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A Ringspot Virus on *Dolichos lab lab**

by

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Synopsis: A study on the transmission, host range and physical properties of a ring spot virus disease of *Dolichos lab lab* observed in Coimbatore is described in this paper. The virus was found to vary considerably from other ringspot viruses in its mode of transmission, Physical properties and host range and the virus is hence considered to be different and reported as new record in India.

Introduction: *Dolichos lab lab* L., is an important pulse crop in Madras State. During August, 1965 a ring spot disease was observed on *D. lab lab* (Fig. 1) in the experimental plots of the Millet Breeding Station, Agricultural College and Research Institute, Coimbatore. The disease was found to affect both the garden (*D. lab lab* L., var. *typicus* Prain) as well as the field beans (*D. lab lab* L., var. *lignosus* Prain). The ring spots were about 2·5 mm in diameter with concentric rings surrounding an island of apparently normal tissue. On older leaves the rings appeared to fade away giving the leaves more or less a mosaic appearance.

So far, only a few viruses have been described as naturally occurring on *Dolichos*. Nour and Nour (1962) reported a mosaic disease of *Dolichos lab lab* (hyacinth bean) in Northern Sudan. A ring spot mottle virus was reported to occur in Peking by Cheo and Tsai (1959). In India Capoor and Varma (1948) reported from Poona a mosaic disease on *D. lab lab*, which they described as '*Dolichos* enation mosaic'. In the year 1950 the same authors reported the occurrence of another disease on *D. lab lab* from Poona which they named '*Dolichos* yellow mosaic'. In India apart from these two records, there appears to be no other report on the natural occurrence of virus diseases on this crop.

Studies were carried out on the transmission, host range and physical properties of the virus and the results obtained are described in this paper.

Materials and Methods: *Sap transmission:* The virus was cultured and maintained on *Nicotiana tabacum* var *white burley* and used when needed. Infected leaves were macerated in a sterile pestle and mortar, adding one ml of 0·1 M phosphate buffer at pH 7 to 1 gm of leaves. The pulp obtained was strained through a muslin cloth. Inoculations were done by gently rubbing the upper surface of leaves with the forefinger wet with inoculum. The leaves were dusted

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with carborandum powder of 600 mesh fineness prior to inoculation. For all inoculation studies, only the young, vigorously growing plants raised from seed in the insect proof glass house were used.

Insect transmission: Insect transmission tests were taken up with the aphids *Myzus persicae* Sulz. *Aphis gossypii* G. and *Aphis craccivora* K. In transmission studies, the schedule of three hours pre-acquisition starvation, one to three hours acquisition feeding and three, six and twenty four hours test feeding was tried.

PHYSICAL PROPERTIES: Physical properties of the virus were tested on the local lesion host plant *Chenopodium album* L. The freshly expressed juice from young leaves of diseased plants of *Nicotiana tabacum* L. var. *white burley* was used as inoculum for all studies.

1. Dilution end point: Serial dilutions of the infective sap were made by using sterile distilled water. For each dilution five leaves of the test plant were inoculated. The plants were kept under observation for one week.

2. Thermal inactivation point: Three milli litre aliquots of the infective sap were taken in thin-walled glass tubes of uniform size and were then subjected to different temperatures ranging from 30°C to 80°C for 10 minutes in a thermostatically controlled water bath. Immediately after exposure to the above temperatures the sap was cooled by immersing the tube in ice-cold water. The sap subjected to each temperature was inoculated on to five leaves of the test plant. The unheated sap was inoculated on control plants. The test plants were kept under observation for a week.

3. Longevity in vitro: The standard extract of the sap stored in stoppered bottle in dark at room temperature (21°C — 25°C) was tested for infectivity at intervals of one hour for eight hours. Five leaves of the test plant were inoculated for every hour and the plants were kept under observation for a week.

Results: Transmission: The virus was easily transmitted by sap and it was found to infect plants belonging to the families, Leguminosae, Cucurbitaceae, Compositae, Solanaceae, Amarantaceae, Chenopodiaceae and Commelinaceae. The insect vectors *Myzus persicae* Sulz., *Aphis gossypii* G. and *Aphis craccivora* K. did not transmit the virus.

Host range and symptomatology: *Dolichos lab lab* L. var. *typicus* Prain (garden bean) and *D. lab lab* L. var. *lignosus* Prain. (field bean (Fig. 2): The first symptoms on inoculated leaves appear as a few fine concentric chlorotic rings in about four to five days. Systemic symptoms develop in about ten to twelve days. In the newly developed young leaves chlorotic rings appear, often two or more being arranged concentrically. The centre of the spot consists of the primary ring and its island of encircled tissue. Either the tissue in the centre or the concentric chlorotic rings turn necrotic. The primary ring varies from 2–3 mm in diameter.

