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Anatomy and Biochemical Study of Collar Rot Resistance in Eggplant

M. M. Hasan[1*] and M. B. Meah[2]

[1]Advanced Seed Research and Biotech Centre, Dhaka, Bangladesh.
[2]IPM Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Bangladesh.

Authors' contributions

This work was carried out in collaboration between both authors. Author MMH designed and performed the study, managed the literature searches and wrote the first draft of the manuscript. Author MBM managed the analysis and supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

Disease reaction of three eggplant varieties (BAUBegun-1, BAUBegun-2 and Dohazari G) to collar rot (*Sclerotium rolfsii*) at early flowering stage, as well as the anatomy and biochemical effects of the infection on the collar region of the plant was studied. The plants were inoculated following soil inoculation technique, using barley culture of the pathogen. All the varieties were infected, with percentage infection ranging from 62.50 to 100%. Varieties varied in percent mortality (0.00 - 100). Plants of the eggplant variety BAUBegun-2, although infected, all regenerated and were graded resistant. The varieties Dohazari G and BAUBegun-1 were graded as susceptible. Anatomy and biochemical constituents, namely total phenols, ascorbic acid, total sugar, reducing sugar and Ca-oxalate contents of the collar region were investigated. BAUBegun-2 was characterized with thick cuticle, thick epidermal cells, many trichomes and smaller intercellular spaces in the cortex which could have restricted the entry of *S. rolfsii* into the cell. A higher level of biochemical activities was observed in eggplant var. BAUBegun-2. There was a clear correlation between anatomical

**Corresponding author: Email: hasan_vi@yahoo.com;*

features and biochemical constituents, and collar rot incidence. Anatomical features and biochemical constituents, as detected to be responsible for the resistance, could be used for the development of superior variety with resistance to collar rot.

Keywords: Eggplant; collar rot; anatomy; biochemical constituents.

1. INTRODUCTION

The eggplant or brinjal (*Solanum melongena* L.), a plant of the family *Solanaceae* and genus *Solanum* is one of the most common, popular and principal vegetable crops grown in Bangladesh and other parts of the world. Due to its low calorific values (24 kcal 100 g^{-1}) and high potassium content (200 mg 100 g^{-1}), it is suitable for diabetes, hypertensive and obese patients [1].

Eggplant is a cheap source of carbohydrate and vitamins [2]. Except peak periods, the market price of eggplant compared to other vegetables remains high which encourages farmers in eggplant cultivation, but there remains a need of better cultivation and information on disease resistant varieties. So there is a need to improve its cultivation to meet the market demands.

In Bangladesh, eggplant suffers from 12 diseases of which collar rot caused by *Sclerotium rolfsii* Sacc. is one of the most important and damaging. The sclerotial bodies of the fungus remain in the dormant condition in the soil or plant debris causing pre and post-emergence death of the plants [3]. Crop loss due to collar rot disease is quite evident.

S. rolfsii is chiefly soil-borne and prefers wet environment, especially water-logged conditions. As the seasons Kharif-I and Kharif-II of Bangladesh are wet and eventually cause water logging in low lying areas, the incidence of collar rot has been found higher in Kharif-II season than in Rabi season [4]. The pathogen *S. rolfsii* inflicts severe damage right from germination till harvest of the crop in all seasons. It colonizes soft tissues and causes rots of tissues adjacent to soil. The pathogen attacks the portion of plant adjacent to soil level termed `collar zone´ causing death by disrupting translocation of nutrients from the top to the root zone [5].

Due to collar rot, the whole plant collapses either at the seedling stage or at the mature stage. In the seedling stage, the death of seedlings can be supplemented by gap filling but in the case of mature plants, this is not possible. As a consequence, collar rot becomes a very destructive disease leading to irreparable loss of the eggplant [5].

The loss caused by this deleterious fungus was reported to be around 60 to 100% and has become a major threat to eggplant cultivation. At present, eggplant cultivation is virtually impossible without pesticide application. Eggplant growers of Bangladesh mostly depend on fungicides to keep crop production steady. Farmers use a wide range of organocarbamate, organophosphorus and synthetic compounds with various spray formulation advocated from time to time against diseases of eggplant [6].

To avoid dependence on chemical control, screening eggplant germplasms resistant to the collar rot pathogen is essential. Information on the genetic variation in respect to disease resistance is of great value in enabling the breeder to use the best genetic stock for improvement in a breeding programme. Continued screening of eggplant cultivars collected from different regions of Bangladesh revealed the existence of resistance against *S. rolfsii* in Integrated Pest Management (IPM) Laboratory with accession number IPM Lab-09 (later designated as BAUBegun 2). Here, the basis of resistance to *S. rolfsii* in BAUBegun 2 was investigated.

Resistance is possible through anatomical structures like parenchyma cells, epidermic layers etc. that regulate the entry of fungi into plant tissue and movement of the fungi inside the host [7]. Anatomical features are built-in structures either pre-existing or self-generated by plants in response to invading pathogens. Biochemical activities regulated by constituents like phenols, ascorbic acid, reducing sugar and Ca-oxalate also may confer resistance to invading pathogens [5]. Identification of eggplant varieties with such characters will be of immense importance to the programme for developing resistance in the host. Very little work has been done in this area in Banglaesh or abroad [5,8]. Therefore, the present research work was designed to investigate the anatomical features and biochemical constituents conferring resistance to collar rot pathogen *S. rolfsii.*

2. MATERIALS AND METHODS

The study was carried out at the Plant Disease Diagnostic Clinic, Field Laboratory of the Department of Plant Pathology and laboratory of the Department of Crop Botany, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh.

