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Study of comparison of level of resistance in different varieties of rapeseed mustard (*Brassica campestris*) and determination of Polyphenol content (in normal and infected) plant

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Abstract
Resistance to several fungal plant pathogen has been ascribed to higher concentrations of fungitoxic phenolic substance and their oxidation products and to increased polyphenol oxidase (PPO), activity which generally, but no invariably, results from infection. When the progress of some pathogen is checked, phenolic compounds develop in abundance within vacuoles of adjacent cells.

Keywords: polyphenol, fungitoxic, PPO, Quinones

Introduction

Plant react to bacterial or fungal infection in the same way as animal react to microorganism or viral infections by producing antibodies however this possibility is unlikely of antibody formation. (Chesters 1933). The aromatic substances, such as polyphenol, phenolic glucosides, flavonoides, anthocyanins, aromatic amino acids and coumarin derivative tend to accumulate in and around infected plant.

The activity of polyphenol oxidase (PPO) seems to be important because it can oxidize phenolics to qinones which may be more fungitoxic. PPO produced by the pathogen may oxidize the host polyphenols to more highly fungitoxic substance which may prevent further development of pathogen. Germinated uredospores of *Puccinia graminis* release phenols and PPO (Farkas and Ledingham, 1959). Resistance of barley plants to *Erysiphe graminis* appears to be associated with the collapse of the mesophyll cells with release of a phenolic substance which accumulates around haustoria and inhibits further development of fungus. (Scott, *et al*., 1957).

Rapeseed mustard (*Brassica campestris*) oilseed crops are important sources of edible oil in Indian diet. It belongs to family Brassicacae and is cultivated on 6.86 mha in Rabi season (Sep/Oct-Mar/Apr), in India respectively.

The major rapeseed mustard growing states are Haryana, Madhya Pradesh, Rajasthan and U.P., together contributing 80.0 and 80.7 percent to the total national hectare and production respectively. Rajasthan has the largest hectare (2.99mha) and production (2.47 mt) which corresponds to 45.4 and 42.9 percent of total rapeseed mustard cropped area and production.

The current production of rapeseed mustard in India is 6.94 mt. According to a most conservative estimate, the domestic demand and supply gap in the edible oil stands at 1.5 mt per annum, and it is estimated that 58 mt. Of this total oil-seed production, the share of rapeseed mustard would be around 24.2 mt. to produce an additional quantity of more than 17

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mt. in about years, realizing that there is very little scope of horizontal expansion of the crop, only practical option is the vertical growth of the crop, i.e., increasing yield.

To achieve the target envisaged, one strategy which is proposed for increasing the production of rapeseed mustard is- Adoption of technology package replacement of old/obsolete varieties, and management of insect-pests and diseases.

Pusa Jaikisan (BIO-902) of Indian mustard is the first somaclonal variety developed through tissue culture, which is one of the variety used for experimental work.

Alternaria brassicicola (Schw.) Wiltshire is the causal organism of the disease alternaria leaf spot of crucifers. The fungus attack mustard, cabbage, cauliflower and knoll-knoll. Spores and mycelium can also survive in diseased plant debris, conidia are disseminated by wind. Small dark colored circular lesions develop on leaves infected by *A. brassicicola*. Concentric rings may develop in lesions. Linear spots form on petioles, pods and stem.

Though the mechanisms of defense have been worked out in many plants but many economically important plants have been ignored. The proposed work aims to study the plant microbe interaction in Rapeseed mustard *(Brassica campestris* family Brassicacae), and the objectives will be determination of Polyphenol content in normal and infected plants using spectrophometric analysis of the two varieties.

Materials and Methods

Plant Material and Pathogen Culture

a) Maintenance of plant system

Seeds of BIO-902 and PCR-15 variety of Brown sarson i.e., Rapeseed *(Brassica campestris*) var. brown sarson were procured from krishi vigyan Kendra. Seeds were surface sterilized with 0.1%HgCl2 and washed several (2-3 times) in sterile distilled water. Seeds were sown on sieved and oven sterilized garden soil in plastic pots (60). So that at least 10-13 plants could grow in each pot.

