Original article

Assessing high-yielding cowpea varieties for bacterial blight resistance in Bangladesh: A step towards an environment-friendly and sustainable solution

Sumitra Saha [a,*], Ismat Jahan Romi [a], Fahmida Khatun [a], Biplob Kumar Saha [b], Muhammad Shahidul Haque [a], Nihar Ranjan Saha [a]

[a] Department of Biotechnology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[b] Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

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ABSTRACT

Cowpea is well-known worldwide for its high protein content, versatile use, and adaptability. However, it is devastatingly affected by bacterial blight disease caused by Xanthomonas axonopodis pv. vignicola (Xav). The present study was designed to assess ten high-yielding cowpea varieties for bacterial blight resistance in two contrasting cropping seasons in Bangladesh. The varieties were evaluated using seed and stem inoculation with Xav bacteria, followed by phenotypic and molecular characterisation. The varieties were morphologically assessed using nine disease-related qualitative and quantitative traits, and genetic variations were investigated through nine SSR markers. Disease development varied significantly (P = 0.05) among the varieties. Substantially higher disease incidence was observed in the Kharif season compared to the Rabi season. Felon local, Dark Green-28, and Dark Green-1028 varieties were resistant in both seasons. On the other hand, BARI Felon-1 was highly susceptible to susceptible in both seasons as infections were over 50%. Moreover, plant height, leaf area, branch number, and leaf number significantly differed among the varieties. Besides, in the molecular study, polymorphism information content and Nei's gene diversity were detected as 0.3658 and 0.4089, respectively. Kegornatki showed the highest genetic variation vs Dark Green-1028. The UPGMA dendrogram segregated the ten cowpea varieties into two main clusters. This study revealed that three high-yielding varieties, viz., Dark Green-28, Dark green 1028, and Felon local, were resistant to bacterial blight and showed better performance in morpho-molecular characterisation. Therefore, these varieties can be integrated into future cowpea breeding programmes to develop cultivars that can control the high pressures of Xav.

1. Introduction

Cowpea (*Vigna unguiculata L.*) is a legume crop widely regarded as the *"poor man's meat"* in less developed countries like Bangladesh due to the high protein contents in leaves (23–40%), pods (18–25%), and grains (23–29%) (Sebetha et al., 2010; Dakora and Belane, 2019; Weng et al., 2019). In Bangladesh, cowpea is cultivated for grains (shelled green or dried), pods, or leaves used for hay, silage, and green manure (Rakibuzzaman et al., 2019). It is usually the first crop collected before the cereal crops are ready and therefore is referred to as a *"hungry-season crop"* (Carvalho et al., 2017, Ahmed et al., 2018). Worldwide over 12 million ha of land are under cowpea cultivation, with gross production of about 6.9 million tons of grains (Durojaye et al., 2019). The total area under cowpea production in Bangladesh in 2017–18 was about 3200 ha, and the total grain production was approximately 3500 tons (BBS 2018). Unfortunately, the area under cultivation and yield of cowpea are badly affected by biotic and abiotic stressors worldwide, including in Bangladesh (Ehlers and Hall 1997, CABI, EPPO 2007, EPPO 2022). Among the biotic stressors, bacterial blight is one of the most devastating seed-borne diseases in cowpea caused by Xanthomonas axonopodis pv. Vignicola (Xav),

* Corresponding author.
E-mail addresses: sumitra@bau.edu.bd (S. Saha), rumiafccl@gmail.com (I.J. Romi), fahmida.meem@bau.edu.bd (F. Khatun), bksaha@bau.edu.bd (B.K. Saha), haquems@bau.edu.bd (M.S. Haque), nihar.saha47303@bau.edu.bd (N.R. Saha).

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formerly *X. campestris* pv. *Vignicola* (Agbicodo et al., 2010). Cowpea bacterial blight (CoBB), also known as canker, is prevalent in all cowpea growing areas with much rain and wind, responsible for the quicker spreading of these bacteria (Kotchoni et al., 2007; Durojaye et al., 2019). In addition, crop debris and weeds can also be inoculum sources (Sikirou and Wydra, 2008). The significant impact of *Xav* infection can be seen on the leaves based on the susceptibility of the genotype, which can cause the complete loss of green leaves. Pods, seeds, and stems can also be affected based on the severity (Durojaye et al., 2019). It can cause severe grain yielding loss and a considerably decrease production rate (Agbicodo et al., 2010).

The first symptom of CoBB appears as reddish and wrinkled cotyledons of seedlings developing from infected seeds (Ganiyu et al., 2017). The early necrotic lesion is formed on leaves, and later, the stem is affected. Subsequently, the pathogen reaches the vascular bundles, and the disease becomes systemic, resulting in infection in the growing tip and killing the plant (Agbicodo et al., 2010). The leaf's secondary infection appeared as light yellow circular spots of 4 to 10 mm in diameter and scattered on the lamina. The centre of these spots is necrotic and brown with red veins. Deep green or water-soaked streaks on pods are formed. Such pods become yellow and shrivel. The diseased pods produce smaller, wrinkled, and infected seeds (Agbicodo et al., 2010). These infected seeds serve as the primary inoculum source, and the secondary spread has occurred through rains, wind, and insects (Durojaye et al., 2019).

To successfully manage any disease under normal conditions, sanitation, eradicating primary sources, and chemical protection at the initial stages are recommended measures (Sikirou and Wydra, 2008). However, these measures are not enough whenever an outbreak of disease occurs. Besides, most Bangladeshi farmers are unaware of the suitable pesticides with proper doses to control or prevent this devastating bacterial disease of cowpea (Uddin et al., 2020). Severe health risks arise from farmers' exposure to pesticides when mixing, applying, or working in treated fields. Moreover, chemicals are sometimes too expensive for smallholders and may not be available (Shi et al., 2016). Hence, a thorough understanding of the disease management strategies and the concrete packages is necessary to effectively address the menace in an environment-friendly way (Emechebe and Lagoke, 2002; Durojaye et al., 2019). Cultivating resistant cowpea varieties is the most practical long-term method for controlling CoBB because it does not harm the environment; the economics of adoption is also minimal (Emechebe and Lagoke, 2002; Durojaye et al., 2019). The evaluation of cowpea varieties for resistance mainly relied on disease incidence and symptoms severity in the fields and greenhouse. Many studies have been done so far to find suitable cowpea varieties for resistance to bacterial blight (Allen et al., 1981; Bua et al., 1998; Agbicodo et al., 2010; Ganiyu et al., 2017; Durojaye et al., 2019). However, no commercial high-yielding cowpea variety with bacterial blight resistance has been released in Bangladesh. Therefore, this research assessed commonly cultivated high-yielding cowpea varieties against bacterial blight through inoculation and morpho-molecular characterisation in two sequential cropping seasons with contrasting weather conditions.

2. Materials and methods

2.1. Experimentals

This study evaluated ten high-yielding and commonly used Cowpea varieties in pot experiments in two contrasting cropping seasons of Bangladesh (*Kharif* 2019 and *Rabi* 2019–2020). One-pot containing three plants was considered as one replicate for each variety. Therefore, three sets of 11 pots were arranged in a randomised block design with three replicates per variety described by Durojaye et al. (2019). Average seasonal temperature, humidity, and precipitation were presented in Appendix 1.

The seeds of commonly used and high-yielding cowpea varieties viz., Dark Green-1028, Dark Green-28, Felon local, BARI Borbati-1, BARI Felon-1, Saba, Kegornatki, Sundori, Lalbenny, and Toki were collected to find the bacterial blight resistance. Table 1 and Appendix II represent the varieties' list with their collection source and approximate yield.

