Received: Jun '23
Revised:
Accepted: Jul '23

© 2023 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

HISTOCHEMICAL STUDIES OF BLACK GRAM GROWN IN TRIBAL BELT OF RAJASTHAN

Jagat Pal Singh[1], Dr. Ram Pal Ahrodia[2]* and Dr. Mahendra Kumar[3]
[1]Professor in Botany, Raj Rishi Govt. College Alwar (Raj.)
[2]Asstt. Prof in Botany, Govt. Nehru Memorial College, Hanumangarh (Raj)
[3]Asstt. Prof in Botany, Govt. Science College, Sikar (Raj)
E-mail: [2]rpahrodia1@gmail.com

ABSTRACT

Vigna is one of the important genera growing among the pulse, forming a major part of the Indian people's diet. Three field surveys were carried out covering all the major crop-growing districts of Rajasthan state. Histochemical studies of fungal-infected seeds were undertaken.

Keywords: - Field survey, Histochemistry, seed-borne pathogen.

INTRODUCTION

Pulses constitute important proteinaceous crops. Several Seed-borne fungi are known to cause considerable loss in the seed content. Bilgrami *et al*. (1976) studied the deterioration of black gram seed in storage conditions by *Aspergillus flavus*. They recorded loss in sugars and organic acid contents but a few amino acids and organic acids were found to be synthesized by *A. flavus*. Deterioration of proteins, total sugars and protein contents was also noted in black gram seeds during infestation by *A. flavus* in thirty-eight cultivators of pulse crops by Premlata Singh, Sita Bhagat and Ahmed (1990). Ibraheem, Okesha and Mhathem (1987) reported a marked decrease in the protein contents of soybean seeds by associated seed-borne fungi. Contrary to this, an increase in protein contents in rice seeds due to helminthosporiose infection was reported by Vidhyasekaran *et al.* (1973). Shukla *et al.* (1988) recorded a decrease in protein contents of Arhar seeds infested with different *Aspergilli*. The amount differed from species to species. *A. flavus* and *A. niger* brought maximum and *A. awamori* and *A. nidulans* minimum alterations in protein contents.

Black gram contains 59.6% carbohydrates. Bilgrami *et al*. (1976) recorded the disappearance of sugars within 5 days of the incubation period. But it again appeared on the 10th and 15th days of incubation. Vidhyasekaran and Govindaswamy (1968) also observed the accumulation of reducing sugars due to seed-borne fungi in paddy seeds. Changes in starch contents of Arhar seeds by fungal infection were also studied by Sinha *et al*. (1981). Maheshwari *et al*. (1984) observed depletion in starch contents of *Coriandrum sativum* infected with *Protomyces macrosporus*.

Faulty storage conditions make the seeds more vulnerable to fungal attacks. Several storage fungi produce aflatoxins which cause great health hazards in human beings. Aflatoxin production in water-soaked black gram seeds by four isolates of *A. flavus* was observed by Reddy and Subbaya (1985). Premlata *et al*. (1990) screened thirty-eight different pulse cultivars against aflatoxin production by *A. flavus*. Two cultivars were found highly resistant against aflatoxin elaboration. They also recorded greater amounts of total phenol and proteins in resistant varieties than susceptible ones of the same pulse crop.

MATERIALS & METHODS

Histochemical methods were applied to study the localisation of various primary constituents and food reserves in the seed. The seeds carrying natural infection of *Rhizoctonia bataticola* and *Fusarium oxysporum* were used for the study. The symptomatic (Heavily infected) and asymptomatic (Healthy) seeds were taken to analyse proteins, lignin and starch. Methods employed for each are dealt with separately.

Total Proteins

Proteins were localised by the mercuric bromophenol blue method (Mazia, Brewer and Alfert, 1953, Ruthmann, 1970, Chapman, 1975).

Preparation of stain

10 mg mercuric chloride and 100 mg bromophenol blue dissolved in 100 ml of distilled water or 95% ethanol.

Procedure

Microtome sections were deparaffinised and passed through a graded series of ethyl alcohol. Sections were stained in the dye solution for 15 min, washed in 0.5% acetic acid for 20 min, further washed in distilled water for 15 min and mounted in glycerine. Protein stain blue.

Starch

Starch was localized by the Iodine-Potassium iodide reaction (Johansen, 1940).

Preparation of stain

2.0 gm. of potassium iodide was dissolved in 100 ml of distilled water and 0.2 gm. of iodine was then added to it.

Procedure

Fresh hand-cut sections were immersed in the prepared solution for 5 min. and mounted in the same.

Starch grains stain blue-black.

