for crude fractionation of substances according to water solubility. These three fractions were completely evaporated in vacuo at 40 °C and dissolved in 25 mL of phosphate buffer (66.7 mM at pH 6.8). Dehusked seeds of IR72 were pregerminated in the dark at 30 °C; seedlings with 5-mm-long radicles were then selected for bioassay. Ten seedlings were placed into a 50-mL beaker containing 5 mL of the test solution. Radicle lengths were measured 72 h after incubation in the dark at 30 °C. A set of seedlings was incubated with phosphate buffer only and used as the control. All measurements were made with five replications.

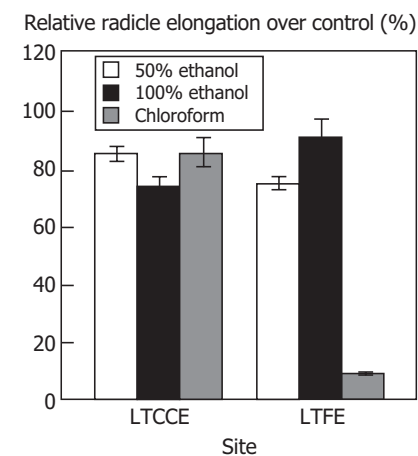
In the LTCCE, radicle elongation

was moderately inhibited in each fraction (see figure). The elongation was least in the 100% ethanol fraction. In the LTFE, the chloroform fraction, which contains the least water-soluble substances, showed drastic inhibitory effects, reducing the elongation to less than 20% of the control. Results suggest the presence of some rooting inhibitors in the soil solution of LTCCE and LTFE plots at IRRI. Tanaka et al (1990) reported inhibitory effects on rice root elongation in the ether fraction. This fraction is identical to the chloroform fraction in this study, from rice soil amended with wheat straw. Major substances in the fraction were identified as two aromatic acids, 2-phenylpropionic acid and 3-phenylpropionic acid. The inhibitory effect was greater in LTFE plots

**Sampling date of soils and management of fields under long-term continuous cropping experiment (LTCCE) and long-term fertility experiment (LTFE), IRRI, Philippines.**

| Site  | Sampling date       | Cropping frequency      | Residue management | Water management     |
|-------|---------------------|-------------------------|--------------------|----------------------|
| LTCCE | 14 DBT <sup>a</sup> | 3 times y <sup>-1</sup> | Remove             | Irrigated year-round |
| LTFE  | 21 DAT <sup>b</sup> | 2 times y <sup>-1</sup> | Incorporate        | Irrigated year-round |

<sup>a</sup>Days before transplanting, 1999 dry season. <sup>b</sup>Days after transplanting, 1999 dry season.



**Inhibitory effects of the soil solution on radicle elongation of IR72, LTCCE and LTFE, IRRI.**

into which plant residues have been incorporated after every harvest than in LTCCE plots from where residues have been removed. Thus, it is assumed that inhibitory substances in the chloroform fraction may be derived from plant residues.

The concentration of the test solution applied for bioassay was 20 times that of the original. Hence, it should be further confirmed whether the growth and yield of rice plants in LTCCE and LTFE plots are directly affected through root development by those substances under practical conditions. The chemical identification of the substances, especially in the chloro-

form fraction, is the subject of another study.

## References

- Cassman KG, de Datta SK, Olk DC, Alcantara J, Samson M, Descalsota J, Dizon M. 1995. Yield decline and the nitrogen economy of long-term experiments on continuous, irrigated rice systems in the tropics. In: Lai R, Stewart BA, editors. Soil management: experimental basis for sustainability and environmental quality. Boca Raton, Florida: CRC Press Inc.
- Tanaka F, Ono S, Hayasaka T. 1990. Identification and evaluation of toxicity of rice root elongation inhibitors in flooded soils with added wheat straw. Soil Sci. Plant Nutr. 36:97–103.

# Yield response of different rice varieties to root damage at the heading stage

Cai Kunzheng, Luo Shiming, and Duan Shunshan, Institute of Tropical and Subtropical Ecology, South China Agricultural University, Guangzhou 510642, China E-mail: kzcai@scau.edu.cn

Root systems play an important role in the absorption and storage of water and nutrients and in the synthesis of certain hormones. Root cutting may increase the growth and yield of rice because the soil disturbance resulting from root cutting has some beneficial effect on soil conditions, or excess root production may reduce shoot growth because it competes for carbohydrates. Moreover, differences among soils, varieties, and management systems exist. To determine whether a large root system is beneficial to yield, we conducted a field experiment on the effect of root cutting at the heading stage in Guangzhou, China, in 1998. The soil was a clay loam, fertilized with 150 kg N, 33 kg P, and 124.5 kg K ha<sup>-1</sup>. Five varieties were grown in the early-season and late-season experiments: Guang-Lu-Ai (short stalk) and Er-Qing-Ai (tall stalk) for the early season (March–July); Pei-Za 72 (hybrid rice), Feng-Ai Zhan 1, and Te-San-Ai for the late season (July–November).

Thirty-day-old seedlings were transplanted at 20 × 20-cm spacing at two seedlings hill<sup>-1</sup>. The root systems of treated blocks were vertically cut 1 cm away from the stalk to a 20-cm depth on one, two, or three sides of the plant (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, respectively) at the heading stage. No cutting was done in the control variety (check). Treatments were arranged in a randomized complete block design with three replications. Block size was 15 m<sup>2</sup>. Root activity was measured in terms of  $\alpha$ -naphthylamine-oxidizing activities with a spectrometer. Yield and yield components were analyzed and recorded at maturity.

Table 1 shows that root cutting at the heading stage significantly increased root activity at the ripening stage in general, irrespective of varieties and treatments. The remaining roots evidently compensated for the ones removed.

In Table 2, one-side cutting (T<sub>1</sub>) increased yield in four varieties (Guang-Lu-Ai, 10.2%; Pei-Za 72, 4.6%; Feng-Ai Zhan 1, 12.8%; and Te-San-Ai, 11.4%) and decreased yield in one (Er-Qing-Ai, 18.2%). The difference was mainly associated with a difference in grains panicle<sup>-1</sup> and percentage filled grains. Two-side cutting (T<sub>2</sub>) and three-side cutting (T<sub>3</sub>) caused

**Table 1. Effect of root cutting at heading stage on root activity ( $\mu$ g  $\alpha$ -NA g<sup>-1</sup> DW h<sup>-1</sup>).**

| Treatment      | Variety <sup>a</sup> |            |           |                |           |
|----------------|----------------------|------------|-----------|----------------|-----------|
|                | Guang-Lu-Ai          | Er-Qing-Ai | Pei-Za 72 | Feng-Ai Zhan I | Te-San-Ai |
| Check          | 43.7 b               | 87.5 b     | 96.4 c    | 59.2 b         | 84.2 b    |
| T <sub>1</sub> | 48.3 b               | 111.8 a    | 108.2 b   | 73.4 a         | 89.3 ab   |
| T <sub>2</sub> | 111.4 a              | 109.9 a    | 123.5 a   | 70.2 a         | 93.4 a    |
| T <sub>3</sub> | 101.7 a              | 102.3 a    | 109.5 b   | 68.4 a         | 90.5 a    |

<sup>a</sup>Numbers followed by different letters in a column significantly differ at P = 0.05.