2.2. Isolation and identification of bacteria

Xav bacteria were isolated from the infected cowpea leaves collected from different regions of Mymensingh district, Bangladesh, following the method described by Sarker et al. (2017). Bacterial colonies from each plate were repeatedly sub-cultured at 28 °C until pure colonies were obtained (Ah-You et al., 2009). Gram staining was done according to standard protocol. The shape and gram staining were observed under a phase-contrast microscope (Olympus CX41RF, Japan) at 40X and 100X (Tabe 2). For the catalase test, a few drops of hydrogen peroxide were added to the surface of 48 h old culture of each isolate on the bacteria growth medium [Lysogeny broth (LB)], following standard protocol. Bubble formation was recorded for catalase activity (Table 2).

2.3. Pathogenicity test

2.3.1. Seed inoculation

For inoculation treatment, 2 g seeds (around 15–20 seeds) per cultivar, including control, were used and immersed in the diluted bacterial solution of *Xav* prepared from infected leaves as described above for 5 min. Before inoculation, the bacterial concentration was adjusted to an optical density (OD) of 0.3 at 600 nm corresponding to 10^6 colony-forming units ml^{-1} (CFU ml^{-1}) with a spectrophotometer (UV–VIS Spectrophotometer T80) (Agbicodo et al., 2010). As no bacterial blight-resistant high-yielding cowpea variety was found in Bangladesh to be used as a control, BARI-Felon treated with the same amount of LB media without bacteria culture was chosen randomly to use as the control. The percentages of seed infection were calculated by dividing the infected seed by the total number of seeds used and multiplying by 100. Inoculated seeds were kept in sterilised Petri dishes covered with cotton clothes in a dark growth chamber at 25 °C

Table 1
Cowpea varieties, sources, and approximate yield of varieties used.

No.	Varieties	Grain yield of Cowpea (ton/ha)	Sources
1	Saba	0.8–0.9	BADC (Bangladesh Agricultural Development Corporation)
2	Lalbenny	0.9–1.0	BADC
3	Dark Green-1028	0.7–0.8	BADC
4	BARI Borboti-1	1.1–1.4 (Uddin et al., 2020 & Haque et al., 2020)	BARI (Bangladesh Agriculture Research Institute)
5	Felon local	0.5–0.6	BADC
6	BARI Felon-1	0.62 (Putul et al, 2021)	BARI
7	Sundori	0.6–0.8	BADC
8	Dark Green-28	0.6–0.7	BADC
9	Kegornatki	0.6–0.7	BADC
10	Toki	0.7–0.8	BADC

Table 2
Identification of bacteria from cultural and morphological characters.

Test	Reactions	Appearance	Remarks	Bacteria
Gram staining	-ve	Small, Rod, Pink colour colony	Observed by microscope (100X) represent gram-negative bacteria	*Xav*
Catalase test	+ve	Bubbles formation	Bubbles formation represents catalase test was positive	

until germination (Appendix III). Seeds were considered germinated when the radicle reached at least 1 cm in length. The percentage of germinated seeds was recorded daily, and final germination was determined after 3–4 days. After planting, pots were kept in the open place of the field laboratory of the Biotechnology department, Bangladesh Agricultural University, Mymensingh, Bangladesh.

2.3.2. Stem inoculation

In *rabi* season, three-week-old cowpea seedlings were inoculated with bacteria on the underside of each leaf node by stem injection (Allen et al. 1981). The bacterial inoculum was prepared from diseased cowpea leaf samples as described earlier. The bacterial concentration was adjusted with a spectrophotometer of 600 nm to 10^6CFU ml^{-1} at 0.3 optical density. The control received the same amount of LB media without bacteria. Before inoculation, CoBB symptoms were not detected in any plants. After inoculation, all plants in each pot were inspected for CoBB symptoms for three weeks. Disease severity was rated visually using a 0–4 scale according to Agbicodo et al. (2010); here, 0 = no visible symptoms developed, 1 = leaf spots covering < 10% leaf area, 2 = blight affecting 10–50% leaf area, 3 = severe blight on > 50% leaf area, and 4 = trifoliate leaf shed (Agbicodo et al., 2010). CoBB severity index was calculated by averaging disease values from all three replicates of each variety and converting score values to the respective percentages described by Durojaye et al. (2019). Varieties were classified as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible based on total disease incidence as follows 0–10 %=Resistant, 11–30%=Moderately resistant, 31–50 %=Moderately susceptible, 51–75 %=Susceptible and 76–100 %=Highly susceptible.

2.4. Planting

During the *Kharif* season, seeds were considered germinated when the radicle reached at least 1 cm in length. The germinated seeds were recorded daily, and final germination was determined after 3–4 days. After germination, all transplanted plant pots were placed in sunlight, and water was sprayed as needed. The cowpea plantlets' survival percentage was recorded at 7, 14, 21, and 28 days after transplanting. Varieties were classified based on disease severity as described above.

2.5. Morphological data analysis

The cowpea varieties were morphologically evaluated through nine disease-related qualitative (leaf colour, leaf appearance, growth habit) and quantitative traits (leaf number, branch number, leaf area, plant height) and disease severity at 7, 14, 21, and 28 days after transplanting for both seasons (appendix V). However, the after-treatment effects of seed and stem inoculations were given importance during the presentation of morphological data. All sample selection and measurements were performed blinded until analysis. Leaf area (LA) was measured following the formula LA (cm^2) = lamina length × maximum width × 0.75 as described by Musa and Usman (2016) for cowpea. One-way analysis of variance (ANOVA) and Duncan's multiple range tests (α = 0.05) was performed using SPSS version 20.0 (Statistical Package for the Social Sciences).

2.6. Molecular screening

2.6.1. Genomic DNA extraction

The cowpea varieties were used to find the molecular diversity. The DNA was isolated from the young leaves of three-weeks old cowpea seedlings using the CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method as described by Rogers and Bendich (1989). Isolated genomic DNA was evaluated qualitatively and quantitatively using the nanodrop spectrophotometer (Appendix VII) and agarose gel electrophoresis.

2.6.2. Amplification of SSR markers by polymerase chain reaction (PCR)

A set of seventeen microsatellite primers screened from literature studies were used in this study. Primers were selected based on band resolution intensity, presence of smearing, consistency within individuals, and potential for population discrimination. Out of seventeen primers, nine primers showed clear polymorphism, used for further analysis. The detailed information on selected primers is given in Table 3. The amplified products were separated using 1.5% agarose in gel electrophoresis. The gel was visualised under a UV illuminator and photographed in a gel documentation unit. The detailed information on selected primers is given in Table 3.

For SSR analysis, a total volume of 10 μl/reaction mixture was made, including 9 μl PCR cocktail [5 μl master mix (Promega), 3 μl Nuclease free water, 1 μl Forward and 1 μl reverse Primers] and 1.0 μl genomic DNA for a single reaction. Then the sample was placed in the 96-well plate and run in a thermocycler (Biometra TOne 96). The reaction mix was preheated at 94 °C for 3 min, followed by 35 cycles of 3 min denature at 94 °C, 1 min annealing at 55–65 °C (based on the annealing temperature of the individual primer), and elongation at 72 °C for 2 min. After the last cycle, a final step was maintained at 72 °C for 7 min to allow complete extension of all amplified fragments, followed by holding at 4 °C until electrophoresis.

2.6.3. Genetic data analysis

Band sizing of the SSR markers was calculated using the molecular weight markers [50 bp (Promega)]. The scores obtained from the SSR marker analysis were pooled to create a single data matrix. These scores were used to estimate population differentiation, genetic distance (Nei, 1972), polymorphic loci, (Nei, 1978) gene diversity, and gene frequency and to construct an unweighted pair group method with arithmetic averages (UPGMA) dendrogram among populations using a POWER MARKER version 3.23 (Liu and Muse, 2005). Polymorphism Information Content (PIC) or expected heterozygosity for each SSR marker was calculated based on Hn = 1-Pi2, where Pi is the allele frequency for the ith allele (Nei, 1972). The varieties were clustered based on genetic similarities using the UPGMA. The cluster analysis and dendrogram construction were performed with NTSYS-PC (version 2.1).

