# Nutritional disorders in rice due to infestation by *Heterodera oryzicola* and *Meloidogyne graminicola*

Y. Seshagiri RAO, Ayyamperumal JAYAPRAKASH and Janaki MOHANTY

*Central Rice Research Institute, Cuttack 753 006, India.*

SUMMARY

Infestation of rice by *Heterodera oryzicola* and *Meloidogyne graminicola* caused reduction in nitrogen, phosphorus, iron, and reducing sugars in shoots and roots. Total sugars, protein, IAA, cytokinin and thiamine were reduced in the cyst nematode infested plants and phenols were reduced with root-knot nematode infested plants. Phenols decreased in shoots and increased in roots due to cyst nematode, while the rootknot nematode caused similar changes in potassium, manganese and magnesium. There was an increase in calcium, sodium, soluble amino-acids, ABA and starch in both shoots and roots of the cyst nematode infested plants and of total sugars, protein, DNA and RNA with the root-knot nematode infested plants. Infestation by both the nematodes caused nutritional disorders limiting the uptake of nitrogen and phosphorus and chlorosis of leaves.

RÉSUMÉ

*Perturbations nutritionnelles chez le riz infesté par* Heterodera oryzicola *et* Meloidogyne graminicola

L'infestation du riz par *Heterodera oryzicola* et *Meloidogyne graminicola* provoque une diminution de la teneur des racines et des parties aériennes en azote, phosphore, fer et sucres réducteurs. *H. oryzicola* diminue les taux de sucres totaux, de protéines, d'acide indol-acétique, de cytokinine et de thiamine, tandis que *M. graminicola* diminue le taux des phénols. Les phénols diminuent dans les racines et augmentent dans les parties aériennes dans le cas de *H. oryzicola*, tandis que *M. graminicola* provoque des modifications similaires pour le potassium, le manganèse et le magnésium. Le calcium, le sodium, les acides aminés solubles, l'ABA et l'amidon augmentent tant dans les racines que dans les parties aériennes dans le cas de *H. oryzicola*, cette augmentation affectant les sucres totaux, les protéines, l'ADN et l'ARN dans le cas de *M. graminicola*. L'infestation par l'un et l'autre des nématodes provoque des perturbations nutritionnelles limitant l'absorption de l'azote et du phosphore, et causant une chlorose foliaire.

Infestation in roots of rice (*Oryza sativa* L.) by sedentary endoparasitic nematodes which compete for vital elements in the stele, causes reduction in grain yield due to the nutritional stress created during and perhaps also after nematosis. Virtually, no galls were developed on roots as a result of infestation by the cyst nematode (*Heterodera oryzicola* Rao & Jayaprakash, 1978) but a few phloem cells coalesced to form syncytia and with no hyperplasia of xylem. Cell walls of endodermis and pericycle near the head of the nematode were greatly thickened due to pectin deposition (Jayaprakash & Rao, 1981). Browning and chlorosis of leaves, reduction in plant height and tiller number, early flowering and partial filling of kernels are the typical symptoms of injury (Jayaprakash & Rao, 1982). Loss in grain yield up to 41.9 per cent was recorded (Ushakumari & Kuriyan, 1981). Infestation by the root-knot nematode (*Meloidogyne graminicola* Golden & Birchfield, 1968) caused distinct galls on roots. Phloem parenchyma cells developed multinucleate coenocytes with abnormal xylem proliferation and the cortical cells were hypertrophied (Jena & Rao, 1977). Yellowing, stunting, reduction in tiller number, delay in flowering by 10 to 17 days and reduction in test weight of grain are symptoms of injury (Israel, Rao & Rao, 1963). Loss in grain yield ranged from 17 to 32 per cent (Biswas & Rao, 1971).

In view of their importance, the etiology of nematosis due to the cyst and root-knot nematodes of rice was investigated by bio-chemical analyses of healthy and nematode infested plants.

## Materials and methods

Steam sterilised field soil (sandy loam) was air dried, placed in plastic trays (75 cm × 6 cm depth) saturated with water and seeds of rice cv. Sattari or IR 8 were sown at 500 per tray in six trays. When sprouts of Sattari were 15 days old, soil in three trays were inoculated to provide 100 infective juveniles of the cyst nematode per sprout. Similarly, sprouts of IR 8 were inoculated with infective juveniles of the root-knot nematode (Rao & Israel, 1972). At 35 days after inoculation, the endoparasites in roots of fifteen plants were estimated (Rao *et al.*, 1971). Inoculated and uninoculated plants of both varieties were taken, the nematodes dissected out from the former and samples were adjusted to provide 10 g fresh weight of shoots or roots for analyses.

