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ORIGINAL PAPER

Resistance assessment and biochemical responses of maize genotypes against *Rhizoctonia solani f.* sp. *sasakii* Exner causing Banded leaf and sheath blight disease

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Abstract
Maize (*Zea mays* L.) is a staple food in many parts of the world. In addition to being used as grain, animal feed, production of corn ethanol, starch, syrup etc., it has been widely consumed as a vegetable in the form of baby corn and sweet corn. Banded leaf and sheath blight (BLSB) is one of the major diseases affecting maize and is caused by *Rhizoctonia solani* f. sp. *sasakii* Exner. In the experiment forty six maize inbreds were screened for their reaction against the BLSB pathogen following artificial inoculation. Two inbred lines namely, EV1417 and CML-323 produced resistant and moderately resistant disease reaction respectively. Biochemical studies indicated the presence of a high level of pre-infectional barriers in the resistant line. Further, higher level of defense related enzymatic activities of peroxidase and poly phenol oxidase were detected in the resistant inbred line. After 48 h of inoculation, in comparison to highly susceptible EV1428, electrolyte leakage was found to be stabilized in EV1417 and CML-323. This study clearly establishes the presence of effective pre-infection barriers and induced biochemical defence mechanisms operating in the resistant inbred line. The identified resistant line EV1417 is further being used in BLSB disease resistance breeding programmes.

Keywords Maize · BLSB · Resistance · Enzymatic activity · Inbreds

Introduction

Maize (*Zea mays* L.) is an important cereal crop, especially in the developing countries owing to its importance as food, feed and source of industrial products. Major researchable constraints that limit the productivity of maize include abiotic and biotic factors such as acid soils, water logging, downy mildews, post-flowering stalk rot, Turcicum leaf blight, banded leaf and sheath blight (BLSB), stem borers and weevils (Gerpacio and Pingali 2007). The BLSB caused by *Rhizoctonia solani* f. sp. *sasakii* Exner (Tel: *Thanatephorus sasakii* (Shirai) Tu and Kimbro), is a very destructive disease of maize gaining economic importance in several hot and humid tropical areas worldwide (Subedi 2015). It was first recorded from Srilanka (Bertus 1927). Presently, the disease is of common occurrence in Nepal, India, Bhutan, Bangladesh, Myanmar, Malaysia, Philippines, Thailand, Vietnam, Kampuchea, Laos, South China and Taiwan (Siva kumar et al. 2000). In India, its incidence has been documented from the states of Himachal Pradesh, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Rajasthan, Jharkhand, West Bengal, Meghalaya, Assam and Orissa (Akhtar et al. 2009, Rani et al. 2013). The pathogen spreads from basal sheath to developing ears causing extensive damage leading to premature drying of cobs (Kumar and Singh 2004). Losses of 10–40% have been estimated by Singh and Sharma (1976) in different cultivars by creating artificial epiphytotics.

Several management options inclusive of mechanical control through stripping of two lower leaves along with the leaf sheath (Sharma and Hembram 1990) and chemical control through foliar application of carbendazim, propicanazol, validamycine etc. (Sharma et al. 2002, Akhtar et al. 2010; Singh and Singh 2011) have been recommended for BLSB disease. However, longer persistence of pathogen inoculum (sclerotia) (Zachow et al. 2011) combined with several other practices like mono-cropping of compact hybrids, higher use of nitrogen fertilizer, high density planting etc. further

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aggravate the severity of the disease thus making it very difficult to manage (Payak and Sharma 1981). In this context, developing disease resistance cultivars provides a promising option for disease management in an effective, economical and environmentally safe manner (Khush and Jena 2009). Having reliable source of resistance is a prerequisite to develop disease resistant cultivars. Very few sources of resistance to BLSB have been reported. Further, the BLSB resistance has too complex nature of inheritance and high degree of genotype x environment (G × E) interaction to be used in maize improvement programmes (Prasanna et al. 2010). Thus, it is important to screen more germplasm to identify stable sources of resistance to BLSB.

