

Asian Resonance

Studies of Mosaic Virus Disease on the Growth and Yield in Pigeon pea Crop



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Abstract

Pigeon pea (Fabaceae) botanical name *Cajanus cajan* Linn. Commonly known as red gram or Arhar dal is an old Indian crop and main source of protein in the Indian food diet .In the present investigation various parameters of the pigeon pea related to growth and yield of the healthy as well as the mosaic virus infected plants were studied.The infected plants were light greenish in colour as compare to healthy plants, which are dark green in colour.The height and size of the stem and the leaves were also reduced in diseased plant.The anatomical studies of leaf-revealed that the size and the shape of the parenchymatous, palisade tissues and spongy cells were also reduced.The flowering was delayed, number and shape and size of the fruits and seeds were also reduced in mosaic infected plants. It is a leguminous crop is rich in proteins than other plants of Fabaceae family .In our present investigation some histological changes observed in diseased leaves that there were reduction in thickness of leaves ,in number of chloroplasts and in size of stomata .The number of stomata were increased in the diseased leaves .In viral infected plants the thickness of leaflet was also reduced. The assimilatory tissues of diseased leaves was reduced in size .Reduction in the percentage of chlorophyll is very high .Carotene and xanthophylls content also decreased, translocation of sugar was reduced there is also reduction of sucrose synthesis as comparison to normal healthy plants.

Keywords:Pigeonpea,Mosaic,Susceptibility,Inoculation,Lesions,Invasion, Implantation,Symptomatology,Transmission,Contamination, Syndroms,Chlorosis,Translucent.

Introduction

Pigeon pea (*Cajanus cajan*.linn) is an important leguminous crop,which is widely distributed in the tropics regions and extensively for its edible seeds.The crop of Arhar is annual or perennial shrub,4 to 10 ft high ,cultivated nearly through out india as a pulse crop.It is often grown for green and cover plantations.Its deep and penetration root system makes it specially valuable as a renovating and contour hedge crop for checking soil erosion.The plant is probably a native of Africa.But it is now grown in almost all the tropical countries of the world , including Africa, America,India,Australia,Hawaii, East & West Indies.In India,it is mainly grown in U.P,Bihar, Maharashtra and TamilNadu. Numerous types of *Cajanus* are known ,differ in height habit time of maturity colour ,shape and size of pods and seeds .It is mainly known as mixed crop,such as jawar, bajra, maize, cotton and other crop and almost all type of soil not deficient in line but it prefer a medium, moist soil and good drainage . In north India it is grown on the alluvial soils of the Indo-Gangetic plain. The crop require no special attention not even weeding.The main crop being usually of shorter duration than *cajanus* is harvested before the other matures.The most common disease of *cajanus* is wilt,coused by the fungus *Fusarium udum* Butl. Our present investigation deals with different aspects of mosaic disease of *Cajanus* viz. indentification of the virus .Symptomatology, transmission ,host range, physical properties and inactivation of virus .Historically ,symptomatically has been most widely used as a criterion for the identification of virus.Transmission plays a significant role in characterization of plant viruses.The seed transmission provide a very effective means of introducing viruses at early stage,giving randomized foci of infection. Sap inoculation is the most useful method for experimental work as most of the basic knowledge of the plant viruses have been obtained by this method .Grafting is another successful method of virus transmission .It become more important when other methods of transmission is fails. Plant viruses in general are "Wound Invades" insect

produces wounds in the plants at the time of biting and in this way they transfer the viruses present in its saliva from one plant to another. Thus virus-vector host relationship assumes great importance. The physical properties viz. dilution end point, thermal inactivation point, longevity in vitro and in vivo and effect of desiccation provide an effective tools has been included as a part of present investigation. Histopathological studies of plants infected with virus have played a significant role in understanding the relation between the virus and host tissues.