**Table 2. Effect of root cutting at heading stage on yield and yield components of different rice varieties.<sup>a</sup>**

| Variety        | Treatment      | Effective panicles (no. m <sup>-2</sup> ) | Grains per panicle (no.) | Filled grains (%) | 1,000-grain weight (g) | Yield (t ha <sup>-1</sup> ) | Yield difference (%) |
|----------------|----------------|-------------------------------------------|--------------------------|-------------------|------------------------|-----------------------------|----------------------|
| Guang-Lu-Ai    | Check          | 300.0 a                                   | 90 a                     | 86.4 a            | 25.9 a                 | 6.0 b                       | 0.0                  |
|                | T <sub>1</sub> | 312.5 a                                   | 94 a                     | 88.9 a            | 25.5 a                 | 6.6 a                       | 10.2                 |
|                | T <sub>2</sub> | 282.5 b                                   | 95 a                     | 88.5 a            | 25.8 a                 | 6.1 b                       | 1.4                  |
|                | T <sub>3</sub> | 292.5 b                                   | 94 a                     | 87.0 a            | 25.4 a                 | 6.1 b                       | 0.6                  |
| Er-Qing-Ai     | Check          | 270.0 a                                   | 155 a                    | 85.1 a            | 23.8 a                 | 8.5 a                       | 0.0                  |
|                | T <sub>1</sub> | 260.0 a                                   | 143 b                    | 78.0 b            | 23.9 a                 | 6.9 b                       | -18.2                |
|                | T <sub>2</sub> | 250.0 a                                   | 144 b                    | 83.2 a            | 23.3 a                 | 7.0 b                       | -17.7                |
|                | T <sub>3</sub> | 270.0 a                                   | 133 c                    | 84.2 a            | 24.3 a                 | 7.4 b                       | -13.3                |
| Pei-Za 72      | Check          | 243.8 a                                   | 255 a                    | 71.0 a            | 19.2 a                 | 8.5 a                       | 0.0                  |
|                | T <sub>1</sub> | 233.3 a                                   | 267 a                    | 74.5 a            | 19.1 a                 | 8.9 a                       | 4.6                  |
|                | T <sub>2</sub> | 247.3 a                                   | 241 b                    | 71.4 a            | 19.3 a                 | 8.2 a                       | -3.1                 |
|                | T <sub>3</sub> | 225.0 b                                   | 234 b                    | 66.7 b            | 19.2 a                 | 6.7 b                       | -20.4                |
| Feng-Ai Zhan 1 | Check          | 246.5 ab                                  | 214 a                    | 63.1 b            | 20.6 a                 | 6.8 a                       | 0.0                  |
|                | T <sub>1</sub> | 247.3 ab                                  | 191 b                    | 78.6 a            | 20.7 a                 | 7.7 a                       | 12.8                 |
|                | T <sub>2</sub> | 236.3 b                                   | 171 c                    | 64.4 b            | 19.9 a                 | 5.2 b                       | -24.5                |
|                | T <sub>3</sub> | 267.8 a                                   | 189 b                    | 52.9 c            | 20.2 a                 | 5.4 b                       | -21.2                |
| Te-San-Ai      | Check          | 207.5 b                                   | 172 a                    | 66.9 b            | 28.9 a                 | 6.9 b                       | 0.0                  |
|                | T <sub>1</sub> | 220.0 a                                   | 169 b                    | 73.3 a            | 28.2 a                 | 7.7 a                       | 11.4                 |
|                | T <sub>2</sub> | 227.5 a                                   | 166 b                    | 69.3 ab           | 28.4 a                 | 7.4 ab                      | 7.7                  |
|                | T <sub>3</sub> | 212.5 a                                   | 180 a                    | 66.1 b            | 27.5 b                 | 7.0 b                       | 0.8                  |

<sup>a</sup>Numbers followed by different letters in a column in the same variety significantly differ at  $P = 0.05$ .

yield increases in two varieties (Guang-Lu-Ai, Te-San-Ai) and yield decreases in the others (Er-Qing-Ai, Pei-Za 72, Feng-Ai Zhan 1).

It can be concluded that light root cutting at the heading stage has no significant effect on rice yield. It can even increase the yield of some varieties. This

may be because root cutting can increase root activity at maturity, which is important in absorbing water and nutrients and delaying plant senescence.

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# Equilibrium moisture content of rice and maize upon drying

T.M. Lando and R.Arief, Research Institute for Maize and Other Cereals (RIMOC), P.O. Box 1173, Makassar, South Sulawesi, Indonesia E-mail: balitjas@upg.mega.net

Cereal grain is ordinarily harvested at moisture contents above safe storage levels; therefore, additional drying is usually needed (Robayo and Pfof 1973). During drying, heat is necessary to evaporate moisture from the grain and a flow of air is needed to carry away the evaporated moisture (Trim and Robinson 1994).

The equilibrium moisture content (EMC) of cereal grain is important in both drying and storage because of its relationship with the relative humidity

(RH) and temperature of the air. Crop grains (including cereals) are hygroscopic and will lose or gain moisture until equilibrium is reached with the surrounding air (Henderson and Perry 1955, Trim and Robinson 1994).

This experiment observed the interaction between water and crop grain by measuring EMC as a function of RH at constant temperature. A plot of the equilibrium RH and moisture content (MC) is known as the moisture curve.

The drying experiment was conducted at RIMOC in Maros, South Sulawesi, Indonesia, to study the equation/curve of EMC by using a mechanical batch-type dryer with a grain bin in a 5-m<sup>2</sup> (2 × 2.5 m) rectangular box, 0.9 m tall, and holding 1–1.5 t of rice or maize at a depth of 0.35 m. The drying air temperature and drying air velocity were 43 °C and 50 m<sup>3</sup> min<sup>-1</sup>, respectively.

