DETECTION OF RICE TUNGRO VIRUS DISEASE

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THE tungro virus infection in rice can be detected by serological techniques which require elaborate procedures and expensive equipment. The present study reports however a simple technique for quick diagnosis of the virus infection.

Iodine and phloroglucinol were tested for their suitability for detection of tungro virus infection. Susceptible ADT 31 rice plants at 10, 20, 30 and 40 days of age were inoculated with viruliferous green leafhopper (GLH) *Nephotettix virescens* Distant. In each treatment 20 plants were inoculated. Then the second leaf from the youngest of each test plant was excised early in the morning (6.30 am) at 5-day intervals after inoculation. The cut ends of the leaves were dipped in 5 ml of iodine solution placed in test tubes. The number of leaves showing dark blue streaks was recorded for each treatment.

Twenty-day-old ADT 31 seedlings were inoculated with viruliferous GLH. After 20 days, free hand sections of leaves (second leaf from youngest), leaf sheath and culm from rice tungro virus (RTV) inoculated and healthy plants were placed in different concentrations of phloroglucinol in 80% ethanol for 10 and 20 min. They were immediately transferred to concentrated HCl for 30–60 s, washed in water and observed in light microscope.

The results indicate that in plants inoculated at 10 days, 17.65% of plants show positive reaction with iodine test at 5 days after inoculation when symptoms of infection are not visible. The percentage increased, as the period after inoculation increased, and reaches a maximum of 88.2 at 15 days after inoculation. In the case of plants inoculated at 20, 30 and 40 days the positive reaction could be seen only after the expression of visible symptoms. The number of plants showing positive reaction decreased with increase in age of plants at inoculation.

Among the different tissues, the vascular elements in RTV-infected and healthy reacted with the phloroglucinol differentially. The comparison of different stain concentration and reaction of different tissues indicate that the vascular tissue in the culm react consistently at stain concentration of 2 and 5%. The difference in the intensity of pink colour in phloem of culm could be considered as a basis for determining tungro infection in rice plants. At 2% concentration, 93% of infected plants reacted differentially, while at 5% concentration, 97% of infected plants could be differentiated.

The present study showed that iodine test was useful in differentiating the healthy and tungro-infected plants based on the development of dark blue streaks in the leaves. The leaves of rice plants infected by tungro virus often became dark blue after treatment with iodine solution[1]. The second leaf (starting from the youngest leaf) was found a suitable tissue giving maximum positive reaction. The healthy leaves (yellow, senescent) gave a negative reaction[2]. However, the starch reaction test is not foolproof and not highly specific to RTV because rice leaves infected with orange leaf disease also showed positive reaction[1].

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CHEMOTAXONOMY OF BASELLACEAE

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ALTHOUGH Basellaceae has received the attention its phytochemistry has not been fully studied. The present investigation on the distribution pattern of phenolic acids is an attempt to assess its taxonomic placement based on the present observations together with the data gathered from the collateral disciplines.

Following the method of Narayana *et al*[1] different phenolic acids have been detected in the vegetative materials of Basellaceae and its putative relatives by two-dimensional chromatography and the observations are recorded in table 1. The paired affinity, group affinity and isolation values (i) and (n) are calculated according to Ellison *et al*[2] (table 2).

The taxa examined in the present study are: *Basella alba* L. and *B. rubra* L. (Basellaceae),

Table 1 *Distribution pattern of phenolic acids*

Phenolic acid	Basellaceae	Aizoaceae	Chenopodiaceae	Portulacaceae
p-hydroxy benzoic acid	+	+	+	
Caffeic acid	+	+	+*	+*
p-Coumaric acid	+	+	+*	+
o-Coumaric acid			+	+
Chlorogenic acid	+		+	+
Ferulic acid	+*	+*	+*	+*
Gentisic acid			+*	+*
Shikimic acid	+			
Sinapic acid	+*	+*		+*
Vanillic acid	+	+	+	
Unknown (hrf) 12/2			+	
-do- 39/5			+	
-do- 48/1		+		
-do- 57/3				+
-do- 65/3		+		
-do- 73/2		+		
-do- 76/3	+		+	+

*From Gobbs (1974)[4].