Viral disease of Arhar crop is of highly economic importance and is highly damaging, therefore control of this disease has become a challenging problem. The plant virus produce visible effect on their host cells. Many natural and synthetic compound also show their effect on intensity and temperature on the virus infectivity were also studied. Damages caused by plant diseases result in serious repercussion on the agricultural production. The problem of assessment of consequent losses, therefore is of primary importance. Throughout the world as it is only through such assessment, rational and effective control measures can be developed and applied. Such appraisal of losses is also essential as by making people aware of losses, public support to the plant protection is made available through organization and institutions.

Aims of the Study

Purpose of study about Mosaic virus disease on Pigeon pea is that as we all know about the great value of pigeon pea (Arhar Dal) as a major source of protein in our Indian food diet as a pulses crop. Due to this virus infection in the crop of pigeon pea there was a major crop loss were estimated few years back. Due to this my interest was lead to me for such curries to know about the total facts about how to protect such crop and how to take prevention and control from such a great loss of the crop. During my investigations and study about such disease have also taken different parameters to conduct our experiment. During my studies I found one of the homeopathic drug shows great results to control over such disease which is also environmental friendly easily available to farmers with in their reach. This crop is leguminous it has much importance in the field of agriculture. It is highly consume by the people due to very sweet taste also rich in protein. It is consumed in various way the most common being cooking with the spices and vegetables. This crop mainly grown as a mixed crops and it can be grown almost in all type of soils not deficient in lime but it prefers a medium moist soil, with good drainage. It is highly adaptable with respect to climate and grow both in dry and moist tropics. Under dry conditions it trends to mature quickly, while under humid conditions it develops a luxuriant vegetative growth and ripens late. It is universal fact, that the healthy crop can only be gathered from the healthy plants. It is therefore, the foremost duty of man to produce healthy plants free from any disease. During our investigation of such disease we study on symptomatology, transmission, host range, physical properties, histopathology, effect

of different buffers on the infectivity of the virus, effect of certain factors on disease developments and inactivation of viruses and crop loss estimates.

Review of Literature

The work on plant viruses began with the work of Mayer in Holland in 1886; demonstrating the disease as readily transmissible and infection he coined the word Mosaik krankheit (Mosaic). Later on Iwanowski 1892 in Germany found that the disease causing entity could pass through the bacteria proof filter. Six year after, Beijerinck (1898)

Working in Holland confirmed the work of both Mayer and Iwanowski. He was the first to use the word virus. As the latin word meaning "poison". Hunger 1902 studied tobacco Mosaic in Sumatra and established several important facts among which was the important role played in plant to plant spread of the disease in field by labourers picking the worms from the plants. Allard 1916 was the first to published extensive series of papers on tobacco mosaic virus. At first little attempts and attention was made to distinguish between cause and effect of the virus and the disease it caused both were referred as the same thing. A distinction was made by adding the word "Virus" to the name of the disease to indicate the cause, for example tobacco mosaic was caused by Tobacco Mosaic Virus (TMV), the name of the disease being now commonly used. Symptomatology study is considered essential for quick orientation and rapid diagnosis of a virus disease in the field (Bos, 1967). But some time the symptoms mislead, because some virus may produced different type of symptoms on the different hosts and different viruses may produce similar symptoms on a host plant. The diversity in symptoms depends on virus host reaction, external conditions and stage of development of host. (Nair & Nene 1974) Change in the colour is the most common and perceptible primary symptoms which may be brought about by the chlorophyll disorder in the tissue resulting in chlorosis (Bos, 1970). Virus infection may induce some pathomorphological changes. Such as stunting (Hagedorn & Walker, 1949) and reduction in size of seeds etc. (Foster et al., 1951; Bos, 1963; Roy, 1974 & Sharma, 1976). Anatomical studies of virus infected plants play a significant role in understanding the development of relationship between the virus and tissues of their hosts. In mottle disease, the lamina of the leaf is thinner mesophyll cells are less differentiated with fewer chloroplasts, therefore, the tissues may become translucent (Mathews, 1970). A significant decrease in thickness of leaflet, length & breadth of palisade cells, number of chloroplast in palisade cells and spongy cells, number of stomata and length, breadth of stomata was recorded by Roy (1974). Abnormal development of phloem & cambium cells observed in "Crismon Clover" infected with wound tumor virus (Lee & Black, 1955). In the diseased shoots found in swollen shoot disease of cocoa abnormal amount of xylem tissue are produced but the cells appear structurally normal (Posnette, 1947). Mechanical inoculation is defined as the process by which infectious virus is manually