Lignin

Lignin was localized by the phloroglucinol-hydrochloric acid test (Purvis, Collier and Walls, 1964).

Preparation of stain

1 gm. of phloroglucinol was dissolved in 100 ml. of 95% ethanol.

Procedure

Fresh hand-cut sections were placed in a 1% solution of prepared phloroglucinol for 2 min. and then treated with hydrochloric acid. The sections were mounted in weak glycerine.

Lignin stains green blue.

RESULTS

Total soluble proteins

Normal seed

The localization of proteins was seen in the aleurone layer and embryo and the maximum stain was seen in cotyledons and embryonal axis. It was not observed in cells of the seed coat (Palisade, hourglass and parenchyma). The cells of cotyledons contained numerous protein bodies arranged compactly and uniform intensity of stain through the tissue. Blue stain in cells of the vascular strand of cotyledons was weaker than other cells.

Infected seeds (Symptomatic)

The cells of cotyledons and embryonal axis showed discrete light or dark-coloured patches. The darkly stained areas contained relatively more protein than the lighter areas. Protein bodies in lighter regions of cotyledons were loosely arranged and showed depleted cell contents. The cells also carried the mycelium of *R. bataticola*.

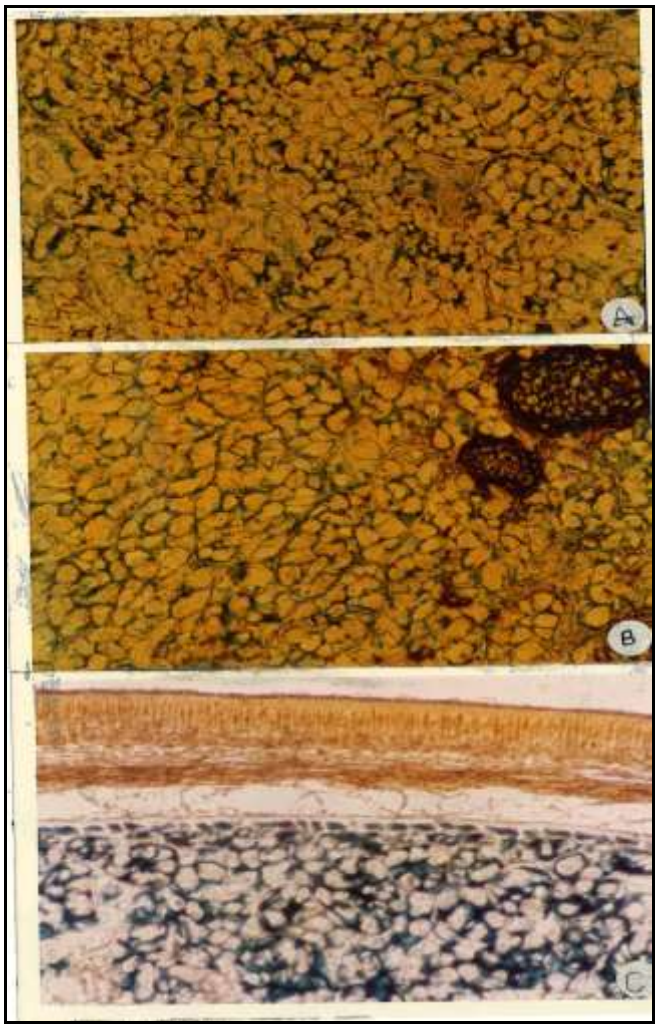
Starch

Normal seeds

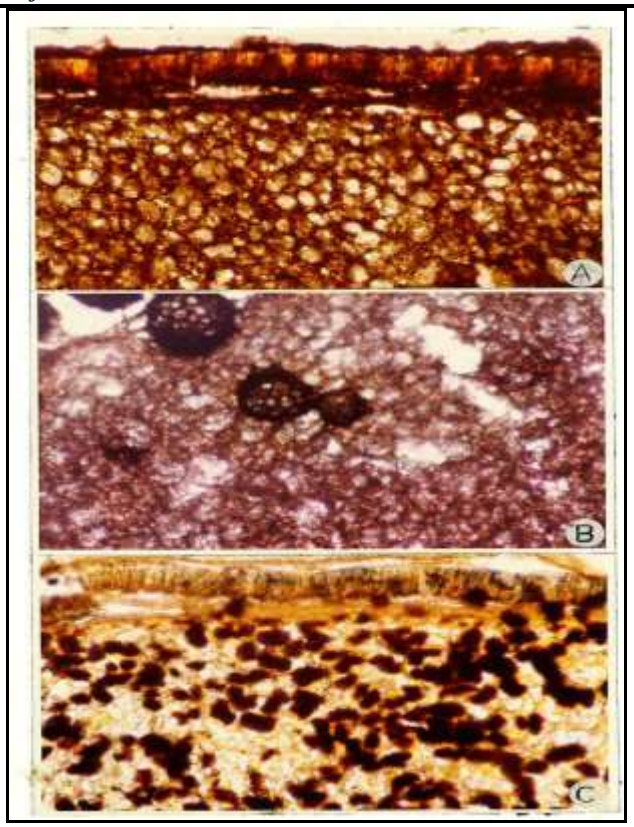
The IKI reaction gave a positive result showing its presence in the cotyledonary cells. The localization of starch grains was maximum in the cells of cotyledons and arranged compactly. Stain reaction was uniform throughout the cotyledons. The average number of starch grains per cell varies from 12-40. All three layers of the seed coat (palisade, hourglass and parenchyma) and aleurone layer showed absence.