Plants use a wide range of physiochemical mechanisms for survival against different challenges driven by biotic stresses like BLSB. These include pre-existing physical and chemical barriers that could be constitutively present in the plant irrespective of pathogen attack and a variety of defense mechanisms that are activated after pathogen attack (Huang et al. 2008). Depending on the genetic resistance present in the genotype, the expressions of different biochemicals vary. In this study, we investigated the reaction of maize inbred lines against *R. solani* f. sp. *sasakii* Exner both under field and controlled conditions. Further, we studied different biochemical changes in resistant, moderately resistant and highly susceptible maize inbreds upon infection with BLSB.

Materials and methods

Plant material

The plant material used in the experiment constituted 46 maize inbred lines. Out of 46 inbreds, 44 were provided by Winter Nursery Centre, ICAR-Indian Institute of Maize Research, Hyderabad, India and two inbreds namely, HKI-1105 and CML-323 were provided by Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (Table 1).

Inoculum preparation

Pure culture of *R. solani* f. sp. *sasakii* Exner was isolated from naturally infested maize plant in the farm of ICAR-IIVR. Typha (*Typha latifolia*) was collected from the water canal nearby where it was growing as a weed. Typha leaf cuttings of 2 cm were autoclaved twice at 24 h interval. The pure culture of the pathogen was inoculated on autoclaved leaf cuttings and incubated at 28 ± 2 °C for 20 days.

Table 1 Grouping of maize inbreds based on Tukey's Honest Significant Difference for BSLB resistance reaction under field inoculation conditions

S.No.	Genotype	Percent disease index	Disease reaction
1	EV1428	97.8 ^{a}	HS
2	EV1472	93.67 ^{ab}	HS
3	EV1474	93.67 ^{ab}	HS
4	EV1470	93 ^{abc}	HS
5	EV1409	93 ^{abc}	HS
6	EV1444	92.5 ^{abcd}	HS
7	EV1452	92 ^{abcd}	HS
8	EV1410	91.67 ^{abcd}	HS
9	EV1469	90.67 ^{abcde}	HS
10	EV1414	90.33 ^{abcdef}	HS
11	EV1457	90.33 ^{abcdef}	HS
12	EV1402	90 ^{abcdef}	HS
13	EV1443	89.33 ^{abcdef}	HS
14	EV1479	89 ^{abcdef}	HS
15	EV1445	88.67 ^{abcdef}	HS
16	EV1477	88.67 ^{abcdef}	HS
17	EV1485	88.33 ^{bcdef}	HS
18	EV1403	87 ^{bcdef}	HS
19	EV1407	87 ^{bcdef}	HS
20	EV1406	86.67^{bcdefg}	HS
21	EV1454	86.67 ^{bcdefg}	HS
22	EV1468	86.33 ^{bcdefg}	HS
23	EV1482	86.33 ^{bcdefg}	HS
24	EV1437	86 ^{bcdefg}	HS
25	EV1465	84.67 ^{bcdefg}	HS
26	EV1412	84.67 ^{bcdefg}	HS
27	HKI-1105	84 ^{cdefg}	HS
28	EV1450	84 ^{cdefg}	HS
29	EV1401	83.07 ^{defg}	HS
30	EV1447	83 ^{defg}	HS
31	EV1484	83 ^{defg}	HS
32	EV1471	82.67 ^{defg}	HS
33	EV1453	82.33 ^{defg}	HS
34	EV1416	82^{efg}	HS
35	EV1442	81.67 ^{efg}	HS
36	EV1455	81.67 ^{efg}	HS
37	EV1461	81.67 ^{efg}	HS
38	EV1446	81.33 ^{efg}	HS
39	EV1451	81.33 ^{efg}	HS
40	EV1478	81 ^{fg}	HS
41	EV1449	77.33 ^{gh}	S
42	EV1411	71.33 ^{hi}	S
43	EV1439	70.33 ^{hi}	S
44	EV1423	62.1^{i}	S
45	CML-323	44 ^{j}	MR
46	EV1417 (IIVRBC-13)	20.17^{k}	R

R = resistant, MR = moderately resistant, S = susceptible and HS = highly susceptible

Means with different superscript letters are statistically different at $p < 0.05$ based on Tukey's Honest Significant Difference