3. Results

3.1. Seed and stem inoculations and planting

There were significant (P < 0.05) differences in CoBB disease severity indices among the examined cowpea varieties in *the Kharif*

Table 3
Details of primers used for molecular characterisation.

| SL	Primer	Forward sequence	Reverse sequence	Annealing temperature	Reference
1	CP5	AGCTCCTCATCAGTGGGATG	CATTGCCACCTCTTCTAGGG	57	Asare, Gowda et al. (2010)
2	CP6	GGGGGAGAGAGAGAGAGAGA	TTCTCCCCCTATGTGGACCT	58	Asare, Gowda et al. (2010)
3	CP7	GAGGAGGAGGAGGATCTGACA	CTTCTGCAGGCTTGTGGTTC	57	Asare, Gowda et al. (2010)
4	VM37	TGTCCGCGTTCTATAAATCAGC	CGAGGATGAAGTAACAGATGATC	54	Potarot (2012)
5	VM31	CGCTCTTCGTTGATGGTTATG	GTGTTCTAGAGGGTGTGATGGTA	55	Shivachi, Kiplagat et al. (2012)
6	VM34	AGCTCCCCTAACCTGAAT	TAACCCAATAATAAGACACATA	52	Shivachi, Kiplagat et al. (2012)
7	VM35	GGTCAATAGAATAATGGAAAGTGT	ATGGCTGAAATAGGTGTCTGA	52	Shivachi, Kiplagat et al. (2012)
8	BMd12	CATCAACAAGGCAGCCTCA	GCAGCTGGCGGGTAAAACAG	52	Shivachi, Kiplagat et al. (2012)
9	BQ481672	ATTTTTGGTGTGCTTTCGTTTAT	TCCGTGGCTTGCTGATTAG	52	Asare, Gowda et al. (2010)
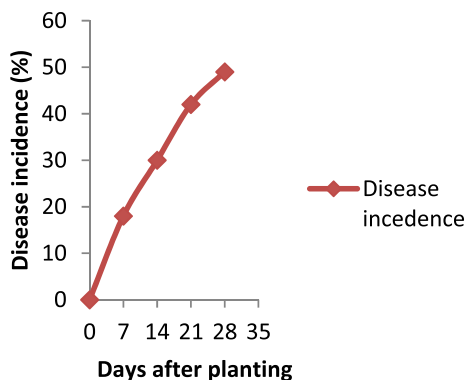
Fig. 1. Percentage of disease incidence on different days after planting during the Kharif and season.

season (Fig. 1). Seed inoculation treatments led to blight symptoms to seedling mortality (Tables 3 and 4). While significantly, no seedling mortality was found in the *Rabi* season before stem inoculation. In the *Kharif* season, after inoculation of seeds, the highest percentage of germination rate was shown by BARI Barbati-1 (97%), which was identical to Kegornatki (97%) whereas the lowest germination was recorded in BARI Felon-1 (80%) (Table 4). On the first evaluation day [7 days after planting (DAP)], Saba and Felon local showed a 100% plantlet survival and fast growth habits (Table 4 and appendix V). Control of seed inoculation treatment also showed rapid growth and a 100% survival rate at 7 DAP. On the other hand, the lowest survival percentage (50%) was shown by BARI Felon, and their growth habit was recorded as moderate (Table 4 and appendix VI). During the second evaluation day (14 DAP), significantly the highest survival percentage was recorded

in Felon local (93%), which was statistically identical to Dark Green-28 (83%), Toki (83%), Sundori (78%), and Kegornatki (78%). On the other hand, the lowest survival rate was found in BARI Barbati-1, and this was identical to BARI-Felon as leaf infections in these two varieties (BARI Barbati-1 and BARI Felon-1) were severe (>50%), so they died their survival percentage was recorded as 0% (Table 4). In the third and fourth evaluation periods (21 and 28 DAP), the highest survival percentage was shown by Felon local (93%) (Table 4). On the other hand, there were no significant differences in survival rates among varieties in *Rabi* season at DAP 21 and 28 before and after inoculation.

Significantly, the highest percentages of Felon Local seeds were infected by blight bacteria than control in seed inoculation during *Kharif* season, whereas the lowest infection was found in Lalbenny (10%), which is statistically identical to Dark Green-1028 (10%) and Dark Green-28 (10%) (Table 5 and Fig. 3). However, no seed infection was developed in BARI Felon-1 and Kegarnati. Interestingly, despite having lower seed infection at 7 DAP, the bacterial blight incidence of BARI Felon-1 was 63%, and in BARI Barbati-1, it was about 75%. They eventually died in progressive days of observation. However, Felon local, Saba, Lalbenny Sundori, Dark green-1028 and 28, and Toki infection percentages were<10% at 7 DAP. Bacterial blight symptoms got prominent almost in all varieties as the days progressed except Felon local (Figs. 1 and 3). Typical symptoms appeared in small brown lesions, which gradually expanded to large necrotic lesions in BARI Barbati-1 and BARI felon-1. Based on disease severity at the end of evaluations (28 DAP), varieties were classified as resistant, moderately resistant, moderately susceptible, and susceptible to CoBB. During *Kharif* season, the Felon local (1%) and Dark-green-28 (2%) were resistant to CoBB. Dark green-1028 and control were moderately resistant, whereas the highly susceptible group was BARI varieties: BARI Felon-1 (100%) and BARI Barbati-1 (100%) (Table 5).

Table 4
Percentage of seed germination and plantlet survival at different days after inoculation (DAI) and DAP of seed inoculated varieties.

Varieties	Germination (%) after treatment with bacterial inoculation during the *Kharif* season	Survive (%) at different DAP of *Kharif* season				*Rabi* season		
		7	14	21	28	Germination (%) during *Rabi* season	Plant survival before treatment at 21 DAP	Survival after stem inoculation at 28DAP
Saba	83de	100^a	66^{bc}	66^{bc}	66^{bc}	80^{cd}	100^a	88.9^a
Lalbenny	84^d	74^{bc}	59^{cd}	59^{cd}	59^{cd}	78^d	100^a	100^a
Dark Green-1028	87^c	67^{bcd}	44de	44de	44de	84^{ab}	100^a	100^a
BARI Barbati-1	97^a	56^{cd}	0^f	0^f	0^f	85^{ab}	100^a	88.9^a
Felon Local	87^c	100^a	93^a	93^a	93^a	79^d	100^a	100^a
BARI Felon-1	80^e	50^d	0^f	0^f	0^f	82^{bcd}	100^a	100^a
Sundori	93^b	78^{ab}	78^{abc}	78^{abc}	78^{abc}	88^a	100^a	100^a
Dark Green-28	84^d	83^{ab}	83^{ab}	83^{ab}	83^{ab}	72^e	100^a	88.9^a
Kegornatki	97^a	78^{ab}	78^{abc}	78^{abc}	78^{abc}	82^{bcd}	100^a	100^a
Toki	82de	83^{ab}	83^{ab}	83^{ab}	83^{ab}	84^{ab}	100^a	100^a
Control	93^b	100^a	34^e	34^e	34^e	88^a	100^a	100^a

Note: A column with the same letters does not differ significantly, whereas the column with the different letters differed significantly at a 5% probability level.

Table 5
Percentage of seed and leaf infection after seed and stem inoculations and planting.