Rapeseed (*B. campestris* var. Brown Sarson) was maintained in green house conditions of temperature 26 degree Celsius and 60% humidity with 12 hours photoperiod.

For experimentation 20 days, and 25 days old plants were chosen for infection in vivo, and same age plant parts (leaves) were harvested for *in vitro* estimation and quantitation.

Maintenance of fungal (pathogen culture)

The conical flask (250 ml capacity) containing sterilized PDA (Himedia) were UV sterilized. Under proper aseptic conditions, in laminar flow hood (YORKO Horizontal, New Delhi). Lyophilized fungal strains were rejuvenated under proper sterilization conditions and cultured in PDA medium at 28 degree C with aerobic conditions (G 24 Environmental incubator shaker, New Brunswick Scientific Co. Inc. Edison, N.J., USA), initially with 120 rpm speed in incubator shaker. Periodic sub culturing in PDA slants was done at intervals of 15-20 days.

Lyophilized *Alternaria brassicicola* (Schw.) Wiltshire (MTCC No. 2102) were obtained from IMTECH, Chandigarh. Strain was activated on PDA medium (potato infusion 200g/lt., dextrose 20g/lt., agar 15g/lt; PH-5.6+/-0.2 at 25 degree C.) autoclaved at 121 degree for 15 lb pressure for 15 MIN.

Preparation of Spore Suspension and Plant Infection

Spore suspension was prepared by the following procedure. The surface of culture mat was scrapped gently with the sterilized inoculation loop under proper aseptic condition i.e., under LAF. Spore suspension was then adjusted to a concentration of 10, 0000 spores/ml using haemocytometer. Plants of 20 and 25 days old were selected for inoculation. For *in vitro* experimentation, plant part (leaves) was kept in Petri-plates, layered with tissue paper. Surface epithelium was injured mildly with abrasives to facilitate pathogen entry. In both, in vivo and *in vitro*, systems were prepared in duplicates, i.e., normal (control or healthy) and infected (inoculated with pathogen). For infection, plants were sprayed with fungal spore suspension (prepared in sterile distilled water) using TLC sprayer.

Determination of polyphenol contents

Extraction of polyphenos.

The 20 and 25 days old plants with control and inoculated sets were used for polyphenol extraction *in vitro* and in vivo. Plants with different post infection incubation periods of 0, 4, 24, 48, and 72 hr were selected. 1 gram of the tissue was taken and homogenized using prechilled mortar and pestle in 10 ml of 75 % methanol. The homogenate was then filtered through two layers of cheese cloth. This filtered homogenate was then centrifuged(Beckman Avanti TM 30) using a fixed angle rotor at 5,000X g for 25 min. the pellet containing the cell wall and debris were discarded and the supernatant containing the polyphenols was collected in a separate test tube and used for further assay.

Determination of polyphenols

The estimation of polyphenols was done by using a modified version of the Bray and Thorpe (1954) [1] method. An aliquot of plant extract (supernatant) 0.5 ml was added to a test tube containing 8.5 ml distill water and 0.5 ml of Folin-ciciocalteau reagent. Blank was prepared with 9 ml distill water and 0.5 ml Folin's reagent. After 3 minutes of incubation reaction mixture was mixed well and incubated at room temperature for 1hr. absorbance was measured at 725 nm using spectrophotometer 106.

Polyphenol content is expressed in OD units/gfw at A 725.

Results and Discussion

Change in Polyphenol Content

Spectrophotoimetric analysis

The polyphenols content of two varieties of 20 d and 25 d old plant at different hrs of inoculation with pathogen in terms of absorbance unit/gram fresh weight at 725 nm is depicted in table no. 1, 2, 3, and 4.

The observation of data embodied in the table shows a general trend of increase polyphenol content in infected plants of both the varieties than the normal ones. The finding is in the agreement with those of Kuc and Rush; Bennet and Wallisgrove, 1994.

Among the different age group of plants, it was seen that in *in vitro* as well as in vivo experiment 20 d give the best response to the microbe with the maximum amount of polyphenol content change. On comparing the polyphenols content of different varieties of Rapeseed, it was observed that polyphenols content of BIO-902 was higher than PCR-15.

