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Effect of total phenolic content (TPC) and activity of phenylalanine ammonia-lyase (PAL) on spot blotch of wheat incited by *Bipolaris sorokiniana*

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Abstract
Spot blotch, caused by *Bipolaris sorokiniana*, is a devastating disease of wheat (*Triticum* spp.) in warm, humid regions of the world. Used to explain the role of total phenolic content (TPC) and phenylalanine ammoniacalase (PAL) activity. Total phenolic content of wheat leaves depending on cultivars, inoculation at different time intervals after inoculation The total phenolic content during inoculation was (120. 78 mg/gm) and it significantly increased as time increased from 24 to 48 hai and decreased suddenly at 72 hai. The yield and TPC value of 121.6 and 121.8 for their leaf phenolic content were observed in cultivars V1 and V2. It was equivalent to each other. Significantly more phenolic content 130.81 was observed in inoculated plants than non-inoculated plants, which gave the lowest phenolic content in wheat leaves. The total phenolic content at different hours after inoculation was 120.78 and it increases significantly when the time increases from 24 to 48 hours after inoculation and decreases after 72 hours after inoculation. PAL activity at challenge was (0.194 units/g) and increased significantly over time from 24 to 48 hai and decreased sharply in 72 hai.

Keywords: *Bipolaris sorokiniana*, total phenolic content, phenylalanine ammonia lyase, spot blotch, *Triticum aestivum*, hour after inoculation (hai)

Introduction
Triticum aestivum is the most important crop in India after rice and is known as the most important cereal in the world and a staple food crop in many regions grown under both irrigated and rainfed conditions. It belongs to family Poaceae (Yadawad *et al.*, 2015) [10]. There are 16 species in the genus Triticum. There are 3 species, namely *Triticum aestivum* L. (bread wheat), *Triticum durum* Desf. (Macroni or durum wheat) and *Triticum dicoccum* Schrank. (Emmer wheat) which is now commonly cultivated. In India, almost 88 percent of the wheat area is bread wheat, 11 percent is macaroni wheat and less than 1 percent is wheat. Wheat provides almost 55 percent of the carbohydrates and 20 percent of the calories in the diet, which is consumed as a staple food by two billion people (36 percent of the world's population). *Bipolaris sorokiniana* (Sacc.) Cobbler is a seed and soil borne pathogen; causes wilt, seedling, leaf spot, black spot of wheat, barley and other small grains and grasses, common root rot of barley and other small grains and grasses (Wiese, 1998). Symptoms develop mainly as dark brown necrotic spots (boat-shaped) on coleoptiles, leaves, crowns, stems and roots with or without a yellow ring. Darkening of the coronal space is a typical symptom. Leaf lesions begin within a few millimeters and extend over 1–2 cm as elongated dark brown spots (Chand *et al.*, 2002) [1]. It is well documented in several plant systems that phenolic compounds such as phytoalexins, phytoanticipins or the physical barrier ie. lignins may play an important role in disease resistance by preventing the colonization of plant tissues. Induction of PAL activity that precedes increases in phenolic content has been observed in response to fungal infection in several systems. An active role of PAL and phenolics in the expression of resistance responses in various crops has been reported (Nicholson and Hammershmidt, 1992) [7]. The aim of this study is to investigate the effects of total phenolic content (TPC) and phenylalanine ammonia-lyase (PAL) activity on wheat spot

caused by *Bipolaris sorokiniana*.

Material and Methods

Research was carried out at Student Instructional Farm (SIF), Main Experimental Station, Wheat pathological laboratory, Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. The experiments were conducted during *Rabi.*

Estimation of TPCs

Flag leaves (1gm) were collected from SIF, ANDUA&T, Kumarganj, Ayodhya (U.P.) where the experiment was conducted during *Rabi* 2018-19 at 24, 48 and 72 hours after inoculation. In all three iterations. 1 g of leaves was taken and mixed with 20 ml (80% alcohol) and then the contents were centrifuged at 1000 g for 15 min. and filtered through Whattman filter paper. Three test tubes were taken, from which 1 ml of distilled water, 1 ml of bile acid standard solution and 1 ml of filtrate were transferred. 1 mL of phenol reagent and 1 mL of sodium carbonate were added and finally the volume was adjusted to 5 mL. All the test tubes were kept for 1hr. at room temperature and finally the intensity of colour was recorded at 750 nm on spectronic 20 against blank reagent.

