Research Article

DETERMINATION OF SOURCE OF RESISTANCE IN WHEAT AGAINST BACTERIAL LEAF STREAK AND MORPHO-BIOCHEMICAL CHARACTERIZATION OF XANTHOMONAS TRANSLUCENS PV. UNDULOSA

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Abstract

Current research effort was directed focusing on evaluating the wheat germplasm for determination of source of resistance against the bacterial leaf streak (BLS) disease and the morpho-biochemical characterization of *Xanthomonas translucens* pv. *undulosa* (*Xtu*), the causative agent of bacterial leaf streak of wheat. The bacterium *Xtu* showed yellow, mucoid, circular and convex shaped growth when cultured on artificial growth media (NA). Gram staining, Catalase, KOH and Kovacs oxidase tests were employed that confirmed the *Xtu* as gram negative (-ve) bacterium. Fifteen wheat varieties/advanced lines were evaluated against BLS disease employing Randomized Complete Block Design (RCBD) to estimate the disease incidence over two years (2023 and 2024). Screening results for both years revealed that only one advanced line (PBG Line 8) expressed highly resistant response while two varieties PBG Line 1 and NARC-2008 exhibited resistant response. Two advanced lines (PBG Line 2 and PBG Line 5) were observed as moderately resistant while, moderately susceptible response was shown by FSD-2008, PBG Line 4, Ujala-2016, PBG Line 3, PBG Line 7 and PBG Line 6. The remaining varieties/advanced lines Umeed-2014, Zincol 2015, PBG Line 9 and PBG Line 10 showed susceptible response. The successful screening of wheat germplasm for resistance to bacterial leaf streak disease is an important source of resistance for breeding programs. Future research should prioritize leveraging this resource to develop enhanced cultivars and strengthen integrated management strategies for bacterial leaf streak disease (BLS). This approach will support the advancement of disease-resistant varieties and promote more effective, sustainable disease management solutions.

1. INTRODUCTION

Wheat (Triticum aestivum L.) is a vital cereal crop that belongs to the family Poaceae and the genus Triticum (Parmar *et al*., 2023). It is the second cultivated cereal crop after maize, followed by rice. It is grown on 219.15 million hectares globally, yielding 808.44 million tonnes (FAO, 2022). In Pakistan, wheat is cultivated on an area of 9.4 million hectares with yield 31.4 million tonnes and contribute 2.2% to GDP and 9.0% to agriculture (GOP, 2024). It has high nutritional value containing carbohydrates, proteins, minerals, fats, and minute quantities of lipids, sugars, vitamins (B, E, thiamine, riboflavin, niacin), phytochemicals (phenolic acids, ferulic acid, lignans, tocopherols, lutein, β-carotene), macro and micronutrients and dietary fibers (soluble and insoluble) (Shewry and Hey, 2015; Awulachew, 2020; Tse *et al*., 2022). It also play an important role in human health because of its medicinal

properties like cure of heart diseases, constipation, diverticulosis, obesity, biliousness, sore throat, hiatal hernia, type-2 diabetes, and breast cancer (Iqbal *et al*., 2022). A variety of by-products were made from wheat such as wheat bran, white flour, bread flour, gluten flour, semolina are used in animal feed, bioethanol, baking, cosmetics, and pharmaceuticals (Kanojia *et al*., 2018; Mato *et al*., 2024).