Samples of 1 g tissues of shoots or roots were cut into bits and boiled in 80 per cent ethanol. The supernatent was filtered and residues were repeatedly extracted to make 500 ml of filtrate (Yoshida *et al.*, 1976). This filtrate was used for estimation of total chlorophyll of foliage and a and b fractions (Arnon, 1959), total sugars (Hodge & Hofreiter, 1962), reducing sugars (Nelson, 1944), soluble amino-acids (Moore & Stein, 1948) phenols (Bray & Thrope, 1954) in mg/g. IAA and cytokinins (Chatterjee, Mondal & Sircar, 1976). ABA (Saavedra & Wain, 1974) and thiamine (Dasgupta & Cadwellader, 1970) were also estimated from the extracts and expressed as µg/g.

Sugar-free residues of the extracts were used to determine the starch (Hassid & Neufeld, 1964). Protein was precipitated in the extract (Racusen & Johnstone, 1961) and estimated in mg/g by modified Lowry's method (Bensadown & Winstein, 1976). Ethanol extracts were made lipid-free by addition of ethyl ether and centrifugation. RNA was precipitated from residues by alkaline hydrolysis (Howell, 1973) and the residues were further treated with 1 N perchloric acid for extraction of DNA fraction. Total RNA (Markham, 1955) and DNA (Burton, 1956) were expressed as µg/g.

Samples of shoots and roots were oven-dried in oven at 80° and powdered for determination of total nitrogen, phosphorus, calcium and sodium (Pattnaik, Misra & Bhadrachalam, 1966), potassium (Jackson, 1973) and iron (Sandell, 1950) and expressed as mg/g. Each chemical constituent in the nematised plant was presented as the percentage decrease or increase over the actual amount in shoots or root tissues of the healthy plant, given in parenthesis, for comparison.

## Results and discussion

### CYST NEMATODE

*Nematode incidence*

Infested plants showed typical symptoms in foliage and roots. The cysts and developing stages of the nematode were 28.3 and 58.5 respectively in root system, well above the threshold level of 34.5 endoparasites per plant (Rao, 1984).

*Chlorophyll, sugars and starch*

Leaf chlorophyll (4.87 mg/g) was reduced by 37.8 % in the nematode infected plant (Fig. 1). Consequently, total sugars in shoots (74.1 mg/g) and roots (34.9 mg/g) were reduced by 18.2 and 33 %. The amount of reducing sugars (54.7 mg/g in shoots and 24.9 mg/g in roots) was decreased by 13.5 and 34.5 % respectively. However, starch (5 mg/g in shoots and 1 mg/g in roots in healthy plants) increased by 77.3 and 68.3 %. Starch accumulation was similar to that obtained in root infestation by the lance nematode, *Hoplolaimus indicus* Sher, 1963 (Ramana, Prasad & Rao, 1976) root-lesion nematode, *Pratylenchus indicus* Das, 1959 (Prasad & Rao, 1978) and the root-knot nematode, *M. graminicola* (Mohanty & Rao, 1978) in rice. Reduction in chlorophyll inhibited photosynthesis (Livine, 1964).

*Proteins, amino acids and nucleic acids*

Total proteins (36.7 and 31.8 mg/g respectively in shoots and roots of healthy plants) were reduced by 36.7 and 31.8 % respectively in shoots and roots (Fig. 1). Soluble anino acids (22.9 and 12.7 mg/g in shoots and roots of healthy plants) had increased by 75.9 and 58.0 %. Enzymatic degradation of plant proteins and reduced photosynthesis caused accumulation of soluble amino acids and also reduction in the total number of tillers in the nematode infected plant. RNA (106.8 and 70 µg/g of shoot and roots) increased by 17.5 % in shoots and decreased by 25 % in the roots of nematode infested plants due to inhibition of protein synthesis and nitrogen metabolism of plants. DNA (14.8 and 15.6 µg/g in shoots and roots) showed a slight increase in shoots of infected plants by 1.3 % and in roots a decrease by 4.5 %. The non-interference by the nematode in DNA metabolism may be one reason for its inability to induce hypertrophy and gall development in the nematised plants.

