SIGNIFICANCE OF VIRUS-INDUCED PROLINE ACCUMULATION IN RICE TUNGRO EPIDEMICS

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ABSTRACT

Preference for tungro [insect-transmitted transitory (semipersistent) virus]-affected rice plants by the vectors (green leafhoppers) in relation to virus-induced proline accumulation was investigated. Tungro-diseased plants of cultivars Taichung Native 1, Latisail, CR 44-955 and Pankhari 203 attracted greater number of leafhoppers than the healthy plants, *albeit* the number of insects attracted by healthy and diseased plants of CR 44-955 and Pankhari 203 was very low. Insect attraction was related to the host sensitivity to the virus and in turn was associated with the degree of disease-induced proline accumulation. However, healthy and tungro-diseased plants of cultivar IR 20 did not differ in the number of insects harboured by them. The epidemiological significance of proline in tungro spread is discussed.

INTRODUCTION

IN transitory[1] (semipersistent[2]) leafhopper-transmitted rice tungro virus disease, recognition of diseased plants by the vectors and their movement towards the virus source to acquire the virus for its subsequent transmission to healthy plants are of prime importance in disease spread. Insects possess extremely sensitive mechanisms for recognising their hosts and are lured to them by host chemicals[3-6] The concentration of chemical attractants such as amino acids and other nitrogenous compounds and plant growth substances are highly influenced by environmental conditions[6] and this would affect the ability of plants to attract the insects

Available evidence shows that amino acids are associated with insect herbivory. Chewing insects (Lepidoptera) possess amino acid receptors[7] and the sucking insects aphids are known to detect amino acids[8]. Proline and valine have been implicated in the chemodetection of host plants by grasshoppers[9]. Nevertheless, this view has recently been questioned[10]. High levels of proline accumulate in drought-stressed plants[11-14]. Recently, we[15] have shown that the green leafhopper-transmitted tungro virus mimics the abiotic stress caused by drought in accumulating free proline in diseased rice leaves. This led us to suggest that high level of proline accumulation in tungro-diseased plants might be due to a deleterious effect of virus infection. If green leafhoppers are also attracted by proline, accumulation of this amino acid under stress situations may have an epidemiological significance in tungro outbreaks. In this paper, we examine the green leafhopper preference for tungro-diseased plants *vis-a-vis* disease-induced proline accumulation.

MATERIALS AND METHODS

Plant materials and growth conditions: Rice (*Oryza sativa* L.) cultivars Taichung Native 1 (TN 1)[16,17], Latisail[17], IR 20[18], CR 44-955[18], and Pankhari 203[16] differing in their sensitivity to tungro virus in that order by producing severe to mild symptoms when infected with the virus, were grown from seeds in galvanized iron trays (50 × 40 × 10 cm) containing 10 kg of alluvial soil mixed with 5 g of $(NH_4)_2SO_4$, 4 g of superphosphate and 4 g of KCl. Each tray contained all the five cultivars grown in rows (one cultivar/row of 10 seedlings). Eight similar trays with plants were maintained.

Virus inoculation: Twenty-five day old plants at 3-4 leaf stage grown in four trays were caged individually in galvanized iron wire net cages and were inoculated by confining two viruliferous green leafhoppers [*Nephotettix virescens* (Distant)] in each cage for 8 hr[19]. The other four trays of seedlings were maintained as control and were similarly treated with non-viruliferous insects.

Proline determination: The symptoms of the disease appeared in the form of interveinal chlorosis on the fifth leaf of the plants, which had emerged fully six days after inoculation. The fifth leaves were collected separately from 10 healthy and 10 virus-infected plants on seven and 14 days after inoculation from two sets of plants. On each assay date, two trays consisting of a set of healthy and inoculated seedlings were used for sampling. Their free proline extracted from two identical samples, each consisting of five leaves; was determined spectrophotometrically[20] in duplicate.

