IN VITRO SELECTION OF ANTHRACNOSE DISEASE RESISTANT CELL LINES BY SCREENING DIFFERENT GENOTYPES OF *BENINCASA HISPIDA*. COGN.

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ABSTRACT: Stem derived calli of four genotypes of Ash gourd susceptible, moderately resistant and resistant plants were screened *in* vitro for the production of Anthracnose (*Colletotrichum lindemuthianum*). Three methods were used to get resistance i.e., direct spore suspension, high(75%) and step wise increase (25%,50%,75%) in culture filtrate concentration of *Colletotrichum lindemuthianum*. After being exposed to spore suspension, the susceptible of calli were noticed and arrested the growth of the callus. However, certain parts of the calli were observed that wre free from fungal attach and seen proliferating in the toxin containing media. Whereas the susceptible calli turned brown resulting in death of the calli. Then the resistant cell lines were isolated and sub cultured for the three passage of two weeks. The percentage of these resistant cell lines varied among the four genotypes of Ash gourd.

Key words: *In vitro,* Anthracnose, resistant cell lines, spore suspension, step wise selection, culture filtrates.

INTRODUCTION:

Anthracnose disease and Powdery mildew diseases (PLATE-1, Fig- 1,2,3) are the most serious diseases of Ash gourd caused by fungal pathogens, which are *Colletotrichum lindemuthianum* and *Erysiphae cichoracearum* respectively. These two diseases cause damage to crop yield. Therefore, the production of resistant cultivar has assumed great importance in reducing crop yield from the diseases.

In the 1970 studies demonstrated that in vitro technique plant cells could be selected for resistance to pathogen and plants with altered response to infection by the pathogen could be regenerated (Carlson, 1973)[1]. Since then many claims have been made about the vast potential of cell culture selection for the development of disease resistant lines (Daub, 1986)[2]. Successful selections have been reported in various crops such as potato to culture filtrates of *phytophthora infestance*, (Behnke, 1980a)[3]. *Brassica napus* to *Phoma lingam*, alfalfa to *fusarium oxysporium sp. Medicaginis,* tobacco to *pseudomonsyringae* and *Alternaria alternate*(Tanutong *et al.,*

1983)[4], sugarcane to *Helminthosporium sacchari,* (Heinz, et al., 1977)[5] rice to *Pyricularia oryzae* (Palit, Ruma et al., 1990)[6] Oats to *Helminthosporium victoriae(*Arcioni, et al., 1987)[7].

In the present study, multiple shoot regeneration from stem derived calli of ash gourd was used for selection of leaf spot resistant(Anthracnose) plants.

MATERIALS AND METHODS:

Induction of callus (PLATE-2, Fig-1,2,3,4**)**

The main objective of the study is to develop are liable tissue culture protocol for screening of disease resistant clones in ash gourd (*Benincasa hispida.* Cogn.) Stem segments of the four genotypes, of ash gourd Co 1, IVAG-502(susceptible),Karikumbala (moderately resistant) and Indu (resistant) were collected from surrounding areas of Karimnagar rural local fields of Karimnagar district in Telangana state. These stem explants were surface sterilized with 0.1% Mercuric chloride for 15 minutes, washed with distilled water. The explants were inoculated onto MS medium (Murashige,T & Skoog,F. *et al.*, 1962[)[8] supplemented with 2.0 mg/l 2,4-D and 1.0 mg/l BAP. The pH of the medium was adjusted to 5.6 and autoclaved for 20 minutes at 15 lbs. The cultures were maintained at 25^0C with 16 hours photoperiod. After two weeks callus was initiated. These calluses free from original explants were used for *in vitro* screening.

Selection of disease resistance cell lines.

Callus cultures were initiated (PLATE-2,FIG. 1,2,3) from different genotypes i.e., Co 1, IVAG-502(susceptible), Karikumbala (moderately resistant) and Indu(resistant) varieties. Four weeks old calli separated from original explants and were inoculated onto MS media supplemented with 2.0 mg/l 2,4-D + 1.0 mg/l BAP and different concentrations of culture filtrates (25%, 50%, 75%). The proliferated callus cultures were transferred (every 2 weeks) to fresh medium containing the same supplements.

Selection procedure:

The selection methods were followed for screening of resistant cell lines.

