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Screening of Brassica Germplasms against *Plasmodiophora brassicae* under Controlled Condition to Find out Resistant Source for Cultivation in Plains and Hilly Areas of West Bengal, India

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A B S T R A C T

Club root of crucifers, a devastating disease of oilseed *Brassicas* and Cole vegetables is very difficult to control through conventional methods in different areas due to ineffective. Cultivation of resistant Brassica germplasms against this disease is very cheapest method to manage this disease. Therefore this screening trial was conducted with different Brassica germplasms under growth chamber to identify the source of resistance against this disease. Generally root hair infection test and pathogenicity test (club formation) were done for this purpose. Least root hair infection was found in black sarson (29.42 %), radish (35.49 %) and toria sarson (38.59 %) compare to other Brassica hosts. No complete resistance to root hair infection was found. In pathogenicity test Cabbage (94.4%), Broccoli (61.6%) and Indian mustard (72.2%) were found highly susceptible in compare to *Brassica rapa* group. White radish (11%) and Cauliflower (16.6 %) showed lowest disease severity than other *Brassica hosta*. But it is found that root hair infection is present to all Brassica and some non-Brassica hosts and it is non-specific and primary phase of club formation. Therefore White radish and Cauliflower could be considered as a source of resistance against this disease based on pathogenicity test.

Introduction

Clubroot disease is an important disease of crucifers caused by soil borne obligate bio-trophic pathogen *Plasmodiophora Brassicae* (Woronin).

It is particularly prevalent in temperate region under diverse soil environmental stress condition and at present it is reported from all the continents of the world. Gradual irreversible wilting, yellowing of leaves and root galling (club formation) on primary and secondary roots of *Brassica* hosts results in total death of plants within a short period of time.

In India, Clubroot is known to occur since 1952 and has spread on cabbage in the Darjeeling Hill in Eastern Himalayan region of West Bengal (Chattopadhyay and Sengupta, 1952). Since early 80's when extensive

cultivation of rape and mustard started in West Bengal this disease was spreading at alarming rate on rape and mustard specially in acidic soil regions i.e., red and lateritic region and Terai region of West Bengal (Laha *et al.*, 1985). At present Club root diseases is the major and only constraint for rape and mustard cultivation particularly in acidic soil regions of West Bengal where crop loss even reached upto 100% and farmers have to stop cultivation of rape and mustard in endemic areas. Most popular widely cultivated Yellow Sarson cultivar *B-9 Brassica rapa (earlier Brassica rapa) var*. yellow sarson, has been found to be highly susceptible. There is a wide difference of virulence and aggressiveness pattern of the pathogen population within the field and between the club root infested fields in different Agro-Climatic regions of West Bengal state. Conventional methods used to manage the disease are application of soil ameliorating agent like liming to increase soil pH, Calcium Cynamide- a pesticides cum cynamide nitrogenous fertilizers, various forms of soil amendments like Boron, Molybdenum, and cultural practices like soil solarization and composting in sick soil. These management practices were found not effective in all the places. Therefore, development of resistant variety is very urgent. There are very few resistant varieties of *Brassica* vegetables and oilseed *Brassicas* commercially available in world market. Therefore the objective of this study was to identify *Brassica* germplasm possessing resistance to *P. Brassicae* pathotypes of West Bengal for cultivation in plains and hill regions.

Materials and Methods

The whole work was performed in the laboratory and growth chamber by using the collected club root disease samples of *Brassicas* and soils from different parts of West Bengal.

Collection of clubs roots

The infected clubbed roots were first collected from the plains of South Bengal districts and Darjeeling hills (Kalimpong subdivision). In plains of South Bengal districts like Birbhum at Bolpur and Lohapur, Bankura districts at Sonamukhi and Sarenga, Murshidabad districts at Khargram and Hooghly districts at Polva. In case of Darjeeling hills (Kalimpong subdivision) from Lower Taschdin, Upper Dungra Chisopani, 11th Mile, R.R.S. (Hill zone) areas and Upper Dungra S. P. Singh Seed Production nursery, belongs to Red-lateritic, Gangetic Alluvial Zone and Hill Agro-Climatic zones of West Bengal respectively. Mature obscure and black coloured clubs developed on cabbage, Cauliflower and Rayosak, and yellow Sarson (B-9) and Torri Sarson susceptible varieties were collected along with sick soils.

