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Artificial Screening for Black Rot Resistance Based on Different Disease Parameter in Early Cauliflower

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India has maximum genetic materials in early cauliflower, which grow in subtropical conditions. Different disease parameters like linear growth, maximum growth rate per day, AUDPC, apparent infection rate and percent diseased area were calculated in artificially inoculated plants. Apparent infection rate is not co-related with the black rot disease incidence and should never be considered during characterization of disease resistance and varietal screening. Based on the above disease parameters Kunwari-18, Phool Gobhi Kunwari, Kataki-7 and BT-10-2 were selected as moderately resistance to black rot in early cauliflower. These lines can be used for black rot prone area and also for black rot disease improvement programme. Considering the qualitative and quantitative parameters, slow rotting resistance cauliflower lines are selected as such for cultivation and would be best suited in integrated disease programme.

KEYWORDS: AUDPC, Black rot, Cauliflower, Inoculation technique, Resistance

Black rot of cauliflower (*Brassica oleracea* var. *botrytis*) caused by *Xanthomonas campestris* pv. *campestris* is a serious disease and prevalent in all agro climatic zones of India. Early cauliflower grown in subtropical type of climate in late kharif season is more prone to black rot. Severity of disease increased tremendously in all types of cauliflower when rain and hailstorm takes place during the cropping season. The disease is harmful in another way also that it effectively predisposes plants towards increased attack of Alternaria blight (Sharma *et al.*, 1991). The bacteria are pathogenic to most of the cruiciferous vegetables. Muhiar and Khlaif (2000) reported that cabbage, cauliflower, broccoli, red cabbage, radish and turnip were susceptible to the pathogen to different degree under artificial inoculation test. Resistance bacterial pathogen is governs by additive and non-additive genes with the latter preponderant (Pandey *et al.*, 1995). Sources of resistant to black rot in cauliflower were reported by many workers but most of them are not commercially grown by Indian farmers due to poor curd quality. MGS 2-3, Pusa Kea and S-445 were resistant to black rot shown to be governed by dominant polygenes but curd quality of these lines was not acceptable (Ram Singh *et al.*, 1987). Later on they have developed a variety Pusa Subhra resistant to black rot and Pusa Showball K-1 field resistant to the disease with good curd quality utilizing the above materials. EC-162587 line as highly resistant to black rot while RSK-1301 and MRS-1 were moderately resistant to black rot with multi disease resistant character (Sharma *et al.*, 1995). Caulivars, Avans and Iglory resistant to black rot contained high amounts of auxins, cytokinines and polyphenols and low amount of gibberellins, whereas the reverse was apparent in the susceptible (Dua *et al.*, 1978). All above the described resistant sources were only available in snowball group of temperate type of cauliflower. But no report is available on the early group and subtropical type of cauliflower. India has maximum genetic diversity for early cauliflowers in whole world. Early cauliflower of Indian type grown in subtropical is becoming very popular throughout the country due to high price and very good adaptability of the local land race. These lines can be also grown in other countries having subtropical type of plant. Therefore, a screening programme is carried out under artificial conditions to find out the resistance source in early group of cauliflower and to categorize pathogen reaction on the basis of different disease parameters.

Materials and Methods

Total sixty-two germplasm lines of early cauliflower comprising advanced lines, local collections, Hazipur collections and varieties were screened artificially against the black rot pathogen. The germplasm lines belong to mainly Kunwari and Kataki group. Experiment was carried out in cropping season of the year 1999~2000.

Sowing plan. All seedlings were grown in nursery beds. Twenty-five days old seedlings were transplanted in small plastic pots containing approximately eight hundred grams normal field soils. These soils were enriched with required fertilizers and organic matters. One seedling of each ger-

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mplasm lines was transplanted in a pot. Pots were fertilized with Hoagland solution whenever required.