Varieties IR42 (rice) and Bisma (maize) were used in this experiment. The

following equation was based on Henderson's equation (1952):

$$1 - RH = e^{-cT EMC^n}$$

where

RH = equilibrium relative humidity (decimal),

EMC = equilibrium moisture content (%),

T = temperature (°C),

c and n = constants, and

e = 2.7182.

To calculate c and n, Henderson's equation was multiplied by ln (Robayo and Pfost 1973):

$$\ln(1 - RH) = -cT EMC^n \ln e$$

$$\ln \ln \frac{1}{1 - RH} = \ln c + \ln T + n \ln EMC$$

if

$$\ln \ln \frac{1}{1 - RH} = y$$

$$\ln c + \ln T = b$$

$$\ln EMC = x$$

$$n = a$$

to get the following equation:

$$y = b + ax$$

By using the least squares method, a, b, r (correlation coefficient between soil moisture content and relative humidity), c, and n can be found.

$$\sum y = b m + a \sum x$$

$$\sum xy = b \sum x + a \sum x^2$$

$$b = \frac{\sum y - a \sum x}{m}$$

$$a = \frac{\sum xy - \frac{\sum x \sum y}{m}}{\sum x^2 - \frac{(\sum x)^2}{m}}$$

$$r = \frac{\sum xy - \left[ \frac{\sum x \sum y}{m} \right]}{\sqrt{\left[ \sum x^2 - \frac{(\sum x)^2}{m} \right] \left[ \sum y^2 - \frac{(\sum y)^2}{m} \right]}}$$

where

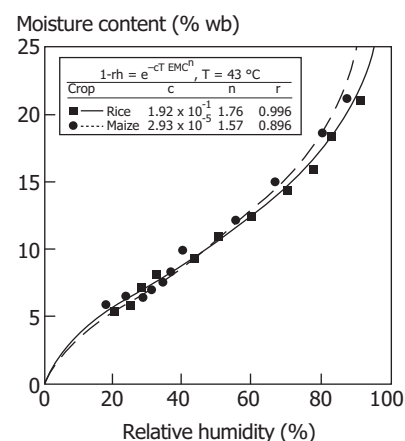
r = coefficient correlation between moisture content and relative humidity

m = drying periods (hours = ii)

At the start of drying, RH for drying rice decreased from 92% to 79%, causing MC to decrease fast from 21.0% to 16.5% (average drying rate = 2.2% h<sup>-1</sup>). For maize, RH decreased from 88% to 67%, resulting in a rapid decline of MC from 21.2% to 15.2% (average drying rate = 2.9% h<sup>-1</sup>). A decrease in RH from 71% to 20% reduced MC in rice further from 14.5% to

5.6% (drying rate = 2.0–0.6% h<sup>-1</sup>), whereas a decrease in RH from 56% to 19% resulted in a decrease in MC in maize from 12.3% to 6.2% (drying rate = 2.2–0.2% h).

From the data in the table, constants c and n could be calculated:  $1.92 \times 10^{-5}$  and 1.76 for rice, and  $2.93 \times 10^{-5}$  and 1.57 for maize constants c and n values depend on the material, which affect the shape and slope of the EMC curve. The EMC for rice and maize is 5.6% and 5.2% at 20% RH, and 15.7% and 16.7% at 75% RH, respectively (see figure).



**Equilibrium moisture curve for rice and maize at 43 °C (569.4 °R).**

## References

- Henderson SM. 1952. A basic concept of equilibrium moisture. *Agric. Eng.* 33(7): 29–32.
- Henderson SM, Perry RL. 1955. *Agricultural process engineering*. New York: John Wiley & Sons, Inc. p 273–301.
- Robayo JF, Pfost HB. 1973. Rice drying rates. MS thesis. Food and Feed Grain Institute, Kansas State University, Manhattan, Kansas (unpubl.)
- Trim DS, Robinson AP. 1994. Drying methods. In: Proctor DL, editor. *Grain storage techniques: evolution and trends in developing countries*. Rome (Italy): FAO. p 89–134.

### Relationship between relative humidity and moisture content at 43 °C.

| Drying period (h) | Grain type          |                        |                     |                        |
|-------------------|---------------------|------------------------|---------------------|------------------------|
|                   | Rice                |                        | Maize               |                        |
|                   | RH <sup>a</sup> (%) | MC <sup>b</sup> (% wb) | RH <sup>a</sup> (%) | MC <sup>b</sup> (% wb) |
| 1                 | 92                  | 21.0                   | 88                  | 21.2                   |
| 2                 | 83                  | 18.3                   | 82                  | 18.2                   |
| 3                 | 79                  | 16.5                   | 67                  | 15.2                   |
| 4                 | 71                  | 14.5                   | 56                  | 12.3                   |
| 5                 | 60                  | 12.7                   | 40                  | 10.1                   |
| 6                 | 50                  | 11.2                   | 37                  | 8.3                    |
| 7                 | 44                  | 9.5                    | 34                  | 7.6                    |
| 8                 | 33                  | 8.1                    | 32                  | 7.1                    |
| 9                 | 29                  | 6.8                    | 30                  | 6.7                    |
| 10                | 25                  | 6.2                    | 24                  | 6.4                    |
| 11                | 20                  | 5.6                    | 19                  | 6.2                    |

<sup>a</sup>RH = relative humidity (%). <sup>b</sup>MC = moisture content (% wb, percent wet basis).

**Aggarwal RM. 2000. Possibilities and limitations to cooperation in small groups: the case of group-owned wells in southern India. *World Dev.* 28(8):1481-1497.**

In recent years, many donors and governments have accepted the view that irrigation systems will serve farmers better if the systems foster participation and cooperation from farmers. This paper explores the determinants of cooperation and the ability to undertake collective action among groups of farmers who collectively own wells in southern India. It was found that the degree of cooperation achieved depends on the activity, even in small groups such as the ones explored in this study (group size is typically 8 people or less). For example, nearly all of these small groups were found to effectively allocate water among group members and undertake routine maintenance activities. This is attributed to the fact that such activities require frequent interaction and because farmers have credible retaliatory strategies in case some farmers do not cooperate (e.g., water can be denied to nonparticipating farmers).

However, it was found that cooperation was limited in expansion activities (i.e., digging new wells), although the groups in this study were small and all had been in existence for at least 10 years. The author hypothesizes that, despite the potential advantages of pooling capital and sharing risks among group members, cooperation was deterred by several factors, one of which is high risk. For example, typically there is just a 50-50 chance of finding water, even with the assistance of a geologist. Also, it can be difficult to obtain electricity to operate the pump even if the drill-

ing is successful, with farmers sometimes having to wait 4 or 5 years for a connection. In addition to risks, there is also a lack of retaliatory strategies that can be used if one party reneges on an obligation during expansion activities. For example, it may be difficult to force another member to pay money if the first drilling unexpectedly encounters heavy rock that requires additional expenses for blasting. These findings suggest that cooperation will be successful only for certain activities, even in settings that are seemingly favorable to the emergence of cooperation (in this case, small, long-established groups).

