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RESPONSE OF FOUR CHICKPEA VARIETIES AGAINST *ASCOCHYTA* BLIGHT AND THEIR HISTOLOGICAL STUDIES

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Abstract

Artificial inoculation with a spore suspension of *Ascochyta rabiei* showed highest leaf and branch infection in Aug424 followed by Pb-1 with maximum resistance in ILC191 followed by CM72. Histological studies showed that thickness of stem epidermis was significantly higher in ILC191. Thickness of stem hypodermis was also greater in ILC191 and CM72 as compared to Pb-1 and Aug424, with minimum thickness of cortical region in Aug 424. The fungus caused severe damage to different tissues of Aug424 and Pb-1 soon after infection as compared to CM72 and ILC191.

Introduction

Chickpea (*Cicer arietinum* L.) grown over 10.4 million hectares with an annual production about 6.8 million tones is an important legume crop of dryland agriculture in Asia, Africa, Central and South America (Anon., 1982). In Pakistan it is cultivated over 1 m hectares with an average yield of 550 kg/ha (Hafiz, 1986). The main constraint limiting chickpea production is the blight disease caused by *Ascochyta rabiei* (Pass) Lab. The disease was first reported in the Indo-Pak subcontinent in 1911 in Attock district and caused serious losses to crop (Butler *et al.*, 1918). Since then the disease has been appearing in alarming proportions in North Western Parts of the country (Nene, 1982).

Various chickpea cultivars have shown varying degree of resistance to Ascochyta blight which necessitates to examine the host parasite interaction and mechanism involved in resistance or susceptibility of chickpea. Histological studies of different crop plants have shown the presence of inhibitory substances on the cuticle (Roberts *et al.*, 1961), thickness of the cuticle and epidermal cell wall (Mence & Hildebrandt, 1961) which might influence germination and/or haustoria formation.

Studies were therefore carried out to examine the anatomical differences and the extent of damage caused by the pathogen in chickpea cultivars possessing variable degree of resistance to Ascochyta blight.

Materials and Methods

Seeds of 4 chickpea cultivars viz., Aug 424, Pb-1, CM72 and ILC191 after seed dressing with Benlate were sown in 12" diam., earthen pots filled with canal sediment. Plants were exposed to natural environmental conditions and irrigated on alternate days. A virulent strain of *Ascochyta rabiei* obtained from chickpea pathology group at NIAB

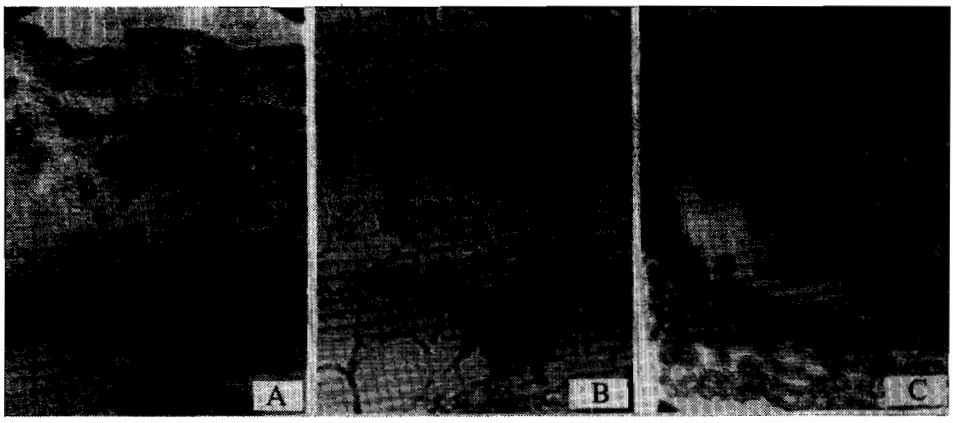

Fig.1. Transverse sections of stem of chickpea variety Aug424 showing the establishment of fungus (Black Stained) in different host tissues (x 160).
A) Control, B) 3 days after inoculation, C) 10 days after inoculation.

was multiplied on chickpea seed meal agar medium. Three month old plants were inoculated with spore suspension ($4.8x10^6$ cfu/ml) from 10 days old culture using a handsprayer to the point of run off. Uninoculated plants served as control. The plants were kept in chambers at 95% R.H. to ensure good infection. The pots were removed after 72 h from the chambers and sprinkled with water three times a day for a few days to maintain humidity. Stem sample from about 2" below the top were collected at 1,2,3,4,6,8,10,12 and 14 days post inoculation period from uninoculated and inoculated branches and preserved in FAA (Ethyl alcohol 95%, 50 ml; glacial acetic acid, 5 ml;