The eggplant cultivars used in the experiment were BAUBegun-1 (IPMLab 31), BAUBegun-2 (IPMLab 09) and Dohazari G (IPMLab 12). Seeds of these cultivars were collected from the IPM and Plant Disease Diagnostic Laboratory, the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh.

Tray soil prepared by mixing soil, sand and well-decomposed cow dung (2:1:1) was sterilized with formalin solution (4%) at 200 ml/cf soil following the procedure of Dhasgupta [9]. One hundred seeds were sown in each tray (37 cm X 29 cm).

The experimental plot prepared properly was cleared of weeds and stubble. Well decomposed cow dung was applied and thoroughly mixed with soil. Before final land preparation, inorganic fertilizers and manures were applied on the basis of fertilizer recommendation guide of Anonymous [10]. Twenty four healthy 28 days old seedlings of each variety were planted in the field maintaining plant to plant distance 75 cm and line to line 100 cm. Irrigation and weeding were done as necessary.

The pathogen was isolated from naturally infected eggplant. The infected collar region with pathogenic structures was collected and repeatedly washed in fresh water and surface sterilized with 10% Clorox for 1 minute followed by three washes in distilled water. Inocula were placed on Acidified Potato Dextrose Agar (APDA) and incubated at 22 ± 2°C for 7 days. The fungus was identified following the keys outlined by Aycock [11] and Barnett [12]. The pathogen was purified and multiplied subsequently through (hypal tip) culture on Potato Dextrose Agar (PDA) for preparation of inocula. Inocula of the pathogen, *S. rolfsii* were prepared in barley culture following the procedure of Babar [5].

Six plants were individually inoculated in each plot in two replications by mixing 10 g of infested barley grain with soil near the plant base and covered with moist cotton [5]. Equal numbers of plants were kept uninoculated control varieties. Inoculation was done in the afternoon to avoid drying of the inoculum; cotton was kept moist by adding water as required.

After inoculation, observations were made regularly. Records were maintained on:

I. Number of plants infected of each variety in each replication,
II. Lesion size (in cm),
III. Variety-wise plant reactions as a part of the response to infection e.g., reduction of base diameter,
IV. Number of plants killed (Mortality).

After inoculation, mortality was recorded up to 20 days and expressed in percentage and the data were analyzed following the Completely Randomized Design.

The tested varieties were placed in various categories of resistance and susceptibility on the basis of mortality percentage using the standard rating scale (Table 1) developed by ICRISAT [13].

2.1 Histopathology

Histopathology of the diseased plants of variety BAUBegun-1, BAUBegun-2 and Dohazari G were studied by sectioning (T.S.). Sectioning was done following the procedure of Babar [5]. Microphotographs of diseased plant tissues were taken.

2.2 Biochemical Study

Total phenol, ascorbic acid, total sugar, reducing sugar, Ca-oxalate, moisture and dry matter content of both susceptible and resistant cultivars were measured.

Table 1. Description of the rating scale for scoring

Scale	Mortality (%)	Reaction
1	0	Resistant (R)
2-3	10 or less	Moderately resistant (MR)
4-5	11-20	Tolerant (T)
6-7	21-50	Moderately susceptible (MS)
8-9	51-above	Susceptible (S)

Estimation of total phenol with Folin-Denis reagent is based on the reaction between phenol and an oxidizing agent phosphomolybdate, which results in the formation of a blue complex [14]. The intensity of the coloured complex was measured in a colorimeter. Ascorbic Acid was determined by following a visual titration method based on the reduction of 2, 6-dichlorophenol indophenol dye. Ascorbic acid was extracted as 6% metaphosphoric acid from infected plants and estimated by titrimatric method [15]. Total sugar content was determined by the anthrone method [16]. Reducing sugar content was determined according to the method of Miller [17] where dinitrosalicylic acid was used for the development of colour. The method of Srivastava and Krishanan [18] was adopted for the determination of oxalate content. Moisture and dry matter on leaf and stem of three cultivars of eggplant were determined by the methods described in the Manual of Analysis of Fruit and Vegetable Products by Ranganna [19].

Data collected from both the field and laboratory experiments were subject to F test following computer statistical package MSTAT-C and Statistical Package for Social Sciences and treatment means were compared through Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Development of Collar Rot Symptoms on Inoculated Eggplant

One day after inoculation of the plants with the barley culture of the pathogen, white mycelial growth was observed on soil surface near the plant base. On the second day, a white mycelial mat was formed which advanced rapidly towards the plant base. Immature white rounded sclerotia were also observed on soil surface near the plant base, which gradually turned brown to black and started to germinate producing white mycelia. Symptoms, as expressed by the plants due to collar rot were exhibited through the development of lesions resulting in characteristic collar rot of the plants, including enhanced wilting, yellowing and leaf fall, and ultimately killing off the plants in susceptible varieties, BAUBegun-1 and Dohazari G (Photo. 1. A - C). In variety BAUBegun-2, plant base showed infection, growth of *S. rolfsii* and slight shredding of the tissues (Photo. 1. D). However, the variety of BAUBegun-2 remained green and produced flowers and fruits as normal.