*Nutrients*

Nitrogen content in shoots and roots of healthy plants was 74.9 and 69.8 mg/g respectively. Cyst nematode infestation caused a reduction by 20.3 and 40.1 % in shoots and roots (Fig. 1). Phosphorus (7.5 and 8.5 mg/g) was reduced by 38.3 and 26.6 % in shoots and roots of affected plants. Deficiency of nitrogen and phosphorus decreased the length of leaf (Ushakumari & Kuriyan, 1982), number of panicles and grains per panicle (Rao, 1984). Potash content (35.5 and 15.9 mg/g in shoots and roots of healthy plants) was reduced by 32.0 and 25.1 %. Reduction in potash decreased the thousand grain weight in nematised plants (Jayaprakash & Rao, 1981). The amount of iron in shoots and roots of healthy plants (19.0 and 14.7 mg per g) was reduced by 16.3 and 27.9 % in roots and roots of the infected plants. Deficiency of iron, an essential element for production of leaf chlorophyll, caused chlorosis. Calcium (19.0 and 10.6 mg/g in shoots and roots of healthy plants) had increased by 22.4 and 30.7 % due to nematode infestation. Similarly, sodium (4.3 and 1.9 mg/g) had increased by 27.1 and 127.3 % in shoots and roots of rice following infestation by the nematode. This indicated an increase in the absorption as a compensation for the decrease in potassium in order to maintain the cation balance as in the case of potato *(Solanum tuberosum)* infected by the cyst nematode *Globodera rostochiensis* (Trudgill, Evans & Parrot, 1975).

*Phenols*

Total phenols in shoots and roots of healthy plants were 19.5 and 28.4 mg/g, and in the infested plants, the phenols were reduced in shoots by 35.7 % and increased in roots by 31.5 % (Fig. 1). Basipetal translocation of free phenols at sites of nematode attack and establishment in roots, and the interference in the phenol metabolism by the nematode, contributed to the reduction of phenols in the shoots.

*Growth regulators and hormones*

IAA (13.0 and 10.2 microgrammes/g) in shoots and roots of healthy plants) was reduced by 39.2 and 32.4 % (Fig. 1). As gall formation was not essential for the development of the endoparasite, the levels of indole compounds were low, which however caused a general reduction in height of plants infected by the nematode (Kuriyan, 1985). Cytokinins (14.2 and 12.1 μm/g of shoot and root of healthy plants) were also reduced by 63.6 and 43.0 % indicating a disruption in their synthesis and translocation, causing loss of chlorophyll. The ABA levels (0.9 and 0.8 μm/g of shoots and roots in healthy plants) had significantly increased by 3.18 and 2.76 times in shoots and roots of infected plants and this increase caused stunting, destruction of chlorophyll, accumulation of starch and inhibition of plant growth hormones such as IAA and cytokinins (Addicott & Lyon, 1969).

*Vitamins*

Thiamine (Vitamin B 1) content in shoots and roots of healthy plant was 14.5 and 11.0 μm/g. Thiamine was reduced by 40.8 and 47.3 % in shoots and roots of nematode affected plants (Fig. 1). Disturbance in the normal synthesis of thiamine in roots and its translo-