Production of the wheat in terms of both quality and quantity is hampered by different biotic (fungi, bacteria, nematode, virus) and abiotic (drought, temperature, salinity, floods, radiations, pollutants) factors (Hussain *et al*., 2022). Among biotic factors, bacterial leaf streak (BLS), is an emerging global threat to wheat production and is caused by bacterium Xanthomonas translucens pv. undulosa (*Xtu*) (Niri *et al*., 2023; Osdaghi *et al*., 2023). Xanthomonas translucens pv. undulosa (*Xtu*) is a Gram-negative, rod-shaped bacterium ranging from 0.4-0.8 µm by 1.0-2.5 µm (Sapkota *et al*., 2020; Singh *et al*., 2023), non-sporing and naturally sporadic (Ramakrishnan *et al*., 2019). The optimal conditions required by the bacterium to grow properly are 28-30°C and high relative humidity (Kaur, 2021). BLS was first documented on barley in 1917 and then on wheat in USA in 1919 (Jones *et al*., 1917; Smith *et al*., 2019). While, in Pakistan, it was first reported in 1986 by Akhtar and Aslam (1986). It can cause up to 10-40% yield losses globally, depending on host susceptibility and initial inoculum level (Hangamaisho *et al*., 2024). Although the BLS is seed-borne disease, the bacterium also persists on crop debris, weeds, and soil for a limited time-period (Ledman *et al*., 2021). It penetrates the plants through wounds, natural openings like stomata and hydathodes. After it, the pathogen reproduces in the mesophyll tissues and produce exopolysaccharides leading to biofilm formation (Ledman *et al*., 2021). Water-soaked lesions followed by

yellow, brown and necrotic symptoms was seen in response to bacterial infection. Honey-like exudate was oozed out from these lesions, spreading under high humid conditions forming a thin and transparent layer of streak (Mehmood *et al*., 2023).

Various management practices are in exercise for the management of bacterial leaf streak disease of wheat. The quicker and easier method is the use of chemicals (pesticides). However, the usage of chemicals in excess for disease management disrupt plant functions by inducing toxicity leading to oxidative stress and retarded growth of the plants (Hashmi *et al*., 2020). Keeping in view the consequences of excessive usage of chemicals and limitations of other control measures, the most effective approach to manage the disease is the use of resistant varieties despite of few circumstances where these varieties become susceptible because of favorable conditions and may lead to epidemics (Atiq *et al*., 2022). Considering the importance of resistant varieties for the management of the disease, current research efforts was directed to screen out the available wheat germplasm in the field to find out the source of resistance towards BLS of wheat.

2. MATERIALS AND METHODS
Isolation, Purification, Identification and Preservation of Pathogen
Diseased samples were collected from Agronomy Research Area, University of Agriculture Faisalabad (UAF), brought to Molecular Bacteriology Laboratory, Department of Plant Pathology, UAF and stored in refrigerator (PL6500) at 4 ℃ for further study. For the isolation of bacterium, Nutrient Agar (NA) media was prepared by adding 28 g synthetic NA into 1000 mL distilled water and sterilized in an autoclave at 121 ℃ and a pressure of 15 Psi for 15 minutes. The infected samples along with some healthy portions were cut into small pieces (2-3 mm) and surface sterilized by

using 1% sodium hypochlorite (NaOCl) for 30 seconds, followed by two washings with distilled water to remove toxicity due to sodium hypochlorite. Whatman's Filter Paper No. 41 was used to dry the sterilized pieces (Singh *et al*., 2014). After that, pieces were placed in petri plates (9 cm) having NA media with the help of sterilized forceps. Then, petri plates were wrapped and incubated (Heraeus) at 28 ± 2 °C for 24 hours to perceive the bacterial growth. To minimize the chances of contamination, the whole procedure was performed in Laminar Air-Flow Chamber (RTVL1312, Robus UK). The purification of bacteria was accomplished by using streaking method (Figueroa-Bossi *et al.*, 2022). A single bacterial colony was picked through sterile wire loop, streaked on new NA plate, wrapped and incubated (Heraeus) at 28 ± 2 °C. Identification of the pathogen was done based on its morphological traits such as shape, color, growth, colony pattern, and biochemical tests like gram staining, KOH, catalase and oxidase test (Sharma and Singh, 2019). 5 mL nutrient broth (NB) media was prepared for the preservation of pathogen in a test tube. Pure culture was transferred to test tube by employing white tips and then placed in shaking incubator at 28 °C for 24 hours. 1 mL of 50 % glycerol and 1 mL of nutrient broth media with bacterial growth was added in a 2 mL Eppendorf tube. After labelling the Eppendorf tube, it was stored at -18 °C in a refrigerator (VF-1045 CVT). Isolation, purification and preservation of the *Xanthomonas translucens* pv. *undulosa* (*Xtu*) was shown in Fig. 1.