Inoculation procedure. *X. campestris* pv. *campestris* was isolated from the naturally infected plants of same cropping season to get virulence culture. The pathogen was multiplied on nutrient agar medium at 25°C for three days. The culture was carefully scrapped from the medium with the help of sterilized slides. The scrapped bacterial culture was mixed in 100 m*l* sterilized distilled water and shaken vigorously to make a uniform suspension of bacterial cells in the water. The bacterial concentration was counted approximately 1×10^{12} cfu/m*l*. The concentration was visually look like a turbid, translucent and light milky suspension. Thirty-five days old seedlings were inoculated by the bacterial suspension. Upper leaf of each plant was cut approximately two to three millimeters from apical margin by scissors. Cut portion of the leaf immediately dipped in the bacterial suspension. During cutting process, scissors was dipped in bacterial suspension to stick water and bacteria on the cut portion of leaf. The main aim was to avoid formation of air bubbles in mesophyll cells near the cut portion. Bacterial suspension was kept in sterilized petriplates and cutting of leaf was carried out in petriplates itself and quickly dipped in the suspension. This will provide easy penetration of bacterial cells through conducting tissues of leaf and do not allow the penetration of air. Formation of air bubble will interfere with the proper penetration of bacteria during inoculation. In such a way, two upper leaves of each plant was inoculated. Inoculated plants were kept in moist chamber by maintaining 90~95% relative humidity for nine days. Thereafter, all inoculated plants were taken out from moist chamber and kept in screen house condition. The temperature within moist chamber varies from 25~30°C while atmospheric diurnal temperature was 20 to 28°C in screen house. Inoculated plants were observed daily for the disease development. Disease initiated on 8th days but observations on disease progress was recorded from 9th days of inoculation and continued till the inoculated leaf become senescent. Linear growth was measured in millimeters (mm) from cut margin of leaf upto the yellowing of tissue along with the midrib towards basal end of the leaf. The line differentiated between yellow and green tissue on leaf was considered for the measurement. Later on the inoculated leaves were detached from the plant and brought in the laboratory. Each leaf was placed below the close circuit camera of image analyzer. The percent area of disease portion on leaf was directly recorded from computer. Maximum growth rate per day of disease on inoculated leaf was also calculated manually. To record maximum growth rate, four maximum subsequent linear growths in whole disease progress period was considered and average was calculated. Here maximum disease growth means the exponential phase of disease progress curve. Area under disease progress curve (AUDPC) value was calculated between first value after 9th days of inoculation where disease initiated and upto the last value till twenty- third days of inoculation. Total apparent infection rate was calculated (r_c) between the same periods. Sequential apparent infection rate was also calculated between each two subsequent observations. AUDPC and r_c was calculated by the method described by Vander Plank 1963, Johanson and Wilcoxson 1982 and later described by Compbell and Madden 1990 as follows:

$$\text{AUDPC} = \left[\left(\frac{X_{i+1} + X_i}{2}\right)*(t_{i+1} - t_i)\right]$$

Where X_i = the cumulative disease index expressed as a proportion at the *i*th observation

t_i = Time (days after planting) at the *i*th observations.

n = Total number of observations.

$$\text{(i) } r_c = \frac{1}{t_2 - t_1}\log_e\frac{n_2(1-n_1)}{n_1(1-n_2)}$$

Where t_1 = Time (days) during the 1st observations

t_2 = Time during the 2nd observation.

$t_2 - t_1$ = Time interval between two observations and subsequently so on.

n_1 = Per cent disease index value in decimal at corresponding t_1 time.

n_2 = Per cent disease index value at t_2 time.

Pathogen reaction on different variety of early cauliflower was categorized on the basis of mean linear growth. Only four rating grades were given for this particular disease considering the progress of black rot and bacterial invasion in the host as follows:

0~3.0 = Resistant (R)
3.1~7.0 = Moderately Resistant (MR)
7.1~11.5 = Susceptible (S)
>11.5 = Highly Susceptible (HS)

Disease progress curve (DPC) was prepared by selecting three cultivars each from moderately resistant group and highly susceptible varieties to show the comparative disease development in artificially inoculated plants in relation to time.