**Ahn JK, Chung IM. 2000. Allelopathic potential of rice hulls on germination and seedling growth of barnyardgrass. *Agron. J.* 92:1162-1167.**

Scientists have been studying the allelopathic effects of rice as a potential tool for weed control. Rice leaf and straw extracts, decomposing rice straw, and the soil where rice was grown were reported to have allelopathic effects. Barnyardgrass is one of the major weeds in the irrigated rice system, especially in direct-seeded rice. The objectives of this study were to determine the effect of aqueous hull extracts from 91 rice cultivars on barnyardgrass germination and seedling growth and to compare the allelopathic potential of various concentrations of hot and warm water rice hull extracts.

There was a significant difference among 91 rice cultivars in inhibiting barnyardgrass germination using rice hull extracts, ranging from 6% for cultivar Nonglimna 1 to 59% for cultivar SR31. The length and dry weights of roots were more

reduced by hull extracts than those of shoots. The extract from Janganbyeo hull had the greatest inhibition effect on total seedling length (75% reduction) and dry weight (96% reduction) of barnyardgrass seedlings. Warm extracts had a greater inhibitory effect on barnyardgrass germination and seedling growth than hot extracts. The highest concentration (8 g L<sup>-1</sup>) of warm water extract resulted in the greatest inhibition.

These results suggest that rice hull extracts may be a source of natural herbicide, and warm water may extract more allelochemicals than hot water. Since there was a genotypic variation in rice hull extracts for allelopathic potential on barnyardgrass, it is possible to develop rice cultivars with greater allelopathic potential through plant breeding.

**Tu J, Zhang G, Datta K, Xu C, He Y, Zhang Q, Khush GS, Datta SK. 2000. Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis*  $\delta$ -endotoxin. *Nature Biotechnol.* 18:1101-1104.**

**Shu Q, Ye G, Cui H, Cheng X, Xiang Y, Wu D, Gao M, Xia Y, Hu C, Sardana R, Altosaar I. 2000.**

**Transgenic rice plants with a synthetic *cryIAb* gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. *Mol. Breed.* 6:433-439.**

**Ye G, Shu Q, Yao H, Cui H, Cheng X, Hu C, Xia Y, Gao M, Altosaar I. 2001. Field evaluation of resistance of transgenic rice containing a synthetic *cryIAb* gene from *Bacillus thuringiensis* Berliner to two stem borers. *J. Econ. Entomol.* 94:271-276.**

These papers are the first reports published in international journals on field trials of rice genetically engineered with toxin genes from *Bacillus thuringiensis* (Bt). The small-scale trials took place in China in the provinces of Hubei in 1999 (Tu et al 2000), Hainan and Zhejiang in 1998 (Shu

## Notice of clarification

The following corrections and clarifications have been submitted by the authors of the paper "Improvement of conjugation methods for *Xanthomonas oryzae* pv. *oryzae* strain DY89031 to identify *avrXa21* clones", IRRN 25(2):24, by P.K. Sharma, F.G. da Silva, Y. Shen, and P.C. Ronald.

1. R.C. Yadav (Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar 125004 India) should be added as the third author.
2. The title should be changed to "Improvement of conjugation methods for *Xanthomonas oryzae* pv. *oryzae* strain DY89031".
3. The last three sentences should be deleted.

et al 2000), and Zhejiang in 1998 (Ye et al 2001). Tu et al (2000) tested transgenic versions of the CMS restorer line Minghui 63 and a derived hybrid line, Shanyou 63. The *Bt* construct used contained a *cry1Ab/cry1Ac* fusion gene under the control of the rice actin1 promoter. The *Bt* lines were highly resistant to leaffolders and striped stem borer under natural and heavy artificial infestation. The transgenic Shanyou

63 had agronomic characteristics (e.g., plant height, days to flowering, grain filling) similar to those of nontransgenic Shanyou 63. Shu et al (2000) and Ye et al (2001) studied two transgenic lines of the japonica variety Xiushui 11, containing a *cry1Ab* gene under the control of the maize ubiquitin promoter. In laboratory assays, Shu et al (2000) found the lines to

be highly resistant to three stem borers and five foliage-feeding Lepidoptera. Shu et al (2000) and Ye et al (2001) also found the lines to be highly resistant to stem borers and leaffolders under field conditions. However, Ye et al (2001) cite earlier findings that the two *Bt* lines do not perform as well agronomically as the nontransgenic Xiushui 11.



**The California Rice Commission's Library on Rice Straw Utilization: searchable database**  
<http://www.ricestraw.org/library/search.html>

The database is part of the California Rice Commission's Web site and contains a wide array of articles on the use of rice straw. The articles were sourced from institutions such as the University of California Libraries, National Agricultural Library, National Library of Medicine, Feed Resources Database, and the IRRI Database. The site provides a search engine to locate the articles. Each article contains a summary with a complete citation, an abstract, and the country, language, materials, and uses indicated in the article. To facilitate easy access to the articles, researchers are provided with summaries organized according to the following areas of concern: animal feed, research, chemical production, construction materials, energy production, ethanol production, general, harvesting, baling, cubing, miscellaneous specific uses, mushroom cultivation, nutrients, paper, single-cell protein, and soil nutrients.

**Scholarly journals distributed via the World Wide Web (University of Houston Libraries)**  
<http://info.lib.uh.edu/wj/webjour.html>

This site provides links to established Web-based scholarly journals that offer access to English language article files without

requiring user registration or fees. It is linked to numerous international journals indexed alphabetically ranging across many disciplines (e.g., sciences, humanities, literature, etc.). Each journal has a search engine to locate articles. A majority of the articles are written in English. Online issues of the journals can be viewed in full text form.

**Entomology Index of Internet Resources**  
<http://www.ent.iastate.edu/list/>

This essential site for entomologists, based at Iowa State University, USA, is "a directory and search engine of insect-related resources on the Internet." The site provides links to many resources that entomologists and others will find extremely useful, including bibliographies and databases, electronic publications, insect collections, insect photos and drawings, entomology institutions and professional societies, job opportunities, pesticide descriptions and regulations, software, and even insect sounds.

