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Anatomy of three *Piper nigrum* genotypes with respect to *Phytophthora* foot rot disease

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ABSTRACT

Foot rot caused by *phytophthora capsici* is the most serious diseases of black pepper. Though all varieties of black pepper are susceptible to this pathogen, variation do exist concering the degree of tolerance and mechanisms of defense. Anatomical features such as thicker epidermis, smaller no: of epidermal appendages, smaller cortical cells, smaller stele, thicker pericycle cells and smaller vascular bundles and compact arrangement of cells can be attributed to immunity of II -SR Shakthi to *phytophthora capsici* as compared to kalluvally. Observed anatomical differencess between the 3 genotypes can be used for selecting parents for disease resistant breeding .

Key words: Anatomy, *piper nigrum, phytophthora capsici,* Disease Resistance.

INTRODUCTION

Black Pepper is affected by several diseases caused by Fungi, Bacteria, Virus and Mycoplasma, besides nutritional disorder. Crop losses due to diseases and pests are identified as major causes of low productivity of pepper in India (Sarma and Anandraj 1995). The earlier record of diseases of pepper in India was that of Barber (1903, 1905). Butler (1906) also recorded the death of pepper and Rao (1929) isolated phytophthora from diseased pepper *Phytophthora capsici* occurs on all parts of the plant and cause severe economic damage. The symptoms expression depends upon the site of infection and extend of damage (Mammooty 1978, Anandraj and Sarma, 1995). Aerial infection occurs on the summer shoots, foliage, spikes and branches causing blight, spike shedding, defoliation and die back and at times death of the plants. Infection on the runner shoots often reach the collar causing foot rot. There is no effective control medicine to combat this malady.However, IISR Shakthi (Released from IISR, Calicut) is reported to be resistant to phythophthora capsici while black pepper cultivars panniyur-1 and kaluvally reported to be susceptible & tolerant respectively to the disease (Kuch & Khew , 1982 and Sharma Nambiar ,1982) Hence, the present study was aimed at analyzing anatomical basis of disease resistant among susceptible,tolerant and resistant genotypes of black pepper to *phytophthora capsici*.

MATERIAL AND METHODS

The plant specimens for the proposed study (IISR Shakthi, Panniyur -1, Kalluvally) were collected from IISR Experimental Farm, peruvanamuzhi, Calicut, Kerala. Three varieties of P. nigrum VIZ, Panniyur-1 (susceptible) Kalluvally (

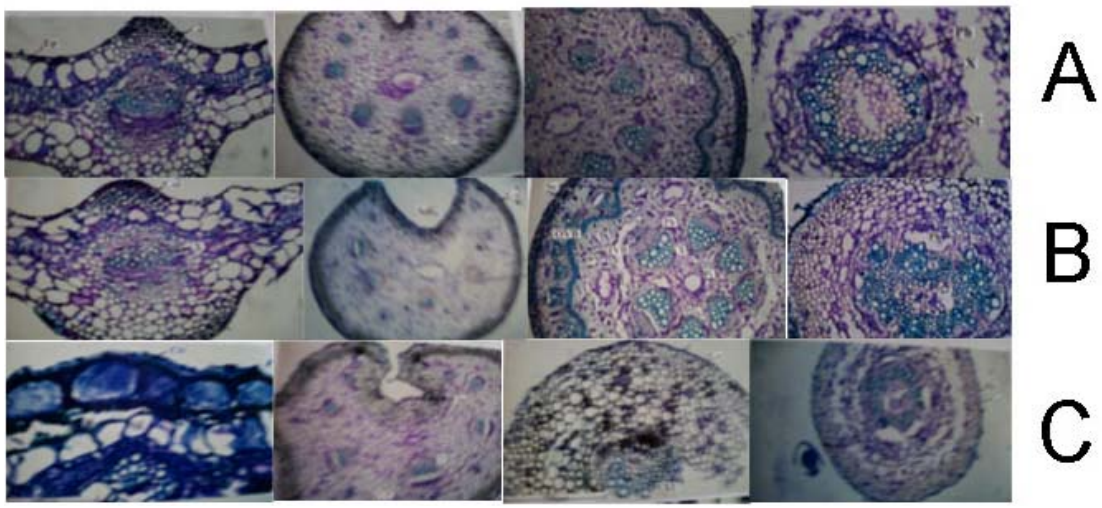
Tolerant) I I SR Shakthi (Resistant) were selected for the study. Rooted cuttings grown in polybags under green house condition (4 month old) were used. Pure culture of *P. Capsici* isolated from infected black pepper leaves were inoculated on carrot agar medium in petriplates and incubated at 27^0 C for optimum growth. Seven day old cultures having profuse growth were used for inoculation . Culture discs of uniform size (5mm dia) were applied on the ventral side of the leaf lamina, stem, petiole after making pin pricks (10 no:s) at the points of contacts. The Mycellium was crushed with the mortar and pestle and was inoculated into soil. The Resistant, susceptible,Infected plant materials were

