

Effect of EM on Germination and Seedling Growth of Rice

U.R. Sangakkara and A.M.U. Attanayake

Department of Crop Science

University of Peradeniya

Peradeniya, Sri Lanka

Abstract.

The effect of solutions of Effective Microorganisms (EM 4) on the germination and seedling growth of rice was studied in laboratory experiments. Freshly harvested seeds of two varieties were sown on germination paper and in sand, using either distilled water, a concentrated solution of EM 4 or dilutions of EM 4 to moisten the substrate. Germination, seedling characteristics, and shoot and root dry weights were evaluated. The concentrated EM solutions had a toxic effect on seed germination and seedling growth. Thus, the percentage of dead seeds and abnormal seedlings increased with this treatment. The concentrated solution also reduced shoot and root dry weights. Dilute solutions of EM 4 had a beneficial effect on all measured parameters. The best germination, seedling characteristics and growth occurred with EM 4 applied after dilution of 1:500 with water. The results are presented in terms of the usefulness of EM 4 in enhancing germination and seedling growth of rice, a crop planted exclusively with seed.

Introduction

Rice (*Olyza sativa* L) is the primary food crop of the tropics (FAO, 1989), and hence is cultivated in a wide range of environments. The crop is established by seed, and a high percentage of germination in the planting material is an important criterion for successful cultivation. Farmers of the developing world obtain their seed for crop establishment from a wide range of sources, because of the non-availability of adequate quantities of good quality seed for planting. These sources range from previous harvests kept for food or seed to seed stocks of neighbors or private traders. Thus, the quality of seed may vary considerably, which could result in heavy expense and crop loss to the smallholder subsistence farmers of the developing world.

Microorganisms have been successfully used in promoting seed germination of many crop species, and this concept could have an important role in agriculture (Kloepper et al., 1986). Studies (e.g., Arsac et al., 1990; Hofte et al., 1991) have used microorganisms such as *Azospirillum* and *Pseudomonas* from natural sources for enhancing germination. The results clearly present a protecting and growth-promoting role of these species in the germination process of many cereals. The causal mechanisms of this phenomenon were attributed to the protection of seeds from external stress factors and diseases during the germination process, when seeds and the emerging radicles and plumules are sensitive to the environment. Other studies (e.g., Zuberi et al., 1991) highlight the use of an aqueous diffusate of *Striga densifolia* on germination and early growth of rice. These reports clearly identify the usefulness of

naturally-occurring microorganisms and biological products in enhancing germination and early growth of very important crop species of the developing world

To ensure that the yield and quality of rice keeps pace with population increases in consumer demand, researchers are exploring new technologies that can benefit germination and early growth of basic food crops. One such technology is Effective Microorganisms (EM), a mixed microbial inoculant of naturally-occurring, beneficial microorganisms to improve soil quality and the growth and development of crops in organic farming. This has been reported from many sources and countries. For example, Sangakhara and Higa (1992) showed yield increases of 20 to 30 percent with EM 4 in organic systems in Sri Lanka. Speculations on the benefits of EM in promoting germination of seeds have been discussed (Higa, 1992) although the mechanisms or modes-of-action have not yet been elucidated. Thus, a study was undertaken to identify the beneficial effects of EM solutions in promoting germination and early growth of two rice varieties in Sri Lanka.

Methodology

The study was conducted in the Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka, during March-April, 1992. Freshly harvested seed (February, 1992) of rice varieties BG 11-11 (new improved variety) and H 4 (old improved variety) were germinated in clean petri dishes (9-cm diameter) containing filter paper (No 597) into which different dilutions or concentrations of EM 4 solutions were placed. The dilutions (i.e., concentrations) of EM stock solution used were as follows: undiluted or diluted to 1:100, 1:250, 1:500, 1:750 or 1:1000 with distilled water. Molasses, 4 g per liter of water, was added as a substrate and also served as a control. The experiment was a completely randomized design with five replicates per treatment. Each replicate had 100 seeds. Germination was recorded at 21 days along with the number of dead and diseased seeds, i.e., seeds with fungi on the surface.