The location of the ring spots seems to bear no difinite relation to the veins. It is not unusual to see the ring spots fade away as the leaves matured. However, the leaves on which the ring spots have become masked appear to be a little thicker and leathery in comparision with normal ones. Occasionally, young leaves that follow the development of two or more symptomless, were observed to exhibit ring spot.

The sap from apparently symptomless leaves produced infection on inoculation showing the presence of the virus.

The stems, flowers and pods of infected plants did not reveal any external symptoms.

The symptomalogy of the virus on several hosts belonging to different families was studied and the observations recorded are summarised in the following table.

Family	Name of the host plant	Incubation period in days	Symptoms
Cucurbitaceae	*Cucurbita pepo*	10—12	Yellowing of veins accompained by the development of ringspots, 1-2 mm in diameter with a central green disc encircled by one or more chlorotic interveinal concentric rings.
Compositae	*Zinnia elegans* Jacq.	6—8	Mosaic mottling of leaves.
Solanaceae	*Nicotiana tabacum* L. var *white burley*	7—8	Mosaic mottling, blistering and distortion of leaves. (Fig. 6.)
	N. glutinosa L.	10—12	Mosaic mottling, slight blistering distortion and filiformity of leaves.
	Capsicum annuum L.	8—12	Vein clearing followed by mosaic mottling of leaves.
	C. frutescens L. var. *baccatum*	25—30	Severe mosaic mottling and distortion of leaves. Portions of leaf tissue turn necrotic and give the leaves a necrotic mottled appearance. There is no veinal necrosis or stem streaking.

Family	Name of the host plant	Incubation period in days	Symptoms
Solanaceae	*Lycopersicon esculentum* Mill.	8—10	Severe mosaic mottling of leaves with yellowish green and green patches, reduction in leaf size, crinkling, distortion and filiformity of leaves. (Fig. 5)
	Solanum nigrum L.	20—25	Slight crinkling of leaves with patches of light green on a dark green background.
	Physalis peruviana L.	10—12	Mosaic mottling is accompained by reduction in size, marginal frilling, distortion, blistering and downward curling of leaves.
	P. floridana Rydb.	15—20	Mosaic mottling of leaves is accompanied by reduction in size, marginal distortion and elongation of leaf tip.
	Nicandra physaloides Gaertn.	12—14	Mosaic mottling and severe distortion of leaves.
	Datura stramonium L.	5—6	Circular chlorotic spots, 2-3 mm in diamater with indistinct concentric rings develop on the inoculated leaves. The primary symptom is followed by mosaic mottling, blistering, distortion and filiformity of leaves about a a month after inoculation. (Fig. 3).
	D. metel L.	5—6	The symptoms are more or less similar as in *D. stramonium*, but the severity is less.
	D. ferox L.	4—6	Concentric, chlorotic rings, 2-3 mm in diameter and line patterns develop on inoculated leaves. Systemic symptoms consisting of mosaic mottlings reduction and distortion of leaves appear in the leaves that develop about a month after inoculation.

Family	Name of the host plant	Incubation period in days	Symptoms
Solanaceae	*Solanum melongena* L.	12—15	More less circular chlorotic patches, 2-4 mm wide develop on inoculated leaves. The margin of the chlorotic spots turn necrotic and form irregular discontinuous rings. Sometimes indistinct, incomplete, necrotic rings develop on the chlorotic patches. The primary symptom is followed by systemic mosaic mottling and fine vein banding of leaves. The symptoms become masked in old plants.
	Petunia hybrida Vilm.	10—12	Vein clearing is followed by mosaic mottling, blistering and slight marginal frilling of leaves.
Amarantaceae	*Gomphrena globosa* L.	30—35	Mosaic symptoms consist of yellowish green and green areas in young leaves. The symptoms tend to become faint in older leaves.
Commelinaceae	*Commelina jacobii* C. Fisch.	8—10	Mosaic mottling of leaves with areas of yellowish green present either as irregular longitudinal patches or as roughly circular spots of 2-3 mm in diameter. The yellowish green areas often extend across the veins. Sometimes incomplete, concentric rings of yellowish green tissues, develop in the circular patches.
	C. benghalensis L.	8—10	Symptoms similar as in *C. jacobii*.
Leguminosae	*Vigna unguiculata* Walp.	2—3	Local necrotic lesions, 0·5 to 1 mm in size, brick red in colour, develop in the inoculated cotlydonary leaves.
Chenopodiaceae	*Chenopodium amaranticolor* Coste & Reyn	2—3	Local necrotic lesions, 1 mm in diameter, dull white in colour, no systemic symptom follows. (Fig. 4).