Note: Name given in parenthesis for EV1417 is ICAR-IIVR ID

Disease screening under field conditions

The research was conducted during monsoon season of 2017-18 at the research farm of ICAR-Indian Institute of Vegetable Research (ICAR-IIVR) (25°10'57.5"N 82°52'19.8"E), Varanasi, India. The experimental design fallowed was a randomized block design with three replications. Ten plants were inoculated in each replication. Row to row and plant to plant distance of 60 and 20 cm respectively, was maintained throughout. Inoculation was done on 60-days-old plants by inserting two infested leaf cuttings of typha grass in the 4th leaf sheath below the cob. Data concerning the appearance of disease was recorded 20 days post inoculation, practicing the scale of Wang and Dai (2001). The disease index (DI) was calculated by the following formula (Wang and Dai, 2001):

$$DI = \frac{\sum (\text{Severityscore} \times \text{No.ofplantatthislevel})}{\text{Totalnumberofobservations} \times \text{highestrating}} \times 100\%$$

Disease score	PDI	Symptom of disease	Reactions
0	0	No disease symptoms	I
1	0.1–20	Disease spots below 4th sheath under ear	HR
3	20.1–40	Disease spots below 3rd sheath under ear	R
5	40.1–60	Disease spots below 2nd sheath under ear	MR
7	60.1–80	Disease spots below 1st sheath under ear	S
9	80.1–100	Disease spots over sheaths under ear	HS

I = Immune, HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

Disease screening under controlled conditions

Based on filed evaluation, a total of nine genotypes were taken to test the disease resistance under controlled conditions. The genotypes included three highly susceptible lines namely, EV1410, EV1428 and EV1455; four susceptible lines namely, EV1449, EV1411, EV1439 and EV1423; one moderately resistant genotype, CML-323; and one resistant genotype, EV1417. Autoclaved soil was used for filling the pots. Two seeds each of the inbred line were sown in a pot. Three pots per replication and three replications were taken up and all the pots were kept in the green house. Inoculation was done using the same method as described above. The plants were kept in a growth chamber under a temperature of 28 ± 2 °C. Humidity of 90 ± 5 °C was maintained with the help of a humidifier (Chen et al. 2013).

Estimation of biochemical parameters

A total of seven biochemical parameters were studied in three germplasm namely, EV1417 (resistant), CML-323 (moderately resistant) and EV1428 (highly susceptible). Three parameters (lignin content, sugar content and phenol content) were studied prior to inoculation and remaining four parameters (protein content, peroxidase activity, polyphenol oxidase activity and electrolyte leakage) were studied during infection period at 0, 24, 48, 72, 96 and 120 h post inoculation. For all biochemical parameters, five biological replicates were examined.

Lignin content

Lignin content was estimated by the Klason method (Emmanuel et al. 2018). Leaf samples collected from EV1417, CML-323 and EV1428 were dried in a hot air oven at 50 °C for 24 h. The dried samples were ground and sieved through 20 mm mesh sieve. Sieved powder of 200 mg was transferred into Erlenmeyer flask and 2 mL of 72% H_2SO_4 was added. Later the sample solution was incubated in water bath at 30 °C for 1 h with frequent stirrings. Distilled water of 56 mL was added to the sample solution and autoclaved at 120 °C for 1 h. The autoclaved, hot, sample solution was filtered through a fritted glass crucible and washed with hot water. The crucible along with its residue was dried at a constant temperature of 105 °C in hot air oven, overnight. The amount of lignin content in the leaf sample was calculated with the following formulae:

$$\%\ \text{ligninweight} = \frac{\text{Driedligninsampleweight(g)} \times 100}{\text{Driedsampleweight(g)}}$$

Total soluble sugar content

Total soluble sugar content was estimated following the phenol-sulphuric method (Krishnaveni et al. 1984). Leaf samples of 100 mg were added to 5 mL of 2.5N HCl in glass tubes to hydrolyze them by keeping it in a boiling water bath for 3 h. The tubes were taken out and cooled down at room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceases. Later, the volume was made up to 100 mL with water and centrifuged. A volume of 0.1 mL solution was taken and made up to 1 mL. Further, 1 mL phenol solution and 5 mL of 96% sulphuric acid were added and shaken well. After 10 min, the content in the tubes was placed in a water bath at 25 to 30^0C for 20 min. The absorbance was recorded at 490 nm against the blank control and percent carbohydrate on dry weight basis in the sample was calculated using the standard curve derived from glucose.