**[www.aec.msu.edu/agecon/fs2/market/contents.html](http://www.aec.msu.edu/agecon/fs2/market/contents.html)**

The Agricultural Market Information Virtual Library contains a vast array of agricultural market information sources available through the Internet. For example, there are links to daily and to yearly market and outlook reports and recent information on commodity prices. These site links are organized by commodity (grains, sugar, fertilizer, palm oil, seafood, cotton, and many others), region/country (including global sites and sites for commodity exchanges), and those that specialize in market analysis or e-commerce. The site also contains information on e-mail and discussion groups, weather information, and agricultural news, among other features. If you are looking for information on agricultural prices and markets for any commodity in the world, this is one of the best places to start your search. The site is written and managed by Jean-Charles Le Vallee, Department of Agricultural Economics, Michigan State University.



## Mapping of rice genome completed

IRRI scientists have expressed excitement about the recent mapping of the rice genome, predicting that this could have a significant impact on a range of previously intractable problems in the developing world.

Syngenta, the world's largest agrochemicals company, has published the rice genome map—the first complete genome of any food crop, beating its rival Monsanto and the public sector International Rice Sequencing Project. “The mapping of the rice genome is a major scientific breakthrough because for the first time scientists have been able to unravel the biological inner workings of one of the most important food crops in the world,” says Ian Johnson, chairman of the Consultative Group on International Agricultural Research (CGIAR) and World Bank vice president.

IRRI Director General Ronald P. Cantrell said that, “As scientists focused on the objective of developing freely available, safe, and sustainable technologies that will

help rice farmers and consumers in the developing world, we see the recent rice genome announcement as highly significant for two key reasons.”

“First, it will make a very big difference in much of the work we do at IRRI, not only in areas such as biotechnology but also by greatly improving the efficiency of research, such as our traditional plant breeding work. Second, we see efforts by the company involved, Syngenta, to make its research available at no cost to subsistence farmers as continuing a promising trend by the corporate sector.”

Dr. Cantrell added that he was especially pleased that rice, the grain that feeds most of the world's desperately poor, was at the forefront of this trend. “We hope that the Syngenta announcement will just be the first of many by private companies that will allow much greater freedom in the transfer of technologies to the developing world. But, if this is to happen, we must allow these companies some way to recover their development costs.”

Dr. Cantrell said that poor rice farmers need and deserve the best science has to offer and that includes technologies

developed by the private sector. “However, while we want to encourage the private sector to invest in research that will help rice farmers and consumers, patent protection should not be allowed to deny poor people access to such much-needed modern technologies,” he added.

The most recent example of this challenge was the development of pro-vitamin A or “golden rice.” After months of negotiations with many parties, the two German inventors, along with Syngenta representatives, recently handed over to IRRI the first golden rice genetic material for further development. Once completed, golden rice will be made freely available to resource-poor farmers in the developing world indefinitely.

“All this is completely new territory to a public research institute such as IRRI, and to an industry such as rice, but with thousands suffering blindness and around a million deaths a year attributed to vitamin A deficiency, we have a moral responsibility to at least investigate whether such strategies could work,” Dr. Cantrell said.

## Gurdev Khush to receive Chinese International Award... and closes out nearly 29 years as PBGB head

Dr. Gurdev Khush was one of the scientists named as a recipient of *The China International Scientific and Technological Cooperation Award for 2001* of the People's Republic of China.

Two Chinese scientists, Prof. Yuan Longping, a long-time collaborator with IRRI on hybrid rice, and Dr. Wu Wenjun, a mathematician with the Chinese Academy of Sciences, were also awardees. Dr. Khush, who was tapped for his contribution to science and technology, and Dr. Wolfgang K.H. Panofsky of the United States, will come to China later in the year to receive their recognition.

Dr. Khush ended his long-standing service as head of the Plant Breed-

ing, Genetics, and Biochemistry (PBGB) Division and leader of various research programs at IRRI.

He has officially retired from these leadership positions, but will continue to serve as IRRI's principal plant breeder until the end of August 2001.

Over nearly three decades since 1 July 1972, Dr. Khush has served as PBGB division head. His commitment, tireless efforts, and significant achievements in improving rice germplasm and developing elite breeding lines and varieties have been well recognized worldwide.

Dr. David Mackill is the new PBGB head.





**Prof. Ingo Potrykus (second from right) and Prof. Peter Beyer (rightmost), inventors of golden rice, with IRRI's Drs. Karabi Datta and Swapan K. Datta (left).**

## Golden rice arrives in Asia

The first research samples of pro-vitamin A-enriched "golden rice" have arrived in Asia. This genetically modified rice, which contains betacarotene and other carotenoids, arrived at IRRI early this year.

IRRI scientists will begin work to further investigate the safety and utility of golden rice in combating vitamin A deficiency (VAD), which is responsible for 500,000 cases of irreversible blindness and 1–2 million deaths worldwide each year.

IRRI's Director-General, Dr. Ronald P. Cantrell said, "The arrival of these initial samples at IRRI allows us to finally start on the required testing processes using local rice varieties. IRRI expects to play a major role in the ongoing golden rice research effort and its eventual introduction to the

world's millions of poor rice farmers and consumers."

The delivery of golden rice from the inventors' laboratories in Europe was possible as a result of the donation of intellectual property licenses from Syngenta Seeds AG, Syngenta Ltd.; Bayer AG, Monsanto Company Inc., Orynova BV, and Zeneca Mogen BV. Each company has licensed free-of-charge technology used in the research that led to the golden rice invention. Subject to further research, initially in the developing countries of Asia, as well as local regulatory clearances, golden rice can then be made available free-of-charge for humanitarian uses in any developing nation.

Prof. Ingo Potrykus, coinventor of golden rice, said, "Vitamin A deficiency remains a very important health problem which no current remedy completely ad-

dresses. Peter Beyer and I are very pleased that these companies have provided essential support to assist our long-held intent of donating this potentially beneficial invention to those countries where Vitamin A deficiency causes hundreds of thousands of cases of irreversible blindness every year."

To further expedite the introduction of golden rice to developing countries, a Humanitarian Board has been established, composed of several public and private sector organizations. The Board has four principal aims:

- to support the inventors in making golden rice freely available to those that need it, consistent with the highest standards of safety assessment
- to ensure the proper investigation of golden rice as one potential solution to vitamin A deficiency
- to support individual developing countries and their national research institutes as they assess their interest in golden rice
- to facilitate information sharing between golden rice projects in different parts of the world.

The Humanitarian Board is chaired by Prof. Potrykus and also includes his coinventor, Prof. Beyer. Other members of the Board are Dr. Ronnie Coffman, Cornell University; Dr. Adrian Dubock, Syngenta AG (Secretary); Dr. William Padolina, IRRI; Dr. Ashok Seth, Rural Development Unit, South Asia Region of the World Bank; and Dr. Gary Toenniessen, Rockefeller Foundation. Dr. Katharina Jenny, Indo-Swiss Collaboration in Biotechnology, ETH, Zurich, is an observer to the Board. Each individual on the Humanitarian Board retains his/her independent advisory status.