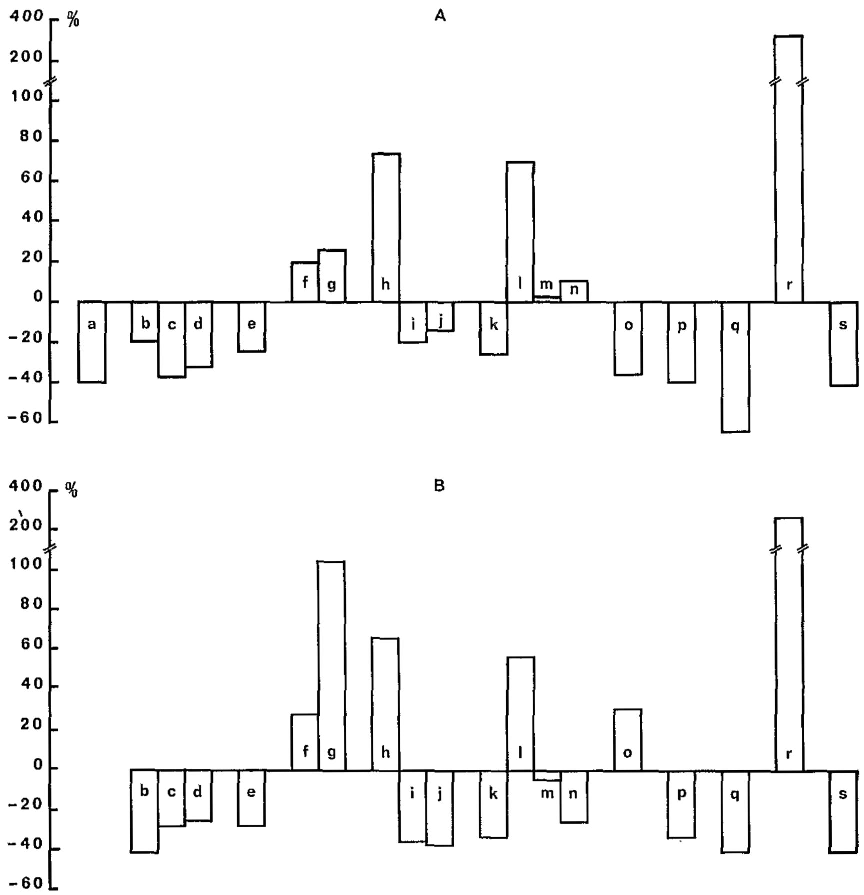


Fig. 1. Changes in the chemical constituents of rice plants, 35 days after inoculation with *Heterodera oryzicola;* A : shoot; B : root. (a = chlorophyll; b = nitrogen; c = phophorus; d = potassium; e = iron; f = calcium; g = sodium; h = starch; i = total sugars; j = reducing sugars; k = total protein; l = soluble amino-acids; m = DNA; n = RNA; o = phenols; p = indol-acetic acid; q = cytokinins; r = IBA; s = thiamine).

cation to roots was found to cause reduction in root growth.

Increase in calcium, sodium, starch and ABA and decrease in nitrogen, phosphorus, potash, iron, total and reducing sugars, protein, IAA, cytokinin and thiamine in both shoots and roots characterised the changes in the nutritional status of rice plants infested by the cyst nematode. Drought conditions in maize (*Zea mays* L.) and viral infection in rice increased ABA synthesis (Saavedra & Wain, 1974). Decrease in phenols of leaves and leaf-sheaths may be one reason for the increase in susceptibility of cyst nematode affected plants to the seedling-blight pathogen, *Sclerotium rolfsii* (Jayaprakash & Rao, 1983).

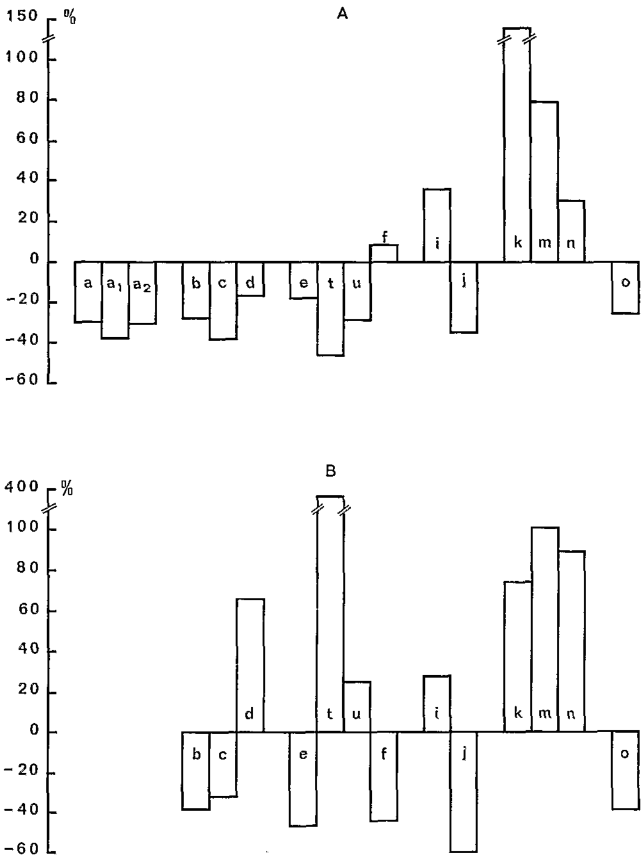


Fig. 2. Changes in the chemical constituents of rice plants, 35 days after inoculation with *Meloidogyne graminicola;* A : shoot; B : root (same legend as Fig. 1; a = chlorophyll a; a = chlorophyl b; t = manganese; u = magnesium).