Infected seeds

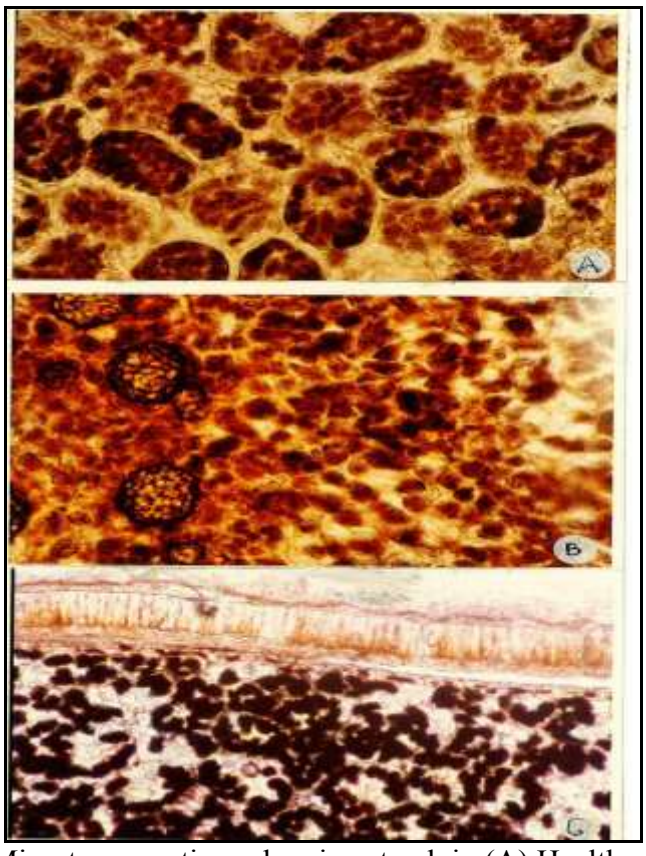
The size of starch grains appeared small, loosely arranged in some places and the cotyledonary cells appeared vacuolated. The average number of starch grains per cell varies from 2-7. The seeds infected with *R. bataticola* and *F. oxysporum* showed almost similar localization of starch in cotyledons.



Microtome sections showing proteins in (A) Healthy Seeds (B) *R. bataticola* infected seeds (C) *F. oxysporum* infected seeds.



Microtome sections showing lignin in (A) Healthy Seeds (B) *R. bataticola* infected seeds (C) *F. oxysporum* infected seeds.



Microtome sections showing starch in (A) Healthy Seeds (B) *R. bataticola* infected seeds (C) *F. oxysporum* infected seeds.

Lignin

Normal seeds

It was tested by the IKI-hydrochloric acid method which showed slight swelling of cell walls indicating its presence in different layers of the seed coat. Cells of cotyledons and embryonal axis showed absence of lignin.

Infected seeds

R. bataticola and *F. oxysporum* infected seeds showed weak staining for lignin, as compared to healthy, in seed coat layers.

REFERENCES

1. Goodmann J J and Christensen C M 1952. Grain storage studies XI. Lypolylitic activities of fungi isolated from stored corn. Cereal chem 29 299-308.
2. Ibraheem S A, Okesha A M and Mhathem K T 1987. Interrelationship between protein and oil content of soybean seed with some associated fungi. J of Aric & Water Resources Res, Pl Production 6 53-66.
3. Reddy M R S and Subbayya J 1981. Mycoflora associated with discoloured shrivelled seed of pant U-30 black gram. Seed & Farms 6 (3) 32-33.
4. Reeve R M 1951. Histochemical tests for polyphenols in plant tissues. Stain Technol 26 91-96.
5. Purvis M J, Collier D C and Walls D 1964. Laboratory techniques in Botany, Butterworths, London.