Analysis of Soil pH

In case of collected sick Soil from plain of North-South Bengal and Darjeeling hills, only pH analysis was made in the laboratory of the Department of Soil Science and Agricultural Chemistry in Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, 741252 under the supervision of Dr. P. K. Patra, to find out favourable soil pH against this disease (Table 1).

Extraction of resting spores and preparation of Inoculums

Fresh, firm and mature clubbed roots were collected and kept in Deep Freezer. Later on, the roots were washed thoroughly, frozen and stored at - 10^{O}C. Spores were obtained by grinding 100g of frozen clubbed roots in 400 ml of sterilized distillate water for 3minutes in a high-speed Warming Blendor. The minced roots were filtered through six (6) layers of cheese cloth and the filtrate was centrifuged at

4230 rpm for seven (7) minutes. The supernatant liquid was discarded and the pellet containing resting spores was suspended in 100 ml distillate water. The centrifugation and washing steps were repeated three (3) times after which the pellet of spores were light grey. After the final wash, spores were thoroughly mixed in 100 ml of sterilized distillate water and shaken in Vortex test tube shaker and the population of resting spores were counted with a bright line haemocytometer and stored at $2^{O}C$ until mixed with the sterilized soil (William. P. H., 1966).

Microscopic observation and spore count

One (1) ml Spore suspension of *P. Brassicae* was taken on the haemocytometer and covered with the cover slip. After that, haemocytometer placed in the Electronic Microscope for spore count with help of 10X Lens. The spore suspension was adjusted to approximately 4×10^{7}.

Tested *Brassica* and Non-*Brassica* hosts

The following *Brassica* and Non-*Brassica* hosts (Table 2) were used for root hair infection tests, Disease reaction test.

Inoculation and Disease reaction test

The whole screening studies of *Brassica* hosts were conducted in the Growth Chamber by maintaining the temperature (22-25^{O}C) and relative humidity >80% for 45 -50 days. Here, three (3) *Brassica oleracea* hosts (Cabbage, Cauliflower and Broccoli), three (3) *Brassica rapa* hosts (Yellow sarson (Binoy), Torri sarson (Agrani) and Local yellow sarson (Jhumka)), One (1) *Brassica juncea* hosts (Indian mustard (Varuna), and Radish (White radish) *Raphanus sativus* were screened to determine the source of resistance among these hosts. The seeds of these hosts were sown 1-2cm deep into 125mm-diameter plastic pots containing sterilized soil (mixture of 1% sand + 1% manure + 2% collected soil sample) to raising the seedling and pH of the sterilized soil adjusted to 5.6, each pots containing 6 seedling, When seeds were germinated the spore suspension of *P. Brassicae* poured though the pipette (10ml) at the base of the each seedling at a concentration of 4×10^{7} @10ml /pot.

Infected root hair count under microscope

The seven (7) days, growing seedlings of *Brassica* and Non-*Brassica* hosts in B.O.D incubator were removed carefully from the soil and roots were thoroughly washed to remove soil particles from the root hairs. The roots were stained with the 1% aceto-carmine solution for 24 hours. For counting the infected root hairs of the each host, the seedling tap roots were laid out under a cover slip on a microscopic slide and infected and non-infected root hairs were counted(Single microscopic view) on both side of the root respectively.

Disease assessment for source of resistance

After 45-50 days, the plants were removed from the pots, the roots washed to free the soil particles and visually rated for clubroot severity on a 4-point scale as follows: 0, no visible root galls; 1, less than 10% roots visibly galled; 2, between 10 and 50% of roots visibly galled and 3, greater than 50% roots visibly galled.

The percentage of disease index (PDI), as used by Dobson *et al.*, (1983),was calculated for each host and used to assign a host reaction type (resistant DI=0, indeterminate 0<DI<33 and susceptible DI $\geq$ 33).

The Disease index (DI) was calculated using the 4-point scale according to the following formula:

$$\mathbf{DI} = \frac{[n0 \times 0] + [n1 \times 1] + [n2 \times 2] + [n3 \times 3]}{N1 \times 3} \times 100$$

Where,

n0= is the number of plants with a clubroot severity rating 0.

n1= is the number of plants with a clubroot severity rating 1.

n2= is the number of plants with a clubroot severity rating 2.

N1 = number of plants tested.

Results and Discussion

Screening results of different *Brassica* germplasms against club root disease of crucifers using different testing process under *in vitro* (growth chamber) condition were discussed below preciously.