Table 2 *Quantified chromatographic data*

	Paired affinity				Group affinity	Isolation values	
	Basellaceae	Aizoaceae	Chenopodiaceae	Portulacaceae		(i)	(n)
Basellaceae	100	66.67	70	66.67	303.34	11.11	5.88
Aizoaceae		100	50	44.44	261.11	33.34	17.64
Chenopodiaceae			100	70.0	290.0	18.18	11.76
Portulacaceae				100	281.11	11.11	5.88

Sesuvium portulacastrum (L) L., *Trianthema triquetra* Roth ex Willd. and *Zaleya decandra* (L.) Burm. f. (Aizoaceae), *Chenopodium* sp., *Salicornia brachiata* Roxb., *Suaeda maritima* (L.) Dumort. and *S. nudiflora* Mosq. (Chenopodiaceae) and *Portulaca oleraceae* L. var. *oleracea*, *P. oleracea* var. *sativa* (Haw.) DC., *P. cryptopetala* Speg. and *P. pilosa* L. (Portulacaceae). They were collected from various locations in Andhra Pradesh. The voucher specimens of these taxa are deposited in the Kakatiya University Herbarium.

In the presence of *p*-hydroxy benzoic, caffeic, *p*-coumaric, ferulic, sinapic, vanillic acids and in the absence of *o*-coumaric, gentisic and three unknown phenolic acids (hrf 12/2, 39/5, 57/3) the Basellaceae and Aizoaceae are related. However, by the presence of chlorogenic, shikimic and an unknown phenolic acid (hrf 76/3) and by the absence of three unknown phenolic acids (hrf 48/14, 65/3, 73/2), the Basellaceae differ chemically from the Aizoaceae.

Although the relationship of Basellaceae to Chenopodiaceae is apparent possessing *p*-hydroxy benzoic, caffeic, *p*-coumaric, chlorogenic, ferulic, vanillic and an unknown phenolic acid (hrf 76/3) and in the negative response to four unknown phenolic acids (hrf 48/14, 57/3, 65/3, 73/2), in the presence of *o*-coumaric, gentisic and two unknown phenolic acids (hrf 12/2, 39/5) and in the absence of shikimic and sinapic acids, the Chenopodiaceae differ from the Basellaceae.

Chemically, the Basellaceae and Portulacaceae resemble one another by sharing the presence of caffeic, *p*-coumaric, chlorogenic, ferulic, sinapic and an unknown phenolic acid (hrf 76/3) and the absence of five unknown phenolic acids (hrf 12/2, 39/5, 48/1, 65/3, 73/2). However, the former differs from the latter in the presence of *p*-hydroxy benzoic, shikimic, vanillic acids and in the absence of *o*-coumaric, gentisic and an unknown phenolic acid (hrf 57/3).

The synthetic numerical chromatographic data (table 2) indicate that the Basellaceae are related to Chenopodiaceae on the one hand and to the Portulacaceae on the other. The paired affinity and the group affinity indices indicate that the Basellaceae

are more allied to Chenopodiaceae, but the isolation values (i) and (n) point out a relationship between Basellaceae and the Portulacaceae. The relatively low group affinity (261.1) and high isolation values (33.34 and 17.64) of Aizoaceae indicate that its relationship with Basellaceae is not as close as between Basellaceae and the other two families.

Despite the fact that all families possess betalains[3,4] in common the numerical analysis based on genomic, serological and ultrastructural investigations reveal that the family Basellaceae is very much nearer to Portulacaceae than to Chenopodiaceae or Aizoaceae[5].

Therefore, it is tentatively suggested that the Basellaceae may be treated as an independent family and should be placed in the vicinity of Portulacaceae and Chenopodiaceae under Caryophyllales.

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HEPARIN FROM SOME BIVALVE MOLLUSCS

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HEPARIN has been obtained from the marine gastropod *Charonia lampas*[1] and from the surf clam *Spisula solidissima*[2-3]. The present note reports heparin from three species of bivalve molluscs, *Katelysia opima*, *Anadara rhombea* and *Crassostrea madrasensis*, common in the estuaries of India.

Table 1 *Heparin activity of the test extracts*

Source of tissue	Units of heparin activity per kg tissue
K. opima	283,320
A. rhombea	74,140
C. madrasensis	14,125

The animals were collected from the Vellar estuary (11°29'N; 79°46'E). The animal meat without the gonad was weighed and minced. The minced tissue was mixed with distilled water and autolyzed for 48 h at 38°C. Extraction and partial purification of heparin were made following the method of Thomas[4]. The test extracts were compared with standard heparin by paper chromatography using a solvent system of water-ethanol-ammonia (39:60:1)[5] and developed in iodine vapour. The activity of the test extracts was determined by azure A assay[6].

The qualitative analysis of heparin in the three bivalves showed similar R_f value close to that of the standard heparin.

The unit of heparin activity per kg of tissue is shown in table 1. *Katelysia opima* showed maximum activity. The activity was low in *C. madrasensis*. *A. rhombea* showed intermediate activity. However, all the three species could be potent sources of heparin if HPLC system is used for purification of test extracts.

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