3.2 Effect of Collar Rot Infection in Eggplant

All the tested varieties were infected within 3-4 days of inoculation of the plants with the pathogen. Percent plant infections among the varieties were not significantly different. Variety BAUBegun-2 had 62.50 percent plant infection, the lowest (Table 2). Uninoculated plants did not develop any infection.

The collar rot symptoms were manifested by lesion developed at collar zone of eggplant. The lesion size was significantly different among the varieties. The variety Dohazari-G produced the largest average size lesions at 2.35 cm and BAUBegun-1 produced 1.92 cm lesions that indicated their susceptibility to collar rot disease. The variety BAUBegun-2 produced the smallest lesion which was 1.19 cm that indicated its resistance to collar rot disease (Table 2).

Base diameters of tested varieties were reduced due to collar rot as compared with uninoculated plants. Percent reduction in base diameter differed significantly among the varieties. The highest reduction in base diameter was recorded 12.43% in the variety BAUBegun-1 and lowest in the variety BAUBegun-2 which was 8.43% (Table 2).

None of BAUBegun-2 plants was killed while all plants of the varieties Dohazari G and BAUBegun-1 were killed. Thus the variety BAUBegun-2 was graded resistant and the other two varieties were graded susceptible (Table 2).

3.3 Histopathology of Collar Rot of Eggplant

Microscopic observation of the cross section of affected collar zone of eggplant revealed that the mycelia of the pathogen on reaching the collar zone of the plants aggregated and settled on cuticular surfaces, adjacent to soil surface from where penetration of cuticles occurred, followed by infection (Photo. 2. A, A'). Cuticles were discolored, and then rotting started with longitudinal expansion towards the upper region along the stem as well as circular progress around the base. From observation, it obviously appeared that the pathogen first multiplied and increased inoculum density through the formation and subsequent germination of sclerotia in order to increase inoculum potential for successful infection of the host.

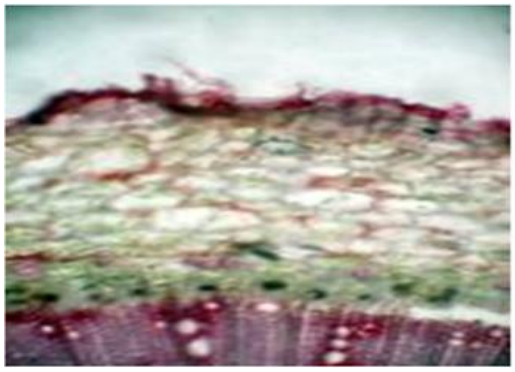
Photograph 1. (A-D). Development of collar rots in eggplant

A. Typical collar rot symptoms with sclerotia at the base of the eggplant
B. Infected eggplant showing wilting symptom (BAUBegun 1)
C. Dohazari
D. Collar rot infected eggplant variety BAUBegun-2 plant remained green after Infection

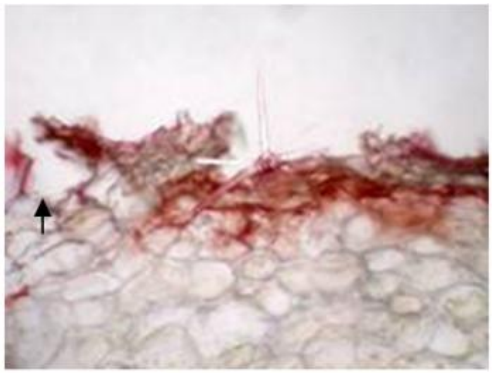
Table 2. Disease reaction of three eggplant varieties against *S. rolfsii* causing collar rot

Sl. no.	Accession no.	Eggplant varieties	% plant infection	Lesion size (cm)	% Reduction in base diameter	Percent plant mortality	Varietal reaction
1	IPM lab-31	BAUBegun-1	100.00	1.92	12.43	100.00	S
2	IPM lab-09	BAUBegun-2	62.50	1.19	8.43	00.00	R
3	IPM lab-12	Dohazari G	100.00	2.35	11.18	100.00	S
LSD (P=0.01)			ND	0.32	1.81	ND	

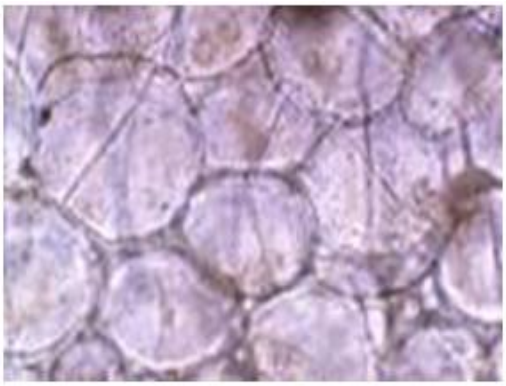
Number of plants counted is 24 in each variety. ND= Not done, S = Susceptible, R =Resistant



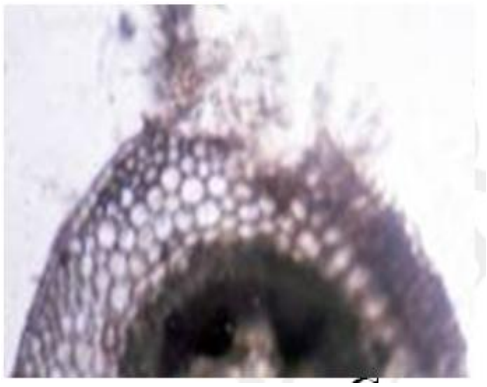
A



A'