Results

Inoculated seedlings of early cauliflower initiated symptoms as translucent tissue near the cut portion of leaf after eight days of inoculation. Characteristic symptoms as yellowing of the leaves were observed on next day. The periodical observation of all the test plants revealed that none of the germplasm line was resistant to black rot disease.

Table 1. Effect of bacterial inoculation on different parameters of disease development and varietal reaction

Variety	Mean linear growth (mm)	Apparent infection rate (r_c)*	AUDPC	Growth rate (mm/day)	Pathogen reaction
Kunwari-18	5.9	–0.041	61.4	1.9	MR
Phol Gobhi Kunwari	4.8	–0.201	53.0	1.9	MR
Kataki-7	6.7	0.084	68.8	1.7	MR
BT-10-2	5.7	–0.16	62.0	1.7	MR
Suryamukhi	8.1	–0.063	91.3	3.8	S
Kunwari-15	9.5	0.184	98.2	4.2	S
Kathmandu (Local)	10.7	0.145	109.9	5.0	S
Kunwari (Laxmi seed)	9.9	0.006	99.8	3.2	S
Hazipur-4	9.9	0.103	104.6	3.9	S
PDVR Early	9.6	0.097	101.1	4.7	S
Aghani-VII	8.9	0.077	90.1	2.5	S
Kataki-3	9.7	0.124	99.1	3.2	S
Phol Gobhi (Kataki)	8.9	0.005	100.7	4.9	S
Manoj Express	10.2	0.131	105.7	2.5	S
Kunwari-7	9.3	0.111	97.0	5.1	S
Kunwari-8	9.5	0.040	107.0	3.3	S
Kunwari-1	9.6	0.070	101.1	4.8	S
Kunwari-13	9.3	0.086	92.7	4.2	S
Kunwari-12	9.4	–0.015	96.7	2.4	S
Kataki-6	9.4	0.012	96.8	4.7	S
Pusi Him Karan	8.9	–0.068	100.1	4.9	S
Kunwari-10	9.7	0.034	131.4	5.6	S
Pusa Deepali	11.3	–0.005	121.5	4.8	HS
Vaishali	11.5	0.031	127.2	6.2	HS
NDC-1	11.1	0.020	120.7	5.1	HS
Kataki-11	11.8	0.110	120.0	6.7	HS
Kataki-9	11.2	0.248	116.9	5.9	HS
Kataki-29	12.4	0.085	130.2	6.7	HS
Kunwari	11.3	0.028	124.0	6.4	HS
Kunwari-4	11.4	0.102	122.5	6.4	HS
Kataki-15	11.3	0.078	105.7	5.0	HS
Early Laxmi	11.9	–0.068	120.8	5.3	HS
Deep Mallika	11.2	0.061	117.8	5.8	HS
Kataki-13	12.8	0.119	132.1	6.5	HS
Aghani-X	12.2	–0.005	130.4	6.2	HS
Aghani-I	13.0	0.081	135.7	6.8	HS

*Indicates apparent infection rate between first observations to last observation.

Out of sixty two lines of early cauliflower only five viz., Kunwari-18, Phool Ghobhi Kunwari, Kataki-7, Kunwari-11 and BT-10-2 were moderately resistant where mean linear growth vary from 4.8 to 7.0 mm. The maximum growth rate during the period of disease development was very low and ranged from 1.7 to 2.4 mm/day. Most of the test lines were either susceptible or highly susceptible to the black rot infection. The mean linear growth of highly susceptible lines was 11.1 to 13.0 (Table 1) while maximum growth rate per day was recorded 5.0 mm to 6.8 mm. The growth rate of black rot was not constants at each day of disease progress. The germplasm lines showing moderately resistant can be selected as such for the cultivation in black rot prone area. A characteristic phenomenon observed in some cauliflower lines in which subsequent linear growth was significantly at par to mean linear growth in all the observations. These lines were categorized as slow rotting resistant varieties. Mean linear growth in these lines was always less than 9.0. Although they were in susceptible group but on the basis of almost constant growth behavior of disease progress, it was considered as slow rotting resistance. Such character of a variety can be exploited in resistant breeding programme. The main advantage of slow rotting resistant variety was delay in disease and loss below the economic levels through out the cropping period. Such character was identified in Kunwari-11, Kataki-8, Kataki-14, Early, Early-1, Aghani-II, Patna Kataki, Kataki (Laxmi). Mean linear growth of slow rotting resistant variety varies from 4.8 to 10.2 (Table 3). Some moderately resistant lines may also show slow rotting resistant but the degree of tolerance to the disease in slow rotting resistance was always less than