ROOT-KNOT NEMATODE

*Nematode incidence*

Infested plants showed typical symptoms on foliage and roots. The number of endoparasites and egg masses were 64.5 and 31.5 per plant, higher than the threshold level of 15 egg masses per plant (Rao, 1984).

*Chlorophyll and sugars*

Total chlorophyll in leaves of healthy plants was 3.7 mg and of a and b fractions were 2.1 and 1.6 mg/g. Chlorophyll was reduced, owing to root-knot nematode incidence, by 30.2 % of total and 39.5 and 32 % of the a and b fractions. Total sugars (5.2 and 2.8 mg/g in shoots and roots of healthy plants) increased by 36.6 and 29.2 % (Fig. 2). Reducing sugars (0.3 and 0.2 mg/g) decreased by 35.5 and 60 %. High starch and total sugars accumulation due to nematosis was reported earlier (Jena & Rao, 1977). Increase of total sugars due to higher metabolism in the hypertrophied cortical and endodermal cells of infected roots, was similar to that observed with root infestation by *Hirschmanniella mucronata* (Mahapatra, 1966) and the root-lesion nematode, *Pratylenchus indicus* (Prasad & Rao, 1981) in rice. Also, reduction in the uptake of nitrogen caused an increase in sugars (Srirdhar & Ou, 1974).

*Proteins and nucleic acids*

The amounts of protein in the shoots and roots of healthy plants were 0.8 and 0.7 mg/g. Proteins increased in shoots and roots of nematode infected plants by 1.42 times and 75 % respectively (Fig. 2) confirming earlier reports (Jena & Rao, 1977). The amount of RNA (1.0 and 0.6 µm/g of shoots and roots of healthy plants) increased by 23.5 and 91.9 %, due to excessive hypertrophy and hyperplasia following nematode establishment and the translocation of this nucleic acid to foliage. Similarly, DNA (0.6 and 0.5 µg/g per g of shoots and roots of healthy plants) had increased by 80.5 and 103.5 % in shoots and roots of nematised plants. The nematode induced galls were reported to contain higher DNA and RNA (Epstein, 1974; Mohanty & Rao, 1978). RNA had increased due to the inhibition of protein synthesis and nitrogen metabolism.

*Nutrients*

Nitrogen (28.2 and 16.9 mg/g of shoots and roots of healthy plants) was reduced by 29.2 and 36.6 % in shoots and roots of infected plants (Fig. 2). Similarly, phosphorus (6.0 and 3.1 mg/g) was reduced by 38.5 and 33.5 % in shoots and roots of nematised plants. The amount of potassium (14.0 mg/g and 3.0 mg/g in shoots and roots of healthy plants) was reduced in shoots by 18.7 % and increased in roots by 67.6 %, in the nematode infested plants, indicating that it inhibited translocation to foliage. Iron content in the shoots of healthy plants was 1.7 and 2.1 mg/g, while in the nematode infested plants, there was a reduction by 19.0 and 45.5 per cent respectively in shoots and roots. Calcium (6.5 mg/g in shoots and 2.3 mg/g in roots of healthy plants) slightly increased in shoots of infected plants by 8.2 % and decreased in roots by 44.4 %. The amount of magnesium (4.8 mg/g in shoots and 1.7 mg/g in roots of healthy plants) was reduced in shoots by 29.5 % and increased in roots by 24.5 %. Similarly, manganese (0.6 mg/g in shoots and 0.1 mg/g in roots of healthy plants) was reduced in shoots by 48.0 % and increased in roots by 3.63 times in the nematised plants perhaps due to low absorption of iron (Pande, 1984). Deficiency of calcium, magnesium and phosphorus in early growth stages of the plants interrupted the normal physiological functioning of roots.

