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Biochemical basis of resistance in different varieties of maize for their relative susceptibility to Northern Corn Leaf Blight (NCLB)

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Abstract

Northern Corn Leaf Blight (NCLB) is most devastating foliar disease of maize in Himalayan region of the world, caused by fungus *Exserohilum turcicum*. Thirty maize genotypes were planted in experimental field of Department of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot, during spring 2017. Disease severity of maize genotypes was assessed by 0-5 points scale. Data on biochemical and antioxidant (Phenolic and flavonoids) traits were recorded. The results showed that genotype Karamat-Bar-25, SZP-13200 had disease rating of 5R followed by NCEV-1530-11 (10R) and marked as highly resistant genotypes while Soan-3, Ghuari-122 had disease rating of 90S followed by Kissan-60 (80S) and evaluated as highly susceptible genotypes within the germplasm. The biochemical assay showed that moisture contents ranged from 6.2%–20.8%, crud protein (3.2%–2.4%), crude fiber (2.96%–26.46%), ash contents (0.13%–4.33%), fat contents (1.1%–4.53%), carbohydrate content (44.08%–85.79%) and total energy (66.49%–320.61% while Antioxidant assay showed that DPPH antioxidant activity ranged 8.8 –29.22%, phenolic contents (17.4 –38.8 mg GAE/g) and flavonoids content (2.59–36.49 GAE/g) respectively. The biochemical changes in resistant and susceptible maize genotypes showed that the biochemical constituents was high in resistant genotypes as compared to infected, while antioxidant scavenging power was also high in resistant genotypes, due to infection it increased drastically. From the current results, it has been hypothesized, that the biochemical-constituents and antioxidant can play role in better metabolic response as it prevents the allocation of metabolic resources to actively defend against the pathogen.

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Introduction

Maize (*Zea mays* L.) is one of the most economically valuable staple food crop of the world (Brutnell *et al.*, 2015). It is the third important cereal crop after wheat and rice in Pakistan (Rahman *et al.*, 2015). The total production and yield of maize grown in various parts of Pakistan is 3.13 million tons and 3264 kg per hectares respectively (Qamar *et al.*, 2017). Maize is an ironic source of vitamins, minerals and dietary fiber (Ullah *et al.*, 2010).

It is reported that maize grains have moisture contents (11.6%-20.0%), ash content (1.10%-2.95%), protein content (4.50%-9.87%), fat content (2.17%-4.43%), fiber content (2.10%-26.70%) and carbohydrates contents of (44.60%-69.60%) (Enyisi *et al.*, 2014a; Ullah *et al.*, 2010). The maize composition has wide difference among its various species and subspecies, which may be due to diverse environmental, topographical and pathological stresses.

Among the various diseases damaging the maize crop, Northern Corn Leaf Blight is one of the most important diseases in maize growing areas of the world. Earlier, this disease was considered as minor, but now it attains the status of major disease in world. Epidemics of NCLB at an early stage causing death of premature blighted leaves and lose their nutritional value as feed and fodder.

Most plants produce a broad range of secondary metabolites and nutrients that are toxic to pathogens, either as part of their normal growth and development or in response to certain biotic stresses. It has been well documented in various path systems that antioxidants like phenolic and flavonoid compounds or physio-chemical barrier, i.e. moisture contents, sugar, carbohydrates, proteins can play an important role in disease resistance, thus preventing colonization of fungus in plant tissues. Rapid accumulations of phenolic compounds at the infection site allow the activation of antioxidants or other stress related substances and slow the growth of the pathogen. However, no single component is

sufficient to determine the resistance in plants since, it is the complex phenomenon. An active role of secondary metabolites and antioxidants in expression of resistant reaction in various crops was reported.

The target should be to identify the biochemical compounds involved in resistance, in order to use them as molecular markers in plant breeding programs or to design appropriate control strategies. Differences in biochemical components, i.e. DPPH antioxidant activity, total phenolic contents, flavonoids contents and other secondary metabolites have been used as markers for preliminary selection of various plants species resistant to different pathogens. These components have been correlated with the defense activities against pathogens in plant species (Thilagavathi *et al.*, 2007).

