An Effective Method for Bacterial Leaf Streak Disease Severity Estimation in Controlled and Field Environments in Small Grains

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Method Article

Keywords: Bacterial Leaf Streak Disease, Wheat, Xanthomonas translucence spp. Infection Assay, Greenhouse

Posted Date: November 24th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-8098615/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Wheat (*Triticum aestivum*) is one of the most economically important crops in the United States. However, over the past two years, wheat production has suffered up to a 40% reduction in final yield due to pathogen infections worldwide. A major emerging threat in the Great Plains and Canadian Prairies, including South Dakota, North Dakota, and Minnesota, is bacterial leaf streak (BLS)/black chaff disease caused by *Xanthomonas translucens* spp., which has led to substantial yield losses in the last decade. Absence of both effective chemical controls and competitive highly resistant varieties makes BLS disease management very difficult. A critical step missing in this process is the establishment of a reliable and reproducible infection protocol for resistance evaluation under both controlled and field conditions. Currently, no protocols are published, and the methods published as part of research manuscripts lack detailed procedures, equipment specifications, and have major drawbacks for applications limited to controlled environment and discrepancies in field disease ratings scales. Therefore, we are presenting here a robust and reproducible BLS disease infection protocol and, disease severity rating scale for estimation of BLS disease in both controlled and field conditions.

Results

After Three days of inoculation with *X. translucens* pv. *undulosa* (*Xtu*), wheat plants developed initial water-soaked symptoms at inoculation sites. Over seven days, symptoms progressed to chlorosis and necrosis, frequently covering entire leaves of highly susceptible genotypes, whereas limited to no symptoms on resistant genotypes. Disease severity was consistently scored on a 1–9 scale, enabling clear differentiation of resistant, moderately resistant, and susceptible genotypes. Pathogen re-isolation confirmed infection fidelity. Field validation at the booting stage produced comparable symptom progression on flag leaves, with severity scored at 7, 14, and 21 days post-inoculation. The same protocol was successfully adapted for *Pantoea ananatis* and *Xanthomonas prunicola*, demonstrating the adaptability of the method. The protocol was repeated across five independent trials and produced reproducible results in both controlled and field environments.

Conclusion

We describe a simple, reproducible, and cost-effective inoculation protocol for evaluating BLS severity in wheat. The method reliably distinguishes resistance responses across environments and can be extended to other bacterial pathogens affecting small grains. Its affordability, accessibility, and reproducibility make it a valuable tool for large-scale germplasm screening and resistance breeding.

Key Features

• A detailed and systemic infection protocol is devised for different cultivars of wheat.

• Plants can screen at seedling and adult-plant stage.

• No specific equipment required.

• Using an inexpensive pipeline to ensure uniform symptoms.

• This protocol is validated for other bacterial species that are reported to cause bacterial leaf streak symptoms on small grains (*Pantoea* spp and *Xanthomonas prunicola* on small grains (wheat and Barley).

Background

The global output of wheat and other cereals (barley, oats and corn) is hampered by bacterial plant disease (1). Bacterial leaf streak (BLS) disease of cereals (wheat, barley, rye, oat, and triticale) is caused by *Xanthomonas translucens (Xt)*. Typical symptoms of BLS include water-soaked, translucent streaks that progress into leaf necrotic lesions. In severe infections, the entire plant shows a blight appearance (2); thus this disease is also called bacterial blight. BLS on the grain heads leads to the darkening of kernels and is named black chaff. In the last decade, BLS incidence has dramatically increased in the Northern Great Plains (3, 4). In severe infections, the yield losses can reach up to 60 % on susceptible varieties (5). On average, in the US, about 14.5 million bushels of wheat are lost due to BLS and Black Chaff, which account for $78.5 million (Crop Protection Network, 2024). In 2018, and 2020 about 24 and 23 million bushels were lost to BLS, which accounted for $121 and $119 million lost in revenues (Crop Protection Network, 2024) (**Figure 2 and Table 1**).

Table 1 Estimated annual yield and economic losses in wheat due to bacterial leaf streak (BLS) in the United States from 2018 to 2024.

Year	% Loss	Bushels Lost	$(USD) Loss	$(USD) Loss per Acre
2018	1.49%	23,585,844	$121,150,030	$3.10
2019	1.01%	18,251,357	$82,875,296	$2.01
2020	1.40%	23,047,060	$119,654,927	$2.98
2021*	0.03%	540,356	$3,491,449	$0.09
2022	0.49%	7,073,301	$64,979,800	$1.56
2023	0.43%	7,023,865	$51,561,573	$1.14
2024	0.46%	8,960,240	$49,751,075	$1.18
TOTALS		88,482,023	$493,464,149	
AVERAGE	0.72%	12,640,289	$70,494,878	$1.72

*An odd year, where due to severe drought, data has not been collected on yield losses.

Existent Infection Methodologies and Disease Severity Estimation

Bacterial leaf streak disease management options are very limited. Several studies used chemicals like silicon (6) and copper containing compound (7) and more recently, 21 plant-protection products were evaluated to control BLS disease (8). However, none of the compounds showed consistent suppression of the BLS disease. Therefore, the most sustainable disease management solution is to develop a resistant variety against *X. translucens*. However, there is lack of detailed, robust, and reproducible protocol available to researchers for disease development. One method involved coating injured grains with the bacterial suspension, but it was considered labor intensive and unsatisfactory for pathogenicity testing (9). Another efficient approach is injecting a suspension into the leaf whorl of young plants (with 4-5 leaves) or into the boot of older plants using a hypodermic syringe. This technique has been validated at CIMMYT is often followed by incubation in a humid chamber (10). Other proposed methods include vacuum application (11). Using a specialized device to inject solution into thin plant leaves, which involves tongue seizing forceps, rubber stoppers and a hypodermic needle and syringe (12). Although leaf clippings and detached leaves have been used for pathogenicity testing using plants, inoculation and subsequent tests are generally considered effective (12). But all these methods are costly and time consuming. Another major issue in disease scoring lies in the inconsistency of rating scales used. The 1 to 5 scale from Acharya et al., (13) considers only water soaking and chlorosis, which does not represent the full range of symptoms observed under greenhouse and field conditions. In contrast, the 1 to 9 scale proposed in this protocol is more descriptive, employed for both seedling and adult-plant stages, and is consistent with the evaluation methods used for other important wheat diseases such as stripe rust, stem rust, spot blotch, and Soil-Borne Mosaic Virus (USDA Hard Winter Wheat Regional Nursery Programs). This broader and standardized approach provides a more reliable basis for disease estimation and would be widely accepted for application by both pathologists and breeders. Therefore, the protocol presented here is very timely, has the potential for wide adaptability and fills an essential knowledge gap for research needs against the bacterial leaf streak disease affecting small grains.

Results and Discussion

Bacterial Leaf Streak Disease Scoring in Controlled Environment

Three days after inoculation with *Xanthomonas translucens* pv. *undulosa* (*Xtu*), the earliest symptoms were observed as water-soaked spots at the site of pathogen entry. By seven days post-inoculation, these lesions progressed into chlorotic areas and eventually developed necrotic tissue. In highly susceptible genotypes, the necrosis could expand to encompass the entire leaf. Symptom development was reliably captured using a 1–9 visual scoring scale (Fig. 3), which allowed differentiation of resistant and susceptible responses. Diseased leaves were collected and brought to the laboratory, where the presence of *Xtu* was confirmed through pathogen isolation, validating that the observed symptoms were indeed caused by the inoculated bacterium. This assay was repeated five times, and the results were highly consistent, demonstrating the robustness and reproducibility of the inoculation method.