Table 2. Percent diseased area on inoculated leaf of cauliflower

Variety	% Diseased area	Av. linear growth (mm)	Reaction
Phool Gobhi-1	9.1	6.7	MR
Kataki-1	16.0	7.0	MR
IIHR-13	25.4	7.8	S
Hazipur-2	17.7	10.4	S
Pakistan-1 (Early)	19.2	10.7	S
Fo_2-Co-4	18.7	10.8	S
Manoj Express	27.9	10.7	S
Kunwari-3	16.0	10.9	S
KT-25	17.9	10.0	S
Punjab Sel-1	25.3	12.6	HS
RSK-301	25.7	12.9	HS
PSB-1	22.2	14.4	HS

the moderately resistant lines.

The inoculated leaves were detached at maximum rotting stage and brought in the laboratory. Computerized image analysis system used for recording the percent area of leaves covered by the disease. It revealed that the image analysis software detect proportionate bright yellow area of disease but not the translucent portion. The area was comparatively less and varies from 9.1% to 16.0% in moderately resistant lines while more (16.0 to 27.9%) in susceptible and highly susceptible varieties. But it was not always a universal facts and no correlation between pathogen reaction and percent diseased area on leaf. For example Kataki-1 a moderately resistant line was covering 16% area by disease but at the same time a susceptible line Kuwari-3 also covered 16% area (Table 2). A highly susceptible line RSK-301 showed 25.7% diseased area while a susceptible line Manoj Express showed more area i.e. 27.3%. This was due to size of the leaf.

Area under disease progress curve. All inoculated leaves were periodically observed for calculating area under disease progress curve. The AUDPC value was recorded low i.e. 53.0 to 68.5 in moderately resistant lines while comparatively high AUDPC value (105.7 to 135.7) in highly susceptible lines. Value of AUDPC increased with the increase in degree of susceptibility. It was dependent on all the value of linear growth recorded from first observation to last observation. It had no direct relation with maximum growth rate per day. It was also observed that at same mean linear growth there was different AUDPC value. The higher AUDPC was recorded in some variety but maximum growth rate per day was accordingly not high and vice-versa was also observed. For example, 5.1-mm growth rate per day (high value) was observed in variety Kunwari-7 but the AUDPC was proportionately low 97.0. Contrary to this, comparatively high AUDPC value 105.7 was recorded with only 2.5 mm/day growth rate in Manoj Express (Table 1). AUDPC value must be calculated for categorizing the pathogen reaction. Disease progress curve of three line from highly susceptible varieties, Kataki-29, Aghani X, Aghani-1 and three moderately resistant varieties Kunwari-18, BT-10-2, Kataki-7 was prepared (Table 1, Fig. 1). The disease progress curve was distinctly different in both the group of varieties (Fig. 1). The curve of moderately resistant lines was very close to X-axis and covered less area under the curve. The peak of disease progress curve and sector wise area was large in highly susceptible lines. In both the cases, the curve initially showing lag phase followed by log phase and than it started declining. The disease progress curve was not always sigmoid. In slow rotting resistant varieties disease progress curve was recorded with more than two crest and peak giving almost double sigmoid disease progress curve (Fig. 2). It indicated that disease development was increased then decreased and again fluctuating to maintain an equilibrium level of disease throughout the period of disease progress.

