Plant Growth-Promoting Activities of 3-Hydroxy-5-Methyl-Isoxazole

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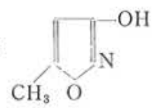
3-Hydroxy-5-methyl isoxazole (HMI, trade name Tachigaren) is a highly effective fungicide against damping-off organisms in rice and sugar beet seedlings, and also acts as an effective plant growth-promoter. The chemical structure is similar to those of tricholomic acid and ibotenic acid, found in mushrooms. The chemical and physical properties of 3-hydroxy-5-methyl isoxazole are as follows;

Chemical structure: 

Empirical formula: $C_4H_5NO_2$
Molecular weight: 99
Physical state and color:
Colorless, needle-shaped crystals
Melting point: 86–87°C
Solubility:
Freely soluble in methanol, ethanol, acetone and other organic solvents. Also very soluble in water
Stability:
Stable in both acidic and alkaline solutions
Odor: None

Effects on growth of rice seedlings

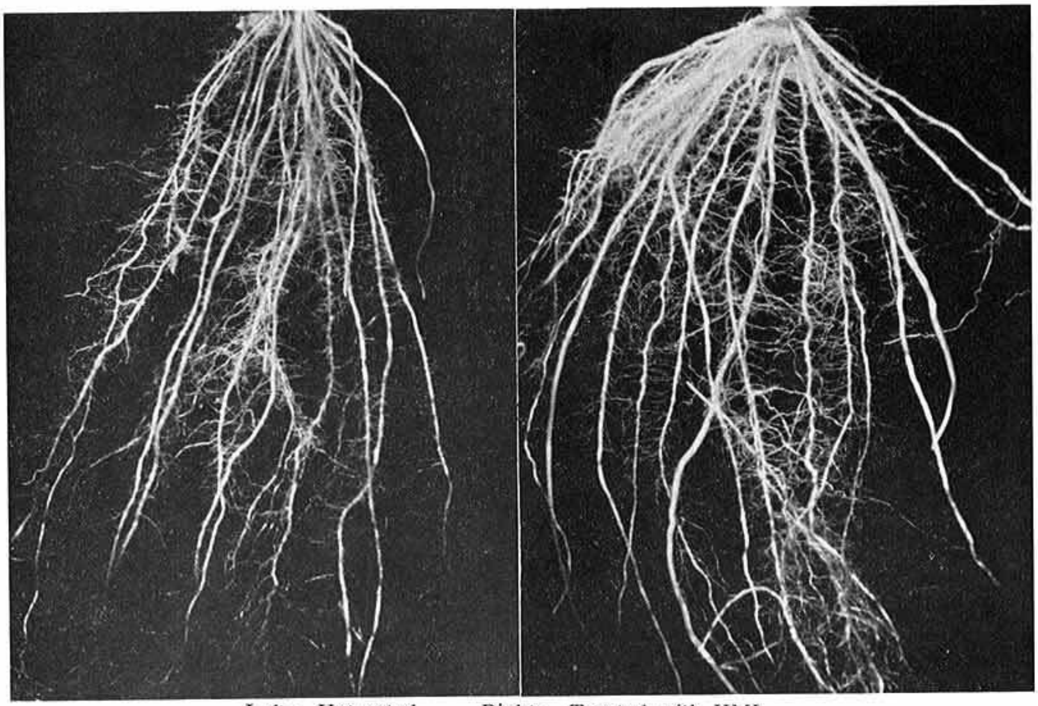
The effect of HMI on the growth of rice (Oryza sativa L. var Nihonbare) seedlings was examined in nursery boxes using non-sterilized or sterilized soil. In both soils, the weight of shoot and root increased in soil treated with HMI as compared with the untreated soil as shown in Table 1 and Plate 1. The increase in weight due to HMI treatment was greater for roots than for shoots. Therefore, these results indicate that HMI has a growth-promoting effect on the rice seedlings.