Varieties	*Kharif* season (2019)						*Kharif* season (2019)		*Rabi* season (2020)	
	Seed infection (%)	Reaction type	Disease incidence at 7 days (%)	Disease incidence at 14 days (%)	Disease incidence at 21 days (%)	Disease incidence at 28 days (%)	Reaction type	Pictorial view of leaf infection in connection with resistance	Disease incidence at 28 days (%)	Reaction type
Saba	20[b]	MR	2[b]	5[de]	30[d]	50[c]	MS		25[a]	MR
Lalbenny	10[c]	R	3[b]	6[d]	30[d]	40[d]	MS		46[a]	MS
Darkgreen-1028	10[c]	R	5[b]	10[c]	20[e]	30[e]	MR		4[a]	R
BARI Barbati-1	20[b]	MR	75[a]	100[a]	100[a]	100[a]	HS		29[a]	MR
Felon Local	60[a]	S	2[b]	1[e]	1[f]	1[f]	R		28[a]	MR
BARI Felon-1	0[d]	R	63[a]	100[a]	100[a]	100[a]	HS		56[a]	S
Sundori	60[a]	S	3[b]	20[b]	35 [cd]	60[b]	S		61[a]	S
Dark green 28	10[c]	R	1[b]	1[e]	2[f]	2[f]	R		29[a]	MR
Kegornati	0[d]	R	5[b]	20[b]	40[bc]	60[b]	S		57[a]	S
Toki	20[b]	MR	6[b]	20[b]	45[b]	50[c]	MS		24[a]	MR
Control	10[c]	R	0[b]	20[b]	30[d]	30[e]	MR		5[a]	R

Here, Disease incidence 0–10 %=Resistant (R), 11–30%=Moderately resistant (MR), 31–50 %=Moderately susceptible (MS), 51–75 %=Susceptible (S), 76–100 %=Highly susceptible (HS).
Note: A column with the same letters does not differ significantly, whereas the column with the different letters differed significantly at a 5% probability level.
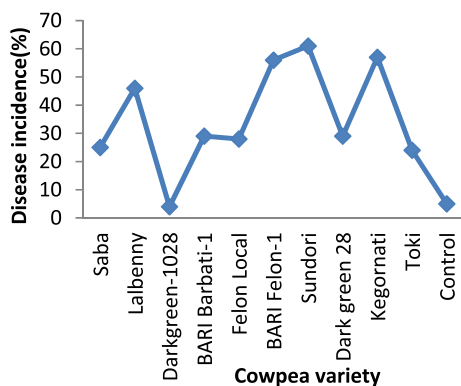

Fig. 2. Disease incidence of Cowpea varieties after 28 days of planting during the Rabi season.

Fig. 3. Individual disease incidence (%) of ten Cowpea varieties after planting in the Kharif season.

On the other hand, the percentage of disease incidence was significantly lower in the *Rabi* season than in the *Kharif* season (Fig. 2). Though there were no significant differences among varieties in disease incidence during *Rabi* season, visually, Dark green −1028 (4%) was found resistant, whereas Felon local (28%) and Dark green-28 (29%) were found moderately resistant in *Rabi* season. Other notable varieties of the moderately resistant group were Saba (25%) and Toki (24%). However, BARI Felon-1 (56%) was found to be consistently susceptible like *Kharif* season with Sundori (61%) and Kegornati (57%) (Table 5).
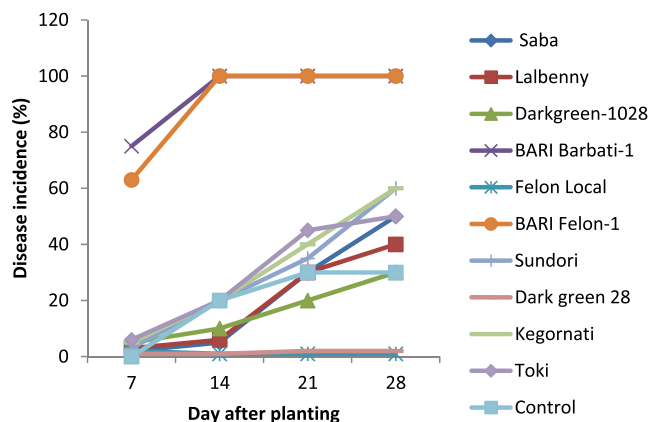
3.2. Morphological characterisations

The varieties were morphologically evaluated through nine disease-related qualitative (leaf colour and leaf appearance) and quantitative traits (leaf number, leaf area, plant height, branch number, and leaf infection percentage). The leaf colour and appearance for all the ten cowpea varieties on different DAP are shown in Table 6, Appendix IV and V. During *Kharif* season, blight disease

Table 6

Quantitative morphological characters of cowpea varieties at different observation days (DAP).

Varieties	Kharif (mid-March to mid-November/2019)												Rabi (mid-November to mid-March/2019–20)	
	Plant height (cm)				Branches				Leaves no.				Plant height (cm)	Leaves no.
	7	14	21	28	7	14	21	28	7	14	21	28	21	21
Saba	15.50[d]	16.17 [g]	19[f]	19[f]	1.00[c]	1.00[d]	1.33 [cd]	2.50[bc]	2.0[b]	7.50[a]	8.50 [cd]	11.50 [cd]	17.66[b]	23[bc]
Lalbenny	22.83[ab]	23.33[b]	27[c]	28.33[b]	2.33[a]	2.67[a]	3.00[a]	3.33[a]	2.0[b]	4.00[c]	7.00[de]	9.0[de]	18.33[b]	28[ab]
Dark Green-1028	22[b]	22.33[c]	24.67[d]	26.67[c]	1.00[c]	1.00[d]	1.00[de]	1.00[e]	2.0[b]	2.33[e]	8.00[de]	10.67[de]	19.66[b]	29[a]
BARI Barbati-1	11[e]	0.0[i]	0.0 [g]	0.0 [h]	1.00[c]	0.0[e]	0.0[f]	0.0 [g]	2.67[a]	0.0[f]	0.0[f]	0.0 [g]	17.33[b]	26[ab]
Felon Local	18.00[c]	18.67[e]	20.0[ef]	21.00[e]	1.00[c]	1.00[d]	1.33 [cd]	1.67[d]	2.0b	3.00[d]	8.00[de]	9.50[de]	26.00[a]	20[c]
BARI Felon-1	10.50[e]	0.0[i]	0.0 [g]	0.0 [h]	1.00[c]	0.00[e]	0.00[f]	0.00 [g]	2.0[b]	0.0[f]	0.0[f]	0.0 [g]	19.33[b]	27[ab]
Sundori	15.50[d]	16.67 [fg]	21.00[e]	24.33[d]	1.00[c]	1.67[c]	1.67[c]	1.67[d]	2.33[ab]	3.50 [cd]	7.00[de]	8.50[e]	18.33[b]	28[ab]
Dark Green-28	23.83[a]	28.33[a]	29.83[b]	31.50[a]	1.00[c]	1.00[d]	0.00[f]	0.00 [g]	2.67[a]	7.67[a]	13.33[ab]	14.0[ab]	18.00[b]	28[ab]
Kegornatki	18.50[c]	21.33[d]	32.33[a]	33.00[a]	2.00[b]	2.33[b]	2.33[b]	2.67[b]	2.33[ab]	5.00[b]	15.50[a]	16.0[a]	19.33[b]	19.33[b]
Toki	15.50[d]	17.00[f]	26.00 [cd]	28.50[b]	1.00[c]	1.00[d]	1.67[c]	2.33[c]	2.00[b]	5.50[b]	10.50[c]	13.5[bc]	19.66[b]	27[ab]
Control	15.67[d]	19.67[d]	24.33[cd]	25.33 [cd]	1.00[c]	1.00[d]	0.67[e]	0.67[f]	1.67[b]	4.00[c]	6.00[e]	6.0[f]	18.66[b]	31[a]

Note: A column with the same letters does not differ significantly, whereas columns with different letters differed significantly at a 5% probability level.

symptoms were observed on leaves of BARI Barbati-1 and BARI Felon-1 at 7 DAP, and their leaf colour was yellow, and leaf appearance was rough (Table 5 and 6). On second evaluation periods (14 DAP), with other varieties, blight disease symptoms on leaves were also observed in Felon local and Dark Green-28 and other varieties (Table 5 and 6). However, the percentage of leaf infection was < 6% for the varieties Felon Local and Dark Green-28. Moreover, no new blight disease symptoms were observed on leaves at 21 and 28 DAP in Felon local and Dark Green-28, and their leaf colours were green. Necrotic spots on leaves were only visible for these varieties on leaf surfaces during the first and second observations.