B

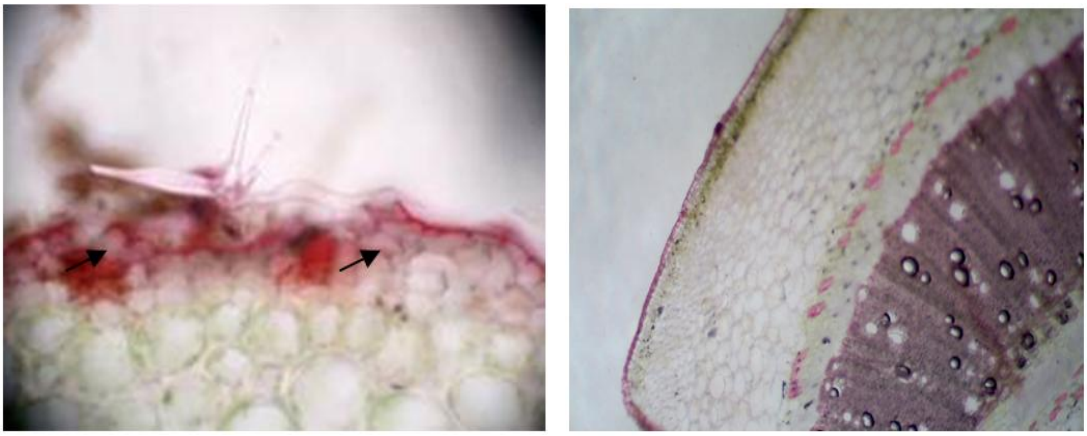


C

D

Photograph 2. (A-D). Transverse section of the infected basal stem of eggplant cvs. BAUBegun-1 and Dohazari G

A. Mycelial aggregation on cuticular surface of the host, Dohazari G
A'. Mycelial aggregation on cuticular surface of the host, BAUBegun-1. Arrow shows epidermal cells loosely attached
B. Spreading of mycelia in between and within the cells
C. Disintegration of Cell wall, degradation of cell substances and disorganization of cortical cell layers
D. An irregular rotting of cells occurred in vascular zone and the pathogen did not enter into the xylem vessels of BAUBegun-1



A B

Photograph 3. Transverse section of the basal stem of eggplant cv. BAUBegun-2 A. infected and B. non-infected. Arrow shows epidermal cells compact

After penetration, pathogen spread occurred both in between (inter-cellular) and within (intra-cellular) the cells, thus covering the entire cell mass of the cortex with an interwoven mycelial network (Photo. 2. B). As a consequence of penetration, cell walls disintegrated, cell substances degraded, cortical cell layers became disorganized (Photo. 2. A, A'-C) and rotten. Rotting of cortical cell layers with large parenchymatous cell was rapid. The progressive invasion slowed when the pathogen reached the vascular tissues. The xylem vessels were clear. The region around the vessels woody parenchyma was distorted. Thus irregular rotting of cells observed in the vascular zone.

Cross sections from diseased stem examined under a microscope showed that the pathogen did not enter into the xylem vessels; the microphotography also reflected the clear xylem of the infected tissues (Photo. 2. D). This indicates that the pathogenic invasion is not systemic.

These changes in the anatomical structures were observed in the infected collar zone of the susceptible varieties BAUBegun-1 and Dohazari G. In BAUBegun-2, the pathogen did not enter into the cortical region. Disintegration of cell, degradation of cell substances and finally rotting occurred only in the epidermal region (Photo. 3).

From the study, it appeared that all the eggplant varieties were infected by the pathogen *S. rolfsii* and there was no significant difference in percent plant infection among the varieties. Siddique [20] also had similar findings while working with 10 eggplant varieties.

The lesion size of collar rot significantly differed in different varieties. This could be explained by the variation in susceptibility of the eggplant varieties. The variation in lesion size after infection indicates differences in plant's capacity to cope with the pathogenic invasion that might be the inherent character of the plants.

Variations in reaction to collar rot among the eggplant varieties were observed at the early flowering stage. Only the variety of BAUBegun-2 showed no mortality so was graded resistant (R). Siddique [20] categorized eggplant variety Mirsarai-1 as resistant to collar rot at early flowering stage and Begum et al. [3] categorized eggplant vars. D. R. Chowdhury and Longla as resistant to collar rot at early stage.

The effect of collar rot on base diameter was quite apparent. The differences in reduction of base diameter may be due to the differences in the susceptibility to collar rot of the varieties. The variety BAUBegun-2 showed the lowest reduction in base diameter.

S. rolfsii entered into the lower part of the stem and destroyed almost all thin-walled living tissues, cortex and phloem in particular. The destruction of cortex and phloem disrupted the conduction of nutrients and water resulting in the death of the plant. The present findings are in agreement with the report of Siddique [20] who studied the pathogenic effect of *S. rolfsii* on some selected

varieties of brinjal and Babar [5] who studied the pathogenic effect of *S. rolfsii* on sunflower and made similar conclusions.