Development of resistant varieties with high phytoconstitutes is the most suitable approach to control the diseases. Generally, resistance in plants against different diseases directly correlated with various biochemical substances such as carbohydrates, sugars, chlorophyll, proteins and antioxidants. (Rashmiet *et al.*, 2017).

However, information on relationship between resistance to Northern Corn Leaf Blight and biochemical parameter in maize is very scanty.

Therefore, the present investigations were started with aimed to identify biochemical parameters associated with different gradients of maize resistance to Northern Corn Leaf Blight.

Materials and methods

Field screening against Northern Corn Leaf Blight (NCLB) was carried out in the experimental field of Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, The University of Poonch Rawalakot. Rawalakot (Latitude 33°51'32.18"N, Longitude 73° 45'34.93"E, Elevation 5500 ft). The research material was comprised of 30 maize genotypes. All the cultural practices were

applied uniformly and evenly to all the experimental units to minimize the experimental error. Data was recorded at different time of growth stages for disease assessment.

Reaction and scoring of disease

All the leaves on infected plants were scored using 0-5 scale adopted by maize pathology unit CIMMYT (2004) as

0 = no visible lesion

1 = one to few scattered lesion on leaves covering up to 10% of leaf area

2 = lesions on leaf covering 11- 25% leaf area

3 = lesions on leaf covering 26-50% leaf area

4 = lesions abundant on leaf covering 51-75% leaf area

5 = lesions abundant on almost all leaf, plant prematurely dried with 76-100% leaf area covered.

According to scale 0 mean complete resistant and 5 being complete susceptible. Based on this rating scale, maize genotypes were classified into four groups namely, resistant (R) genotypes with a score < 2.0; moderately resistant (MR) 2.1-3.0; moderately susceptible (MS) 3.1-3.5 and highly susceptible (S) > 3.5.

Bio-chemical Assay

Bio-chemical constituents in maize genotypes were estimated using the methods described by association of official analytical chemist (AOAC, 2005).

Sample

Maize sample (seed) was dried at 55 °C in the oven. Mortar and pestle is used to grind seeds into powder form. *Zea mays* seed's powder was used for extract formation.

Determination of moisture contents:

Moisture contents were calculated using formula

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{\text{weight of original sample}} \times 100$$

Where, W₂ = weight of sample before drying and W₃ = weight of sample after drying.

Determination of crude protein

Calculations of protein contents was done as follows
Percent protein = % N × Protein factor, Protein factor for wheat flour = 5.7.

Determination of crude fibers

Crude fiber was calculated as per formula described by William and Straky, 1982.

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{\text{weight of original sample}} \times 100$$

Where, W₁ = weight of crucible before ignition, W₂ = weight of crucible after ignition.

Determination of ash

Ash contents was calculated as follows

$$\text{Ash (\%)} = \frac{\text{weight of sample after ashing}}{\text{weight of original sample}} \times 100$$

Crude fats determination

The percentage of crude fat was calculated in the following formula:

$$\text{Crude fat (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where, W₂ = Weight of beaker after fat extraction, Weight of empty beaker = W₁ and Weight of original sample = W₃.

Determination of carbohydrate contents

Carbohydrate contents were measured by following formula. Carbohydrate (%) = 100 - (% moisture + % ash + % protein + % fats + % fibers).

Determination of total energy

Total energy was calculated as follows.

Total energy = % proteins×4 + % fats×9 + % carbohydrates×4

Antioxidant scavenging assay.

Estimation of antioxidative activity by DPPH

The antioxidant activity was measure using the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical as described by Hatano *et al.*, (1988).

The activity was expressed as percentage scavenging of DPPH by the extracts calculated as:

$$\text{DPPH Scavenging activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Estimation of total phenolic compounds:

The total phenolic compounds were analyzed using the FolinCiocalteu method with some modification (Ghafoor and Choi, 2009).

Estimation of total flavonoid compounds

The total flavonoid content was determined with aluminium chloride (AlCl₃) according to a known method (Kosalecet *al.*, 2004) using quercetin as a standard.