Bacterial Leaf Streak Disease Scoring in Field Environment

Field evaluation focused on the flag leaf, which is critical for grain filling and particularly sensitive to pathogen-induced damage. Disease severity was quantified using a standardized 1–9 scale (Fig. 4), where scores 1 indicated resistance and scores 9 indicated susceptibility. Plants with scores of 1–3 exhibited either no visible symptoms or only faint streaks restricted to a small portion of the leaf, indicative of resistant reactions. Moderate susceptibility (scores 4–6) was characterized by distinct water-soaked streaks affecting less than half of the leaf area. Highly susceptible plants (scores 7–9) showed extensive streaking and necrosis, often coalescing into large, blighted areas that covered most or all the flag leaf. The use of this standardized scoring system enabled reliable comparison of disease responses across multiple genotypes and field replicates, providing a clear framework for identifying resistant and susceptible wheat lines.

Applications of the Protocol in Multiple Field Trials of Wheat

We conducted multiple field trials in hard red spring and winter wheat and durum wheat and barley to evaluate disease severity following artificial inoculation with *Xtu*, *Xanthomonas prunicola, Pantoea ananatis*, and *Pantoea agglomerans*. In one of the experiments conducted, we used the same set of wheat genotypes and inoculated across environments to assess the consistency of this protocol, symptoms and host response. In wheat, inoculation with *Xtu* produced characteristic bacterial leaf streak (BLS) symptoms, with water-soaked lesions that later became necrotic and streak-like, particularly under favorable environmental conditions. In the inoculated field plots, clear differences between resistant and susceptible wheat genotypes were observed (Fig. 5A). The resistant and susceptible lines grown side by side demonstrated the accuracy and consistency of the inoculation protocol. Susceptible lines exhibited rapid disease progression, leading to coalesced streaks and premature leaf senescence. In severe cases, entire leaves dried early, resulting in substantial canopy damage and potential yield reduction (Fig. 5B). Moreover, blight-like symptoms extended beyond the foliage, as black chaff appeared on wheat spikes, marked by dark, water-soaked, and necrotic lesions on glumes and awns (Fig. 5C). These symptoms were most pronounced in genotypes displaying high foliar susceptibility to *Xtu*, suggesting systemic infection and pathogen movement within the plant.

In contrast, inoculation with *X. prunicola* had more symptoms compared to *Xtu* and *P. ananatis* which showed the complexity of this disease, *X. prunicola* produced more necrotic spots than translucence streak, while *P. ananatis* symptoms were generally less severe across replicates. While chlorosis and occasional necrotic flecks were observed on *P. ananatis* inoculated wheat genotypes, the extent of coalescing necrotic leaves did not approach to a score of 9. Thus, *P. ananatis* strain tested, failed to induce clear streak symptoms under field conditions, indicating either weak pathogenicity or a role more consistent with opportunistic colonization.

Collectively, these results confirms that *Xtu* remains highly virulent for both wheat and barley in the US, capable of causing both foliar BLS and black chaff on spikes and with new species of Xanthomonas

detected in the US, the BLS disease is complex and emerging in both South and North American continents (14, 15).

Black Chaff Symptoms caused by Bacterial Leaf Streak Disease on spikes

In addition to foliar BLS symptoms, clear manifestations of black chaff were observed on wheat spikes following inoculation with *Xtu*. Symptoms included dark brown to black streaks and blotches on the glumes and awns, with lesions often extending longitudinally along spikelet (Fig. 6A). In severely affected spikes, symptoms intensified, with coalesced streaks and widespread necrosis on glumes and awn, giving a pronounced blackened appearance (Fig. 6B).

The severity of black chaff was positively correlated with foliar susceptibility, as highly susceptible lines exhibiting intense BLS also showed more severe black chaff on spikes. Inoculations with *X. prunicola*, *Pantoea ananatis*, and *Pantoea agglomerans* did not reproduce typical black chaff symptoms, producing either mild discoloration or no visible spike damage. These findings confirm that black chaff is a diagnostic symptom of *X*tu infection in wheat and highlight its importance as a phenotypic marker for assessing susceptibility and yield impact under field conditions.

Application of Developed Methodology in Controlled Environment

The inoculation methodology successfully induced consistent BLS symptoms under greenhouse conditions. Water-soaked lesions appeared within three days of inoculation, progressing to chlorosis and necrosis within 7–10 days. Disease severity, scored on a 1–9 scale, showed a wide range of responses across genotypes. Violin plots (Fig. 7A, B) demonstrated clear separation between resistant, moderately resistant, and susceptible groups. Resistant genotypes were concentrated at lower scores (1–3), while susceptible lines showed higher distributions (7–9). The statistical analysis confirmed these phenotypic differences. In Experiment 1, variation due to treatment was marginally significant ($F = 2.88$, $p = 0.0571$), indicating some experimental influence (**Supplementary Table 3**). However, in Experiment 2, highly significant differences were observed among genotypes ($F = 7.30$, $p < 2e - 16$), with genotype effects explaining most of the variance compared to residual error (**Supplementary Table 4**). These findings demonstrate that while environmental or experimental variation can influence disease expression, the methodology consistently discriminates among resistant and susceptible genotypes under greenhouse conditions.

Field Experiments

Field validation under natural infection pressure supported the robustness of the developed method. Both flag and bottom leaves were evaluated, with bottom leaves generally exhibiting higher severity scores, reflecting natural disease progression (Fig. 8A, B). The bar chart (Fig. 8A) showed that most

genotypes clustered around moderate severity classes (4–6), while violin plots (Fig. 8B) revealed clear distinctions between resistant and susceptible lines.

ANOVA further confirmed significant variation among experimental factors. In the first field trial, strong effects of trial ($F = 67.98$, $p < 2e-16$) and day ($F = 1296.0$, $p < 2e-16$) were detected, with a smaller but significant trial × day interaction ($F = 2.74$, $p = 0.0118$) (**Supplementary Table 5**). These results indicate that disease development was influenced by both environment and scoring time, but the magnitude of genotypic effects remained much stronger. In Experiment II, highly significant differences among wheat lines were observed ($F = 2.13$, $p < 2e-16$), confirming that genetic variability was the primary determinant of BLS severity (**Supplementary Table 6**).

Together, these results demonstrate that the methodology is reproducible in both greenhouse and field conditions, allowing reliable identification of resistant, moderately resistant, and susceptible genotypes. The combined evidence from distribution patterns and ANOVA strongly supports its use in large-scale resistance screening programs.