Total phenol content, Peroxidase enzyme activity and Polyphenol oxidase enzyme activity

Leaf samples of 200 mg were homogenized and crushed in 10 mL ice-cold phosphate buffer (0.1M, pH 6.5) in a pre-chilled mortar-pestle. The homogenized extract was centrifuged at 2 °C at 10,000 rpm for15 min. The clear supernatant was collected in fresh tubes and used for estimation of phenol content, peroxidase and poly phenol oxidase enzyme activities.

Total phenol content

2 mL of plant extract was taken out and kept at room temperature than 2 mL ethanol was added. The tubes were kept in a boiling water bath to facilitate evaporation of all the liquid. The dried residue was dissolved in 5 mL distilled water to prepare aqueous extract. The final assay was performed by mixing 1.0 mL aqueous extract, 2.5 mL distilled water, 0.5 mL Folin-Ciocalteau reagent (1:1 diluted) and 1 mL 35 per cent Na_2CO_3. The tubes were shaken well and allowed to stand at room temperature for 1 h along with the blank. The intensity of blue color was read at 650 nm and content of total phenol (mg/g of fresh wt.) was estimated using standard curve prepared from Catechol (Mallick and Singh 1980).

Peroxidase enzyme activity

The enzyme activity was measured by adding 0.1 mL of plant extract to 3 mL of 0.05 M guaiacol prepared in 0.1 M potassium phosphate buffer (pH 6.5). The reaction was initiated by addition of 0.1 mL 0.8 M H_2O_2, and absorbance was recorded at 470 nm for 3 min at an interval of 30 s. The reaction mixture without enzyme was used as blank. The unit of enzyme activity was expressed as the change in absorbance min^{-1} g^{-1} fresh weight (Shannon et al. 1996).

Polyphenol oxidase enzyme activity

The enzyme activity was measured by adding 0.1 mL of plant extract to 2.5 mL of 0.01 M catechol (in 0.1 M phosphate buffer, pH 6.0). The change in absorbance was recorded at 495 nm after every 30 seconds for 3 min (Bastin and Unluer 1972). The unit enzyme activity was expressed as change in absorbance min^{-1} g^{-1} fresh weight.

Total protein content

Estimation of the total protein was performed employing Lowry's method (Lowry et al. 1951). Leaf samples (0.5 g) were ground in cold extraction buffer. The homogenized samples were centrifuged at 15,000 rpm for 10 min at 4 °C and the supernatant was separated. 0.1 mL of supernatant was taken and the volume was made up to 1 mL by adding 0.9 mL distilled water. Then 5 mL alkaline copper solution was added and mixture was incubated for 10 min at room temperature. Later 0.5 mL Folin-Ciocalteu reagent was added and incubated for 30 min in dark at room temperature. Absorbance was read at 660 nm against blank and protein content (mg/100 g of leaf sample) was determined using standard curve derived from Bovine Serum Albumin (BSA).

Electrolyte leakage

The electrolyte leakage was determined according to Lima et al. (2002). A total of 22 leaf discs (8 mm in diameter) were collected from the leaves per replication. The leaf discs were thoroughly washed in deionized water immediately after being sampled. Then, the leaf discs were left to float on 60 ml of deionized water in a sealed glass for 4 h at 25 °C. After this period, the first value of conductivity (reading one) was obtained using a conductivity meter. Next, the vials were transferred to an oven for 2 h at 90 °C to obtain a new value for conductivity (reading two). The Electrolyte Leakage was obtained by dividing the value of reading one by the value of reading two and expressed as percentage.

Statistical analysis

The data obtained was analyzed in *Agricolae* package in a computing environment R v 30,102 (R Core Team 2017) to get analyses of variance (ANOVA) and multiple comparison test based on Tukey's Honest Significant Difference'.