As morphological characters, LA, leaves number, branches, and plant height was considered on different DAP. The number of leaves and branches was significantly different among the varieties. Leaf number and branch number were increased day by day (Table 6 and Appendix V). The significantly higher branches and leaves were found in Kegornatki and Dark Green-28, respectively, in all observation days in the *Kharif* season. On the other hand, in the *Rabi* season, the highest number of leaves were produced in Dark green-1028 and then in Dark green-28, and their produced leaves were two times bigger than the leaves produced in the *Kharif* season (Table 6). Plant height at different DAPs for all the ten cowpea varieties is shown in Table 6. Significant differences were found in the plant height among the varieties during the *Kharif* season, and it was increased gradually day by day after planting, indicating the average growth of plants (Table 6 and

appendix V). The significantly highest plant height was found in Kegornatki (33.0 cm), and the lowest was found in Saba (19.0 cm) at 28 DAP. However, there were no significant differences in plant height in the *Rabi* season before and after inoculation at 21 DAP and 28 DAP (28 DAP data was not shown as they are not significant) (Table 6).

Moreover, LA was significantly varied among the cowpea varieties at different DAPs. At all observation periods of the *Kharif* season, the largest LA was found in Felon local and Sundori (Fig. 4). On the other hand, there were no significant differences among varieties for LA during the *Rabi* season (Fig. 4).

3.3. Molecular screening

In molecular screening, the UPGMA constructed a dendrogram for the cowpea varieties (Fig. 8) based on Nei's (1972) genetic distance (Figs. 5 to 7). In UPGMA cluster analysis, significant genetic variation was found among the cowpea varieties studied, with a similarity coefficient varying between 0.40 and 1.00. The UPGMA cluster analysis led to the ten varieties into two major clusters: Cluster I and II possessed 3 and 7 varieties, respectively, at a 0.50 cut-off similarity coefficient below which the similarity values narrowed conspicuously (Fig. 8). Cluster 1 consisted of two subclusters (subcluster 1.1 and 1.2); subcluster 1.1 with Sundori and Lalbenny. Another subcluster, 1.2, contained Kagornatki. Cluster 2 comprises three subclusters (Subcluster 2.1, 2.2 and 2.3). Sub-
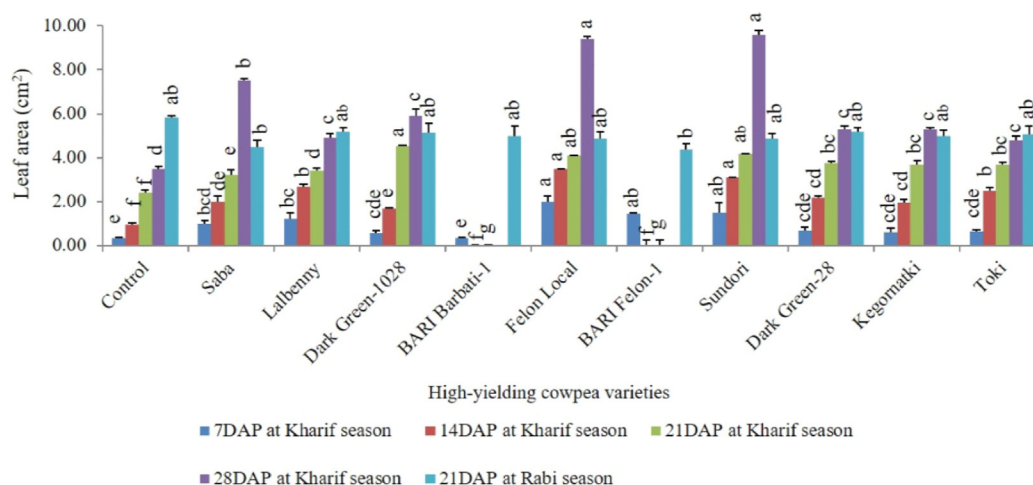

Fig. 4. Variation in leaf area among the cowpea varieties at different DAP in *Kharif* and *Rabi* seasons.
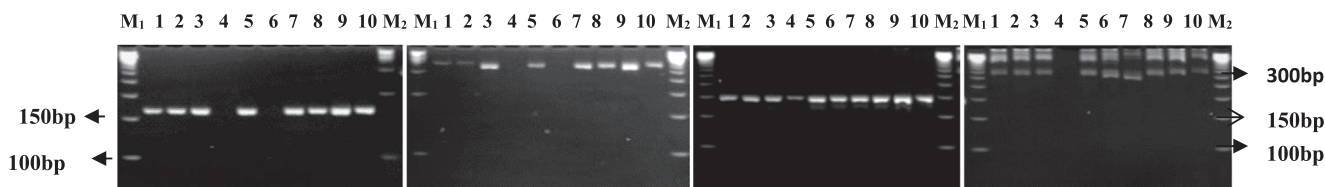
Fig. 5. The allele banding pattern at locus BMd-12, BQ481672, CP5, and CP6 in 10 cowpea varieties. BMd-12 product sizes were 169–171 bp, BQ481672 product sizes were 307–321 bp, CP5 product sizes were 201–204 bp, and CP6 product sizes were 343 bp, respectively. In this Fig.; Variety 1 = Sundori, 2 = BARI Barbati-1, 3 = Lalbenny, 4 = Dark Green-28, 5 = Kegornatki, 6 = Dark Green-1028, 7 = Felon local, 8 = BARI Felon-1, 9 = Saba Barbati, 10 = Toki and M_1 and M_2 = 50 bp.
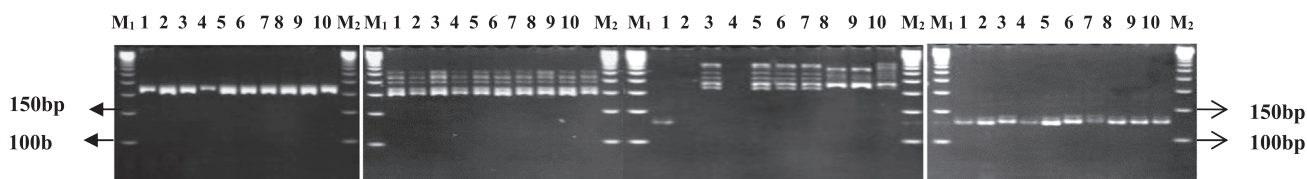

Fig. 6. The banding pattern of allele at locus CP7, VM31, VM34, and VM35 in 10 cowpea varieties. CP7 and VM31 product sizes were 224–230 bp and 201–204 bp, VM34 product sizes were 216–229 bp, and VM35 product sizes were 129–131 bp, respectively. In this Fig.; Variety 1 = Sundori, 2 = BARI Barbati-1, 3 = Lalbenny, 4 = Dark Green-28, 5 = Kegornatki, 6 = Dark Green-1028, 7 = Felon local, 8 = BARI Felon-1, 9 = Saba Barbati, 10 = Toki, M_1 and M_2 = 50 bp.
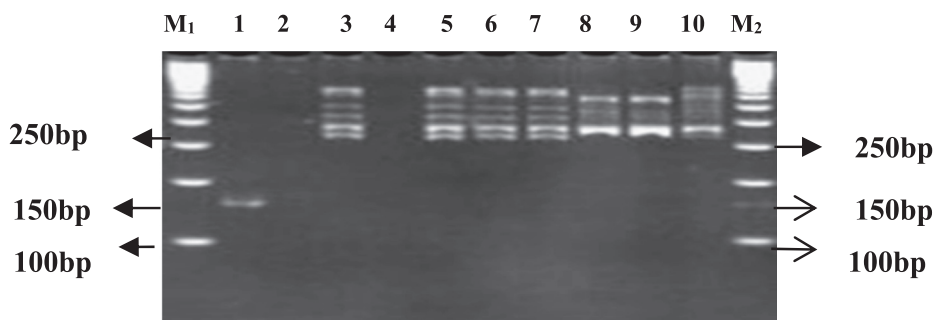