Insect attraction: A different set of plants was used for insect attraction studies on seven and 14 days after

inoculation. On each assay date, two trays consisting of a set of healthy and inoculated seedlings were exposed under natural light conditions inside insect rearing wooden cages (90 × 60 × 90 cm) covered with insect-proof galvanized iron wire mesh containing 300 adult green leafhoppers for 16 hr (from 1600 to 0800 hr). At the end of the exposure to the insects, the number of leafhoppers harboured by individual seedlings was carefuly counted without disturbing the insect colony. The mean number of insects attracted per plant was calculated from two different experiments.

Results and Discussion

Tungro disease of rice initially results in chlorosis leading to discolouration of the leaves from yellow-orange to various shades of orange-red, besides characteristic stunting of plants[21,22]. Seven days after inoculation, the leaves from inoculated plants had just started to exhibit mild chlorosis, while 14 days after inoculation they showed various shades of yellow-orange discolouration depending upon their sensitivity to the virus. Cultivar TN 1 exhibited the maximum intensity of leaf discolouration 14 days after inoculation with virus followed by Latisail and IR 20, while CR 44-955 and Pankhari 203 exhibited mild stunting but did not show any leaf discolouration.

Resistance to the insect vector is genetically independent of the resistance to tungro virus[23]. Cultivar TN 1[16,17] and Latisail[17] are susceptile, while IR 20[24,25], CR 44-955[26] and Pankhari 203[16,27] are resistant to the insect vector. Healthy seedlings of cultivar TN 1 harboured more number of insects followed by IR 20, Latisail, CR 44-955 and Pankhari 203 (Table 1). Leafhopper resistance in rice is believed to be due to presence of toxic substances capable of repelling the insects or absence of feeding stimulus or of certain essential nutrients required by the insects[27]. The cause of leafhopper resistance is also considered to be gustatory rather than olfactory or visual since the insects do not exhibit distinct differences in their alighting behaviour on different varieties, but they do not stay on resistant plants for sustained feeding[28]. This would explain the presence of insects on leafhopper resistant healthy plants. However, the feeding behaviour of leafhopper on resistant Pankhari 203 and susceptible TN 1 is similar[16] and if the cultivars are susceptible to the virus, they may develop the disease even though they are resistant to the vector.

Tungro-diseased plants, excepting IR 20, attracted greater number of green leafhoppers than the healthy plants *albeit* the number of insects attracted especially by both healthy and diseased plants of cultivars CR 44-955 and Pankhari 203 was very low (Table 1). Interestingly, there was no difference between the healthy

Table 1

Influence of tungro disease development on green leafhopper preference for different rice cultivars with various levels of sensitivity to the virus.

(Data are number of insects per plant)

Cultivar	Virus sensitivity*	Days after inoculation: 7 — Healthy	Inoculated	Mean	14 — Healthy	Inoculated	Mean
TN 1	++++	3.3	4.1	3.7	3.8	5.7	4.8
Latisail	+++	1.8	2.9	2.4	1.7	3.6	2.7
IR 20	++	2.6	2.6	2.6	2.3	2.6	2.5
CR 44-955	+	1.5	2.3	1.9	1.6	2.4	2.0
Pankhari 203	+	1.0	1.5	1.3	0.9	1.7	1.3
Mean		2.0	2.7	—	2.1	3.2	—

* Based on severity of the symptoms in terms of leaf discolouration and/or stunting; the plus signs denote the degree of host sensitivity to the virus. L.S.D. at $P=0.05$ between cultivar means = 0.3 and 0.3; treatment means = NS and 0.1; and cultivar x treatment means = NS and NS on 7 and 14 days after inoculation, respectively.

and inoculated plants of cultivar IR 20 in the number of insects harboured by them. Our experiments further showed that insect attraction by rice cultivars was related to virus sensitivity of the plants. The cultivar with maximum sensitivity to the virus attracted more number of insects than those with mild sensitivity. The evidence on proline accumulation gathered by us reveals that the amino acid accumulated in response to virus infection even seven days after inoculation (Table 2). The accumulation of the amino acid increased with the symptom development and was maximum in highly sensitive cultivar TN 1 and least in the tolerant cultivar Pankhari 203.