The total flavonoid contents were expressed in milligram of quercetin equivalents/g extract.

Statistical analysis

Mean separation was done based on the LSD at the 5% probability level. Simple statistics and numerical taxonomic techniques were utilized for data analysis with the help of computer software 'Statistica' (www.statsoft.com).

Results and discussion

Reaction and scoring of Disease

Under field conditions the genotypes Kramat-Bar-25, SZP-13200 and NCEV-1530-11 displayed the disease severity of 5R, 5R, 10 R respectively and showed resistance type reaction against Northern Corn Leaf Blight.

While genotypes Soan-3, Ghuari-122, Kissan-60 and Iqbal-68 showed susceptible type response and reaction with rating values 9oS, 9oS and 8oS, respectively.

Table 1. Response of maize genotypes with various susceptibility to NCLB.

O	Highly resistant	No visible infection.
R	Resistant.	Necrotic areas with or without minute uredia.
MR	Moderately resistant	Small uredia present surrounded by necrotic areas
MS	Moderately susceptible	Medium uredia with no necrosis but possibly some distinct chlorosis.
S	Susceptible	large uredia and little or no chlorosis present severity

Genotypes Jalal-29, Sarhad White-27, NCEV-1530-1 NCEV-1530-3 NCEV-1530-6, NCEV-1530-7, NCEV-1530-10, NCEV-1530-12, NP-1, NP-2 showed moderately susceptible type reaction. Whereas genotypes Azam-140, Sadaf-141, Golden-199, Pahari-68, Aziz-2003, NCEV-1530-2, NCEV-1530-4, NCEV-1530-5, NCEV-1530-9, NCEV-1530-12 NARC-W, NP-3, TP-1217, Rakaposhi showed moderately resistant reaction under field screening conditions (Table 2).

Bio-chemical studies

Maize is credible source of variety of nutritional constituents like proteins, carbohydrates, minerals, vitamins and antioxidant compounds including phenolic and flavonoids. Moisture content of 30 maize genotypes is shown in Table 3. The percentage moisture content was in the range of 6.2–20.8%. Maximum moisture content was shown by genotype

Soan-3 (20.8 %), Ghuari-122 (20.8%) and Kissan-60 (19.9%) whereas minimum was shown by genotypes Karamat-Bar-25, NCEV-1530-11 and SZP-13200 (6.2%), (7.8%) and (8.9%) respectively.

The resistant genotypes against Northern Corn Leaf Blight showed minimum moisture content whereas genotypes which are susceptible towards NCLB indicated high value for moisture contents. The moisture content of maize genotypes .obtained for this study varies from genotype to genotype with various gradient to NCLB susceptibility, which similar to the findings of Enyisi *et al.* (2014) who reported a similar value of 11.6% – 20%) moisture contents of maize grown in Nigeria. The moisture content of the maize in the current study is consistent and slightly higher in susceptible genotypes than the resistant genotypes.

Table 2. Disease Scoring and Disease Reaction.

Sr. no.	Genotypes	SD	RD	Sr. no.	Genotypes	SD	RD
1	Azam-140	50	MR	16	NCEV-1530-5	40	MR
2	Ghuri-122	90	S	17	NCEV-1530-6	60	MS
3	Jalal-29	80	MS	18	NCEV-1530-7	60	MS
4	Kissan-60	80	S	19	NCEV-1530-9	20	MR
5	Sadaf-141	60	MR	20	NCEV-1530-10	40	MS
6	Sarhed white-27	90	MS	21	NCEV-1530-11	10	R
7	Golden-199	50	MR	22	NCEV-1530-12	15	MR
8	Iqbal-78	20	MR	23	NARC-W	30	MR
9	Karamat Bar-25	5	R	24	NP-1	80	MS
10	Pahari-68	50	MR	25	NP-2	40	MS
11	Aziz-2003	10	MR	26	NP-3	25	MR
12	NCEV-1530-1	70	MS	27	TP-1217	60	MR
13	NCEV-1530-2	60	MR	28	SZP-13200	5	R
14	NCEV-1530-3	40	MS	29	Rakaposhi	20	MR
15	NCEV-1530-4	20	R	30	Soan-3	90	S

SD = Disease scoring and RD = disease response.