Root hairs infection test to identify source of resistance

The activity of *Plasmodiophora Brassicae* Wor. in the soil was measured simultaneously by counts of root-hair infection on *Brassica* seedlings and percentage clubbing of older plants. The pathogenesis of clubroot, a disease of crops caused by the fungus *Plasmodiophora Brassicae*, starts with infection of the root hairs. The root hair infection test was conducted on all the *Brassica* hosts and also on non-*Brassica* crop plants. The test was conducted on popular commercial varieties of *Brassica* vegetables and oilseed *Brassicas* and non-*Brassicas*. Zoo-sporangia of *Plasmodiophora Brassicae* was observed on all the root hairs of tested *Brassica* vegetables and oilseeds. The rate of root hair infection varies with the different *Brassica* hosts but no statistically significant result has been obtained among the different *Brassica* germplasms. Most of the susceptible *Brassica* host cabbage showed higher rate of root hair infection in compare to Black sarson, Radish and Toria sarson (Table 3).

When compared with the standard susceptible hosts Cabbage and Yellow sarson, both higher and lower resistance to root hair infection was found in the accessions of the different *Brassica* species. No complete resistance to root hair infection was found.

Over the accessions studied, there was no correlation between the plant resistance estimated from green house tests and the resistance to root hair infection of seedlings. The resistance of all accessions must at least partly be caused by other mechanisms which operate after the root hair plasmodia are formed. The expression of genetic resistance usually observed during club formation.

Although root hair infection is compulsory and first stage in pathogenesis but there is no relation between root hair infection and club formation. All the non-*Brassica* hosts did not respond to root hair infection (Table 4).

Pathogenicity test of *Plasmodiophora Brassicae* (woronin) on different *Brassica* germplasms under controlled condition

The primary phase of colonisation i.e., roots hair infection by primary zoospore produce zoo-sporangia on epidermis of root had been observed on all *Brassica* hosts. Root hair infection is common to all *Brassicas* and some non-*Brassicas* showed root hair infection which is non-specific and not directly related to pathogenicity or club formation. The present experiment was conducted in the growth chamber under controlled condition to observe disease reaction/rate of club formation on different *Brassica* germplasms by using inoculums from cabbage infected clubbed root in Kalimpong.

Table.1 Characterizations of Sick Soils

Sl. No.	Location	Soil pH
	Plain of South Bengal	
1	Nalhati (Birbhum) Lohapur	6.5
2	Bolpur (Birbhum)	6.5
3	Sonamukhi (Bankura)	5.6
4	Sarenga (Bankura)	5.7
5	Khargram (Murshidabad)	6.6
6	Polva(Hooghly)	6
	Darjeeling hills	
	(Kalimpong Subdivision)	
7	Lower Taschdin	6.2
8	Upper Dungra Chisopani, 11th Mile	6
9	R.R.S. (Hill zone) areas	6.5
10	Upper Dungra S. P. Singh Seed Production Nursery	6.5

Table.2 Tested *Brassica* & non *Brassica* hosts

Sl. No.	*Brassica* hosts used	crops
1	*Brassica oleracea* group	Cabbage, Cauliflower and Broccoli
2	*Brassica rapa* group	Yellow sarson (Binoy), Torri sarson (Agrani) and Local yellow sarson (Jhumka)
3	*Brassica juncea* group	Indian mustard (Varuna), Black Sarson and Rayosak
4		Radish (White radish) *Raphanus sativus*
5	Non-*Brassica* hosts used	Rice, Linseed and Bengal gram

Table.3 Screening of Cole vegetable *Brassica* and oilseed *Brassica* hosts based on root hair infection

Host	No. of root hair	No. of Inf.R.hair	% of R. hair inf.
Black Sarson	**42.00**	**12c**	**29.42c**
Broccoli	**47.00**	**20.67bc**	**42.64bc**
Cabbage	**44.67**	**39.67a**	**88.53a**
Cauliflower	**74.33**	**38.33ab**	**53.62b**
Indian mustard (Varuna)	**61.00**	**49a**	**81.56a**
Local yellow sarson (Jhumka)	**55.00**	**45.33a**	**84.40a**
Radish (White radish)	**40.33**	**14.33c**	**35.49c**
Tori sarson (Agrani)	**40.33**	**15.67c**	**38.59c**
Yellow sarson (Binoy)	**44.67**	**37.67a**	**82.45a**
SEM	**8.18**	**5.70**	**4.92**
CD at 5%	**NS**	**16.95**	**14.62**