introduced into a living organism by mechanical trauma to facilitate initiation of infection in potentially infective cells. (Kado, 1972). The process involves the introduction of infective virus or viral RNA into a wound made through the plant surface (Mathews, 1970). Most of our basic knowledge of plant viruses has resulted from sap inoculation.

Principal identified inhibitors are proteins (Baroden, 1964), tannins (Chewb & Linder, 1964) & enzyme (Kado, 1964). Served substance have been reported which facilitate the virus infection when applied mechanically. Abrasive are the most universally accepted supplements to the virus inoculum. First abrasive used was the sand (Fajardo 1930) but now carborundum (Beraha et al, 1955) or celite (Yarwood, 1968) are used most widely. It was concluded that the incorporation of auxin accelerated the movement of virus and change the direction of the virus particles being passively swept away along with the hetroauxins. Regarding the mode of action of growth regulators, there are several contradictory views. Beside these growth regulators. Antibiotic, different inhibitors, seed extracts, homeopathic drugs were used for different experimental work. Some environmental factors such as temperature, light intensity, Ultra-violet, UV-exposure were also affected mosaic disease in different ways. There was a significant decrease in yield in terms of plant, seeds per pod & 1000 grains cut in pre-bloom infected plants. It was observed that earlier the infection, greater the loss, which was mainly attributed to a reduction in the number of pods. The reduction was greater in plants inoculated early during development.

Experimental Part

Reagents

All reagents used for present investigation work are as follows

Fungicides

used for 1, 6, 12, 24 hours

- ~ Nitrosul
- ~ Hexathane
- ~ Pentavax
- ~ Aureofungin

Growth Regulators

Used for 1, 6, 12, 24 hours

- ~ IAA (Indole acetic acid)
- ~ GA (Gibberellic acid)
- ~ NAA (Nephthalene acetic acid)
- ~ 2,4-D (2,4-Dichlorophenoxy acetic acid)

Plant Extracts

Different parts of different plants also used for our present investigation.

- ~ Argemone maxicana (seeds)
- ~ Ocimum sanctum (leaves)
- ~ Allium cepa (bulbs)
- ~ Opuntia (Phylloclade)

Antibiotics

Many antibiotics were used in different level of quantities (10, 50, 100, 500, 1000 ppm)

- ~ Chloromycetin
- ~ Ledermycetin

~ Streptomycin

Homeopathic Drugs

Few of them also used in different amounts such as 10, 50, 100, 500, 1000 ppm.

~ Gentamycin

~ P lumbum-30

~ Vaccinium-30

~ Thuza-30

~ Arsenic-30

Purine and Pyrimidine base

(100, 250, 500 ppm)

~ 2-Thiouracil

~ 8-Azaguanine

~ 5-Nitrouracil

~ 6-Azauracil

Buffers Solutions

(1 ml/gm leaf)

~ Phosphate Buffer

~ Sodium citrate Buffer

~ Phosphate Ascorbic Acid Buffer

~ Citric Acid Phosphate Buffer

~ Sodium Borate Buffer

All the investigation conducted for present test different samples were collected from different fields from different locality and in green house than preparation of slides observed under electron microscope to compare our results from different work.

Equipments

Requirement of equipments for the experiment are section cutting box, electron microscope, chemicals for staining cutting sections, sterilized chamber for work, magnifying glass, burner, flask, holder for tube, test tubes, pipettes, test plants (healthy & diseased) and laboratory to compile work.