Family	Name of the host plant	Incubation period in days	Symptoms
	C. quinoa Willd	2—3	Reaction similar as in *C. amaranticolor*.
	C. album L.	2—3	Necrotic, circular, local lesions 1-2 mm in diameter dull white in colour. No systemic symptom follows. (Fig. 4).
	C. murale L.	3—4	Local lesions, 1-2 mm in diameter light brown in colour. No systemic symptom follows. (Fig. 4).

The virus did not infect the following plants.

Malvaceae : *Althaea rosea* Cay.

Leguminosae : *Canavalia ensiformis* A. C.; *Cyamopsis psoraloides* Dc. *Dolichos biflorus* L.; *Phaseolus mungo* L.; *P. aureus* Roxb.

Amarantaceae : *Alternanthera echinata* Smith; *Amaranthus caudatus* L.

Euphorbiaceae : *Ricinus communis* L.

Gramineae : *Urochola panicoides* Beauv.

PHYSICAL PROPERTIES: 1. Dilution end point: The virus retained infectivity at a dilution of 1:100 but was inactivated at 1:1000 dilution. The results are furnished in table below.

S. No.	Dilutions	No. of leaves inoculated	Average No. of local lesions produced per leaf
1.	1:10	5	15
2.	1:100	5	6
3.	1:1,000	5	0
4.	1:10,000	5	0
5.	Control (undiluted sap)	5	34

2. Thermal inactivation point: The virus withstood exposure to a temperature of 40°C and was inactivated at 50°C. The results are given in table below.

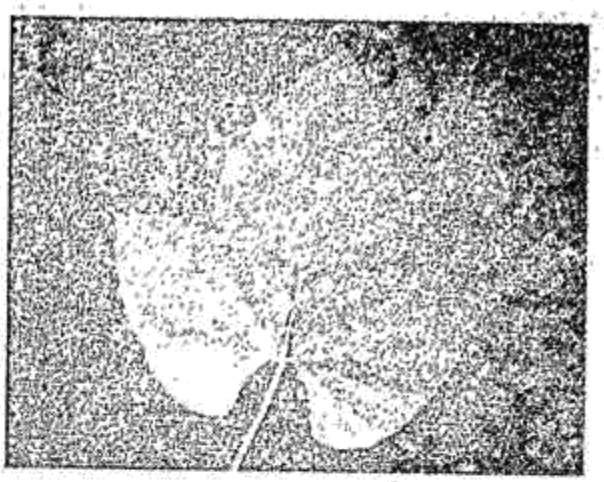

FIG. 1. Ring spot symptom as seen in the field on *Dolichos-lab lab.*

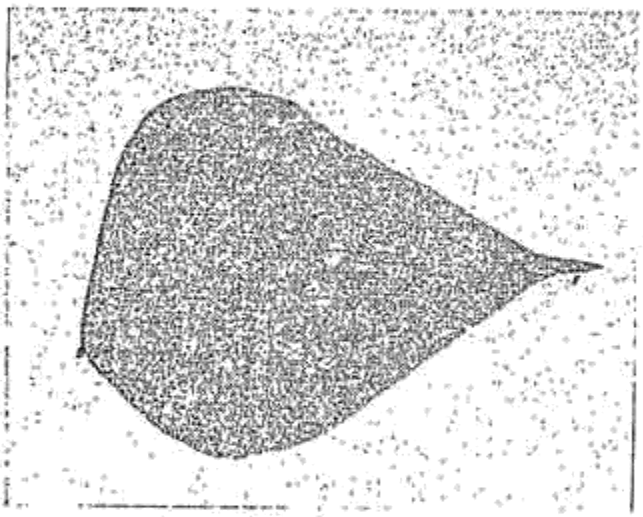

FIG. 2. Ring spot symptom on *Dolichos-lab lab* on transmission.