*Data bearing same alphabet are statistically at par on the basis of Duncun's multiple range test (DMRT)

Table.4 Screening of non-*Brassica* hosts based on the root hair infection

Sl. No.	Host	No. of root hair	No. of inf. root hair	% of root hair inf.
1	Rice (*Oryza sativa*)	43	0	-
2	Linseed (*Linum usitatissimum*)	46	0	-
o	Bengal gram (*Pisum sativum*)	52	0	-

Table.5 Screening of Oilseeds and Cole vegetables *Brassica* hosts based on PDI

HOST	Mean (PDI)
Broccoli *B.oleraceae*	61.6(51.71)c
Cabbage *B.oleraceae*	94.4(76.31)a
Cauliflower *B.oleraceae*	16.6(24.04)f
Indian Mustard *B.juncea* (Varuna)	72.2(58.18)b
Local Yellow Sarson *B.rapa* (Jhumka)	50(45.00)d
Radish (White radish)	11(19.37)g
Torri Sarson *B.rapa* (Agrani)	44.4(41.78)e
Yellow Sarson *B.rapa* (Binoy)	50(45.00)d
SE(m)	1.74
CD at 5%	5.11

*Data in the parentheses are angular transformed data

*Data bearing same alphabet are statistically at par on the basis of Duncun's multiple range test (DMRT)

Fig.1 Relationship between Root hair infection (%) and PDI (%)

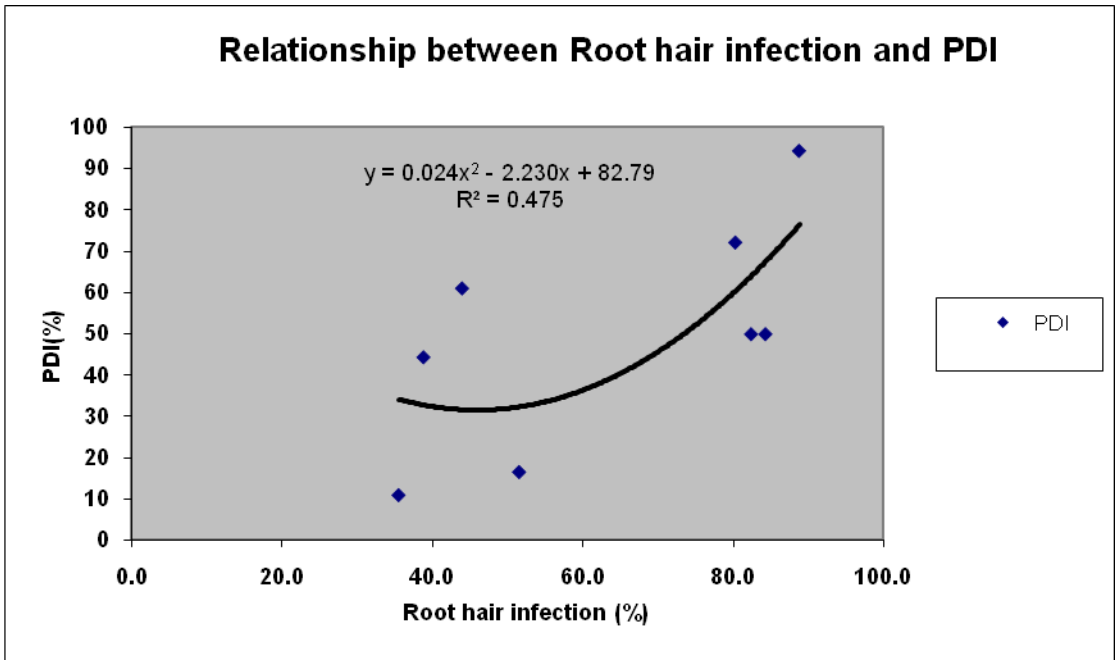
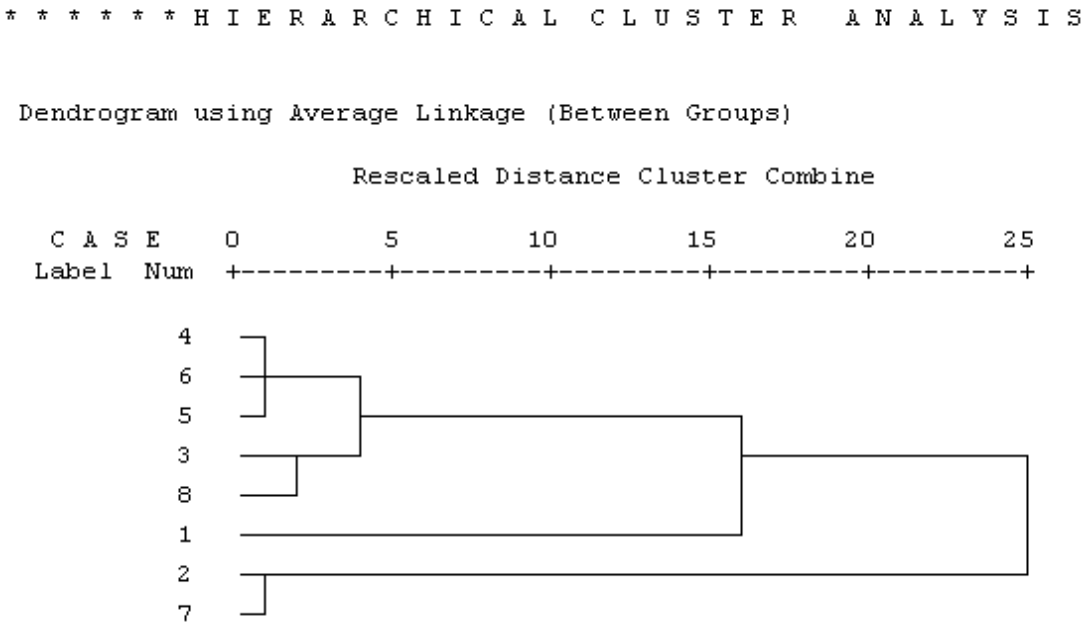