In BAUBegun-1 and Dohazari G, the pathogen, *S. rolfsii* entered into the plant body through a few sites located over the entire epidermal circumference. In case of massive attack, a number of epidermal cells were bulged out and decreased in wall thickness and protoplasmic consistency. Ultimately, all these epidermal cells were disorganized. In such a situation also, the hyphae of the pathogen had been found to take bi-directional paths, adaxial and lateral. Due to the lateral pathogenic action, the intact epidermis on other sides of the penetrating point had been found to be separated from the adjoining adaxial cortex.

The present finding differs with the report of Siddique [20] who studied pathogenic effect of *S. rolfssi* on some selected varieties of eggplant. According to him, the pathogenic mycelia attack the collar region of the plant and progress longitudinally. Adaxial and lateral paths of the pathogenic hyphae were not detected there. From that report, one gets an impression that the epidermis is affected in the form of a ring which progresses only in the upward direction.

From the present investigation, it cannot be said whether the hyphae of *S. rolfsii* enter into the plant body through some holes, cracks or ruptures as exist on the cuticle and abaxial wall of epidermis, or the hyphae secrete some enzymes which soften or perforate the cuticle and cell wall [21]. Many fungi enter into the plant body through crack, lesions formed on the epidermal wall due to the stress of secondary growth [22]. Further investigation is required to ascertain the same and establish this fact.

In phloem, the sieve elements (sieve tube member and companion cell) and phloem parenchyma (axial and ray) of both primary and secondary in origin had been found to be thin walled, and were easily accessible to the pathogen. Being next to the middle cortex, tissues of phloem were vulnerable to the fungal attack. Similar observations were made by a number of researchers [20,21]. Young phloem parenchyma (axial and ray) and companion cells had been found to be easily destroyed in eggplant stem by the pathogen, *S. rolfssi.* Along with companion cells, the sieve tube members were disorganized resulting in serious disruption of food transport mechanism, causing death of the plant at length. A similar report was made by Siddique [20].

Due to collar rot, disintegration and rotting of basal cuticle tissues disturbed translocation led to a nutritional deficiency in plants which hampered normal physiology in sensitive varieties. The base diameter was reduced due to the destruction of basal cuticles, a symptom from which the plants could not recover from.

The pathogen failed to enter into the cortex of BAUBegun-2. The thick cuticle, compact epidermal cells and small abaxial cells of the cortex with smaller intercellular spaces apparently restricted penetration by the invading pathogen.

3.4 Biochemical Study

Biochemical analysis indicated a differential status of compounds such as total phenol, ascorbic acid, total sugar, reducing sugar and Calcium oxalate content in the infected and non-infected cultivars of eggplant.

Phenol content was highest in the leaf and stem of the cultivar BAUBegun-1 and second highest was in the leaf of the cultivar Dohazari G. The lowest amount of phenol was present in the leaf of the cultivar BAUBegun-2 (Table 3). There were no significant differences among the cultivars in ascorbic acid content of the leaves. However, stems of cultivar BAUBegun-2 had the highest content of ascorbic acid followed by that in the stem of cultivar BAUBegun-1. The amount of total sugars was highest in both the leaves and stem of cultivar BAUBegun-2. The second highest amount of total sugar was detected in the leaves of cultivar BAUBegun-1. Cultivar Dohazari G has the lower contents of total sugar all through (Table 3). Leaves and stem of BAUBegun-2 had the highest content of reducing sugar. The second highest amount of reducing sugar was detected in cultivar Dohazari G. BAUBegun-2 had the higher content of Ca-oxalate in both leaves and stem. The cultivars BAUBegun-1 had the lowest Ca-oxalate content (Table 3). Dry matter contents were highest in both leaves and stem of cultivar BAUBegun-2. Second highest amount of dry matter was found in Dohazari G. The highest moisture (%) was recorded in the leaf of the cultivar BAUBegun-1 and stem of Dohazari G. The cultivars BAUBegun-2 carried the lowest moisture percentage (Table 3). The cultivars BAUBegun-1, BAUBegun-2 and Dohazari G were statistically

identical in terms of containing moisture (%) in stem (Table 3).

3.5 Relationship between Anatomy and Biochemical Constituents and Collar Rot Resistance in Eggplants

BAUBegun-1 is characterized with thin cuticle, few trichomes, loosely attached epidermal cells and large intercellular spaces. Phenol contents in the stem were higher; while ascorbic acid, reducing sugar, Ca-oxalate and dry matter content are lower. Moisture content is higher. The variety was graded susceptible to S. rolfsii (Table 4).

The variety BAUBegun-2 was characterized with a thick cuticle, thick epidermis, many trichomes and smaller intercellular spaces which might have restricted the entry of the pathogen. Ascorbic acid, reducing sugar and Ca-oxalate contents were higher in the stem of this variety, though phenol contents were lower. Dry matter (%) was higher and moisture content was lower than the other two varieties. Thus the variety was graded resistant (Table 4).

On the other hand, the variety Dohazari G was graded susceptible to *S. rolfsii* though it contained a higher amount of phenols. The variety Dohazari G has the highest amount of moisture and lower amount of dry matter (Table 4).

Higher phenol content in the diseased cultivars BAUBegun-1 and Dohazari G as observed may be due to the production of phenolic acids by the pathogen *in vitro* and those in the infected plants, this agrees with Aggarwal and Mehrotra [23], who reported alterations in phenolic compounds in Colocasia due to *Phytophthora colocasiae* infection.