The second study evaluated the effect of selected concentrations of EM on seedling growth of the two rice varieties. Thus, uniform seeds from the same source were planted in trays containing washed river sand (particle size diameter 0.7 to 1.5 mm). The sand in each tray was moistened with the respective EM solution or distilled water prior to planting the seeds at a depth of 1 cm. The moisture content of the sand in each tray was maintained with its respective solution (i.e., EM concentration). The experiment was a completely randomized design with four replicates, each containing 50 seeds.

Germination was determined at 30 days after planting, along with the number of diseased and abnormal seedlings as described by ISTA (1987). Thereafter, 15 healthy seedlings were carefully removed from each tray, washed and dried at 80°C for 48 hours to determine the dry weight of shoots and roots.

Because of the similarity in the response of both varieties, the data were pooled for statistical analysis. The analysis used a general linear model procedure to determine treatment differences

Results and Discussion

Viable seeds of rice germinate in 7 to 10 days, and the process is primarily influenced by seed characteristics and moisture (de Datta, 1981). The application of different concentrations of EM had no effect on this process in the selected rice varieties, and the seeds showed a 25-percent germination rate within 7 to 8 days (Table 1). Thus, even the concentrated solutions of EM did not totally suppress the germination process in the selected rice varieties.

Table 1. Effect of Various Concentrations of Effective Microorganisms (EM 4) on Germination of Rice Seed.

| Dilution of EM | Germination to 25% (days) | Germination at 21 Days (%) | Dead Seeds (%) | Diseased Seeds (%) |
|-----------------|---------------------------|----------------------------|----------------|--------------------|
| Undiluted | 8 | 47.4 | 50.6 | 2 |
| 1:100 | 7.9 | 65.4 | 32.8 | 1.7 |
| 1:250 | 7.4 | 85.8 | 12.9 | 1.3 |
| 1:500 | 7.4 | 89.8 | 9.4 | 0.7 |
| 1:750 | 7.9 | 82.4 | 14.6 | 3 |
| 1:1000 | 8.2 | 79.8 | 15.4 | 4.7 |
| Control (Water) | 8.4 | 76.8 | 16.4 | 6.9 |
| Sx | 1 | 5 | 2.1 | 0.3 |

Seed germination was determined on the basis of radicle emergence.

Germination percentages at 21 days illustrate a significant effect of EM concentration on total emergence (Table 1). Application of the undiluted EM stock solution reduced final emergence significantly, indicating a toxic effect. However, the actual mechanism or mode-of-action of EM, whether on the seed or on the emerging radicle, was not determined.

Increasing dilution of the EM (i.e., lower concentration) enhanced germination at 21 days. The maximum germination was observed with a dilution of 1 :500 and declined with further reductions in EM concentration. Although the germination of seeds treated with water alone was reduced, it was 29 percent greater than when undiluted EM was applied.

The incidence of dead seeds declined with increasing dilution. Again, the lowest number of dead seeds were observed at a dilution of 1 :500, which also produced maximum germination. Thus, as expected, there was a significant negative correlation ($r = 0.85$) between germination and the percentage of dead seeds.

The control treatment had 16 percent of dead seed (Table 1), which is common for rice grown under farm conditions. In contrast, EM reduced the incidence of dead seed; at a dilution of 1 :500, the percentage of dead seed was reduced by 7 percent, which is a substantial benefit to the farmer purchasing seed from other sources or using his consumable seed stocks. The data also show a close relationship to similar studies where other microbial solutions have been used to promote emergence of cereals (e.g., Kloepper et al., 1986; Iswandi et al., 1987; Hofte

et al., 1991). The reduction in the numbers of dead seed with the application of dilute solutions of EM 4 may be due to the possible activation of germination in dormant seeds. These seeds are generally classified as dead seed.