Fig.2 Cluster analysis of the different *Brassica* based on the result of PDI %



This secondary phase of colonization by secondary zoospores following root hair infection colonizes cortical tissue results in formation of clubbed roots. Resistant and susceptibility of the hosts depend on nature and intensity of club formation. The result of club formation study showed distinct differences of resistance between different species of *Brassicas* (Table 5). Among the germplasms of *Brassicas*, *Brassica oleraceae* (Cabbage. Broccoli) and *Brassica juncea* (Indian Mustard) were found highly susceptible in compare to *Brassica rapa* group. White radish (*Raphanus sativus)* showed lowest disease severity value (11%). Among *Brassica oleraceae* group cabbage (94.4%) showed maximum disease intensity in compare to Cauliflower (16.6%) and Broccoli (61.6%). Presence of different pathotypes is very pronounced with the difference of pathogenicity on different *Brassica* hosts. Pathotype infecting Cabbage is more predominant in Kalimpong which is virulent to *Brassica oleraceae* and *Brassica juncea* group. The race/ pathotype virulence of *Plasmodiophora Brassicae* depend on exposure of *Brassica* hosts as cabbage is most popular and most widely cultivated *Brassica* species. On the other hand white radish and cauliflower may act as source of resistance for *Brassica* vegetables cultivated in Kalimpong due to tolerance of these two germplasms.

Relationship between Root hair infection and club formation and clustering of *Brassica* hosts based on pathogenicity test result

The relationship between root hair infection and club formation is not very well understood. Although root hair infection is mandatory for club formation but intensity of root hair infection is not related to pathogenicity. In the present experiment r2 value is less than 0.5 which is polynomial in nature. The disease severity (P.D.I. %) and rate of club formation is distantly related. The Dendrogram showing pathogenicity relationship between different oilseeds and Cole vegetable *Brassica* hosts. This cluster

analysis grouped into the three different groups. In one group belongs Cabbage and Radish and in second group belongs only Cabbage (*Brassica oleracea*) and last one is the third group, it divided into two sub-group in A belongs Broccoli and Indian mustard (Varuna) and in B belongs Yellow sarson (B-9), Torri sarson (Agrani) and Local yellow sarson (Jhumka). It is very difficult to identify predominant race/pathotype with flexible *Brassica* hosts. More stable and standardized homogenous host is required for screening of *Brassica* hosts for identification of source of resistance.

Different *Brassica* germplasms were screened against *Plasmodiophora Brassicae* under in *in vitro* condition. But it is found that there is no complete resistance against this pathogen from root hair infection and club formation test along with different result in this two tests. Black sarson, white radish and broccoli exhibit less root hair infection in comparison to other *Brassica* germplasms but white reddish and cauliflower exhibit less club formation. Therefore it can be suggest that these *Brassica* germplasms may be cultivated against club root disease of crucifers.

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