Pathogenicity Test
Koch's postulates were followed for the confirmation of pathogen. For this purpose, bacterial aqueous suspension @ 1×10^6 CFU/mL was prepared. A moderately susceptible variety (FSD-2008) was inoculated using spray method in the daytime when the maximum number of stomata is opened. One plant remained uninoculated and used as control. After the expression of symptoms, the pathogen was reisolated from the infected plants and cultured on artificial media (NA). The bacterial colonies were compared with the parental pathogen based on morphological characteristics.

Evaluation of wheat germplasm against bacterial leaf streak disease (BLS) for two years 2023-2024
For the screening of the wheat varieties/advanced lines against the bacterial leaf streak of wheat, fifteen varieties/advanced lines viz PBG Line 1, PBG Line 2, PBG Line 3, PBG Line 4, PBG Line 5, PBG Line 6, PBG Line 7, PBG Line 8, PBG Line 9, PBG Line 10, NARC-2008, FSD-2008, Ujala-2016, Zincol-2015, and Umeed-2014 were grown at Research Area, Department of Plant Pathology, UAF by maintaining the distance between plant to plant and row to row of 30 cm (1 ft) and 22.86 cm (0.75 ft) respectively under the complete randomized block design (CRBD). All agronomic practices were employed for the better growth of plants. The pathogen *Xtu* 5 ml was applied by using the spray method and then after the appearance of disease symptoms, the rate of disease incidence was recorded by using the formula described by Seem (1984). Response of wheat varieties/advanced lines was assessed based on disease rating scale (Table 1).

$$\text{Disease incidence (\%)} = \frac{No.\ of\ Diseased\ Plants}{Total\ No.\ of\ Plants\ Observed} \ x\ 100$$
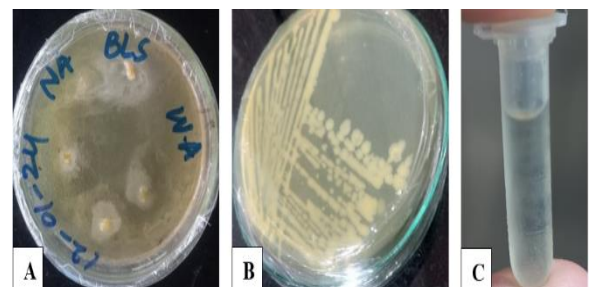

Fig. 1. Isolation (a); Purification (b); Preservation of Pathogen (c)

Table 1. Disease Rating Scale

Disease Rating	Disease Incidence (%)	Disease Response
1	1-5	Highly Resistant
2	6-15	Resistant
3	16-25	Moderately Resistant
4	26-50	Moderately Susceptible
5	51-75	Susceptible
6	>75	Highly Susceptible

3. RESULTS

### Identification of bacteria based on morphological characteristics

Identification of *Xanthomonas translucens* pv. *undulosa* cultured on artificial media (NA) was done based on morphological characteristics. The results expressed that pathogen exhibited yellow, mucoid, circular and convex shaped growth as demonstrated in Table 2.

Table 2. Morpho-cultural characteristics of *X. translucens* pv. *undulosa*

Morphological features	Key findings
Color	Yellow
Elevation	Convex
Margin	Entire
Surface	Shiny
Cell Shape	Rod
Size	0.4-0.8 µm to 1.0-2.5 µm