Reagents for TPC

1. Folin-ciocalteu reagent (FCR).
2. 80% Ethanol.
3. 20% Sodium carbonate.

$$\text{TPC (mg./100g.)} = \frac{\text{Amount of gallic acid from standered}}{\text{O. D. of known gallic acid solution}} \text{x} \frac{\text{sample O. D.}}{\text{sample weight}} \text{x} \frac{\text{vol. made up}}{\text{vol. of aliquot}}$$

Estimation of phenylalanine ammonia-lyase (PAL)

Flag leaf was collected from the three replications in the field after inoculation. PAL activity was analyzed as the rate of conversion of L- phenylalanine into trans- cinnamic acid at 270nm UV- spectrophotometer. Sample was prepared by crushing 100mg plant tissue in a 50 µl buffer (100 mM sodium borate buffer, 1.4mM β-mercaptoethanol and 1% PVPP). After homogenization samples were centrifuged at 10000 rpm for 5 minutes and supernatent was stored at -20˚C for further analysis. This supernatent was treated as crude enzyme extract. 50µl of enzyme extract were treated with 0.5ml of 0.1M trisodium borate buffer (pH 8.5) and 0.5 ml of 12 mM L-phenylalanine in the same buffer. The volume of reaction mixture was made up to 3ml with deionized H_2O and immediately mixed by inversion and recorded the increase in absorbance at 270 nm for approximately 5minutes. The samples were prepared in duplicate for each analysis and the mean value of the ΔA270nm/minute was obtained using the maximum linear rate for both the test and blank. Units/ml= (ΔA_{270nm} /min Test - ΔA_{270nm}/min Blank) (df) / (19.73) volume of Enzyme. Where ΔA is the change in absorbance, (df1), 19.73 is the mili molar extinction coefficient of trans-cinnamate at 270 nm.

Statistical analysis

The statistical analysis of field experiment was done by the method randomized block design (RBD). The significance of treatment difference was tested by variance ratio test at 5 per cent level of probability.

Result and Discussion

Total phenolic content (TPC) (mg/g)

The total phenolic content of wheat leaves according to cultivar, different time intervals of inoculation after inoculation are shown (Table-1). The study was almost similar to obtain phenolic content of these leaves and TPC values of 121.6 and 121.8 for cultivars V1 and V2. It was equivalent to each other. Significantly more phenolic content 130.81 was observed in inoculated plants than non-inoculated plants, which gave the lowest phenolic content in wheat leaves. The total phenolic content at different hours after inoculation was 120.78 and it increases significantly when the time increases from 24 to 48 hours after inoculation and decreases after 72 hours after inoculation. The study was almost similar to obtain phenolic content of these leaves and TPC values of 121.6 and 121.8 for cultivars V1 and V2. Significantly more phenolic content 130.81 was observed in inoculated plants than non-inoculated plants which gave lowest phenolic content in wheat leaves, total phenolic content at different hours after inoculation was 120.78 and it increases significantly with increasing time from 24 to value 48 h after inoculation and decline 72 h after inoculation Chand and Joshi (2013) [1], Mali *et al.* (2017) [5]. TPC levels in wheat are correlated with host resistance to several Alternaria blight diseases (Mishra *et al.*, 2011) [6]. Growth stages can also affect the development of spot resistance in wheat and if left untreated, early maturing genotypes appear more susceptible than late maturing genotypes on a given day (Joshi and Chand, 2002) [4].

Table 1: Estimation of TPC in inoculated and un-inoculated leaves of wheat

Total Phenolic Content (mg g^{-1})						
Varieties	**Treatment**	**Mean of TPC (mg g^{-1}) fresh weight in leaves sampling interval (hrs)**				
Sonalika (V_1)		**0 hai**	**24 hai**	**48 hai**	**72 hai**	**Mean**
Inoculated (I0)	T_1	128	143	196	54	130.25
Uninoculated (I_1)	T_2	112.10	124	174.20	41	112.83
Raj 4015 (V_2)						
Inoculated (I0)	T_3	129	145	195	56	131.25
Uninoculated (I_1)	T_4	114	123	172	40.10	112.45
Mean		120.77	133.75	184.30	47.78	