Results

Reaction of maize genotypes to BLSB Disease

After inoculation, nearly all maize inbred lines showed BLSB symptoms under both field and controlled conditions. Analysis of BLSB disease index among inbreds showed that a significant difference exists among the maize inbreds included in the study. Based on Tukey's Honest Significant Difference, maize inbreds were classified into significantly different groups (Tables 1 and 2). None of the genotypes showed either an immune or highly resistant reaction to the disease. Inbred line EV1417 gave resistant reaction while inbred line CML-323 showed moderately resistant reaction. Inbreds namely, EV1411, EV1423, EV1439 and EV1449 produced susceptible reaction whereas the remaining lines gave highly susceptible reaction. Under controlled conditions, only EV1417 gave resistant reaction. Maize inbreds, EV1423

Table 2 Grouping of maize inbreds based on Tukey's Honest Significant Difference for BSLB resistance reaction under controlled conditions

S.No.	Genotype	Percent disease index	Disease reaction
1	EC1428	95.67[a]	HS
2	EC1410	95[a]	HS
3	EV1439	90.67[ab]	HS
4	EV1455	88.67[abc]	HS
5	EV1449	84[bc]	HS
6	EV1411	82.67[c]	HS
7	EV1423	50[d]	MR
8	CML-323	42.33[d]	MR
9	EV1417	22.33[e]	R

R = resistant, MR = moderately resistant and HS = highly susceptible

Means with different superscript letters are statistically different at $p < 0.05$ based on Tukey's Honest Significant Difference'.

and CML-323 developed moderately resistant reaction while other genotypes under controlled conditions showed highly susceptible reaction.

Estimation of biochemical parameters

Total phenol, lignin and sugar content

In this study, phenol, lignin and sugar content were estimated prior to pathogen inoculation (Fig. 1). Comparatively, resistant inbred line EV1417 had the highest total phenol content (mg/g fresh weight) followed by moderately resistant CML-323 and the lowest content was found in highly susceptible EV1428. A similar trend was found in case of percent lignin content on dry weight basis i.e., the highest lignin content was present in EV1417 followed by CML-323 and the lowest in EV1428. On the other hand, sugar content (percent soluble sugar) followed a reverse trend with the highest sugar content present in EV1428 then followed by CML-323 and EV1417.

Response of defence related enzymes peroxidase and polyphenol oxidase

The activity of both the enzymes increased continuously up to 72 h post inoculation, in all the genotypes. The rate of increase of enzyme activity was quite linear for polyphenol oxidase in comparison to peroxidase (Figs. 2 and 3). For both enzymes, the rate of increase in enzyme activity was highest in EV1417 followed by CML-323 and EV1428. Activity of both the enzymes started to decrease from 72 h post inoculation in all the genotypes.

Protein content

In un-inoculated plants, the protein content for EV1428 plants was significantly lower than that for EV1417 and CML-323 plants. In inoculated EV1428 plants, protein content decreased continuously with time. In CML-323 plants, the percent protein content increased from the time of inoculation to 72 h after inoculation and started declining from 72 h up to 120 h after inoculation. A sharp increase in the percent protein content was observed within 24 h in EV1417 after inoculation. The higher protein content was maintained up to 48 h after inoculation, started decreasing up to 72 h and then started increasing from 72 h onwards up to 120 h after inoculation (Fig. 4).

Electrolyte leakage

During the initial infection period, electrolyte leakage increased in all the three inbred lines. With the progress of the disease, electrolyte leakage increased continuously in susceptible EV1428 inbred line. In CML-323, electrolyte leakage increased up to 48 h after inoculation which decreased with time. In EV1417, electrolyte leakage shot up very fast in between 24 and 48 h after inoculation, decreased sharply from 48 to 72 h, it remained stable from 72 to 120 h and was higher than CML-323 and lower than EV1428 at 120 h after inoculation (Fig. 5).