Identification of bacteria through Biochemical tests

The biochemical characterization of *X. translucens* pv. *undulosa* was done for the identification of bacterium. The gram staining test is of paramount importance for the initial identification of bacterium. The bacterium cell wall color following gram staining test was red which expressed that the bacterium exhibited gram -ve behavior as shown in Fig. 2A. KOH test result indicated
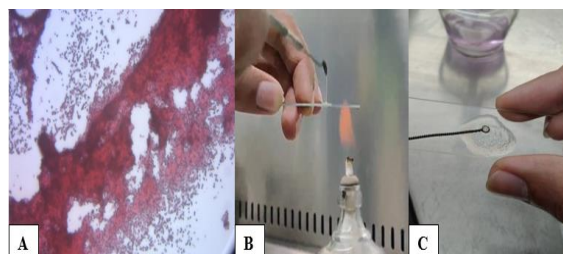
Fig. 2. Gram staining test (a); KOH test (b); Oxidase test (c)
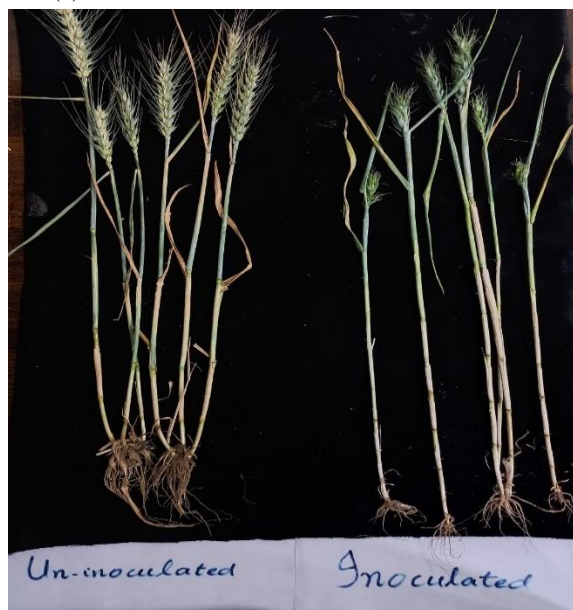
Fig. 2. Inoculated and uninoculated wheat plants with *Xanthomonas translucens* pv. *undulosa*

a clear mucoid thread which is typical identification of gram -ve bacteria as shown in Fig. 2B. Bubble formation was observed during catalase test which showed gram -ve behavior of bacterium as expressed in Fig. 2C. The key findings of the biochemical tests for the identification of pathogen were shown in Table 3.

Table 3. Biochemical characterization of *X. translucens* pv. *undulosa*

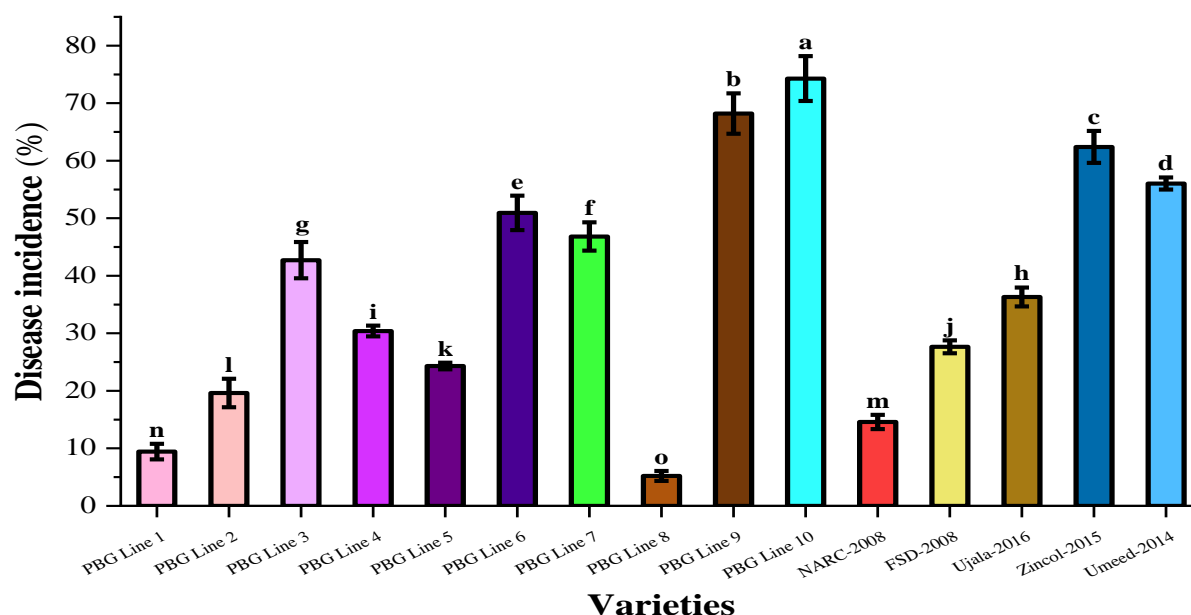| Biochemical characterization	Key findings
Gram Staining test	- (negative)
Catalase test	+ (positive)
KOH test	+ (positive)
Kovacs Oxidase test	- (negative)