The incidence of diseased seed was significantly reduced by EM. The decline in the percentage of dead seed was approximately 5 and 6 percent with applications of the undiluted solution or 1 :500 dilution as compared with the control. The relationship between the incidence of diseased seed to germination percentage was similar to that of the incidence to dead seed. Thus, when EM is used in an undiluted form, it appears to reduce the activity of disease-causing organisms. This again presents interesting results that warrant further study on causal organisms and mechanisms. The data also clearly confirm the earlier reports (e.g., Kaiser et al., 1989) about the use of microorganisms for disease control in emerging seedlings of chickpea.

The lowest percentage of healthy seedlings suitable for planting and the highest number of abnormal seedlings were observed when seeds were treated with the undiluted solution of EM 4 (Table 2). This illustrates that very high concentrations of EM 4 can produce abnormalities in rice seedlings. The abnormal seedlings are not suited to field establishment. Diluting the concentrated stock solution of EM 4 to 1 :500 increased the number of healthy seedlings and decreased the number of abnormal seedlings. The number of diseased seedlings (determined on the basis of the presence of fungi) also decreased at the 1 :500 dilution of EM 4, which may be attributed to interactions between the disease-causing microorganisms and certain EM cultures.

Table 2. Seedling Characteristics of Rice as Affected by Various Concentrations of Effective Microorganism (EM 4).

| Dilution of EM | Normal Seedlings (%) | Abnormal Seedlings (%) | Diseased Seedlings (%) |
|-----------------|----------------------|------------------------|------------------------|
| Undiluted | 60.8 | 33.3 | 5.8 |
| 1:100 | 81 | 13.8 | 5.2 |
| 1:250 | 86.8 | 8.4 | 4.8 |
| 1:500 | 92.8 | 4.2 | 3 |
| 1:750 | 85.8 | 5.7 | 8.5 |
| 1:1000 | 79 | 11.9 | 9.2 |
| Control (Water) | 74.6 | 14.2 | 11.2 |

Seedling characteristics were determined after 21 days, based on descriptions of ISTA (1987).

Increasing the dilution beyond the ratio of 1 :500 reduced the percentages of healthy seedlings, with corresponding increases in numbers of abnormalities. However, the numbers of abnormal seedlings did not exceed 15 percent even with water (control); also, the percentage of diseased seedlings was greater in the control than in the EM solutions. Dilution of 1 : 1000 also reduced the numbers of diseased seedlings significantly when compared with the control. Thus, the study suggests that EM 4 can be used beneficially at a dilution of 1 :500 to enhance

seedling germination and emergence of rice. The ability of EM 4 to reduce the incidence of diseased seedlings is also noted even though undiluted or high concentrations produce abnormalities.

The effect of EM on shoot and root growth of rice seedlings at 30 days (Table 3) is a quadratic relationship. The effects are best presented by the equations $Y = 0.85 + 0.59X - 0.042X^2$ (± 0.024 , $n = 35$; $R^2 = 0.86$) and $Y = 0.51 + 0.29X - 0.036X^2$ (± 0.032 , $n = 35$; $R^2 = 0.71$) for shoots and roots, respectively. The data suggest that EM influences shoot growth to a greater extent than root growth, as seen in the dry weight tissue response with decreasing concentration. The data show that the best shoot and root growth occurs at a dilution of 1:500, and therefore, is similar to the seedling responses. Plants grown in the presence of undiluted EM 4 produce the lowest shoot and root dry weights which are less than that of the control. Thus, the clear relationship between germination and seedling characteristics, and dry matter accumulation of shoots and roots illustrate the value of using dilute solutions of EM 4 (e.g., 1 :500) to increase the number of healthy plants available for the establishment of a rice crop from scarce sources of seed.