Resistant variety tolerant variety

	Parts	**Resistant Variety**		**Tolerant Variety**
I.	**LEAF**	Thickness of the midrib	650µm thick	720 µm thick
		Cuticle of the midrib (adaxial side)	5 µm thick	very thin
		Cuticle of the abaxial side	8 µm thick	very thin
		Adaxial epidemis thicknees	12µm thick	20 µm
		Abaxialepidemis thickness.	10 µm thick	7 µm thick
		Leaf palisade tissue height	30 µm	20 µm
		Palisade cells	Compact	loosely arranged
		Spongy mesophyll tissue	2.3 layered	layered
		Spongy mesophyll tissue	Compact	loosely arranged
		Ground tissue of the petiole (Angolan, compact, thick walled)	Ground tissue with circular cells, less compact & this walled.	
		Sclerenchymatous bundle laps wide & thick walled	Bundle caps- narrow and thin walled	

STEM		
Epidermal layer – 25 µm thick & cuticle – 8 µm thick		Epidermal layer 20 µm thick cuticle – 5 µm thick.
Hypodermal scteremchyma.		Hypodermal sclerenchyma cylinder – 90 µm thick, cells wide, walls loss thick.
Cyliader – 100 µm thick; cells thick walled with narrow lumen.		
Corticl zone narrow – 50 µm wide, with small, compact cells.		Cortical zone – 100 µm wide, cortical cells wide & thin walled.
Outer vascular bundles more in no:, large in size (24 bundles; 150 x 250 xylem –		Outer vascular bundles less in no: (about 15 bundles) smaller in size (100 x150 µm m)., with parenchymatons cells in the bundle and thin walled vessels.
Sclerenchyma and thick walled vessels.		
Cortical sclerenchymma cylinder 5 or 6 layered, cells with walls		Corticl sclernchyma cylinder 3 layered, cells – thin walled.
Inner (central) vascular bundles larger, 300 x 350 µm thick, vessels 70mm wide,		Central vascular bundles smaller in size, 250 x 350 µm thick, vessels 40 µm wide.
ROOT:-The rhizodermis with subcised outer walls is well preserved; the cortical zone is narrow with fair thick walled compact cells. The stele has wide thick walled Xylem elements as well as thick walled fibres.		The rhizodermis bears close root hairs, the cortex is wide, cells having thin walls and less compact. The stele consists of narrow, thin walled scattered xylem elements & dense xylem fibres.

cut and fixed in FAA.(Formalin 5 ml + Acetic acid 5ml + 70% Ethyl Alcohol – 90ml).After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary -Butyl alcohol as per the schedule given by Sass, (1940). Infiltration of specimens was carried by gradual addition of paraffin wax (melting point (58 – 60^0) until TBA solution attained super saturation. The specimens were cut into paraffin Blocks, which were sectioned with the half of Rotary Microtome. The sections were stained with Toluidine blue as per the method published by O' Brien et al (1964). Microscopic descriptions of tissues are supplemented with Micrographs. Photographs of different Magnification were taken with NIKON LABPHOTO2 Microscopic units. Magnifications of the figures are indicated by scale bars. Descriptive terms of the anatomical features are given in the standard Anatomy Books (Esau, 1964).



Anatomy of A (Tolerant), B (Resistant) C (Infected) Sections of *Piper nigrum*

Plate 1: Comparison of anatomical features of resistant and tolerant variety

Structural changes of the infected organs of pepper

Leaf

The infected leaf exhibits thick continuous layers. Cleistocarps, surrounded all around by thick dark covering. The cleistocarps are in different stages of development, they are on the adaxial surface of the leaf. In some of the first bodies, there is an apical pole through which conidia are likely to be released. This conidiophores are thicker and dense. The conidia are elliptical and elongated. The subepidermal cells break from the epidemis and become crumbled. The mesophyll tissues are also distorted due to the formation of the first bodies on the epidemis.

Petiole

The infection occurs in the adaxial groove of the petiole. The lateral borders of the groove are wounded. The cells along the borders undergo wound healing process by producing this zone of small suberised cells. This infection seems to penetrate the cells along the concavity so that some of the cells are crushed and others become dark and compressed. Since the infected is in initial stage, interior tissue and vascular strands are not altered significantly.

Stem

In a young stem, the critical zone consists of only parenchymatous tissue and no sclerenchyma cylinder is seem. So the inoculated pathogen has spread into wide corticial tissue, which become dark and crushed. This stimulates poliferation and hyperplasia of the inner cortical cells adjacent to vascular bundles.