Apparent infection rate. This was calculated for all the test lines. The total infection rate between first observation to last observation revealed that it has no relation with either AUDPC or pathogen reaction. The value var-

Table 3. Periodical disease development in slow rotting resistance varieties of cauliflower

Variety	Linear growth (mm)										AUDPC	r_c
	Days after inoculation											
	9	10	11	12	13	14	16	17	18	Mean		
Kunwari-11	4.0	5.2	5.5	7.0	8.3	8.6	10.8	9.4	8.2	7.5	72.5	0.043
Kataki-8	2.3	2.5	4.5	8.4	9.2	9.5	12.0	9.6	7.6	7.3	74.6	0.075
Kataki-14	3.6	4.2	7.2	8.4	11.3	10.8	10.4	12.8	6.4	8.3	86.3	0.060
Early	5.0	7.6	8.8	10.4	10.5	9.6	12.0	12.8	8.8	10.2	105.4	0.061
Early-1	4.8	5.6	6.5	8.4	14.0	10.3	11.2	11.8	7.6	8.9	90.8	0.049
Aghani-II	4.5	4.9	7.6	8.2	8.7	11.1	10.0	12.0	10.0	8.9	90.4	0.077
Kataki (Laxmi)	3.9	5.1	9.7	10.0	12.8	12.0	5.6	2.6	4.8	7.4	82.4	0.022
Phool Gobhi Kunwari	2.6	4	4	7.0	9.5	7.6	6.4	1.2	1	4.8	53.0	-0.201
Patna Kataki	2.4	5.1	6.3	9.6	11.6	12.5	9.3	7.7	5.0	7.7	82.4	0.075

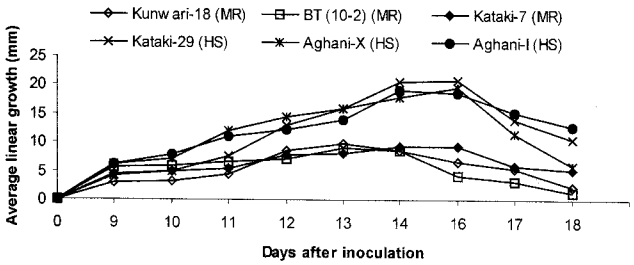

Fig. 1. Disease progress curve of black rot in artificially inoculated plants of early cauliflower.

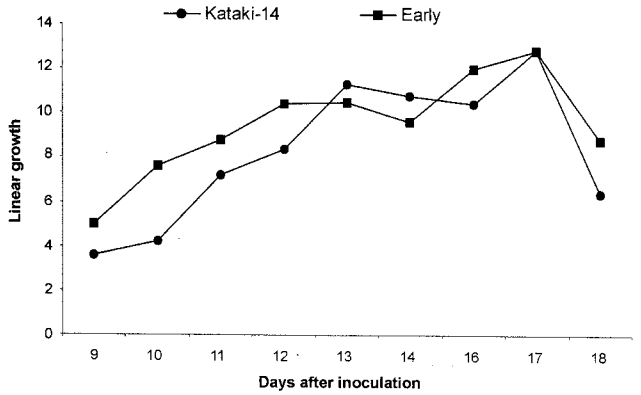

Fig. 2. Double sigmoid disease progress curve in slow rotting resistant lines.

ies from lowest of 0.005 to maximum of 0.248 (Table 1). Therefore total infection rate should not be used for any interpretation of pathogen reaction of black rot under artificial inoculation conditions. The sequential infection rate between two subsequent observations is also not informative for disease like black rot where chance of air borne inoculum is negligible. It was also observed that many times the value of total apparent infection rate was negative. The negative value recorded only in those varieties where linear growth was more in first observation than the last observation.

Discussion

The study reveal that there is no resistant line but tolerant lines are available in early group of cauliflower belongs to Kunwari and Kataki group for black rot. These groups of cauliflower are mainly grown in rainy season. They are severely affected by black rot disease due to high temperature, high relative humidity and rainfall during vegetative growth stage of the crop. The moderately resistant lines can be grown in such situation. Considering the good qualitative and quantitative characters, slow rotting resistance lines are equally effective. Preference of slow rotting lines in integrated disease management is best suited due to most of the acceptable characters and durable resistance under epidemic condition. Although these lines are coming under susceptible group but the AUDPC and mean linear growth is less and very close to moderately resistant lines. The economic loss in such lines was very less. Genetic make up of these lines shows tolerance to bacteria because it does not allow fast multiplication and disease progress in the host. The degree of resistance in slow rotting resistant lines is slightly less than moderately resistant lines and more than susceptible lines. Maximum growth rate per day of black rot was less in moderately resistant cauliflower as compared to susceptible and highly susceptible lines. It indicates that the maximum possible growth of the disease in host can take place upto that much extent if congenial weathers prevail.