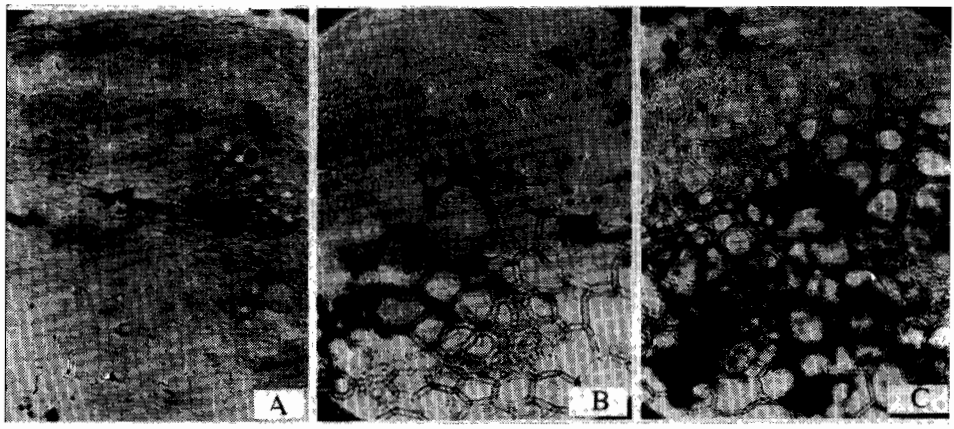

Fig.2. Transverse sections of stem of chickpea variety Pb-1, showing the destruction of host tissues by fungal invasion which is maximum 10 days after inoculation. (x 160).
A) Control, B) 3 days after inoculation, C) 10 days after inoculation.

Table 1. Reaction of chickpea cultivars to infection by *Ascochyta rabiei*.

Varieties	infection		Thickness of steam		
	Leaf	Branch	Epidermis	hypodermis	cortical region
AUG424	93.81a	97.57a	8.81b	19.98b	61.30c
Pb-1	65.49b	89.46b	8.81b	19.58b	75.71b
CM72	40.33c	45.22c	9.20ab	34.45a	98.31a
ILC191	26.84d	46.02c	9.79a	34.47a	73.24b

Means not sharing a letter in common differ significantly as rated by DMRT.

Formaldehyde 37-40%, 10 ml; water, 35 ml) until processed for preparing blocks for microtomy and anatomical studies. Data for leaf and branch infection was collected 2 weeks after inoculation. Transverse sections 10 μm thick were stained with Iron hematoxylin following the procedure described in Botanical Microtechnique (SASS, 1958) and examined under a light microscope.

Results and Discussion

Highest leaf and branch infection (Table 1) observed in chickpea cv. Aug.424 indicated its greater susceptibility to Ascochyta blight. Pb-1 closely resembled Aug424 both in leaf and branch infection. Maximum resistance to leaf infection was observed in ILC191, where 27% leaves were infected. The two resistant cultivars viz., ILC191 and CM72 had non-significant differences in branch infection. Varying degree of leaf infection in chickpea might be due to secretion of more malic acid by resistant cultivars than the susceptible cultivars (Hafiz, 1952). Greater infection on branches than on leaf may be attributed to more phenolic synthesis in leaves than branches (Vir & Grewal, 1974).

Highest thickness of stem epidermis (Table 1) was observed in ILC191 followed by CM72. ILC191 showed significant differences ($p<0.05$) from Aug.424 and Pb-1 and was almost similar to CM72. Thickness of stem hypodermis (Table 1) was more ($p<0.01$) in both the resistant cultivars (ILC191 and CM72) than susceptible cultivars (Aug424 and Pb-1). Thickness of stem hypodermis may contribute to host resistance by protecting the cortical and vascular tissues from fungal attack by providing a shielding effect. Highest thickness of cortical region (Table 1) was observed in CM72, which was significantly different ($p<0.01$) from other cultivars, while Aug424, the most susceptible cultivar showed a small cortical region.