Discussion

The lack of a reliable and reproducible inoculation protocol has long been a major constraint in advancing research on bacterial leaf streak (BLS) and black chaff of small grains. The inability to consistently reproduce infections under controlled conditions has limited progress in elucidating host-pathogen interactions, identifying resistance sources, and accelerating breeding for resistant cultivars. Although BLS has been recognized in the United States for several decades, it has emerged as a serious threat in the Northern Great Plains in recent years (16). Despite increasing importance, reproducible inoculation methods remain challenging, with most previous approaches such as needleless syringe or vacuum infiltration and leaf clipping, requiring specialized facilities and operator skill while yielding variable amounts of bacteria inoculum, disease severity outcomes, labor intensive and time-consuming (3, 7). The disease rating scales currently published consider only leaf water-soaking and chlorosis and do not account for necrosis symptoms. Such inconsistency in both inoculation protocols, disease severity and rating scales has hindered the fulfillment of Koch's postulates and slowed systematic screening for resistance. Earlier studies demonstrated that inoculation method, bacterial concentration, and plant growth stage significantly influence symptom development and disease severity. Syringe or vacuum infiltration methods force bacterial entry into leaf apoplast but often cause mechanical injury and fail to represent natural infection capabilities of bacterial strains. Conversely, spray inoculations more closely mimic field conditions but often result in uneven droplet distribution and poor bacterial adherence to the leaf surface, particularly when surfactants are absent. Environmental parameters such as humidity, temperature, and dew duration after inoculation also critically affect lesion formation [1, 3]. In several reports, bacterial suspensions below 10^5 CFU mL^{-1} failed to produce consistent symptoms, whereas higher concentrations (≥ 10^6 CFU mL^{-1}) induced typical streaking in susceptible cultivars (13). These findings underscore the importance of standardizing inoculum preparation, delivery, and environmental conditions for reliable infection establishment.

The air-pressure spray inoculation method described in this study addresses all of these limitations by providing a scalable and reproducible approach suitable for both controlled and field environments. Incorporating a non-ionic surfactant into the bacterial suspension ensures uniform wetting and adhesion of droplets to leaf surfaces, facilitating bacterial penetration through stomata and cuticular openings. The controlled air pressure allows consistent droplet size and uniform distribution of inoculum across leaves and spikes, producing reproducible symptom development across genotypes. In susceptible cultivars, water-soaked lesions typically appeared three days post-inoculation and progressed to chlorosis and necrosis within seven days, whereas resistant lines exhibited minute, restricted lesion expansion. This consistency supports the method's utility for screening germplasm and differentiating resistance levels. Notably, when applied at booting, the protocol also successfully reproduced black chaff symptoms on spikes, providing a comprehensive assessment of both foliar and reproductive stage susceptibility, a feature absent from many previous studies (7, 10).

The broader applicability of this inoculation system was demonstrated by its successful adaptation to other bacterial pathogens, including *X. prunicola* and *P. ananatis.* While *Xtu* remains the primary causal agent of severe BLS in U.S. wheat, emerging reports of *X. prunicola* and *Pantoea* species highlight a complex disease etiology (3). The adaptability of the current protocol to multiple pathogens emphasizes its flexibility and potential use for comparative pathogenicity testing and cross-host inoculation studies in small grains. A further contribution of this work is the standardization of disease scoring. Previous studies often employed a 1–5 scale that captured early water soaking and chlorosis but failed to represent advanced necrotic or spike symptoms (17). The 1–9 scale implemented here provides higher resolution and aligns BLS assessment with scoring systems used for other major wheat diseases such as stripe rust, stem rust, soil-borne mosaic virus and spot blotch. This harmonization enables comparative evaluation of resistance across diseases and improves the integration of phenotypic data into practical applied pathology and breeding. Despite these advantages, the method requires careful management of inoculum quality, environmental humidity, and plant growth stage to ensure optimal results. Field disease severity was greatest when inoculation occurred at booting, suggesting that physiological and anatomical changes during spike emergence influence bacterial colonization. This observation aligns with previous reports that host developmental stage affects infection success and symptom expression (10, 17). Further refinement is needed to define the latent epiphytotic period, quantify bacterial proliferation within tissues during pathogenic stage, and assess the influence of environmental variability across locations.

Overall, the air-pressure spray inoculation method represents a significant methodological advance for BLS and black chaff research. Its reproducibility, scalability, and adaptability make it suitable for both fundamental studies on *Xanthomonas translucens* pathogenesis and large-scale resistance screening in pathology and breeding research. Moreover, the approach can be extended to related cereal hosts and emerging bacterial pathogens, positioning it as a versatile and standardized tool for the wider plant research community.

Conclusions

This study establishes a reliable, reproducible, and cost-effective assay for bacterial leaf streak (BLS) evaluation in wheat. The protocol consistently reproduced characteristic symptoms under both greenhouse and field conditions, enabling clear differentiation between resistant and susceptible genotypes using a standardized 1 to 9 scale. Multiple repetitions confirmed the robustness, accuracy and adaptability of this method, not only for *Xtu* but also for related pathogens such as *X. prunicola* and *P. ananatis*. Unlike earlier approaches that were not accurate, labor-intensive, or dependent on specialized equipment, this assay is accurate, simple, scalable, and efficient for large germplasm screening. By integrating greenhouse and field evaluations, the protocol provides a powerful tool to accelerate discovering key components of the pathogen effectors, host resistance genes, plant-bacterial interactions, yield loss estimation, and breeding efforts aimed at developing BLS-resistant varieties. Importantly, it also demonstrates potential utility for other cereals and bacterial diseases, broadening its impact on cereal pathology and crop improvement.

Key Implications

The development of this simple yet robust assay directly benefits plant biologists, breeders, pathologists, and agronomists by offering an efficient way to screen wheat germplasm for resistance against BLS. Its flexibility across pathogens and cereal crops provides a unified framework for studying streak-like bacterial diseases, which are increasingly threatening cereal production worldwide. Most importantly, this protocol bridges-controlled environment and field evaluations, ensuring reliable disease phenotyping that can accelerate research. Ultimately, the adoption of this method will contribute to accelerated breeding for BLS resistance, sustainable wheat production, reduced yield losses, and enhanced global food security.

Material and Methods

Xtu Isolation and Characterization

The diseased samples were collected from the Volga SDSU wheat field station and the Dakota Lakes Research Farm on July 9th, 2023. The diseased samples were cut into small pieces and surface sterilized with 10% bleach and 70% ethanol, followed by ddH2O washing (7). The leaf was crushed into ddH_2O and with the help of a sterile loop, streaked on nutrient agar (NA) (**Supplementary Table 1**) and kept in an incubator for 2 days at 28°C. The single yellowish colony streaked separately on NA plates for pure culture. The DNAs were extracted from all single colonies by using the protocol of (18) for Multiplex primer amplification specific to *Xanthomonas translucence* spp. and 16S rRNA gene sequencing.

Culture Preparation and Greenhouse Inoculation

After the confirmation of *Xtu AL-1029* (Accession No. PQ524066) (19) the secondary culture was grown in NA media supplemented with 10% sucrose. Bacterial cells were then suspended in 1X phosphate saline buffer (PBS) (pH 7.4) to prepare an inoculum. To achieve an optical density (OD_{600}) of 0.4, or

around 1×10^8 cfu/ml, the cell concentration was adjusted. Four drops of Tween-20 (polyoxyethylene sorbitan monolaurate, Sigma-Aldrich) were added as a surfactant to the bacterial solution before inoculation. The plants were grown in 9-inch cones with three replications, and each replication had two plants, total six plants were inoculated after 15 days of germination. The bacterial solution was equally sprayed over the plants till runoff for the real inoculation procedure. A spray cannon connected to an air pump with around 20psi of pressure. Following this, the infected plants were kept at room temperature and subjected to a 12-hour photo period in misting chambers for two days. The plants were moved and placed on flow trays within the greenhouse room at 80% humidity for seven days. The assessment of disease development was scored by a scale from 1 to 9 (1 for highly Resistant and 9 for highly susceptible), with an estimation made of the proportion of the plant area affected by the disease.