Green leafhoppers prefer tungro-diseased plants in fields[29,30] and in cages[31] over healthy plants. The insect attraction by diseased plants has been attributed to the yellow colour of the diseased leaves[31]. Although colour influences the early stages of insect orientation, it may not be exclusively responsible for insect attraction[32]. Based on the fact that the diseased plants of highly sensitive cultivar TN 1, which did not yet develop typical yellow-orange discolouration of the leaves seven days after inoculation, attracted more insects than the healthy plants; we believe that apart from colour effect, a chemoattractant, presumably proline synthesized and accumulated due to virus-host interaction might be responsible for the attraction of insects.

It has been reported[25,31,33] that cultivar IR 20 is a poor source of virus. This may be due to the fact that the insects do not visit the diseased IR 20 plants frequently to acquire the virus. Perhaps, the lower preference (nonpreference) of cultivars IR 20, CR 44-955 and Pankhari 203 might be due to their antibiosis to the insects. Even under diseased conditions, these cultivars might have repelled the green leafhoppers from feeding, despite the increase in their proline content due to virus infection.

Additionally, as the virus is transitory[1] (semipersistent[2]) in its vectors which remain viruliferous for a maximum period of five days after becoming infective[34], it is essential that the leafhoppers repeatedly feed on diseased plants to serve as effective vectors of the virus[35]. Proline that accumulates in tungro-diseased rice plants may lure the green leafhoppers to revisit the diseased plants frequently and thus encourage them to acquire the virus repeatedly and transmit it to adjacent healthy plants. Admittedly, this would enhance the population of viruliferous insects and may result in the spread of the disease in serious epidemics. Further, our earlier results[15] have shown that a drought tolerant rice cultivar which has a greater capacity to accumulate proline is most sensitive to the virus than a susceptible cultivar. As we are led to believe that proline accumulation in tungro syndrome might be due to a harmful effect caused by

TABLE 2

Proline content of healthy and tungro-diseased leaves of rice cultivars differing in their sensitivity to the virus.

(Data are μmol of proline per gram fresh weight)

		Days after inoculation					
		7			14		
Cultivar	Virus sensitivity*	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
TN 1	++++	0.15	0.23	0.19	0.17	1.09	0.63
Latisail	+++	0.10	0.18	0.14	0.16	0.93	0.55
IR 20	++	0.11	0.16	0.14	0.18	0.55	0.37
CR 44-955	+	0.09	0.12	0.11	0.17	0.41	0.29
Pankhari 203	+	0.08	0.10	0.09	0.14	0.22	0.18
Mean		0.11	0.16	—	0.16	0.64	—

* Based on severity of the symptoms in terms of leaf discolouration and/or stunting; the plus signs denote the degree of host sensitivity to the virus. L.S.D. at $P = 0.05$ between cultivar means = 0.0001 and 0.0004; treatment means = 0.02 and 0.04; and cultivar x treatment means 0.03 and 0.05 on 7 and 14 days after inoculation, respectively.

the virus infection, there is every likelihood that if tungro occurs in water-stressed locations, it might be more severe, since the metabolic system of water-stressed plants is already conditioned to accumulate this amino acid.