The effect of HMI on the growth of rice roots was examined under solution culture at various concentrations. The elongation of roots was inhibited at high concentrations of

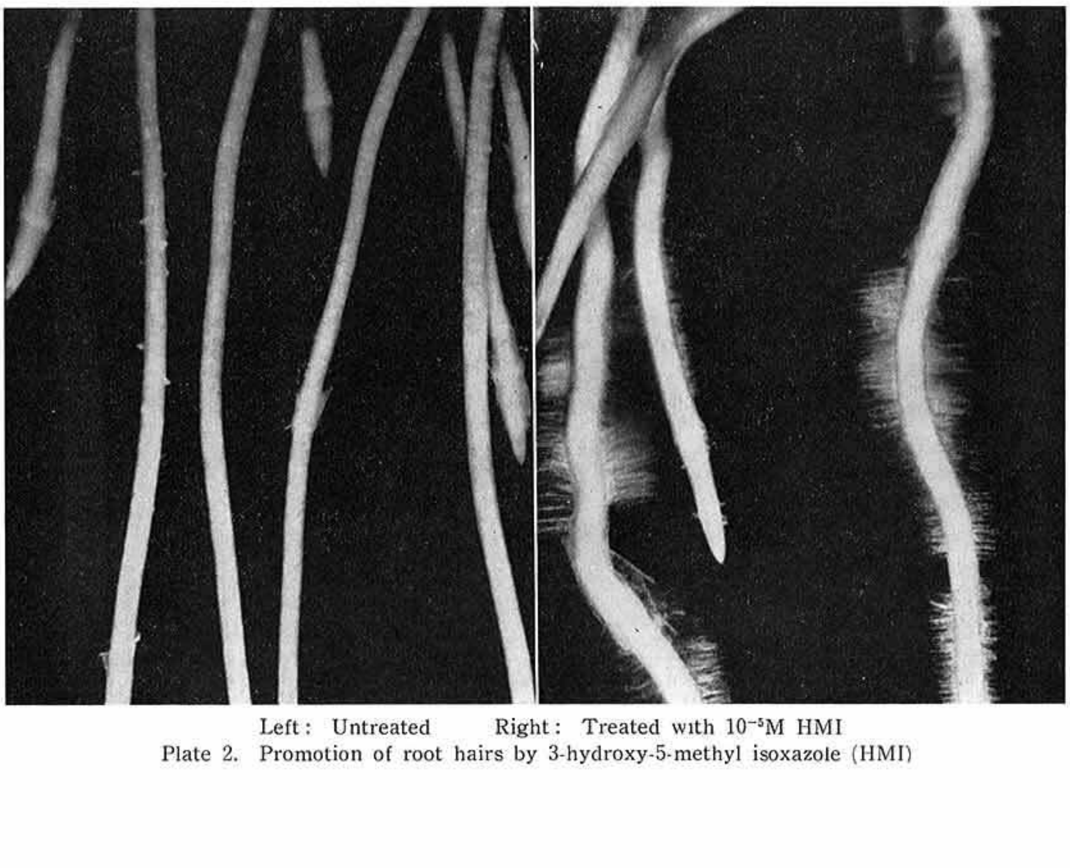
Table 1. Effect of 3-hydroxy-5-methyl isoxazole (HMI) on growth of rice seedlings in non-sterilized and sterilized soils

Treatment	Plant height (cm)	Root length (cm)	Fresh weight (mg)	
			Shoot	Root
Non-sterilized soil				
control	12.4	5.7	86	21
treated	15.4	6.7	99	29
Sterilized soil				
control	14.7	6.8	89	25
treated	15.2	6.8	95	34

* About 7,500 rice seedlings were grown densely at 18–13°C in a nursery box (30×60×3 cm). Five hundred ml solution containing 0.15 g of HMI per nursery box was applied to the soil immediately after sowing in the treated plot. Measurements were taken at 20 days after sowing. Numbers represent average of the value for 20 individuals



Left: Untreated Right: Treated with HMI
Plate was taken 40 days after sowing
Plate 1. Promotion of root growth by 3-hydroxy-5-methyl isoxazole (HMI)

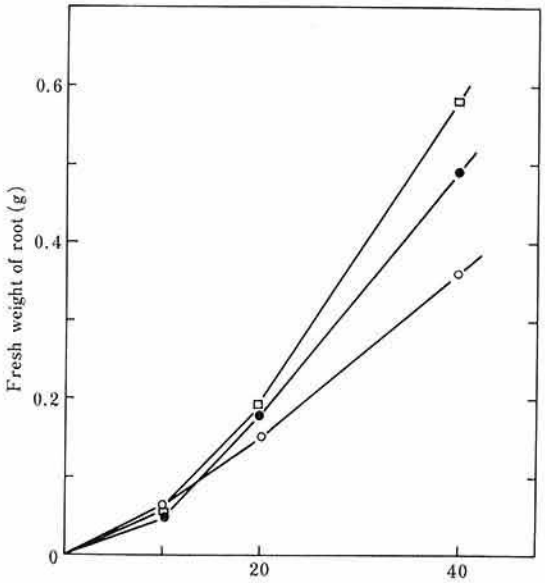


Left: Untreated Right: Treated with 10^{-5}M HMI
Plate 2. Promotion of root hairs by 3-hydroxy-5-methyl isoxazole (HMI)

10^{-3} to 10^{-2} M HMI, while the development of root hairs was increased at concentration of 10^{-5} to 10^{-4} M (Plate 2).