V_1	121.60	Inoculated	130.81
V_2	121.85	Uninoculated	112.64
SEm±	1.93	SEm±	1.93
CD	NS	CD	5.56

Phenylalanine ammonia lyase (PAL) (unites/g)

Phenylalanine ammonia lyase in wheat leaves as influenced by varieties, inoculation different intervals after inoculation have been presented in Table-2 it is evident from data presented. Study was almost similar for production of PAL in their leaves and PAL of 0.209 and 0.212 was noted in variety V1 and V2 respectively. Significantly more PAL of 0.215 was recorded in inoculated plants that of un-inoculated one which gave least PAL in wheat leaves, PAL at different intervals of hours after inoculation was 0.222 and 0.380 it is increased significantly with increase of time from 24 to 48 hours after inoculation and decline at 72 hours after inoculation. Study was almost similar for production of PAL in their leaves and PAL of 0.209 and 0.212 was noted in variety V_1 and V_2 respectively. Significantly more PAL of 0.215 was recorded in inoculated plants that of un-inoculated one which gave least PAL in wheat leaves, PAL at different intervals of hours after inoculation was 0.222 and 0.380 it is increased significantly with increase of time from 24 to 48 hours after inoculation and decline at 72 hours after inoculation. Chand and Joshi (2013) [1] also reported an average TPC of 133.5 mg/g fresh weight for 24 sharks, PAL of 248.8 µmol cna mg/g fresh weight, and 10% significantly lower lignin in susceptible genotypes. Mali *et al.,* (2017) [5] also reported induction of PAL and POX activity in resistant cultivars NIDW 295 (356.7%, 184.3%), PDW 314 (253.6%, 156.6%), while minimal response was observed in susceptible cultivars. PAL catalyzes phenylalanine to trans-cinnamic acid, the first step in phenylpropanoid biosynthesis, resulting in a diverse group of plant secondary metabolites, including lignins, phytoalexins, and flavonoids (Hahlbrock and Scheel, 1989) [3]. Overexpression of PAL in tobacco produced significantly fewer and smaller lesions after infection with the virulent fungal pathogen Cercospora nicotianae (Shadle *et al.,* 2003) [8].

Table 2: PAL activity in inoculated and uninoculated leaves of wheat

Phenylalanine Ammonia Lyase (Units g^{-1})						
Varieties	**Treatment**	**Mean of PAL (Units g^{-1}) fresh weight in leaves sampling interval (hrs)**				
Sonalika (V_1)		**0 hai**	**24 hai**	**48 hai**	**72 hai**	**Mean**
Inoculated (I0)	T_1	0.195	0.228	0.383	0.047	0.213
Uninoculated (I_1)	T_2	0.191	0.214	0.374	0.042	0.205
Raj 4015 (V_2)						
Inoculated (I0)	T_3	0.197	0.231	0.387	0.051	0.217
Uninoculated (I_1)	T_4	0.192	0.216	0.377	0.043	0.207
Mean		0.194	0.222	0.380	0.046	

V_1	0.209	Inoculated	0.215
V_2	0.212	Uninoculated	0.206
SEm±	0.002	SEm±	0.003
CD	0.006	CD	0.009

Conclusion

In conclusion, the investigation into the total phenolic content (TPC) and Phenylalanine ammonia lyase (PAL) levels in wheat leaves across different cultivars and time intervals after inoculation reveals significant insights. The TPC values showed comparable results between cultivars V1 and V2, indicating minimal variation in phenolic content. However, there was a notable increase in phenolic content in inoculated plants compared to non-inoculated ones, emphasizing the role of inoculation in enhancing phenolic levels. Moreover, TPC levels exhibited a temporal pattern, peaking at 48 hours post-inoculation and declining thereafter. This temporal variation underscores the dynamic nature of phenolic accumulation in response to inoculation. Additionally, PAL levels mirrored the trends observed in TPC, further validating the connection between these two parameters. Studies by Chand and Joshi (2013) [1] and Mali *et al*. (2017) [5] provide additional context, highlighting the significance of TPC and PAL in host resistance against fungal pathogens. The findings underscore the importance of understanding the dynamics of phenolic metabolism in wheat leaves, offering potential insights into enhancing plant defense mechanisms against diseases.

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