Field Inoculation Assay

The same protocol was used for inoculating the field trial. Inoculation was carried out at the wheat booting stage using an air-pressure sprayer, without the addition of carborundum powder. Disease severity was assessed at 7th, 14th, and 21st days post-inoculation (**Supplementary Fig. 1**), scoring the flag leaf on a 1–9 BLS disease scale. The same method was applied to screen *Xanthomonas prunicola* (15) and *Pantoea ananatis* (20) in the field. Since BLS is a disease complex, this protocol can be reliably used to evaluate all bacterial pathogens that produce streak-like symptoms on small grains plants. In summer 2024 and 2025, more than 1000 row plots of winter, spring and durum wheat and winter barley were inoculated with *AL-1029 Xtu* strain by following the same protocol. There was one repeated experiment in 2024 and 2025 where the same set of genotypes was inoculated with *Xtu*, *P. ananatis* and *X. prunicola* by following the same protocol.

Development of the new Methodology

Here, we provide a detailed, step-by-step procedure for small-scale, repeatable BLS infection testing in wheat. The procedure was created to treat wheat that had been infected with *Xanthomonas translucence* spp. It comprises a pre-propagation and simple harvesting process to generate a large enough quantity for an infection trial. The technique may be quickly established because of the thorough process of knowledge and ease of handling. The necessary tools are easily accessible and reasonably priced. In addition, we show that a particular resistance phenotype may be evaluated by combining our infection strategy with macroscopic and microscopic assessment techniques. Finally, the procedure is easily adaptable to various host plants and bacterial and fungal diseases.

- Diseased wheat leaves showing typical streak and necrosis symptoms were collected from the field (Fig. 9A).
- Collected leaves were cut into small pieces, surface-sterilized to eliminate epiphytic microbes, and macerated in 500 µL sterile water. The suspension was streaked onto nutrient agar (NA) plates and incubated at 28°C for 2 days (Fig. 9B).
- Single colonies were sub-streaked onto fresh NA plates to establish pure cultures; a process repeated three times to ensure purity. The identity of *X. translucens pv. undulosa* (*Xtu*) was

confirmed to be using published multiplex primers (2) and agarose gel electrophoresis (Fig. 9C).

- Pure *Xtu* cultures were streaked onto NA plates supplemented with 10% sucrose and incubated for 5–7 days to obtain maximum bacterial growth prior to inoculation (Fig. 9D).
- A 1× phosphate-buffer saline (PBS) solution (**Supplementary Table 2**) was prepared and autoclaved. Bacterial colonies were scraped from the NA plates, resuspended in 1X PBS, and adjusted to an optical density (OD_{600}) of ~ 0.4, corresponding to 1 × 10^8 cfu/mL. Four drops of Tween-20 were added per 100 mL of inoculum (Fig. 9E).
- Wheat plants were grown in 9-inch cones, each containing two seeds, with three replications (total: 6 plants per treatment). Plants were maintained under controlled conditions (23–25°C, 14 h light/10 h dark photoperiod) for 15 days, with adequate watering and fertilizer (Fig. 9F).
- Inoculation was performed 15 days after germination. The prepared inoculum was applied evenly to the plants using an air sprayer set at 25 psi (Fig. 9G).
- Inoculated plants were incubated in a humidity chamber at 25°C with 100% relative humidity and a 14 h light/10 h dark cycle for 48 h (Fig. 9H).
- Following incubation, plants were transferred to a transparent tent maintained at ~ 80% relative humidity with the aid of a humidifier, where they remained for 7–10 days to allow disease development (Fig. 9I).
- Disease severity was assessed 7 days post-inoculation using a 1–9 rating scale (Fig. 4) initial symptoms were gummy like exudates produced on leaf surface (Fig. 9J, K), these exudates progressed into translucence streak (Fig. 9L) which later turned necrotic lesion (Fig. 9M).

Abbreviations

BLS	Bacterial leaf streak
Xtu	*Xanthomonas translucence* pathovar *undulosa*
Xp	*Xanthomonas prunicola*
PBS	Phosphate buffer saline
cfu	Colony forming unites
AL1029	Ameen Lab strain 1029
DPI	Day post Inoculation
NA	Nutrient agar
SDSU	South Dakota State University

Declarations

Acknowledgements: We thank Mr. Jack Ingemansen, Steve Stein, Cody Hall, Chris Nelson, Julie Thomas and Dr. Dalitso Yabwalo for help with field planting, preparations and management of research plots.

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Contributions

MA planned and designed the experiments, conducted research, analyzed the data, prepared figures and wrote the manuscript. GA, MA and KG develop the protocol. MA and TP conducted the field trials and collected data. GA and KG gathered funding for the projects. SS, TP, KG and GA reviewed the manuscript, edited and provided the ideas for improvement of the technique and manuscript.

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Funding

This study was carried out with the support of the Agricultural Experimentation Funding SD00H754, SD00H717, and South Dakota Wheat Commission funding SA2400069 and SA2500056.

Ethics Declarations

Ethics approval and consent to participate

Not applicable

Consent for Publication

All the authors have reviewed and edited this manuscript and agreed to submit it to the Plant Method Journal for publication. This manuscript have not been submitted to any other journals and has not published in any other journal.

Competing interests

The authors declare that there are no competing interests.