For such investigation samples of healthy and diseased plants were collected from the two different fields of village Patholy near Agra, U.P. and some samples were also collected from the city of Agra at different intervals. Also grafted the same plant in the green house which was totally sterilized by sprayed to kill any type of contaminations.

The symptoms were suspected to be viral in origin, therefore the diseased leaves and some twigs were collected and brought to the laboratory where leaf graft and mechanical inoculation were done on the healthy seedlings of Cajanus in 4-5 leaves stage. The symptoms were readily transmitted in both cases and no cellular organism was found to be associated with it, thus confirming the viral nature of the disease.

Results

Protection of plants from pathogens requires the presence of barrier between the host and the pathogen and this barrier may be of any or several types few of them shown by tables below.

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The Incubation Period and The Nature of Symptoms Were Recorded in Each Case. The Results are Shown in Table No.1.

Table.No.1

S. no	Temperature in degree`c	Incubation period (in days)	Nature of symptoms produced
1.	10	-----	No disease symptoms
2.	18	22-24	Clear chlorotic spots appeared on young apical leaves.
3.	26	14-16	Chlorosis & reduction in the size of leaves.
4.	38	17-19	Severity decrease, chlorosis stunting 7 bushy appearance of plants

Light is known to play a decisive role in the development of plant and other biosynthesis .The effect of duration oexposure to light on disease development & incubation period of AMV (Arhar Mosaic Virus).Shown in table no. 2

Table No.2

S.No	Light Duration(inhours)	Incubation period
1	5	16-18
2	9	13-15
3	15	13-14
4	24(darkness)	Plant did not survive upto syptoms expression .

The effect of Ultra violet light was studied in vitro by exposing the standard inoculums to UV-radiation for different length of time ranging from 5 to 100 minutes.

Effect of UV-radiation on infectivity of virus AMV in vitro .Shown in table no.3

Table No. 3

S.No.	UV-Exposure in minutes	Lesions per 40 leaves (+SEM)*		% Inhibition
		Control	Treated	
1.	5	360+6	350+5	2.70
2.	20	350+5	250+4	28.57
3.	60	310+5	26+2	91.61
4	100	340+5	00+0	100

*(SEM-Standard Error Mean)

No.of lesions produced By inoculums containing inhibitor % Inhibition = 100- -----x100

No. of lesions produced by Control inoculums

Due to this equation we calculate the actual and maximum percentage of UV-Exposure on plants.

Effect of various fungicides at different ppm on the infectivity of AMV in vitro .Shown below in table no.4.

Table No.4

S.No.	Fungicides (conc.Ppm)	Lesions per 40 leaves (+SEM)		Incubation Period (in Days)	%inhibit-ion
		control	Treated		
1	Nitrosul				
	100	360+6	325+5	11	9.72
	250	360+6	270+4	12	25.00
	500	360+6	45+2	13	87.50
2.	Pentavax				
	100	360+6	320+3	12	11.11
	250	360+6	255+3	13	29.16
	500	360+6	85+2	13	76.38

Effect of growth regulators on the infectivity of AMV in vitro .Shown in the table no.5 where each reading is means of two replications.

Table No 5

S. NO.	Growth Regulators (Conc.ppm)	Lesions /40 leaves(+SEM)		% Inhibition
		Control	Treated	
1.	IAA 100	320+3	160+2	50.00
	250	320+3	145+2	85.93
	500	320+3	8+2	97.50
2.	NAA 100	PHYTOTOXIN	-	-
	250		-	-
	500		-	-

Effect of different antibiotics at different ppm. Concentrations on the infectivity of AMV in vitro Shown in table no.6

Table No.6

S.N.	Antibiotics	Concentration (In ppm)	Lesions /40 leaves (+SEM)		% Inhibition
			Control	Treated	
1	Chloromycetin	10	360+3	340+3	5.75
		100	340+2	135+2	
		1000	360+3	0+3	
2.	Streptomycin	10	350+3	350+3	0.00
		100	360+3	285+3	20.83
		1000	360+3	72+3	78.33