Pathogenicity Test

Fig. 4. Response of wheat varieties/advanced lines against bacterial leaf streak of wheat under field conditions along with disease incidence rate during 2023

Table 4. Response of wheat varieties/advanced lines against bacterial leaf streak of wheat under field conditions during 2023

Sr. #	Variety	Disease incidence (%)	Disease Rating	Disease Response
1	PBG Line 8	5.2033 o	1	Resistant
2	PBG Line 1	9.4367 n	2	Resistant
3	NARC-2008	14.587 m	2	Resistant
4	PBG Line 2	19.623 l	3	Moderately Resistant
5	PBG Line 5	24.307 k	3	Moderately Resistant
6	FSD-2008	27.653 j	4	Moderately Susceptible
7	PBG Line 4	30.393 i	4	Moderately Susceptible
8	Ujala-2016	36.317 h	4	Moderately Susceptible
9	PBG Line 3	42.713 g	4	Moderately Susceptible
10	PBG Line 7	46.830 f	4	Moderately Susceptible
11	PBG Line 6	50.930 e	4	Moderately Susceptible
12	Umeed-2014	56.033 d	5	Susceptible
13	Zincol-2015	62.393 c	5	Susceptible
14	PBG Line 9	68.200 b	5	Susceptible
15	PBG Line 10	74.280 a	5	Susceptible
LSD		**2.31**		

Purified bacterial colonies of *Xtu* were multiplied and inoculated in wheat plants for the confirmation of the pathogen. After 8-10 days of inoculation, disease symptoms were observed while control plants remained symptomless as shown in Fig. 3. On comparison with parental infected leaves, resemblance in the symptoms was observed. Again, the pathogen was reisolated from these infected plants and proved as *X. translucens* pv. *undulosa* based on morphological characteristics.
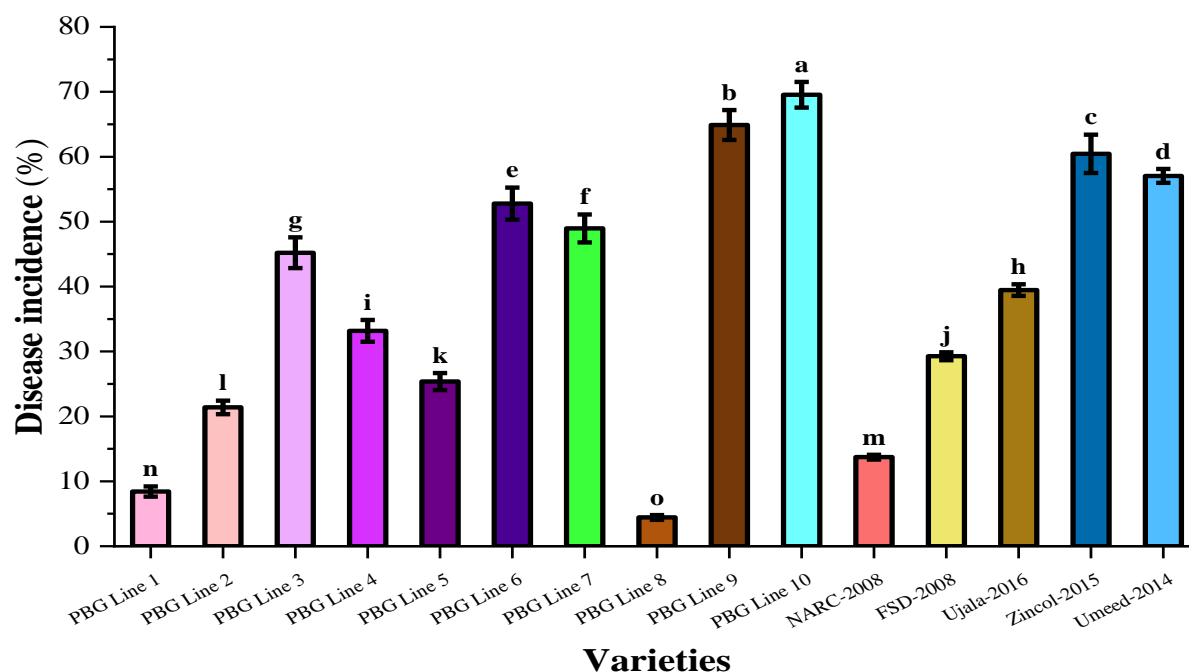
Fig. 5. Response of wheat varieties/advanced lines against bacterial leaf streak of wheat under field conditions along with disease incidence rate during 2024