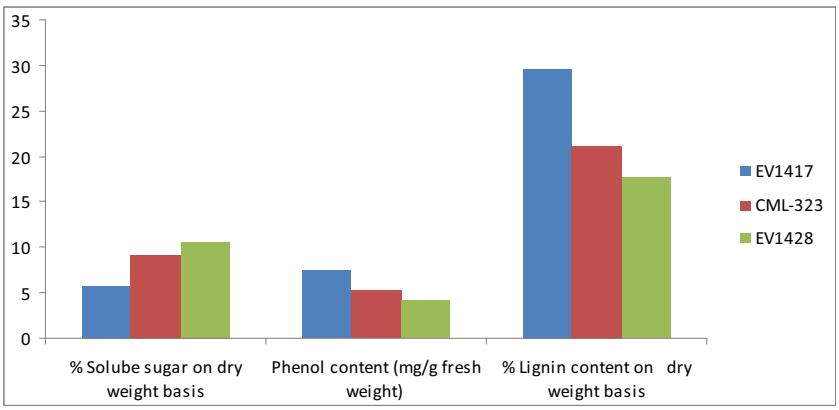

Fig. 1 Comparative content of total phenol, lignin and sugar among the maize inbred lines

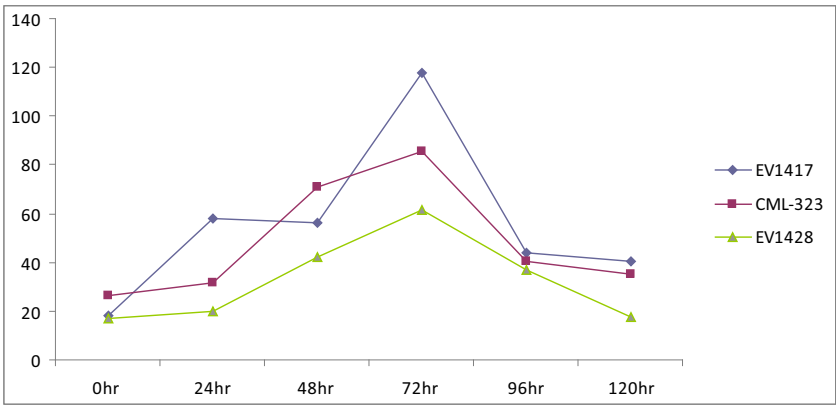

Fig. 2 Compariative peroxidase activity (min − 1 g − 1 fresh weight) in the maize inbred lines

Discussion

The banded leaf and sheath blight (BLSB) caused by *R. solani f.* sp. *sasakii* Exner is a very destructive disease of maize. Non-availability of widely adapted and stable source of resistance to BLSB is one of the main limiting factors for its resistance breeding. The present study was conducted to identify resistance sources for BLSB and to study changes in some biochemical parameters in maize leaves infected with the fungus.

Consequently, field inoculation technique was used to evaluate resistance reaction of the 46 maize inbred lines. Field inoculation technique allows satisfactory disease epiphytotic development and is widely used for the evaluation of a large number of germplasms in the field (Ahuja and Payak 1981). Further, results of field evaluation were reconfirmed through further screening of some selected inbreds under controlled conditions. Inserting the typha cutting with pathogen at junction of sheath and leaf can provide optimum level of inoculum and do not fall away with strong wind or heavy rain. Inoculating with *Typha* cuttings was easy and fast.

Under both field and controlled conditions, inbred line EV1417 produced resistant reaction while inbred line CML-323 displayed a moderately resistant reaction. EV1423 gave susceptible reaction under field conditions whereas under controlled conditions it gave moderately resistant reaction against the disease. This change in disease reaction in EV1423 may be due to difference in G × E interaction. In case of BLSB, limited variation for its resistance in maize (Sharma et al. 2002) is the main limiting factor for an effective resistance breeding programme. National programmes in different countries like India, China, Indonesia, and Philippines are making efforts towards screening for BLSB resistance. In India, the All India Coordinated Research Project (AICRP) on maize evaluated both inbreds and hybrids for their reaction against BLSB and identified lines with a moderate level of resistance (Sharma et al. 2003; Sharma et al. 2005; Anshu et al. 2007; Madhavi et al. 2012). In addition to limited variation for the disease resistance in maize, complex nature of inheritance has been reported. Zhao et al. (2006a, b) from China reported four resistance QTLs accounted for only 3.72–10.35% of the phenotypic variation responsible for the disease resistance. Garg et al. (2009) from India reported three QTLs governing BLSB resistance with significant epistatic interactions.