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Figures

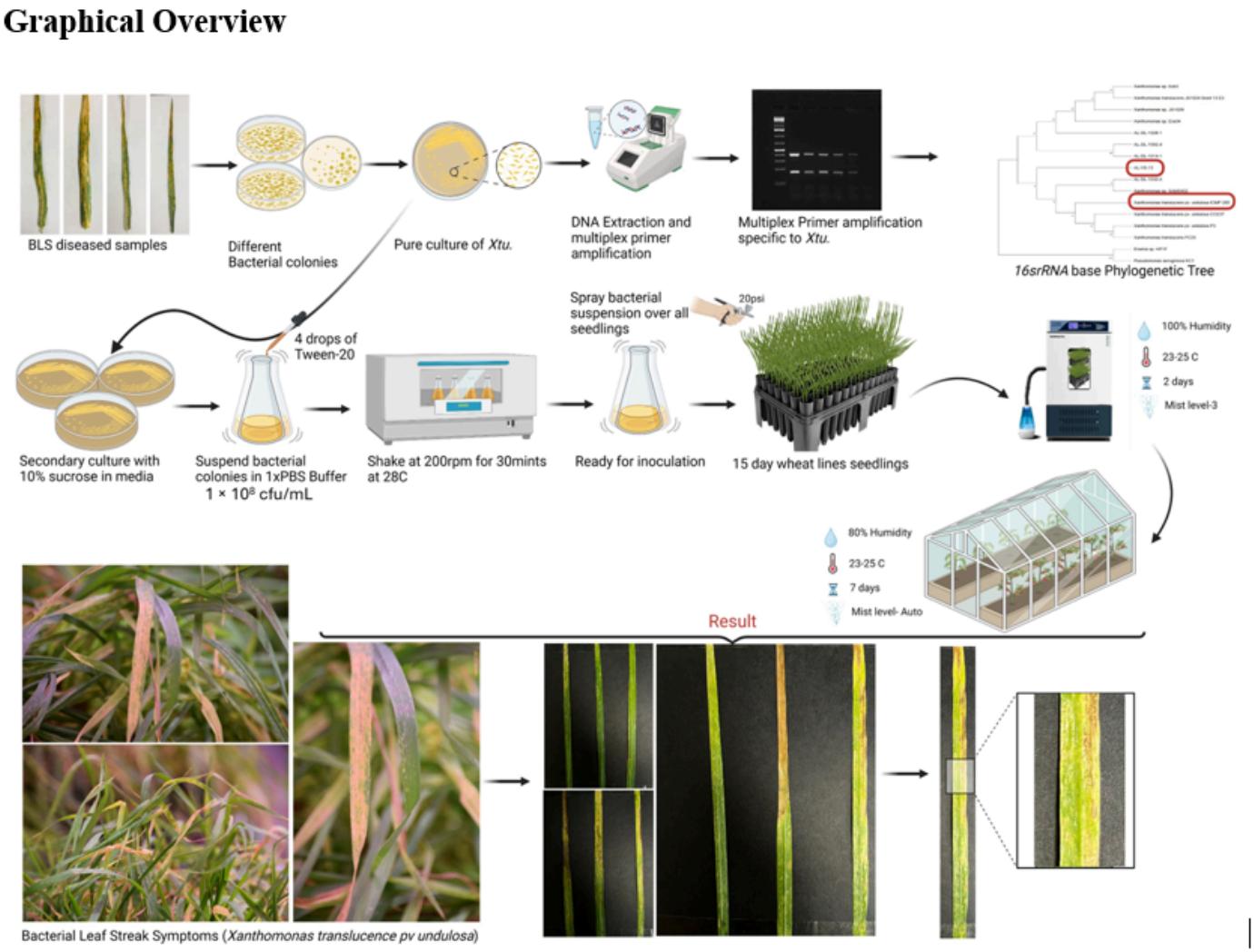

Figure 1

Graphical overview of infection assay for *Xanthomonas translucence* pv. *undulosa* under controlled Environment

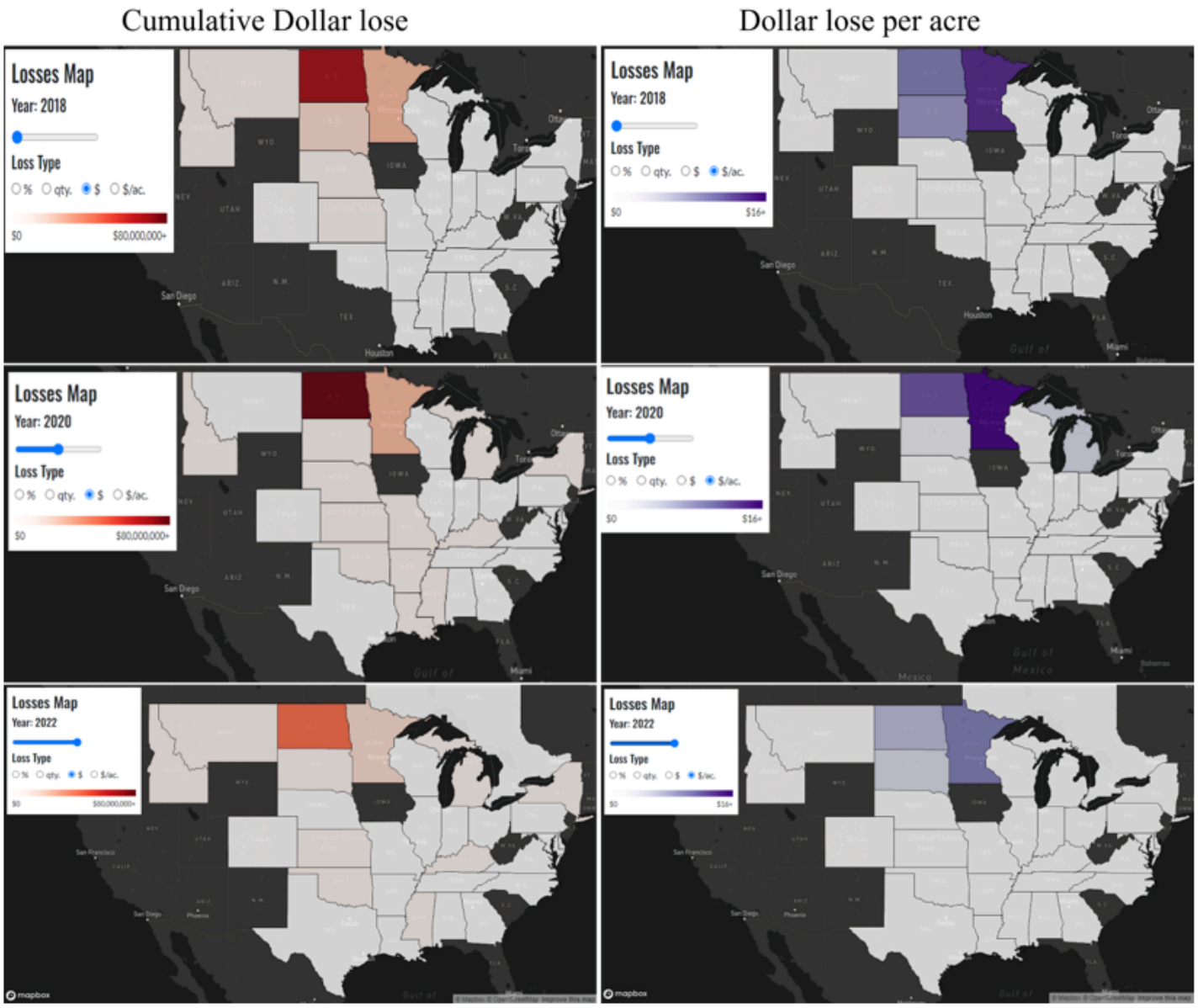

Figure 2

Distribution of crop losses across U.S. states in 2018, 2020, and 2022. Maps on the left show economic losses (USD), while maps on the right display losses per acre ($/ac). Loss intensity is represented by color gradients, with darker shades indicating higher losses (Crop Protection Network, 2024).