Direct selection on high toxin concentration :

In this method of selection, the calli were inoculated onto MS medium (2.0 mg/l 2,4-D + 1.0 mg/l BAP) supplemented with high concentration (75%) of culture filtrate. Healthy looking calli were selected and sub cultured for two passages of three weeks duration each containing with or without culture filtrate.

Step wise selection by increasing culture filtrate concentration:

In this method, the calli were chosen by adapting the calli to lower concentration of culture filtrate (25%) and then transferred to higher concentration (50% and 75%) calli resistant to lower

concentration of culture filtrate (25%) were selected and utilized for subsequent transfers. Calli were maintained for three passages of three weeks duration each onto 50%,75% culture filtrate media. Healthy calli were subsequently separated and maintained in fresh media.

Direct challenging selection with spore suspension:

Calli were transferred onto MS medium supplemented with 2.0 mg/l 2,4-D + 1.0 mg/l BAP and inoculated with spore suspension ($2x10^3$ spores/ml). Cultures were maintained in continuous light. The growing calli over the fungal hyphae free from (mycelial growth) were selected. The selected resistant portions of calli were sub cultured onto MS media for two passages of three weeks each.

RESULTS AND DISCUSSIONS:

In the screening procedures, the calli were screened for Anthracnose disease resistance in two different ways. Calli initiated from four different genotypes were subjected to a biotic stress i.e., to fungal spore suspension ($2x10^3$ spore/ml) and incubated for 2 to 3 weeks. After being exposed to the spore suspension, the susceptibility of the calli were noticed from the growth mycelia which completely arrested the growth of the callus cultures whereas small portion of calli free from fungal attack were observed. The percentage of resistant calli was ranged from 3.17% to 16.91% among the four genotypes (Table). These resistant portion were isolated and transferred to MS medium and subjected to fungal spore suspension for two successive passages. The calli of four genotypes were also screened for Anthracnose disease resistant against culture filtrate in two different ways by direct screening, calli were inoculated on MS medium containing high concentration of culture filtrate (75%). After 2 to 3 weeks of the inoculation in toxin supplemented media, susceptible calli were turned brown resulting in death of calli. Whereas some calli were seen proliferating in toxin containing media, Calli that were growing on culture filtrate were isolated and transferred onto a medium containing the same concentration of culture filtrate for two passages.

The percentage of resistant calli ranged from 3.33% (Co 1) to 16.66% (Karikumbala) (Table). By step wise increase of culture filtrate, calli were inoculated on MS medium (2.0 mg/l 2,4-D+ 1.0 mg/l BAP) with 25% culture filtrate. Calli that were growing on 25% culture filtrate were transfer on MS basal medium (without stress) and then sub cultured on higher concentration of culture filtrate i.e., 50% and 75% calli were growing for two passages with 2-3 weeks duration in each treatment. The percentage of resistant calli ranged from 6.42% (Co 1) to 28.57% (Karikumbala)(Table 1). From these two methods later method gave more number of resistant cell lines.

Plant cell and tissue cultures can be efficiently exploited for selection of disease resistant plants. Selection for leaf spot disease resistant plants was carried out in the present investigation using *in vitro* techniques. The development of an in vitro selection program depends on two important factors, i. e., an effective selective agent and cell to plant regeneration. The use of pathogen spores is a most effective method for screening and isolating disease

resistant varieties. However, this approach may involve several difficulties. Even though the problem can be eliminated to certain extent by directly subjecting the plant cell with pathogen than its incorporation in the nutritive media (Sacristan, 1982)[9].

In the present study for *in vitro* selection of variants, the disease resistance can be expressed in cultured cells to which the selection procedure is applied. Susceptibility of the calli was expressed in the control calli of anthracnose after three weeks of inoculation with the pathogen. Proliferation of calli was completely arrested due to growth of the fungal mycelium. However resistance reaction was expressed by small sectors of calli seen growing over the fungal mycelium which were free from the fungal growth observations were made in response to pathogen attack in a number of parasites including *phoma lingam* (Sacristan 1985)[10], *Phytopthora infestance* (Helgeson,1983)[11].

CONCLUSION:

In conclusion, the present studies on in vitro screening of callus against culture filtrate offer a potential source for selection of resistant cell lines. In vitro selection studies demonstrated that, cell and tissue culture techniques would supplement conventional plant breeding programs for the production of disease resistant plants in ash gourd.