The present results correspond with Alozie et al. [24] who found *Colletotrichum gloeosporioides (Glomerella cingulata)* infected yam (*Dioscorea alata*) to have higher phenol content than in healthy material, and Gupta and Kaushik [25] reported total phenol content was higher in Alternaria blight diseased leaves and siliquae walls of mustard compared to healthy leaves of mustard. The increase in total phenol content in susceptible cultivars BAUBegun-1 and Dohazari G, after infection, reflected the response of the host to check the attack of the pathogen and is confirmed by reports of Barua and Das [26] in fruit rot resistant chilli and Kumar and Balasubramanian [27] in *Puccinia arachidis* inoculated groundnut.

Jindal [28] and Kumar et al. [29] found (other than eggplant) resistant cultivars contained higher levels of total phenol which are contradictory to the results of the present study. But it may be possible that resistant cultivars contained a higher level of total phenol and phenol content increased in susceptible cultivars after infection.

BAUBegun-2 had the highest amount of ascorbic acid in the stem and correspondingly the lowest disease incidence. There are reports that plants having a higher amount of ascorbic acid are less susceptible to pathogenic attack. The present findings are supported by Zhang et al. [30] who found high contents of ascorbic acid in sweet potato which was characterized by high resistance to *Fusarium oxysporum*, Wang et al. [31] reported that the resistant cultivar of watermelon to *Fusarium oxysporum* f. sp. *niveum*, maintained a higher content of ascorbic acid than the susceptible cultivar, Gong et al. [32] developed total disease resistant cucumber variety containing higher amount of ascorbic acid than that of the control and Kalarani et al. [33] found resistant genotype of chilli against fruit rot caused by *Colletotrichum capsici* contained higher ascorbic acid than susceptible ones.

The information obtained through biochemical analysis corroborate with the field records where BAUBegun-1 and Dohazari G were found more susceptible. Schaaf [34] revealed ascorbic acid as an additional potent promoter-stimulating agent related to pathogen defence in transgenic tobacco plants

The lowest amount of total sugar and maximum disease was found from the cultivar Dohazari G followed by cultivar BAUBegun-1 but cultivar BAUBegun-2 had the minimum disease and a higher amount of total sugar both in stem and leaf. These results consented to the proposal of Chattopadhyay [35], who found total sugar content fell more rapidly in susceptible mustard plant to *Alternaria brassicae* and depletion of sugars were greater in lesions than in lesion free areas of infected leaves, Khirbat and Jalali [36], who observed total sugar content in susceptible genotype of chickpea to *Ascochyta rabiei* were low and Kumar et al. [29], who showed *Albugo candida* infection caused a greater decrease in total sugar of susceptible compared to resistant varieties of mustard.

Table 3. Biochemical compounds, dry matter and moisture content in three cultivars of eggplant

Cultivar	Phenol (mg/100gm sample)		Ascorbic acid (mg/100gm sample)		Total sugar (mg/100gm sample)		Reducing sugar (mg/100gm sample)		Ca-oxalate (mg/100gm sample)		Dry matter (g/100 gm sample) (%)		Moisture (g/100 gm sample) (%)	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
BAUBegun 1	136.50	97.50	5.92	2.69	1608.15	1308.45	810.00	540.50	261.28	222.85	16.24	20.80	83.67	79.43
BAUBegun 2	79.55	72.25	5.75	4.55	1807.85	1308.45	944.50	928.00	268.96	299.70	18.06	22.83	81.69	76.78
Dohazari G	118.00	88.25	5.39	2.87	1278.20	1008.85	877.50	802.00	268.93	245.91	17.77	21.16	81.76	80.48
Control	82.87	87.96	5.65	4.25	1723.42	1254.98	841.09	804.00	263.58	247.09	18.10	21.90	82.33	82.15
LSD (P=0.01)	1.28	0.29	NS	0.005	45.27	46.13	31.09	30.05	1.01	(P=0.05) 24.24	0.52	NS	0.57	NS

Table 4. Relationship between varietal disease reactions to anatomical structures and biochemical constituents in eggplant

Eggplant variety	Anatomical structures of stem	Biochemical composition of stem							Reaction to Collar rot *S. rolfsii*
		Phenol (mg/ 100g)	Ascorbic acid (mg/ 100g)	Total sugar (mg/ 100g)	Reducing sugar (mg/ 100g)	Ca-oxalate (mg/ 100g)	Dry matter (g/100g) %	Moisture (g/100g) %	
BAUBegun 1	Cuticle thin, Trichomes few, Epidermal cells loosely attached, Abaxial cells of the cortex are large with more intercellular spaces.	97.50	2.69	1308.45	540.50	222.85	20.80	79.43	Susceptible
BAUBegun 2	Cuticle thick, Trichomes many, Epidermal cells compact, Abaxial cells of the cortex are smaller with smaller intercellular spaces.	72.25	4.55	1308.45	928.00	299.70	22.83	76.78	Resistant
Dohazari G	Cuticle thin, Trichomes few, Epidermal cells not compact, Abaxial cells of the cortex are large with more intercellular spaces.	88.25	2.87	1008.85	802.00	245.91	21.16	80.48	Susceptible

The ability of resistant variety BAUBegun-2 to prevent greater loss of total sugar upon infection appeared important for resistance. The ability of resistant plants to prevent greater loss in phenylalanine ammonia lyase, phenolics, proline and total sugar upon infection appeared important for resistance and is supported by Chakrabarty et al. [37], who found similar results in cotton resistance to grey-mildew.