## Meetings, conferences, and symposia in 2001/2002

### IFPRI conference

The International Food Policy Research Institute (IFPRI) will hold its 2020 International Conference titled *Sustainable food security for all by 2020: from dialogue to action*, on 4-6 September 2001 in Bonn, Germany. This conference will focus on the most important emerging issues shaping

future food prospects and priorities for action to assure a food-secure world.

### ICLD3, Rio de Janeiro

The *3rd International Conference on Land Degradation (ICLD3)* will be held in Rio de Janeiro on 17–21 September 2001. ICLD3 will issue its 3rd announcement with details of the scientific program of the conference, registration, payment, and postconference tours. For more informa-

tion about the conference, click on [www.cnps.embrapa.br/ICLD](http://www.cnps.embrapa.br/ICLD).

For inquiries, please contact

Dr. Beáta Madari  
ICLD3 Conference Secretary  
Embrapa Solos  
Rua Jardim Botânico, 1024  
22460-000 Rio de Janeiro, RJ, BRAZIL  
Tel/Fax: (+55 21) 294 8039  
Tel: (+55 21) 274 4999  
Fax: (+55 21) 274 5291  
E-mail: [icld3@cnps.embrapa.br](mailto:icld3@cnps.embrapa.br)

## **Rice-wheat symposium**

An international symposium on *Improving the productivity and sustainability of rice-wheat systems* will be held at the 2001 Annual Meetings of ASA-CSSA-SSSA, Charlotte, North Carolina, on 21-25 October. This symposium is organized by the division of International Agronomy-A6, IRRI, and the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico.

The symposium is devoted to discussing the challenges facing the sustainability of rice-wheat systems, reviewing emerging technologies in rice and wheat, and their integration into the systems, and identifying knowledge gaps and priorities for future research.

For further information, please contact

J.K. Ladha, IRRI, DAPO 7777  
Metro Manila, Philippines  
e-mail: j.k.ladha@cgiar.org.

## **International conference on agricultural science and technology**

The *International Conference on Agricultural Science and Technology* (ICAST) will be held in Beijing, China, on 7–9 November 2001.

Aimed to promote international cooperation in agricultural science and technology, ICAST is initiated and sponsored by the Ministry of Science and Technology, Ministry of Agriculture, Ministry of Education, and the State Administration of Foreign Experts Affairs of China. International organizations, such as the World Bank, United Nations Development Programme (UNDP), United Nations Educational, Scientific, and Cultural Organization (UNESCO), Food and Agriculture Organization of the United Nations (FAO), World Food Programme (WFP), and Asian Development Bank have been invited to cosponsor and address the conference.

ICAST will provide an opportunity to review achievements and lessons learned in the 20th century; to exchange views on policy and experiences in developing agricultural science and technology from different countries; and to envision the global mission for the new century, through innovation and cooperation on agricultural science and technology around the world.

ICAST covers most disciplines of agricultural science and technology: governmental forum, sustainable agriculture, agricultural biotechnology, postharvest management, agricultural information technology, resource and environment, and agricultural business.

## **Impacts of agricultural research and development conference**

The Standing Panel on Impact Assessment (SPIA) of the Technical Advisory Committee (TAC) of the CGIAR and CIMMYT invite all interested professionals to an *International Conference on Impacts of Agricultural Research and Development: Why Has Impact Assessment Research Not Made More of a Difference?* on 4–7 February 2002, at the Meliá Confort Hotel, San José, Costa Rica.

This conference will assemble researchers and other professionals with an interest in documenting and measuring the impact of international agricultural research. This 3-day conference will consist of presentations of contributed and invited papers as well as panel/working group discussions. Participants will highlight experiences and case studies of impact measurement in the following areas: agricultural productivity, equity, poverty, social health and nutrition, the environment, institutions, and human capital.

Check the conference Web site at [www.cimmyt.org/research/economics/impacts](http://www.cimmyt.org/research/economics/impacts) for updates on registration, instructions for preparing abstracts and papers, the program, and related information. You may also contact [impacts@cgiar.org](mailto:impacts@cgiar.org) for additional information.

## **ISCO 2002**

The 12th conference of the International Soil Conservation Organization (ISCO 2002) will be held in Beijing, China, on 26–31 May 2002. This conference will allow international soil and water conservation experts to find a common forum of participation and discussion.

Researchers, educators, sociologists, administrators, and representatives of public institutions and nongovernment organizations will exchange experiences about the

task of conserving soil and water resources and about the development of sustainable forms of land use. Excursions during, before, and after the conference will put the participants in contact with different aspects of a country rich in natural resources, with extremely contrasting regions and diverse productive systems, and exposed to important risks of degradation and desertification.



## **International Rice Congress Beijing, China, 2002**

The inaugural *International Rice Congress* will take place in 2002 to address issues related to rice research, production, processing, and consumption, as well as the sustainable improvement of the livelihood of rice farmers and consumers (see advertisement on next page). During the Congress, there will be simultaneous conferences and exhibitions based on the interconnected themes of "Innovation, Impact, and Livelihood."

The Congress—being jointly organized by the Chinese Academy of Engineering (CAE), the Chinese Academy of Agricultural Sciences (CAAS), and IRRI—will be held in Beijing, China, 16–20 September 2002.

The Congress will consist of plenary, concurrent, and poster sessions that are part of three major events including a scientific conference, a rice commerce conference, and a technology and mechanization exhibition.

More information will be made available soon with the inauguration of the IRC2002 Web site.



## Introduction

The inaugural International Rice Congress will take place in 2002 to address issues related to rice research, production, processing, trade, and consumption, as well as the sustainable improvement of the livelihood of rice farmers and consumers. During the Congress, there will be simultaneous conferences and exhibitions based on the interconnected themes of "Innovation, Impact, and Livelihood."

The Congress—being jointly organized by the Chinese Academy of Engineering (CAE), the Chinese Academy of Agricultural Sciences (CAAS), and the International Rice Research Institute (IRRI)—will be held in Beijing, China, 16-20 September 2002.