The elongation of roots was initially inhibited in the nursery boxes where the soil was treated with high concentrations of HMI. This response was similar to that was observed at high concentrations in solution culture.

However, root elongation recovered in later stages of seedlings development, and the seedlings in the treated nursery boxes showed greater root growth than those in untreated boxes (Fig. 1).



○—○ : control
●—● : 0.30 g of HMI per nursery box (30×60×3 cm)
□—□ : 0.15 g of HMI per nursery box

Fig. 1. Effect of 3-hydroxy-5-methyl isoxazole (HMI) on root growth of rice seedlings

The effect of HMI on the growth of roots was also observed in other plant species. HMI was observed to promote root growth in sweet potato, taro, shallot, lotus, carrot, strawberry, onion, tea, tomato, eggplant, cucumber and chrysanthemum, when HMI was applied to the soil after sowing or planting. In general, the application of HMI at about $0.9 g/m^2$ promoted the development of branched roots and root hairs as well as the growth of roots.

Influence on the function of roots

Fig. 2 shows a comparison of α-napthylamine-oxidizing activity in roots from HMI-treated and control plots. It is evident from this figure that the oxidizing activity of roots from the HMI-treated plot was higher than

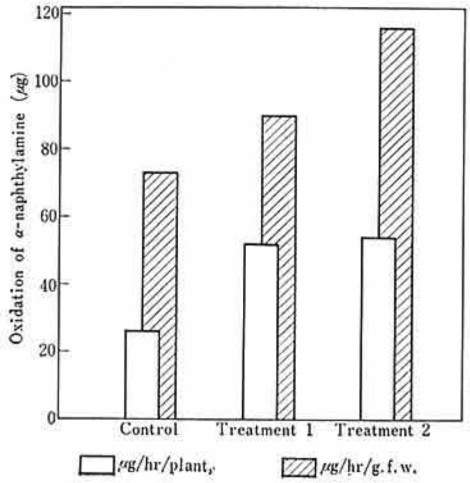


Fig. 2. Effect of 3-hydroxy-5-methyl isoxazole on α-naphthylamine oxidizing activity in rice roots 20 days after sowing

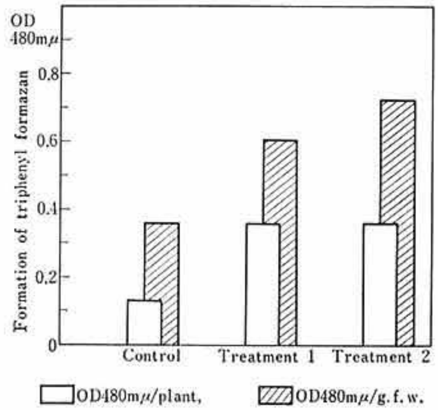
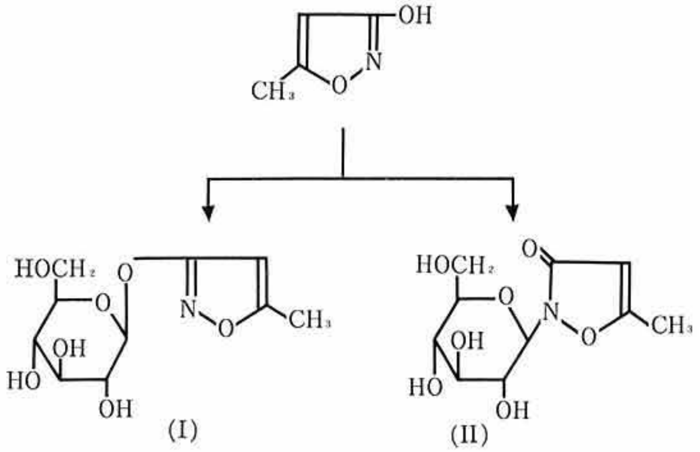


Fig. 3. Effect of 3-hydroxy-5-methyl isoxazole on 2, 3, 5-triphenyl tetrazolium chloride (TTC) reducing activity in rice roots 20 days after sowing



(I) 3-(β-D-glucopyranosyloxy)-5-methyl isoxazole (0-β-glucoside)
(II) 2-(β-D-glucopyranosyl)-5-methyl-4-isoxazolin-3-one (N-β-glucoside)

Fig. 4. Metabolism of 3-hydroxy-5-methyl isoxazole in higher plants[1)]

that of the control when expressed on a per plant basis or per gram fresh weight of roots.