There are reports where conidia of *A. rabiei* penetrate the host tissue at the juncture of epidermal cells rather than stomata (Pandey *et al.*, 1987). Advancement of the fungus and consequent tissue damage varied in different cultivars indicating their variable resistance to fungal attack. Aug424 was found to be most susceptible and showed greater damage of cortical and pith tissues (Fig.1) at 3 days post inoculation (dpi) period. Some of the vascular bundles were also damaged at later stages of

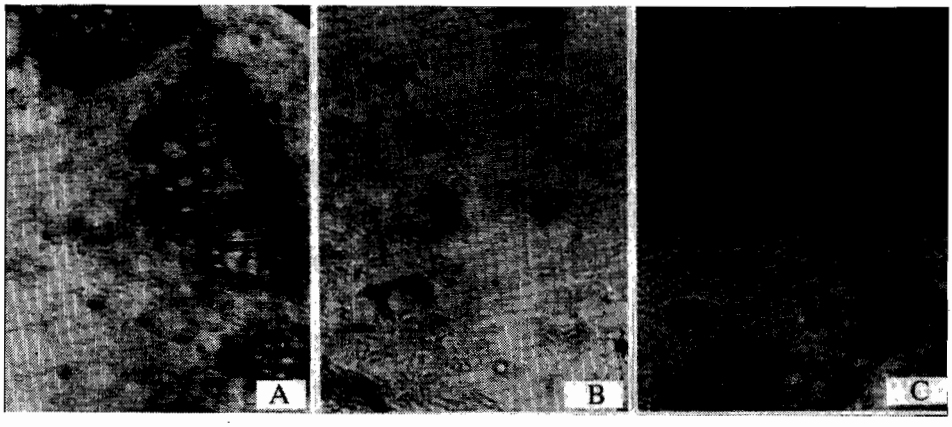

Fig.3. Transverse sections of stem of chickpea variety CM72. The fungus caused less damage as compared to susceptible varieties i.e. Aug424 and Pb-1. (x 160).
A) Control, B) One week after inoculation, C) Two weeks after inoculation.

infection. A similar pattern of fungal infection was observed in Pb-1 (Fig.2). Disease symptoms in both the resistant cultivars (Fig.3 & 4) appeared later than in susceptible cultivars. Fungal invasion and establishment in cortical and underlying tissues was observed at 7 dpi. Tissue damage was less as compared to susceptible cultivars. However, pith and cortical tissues were heavily damaged at 12-14 dpi. Lignified tissues were damaged comparatively to a less extent.

Resistance to fungal invasion may be explained on some structural and biochemical basis such as phenolic content and structural defensive barriers that is papillae and

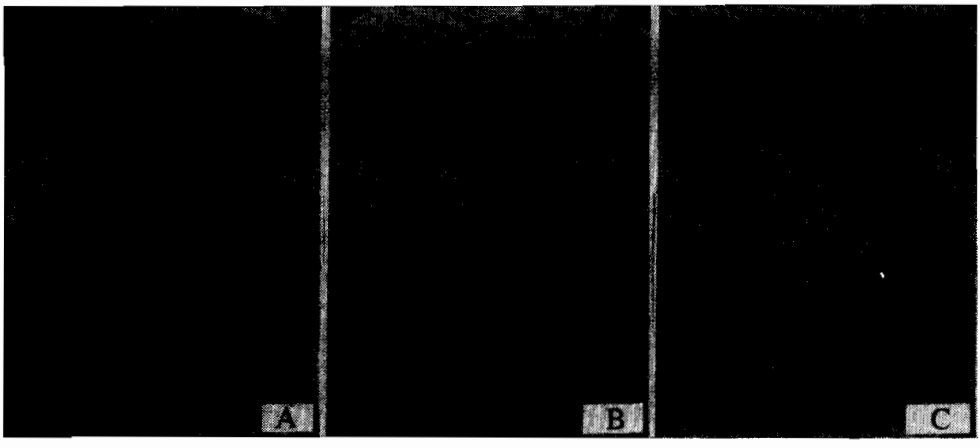

Fig.4. Transverse sections of chickpea variety ILC191. The fungus caused minimum damage as compared to other varieties. (x 160).
A) Control, B) One week after inoculation, C) Two weeks after inoculation.

modified cortical cell walls (Brammall & Higgins, 1988). Thickness of cuticle and cell wall, lignification or suberization of cell walls, constitutive toxic compounds and induced antimicrobial substances, especially phtoalexins might constitute the structural and biochemical basis of plant resistance (Kyostio & Sirkka. 1988).

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