The lower moisture content is important as it enables long storage by minimizing fungal contamination and spoilage of the maize and maize products. Results showed that the percentage crude protein was in the range of 3.2– 2.4%. Maximum crude protein was observed in resistant genotype SZP-13200 was 12.4%, Karamat-Bar-25 (11.8%) and NCEV-1530-11 (11.2%) and minimum crude protein was shown by susceptible genotypes i.e. Soan-3 (3.2%), Ghuari-122 (4.1%) and Kissan-60 (4.3%) respectively. Similar result was reported by Micic *et al.* (2015) as protein of 13 maize populations within the range of 10.58%–12.45%.

The percentage crude fiber was in the range of 2.96–26.46%. Maximum crude fiber value in genotype Karamat-Bar-25 was (26.46%), NCEV-1530-11 (25.22%) and SZP-13200-11 (25.17%) respectively. While minimum crude fiber value was shown by genotypes Soan-3 (2.96%), Ghuari-122 (3.52%) and Kissan-60 (6.43%) respectively. Similar results were reported by Qamar *et al.* (2017) in white maize flour crude fiber was in range of 7.82 – 12.02%. Percentage ash content was in the range of 0.13 – 4.33%. Maximum ash content was observed in genotype SZP-13200-11 was (2.8%), Karamat-Bar-25 (2.33%) and

NCEV-1530-11 (2.2%) and minimum was observed in genotypes Soan-3 (0.13%), Kissan-60 (0.25%) and Ghuari-122 (0.68%) respectively. Current results are supported by Qamar *et al.* (2017), who reported ash content of maize flour within the range of 1.4–2.6%. Fat content of 30 maize genotypes is shown in Table 3. The percentage fat content was in the range of 1.1–4.53%. Maximum fat content was observed in genotypes NCEV-1530-11 (4.53%) SZP-13200-11 (4.49%) and Karamat-Bar-25 (4.41%) while minimum fat content value was shown by genotypes Soan-3 (1.17%), Ghuari-122 (1.23%) and Kissan-60 (1.1%).

The current results are supports by finding of Kataria (2014), according to their results fat contents was in range of 0.9 – 4.47%. Percentage carbohydrate content was in the range of 44.08–85.79%. Maximum carbohydrate content value in genotype Karamat-Bar-25 was (85.79%), SZP-13200-11 (77.45%) and NCEV-1530-11 (70.77%) respectively. The genotypes which are susceptible towards NCLB showed minimum carbohydrate content value as Soan-3 (44.08%), Kissan-60 (44.52%) and Ghuari-122 (44.83%). Similar results were reported by Ndukwe (2015). Proximate composition shows carbohydrate content in the range of (68.73±0.05e -72.17±0.01 a).

Table 3. Bio-chemical constitutes in maize genotypes with various susceptibility towards Northern Corn Leaf Blight.