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1. Ling, K. C. and Tiongco, E. R., In *Leafhopper vectors and plant disease agents*, (eds) K. Maramorosch and K. F. Harris, Academic Press, New York, London, 1979, p. 349.
2. Harris, K. F., In *Leafhopper vectors and plant disease agents*, (eds) K. Maramorosch and K. F. Harris, Academic Press, New York, London, 1979, p. 217.
3. Jacobson, M., *Annu. Rev. Entomol.*, 1966, **11**, 403.
4. Jacobson, M. and Beroza, M., *Science*, 1963, **140**, 1367.
5. Hanover, J. W., *Annu. Rev. Entomol.*, 1975, **20**, 75.
6. Levins, R. and Wilson, M., *Annu. Rev. Entomol.*, 1980, **25**, 287.
7. Schoonhoven, L. M., *Nature*, (*London*), 1969, **221**, 1268.
8. van Emden, H. F. and Way, M. J., In *Insect/plant relationships*, (ed.) H. F. van Emden, *Proc. of Symp. Royal Ent. Soc.*, London, Blackwell Scientific Publications, Oxford, 1973, p. 181.
9. Haglund, B. M., *Nature* (*Lodnon*), 1980, **288**, 697.
10. Bright, S. W. J., Lea, P. J., Kuch, J. S. H., Woodcock, C., Hollomon, D. W. and Scott, G. C., *Nature* (*London*), 1982, **295**, 592.
11. Singh, T. N., Aspinall, D. and Paleg, L. G., *Nature, New Biol.*, 1972, **236**, 188.
12. Blum, A. and Ebercon, A., *Crop Sci.*, 1976, **16**, 428.
13. Bogess, S. F., Aspinall, D. and Paleg, L. G., *Aust. J. Plant Physiol.*, 1976, **3**, 513.
14. Mali, P. C. and Mehta, S. L., *Phytochemistry*, 1977, **16**, 1355.
15. Mahanty, S. K. and Sridhar, R., *Physiol. Plant.*, 1982, **56**, 89.
16. Ling, K. C., *Philippine Phytopathol.*, 1968, **4**,21.
17. *International Rice Research Institute, Annu. Rep. for 1980*, Los Banos, Philippines, 1981, p. 153.
18. Rao, G. M. and Anjaneyulu, A., *Oryza*, 1980, **17**, 210.
19. Sridhar, R., Reddy, P. R. and Anjaneyulu, A., *Phytopathol. Z.*, 1976, **86**, 136.
20. Bates, L. S., Waldren, R. P. and Teare, I. D.,*Plant Soil*, 1973, 39, 205.
21. Ling, K. C., *Rice virus diseases*, Int. Rice Res. Inst., Los Banos, Philippines, 1972.
22. Ou, S. H., *Rice diseases, Commonw. Mycol. Inst. Kew, Surrey*, England, 1972.
23. Athwal, D. S., In *Rice, science and man. Int. Rice Res. Inst.*, Los Banos, Philippines, 1972, p. 41.
24. Kush, G. S., *Adv. Agron.*, 1977, **29**, 265.
25. *International Rice Research Institute, Annu. Rep. for 1973*, Los Banos, Philippines, 1974, p. 9 and 117.
26. Rao, G. M., *Virus-vector-host relationships of rice tungro virus.*, Ph.D. Thesis, Utkal Univ., India, 1977.
27. Pathak, M. D., Cheng, C. H. and Fortuno, M. E., *Nature*, (*London*), 1969,**223**, 502.
28. Pathak, M. D., In *Rice breeding. Int. Rice Res. Inst., Los Banos*, Philippines, 1972, p. 325.
29. *International Rice Research Institute, Annu. Rep. for 1971*, Los Banos, Philippines, 1972, p. 117.
30. *International Rice Research Institute, Annu. Rep. for 1976*, Los Banos, Philippines, 1977, p. 149.
31. *International Rice Research Institute, Annu. Rep. for 1972*, Los Banos, Philippines, 1973, p. 121 and 163.
32. Beck, S. D., *Annu. Rev. Entomol.*, 1965, **10**, 207.
33. *International Rice Research Institute, Annu. Rep. for 1974*, Los Banos, Philippines, 1975, p. 179.
34. Ling, K. C., *Phytopathology*, 1966, **56**, 1252.
35. Bae, S. H. and Pathak, M. D., *J. Econ. Ent.*, 1969, **62**, 772.