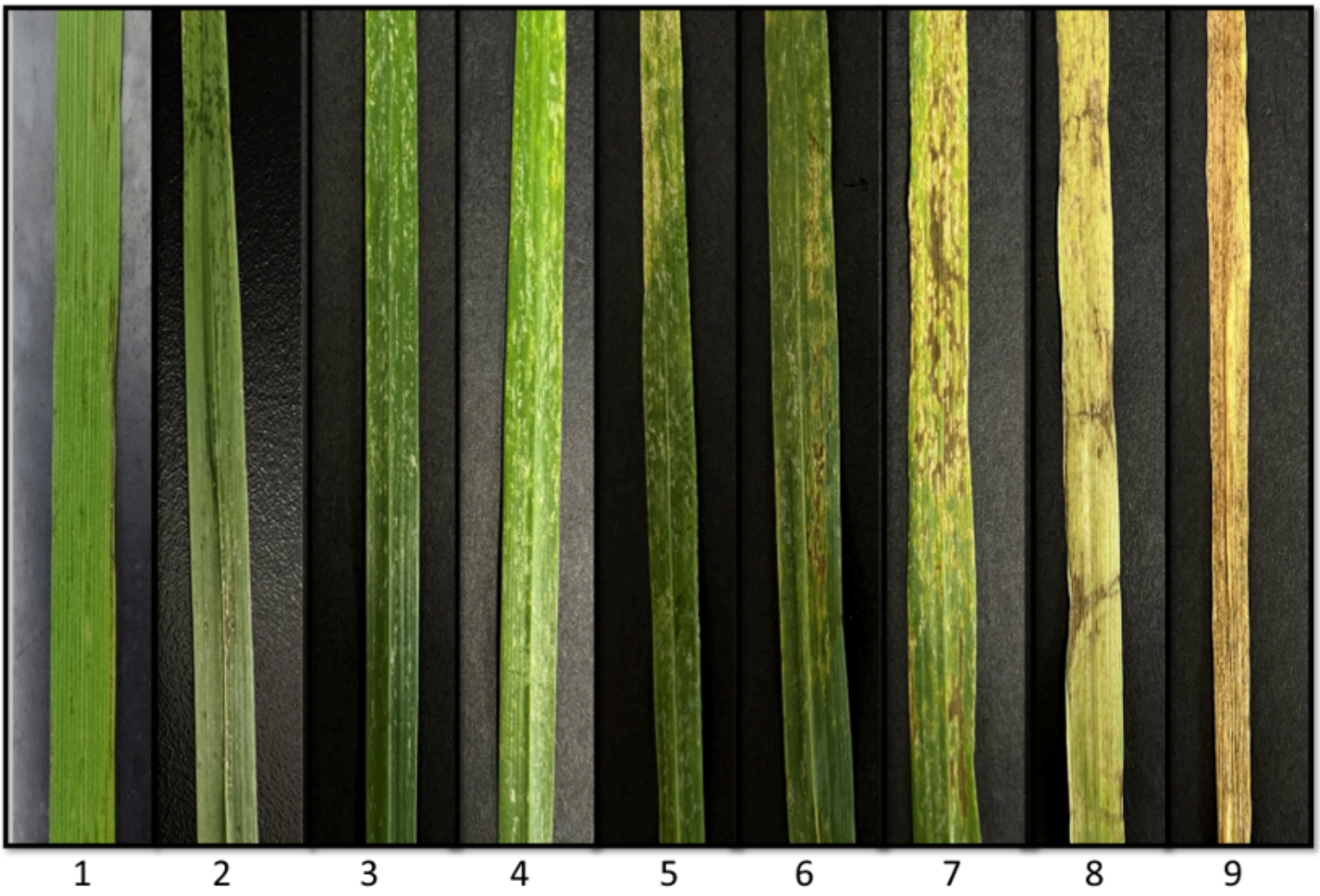

Figure 3

Disease severity ranging from 1 to 9 of third leaf of wheat plant after 15 days of inoculation. 1 indicate the resistant and 9 indicate the susceptible against *Xanthomonas translucens* pathovar *undulosa.*

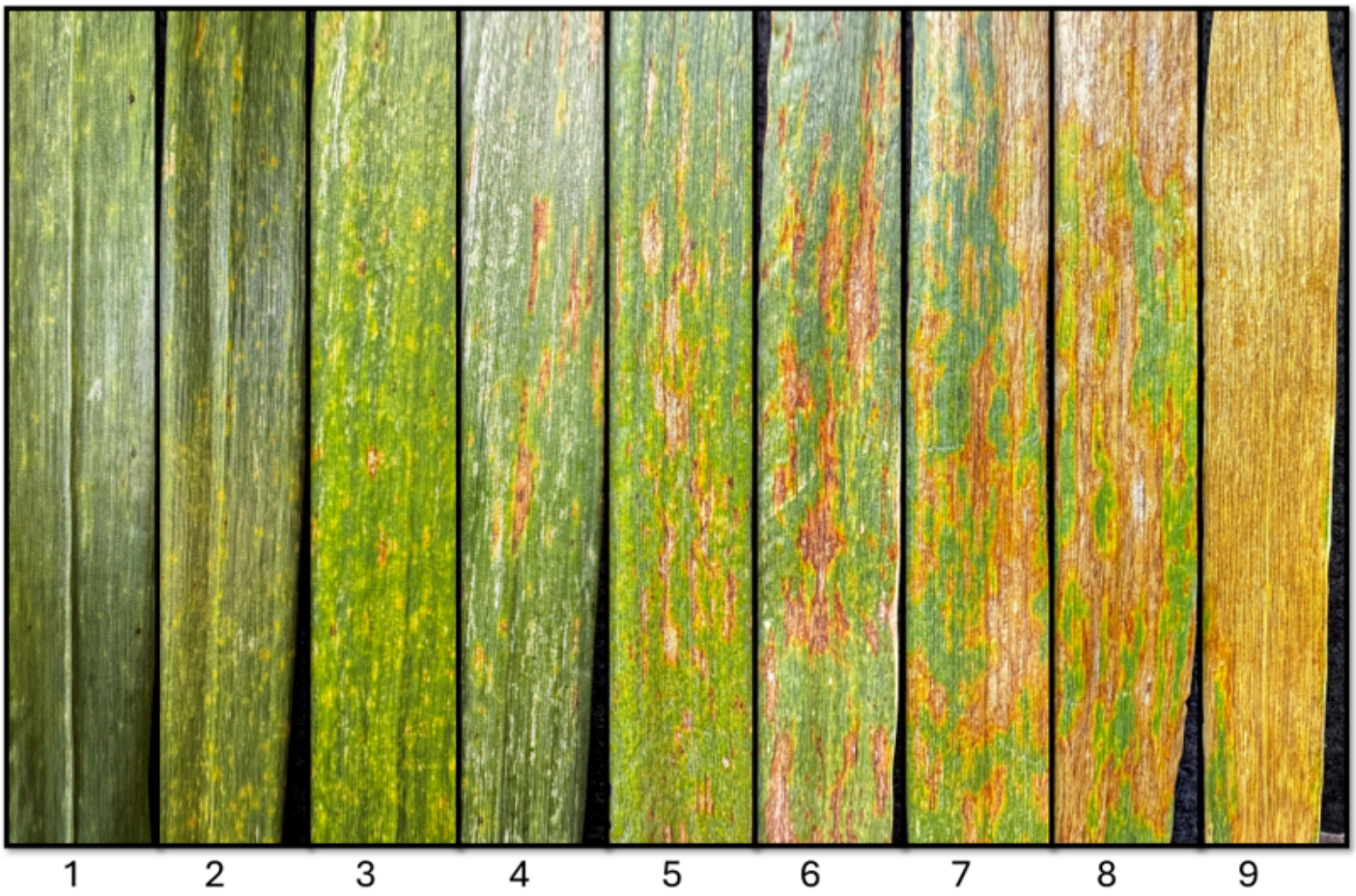

Figure 4

Bacterial Leaf Streak disease severity ranging from 1 to 9 of flag leaf of wheat plant after 21st days of inoculation. 1 indicate the resistant and 9 indicate the susceptible against *Xanthomonas translucens* pv *undulosa.*