Table 5. Response of wheat varieties/advanced lines against bacterial leaf streak of wheat under field conditions during 2024

Sr. #	Variety	Disease incidence (%)	Disease Rating	Disease Response
1	PBG Line 8	04.43 o	1	Highly Resistant
2	PBG Line 1	08.43 n	2	Resistant
3	NARC-2008	13.72 m	2	Resistant
4	PBG Line 2	21.38 l	3	Moderately Resistant
5	PBG Line 5	25.36 k	3	Moderately Resistant
6	FSD-2008	29.26 j	4	Moderately Susceptible
7	PBG Line 4	33.18 i	4	Moderately Susceptible
8	Ujala-2016	39.46 h	4	Moderately Susceptible
9	PBG Line 3	45.21 g	4	Moderately Susceptible
10	PBG Line 7	48.95 f	4	Moderately Susceptible
11	PBG Line 6	52.77 e	4	Moderately Susceptible
12	Umeed-2014	57.04 d	5	Susceptible
13	Zincol-2015	60.45 c	5	Susceptible
14	PBG Line 9	64.88 b	5	Susceptible
15	PBG Line 10	69.54 a	5	Susceptible
LSD		**2.84**		

Response of wheat varieties against bacterial leaf streak disease during the years 2023 and 2024

Fifteen varieties/advanced lines were screened against the bacterial leaf streak (BLS) of wheat caused by *Xanthomonas translucens* pv. *undulosa* in the Research Area, Department of Plant Pathology, UAF during 2023 and 2024. First year results expressed that among the evaluated varieties, PBG Line 1 and NARC-2008 showed resistant response against the disease with the disease incidence of 9.43 and 14.58 % respectively (Disease rating 2). Only one advanced line, PBG Line 8 expressed highly resistant response against the disease by showing 5.20 % disease incidence (Disease rating 1). Two advanced lines (PBG Line 2 and PBG Line 5) were observed as moderately resistant response with the disease incidence of 19.623 and 24.307 %, respectively (Disease rating 3). The moderately susceptible response was shown by FSD-2008, PBG Line 4, Ujala-2016, PBG Line 3, PBG Line 7 and PBG Line 6 with disease incidence rate of 27.653, 30.393, 42.713, 46.830 and 50.930 %, respectively (Disease rating 4). Remaining four varieties/advanced lines Umeed-2014, Zincol 2015, PBG Line 9 and PBG Line 10 with the disease incidence rate of 56.033, 62.393, 68.200 and 74.280 %, respectively exhibited susceptible response (Disease rating 5). The minimum and maximum disease incidence was expressed by PBG Line 8 and PBG Line 10 with the disease incidence rate of 5.203 and 74.280 % respectively as shown in Fig. 4 and Table 4.