Fig. 7. Banding pattern of allele at locus VM37 in 10 cowpea varieties with product size 277 bp. In this Fig.; Variety 1 = Sundori, 2 = BARI Barbati-1, 3 = Lalbenny, 4 = Dark Green-28, 5 = Kegornatki, 6 = Dark Green-1028, 7 = Felon local, 8 = BARI Felon-1, 9 = Saba Barbati, 10 = Toki, M_1 and M_2 = 100 bp.
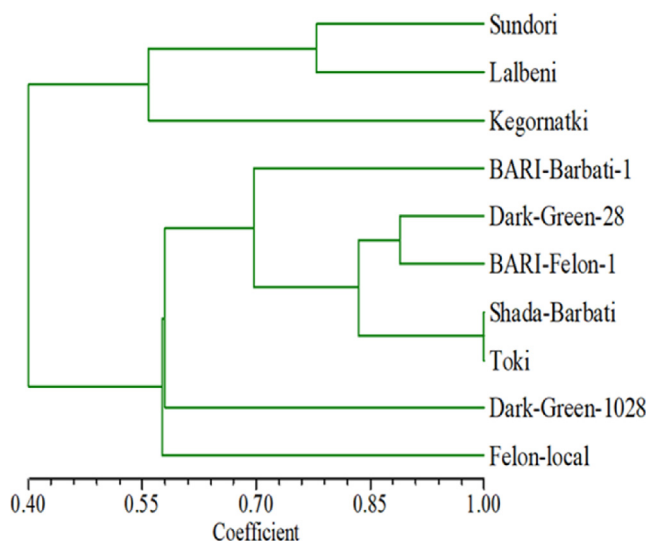

Fig. 8. UPGMA for ten cowpea varieties showing the genetic similarity.

cluster 2.1.1 consisted only of BARI Barbati-1, whereas cluster 2.1.2 consisted of two subclusters, subcluster 2.1.2.1 and subcluster 2.1.2.2; Shada Barbati and Toki belonged to subcluster 2.1.2. Subcluster 2.1.2.1 consisted of the Dark Green-28, and the 2.1.2.2

cluster consisted of BARI Felon-1. On the other hand, subcluster 2.2 comprised Dark Green-1028 only. Cluster 2.3 consisted of Felon local.

Pair-wise comparison value of (Nei, 1972) genetic distance (D) between varieties was computed from data of 9 primers and ranged from 0.1178 to 2.1972 (Table 7). The highest genetic distance indicated that genetically, they were diverse compared to lower genetic distance values. The highest genetic distance (2.1972) was observed between the variety Dark Green-1028 and Kegornatki. However, the variety Saba Barbati and Toki had negligible genetic distance. This experiment found that the CP6 and VM34 primers resulted in 1 and 3 null alleles, respectively, with an average of 0.7 null alleles in 10 accessions (Table 8). The locus that showed the highest frequency of null alleles was VM34 (nulls detected in four varieties). The most common allele frequency at each locus ranged from 0.40 in BQ481672 (Fig. 5) to 1.0 in VM31 (Fig. 7), with a mean frequency of 0.70 (Table 8). The major alleles at different loci ranged from 131 bp for VM37 (Fig. 7) to 343 bp for CP6 (Fig. 5).

The highest gene diversity (0.70) was observed in loci BQ481672 (0.70) (Fig. 5), and the lowest gene diversity (0.18) was observed in loci VM35 (Fig. 6) with a mean diversity of 0.4089 (Table 8). Markers with fewer alleles showed lower gene diversity than those witnessing the higher number of alleles revealed higher gene diversity.

The PIC value is often used to measure a genetic marker's informativeness for the studies. The PIC values ranged from 0.1638 in

Table 7
Genetic distance of cowpea varieties based on nine microsatellite alleles.

Cowpea varieties	Sundor	BARI Barbati-1	Lalbenny	Dark-Green-28	Kegornatki	Dark Green-1028	Felon local	BARI-Felon-1	Saba Barbati	Toki
Sundori	0									
BARI Barbati-1	0.8109	0								
Lalbenny	0.2513	0.4055	0							
Dark Green-28	0.8109	0.4055	0.4055	0						
Kegornatki	0.4055	1.0986	0.8109	1.5041	0					
Dark Green-1028	1.5041	0.5878	0.8109	0.4055	2.1972	0				
Felon local	1.5041	0.8109	0.8109	0.4055	1.0986	0.5878	0			
BARI Felon-1	1.0986	0.5878	0.5878	0.1178	1.5041	0.5878	0.4055	0		
Saba Barbati	0.8109	0.2513	0.4055	0.1178	1.5041	0.5878	0.5878	0.2513	0	
Toki	0.8109	0.2513	0.4055	0.1178	1.5041	0.5878	0.5878	0.2513	0.0000	0

Table 8
Summary statistics of 9 SSR markers found among ten cowpea varieties.

Marker	Allele No	Null allele	Major Allele		Gene Diversity	PIC
			Frequency	Size (bp)		
BMD12	3	2	0.7000	171	0.4600	0.4102
BQ481672	4	2	0.4000	307	0.7000	0.6454 *
CP5	2	0	0.8000	201	0.3200	0.2688
CP6	4	1	0.7000	343	0.4800	0.4500
CP7	2	0	0.8000	224	0.3200	0.2688
VM31	1	0	1.0000	200	0.0000	0.0000
VM34	3	3	0.4000	216	0.6600	0.5862
VM35	2	0	0.9000	131	0.1800	0.1638 *
VM37	3	0	0.6000	277	0.5600	0.4992
Mean	2.6667	0.7	0.7000	–	0.4089	0.3658

In this table, having * means they are significantly different.

VM35 to 0.6454 in BQ481672 (Table 8), with an average value of 0.3658 (Table 8). PIC values also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study. The allele size and the number of alleles themselves were also positively correlated.

4. Discussion

This study assessed commonly used high-yielding cowpea varieties for bacterial blight resistance using combined inoculations (seed and stem) and morpho-molecular characterisation approach to offer sustainable and environment-friendly cowpea production in Bangladesh. Bacterial-blight infected leaves were collected, and *Xav* was isolated and characterised for this inoculation study. This result has confirmed the pathogenesis of bacterial blight infection in leaves.

CoBB is a seed-borne disease (Allen et al., 1981), and wind-driven rain and insects can spread the pathogen. Therefore, to determine the varieties resistant to CoBB, seeds of ten cowpea varieties were treated with isolated *Xav* bacteria in *Kharif* seasons. Three varieties, viz., Feclon local Dark green-1028 and Dark green-28, were resistant to CoBB in both seasons, and their seedling mortalities were significantly low compared to other varieties. The seed inoculation technique for evaluating seedling mortality was proven helpful since distinct syndromes of bacterial blight (seedling mortality, stem canker and foliar blight) were recognised. By this, BARI varieties were found susceptible to highly susceptible to CoBB. Allen et al. (1981) also found seed inoculation advantageous in establishing the relationship between the resistance of different plant parts within cowpea varieties. The seedling mortalities in the uninoculated control were probably due to the seed-borne nature of *Xav* since control was randomly selected (as no high-yielding resistant variety was found) and also as unsterilised soil was used, so soil-borne contamination cannot be ignored (Durojaye et al., 2019).

Although the seed is the primary inoculum source of *Xav* (Allen et al., 1981; Ganiyu et al., 2017*)*, we also used stem inoculation to detect resistance to CoBB in *Rabi* season. The disease incidences were significantly lower in the *Rabi* season, and no statistically significant differences were found among varieties. The interaction observed between the cowpea varieties and inoculation techniques indicate that the inoculation mode influences disease incidence, but recurrent disease development depends on cultivar susceptibility (Bua et al., 1998). However, considering the seasonal variation, visually, in *Rabi* season, Felon local, Dark green-1028 and Dark green-28 showed resistance against bacterial blight. The reason for disease incidence variation could be due to differences in environmental conditions between the seasons (weather data in Appendix 1), as wind and rainfall spread bacteria from diseased plants by raindrops, plant to plant contact, and insect transmission (Moretti et al., 2007; Sikirou and Wydra 2008; Ganiyu et al., 2017; Durojaye et al., 2019). Here, it is essential to mention that *Kharif* is the primary season of cowpea cultivation in Bangladesh (Ahmed et al., 2018). Moreover, as a seed-borne disease, seed inoculation treatment can provoke the bacterial load to spread. Gitaitis and RD (1982) found significantly higher disease incidence in cultivars grown from heavily bacteria-infested seeds.