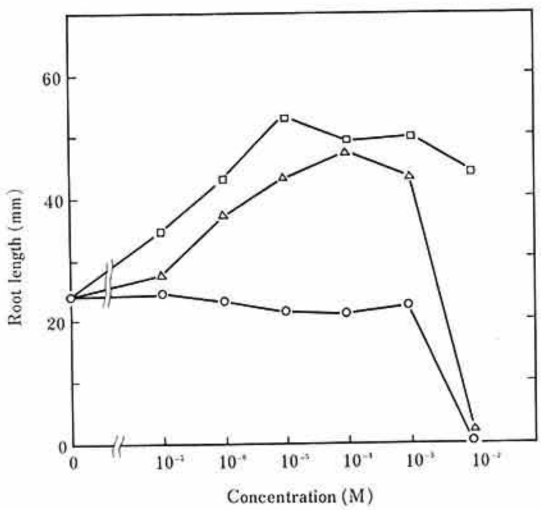
The 2, 3, 5-triphenyltetrazolium (TTC)-reducing activity of roots was also examined, as an indicator of the physiological activity of roots. The results in Fig. 3 indicate that TTC-reducing activity in plants from the HMI-treated plot was considerably higher than plants from the control plot.

It is known that the occurrence of Murenae (physiological damping-off) is closely related to the physiological activity of roots. Roots from Murenae-affected plants show low α-naphthylamine-oxidizing activity.

The results mentioned above indicate that HMI stimulates physiological activity of roots while preventing the infection of roots by the damping-off organism. The increased physiological activity may also prevent the occurrence of Murenae in seedlings from the HMI-treated plots.

Metabolites of HMI and growth of roots

HMI is absorbed by plant roots and translocated acropetally to the shoots of plants. In plants HMI is readily converted to its N-β-glucoside and O-β-glucoside derivatives, shown in Fig. 4. The two metabolites were determined about their effect on the growth



○—○ : HMI
△—△ : O-β-glucoside
□—□ : N-β-glucoside

Fig. 5. Effect of 3-hydroxy-5-methyl isoxazole and its two metabolites on root growth of rice seedlings

Rice seeds were germinated and five seeds each were transferred to a small test tube (2.5×6.0 cm) containing 2 ml of HMI or its metabolites. The test tubes were kept at 30°C under constant light (4,000 lux). Measurements were made at 6 days after sowing.

of rice seedlings. The growth effects of the metabolites were similar to those observed when rice seedlings were treated with HMI.

The metabolites promoted root growth and development of root hairs, and increased the oxidizing activity of roots. However, the response of roots to metabolite concentration was very significant.

As shown in Fig. 5, N-β-glucoside and O-β-glucoside caused a marked elongation of roots for a short 6-day period after germination in water culture. HMI and O-β-glucoside inhibited the growth of roots notably a high concentration of 10^{-2} M, while N-β-glucoside acted promotively at that concentration.

The results strongly suggest that the growth-promoting effect of HMI is possibly due to the conversion of the compound to two kinds of glucosides, especially to N-β-glucoside. It is known that O-β-glucoside is antifungal, but N-β-glucoside is not. Both metabolites, however, seem to be sufficient in promoting the growth of roots.

Geotropic curvature of rice seedlings and HMI

In general, the horizontally placed rice seedlings gradually revert to a nearly vertical position. This response to gravity is referred to as the geotropic curvature of rice seedlings. The extent of this response by rice seedlings is important in determining their physiological status which influences future growth and development. The HMI-treated seedlings had a high geotropic curvature response (Plate 3).

The effect of HMI on the geotropic curvature could also be observed when untreated control seedlings were soaked in HMI solution. The rate of geotropic curvature response of rice seedlings increased with increasing HMI dosage.

