

HISTOCHEMICAL ALTERATIONS IN CLUSTER BEAN SEEDS INFECTED WITH *COLLETOTRICHUM DEMATIUM* AND *FUSARIUM OXYSPORUM*

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Infection of *Colletotrichum dematium* and *Fusarium oxysporum* affected important seed reserves in cluster bean. Histochemical changes revealed a decrease in protein and gum content; increase in lipids and tannins while starch and insoluble polysaccharides could not be localized. The comparative study showed that *F. oxysporum* is more aggressive than *C. dematium* and caused more deterioration.

Keywords : Cluster bean; *Colletotrichum*; *Fusarium*; Histochemistry.

Introduction

Cluster bean or guar (*Cyamopsis tetragonoloba*) is an important legume crop grown mainly for vegetable, fodder and industrial purposes. It is a rich source of protein and galactomannose gum, latter being utilized in various industrial processes.

Colletotrichum dematium and *Fusarium oxysporum* are two important pathogens associated with guar seeds¹. Changes in primary metabolites and deterioration in seed quality by associated seed-borne mycoflora has been reported in other legumes viz. groundnut, soybean and pigeon pea seeds²⁻⁴. But there is no such information available on guar seeds hence the study was undertaken.

Materials and Methods

Seeds categorised into asymptomatic and symptomatic; fixed in 70% alcohol, dehydrated through TBA series and embedded in paraffin wax. Sections were cut at 9-10 μ . For histochemical localization, standard methods described for proteins⁵, lipids⁶, starch⁷, insoluble polysaccharides⁸, tannins⁹ and gum¹⁰ were followed.

Results and Discussion

Histopathology of cluster bean seeds infected individually by *C. dematium* and *F. oxysporum*

indicated that infection of these two pathogens is confined to seed coat (extraembryal) in asymptomatic while invaded all the seed components including embryo (intraembryal) in symptomatic seeds. The cells of embryo underwent lysis and showed signs of stress¹¹.

The changes in various reserves of guar seed caused by seed-borne infection of both fungi were assessed qualitatively by histochemical techniques. Protein is an important constituent of guar seed. Its localization was maximum in outer endosperm, cotyledons and embryal axis in the normal asymptomatic seeds. The cells contained well formed, prominent protein bodies giving a positive stain reaction (Fig.1). The intensity of stain was very high in asymptomatic seeds whereas in infected seeds weak to negative stain reaction was observed thus revealing loss in protein content (Fig.2). In the portion of cotyledons with maximum infection towards distal end of seed, the cells were vacuolated and completely unstained with no protein bodies (Fig.3). Vijayakumar¹² also noticed distorted and less densely stained proteinoplasts in groundnut leaves infected by *Cercospora arachidicola*. In the seed infected by *F. oxysporum*, intensity of stain



Fig. 1. Part of cotyledons of asymptomatic seed showing protein bodies in palisade cells X 250.

Fig. 2. Symptomatic seed showing vacuolated cells X 250.

Fig. 3. Distal end of cotyledons showing weakly stained cells X 125.

was much lower than that of seed infected by *C. dematium* thus indicating higher loss due to the former.

Lipids were localized as red globules in cells of cotyledons and embryal axis of the infected seeds whereas tissues of asymptomatic seeds remained completely unstained thus showing an increase in total lipids after infection. Mycelium of both fungi and stromata of *C. dematium* also gave positive reaction for lipids. This suggests that increase in oil could be due to the synthesis of lipid in fungal propagules as also reported by Shivpuri *et al.*¹³ in mustard seeds. The intensity of stain was higher in tissues of seed infected by *F. oxysporum*.

The present study indicated a negative reaction for starch both in asymptomatic and symptomatic seed tissues revealing its insignificant presence or absence in guar seed. Similar conclusions were drawn for total insoluble polysaccharides.

Gum content, estimated in form of soluble sugars decreased due to infection of *F. oxysporum*. It is stored in endosperm as galactomannose and a decrease in its content may decrease amount of total soluble sugars thus affecting the seed quality. An increase in reducing sugars due to several fungi including *F. oxysporum* was reported².

The infected seeds contained higher amount of tannins in comparison to asymptomatic seeds. The maximum stain reaction (bright red colour) was observed in counter palisade of hilum in seed coat. The presence of tannin contents has been correlated with degree of resistance of host¹⁴.

Present study gives ample proof that infection of *C. dematium* and *F. oxysporum* considerably affects the important seed reserves resulting into poor seed quality. These changes were conspicuous in seeds infected by *F. oxysporum* in comparison to *C. dematium* infected and asymptomatic seeds hence such seeds should be abandoned from seed lots.

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