Disease incidence from all varieties was also recorded during the year 2024. Second year results expressed that among the evaluated varieties PBG Line 1 and NARC-2008 showed resistant response against the disease with the disease incidence of 8.43 and 13.72 % respectively (Disease rating 2). While only one advanced line PBG Line 8 expressed highly resistant response with disease incidence of 4.43 % (Disease rating 1). Two advanced lines (PBG Line 2 and PBG Line 5) were observed as moderately resistant response with the disease incidence of 21.38 and 25.36 %, respectively (Disease rating 3). The moderately susceptible response was shown by FSD-2008, PBG Line 4, Ujala-2016, PBG Line 3, PBG Line 7 and PBG Line 6 with disease incidence rate of 29.26, 33.18, 39.46, 45.21, 48.95, 52.77 %, respectively (Disease rating 4). The remaining four varieties/advanced lines Umeed-2014, Zincol 2015, PBG Line 9 and PBG Line 10 with the disease incidence rate of 57.04, 60.45, 64.88, 69.54%, respectively showed susceptible response (Disease rating 5). The minimum and maximum disease incidence was expressed by PBG Line 8 and PBG Line 10 with the disease incidence rates of 4.43 and 69.54 % respectively as shown in Fig. 5 and Table 5.

4. DISCUSSION

Identification of the bacterium *Xanthomonas translucens* pv. *undulosa* was done based on morphological and biochemical characterization. The results showed that *Xtu* exhibited yellow, mucoid, circular and convex shaped growth. The biochemical tests revealed that gram staining and Oxidase test showed negative response while catalase and KOH test showed positive response indicating that *Xtu* exhibited gram -ve behavior. The findings of the current research are endorsed by the outcomes of Iqbal *et al*. (2014), who identified *Xtu* based on biochemical tests. Screening of available germplasm of wheat is the need of the time to detect the genotypes with anticipated attributes like disease resistance, compliance, high yield and quality. It makes sure that only the most vigorous and well-improved cultivars are established and marketed, which is the need of the hour to improve agricultural sustainability, fulfilling the governing criteria, and enlightening the food security.

So, keeping in view the prominence of screening trials, 15 advanced lines/varieties were evaluated against bacterial leaf streak of wheat. Among these varieties/advanced lines, only one advanced line (PBG Line 8) showed highly resistant response with minimum disease incidence. While highly susceptible response was exhibited by PBG Line 10. Out of the remaining varieties/advanced lines, 2, 2, 2, 6, and 3 varieties/advanced lines revealed resistant, moderately resistant, moderately susceptible, and susceptible expression towards bacterial leaf streak respectively. A significant contrast in the morphological characteristics of resistant and susceptible wheat varieties/advanced lines, was observed during screening tests. The resistant varieties expressed thicker cuticles, acting as a physical barrier against the phytopathogens and well-established venations which enhanced the uptake of nutrients and water translocation as compared to susceptible ones. The results of the present study are supported by the work of Favaro *et al*., (2020), who determined the role of cuticle on the resistance. Higher lignin content provides structural support and strengthens the cell wall that makes it harder for the pests to enter the host plant. These findings are aligned with the work of Li *et al*., (2022a), who reported that lignin contents were higher in resistant varieties as compared to susceptible one. Similarly, deeper roots were observed in the resistant varieties, offering resistance against biotic stress and maintaining the uptake of nutrients and water under deficit conditions. These outcomes are in line with the study of Li *et al*., (2022b), who demonstrated the effect of deeper roots in the resistance of plants towards plant diseases. There was no significant difference in the brightness of the symptoms in both susceptible and resistant varieties but there is difference in streak length, which was less in resistant compared to susceptible one. These

results are consistent with the work of Kandel *et al*., (2012). In comparison to susceptible varieties, resistant ones expressed higher grain weight. Limited photosynthetic area in the leaves of susceptible varieties during grain filling reduces carbohydrate production, resulting in lower starch accumulation in the grains (Prathap *et al*., 2019).