Like many plant species, maize has pre-existing physical, chemical barriers and diverse arrays of defense mechanisms

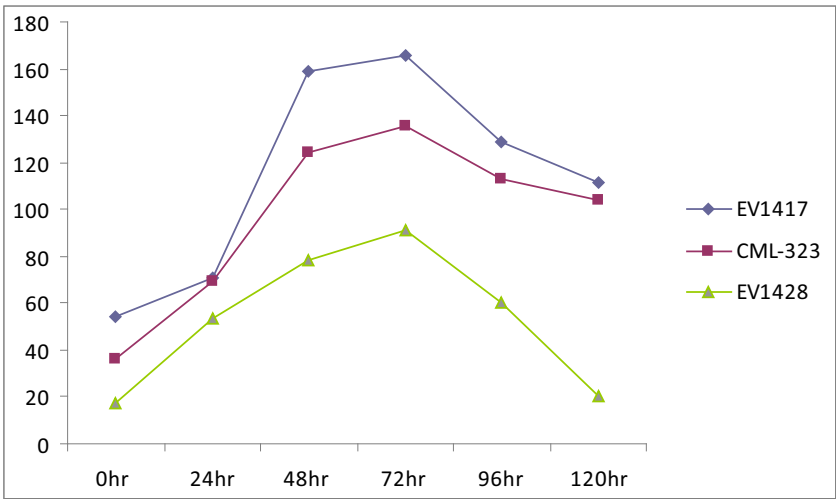

Fig. 3 Comparative polyphenol oxidase activity (min − 1 g − 1 fresh weight) in the maize inbred lines

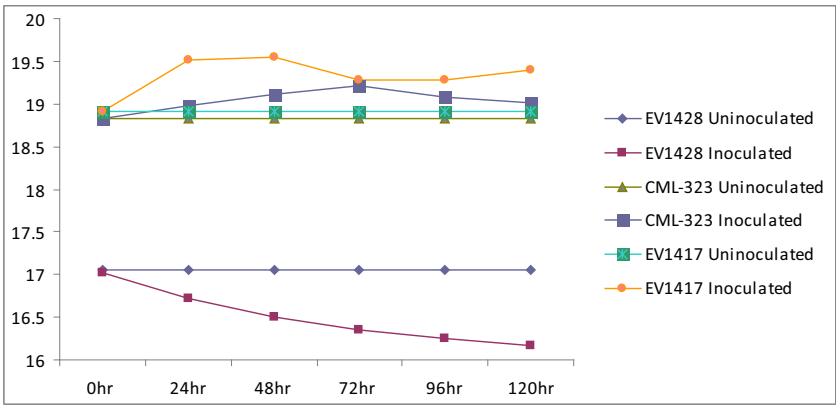

Fig. 4 Changes in protein content (mg/100 g sample) in un-inoculated and inoculated leaves

activated during a pathogen attack (Huang, et al. 2008) and influence disease reaction of different genotypes. Role of different cell wall components in disease resistance have been reported by a number of researchers (Bell 1981; Friend 1981; Nicholson and Hammerschmidt 1992; Matern et al. 1995). Presence of lignin and phenols in host cell walls acts as a physical barrier against fungal penetration (Matern et al. 1995; Grabber et al. 1998). Higher phenol and lignin contents in resistant EV1417 might have acted as a barrier against fungal penetration. When a plant tissue is low in sugar, it becomes more susceptible to certain diseases and less susceptible to others (Horsfall and Diamond 1957). In the present study, resistant genotypes recorded lower sugar levels and susceptible genotypes have higher sugar levels indicating BLSB to be a high-sugar disease. Even though, sugars have a role as signalling molecules in defence pathways, (Morkunas and Ratajczak, 2014) a high-sugar level does not always boost the immune system in plants. *R. solani* is a necrotrophic fungus with a wider host range. Data on metabolic processes that promote necrotrophic fungal development in plant tissues is scarce (Dulermo et al. 2009). In case of necrotrophic fungi, there is no proven general trend suggesting that, higher carbohydrate levels in host plants lead to either more resistant due to enhanced defence or conversely susceptible due to higher sugar availability for the pathogen (Lecompte et al. 2017).