Cultivar BAUBegun-2 (resistant) had minimum disease and higher amount of reducing sugar but BAUBegun-1 (susceptible) had the lowest amount of reducing sugar and maximum disease followed by cultivar Dohazari G. Similar results were found by Deeb et al. [38] in barley infected by *Helminthosporium sativum* where reducing sugar concentration were higher in non-inoculated susceptible varieties, seedlings and adults, and infection reduced reducing sugar concentration in susceptible plants compared with controls. Sindhan et al. [39] found in mungbean cercospora leaf spot where reducing sugar content were higher in healthy leaves of susceptible genotypes than of resistant ones and their amount decreased in diseased leaves of resistant and susceptible genotypes. Shete and Munjal [40] also showed that reducing sugar levels in the leaves of susceptible and resistant mungbean cultivars decreased under the pathogenesis of powdery mildew. Such a decline in the content of sugars was more pronounced in the susceptible cultivars than in the resistant ones.

The higher amount of Ca-oxalate in healthy tissues of eggplant stem as found in the present investigation was supported by the report from Tarabeih and Menoufi [41] who found that plants grown in CaCO3 rich soil were more resistant to collar rot by *S. rolfssi.* This may be explained as the oxalic acid carried by the fungus is being tied-up as calcium oxalate and the fungus becoming inactive. Secondly, high level of CaCO3 causes an increase in soil pH which becomes less suitable for the growth and development of *S. rolfsii* as it thrives well in soil pH 5.0. Similar views have been expressed by Babar [5] who found a higher amount of Ca-oxalate in collar rot resistant cultivars of sunflower.

The lower percentage dry matter content was found in the leaf of highly susceptible cultivar BAUBegun-1. Percent dry matter content was highest in the leaf of resistant cultivar BAUBegun-2.

Percentage moisture was higher in the leaves and stem of highly susceptible cultivars BAUBegun-1 and Dohazari G. While it was lower in resistant cultivar BAUBegun-2. Dry matter content confers stoutness of the plant body whereas moisture makes it succulent. Therefore, varieties with higher dry matter content and lower moisture show resistance to invading pathogen. The findings of the present study are in line with these views but do not agree with Gong *et al.* [32] who found water content 0.5% higher in Xiangpuanggua 5, a new cucumber (*Cucumis sativus*) F1 hybrid compared with those of control variety Xianghuanggua 2 having disease resistance also higher than that of the control.

4. CONCLUSION

Anatomical features and biochemical constituents as analyzed in the present study are indicative of the resistance of eggplant var. BAUBegun 2 to collar rot pathogen *S. rolfsii.* BAUBegun 2 is already released as a collar rot resistant variety. The characteristic resistant features of BAUBegun 2 could be transferred to cultivated eggplant varieties through conventional breeding and also through molecular technique. There was a clear correlation between anatomical features and biochemical constituents and collar rot incidence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prabhu M, Natarajan S, Veeraragavathatham D, Pugalendhi L. The biochemical basis of shoot and fruit borer resistance in interspecific progenies of brinjal (*Solanum melongena*). EurAsian Journal of BioSciences. 2009;3:50-57.
2. Anonymous. Nutritional value of native foods and vegetables. Food and Nutrition Institute. University of Dhaka, Bangladesh. 1980;55.
3. Begum SN, Chowdhury BC, Ahamed HU. Screening of brinjal varieties for resistance to *S. rolfsii*. Abst. 1st National Conf. Plant Pathology, Held at Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. 1985;12.
4. Meah MB. Field screening of oilseeds and pulse diseases. Bull. Crop Diversification