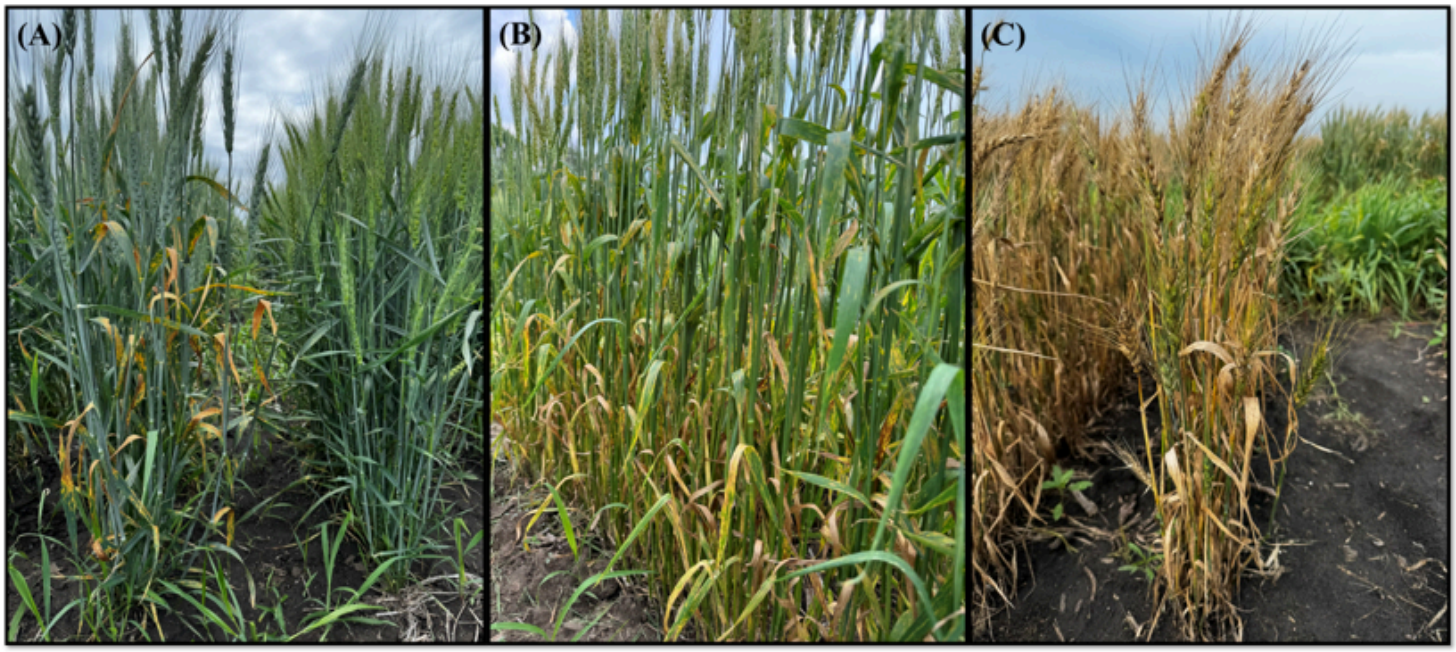

Figure 5

Inoculated wheat field plot showing the Bacterial Leaf Streak symptoms. (A) Comparison of resistant and susceptible wheat genotypes showing the accuracy of the protocol. (B) Moderately resistant wheat genotype (C) Blight-like appearance on susceptible wheat genotype.

Figure 6

Black chaff symptoms on wheat spikes following inoculation with *Xanthomonas translucens* pv. *undulosa*. (A)Early-stage black chaff showing streaking and blotching on glumes and awns. (B) Severe black chaff with extensive dark streaking and coalesced necrosis on spike tissues.

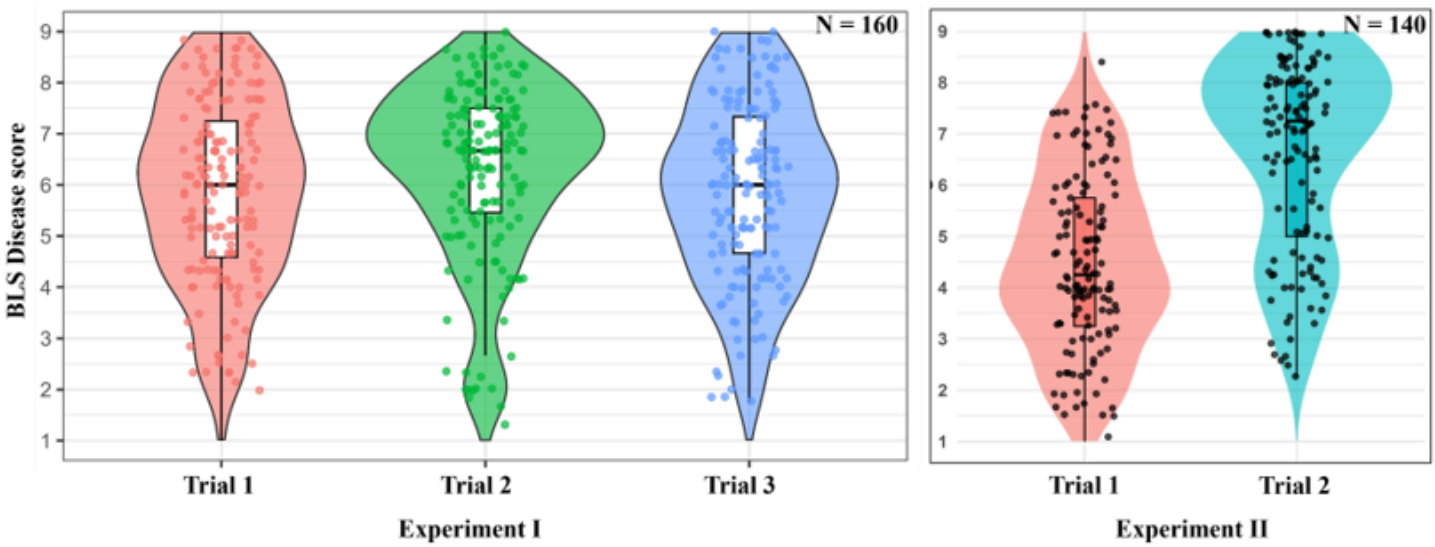

Figure 7

Distribution of bacterial leaf streak (BLS) disease severity scores in controlled environment represented by violin plots with embedded boxplots. The violin shape indicates the density of observations across the 1–9 rating scale, while individual data points are overlaid to show variation within groups. N shows the number of genotypes used for testing.