Effect of purine & pyrimidine base on the infectivity of AMV in vitro in given table no.7

Table No.7

S.No	Purine & Pyridine base ppm	Lesions/40 leaves		% Inhibition	
		Control	Treated		
1	2-Thiourcil				
		100	360	166	53.88
		250	350	55	84.36
		500	350	0	100.00
2	6-Azauracil				
		100	370	141	61.82
		250	340	47	86.17
		500	340	0	100.00

Effect of different homeopathic drugs at different ppm concentration on the infectivity of AMV in vitro in given table no.8

S.No.	Homeopathic Drugs	Concentration in ppm	Lesions /40 leaves		% Inhibition
			Control	Treated	
1.	Plumbum-30	10	330+3	248+3	24.84
		100	310+3	125+3	59.67
		1000	300+2	32+3	89.33
2.	Thuza-30	10	280+2	210+2	25.00
		100	300+2	142+3	52.66
		1000	310+3	62+3	80.00

Infectivity of the Virus Using Different Buffers are Also Shown In Table No.9.Its Shows That How Infectivity of Diseases Increases or Decreases With Use of Buffers.

Table No.9

S.No	Buffers	Plant Inoculated	Leaves Inoculated/ plants	Total Inoculated	Local lesion \pm SEM
1.	Phosphate Buffer	10	4	40	280+6
2.	Sodium Citrate Buffer	10	4	40	200+5
3.	Citric Acid Phosphate Buffer	10	4	40	100+5
4.	Sodium Borate Buffer	10	4	40	60+1

Effect of various plant extracts on the infectivity of the AMV in vitro.Shown in table no.10

S.No	Name of Plants	Plant Part Used	Lesions /40 leaves(+SEM)		% Inhibition
			Control	Treated	
1.	Argemone maxicana	Seeds	360+6	240+6	33.33
2.	Ocimum sanctum	Leaves	250+6	208+6	16.80
3.	Allium cepa	Bulbs	370+6	170+6	54.05
4.	Opuntia	Phylloclade	360+6	102+6	71.66

The above data shown in different tables indicates that there are lots of changes occurs when the healthy plant of Pigeon pea get infected with AMV(Arhar Mosaic Virus).

Conclusion

Arhar or Pigeon pea (*Cajanus cajan* L) is an economically important and widely cultivated crop.It is one of the most edible seed and pulse crop in India.It is consumed in various ways,the most common being cooking with species and vegetables.

Typical mosaic symptoms in leaves developed due to virus invasion under present study remained unchanged

In field or in glass house conditions. These were light yellow patches intermingled with dark green area on young apical leaves .Which later on developed

Chlorosis , vein clearing and occasional rings also appeared .The present conclusion is mainly based on the results obtained from the studies made under various investigations with different substances .Arhar is sown as a mixed crop.It is cultivated mainly as kharif crop in most part of the country used for edible purpose. Which is rich with vitamins and proteins.During present work the vein clearing was observed in Pigeon pea plants which affected by mosaic disease in field as well as in glass house conditions.Occasionally rings also appeared on leaves and later on three rings gradually increased in size and changed into chlorotic patches.Similarly rings were also observed in many viruses.

The pathological relationship between the virus host combination was easily established as the whole of the disease syndroms,which are mainly observed in fields was established on the pigeon pea plants in glass house conditions by mechanical inoculation.

Symptoms will for long provide the main basis for identification and for distinguishing between different virus host reaction,external condition and stage of development of the host.

Much work has been done in relation to disease appraisal and crop loss estimate due to different disease and determine on this subject has been reviewed by Vallega & Cliarappa 1964, Chennulu et.al.,1966; Singh 1970, Blaszczyk et.al.,

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1979; Singh, 1981 7 MITTAL 1985.It is well known that viral diseases are most destructive and they cause tremendous losses to the agricultural output.

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