Programme, Department of Agricultural Extension, Dhaka. 1994;12.
5. Babar HM. Studies on collar rot of sunflower. Ph. D. Thesis, Dept. Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. 1999;153.
6. Satpathy JM. Foliar spray treatment to control vegetables crop pest. Pesticides. 1964;1:29-30.
7. Agrios GN. Plant pathology. Fifth Edition. Elsevier Academic Press. California, USA. 2005;210.
8. Rahman H, Hossain MD, Meah MB. Biochemical changes in pod constituents of mustard due to infection of Alternaria blight (*Alternaria brassicae*). Bangladesh Journal of Plant Pathology. 1997;13(1&2): 1-4.
9. Dhasgupta MK. Principles of plant pathology. Allied Publisher Private Limited. New Delhi, India. 1988;700.
10. Anonymous. Fertilizer recommendation guide. Bangladesh Agricultural Research Council, Dhaka. 1997;70-71.
11. Aycock R. Stem rot and other diseases caused by *S. rolfsii*. Tech. Bull. No. 174. Agric. Expt. Station, North Carolina State University, Raleigh. 1966;202.
12. Barnett HL. Illustrated genera of imperfect fungi. Second Edition. Burgess Publishing Co. Minneapalis, Minn. 1960;225.
13. Nene YL, Haware MP, Reddy MOV. Chickpea diseases, resistance screening techniques. Information Bull. No. 10. ICRISAT, Hyderabad, India. 1982;10.
14. Swain T, Hillis WE. The phenolic constituents of *Prunus domestica* 1. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 1959;10:63-68.
15. Reo JH. Chemical determination of ascorbic acid dehydroascorbic acid and diketogluconic acid. Methods of Biochemical Analysis. 1954;1:115-139.
16. Jayaraman J. Laboratory manual in biochemistry. (1st Ed.). Wiley Eastern Ltd, New Delhi; 1981.
17. Miller GL. Use of DNS reagent for the determination of glucose. Analytical Chemistry. 1972;31:426-428.
18. Srivastava SK, Krishnan PS. Oxalate content of plant tissue. Journal of Scientific & Industrial Research. 1959;18(8):146-148.
19. Ranganna S. Manual analysis of fruits and vegetable production. Tata Mc Graw-Hill Publishing Co. Ltd. New Delhi. 1979;1-20.
20. Siddique MAB. Study on varietal reactions of brinjal to foot rot and its control through chemicals and organic soil amendments. MS Thesis, Dept. of Plant Pathology. Bangladesh Agricultural University, Mymensingh. Bangladesh. 1997;119.
21. Chan YH, Sackston WE. Penetration and invasion of sunflowers by *S. bataticola*. Canadian Journal of Botany. 1973;51(5): 999-1002.
22. Esau K. Plant anatomy. John Wiley, New York; 1965.
23. Aggarwal A, Mehrotra RS. The role of phenolic substances in leaf blight of *Colocasia esculenta* caused by *Phytophthora colocasiae*. Journal of the Indian Botanical Society. 1987;66(3-4): 272-274.
24. Alozie SO, Nwankiti AO, Oti E. Source of resistance in anthracnose blotch disease of water yam (*Dioscorea alata*) caused by *Colletotrichum gloeosporioides* Penz. 2. Relationship between phenol content and resistance. Beitrage zur Tropischen Landwirtschaft und Veterinarmedizin, 1987;25(1):55-59.
25. Gupta SK, Kaushik CD. Metabolic changes in mustard leaf and siliqua wall due to the infection of Alternaria blight (*Alternaria brassicae*). Cruciferae Newsletter. 2002; 85-86.
26. Borua I, Das P. Changes in activities of polyphenol oxidase, acid phosphatase and phenol content in developing chilli varieties susceptible and resistant to *Colletotrichum capsici*. Crop Research Hisar. 2000;19(2): 230-234.
27. Kumar ALR, Balasubramanian P. Induction of phenols in groundnut rust resistance. International Arachis Newsletter. 2000;20: 55-57.
28. Jindal PC. Biochemical disease resistance studies against anthracnose of grape (*Sphaceloma ampelinum* De Barry). Haryana Journal of Horticultural Sciences. 2000;29(1&2):11-14.
29. Kumar R, Thakral NK, Gupta SK, Kaushik CD. Alterations in sugars and phenols in *Brassica juncea* during interaction with white rust (*Albugo candida*). Cruciferae Newsletter. 2002;24:91-92.
30. Zhang ZY, Chen BQ, Wu WM, Yang LM, Cai JR, Zeng J, Wu WM, Zeng J. Breeding and identification of new sweetpotato variety Longshu 3. Acta Agriculturae Universitatis Jiangxiensis. 2003;25(2):174-177.

31. Wang JM, Hao C, Guo CR, Zhang ZG, He YC. Biochemical and physiological changes of three watermelon cultivars infested with *Fusarium oxysporum* f. *sp. niveum*. Agricultural Sciences in China. 2002;1(11):1204-1210.
32. Gong CZ, Liu JX, Xiao XL, Huang QL, Huang FL. Selection of a new cucumber (*Cucumis sativus*) F1 hybrid Xianghuanggua No. 5. China Vegetables. 2001;26-27.
33. Kalarani MK, Thangaraj M, Ramanathan A, Sivakumar R, Mallika V. Physiological and biochemical aspects of blast resistance in finger millet (*Eleusine coracana Gaertn*). Crop Research Hisar. 2002;23(3):526-531.
34. Schaaf J, Walter MH, Hess D. Primary metabolism in plant defense. Regulation of a bean malic enzyme gene promoter in transgenic tobacco by developmental and environmental cues. Plant Physiology. 1995;108(3):949-960.
35. Chattopadhyay AK. Relationship of phenols and sugars in Alternaria blight resistance of rapeseed mustard. Indian Journal of Mycological Research. 1989;27(2):195-199.
36. Khirbat SK, Jalali BL. Biochemical basis of resistance to chickpea Ascochyta blight. Legume Research. 1999;22(1):46-50.
37. Chakrabarty PK, Mukewar PM, Kumar VS, Raj S. Biochemical factors governing resistance in diploid cotton against grey mildew. Indian Phytopathology. 2002; 55(2):140-146.
38. Deeb AA, Ahmed KGM, Ghobrial E, Elian MI. Biochemical changes associated with resistance to spot blotch disease of barley. Phenolic compounds and sugars. Agricultural Research Review. 1987;65(2): 181-190.
39. Sindhan GS, Parashar RD, Hooda I. Sources of resistance to *Cercospora* leaf spot in mungbean and biochemical parameters for resistance. Journal of Mycology and Plant Pathology. 1999;29(1): 130-132.
40. Shete JD, Munjal SV. Involvement of sugars and lignins in powdery mildew reactions of mungbean cultivars. Journal of Maharashtra Agricultural Universities. 2002;27(2):229-231.
41. Tarabeih AM, Al-Menoufi. Effect of calcium carbonate on sunflower foot-rot disease caused by *Sclerotium rolfsii* Sacc. Alexandria Journal of Agricultural Research. 1987;32(1):325-332.

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