*Phenols*

The phenol content of shoots and roots of healthy plants was 0.1 and 0.3 mg/g respectively. Nematode infected plants showed reduction of total phenols by 25 and 38 % in shoots and roots (Fig. 2) as with infestation by the root nematode *Hirschmanniella oryzae* in rice (Jayaprakash, Rao & Mohandas, 1981). For the same reason, the nematised plants were more susceptible to the leaf blast pathogen, *Pyricularia oryzae* (Israel, Rao & Rao, 1963).

Plants affected by the root-knot nematode showed a general increase in total sugars, proteins, DNA and RNA and reduction in nitrogen, phosphorus, iron, reducing sugars and phenols in both shoots and roots. Calcium increased in shoots, and potash, manganese and magnesium increased in roots. Manganese toxicity caused stunting of plants. Possibly the nematode caused specific changes in the nutritional status of plants which resulted in chlorosis, stunting and the associated symptoms on foliage.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. B. Souchaud, ORSTOM Principal Assistant, for having drawn the definitive illustrations.

## REFERENCES

ADDICOTT, F. T. & LYON, J. L. (1969). Physiology of abscisic acid and related substances. *A. Rev. Plant Physiol.*, 20 : 139-164.

ARNON, D. L. (1959). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulagaris. Plant Physiol.*, 24 : 1-5.

BISWAS, H. & Rao, Y. S. (1971). Studies on nematodes of rice and rice soils. II. Influence of *Meloidogyne graminicola* on yield of rice. *Oryza*, 8 : 101-102.

BRAY, H. G. & THORPE, W. V. (1954). Analysis of phenolic compounds of interest in metabolism. *Math. Biochem. Anal.*, 1 : 27-52.

BURTON, K. (1956). A study of the condition and mechanism of diphenyl amine reaction for colorimetric estimation of deoxyribonucleic acid. *Biochem. J.*, 62 : 315-323.

CHATTERJEE, A., MONDAL, R. K. & SIRCAR, S. M. (1976). Changes in the levels of growth substances during grain filling of rice. *Indian J. Pl. Physiol.*, 19 : 254-268.

DASGUPTA, V. & CADWALLADER, D. E. (1970). *Methods in enzymology. Vol. XVIII.* New-York, Acad. Press, 688 p.

EPSTEIN, E. I. (1974). Biochemical changes in terminal root galls caused by an ectoparasite *Longidorus africanus*. Nucleic acids. *J. Nematol.*, 6 : 48-52.

HASSID, W. Z. & Newfeld, E. F. (1964). Quantitative determination of starch in plant tissues. In : Whistler, R. L., Smith, R. J., BeMiller, J. N. & Wolfrom, M. L. (Eds). *Methods of carbohydrate chemistry* IV., New-York, Academic Press : 33-36.

HOWELL, H. S. (1973). The isolation and analysis of DNA from eukaryotic cells. In : Chrespeels, M. J. (Ed). *Molecular techniques and approaches in developmental biology.* New-York, Wiley Inter Sci. : 124-127.

ISRAEL, P., RAO, Y. S. & RAO, Y. R. V. J. (1963). Investigations on nematodes of rice and rice soils. *Oryza*, 3 : 125-128.

JACKSON, M. L. (1973). *Soil chemical analysis.* New Delhi, Prentice-Hall of India, 498 p.

JAYAPRAKASH, A. & Rao, Y. S. (1981). Histopathological changes in rice due to the root infestation by the cyst nematode *(Heterodera oryzicola). Oryza* 18 : 233-235.

JAYAPRAKASH, A. & RAO, Y. S. (1982). Effect of *Heterodera oryzicola* incidence on growth of rice. *Oryza*, 19 : 60-61.

JAYAPRAKASH, A., RAO, Y. S. & MOHANDAS, C. (1981). Biochemical changes in rice associated with the rice root nematode, *Hirschmanniella oryzae* infestation. *Curr. Sci.*, 50 : 186-187.

JENA, R. N. & RAO, Y. S. (1977). Nature of resistance in rice *(Oryza sativa)* to root-knot nematode *(Meloidogyne graminicola).* II. Histopathology of nematode infestation. *Proc. Indian Acad. Sci.*, 86 B : 87-91.

KURIYAN, A. J. (1985). *Biennial Rept. of All India Coord. Res. Proj. on Nematode pests of agricultural crops and their control* : 16-22 [Mimeogr.].

LIVINE, A. (1964). Photosynthesis in healthy and rust affected plants. *Pl. Physiol.*, 39 : 614-661.