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TABLE

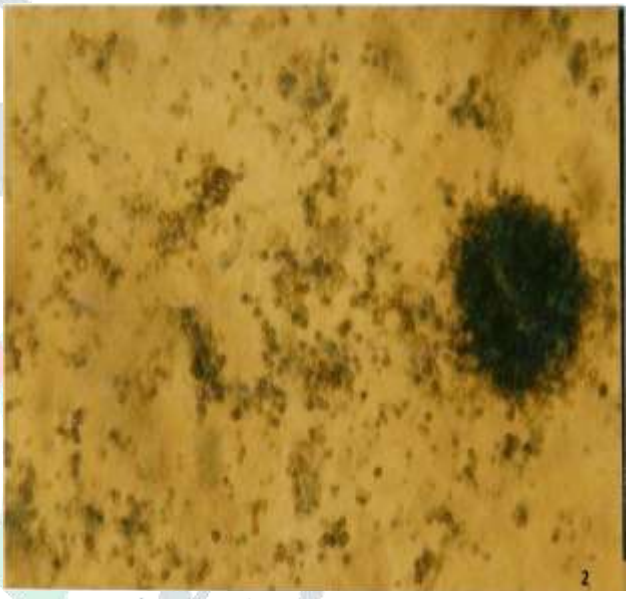
Isolation of cell lines resistant to *Colletotrichum lindemuthianum* by screening genotypes of *Benincasa hispida*.cogn.

Genotype	Type of treatment	No. of calli inoculated	No. of calli growing	Percentage of calli growing
Co 1 (susceptible)	*Control	125	125	100
	*On high conc. Of culture filtrate(75%)	120	4	3.33
	*Directly challenging with spore suspension	129	7	5.42
	*Step wise increase in culture filtrate(25%,50%,75%)	140	9	6.42
IVAG-502 (susceptible)	*Control	115	115	100
	*On high conc. Of culture filtrate(75%)	140	20	14.28
	*Directly challenging with spore suspension	136	23	16.91
	*Step wise increase in culture filtrate(25%,50%,75%)	130	24	18.46
Karikumbala (moderatrly resistant)	*Control	120	120	100
	*On high conc. Of culture filtrate(75%)	90	15	16.16
	*Directly challenging with spore suspension	85	14	16.47
	*Step wise increase in culture filtrate(25%,50%,75%)	70	20	28.57
Indu (resistant)	*Control	110	110	100
	*On high conc. Of culture filtrate(75%)	122	5	4.09

	*Directly challenging with spore suspension	126	4	3.17
	*Step wise increase in culture filtrate(25%,50%,75%)	130	9	6.92

*MS medium with 2 mg/l 2,4-D+1.0 mg/l BAP.

PLATE I



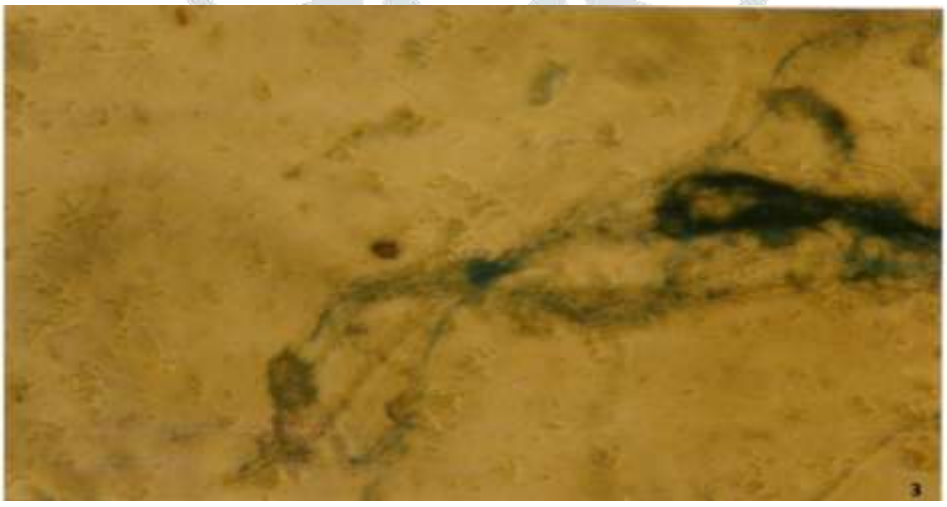


PLATE II