In Bio-902 maximal increase in polyphenol content was observed after 24 hrs. Of inoculation in case of both *in vitro* and in vivo experiments. In general, there was gradual increase in polyphenols content up to 24 hrs. Followed by gradual decrease up to 72 hrs. This shows that BIO-902

variety rapeseed (mustard) shows maximum resistance after 24 hr of inoculation against *Alternaria brassicicola.*

In PCR-15 increase in polyphenols content was observed after 4 hrs of inoculation in case of both *in vitro* and in vivo experiments, there was gradual decrease in polyphenols content up to 72 hrs.

Increase in polyphenols content is indication of development of resistance. Phenylalanine ammonialyse (PAL) is the first enzyme of phenyl propanoid metabolism in higher plants and it has been suggested to play a significant role in regulating the accumulation of phenolics (Massala *et al*., 1980). Since the production of phenolic compounds depends upon PAL activity (Graham and Graham, 1991), increase phenolic synthesis in infected Rapeseed (mustard) plants may be due to increased activity of PAL.

The present study reveals the accumulation of polyphenol in the fungal elicited plants. The aim of present study was to compare the level of resistance in two varieties of rapeseed. Based on finding it was found that both the varieties showed resistance against infection with *A.brassicicola*. Bio-902 was found somewhat more resistant than PCR-15. Plants respond with an increase in polyphenol contents accompanied by rise in PAL activity.

Aforementioned facts, though present a biochemical aspect of the picture, in to, still a lot is to be done from the point of view of genetic engineering i.e. to produce "disease resistant" transgenic plant varieties.

Table 1: Quantitative changes in Polyphenol content in excised leaves (*in vitro*) of 2 d old plant of different varieties of *Brassica campestris* after infection with *Alternaria brassicicola*.

Variety	Hrs. after inoculation	Polyphenol content A725/gfw		% change
		Normal	Infected	
BIO-902	0	0.60	0.60	-
	4	0.71	0.78	9.8
	24	0.67	0.95	42.5
	48	0.77	0.87	12.9
	72	0.51	0.55	4.0
PCR-15	0	0.75	0.79	5.3
	4	0.88	1.07	22.18
	24	0.85	0.89	4.7
	48	1.01	1.04	3.8
	72	0.90	0.90	-

Table 2: Quantitative changes in Polyphenol content in intact leaves (in vivo) of 20 d old plant of different varieties of *Brassica campestris* after infection with *Alternaria brassicicola*.

Variety	Hrs. after inoculation	Polyphenol content A725/gfw		% change
		Normal	Infected	
BIO-902	0	0.58	0.62	6.8
	4	0.71	0.79	11.2
	24	0.62	0.92	48.3
	…..	……..	……..	……
	72	0.79	0.81	2.5
PCR-15	0	0.39	0.40	2.5
	4	0.48	0.66	37.05
	24	0.36	0.42	16.66
	……..	…….	…….	……
	72	0.78	0.81	2.5

Table 3: Quantitative changes in Polyphenol content in excised leaves (*in vitro*) of 25 d old plant of different varieties of *Brassica campestris* after infection with *Alternaria brassicicola*.

Variety	Hrs. after inoculation	Polyphenol content A 725/gfw		% change
		Normal	Infected	
BIO-902	0	1.06	1.08	1.9
	4	1.22	1.37	7.6
	24	1.29	1.72	33.3
	48	1.33	1.43	9.3
	72	1.24	1.27	2.4
PCR-15	0	1.03	1.04	-
	4	1.09	1.32	21.1
	24	0.98	1.10	12.24
	48	1.13	1.17	3.53
	72	1.20	1.22	1.67

Table 4: Quantitative changes in Polyphenol content in intact leaves (in vivo) of 25 d old plant of different varieties of *Brassica campestris* after infection with *Alternaria brassicicola*.

Variety	Hrs. after inoculation	Polyphenol content A725/gfw		% change
		Normal	Infected	
BIO-902	0	0.96	0.99	3.1
	4	1.04	1.13	8.75
	24	1.33	1.88	41.72
	72	1.22	1.28	5.4
PCR-15	0	1.00	1.02	2.4
	4	0.83	1.08	30.12
	24	1.08	1.17	8.33
	……	……	……	……
	72	0.93	0.96	3.4

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