Table 3. Effect of EM 4 Solutions on Shoot and Root Dry Weights of Rice at 30 Days after Planting.

| Dilution of EM | Shoot Weight (mg) | Root Weight (mg) |
|-----------------|-------------------|------------------|
| Undiluted | 85 | 79 |
| 1:100 | 149 | 141 |
| 1:250 | 165 | 159 |
| 1:500 | 196 | 175 |
| 1:750 | 158 | 126 |
| 1:1000 | 145 | 120 |
| Control (Water) | 120 | 116 |
| Sx | 4.54 | 8.65 |

Function curves for shoot weight and root weight changes in relation to EM concentration:

Shoots: $Y = 0.85 + 0.59x - 0.042X^2$ ($R^2 = 0.86$)

Roots: $Y = 0.51 + 0.29X - 0.036X^2$ ($R^2 = 0.71$)

EM 4 is a microbial inoculant containing mixed cultures of naturally-occurring beneficial microorganisms which are commonly found in most agricultural ecosystems. Thus, the use of EM 4 has no adverse impact on productivity. In contrast, the benefits of application are significant as illustrated by this study. Based on results of studies using pure cultures of specific microorganisms (e.g., Arsac et al., 1990), EM 4 may have a beneficial role in alleviating seed problems for the small landholder rice farmers by increasing the potential seedling numbers that can be obtained from scarce seed resources. However, the mechanisms of this process need elucidation.

Acknowledgment

Gratitude is expressed to Mr.H.L.Dayanada for the supply of seeds.



All references herein to EM™ or Effective Microorganisms™ mean the specific technology discovered by Dr. Teruo Higa that is exclusively manufactured, marketed and distributed by EM Research Organization, Inc. and its licensees under the brand name EM•1®.

References

- Arsac, J.F., C. Lamothe, D. Mulard, and D. Fages. 1990. Growth enhancement of maize (*Zea Mays* L.) through *Azospirillum lipoferum* inoculation: Effect of plant genotype and bacterial concentration. *Agronomie* 10:640-654.
- de Datta, S.K. 1981. Principles of Rice Production. Wiley and Sons, New York, N.Y., USA.
- FAO. 1989. Production Yearbook of the Food and Agriculture Organization. FAO, Rome, Italy.
- Higa, T. 1992. Personal Communication. University of the Ryukyus, Okinawa, Japan.
- Hofte, M., J. Boelens, and W. Verstraete. 1991. Seed protection and promotion of seedling emergence by the plant growth beneficial *Pseudomonas* strains 7NSK2 and ANP15. *Soil Biology and Biochemistry* 23:407-410.
- ISTA. 1987. Handbook of Vigour Test Methods. 2nd Edition. F. Fiala (ed.). International Seed Testing Association, Zurich, Switzerland.
- Iswandi, A., P. Bossier, J. Vandenabeele, and W. Verstraete. 1987. Relations between soil microbial activity and the effect of seed inoculation with the Rhizo-pseudomonas strain 7NSK2 on plant growth. *Biology and Fertility of Soils* 3:147-151.
- Kaiser, W.J., R.M. Hannan, and D.M. Weller. 1989. Biological control of seed rot and preemergence damping-off of chickpea with fluorescent *Pseudomonas*. *Soil Biology and Biochemistry* 21:269-273.
- Kloepper, J.W., F. Scher, M. Laliberle, and G.B. Tippin. 1986. Emergence promoting rhizobacteria: Description and implications for agriculture. p. 155-164. In T.R. Swinburne (ed.) *Iron, Siderophores and Plant Diseases*. Plenum Press, New York, N.Y., USA.
- Sangakkara, U.R., and T. Higa. 1992. Effective microorganisms for organic agriculture : A case study from Sri Lanka. p. 151-155. In U. Köpke, and U. Schuiz (ed.) *Proceedings of the 9th IFOAM Conference, Tholey-Theley, Germany*.
- Zuberi, M.I., G.P. Ghosh, and P.C. Roy. 1991. Effect of aqueous diffusates of *Striga densi-folia* Benth. on germination and early seedling growth of rice. *Bangladesh Journal of Botany* 20:93-95.