Different studies on biochemical changes during pathogenesis revealed changes in production levels of enzymes like peroxidase, polyphenols (Mondal et al. 2012; Jiang et al. 2009). In all three inbreds, the activity of both defense-related enzymes (peroxidase and polyphenol oxidase) increased continuously up to 72 h after inoculation which may be due activation of pattern triggered immunity (PTI) (Zipfel 2009). The rate of increase in their enzymatic activity was observed to be higher in resistant inbred lines. Post 72 hours after inoculation the enzymatic activity decreased in all inbreds irrespective of level of resistance but resistant inbred EV1417 maintained a higher level of activity in comparison to susceptible and moderately resistant inbreds. Increase in peroxidase activity was reported when maize tissues were challenged by *R. solani* (Li et al. 2009). From the results it may be interpreted that, in EV1417 and CML-323, the increase in activity of defence enzymes was above the required thresh hold for effective activation of down stream defence activities leading to effective contain meant of the pathogen. The difference between the genotypes with respective PO, PPO and protein content makes them differ in the disease reaction also. The oxidative enzymes were shown to increase their toxicity by oxidizing phenolics and related compounds. Theses enzymes play active role in inhibiting mycelial elongation, penetration, colonization and in spore producing fungi, which may inhibit spore germination too (Usenik et al. 2004). These enzymes are well

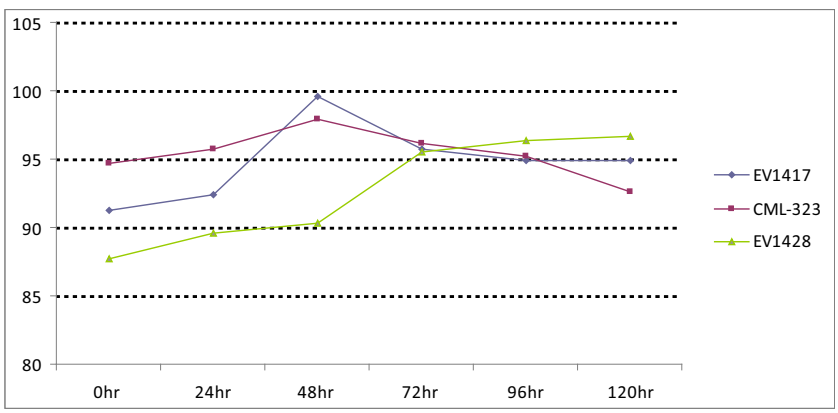

Fig. 5 Changes in electrolyte leakage after inoculation

known for their association with the browning of host tissues (Khan et al. 2001).

Once exposed to any kind of stress, whether biotic or abiotic, plants respond by producing higher levels of defense related proteins (Broz et al. 2010). To recognize pests and counter their attacks, plants employ active, passive or both defence mechanisms (Houterman et al. 2008). In both mechanisms, proteins play a greater role in plant defense against the invading pathogen. In consequence, susceptible inbred EV1428 has low protein content than the resistant inbreds (EV1417 and CML-323) and its protein content continuously decreased after inoculation. In resistant genotypes, the protein content increased after inoculation illustrating the synthesis of new proteins involved in disease resistance mechanisms. Among different proteins, resistance proteins are the most effective weapons plants possess against pathogen invasion as they can recognize the corresponding pathogen effectors or associated proteins to activate plant immune response (Demissie 2017).

Electrolyte leakage indicates stress response of plant tissues. This test is widely used as an indicator of plant stress tolerance including pathogen attack (Blatt et al. 1999). Susceptible inbred had greater electrolyte leakage than resistant inbreds. Recovery from the electrolyte leakage during the infection period was observed in resistant germplasm indicating the active role of the host in the defence. Further, by reducing the electrolyte leakage, availability of nutrients to the pathogen was also checked which further reduced growth and development of the pathogen (Cqmstock and Schefer 1971).

Conclusions

The most important manifestation from this work was the identification of BLSB resistant maize inbred line EV1417. The genotype showed resistance reaction in field epiphytotic conditions as well as under controlled conditions. Further, biochemical studies revealed the presence of high level of pre-infectional barriers and activation of post infectional defence-related activities in the resistant maize inbred. The identified resistant line is being used in the development of different populations to study genetics involved in the resistance mechanism and simultaneously transfer the resistance to susceptible superior sweet corn genotypes.

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Compliance with ethical standards

Conflict of interests Authors declares that they have no conflicts of interest in this publication.

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