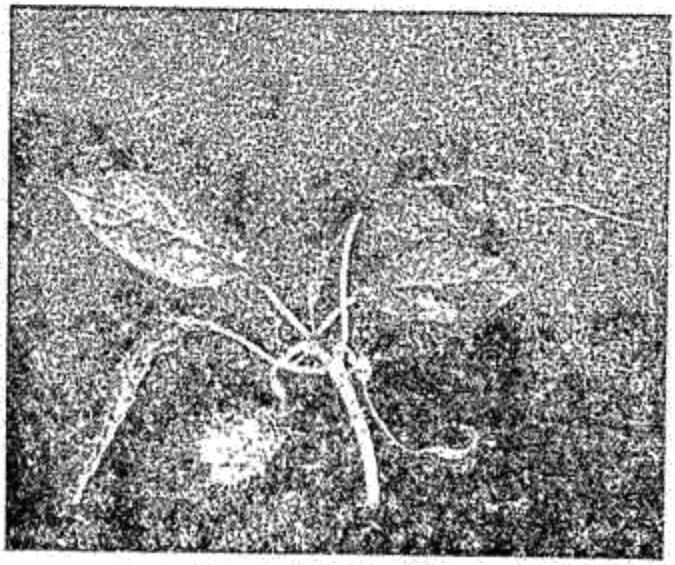

FIG. 3. Symptoms on *Datura stramonium.*

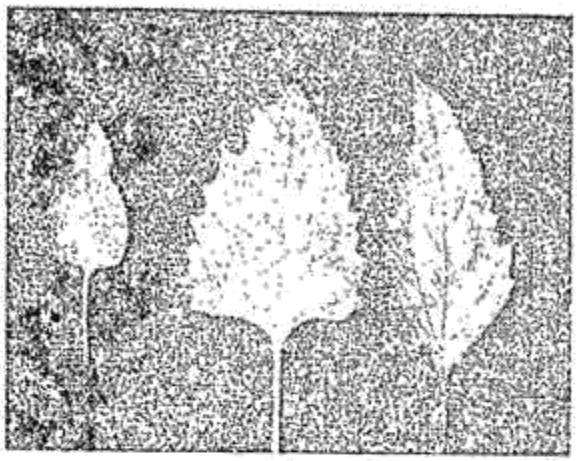

FIG. 4. Local lesions on *Chenopodium album*; *Chenopodium murale* and *Chenopodium amaranticolor*.

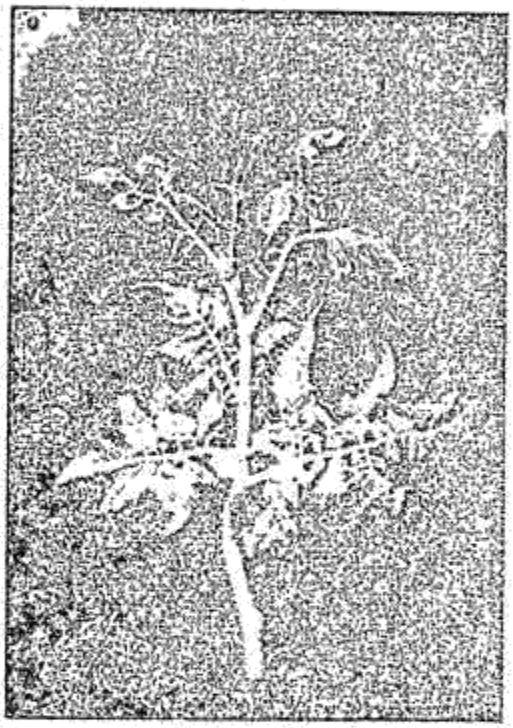

FIG. 5. Symptoms on *Lycopersicon esculentum*.

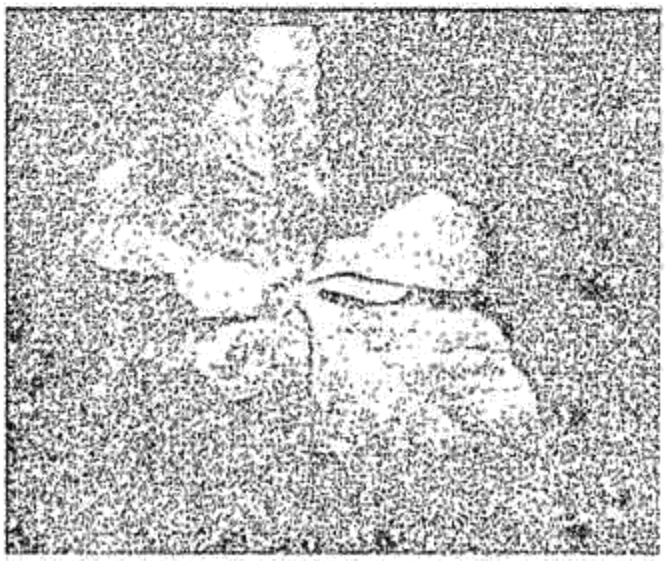

FIG. 6. Symptoms on *Nicotiana tabacum* var. *White Burley*.