## Congress format and scientific program

The Congress will consist of plenary, concurrent, and poster sessions that are part of three major events including

- **A Scientific Conference**

### Themes

- Application of genomics, bioinformatics, and modern plant breeding approaches in rice improvement
- Integrated pest and weed management for yield stability and environmental protection
- Sustaining the natural resource base under intensified rice cropping systems
- Mechanization and postharvest technology for maximum profitability
- Impact of rice research on food security, human nutrition, and poverty alleviation

- Future of information technology and systems networks for enhanced rice productivity
- **A Rice Commerce Conference**
- **A Technology and Mechanization Exhibition**

- International farm machinery and postharvest equipment displays
- Farmer technology exhibits

### Satellite meetings

Satellite meetings/workshops on topics of interest will be organized at the request of delegates and organizations.

### Field trips and study tours

Educational excursions for delegates are being scheduled during and after the Congress.

### Accommodations

A range of accommodations is being reserved for delegates to the Congress. Details will be available in the 2<sup>nd</sup> announcement in November.

### Family and guest program

A full program of interesting visits in and around Beijing is being planned for guests and family members of delegates.

### Beijing

Beijing—a city with a unique history and culture—has been the capital of six dynasties and home to 34 emperors who ruled China's vast territory. Such a rich history has bequeathed many famous historic sites of great aesthetic and cultural value. The Great Wall, a stupendous 2000-year-old engineering project, winds its way along the mountains and valleys bordering northern Beijing. The Forbidden City represents the diversity and splendor of the Chinese culture. September is the golden season in Beijing.

## Financial support for delegates

Participants from developed countries should provide their own funding. The organizing committee will arrange for the partial funding of selected participants from developing countries. Female rice researchers are encouraged to submit abstracts.

### 2<sup>nd</sup> announcement

The 2<sup>nd</sup> announcement to be published in November 2001 will call for papers.

Please fill in the pre-registration form in this 1<sup>st</sup> announcement so that your name will be added to our database for sending you additional information in the 2<sup>nd</sup> announcement.

The deadline to submit the pre-registration form is **15 October 2001**.

Because of the high cost of producing and mailing circulars, the 2<sup>nd</sup> announcement will only be mailed to those who notify us of their interest.

Keep up-to-date on the Congress by checking the IRC2002 Web Site at [www.cgiar.org/irri/irc2002/index.htm](http://www.cgiar.org/irri/irc2002/index.htm).

### International Rice Congress 2002

**16-20 September 2002  
Beijing, China**

To receive further information on the Congress, please complete and return this form (once only, via email or fax or online at the IRC2002 Web Site) to

Dr. T.W. Mew, IRC2002, IRRI, DAPO 7777, Metro Manila, Philippines  
E-mail: [t.mew@cgiar.org](mailto:t.mew@cgiar.org) Fax: (63-2) 891-1292; 845-0606; 761-2404; 761-2406

## PRE-REGISTRATION FORM

Title: Prof/Dr/Mr/Ms/Mrs \_\_\_\_\_

Name: \_\_\_\_\_  
(Surname)

Nationality: \_\_\_\_\_  
(MI)

Organization: \_\_\_\_\_

Address: \_\_\_\_\_

Code: \_\_\_\_\_

Country: \_\_\_\_\_

Telephone: \_\_\_\_\_ Fax: \_\_\_\_\_

Email: \_\_\_\_\_

I PLAN TO ATTEND THE CONGRESS ☐

I PLAN TO SUBMIT AN ABSTRACT:

Yes \_\_\_\_\_ No \_\_\_\_\_

Circle one: Oral \_\_\_\_\_ Poster \_\_\_\_\_

I AM PRIMARILY INTERESTED IN THE FOLLOWING THEMES:

- ☐ Application of genomics, bioinformatics and modern plant breeding approaches in rice improvement
- ☐ Integrated pest and weed management for yield stability and environmental protection
- ☐ Sustaining the natural resource base under intensified rice cropping systems
- ☐ Mechanization and postharvest technology for maximum profitability
- ☐ Impact of rice research on food security, human nutrition, and poverty alleviation
- ☐ Future of information technology and systems networks for enhanced rice productivity

I AM INTERESTED IN DURING/POST-CONGRESS TOURS: During ☐ Post ☐

I AM INTERESTED IN PREPARING AN EXHIBIT FOR THE CONGRESS Yes ☐ No ☐

IRRN welcomes three types of submitted manuscripts: research notes, mini reviews, and "notes from the field." All manuscripts must have international or pan-national relevance to rice science or production, be written in English, and be an original work of the author(s), and must not have been previously published elsewhere.

## Research notes

Research notes submitted to IRRN should

- report on work conducted during the immediate past 3 yr or work in progress
- advance rice knowledge
- use appropriate research design and data collection methodology
- report pertinent, adequate data
- apply appropriate statistical analysis, and
- reach supportable conclusions.

**Routine research.** Reports of screening trials of varieties, fertilizer, cropping methods, and other routine observations using standard methodologies to establish local recommendations are not ordinarily accepted.

**Preliminary research findings.** To reach well-supported conclusions, field trials should be repeated across more than one season, in multiple seasons, or in more than one location as appropriate. Preliminary research findings from a single season or location may be accepted for publication in IRRN if the findings are of exceptional interest.

Preliminary data published in IRRN may later be published as part of a more extensive study in another peer-reviewed publication, if the original IRRN article is cited. However, a note submitted to IRRN should not consist solely of data that have been extracted from a larger publication that has already been or will soon be published elsewhere.

**Multiple submissions.** Normally, only one report for a single experiment will be accepted. Two or more items about the same work submitted at the same time will be returned for merging. Submitting at different times multiple notes from the same experiment is highly inappropriate. Detection will result in the rejection of all submissions on that research.

**Manuscript preparation.** Arrange the note as a brief statement of research objectives, a short description of project design, and a succinct discussion of results. Relate results to the objectives. Do not include abstracts. Up to five references may be cited. Restrain acknowledgments. Limit each note to no more than two pages of double-spaced typewritten text (approximately 500 words).

Each note may include up to two tables and/or figures (graphs, illustrations, or photos). Refer to all tables and figures in the text. Group tables and figures at the end of the note, each on a separate page. Tables and figures must have clear titles that adequately explain contents.

Apply these rules, as appropriate, to all research notes:

## Methodology

- Include an internationally known check or control treatment in all experiments.
- Report grain yield at 14% moisture content.
- Quantify survey data, such as infection percentage, degree of severity, and sampling base.
- When evaluating susceptibility, resistance, and tolerance, report the actual quantification of damage due to stress, which was used to assess level or incidence. Specify the measurements used.
- Provide the genetic background for new varieties or breeding lines.
- Specify the rice production systems as irrigated, rainfed lowland, upland, and flood-prone (deepwater and tidal wetlands).
- Indicate the type of rice culture (transplanted, wet seeded, dry seeded).