In the old stem, where there is a cortical cylinder of sclerenchyma cells, the entry of the pathogen occurs by breaking sclerenchyma cylinder and later infection spreads to the outer cortical tissues. The fungal pathogen seems to be intra cellular. The fungal organism is found occluding the cell cavity of the cortex.

During advanced stage of infection, the critical paramchyma cells on the pith, in the medullary rays, cortex and within the vascular bundles get collapsed into dark amorphous structures. However, there is no evidence of presence of fungal pathogen to the xylem elements of sclerenchyma cells.

Roots

In the root entry of the pathogen is through an injury made in the cortical region. It spreads in the cortical cells and then enters into the stele. More and more cortical cells become invaded by the pathogen and the cells are turned into dark structure. The most obvious effects of the pathogen is manifested in the xylem elements. The xylem elements are occluded by dark, amorphous substances which evidently blocks the flow of water to the aerial system of the plant.

CONCLUSION

The comparative account of the anatomical features of resistant variety and susceptible variety reveals certain structural differences between these two. Previous studies by Marks and Mitchell (1971), Miller and Maxwell (1984), Philips etal (1987), Hamalavi etal (1995), and Markose (1996), on various host plant infested by phytophthora have established that thicker epldermis, smaller no: of epidermal appendages, smaller cortical cells, smaller stele, thicker pericyclic cells, vascular bundles and compact arrangement of cells can be attributed to phytophthora infection.

In the present study we have obtained results similar to those mentioned above in the resistant variety of *piper nigrum* as compared to susceptible variety . physical barriers found in the resistant variety seams to play a pivotal role in resisting fungal infestation. That it can be concluded that anatomical differences observed in 3 *piper nigrum* genotypes can be used for screening various genotypes against tolerance / resistant to *phytophthora capsici* The selection of tolerant / immune genotype via screening will be helpful to evolve resistant / tolerant plant and also on the selection of plants for further breeding.

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REFERENCES

1. Anandaraj M. Sharma, Y.R., Diseases of black Pepper (*Piper* nigrum.L.) and their management. J. *Spices and Aromatic Crops*, **4**: 17-23 (1995).
2. Barber. C.A., Pepper diseases in the Waynad. *Trop. Agric.*, (Colombo) **22**: 805-807 (1903).
3. Bulter. R.J. (1906) The wilt disease of Pigeon pea and Pepper. *Agric. J. India,* 1,25-26.
4. Easu. K., *Plant Anatomy* John Wiley and Sons. New York., 767. Easu. K., (1979). *Anatomy of seed Plants*. John Wiley and Sons. New York (1964).
5. Hamalawl. Z.A., J.A. Menge and F. B. Guillemot., Infection court and factors affecting the expansion of stem canker of avocado caused by *Phytophthora citracola*. Pl. Dis. **79**: 384-88 (1995).
6. Kueh. T.K. and K.L. Khew., A screening technique useful in selecting for resistance to black pepper *Pyytophthora palmivora. Malayasian Agri.* J. **54**: 39-45 (1980).
7. Markose. B.L., Genetic and blochemical bases of resistance to bacterial wilt in chilli. Ph. D. (Hort.) thesis, Kerala Agricultural

University, Thrissur, Kerala (1996).

8. Marks. G.C., and J.E. Mitchell., Penetration and infection of alfalfa roots by *Phytophthora* megasperma and pathological anatomy of Infected roots. *Canadian. J. Bot*. **49**: 63-67 (1971).
9. Miller. S.A. and D.P. Maxwell., Light microscopic observations of susceptiable, host resistant and nonhost resistant interactions of alfalfa with *Phytophthora megasporma. Canadian J. Bot* **62**: 109-116 (1984).
10. Mammootty. K.P., Quick wilt diseases of pepper (*Piper* nigrum Linn.) -1. *Symptomatological studies on the quick wilt diseases of pepper.* M.Sc. (Agri,) Thesis, Kerala Agri. University, 87 (1978).
11. O'Brien. T.P., Feder. N. and Mc. Cull. M.E., Polychromatic Staining of Plant Cell walls by toludine blue- O. *Protoplasma;* **59**: 364-373 (1964).
12. Phillips. D. B.R. Grant and G. Weste., Histological changes in the roots of an avocado cultivar Duke-7. Infected with *Phytophthora cinnamont.* Phytopathology. **77**: 691-698 (1987).
13. Rao. V.M.K., *Annual Report* for 1927-28, Department of Agri., Mysore 19 (1929).
14. Sharma. Y.R. and K.K.N. Nambiar., Foot rot disease of black pepper (*Piper nigrum* L.) Proceedings of the Workshop on *Phytophthora Diseases of Tropical Cultivated Plants.* (Nambiar. K.K.N.) CPCRI, Kasargode, Kerala, 209-224 (1982).