Genotypes	MC	CP	CF	AC	FC	CC	TE	AA	PC	fc
Azam-140	16.4±0.49	6.5±0.17	23.22±0.68	1.47±0.88	2.25±0.67	50.16±0.89	246.89±0.04	22.53±0.08	23.3±0.37	21.45±0.12
Ghauri-122	20.8±0.11	4.1±0.37	3.52±0.23	0.68±0.37	1.23±0.66	44.83±0.67	89.34±0.04	11.44±0.77	17.4±0.11	2.59±0.23
Jalal-29	17.9±0.67	5.6±0.69	23.11±0.19	1.45±0.37	2.4±0.66	49.54±0.44	242.16±0.88	26.46±0.09	23.8±0.66	17.22±0.66
Kissan-60	19.9±0.04	4.3±0.48	6.43±0.67	0.25±0.58	1.1±0.05	45.52±0.45	77.23±0.08	12.12±0.04	18±0.38	5.65±0.03
Sadaf-141	16.3±0.67	6.4±0.13	20.44±0.99	1.55±0.22	1.13±0.56	54.18±0.34	252.49±0.03	28.96±0.44	21±0.03	9.85±0.14
Sarhed white-27	16.7±0.89	6.9±0.79	18.43±0.45	1.12±0.37	1.97±0.04	55.87±0.26	268.81±0.87	24.46±0.54	22.5±0.35	27.51±0.06
Golden-199	16.8±0.62	7.1±0.78	19.45±0.33	1.58±0.99	2.5±0.03	50.13±0.28	251.44±0.29	16.54±0.66	26.7±0.48	20.18±0.07
Iqbal-78	16.4±0.34	6.3±0.57	16.48±0.56	1.21±0.35	3.41±0.49	55.2±0.38	276.69±0.37	14.08±0.56	23.3±0.38	19.56±0.47
Karamat Bar-25	6.2±0.47	11.8±0.87	26.46±0.23	2.33±0.27	4.41±0.45	85.79±0.28	320.61±0.03	29.4±0.08	38.8±0.38	36.49±0.23
Pahari-68	17.4±0.21	6.8±0.36	11.45±0.12	1.47±0.38	3.11±0.67	60.78±0.09	291.31±0.05	26.4±0.03	23.3±0.48	15.76±0.02
Aziz-2003	16.3±0.27	6.9±0.39	10.46±0.89	1.48±0.28	2.55±0.34	60.78±0.02	293.67±0.45	16.9±0.06	23.2±0.04	26.93±0.05
NCEV-1530-1	16.7±0.68	9.2±0.58	21.77±0.09	1.33±0.22	2.32±0.22	45.68±0.09	244.4±0.67	22.88±0.67	24.5±0.37	16.93±0.09
NCEV-1530-2	14.9±0.19	9.6±0.96	22.76±0.78	1.29±0.11	2.11±0.52	47.34±0.04	246.75±0.07	24.56±0.03	27.9±0.38	18.86±0.12
NCEV-1530-3	13.4±0.37	9.2±0.94	20.13±0.37	1.23±0.38	1.95±0.03	53.73±0.55	265.737±0.45	13.16±0.94	31.7±0.38	15.62±0.02
NCEV-1530-4	14.6±0.81	10.3±0.56	23.58±0.45	1.74±0.47	1.95±0.78	46.83±0.56	246.07±0.56	15.66±0.07	36.1±0.47	18.54±0.01
NCEV-1530-5	14.8±0.03	9.4±0.97	21.98±0.67	1.64±0.48	2.11±0.56	47.56±0.23	239.11±0.57	24.29±0.03	32.1±0.37	18.61±0.07
NCEV-1530-6	12.6±0.49	9.4±0.48	13.12±0.56	1.51±0.99	3.51±0.09	48.56±0.59	251.27±0.56	12.67±0.98	19.8±0.46	21.93±0.08
NCEV-1530-7	18.56±0.38	10.4±0.27	24.77±0.08	1.41±0.47	3.21±0.45	50.31±0.38	273.73±0.57	25.7±0.55	23.9±0.45	14.18±0.05
NCEV-1530-9	17.6±0.37	6.5±0.22	16.34±0.03	1.61±0.45	3.14±0.35	47.83±0.39	269.81±0.57	17.95±0.56	21.1±0.12	18.35±0.19
NCEV-1530-10	17.5±0.39	8.4±0.37	19.96±0.55	1.42±0.48	3.19±0.35	49.53±0.66	260.43±0.47	15.66±0.67	36.9±0.04	22.18±0.03
NCEV-1530-11	7.8±0.81	11.2±0.68	25.22±0.09	2.2±0.26	4.53±0.36	70.77±0.56	308.33±0.37	29.22±0.66	38.8±0.67	27.44±0.08
NCEV-1530-12	10.3±0.79	5.7±0.38	14.79±0.04	1.09±0.38	4.21±0.36	61.91±0.45	302.51±0.45	19.89±0.77	18.7±0.05	23.17±0.57
NARC-W	19.4±0.48	6.4±0.37	13.24±0.28	1.22±0.11	3.1±0.55	57.66±0.34	284.14±0.68	19.19±0.77	25.4±0.60	18.46±0.34
NP-1	18.3±0.27	9.4±0.17	11.83±0.78	1.88±0.24	3.79±0.67	52.7±0.66	267.44±0.37	30.63±0.66	22.7±0.67	19.63±0.33
NP-2	19.3±0.12	9.8±0.27	13.23±0.67	1.78±0.34	2.52±0.67	52.17±0.88	270.56±0.46	26.76±0.39	28.1±0.45	13.75±0.85
NP-3	19.3±0.28	8.5±0.42	11.98±0.46	1.72±0.67	1.54±0.56	56.96±0.99	275.7±0.56	15.23±0.78	34.2±0.45	14.67±0.02
TP-1217	19.4±0.46	7.3±0.46	9.78±0.67	1.35±0.56	1.56±0.46	60.61±0.89	285.68±0.09	25.17±0.67	31.1±0.76	20.33±0.04
SZP-13200	8.9±0.37	12.4±0.67	25.17±0.56	2.8±0.78	4.49±0.76	77.45±0.44	313.45±0.45	29.22±0.68	37.9±0.34	30.98±0.46
Rakaposhi	18.1±0.98	5.3±0.46	23.33±0.47	0.98±0.67	2.33±0.87	51.56±0.78	242.34±0.78	16.02±0.04	34.1±0.98	20.69±0.27
Soan-3	20.8±0.47	3.2±0.47	2.96±0.48	0.13±0.44	1.17±0.56	44.08±0.11	66.49±0.99	8.8±0.22	17.5±0.78	7.22±0.68