Xav was isolated by Durojaye et al. (2019) from infected leaves and used as bacterial inoculum for leaf inoculation to identify bacterial blight-resistant cowpea varieties up to 22 days after inoculation. They found cowpea landrace accession TVu 58, TVu 64, and TVu completely resistant to CoBB. However, the cowpea varieties are not similar to the present study. Moreover, twenty-six cowpea lines were field evaluated to find the bacterial blight resistance for two seasons in Uganda following inoculation by spraying (Bua et al., 1998). They found lines IV 1075 and Icirikukwai as resistant for both seasons (Bua et al., 1998). However, to our understanding, this was the first effort in Bangladesh where inoculation studies were combined with morpho-molecular characterisation to assess resistance against the bacterial blight of high-yielding cowpea varieties. Felon local showed resistance in this study could be that

it evolved its high resistance mechanism like infection-induced immune against bacterial blight like other local varieties. Therefore, despite having higher infection in seed inoculation, it has become a resistant variety. Moreover, these resistant varieties could evolve *Xanthomonas transcription* activator-like (TAL) effectors binding site as an executioner to induce hypersensitive host cell death when up-regulated executor resistance genes (Hummel et al., 2012; Dangl et al., 2013).

Besides, this research suggests that bacterial inoculation affects germination percentage and survival percentages. Furthermore, the findings of the morphological study (Plant height, leaf length) (Table 6 and Appendix IV and VI) suggested the diversity present among the cowpea varieties. The lower plant height of Felon local could be its local origin. Moreover, the lower leaf area in *Kharif* than in *Rabi* season on the same observation day could be due to the seed inoculation effect.

This research found leaf infections after 7 DAP during the Kharif season. However, no new infection was observed at 28 DAP in both seasons. This finding is aligned with Gitaitis and RD (1982) observation. They also did not find any infection after 30 days in pea. Allen et al. (1981) suggested that plants should be inoculated at an early growing stage to assess resistance to bacterial blight properly. This can be due to young leaves being more susceptible to bacterial blight. Also can be that the cowpea plant gradually produces a defence mechanism against bacterial blight. This finding confirms Gitaitis and RD (1982) early observations about cowpea's defence response mechanism to X. *axonopodis pv. Vignicola.* Leaf number, branch number, leaf area and plant height were increased after planting. They were significantly different among the studied varieties (P < 0.05). These results agree with the result of Rambabu et al. (2016). They also reported that morphological characters are highly variable with time. Though resistant varieties produced more leaves than others, the lower number of leaves in the *Kharif* season could result from inoculation or a plant's defence mechanism, as leaves are the main target of bacterial blight. In this research, LA of different varieties was measured as an indicator of adaptation to the changed conditions due to bacterial blight (Musa and Usman, 2016). Significantly, the largest LA was found in Felon Local, indicating that this variety may better adapt to the changing conditions of bacterial blight, which could impact the photosynthesis capacity of the plant. Goodwin (1992) found that the photosynthetic capacity of leaves decreased by 50% when only 15–20% leaf area of beans got infected by bacterial blight.

Additionally, in molecular screening, the number of alleles per locus ranged from 1 to 4, with 24 alleles generated from the SSR primers. Polymorphism information content (PIC) and Nei's (1972) gene diversity were detected as 0.3658 and 0.4089, respectively. Kegornatki showed the highest genetic variation vs Dark Green-1028, and the lowest genetic variation was found between Saba Barbati and Toki. In the present study, genetic distances among cowpea varieties were also higher (2.19). More importantly, the UPGMA dendrogram segregated the ten cowpea varieties into two main clusters. Cluster I and II possessed 3 and 7 varieties, respectively. Khan et al. (2015) analysed the genetic diversity of six BARI Cowpea germplasms in Bangladesh using 3 pairs of RADP markers. In agreement with the present study, six germplasms of cowpea are segregated into two main clusters; the main clusters are further divided into sub-clusters, where BARI-Barbati-1 and BARI Felon-1 were clustered in cluster 1, and sub-sub-cluster I and II. Moreover, Ali et al. (2015) characterised 252 cowpea accession from different locations of Sundan using 14 SSR markers. They found a dendrogram of these varieties based on SSR polymorphism divided into three major clusters with only 8% variation among the population.

On the other hand, Asare et al. (2010) measured the genetic distance and relationship among 22 local cowpea varieties and

inbred lines collected throughout Senegal using 49 SSR primer combinations. Their evaluated varieties clustered in two major groups where Cluster 2 was the biggest with 14 varieties. Cluster 2 was the biggest, with 14 varieties, further separated into several subclusters. Based on the dendrogram constructed in the present study and the earlier investigations, it can be concluded that clusters in the dendrogram can be varied on genotypes evaluated and markers used. However, all these studies concluded that the SSR marker is the most powerful among all markers to find genetic diversity and could be used to estimate the genetic diversity of cowpea (Asare et al., 2010; Tan et al., 2012; Ali et al., 2015).

5. Conclusion

Millions of people across Bangladesh and other regions rely on cowpea as a primary source of food and income. Based on pathogenesis and morpho-molecular studies, the present study revealed that high-yielding varieties like Felon local, Dark Green-1028, and Dark green-28 might be resistant to bacterial blight and have genetic diversity. Of course, to our understanding, this was the first attempt to evaluate commonly used high-yielding cowpea varieties for bacterial blight resistance by a combined approach of inoculation and morpho-molecular characterisation. Therefore, our identified varieties with superior genetic backgrounds could lead to identifying genes and single nucleotide polymorphisms (SNP) associated with resistance to CoBB. Future work evaluating resistance to CoBB in other sets of germplasm can consider Felon Local, Dark green-1028, and Dark green 28 as resistant to CoBB. In addition, these high-yielding resistant varieties could be integrated into traditional and molecular-assisted breeding programs to develop cultivars that can resist the high pressures of *Xav*. High-yielding varieties with CoBB resistance could reduce losses associated with the disease and enhance cowpea production in a sustainable and environment-friendly manner. Of course, more research work under various climatic and soil conditions with more varieties will be needed to validate this combined approach to bacterial blight management of cowpea.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103365.

References

Agbicodo, E.M., Fatokun, C.A., Bandyopadhyay, R., Wydra, K., Diop, N.N., Muchero, W., Ehlers, J.D., Roberts, P.A., Close, T.J., Visser, R.G.F., van der Linden, C.G., 2010. Identification of markers associated with bacterial blight resistance loci in cowpea [*Vigna unguiculata* (L.) Walp.]. Euphytica 175 (2), 215–226.

Ah-You, N., Gagnevin, L, Grimont, P.A., Brisse, S., Nesme, X., Chiroleu, F., Ngoc, L.B.T., Jouen, E., Lefeuvre, P., Vernière, C., 2009. Polyphasic characterisation of xanthomonads pathogenic to members of the Anacardiaceae and their relatedness to species of Xanthomonas. Int. J. Syst. Evol. Microbiol. 59 (2), 306–318.

Ahmed, R.N., Haque, M.A., Ahmed, K.S., Uddin, M.M., Khan, M.A.M., 2018. Screening of yard long bean genotypes for resistance to legume pod borer, *Maruca vitrata* F. J. Bangladesh Agric. Univ. 16 (3), 424–428.

Ali, Z.B., Yao, K.N., Odeny, D.A., Kyalo, M., Skilton, R., Eltahir, I.M., 2015. Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] accessions from Sudan using simple sequence repeat (SSR) markers. Afr. J. Plant Sci. 9 (7), 293–304.

Allen, D.J., Nebane, C.L.N., Raji, J.A., 1981. Screening for resistance to bacterial blight in cowpea. Trop. Pest Manage. 27 (2), 218–224.