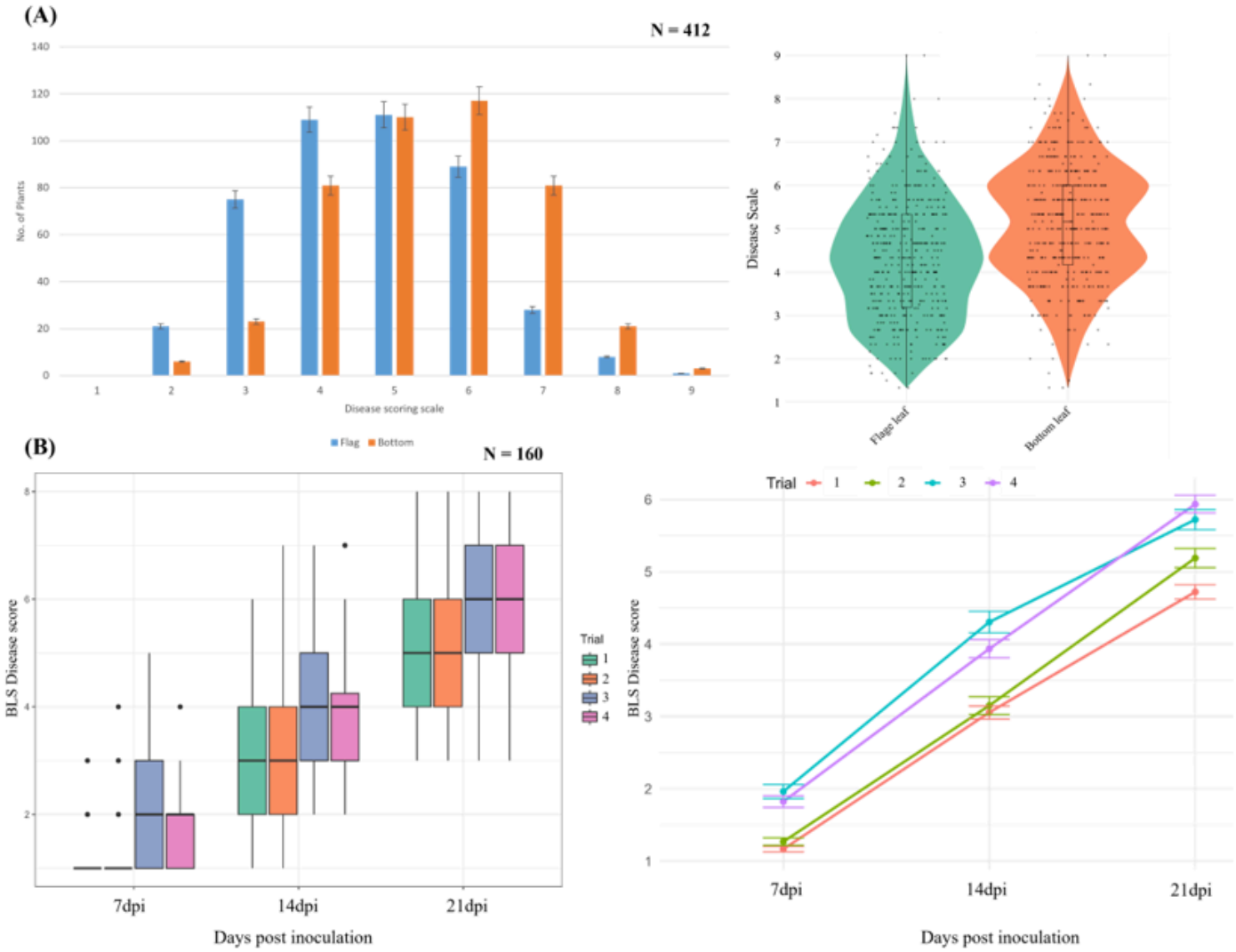

Figure 8

Field evaluation of bacterial leaf streak (BLS) severity in wheat. (A) Violin plots and frequency distribution of disease scores (1–9 scale) recorded on flag and bottom leaves across genotypes, showing differential levels of resistance and susceptibility in first experiment. (B) Boxplots and line graph comparing disease scores across independent trials, illustrating consistency and variation in disease responses for 2nd experiment. N shows the number of genotypes used for the BLS disease screening.

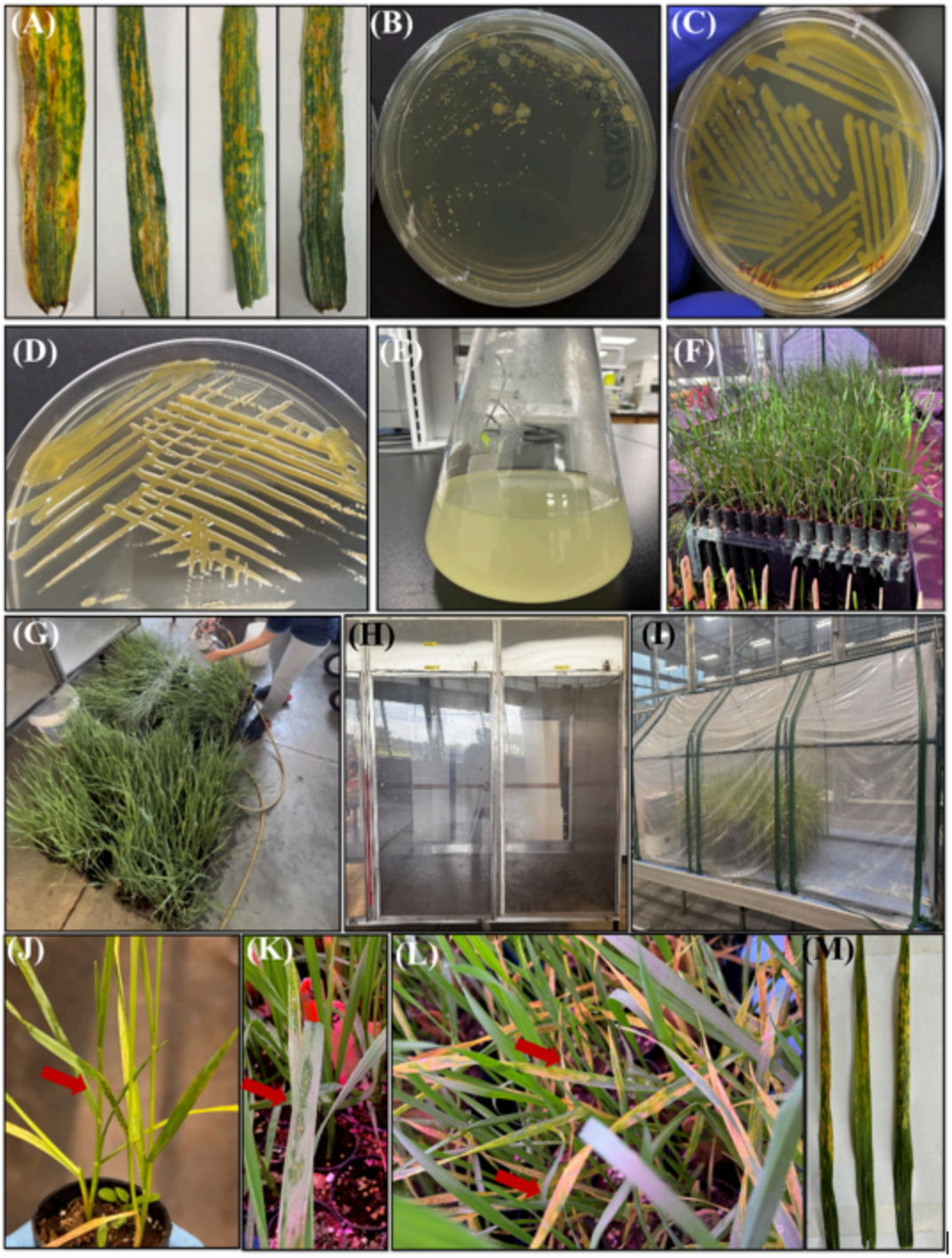

Figure 9

Stepwise workflow for bacterial leaf streak (BLS) inoculation and infection assay in wheat.

(A)Wheat leaves showing typical BLS symptoms with chlorotic and necrotic streaks. (B) Initial bacterial colonies from field-infected leaf tissue plated on nutrient agar. (C–D)Purified single colonies of *Xtu* obtained by streak plating. (E) Liquid culture of *Xtu* prepared in PBS buffer for inoculum. (F) Healthy wheat seedlings grown under controlled conditions prior to inoculation. (G)Inoculation of wheat plants using foliar spray with bacterial suspension. (H–I) High-humidity chambers used to maintain favourable

conditions for infection and disease development. (J–L) Early symptom development (red arrows) showing water-soaked lesions and streak formation on leaves. (M) Representative wheat leaves showing advanced BLS symptoms used for disease scoring.