S. No.	Temperatures	No. of leaves inoculated	Average No. of local lesions produced per leaf
1.	30°C	5	15
2.	40°C	5	11
3.	50°C	5	0
4.	60°C	5	0
5.	70°C	5	0
6.	80°C	5	0
7.	Control (unheated sap)	5	23

3. Longevity *in vitro*: The virus gradually lost its infectivity at every hour of interval and was rendered completely innocuous after four hours of storage.

The results are given in the following table:

S. No.	Duration in hours	No. of leaves inoculated	Average number of local lesions produced per leaf
1.	1	5	9
2.	2	5	6
3.	3	5	3
4.	4	5	3
5.	5	5	0
6.	6	5	0
7.	7	5	0
8.	8	0	0
9.	Control (fresh sap)	0	16

Discussion: The ring spot of *Dolichos lab lab* L studied here vary considerably from the ring spot virus of *D. lab lab* and *Phaseolus vulgaris* reported from places outside India. It also differs from other ring spots described on many host plants like tobacco, tomato, beet, iris, carnation etc., from several countries in their host range and physical properties (Travis, 1957; Yarwood, 1957; Kristensen, 1957; Doolittle and Zaumeyer, 1953; Benedict, 1955; Hollings and Stone, 1965). Hence, a detailed study of the ringspot virus on the host range, symptomatology and physical properties was under taken.

Cheo and Tsai (1959) have reported a virus on *D. lab lab* in Peking, causing ringspot mottle. The virus reported by them was aphid transmissible, while the

virus described here was not transmitted by aphids. It was also observed by them that the virus had a restricted host range and infected *Nicotiana glutinosa* only, if it has passed through broad bean to which it was sap transmissible. This was in contrast to the virus described here, which has a wide host range and was found to infect plants belonging to different families—Leguminosae, Cucurbitaceae, Compositae, Solanaceae, Amarantaceae, Chenopodiaceae and Commelinaceae. Further, certain variations were also observed with regard to physical properties. The virus from Peking was reported to have a dilution end point of 1 : 10000 and thermal inactivation point of 50—60°C. The virus under study was found to have a dilution end point between 1 : 100—1 : 1000 and thermal inactivation point between 40°C to 50°C.

In Germany, Quantz (1955) reported a ring spot, sometimes developing circular necrotic lesions on the younger pinnate leaves of *Phaseolus vulgaris* and it was attributed to tobacco ringspot virus. The virus under study resembles the one reported from Germany and also the tobacco ring spot virus (Wingard, 1928) in having a wide host range. But the virus described here does not produce any ring spot symptoms on tobacco and produced only mosaic mottling. The ring spot virus reported by Quantz (1955) and the tobacco ring spot virus described by Wingard (1928) have a dilution end point of 1 : 1000 to 1 : 10,000, thermal inactivation point of 60—65°C and longevity *in vitro* for three days. But the present virus has comparatively, a low dilution end point (1 : 100—1 : 1000) and thermal inactivation point (40—50°C). It is also of interest to note that the stability of this virus is very low and was completely rendered innocuous after four hours storage at room temperature of 21°—28°C.

The ring spot virus observed at Coimbatore, thus appears to vary considerably from the other ring spot viruses in its transmission, physical properties and host range. It is, therefore, considered to be a different virus and reported as a new record in India.

Summary: A ringspot virus disease of *Dolichos lab lab* L. observed in Coimbatore has been described. The virus was found to be sap transmissible. The aphid species *viz, Myzus persicae* Sulz., *Aphis gossypii* G. and *Aphis craccivora* K. did not transmit the virus. The host range of the virus was found to be fairly wide and it infected plants belonging to the families, Leguminosae, Cucurbitaceae, Compositae, Solanaceae. Amarantaceae, Chenopodiaceae and Commelinaceae. The dilution end point of the virus was found to be between 1 : 100—1 : 1000 and thermal inactivation point between 40°C—50°C. The stability of the virus was found to be low and it lost its infectivity after four hours of storage at room temperature (21°C—28°C). The virus has been compared and discussed with the ring spot viruses, reported from other countries. The virus was found to vary considerably from the other ringspot viruses in its mode of transmission, physical properties and host range. Hence, the virus is considered to be different and reported as a new record in India.

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* Originals not seen.