Asare, A.T., Gowda, B.S., Galyuon, I.K.A., Aboagye, L.L., Takrama, J.F., Timko, M.P., 2010. Assessment of the genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm from Ghana using simple sequence repeat markers. Plant Genet. Resour. 8 (2), 142–150.

BBS (2018). "Yearbook of agricultural statistics of Bangladesh." M. o. P. Bangladesh Bureau of Statistics (BBS). Government of the People's Republic of Bangladesh.

Bua, B., Adipala, E., Opio, F., 1998. Screening cowpea germplasm for resistance to bacterial blight in Uganda. Int. J. Pest Manage. 44 (3), 185–189.

CABI, EPPO (2007). "Xanthomonas axonopodis pv. phaseoli. [Distribution map]". In: Distribution Maps of Plant Diseases, Wallingford, UK: CABI. Map 401 (Edition 4). DOI:10.1079/DMPD/20073069783.

Carvalho, M., Lino-Neto, T., Rosa, E., Carnide, V., 2017. Cowpea: a legume crop for a challenging environment. J. Sci. Food Agric. 97 (13), 4273–4284.

Dakora, F.D., Belane, A.K., 2019. Evaluation of protein and micronutrient levels in edible cowpea (*Vigna unguiculata* L. Walp.) leaves and seeds. Front. Sustain. Food Syst. 3 (70).

Dangl, J.L., Horvath, D.M., Staskawicz, B.J., 2013. Pivoting the plant immune system from dissection to deployment 341, 746–751.

Durojaye, H.A., Moukoumbi, Y.D., Dania, V.O., Boukar, O., Bandyopadhyay, R., Ortega-Beltran, A., 2019. Evaluation of cowpea (*Vigna unguiculata* (L.) Walp.) landraces to bacterial blight caused by *Xanthomonas axonopodis* pv. vignicola. Crop Prot. 116, 77–81.

Ehlers, J.D., Hall, A.E., 1997. Cowpea (*Vigna unguiculata* L. Walp.). Field Crops Res. 53 (1-3), 187–204.

Emechebe, A. and S. Lagoke (2002). "Recent advances in research on cowpea." Challenges and opportunities for enhancing sustainable cowpea production 28: 94

EPPO (2022). "EPPO Global database". In: EPPO Global database, Paris, France: EPPO. 1 pp. https://gd.eppo.int

Ganiyu, S.A., Popoola, A.R., Owolade, O.F., Fatona, K.A., 2017. Control of common bacterial blight disease of cowpea (*Vigna unguiculata* [L.] Walp) with certain plant extracts in Abeokuta, Nigeria. J. Crop Improve. 31 (3), 280–288.

Gitaitis, R. and G. RD (1982). "Detection of *Xanthomonas campestris* pv. vignicola in southern pea seed.".

Goodwin, P.H., 1992. Effect of common bacterial blight on leaf photosynthesis of bean. Can. J. Plant Pathol. 14 (3), 203–206.

Haque, M. P. H., Hossain, M. A., Biswas, S. K., & Chowdhury, S. K. H. (2020). "Impact of Irrigation at Different Growing Stages on Yield, Crop Water Productivity, and Profitability of Cowpea (Vigna unguiculata L.) in Bangladesh." *Journal of Agricultural Science & Engineering Innovation (JASEI)[US ISSN 2694-4812]*, *1*(1), 15-22.

Hummel, A.W., Doyle, E.L., Bogdanove, A.J., 2012. Addition of transcription activator-like effector binding sites to a pathogen strain-specific rice bacterial blight resistance gene makes it effective against additional strains and against bacterial leaf streak. New Phytol. 195 (4), 883–893.

Khan, M.T., Reza, M.O.H., Khan, M.A., Haque, M.S., Islam, M.S., Khan, M.B., 2015. Genetic diversity analysis of cowpea by RAPD markers. Int. J. Innov. Appl. Stud. 10 (2), 459.

Kotchoni, O., Torimiro, N., Gachomo, E., 2007. Control of Xanthomonas campestris pv. Vignicoia in cowpea following seed and seedling treatment with hydrogen peroxide and N-heterocyclic pyridinium chlorochromate. J. Plant Pathol., 361–367

Liu, K., Muse, S.V., 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21 (9), 2128–2129.

Moretti, C., Mondjana, A.M., Zazzerini, A., Buonaurio, R., 2007. Occurrence of leaf spot on cowpea (*Vigna unguiculata*) caused by *Xanthomonas axonopodis* pv. vignicola in Mozambique. Plant. Pathol. 56 (2), 347.

Musa, U.T., Usman, T., 2016. Leaf area determination for maise (Zea mays L.), okra (Abelmoschus esculentus L.) and cowpea (*Vigna unguiculata* L.) crops using linear measurements. J. Biol., Agric. Healthcare 6 (4), 103–111.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106 (949), 283–292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 (3), 583–590.

Potarot, S., 2012. Inheritance of resistance to cowpea aphid (*Aphis craccivora* Koch.) in yardlong bean and cowpea by microsatellite markers. Prince of Songkla University.

Putul, F.B., Khan, A.R., Hossain, M.S., Mahmud, A., Khaliq, Q.A., Ahamed, T., 2021. Growth and yield of cowpea as influenced by different phosphorus levels. Bangladesh Agro. J. 24 (1), 25–36.

Rakibuzzaman, M., Mahato, A., Husna, M., Maliha, M., Jamal Uddin, A., 2019. Influence of natura one and neem oil on growth and yield of brinjal (*Solanum melongena*). J. Biosci. Agric. Res. 20 (02), 1694–1699.

Rambabu, E., Reddy, K.R., Kamala, V., Saidaiah, P., Pandravada, S., 2016. Genetic divergence for quality, yield and yield components in Yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. sesquipedalis Verdc.]. Legume Res.- Int. J. 39 (6), 900–904.

Rogers, S.O., Bendich, A.J., 1989. Extraction of DNA from plant tissues. Springer, Plant molecular biology manual, pp. 73–83.

Sarker, S., Sultana, N., Aminuzzaman, F., 2017. Biochemical characterisation of Xanthomonas axonopodis pv. malvacearum isolated from infected cotton plant and it is in vitro sensitivity against some selected chemicals. Adv. Res., 1–10

Sebetha, E., Ayodele, V., Kutu, F., Mariga, I., 2010. Yields and protein content of two cowpea varieties grown under different production practices in Limpopo province, South Africa. Afr. J. Biotechnol. 9 (5), 628–634.

Shi, A., Buckley, B., Mou, B., Motes, D., Morris, J.B., Ma, J., Xiong, H., Qin, J., Yang, W., Chitwood, J., Weng, Y., Lu, W., 2016. Association analysis of cowpea bacterial blight resistance in USDA cowpea germplasm. Euphytica 208 (1), 143–155.

Shivachi, A., Kiplagat, K., Kinyua, G., 2012. Microsatellite analysis of selected Lablab purpureus genotypes in Kenya. Rwanda J. 28, 39–52.

Sikirou, R., Wydra, K., 2008. Effect of intercropping cowpea with maise or cassava on cowpea bacterial blight and yield. J. Plant Dis. Prot. 115 (4), 145–151.

Tan, H., Tie, M., Luo, Q., Zhu, Y., Lai, J., Li, H., 2012. A review of molecular makers applied in cowpea (*Vigna unguiculata* L. Walp.) breeding. J. Life Sci. 6 (11), 1190.

Jamal Uddin, M., Sami Ur Rashid, M.d., Begum, M.E.A., 2020. Adoption impact of improved cowpea variety in selected areas of chattogram district of Bangladesh. Int. J. Sustain. Agric. Res. 7 (1), 44–55.

Weng, Y., Qin, J., Eaton, S., Yang, Y., Ravelombola, W.S., Shi, A., 2019. Evaluation of seed protein content in USDA cowpea germplasm. HortScience 54 (5), 814.