HMI showed no auxin activity in the rice lamina joint test. However, the auxin activity of indoleacetic acid was increased in the presence of HMI, as shown in Fig. 6.

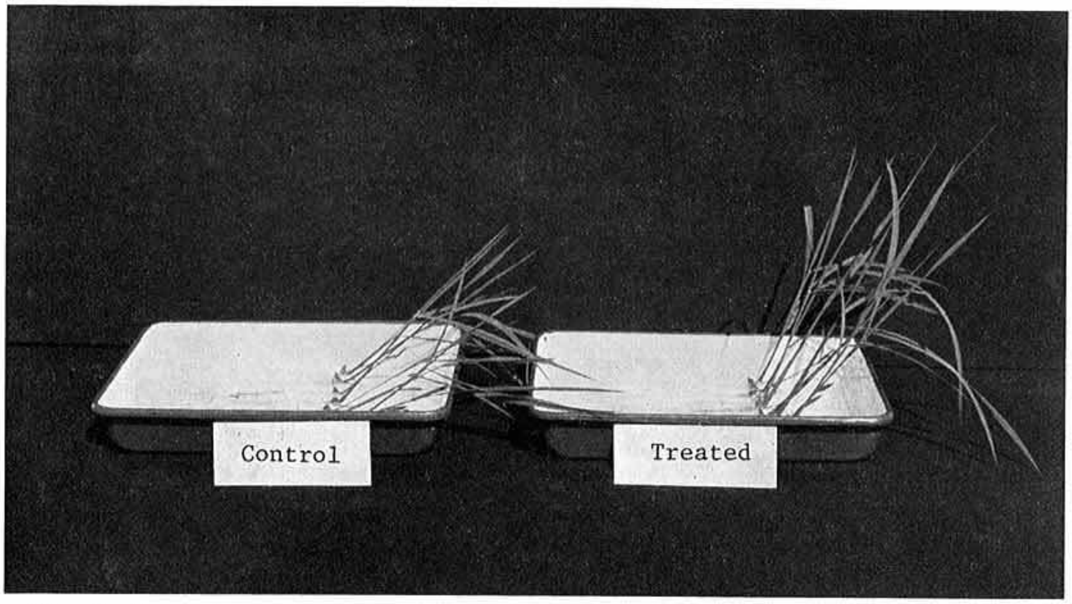


Plate 3. Effect of 3-hydroxy-5-methyl isoxazole on the negative geotropic curvature of the horizontally placed rice seedlings

Immediately after sowing, 7.5 mg of HMI per 500 ml beaker was applied to the soil. Rice seedlings were horizontally placed at 30°C. Plate was taken 3 days after seedlings were placed in a horizontal position

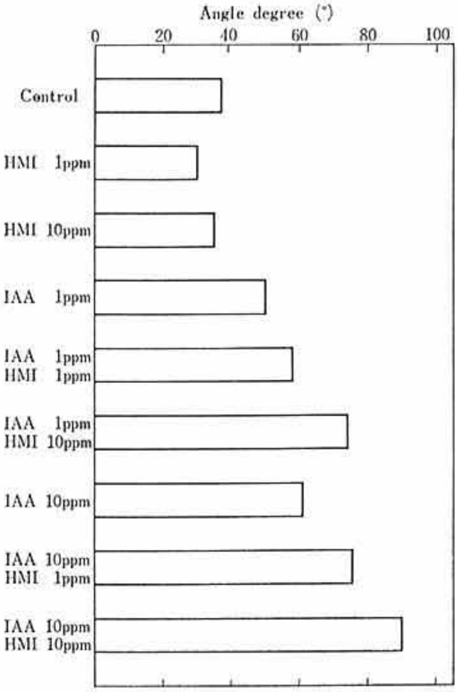


Fig. 6. Effect of 3-hydroxy-5-methyl isoxazole (HMI) on the auxin-induced inclination of lamina in etiolated 2nd leaves of rice
Segments consisted of lamina joint with intact lamina and 2 cm of sheath were treated in various solutions of IAA and HMI after pre-treatment for 24 hours by deionized water. and the inclination of lamina was measured at 48 hours after treatment

Therefore, the increased geotropic curvature response of rice seedlings caused by HMI may be due to a synergistic effect of HMI on endogenous auxin.

It seems that the increased response of rice seedlings caused by HMI is a very important factor to be considered in mechanical transplanting of rice seedlings.