Some selected lines of cauliflower have been analyzed with image analyzer. Result indicates that disease area covered on leaf is less in moderately resistant lines while more in highly susceptible lines. In many lines the percent diseased area is not corresponding to the pathogen reaction grade. For example, Kataki-1 is a moderately resistant and Kuwari-3 is a susceptible line but both the having only 16% diseased area (Table 2). It indicates that the size of leaf in Kataki-1 is large as compared to Kunwari-3. The size of leaf depends upon physiological, genetical and nutritional condition of the plant. The leaf having larger size is showing smaller percent area covered by the disease with respect to total area of that particular leaves during image analysis. In this condition the linear growth of disease portion is more and hence recorded as highly susceptible line. But the area of diseased portion is less in comparison to total leaf area due to larger size of leaf. Similarly Manoj Express show 27.9% leaf area infected by black rot while PSB-1 is showing only 22.2% diseased area (Table 2). It entirely depends on the size of leaf. Larger the size of leaf but higher the liner growth will give less percent diseased area and vice-versa. The line showing more percent area but bearing small size of leaf is certainly more susceptible because the damaged area is more in per unit area of the leaf. Therefore, while characterizing the pathogen reaction of black rot, percent disease area of leaf should also be considered depending upon availability of the image analysis system. However, AUDPC value is more reliable than percent diseased area because the later will be calculated once or twice only after detaching the leaves from plant while former will consider all observations from disease initiation to declining phase. Same way germplasm showing more linear growth but less percent area of disease is due to large size of leaf should also consider for the screening of black rot resistance. There will be less economic loss in such lines due to more photosynthetic area.

Periodical disease progress was recorded from date of disease initiation to the declining of disease for AUDPC. In general AUDPC value increases with increase of mean

linear growth. However, it is entirely dependent upon the subsequent value of linear growth at each observation during the whole period of disease development. In Kunwari (Laxmi seed) and Hazipur-4, in spite of some mean linear growth the AUDPC was 99.8 in former line while 104.6 in later. It is because of the different value recorded in periodical disease progress period. The sector wise area covered in disease progress curve was different in both the cases. Interestingly, the disease progress curve in many lines particularly slow rotting resistant lines is declining after 13 days of inoculation and further increased within next two days. This leads to somewhat double sigmoid curve with more than two peak and crest (Rotem, 1994) reported three pecks in disease progress curve (DPC) for *A. dauci* on carrot grown under conditions highly favorable to disease. The reason is very clear for either double sigmoid or zigzag type of sigmoid curve. The diseased portion of leaf tissue close to inoculation (margin) is become more senescent than the distant side (close to healthy tissue). A stage comes when these senescent portions become dried, papery and started to shrink. Soon these portions break from the yellow tissues and fall down. During this stage the total linear distance from shrinked papery portion to newly developed translucent portion is become less than the previous observation. Some time if weather condition in net house is unfavorable than bacterial growth and disease development slow down. These two factors lead to sudden declining in linear growth. Further in subsequent days bacterial growth and disease progress increase which will give increase in linear growth. This increase is higher than original value prior to declining. Rotem *et al.*, 1988 also reported that misleading shapes of disease progress curve for *A. macrospora* on cotton where disease intensity is masked by the shedding of infected leaves. In case of cauliflower line 'Early' the value is lower than the just preceding value and also it is observed in Kataki-8. This phenomenon depends on genetic character of the variety. The total apparent infection rate has no relation with mean linear growth. It only depends on value of first and last observation and become negative whenever the last value is less than the first observation.

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