MC=Moisture contents, CP=Crude protein, CF= crude fiber, AC=Ash contents, FC=Fat contents, CC=Carbohydrate contents, TE=Total energy, AA=Antioxidant activity, PC=Phenolic contents, fc=Flavonoids contents.

The percentage total energy was in the range of 66.49–320.61% (Table 3). The resistant genotypes against Northern Corn Leaf Blight showed maximum total energy as Karamat-Bar-25 was (320.61%), SZP-13200-11 (313.45%) and NCEV-1530-11 (308.33%) respectively. Whereas minimum total energy was shown by genotypes Soan-3 (66.49%), Ghauri-122 (89.34%) and Kissan-60 (77.23%) which are susceptible towards Northern Corn Leaf Blight. Analysis of bio-chemical constituents revealed that

there was variation in bio-chemical constitues across both the resistant, moderately resistant and susceptible maize genotypes. The percentage crude protein, percent crude fiber, percentage ash content, carbohydrate content, fat content and total energy of maize in the current study was found higher in resistant genotypes and low in quantity in susceptible genotypes. Therefore, studying the mechanisms at the biochemical levels is expected to give a better understanding about resistance operating in maize

against *Exserohilum turcicum*. In case of thirty genotypes with differed in their resistance levels, biochemical constituents was varied more in resistant and moderately resistant as compared to susceptible and highly susceptible genotypes. In contrary to the present findings Arjunan *et al.* (1976) found more protein nitrogen in infected sorghum leaves, infected by *Exserohilum turcicum* compare to healthy leaves. The decrease may be due to degradative activity. The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis particularly, during incompatible reaction. All the biochemical constituents estimated except moisture contents were less in infected plants compare to healthy plants later on gradually reduced. These constituents plays significant role in imparting resistance in maize against Northern Corn Leaf Blight. *Exserohilum turcicum* blighted the infected whole leaf and reduced their photosynthetic area which results into reduction of biochemical compounds and loss their nutritive value as a food, feed and feeder.