Low temperature injury and HMI

Plate 4 shows the effect of HMI on the low temperature injury of rice seedlings.

HMI was applied immediately after sowing to the treated plot. After 2 weeks, both HMI-treated and control plots were exposed to 10°C day and 5°C night temperature for 3 days. The younger upper leaves of plants in the HMI-treated plot remained green but lower leaves were damaged by the cold treatment. On the contrary, all leaves of the seedlings grown in the control plot turned yellow and wilted. This shows very clearly that HMI treatment increases cold resistance in rice.

This improvement in the physiological conditions of HMI-treated plants may be due to the effect of HMI on the growth and activity of roots.

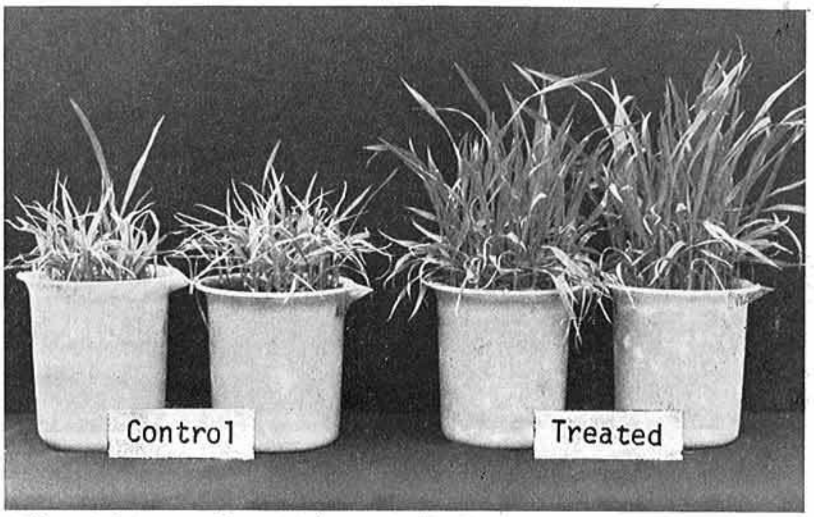


Plate 4. Effect of 3-hydroxy-5-methyl isoxazole (HMI) on growth of rice seedlings exposed to low temperature. The picture was taken 2 weeks after low temperature treatment

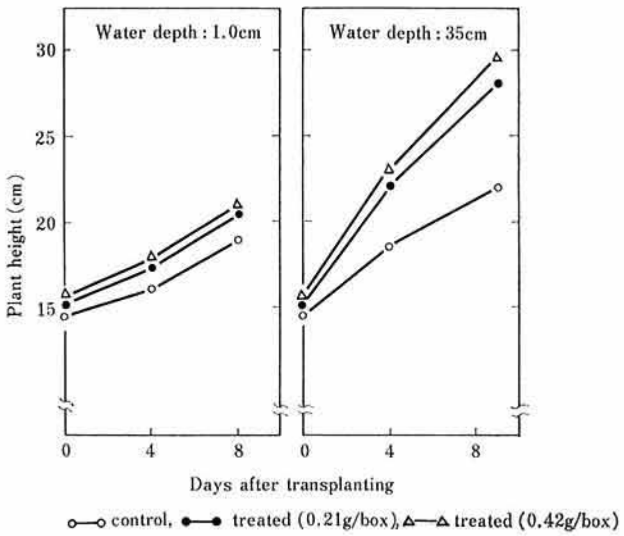


Fig. 7. Effect of 3-hydroxy-5-methyl isoxazole on the increase in plant height of rice seedlings under overhead flooding condition. Values are the means of 20 individuals

Overhead flooding damage, herbicide injury and HMI

It was observed that the pre-treatment of rice seedlings with HMI promoted the shoot elongation, compared with untreated seedlings, under overhead flooding condition (Fig. 7). A drastic reduction in overhead flooding damage was observed when rice seedlings were pre-treated with HMI.

The pre-treatment of rice seedlings with HMI also reduced the phytotoxic action of five of seven herbicides applied three days after transplanting. The five herbicides whose phytotoxic action was reduced were simetryne, MCC-MCP, NIP (nitrofen), CNP and DCPA (propanil).

The reduced submergence damage and herbicidal injury observed in rice seedlings may be attributed to increased seedling vigor induced by HMI. The improved physiological condition in these rice seedlings may increase their resistance to unfavorable circumstances.

References

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