MAHAPATRA, N. K. (1976). Morphology, digestive and reproductive physiology of *Hirschmanniella mucronata* in rice. *Ann. Rept, CRRI, Cuttack*, 113 p.

MARKHAM, R. (1955). Nucleic acids, their compounds and related compounds. In : Peach, K. & Tracy, M. V. (Eds). *Methods of Plant analysis* IV. Berlin, Springler Verlag : 246-303.

MOHANTY, J. K. & RAO, Y. S. (1978). Biochemical perturbations in roots of rice following infestation by the root-knot nematode *(Meloidogyne graminicola). Symp. Host parasite relationships, Univ. Madras* : 17-18 [Mimeogr.].

MOORE, S. & STEIN, W. H. (1948). Photometric ninhydrin method for use in the chromatography of aminoacids. *J. Biol. Chem.*, 176 : 367-388.

NELSON, N. (1944). A photometric adoption of Somogyi method for the determination of glucose. *J. Biol. Chem.*, 153 : 375-380.

PANDE, H. K. (1984). Perspectives of lowland rainfed rice production in India by 2000 AD. *CRRI, Cuttack*, 51 p. [Mimeogr.].

PATNAIK, S., MISRA, C. B. & BHADRACHALAM, A. (1966). The analysis of mineral nutrients in plants. *Lab. Manual. CRRI, Cuttack*, 30 p. [Mimeogr.).

PRASAD, J. S. & RAO, Y. S. (1978). Metabolic changes in rice plant (*Oryza sativa* L.) induced by the root-lesion nematode (*Pratylenchus indicus* Das). *Proc. Symp. Host Parasite Relationships, Univ., Madras* : 19 [Mimeogr.].

PRASAD, J. S. & RAO, Y. S. (1981). Histopathology of rice roots infected with *Pratylenchus indicus* Das. *Riv. Parassit.*, 42 : 475-481.

RAMANA, K. V., PRASAD, J. S. & RAO, Y. S. (1976). Biochemical cchanges in roots of rice infected by the lance nematode, *Hoplolaimus indicus* Sher. *Indian J. exp. Biol.*, 14 : 216-217.

RAMANA, K. V., PRASAD, J. S. & RAO, Y. S. (1976). Biochemical changes in roots of rice infected by the lance nematode, *Hoplolaimus indicus* Sher. *Indian J. exp. Biol.*, 14 : 216-217.

RAO, Y. S. (1984). *Nematode problems in submerged soils and their management.* Haryana Agric. Univ., Hissar, 12 p. [Mimeogr.].

RAO, Y. S. & BISWAS, H. (1973). Evaluation of yield losses in rice due to the root-knot nematode. *Indian J. Nematol.*, 2 : 72-76.

RAO, Y. S. & ISRAEL, P. (1972). Influence of inoculum density on the final population of the rice root-knot nematode. *Indian J. Nematol.*, 2 : 72-76.

RAO, Y. S., ISRAEL, P., RAO, V. N., RAO, Y. R. V. J. & BISWAS, H. (1971). Studies on nematodes of rice and rice soils. III. Extraction methods and sampling. *Oryza*, 8 : 65-74.

SAAVEDRA, A. L. & WAIN, R. L. (1974). Abscisic acid levels in relation to drought tolerance in varieties of *Zea Mays. Nature*, 251 : 716-717.

SANDELL, E. B. (1950). Iron. In : *Colorimetric determination of traces of metals.* New-York, Interscience Publishers : 95-103.

SRIDHAR, R. & OU, S. H. (1974). Biochemical changes associated with the development of resistant and susceptible types of rice blast lesions. *Phytopathology*, 2 : 79.

TRUDGILL, D. L., EVANS, K. & PARROTT, D. M. (1975). Effects of potato cyst nematode on potato plants. I. *Nematologica*, 21 : 169-182.

USHAKUMARI I. A. & KURIYAN, A. J. (1982). Cyst nematode *Heterodera oryzicola* on rice in Kerala. I. Estimation of losses. *Indian J. Nematol.*, 11 : 106 [abstr.].

YOSHIDA, S., FORNO, D. A., COOK, J. H. & GOMEZ, K. A. (1976). *Laboratory Manual for Physiological Studies on Rice.* IRRI, Manila, Philippines, 82 p.

*Accepté pour publication le 28 août 1987.*