Antioxidant Scavenging assay

Free radical scavenging %age profile of maize genotypes is shown in Table 3. The percentage of antioxidant activity was in the range of 8.8 –29.22%. Maximum antioxidant activity value in genotype NCEV-1530-11 (29.22%) SZP-13200-11 (29.22%) and Karamat-Bar-25 was (29.4%), while minimum was observed in Soan-3 (8.8%), Ghuari-122 (11.44%) and Kissan-60 (12.12%). The results are match with the results of Khampas *et al.* (2013) their finding showed that DPPH scavenging activity ranging from 15.7% to 34.9% in dry stage kernels. Total phenolic contents of maize genotypes in mg GAE/100g are displayed in the Table 3. Total phenolic contents was in the range of 17.4 –38.8 mg GAE/g. Maximum phenolic contents was recorded in genotype NCEV-1530-11 (38.8 mg GAE/g), SZP-13200-11 (37.9 mg GAE/g) and Karamat-Bar-25 was (38.8 mg GAE/g) while minimum was shown by genotypes Soan-3 (17.5 mg GAE/g), Ghuari-122 (17.4 mg GAE/g) and Kissan-60 (18 mg GAE/g). Similar finding was reported by Yogesh *et al.* (2014). They noticed that total phenolic

contents were in range of 66.9±3.4 to 248.6±0.67mg/g. Total flavonoid contents were in the range of 2.59 GAE/g –36.49 GAE/g (Table 3). Maximum flavonoid contents value in genotype NCEV-1530-11 (27.45 GAE/g) SZP-13200-11 (30.9 GAE/g) and Karamat-Bar-25 was (36.49 GAE/g), respectively. Minimum flavonoid was shown by genotypes Soan-3 (7.22 GAE/g), Ghuari-122 (2.59 GAE/g) and Kissan-60 (5.65 GAE/g). The resistant genotypes against NCLB showed maximum flavonoid contents whereas genotypes which are highly susceptible towards NCLB indicated least flavonoid contents. Rahman *et al.*, 2014 noticed the total flavonoids contents ranged from 2.31 mg CAE/g-8.40 mg CAE/g. Their results are match with current results. Maximum antioxidant DPPH scavenging activity, phenolic and flavonoids compounds was observed in highly resistant genotypes as compared to moderately susceptible and highly susceptible genotypes is the indication of activation of defense response provided by these compounds to the pathogen. Despite of estimation of antioxidants, phenolic and flavonoid contents and their diversity characterization and alliance pattern with Northern Corn Leaf Blight, present investigation also reveals a strong correlation between antioxidant efficiency (AE), total phenolic contents (TPC) and total flavonoid content. As phenolic and flavonoid contents contribute more towards antioxidant activity, its increasing value increase the free radical trapping percentage. The high phenolic and flavonoid content in resistant genotypes may be due to more sugar as it acts as precursor for synthesis of antioxidants agents during pathogen infection.

The results are in confirmation with the findings of Rashmi *et al.*, (2017). Similarly the variation in biochemical constituents was also observed in maize genotypes with different resistance levels. The results showed that reduction in these compounds was observed after pathogen infection but more reduction was recorded in the susceptible genotypes than that of resistant genotypes. Peltonen and Karjalainen (1995) reported that phenyl alanine activity got enhanced in the leaves of resistant cultivars of barely at 24–32

and 40 hours after inoculation. of *B. sorokiniana*. Phenylalanine ammonia-lyase (PAL) being the first step in the phenylpropanoid biosynthesis pathway and also plays an important role in biosynthesis of the different families of phenolics such as coumarins, flavonoids, lignin and their derivatives and also with the level of synthesis of the phenolic compound. The induction of antioxidant activity preceding an increase in the phenolic and flavonoids content, in response to fungal infection (Mazeyratet *al.*, 1999; Pereira *et al.*, 1999). Fortification of the cell walls by the intensification of lignin and the accumulation of cell wall bound phenolic compounds to many plant pathogens was reported (Niemann *et al.*, 1991). Most of the nutritionally important Bio-chemical constituents are found in maize, bran is a factor to be estimated with notably in milling of maize. Eating whole maize grain is found to be more beneficial as the removal of the bran to make flour may have resulted in removing the vital component of the maize.

Conclusion

The current study suggests that the maize (*Zea mays* L.) seed extracts have considerable Bio-chemical constituents which have nutritional value as a food, feed and forage and antioxidant activity, (DPPH scavenging power, Phenolic and flavonoids contents) which may be helpful in preventing or slowing the progress of blighting of NCLB and enhance the mechanism of resistance in plants against fungal attack. Resistant and moderately resistant genotypes may also be helpful for their selection as improved genetic source with significant phytoconstituents for future breeding program.

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