## Terminology

- If local terms for seasons are used, define them by characteristic weather (dry season, wet season, monsoon) and by months.
- Use standard, internationally recognized terms to describe rice plant parts, growth stages, and management practices. Do not use local names.
- Provide scientific names for diseases, insects, weeds, and crop plants. Do not use local names alone.
- Do not use local monetary units. Express all economic data in terms of the US\$, and include the exchange rate used.
- Use generic names, not trade names, for all chemicals.
- Use the International System of Units for all measurements. For example, express yield data in metric tons per hectare ( $\text{t ha}^{-1}$ ) for field studies. Do not use local units of measure.
- When using acronyms or abbreviations, write the name in full on first mention, followed by the acronym or abbreviation in parentheses. Use the abbreviation thereafter.

- Define any nonstandard abbreviation or symbol used in tables or figures in a footnote, caption, or legend.

## Mini reviews

Mini reviews should address topics of current interest to a broad selection of rice researchers, and highlight new developments that are shaping current work in the field. Authors should contact the appropriate editorial board member before submitting a mini review to verify that the subject is appropriate and that no similar reviews are already in preparation. (A list of the editors and their areas of responsibility appears on the inside front cover of each IRRN issue.) Because only 1-2 mini reviews can be published per issue, IRRN will require high quality standards for manuscripts accepted for publication. The reviews should be 2000-3000 words long, including references. Refer to the guidelines for research notes for other aspects of writing and content.

## Notes from the field

Notes from the field should address important new observations or trends in rice-growing areas, such as pest outbreaks or new pest introductions, or the adoption or spread of new crop management practices. These observations, while not the result of experiments, must be carefully described and documented. Notes should be approximately 250 words in length. Refer to the guidelines for research notes for other aspects of writing and content.

## Review of manuscripts

The IRRN managing editor will send an acknowledgment card or an email message when a note is received. An IRRI scientist, selected by the editorial board, reviews each note. Depending on the reviewer's report, a note will be accepted for publication, rejected, or returned to the author(s) for revision.

## Submission of manuscripts

Submit the original manuscript and a duplicate, each with a clear copy of all tables and figures, to IRRN. Retain a copy of the note and of all tables and figures.

Send manuscripts, correspondence, and comments or suggestions about IRRN by mail or email to:

The IRRN Managing Editor  
IRRI, DAPO Box 7777  
Metro Manila  
Philippines  
Fax: (63-2) 845-0606  
E-mail: k.s.lopez@cgiar.org

**New!**

## Rice: Nutrient Disorders and Nutrient Management (handbook and CD)

By Achim Dobermann and Thomas Fairhurst

International Rice Research Institute and Potash and Phosphate Institute

Thirty years ago, persuading rice farmers to use modern varieties and accompanying fertilizer inputs was easy because the results, in terms of yield increases, were often spectacular. Simultaneously, governments invested heavily in fertilizer subsidies, improved irrigation facilities, infrastructure, and rice price support mechanisms that made rice intensification (increased input use, increased number of crops per year) economically attractive. Further improvements in rice productivity, however, are likely to be much more incremental and "knowledge-based." Future yield increases will mostly result from the positive interactions and simultaneous management of different agronomic aspects such as nutrient supply, pest and disease control, and water.

In many countries, fertilizer and other input subsidies have already been removed and it is likely that, in the future, the maintenance of irrigation facilities will increasingly become the responsibility of farmers rather than governments. This means that to achieve the required future increases in rice production, extension services will need to switch from distributing prescriptive packets of production technology to a more participatory or client-based service function. Such an approach requires greater emphasis on interpreting farmers' problems and developing economically attractive solutions tailored to each farmer's objectives. Yet extension services are generally ill-prepared for such a change.

*Rice: Nutrient Disorders and Nutrient Management* is a handbook that provides a guide to detecting nutrient deficiency and toxicity symptoms and managing nutrients in rice grown in tropical and subtropical regions. Some background information is included on the function of nutrients in the

rice plant and possible causes of nutrient deficiencies, together with a description of nutrient deficiency symptoms, the effect of nutrient deficiency on plant growth, and the effect of flooding on nutrient availability. Estimates of nutrient removal in grain and straw have been included to help researchers and extension workers calculate the amount of nutrients removed from the field under different management systems. Strategies for preventing and treating nutrient deficiencies are described.

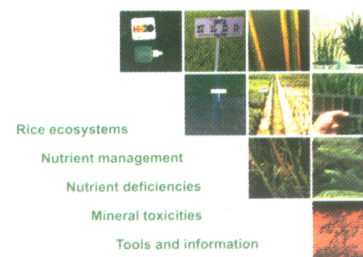
In most tropical and subtropical regions, rice farms are small, nutrients are managed "by hand," and farmers do not have access to more resource-demanding forms of nutrient management such as soil and plant tissue testing. Therefore, we describe a new approach to calculate site-specific nutrient management recommendations for N, P, and K in lowland rice. The concept described is based on ongoing on-farm research in the Mega Project on Reversing Trends in Declining Productivity in Intensive, Irrigated Rice Systems, a collaborative project between IRRI and researchers in China, India, Indonesia, the Philippines, Thailand, and Vietnam. As this work progresses, a more complete approach for site-specific nutrient management will evolve.

The main targets of the handbook are the irrigated and rainfed lowland rice systems because these systems account for about 80% of the total harvested area of rice and 92% of global rice production. Where appropriate, additional information has been included for upland rice or rice grown in flood-prone conditions. This publication and the accompanying CD will help increase the impact of new approaches to nutrient management at the farm level.

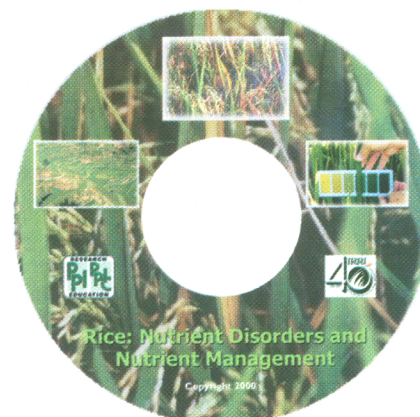
For more information, contact: [tfairhurst@ppi-ppic.org](mailto:tfairhurst@ppi-ppic.org) or [e.hettel@cgiar.org](mailto:e.hettel@cgiar.org)

## Rice

### Nutrient Disorders & Nutrient Management



Achim Dobermann and Thomas Fairhurst



To order, send an e-mail to:  
[e.ramin@cgiar.org](mailto:e.ramin@cgiar.org)

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INTERNATIONAL RICE RESEARCH INSTITUTE

DAPO Box 7777, Metro Manila, Philippines

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