The activity of the biochemical compounds determined the resistance source in wheat germplasms. Resistant varieties regulated the activity of defense related enzymes such as peroxidases, chitinases, and polyphenol oxidases after the attack of pathogen. Higher activity of β-1,3-glucanases breaks down the pathogen's cell wall and prevents the proliferation of the bacterium in the resistant ones, while susceptible varieties are not able to trigger the initial response towards the pathogen (Riseh *et al*., 2023). Secondary metabolites like phenolics, phytoalexins, lignin, flavonoids are responsible for the reinforcement of the cell wall, activation of antioxidant defense system that limited/retarded the penetration of the pathogen, are produced in higher amounts in the resistant varieties while limited production in susceptible ones make them vulnerable to the pathogen attack due to weaker cell wall (Ninkuu *et al*., 2023; Saini *et al*., 2024). Pathogen attack induces alterations in the biochemical profiling of the plants. Rapid and controlled ROS (reactive oxygen species like hydrogen peroxide, superoxide) production was observed in the resistant varieties while delayed and uncontrolled in susceptible ones. Increased level of SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) in the resistant wheat varieties scavenge the ROS efficiently preventing the oxidative stress to plants while still inhibiting the pathogen's proliferation (Hasanuzzaman *et al*., 2020). Induction of pathogenesis-related (PR) proteins such as chitinases, thionins and

glucanases were high and quick in resistant varieties that inhibited the pathogen growth by degrading their cell wall and pathogen enzymes, while in susceptible varieties, PR proteins were observed at lower levels that limited the defense system of these ones (Santos and Franco, 2023). System acquired resistance (SAR) is developed due to the activation of salicylic acid (SA) in the resistant varieties which helps in the accumulation of PR proteins leading to the robust defense mechanism (Saleem *et al*., 2021). In case of resistant varieties, there are specific resistance genes (R-genes) that recognize the bacterial pathogen's effectors and regulate the plant defense mechanism which led to the localized cell death and inhibit the spread of the disease. Resistant varieties having R-genes sustain them under favorable environmental conditions such as low relative humidity and temperature, inhibit pathogen dispersal and growth (Deppotter and Doehlemann, 2020). Hypersensitive response (HR) is triggered in the resistant varieties which leads to the localized cell death resulting in the limited supply of nutrients to the pathogen for their survival while there is delayed HR response in susceptible ones facilitating the bacterium to proliferate in plant tissues (Lukan *et al*., 2022).

A significant difference in the agronomic attributes of the resistant and susceptible varieties was observed during trial. Resistant varieties have higher yield under adverse climatic conditions because these varieties can endure stress, adopt the environment and result in less crop losses as compared to susceptible ones (Manghwar *et al*., 2021). Growth stages such as flowering, tillering and grain filling takes place uniformly to ensure faster crop maturation in the resistant ones as compared to susceptible varieties. These varieties grow taller and have optimal spike length due to the efficient resource use, effective planting strategies, and genetic traits that enhance their adaptability to environment and suppress phytopathogens. Agronomic attributes such as plant height, spike length, grains weight, stomatal conductance, root and shoot length are limited in susceptible varieties because the maximum energy is consumed by them to trigger the defense mechanism against the pathogen attack which reduces the growth and development parameters of the crop (Mirani *et al*., 2024).

5. CONCLUSION

The screening of fifteen varieties/advanced lines for genetic resistance to bacterial leaf streak disease (BLS) of wheat caused by *Xanthomonas translucens* pv. *undulosa* was done in this experiment. It is concluded that only one advanced line (PBG Line 8) showed a highly resistant response, while PBG Line 1 and NARC-2008 exhibited resistant response. Two advanced lines (PBG Line 2 and PBG Line 5) were observed as moderately resistant while, moderately susceptible response was shown by FSD-2008, PBG Line 4, Ujala-2016, PBG Line 3, PBG Line 7 and PBG Line 6. The remaining four varieties/advanced lines Umeed-2014, Zincol 2015, PBG Line 9 and PBG Line 10 showed susceptible response. Recent findings offer a valuable source of resistance for breeding programs and highlight the need for further research to develop enhanced cultivars and effective management strategies for bacterial leaf streak (BLS) in wheat.

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